# ANTIMICROBIAL ACTIVITY OF NATURAL SUBSTANCES

Edited by

**Tomasz M. Karpiński**

**and**

**Artur Adamczak**

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# Antimicrobial activity of natural substances

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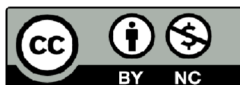


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# Antibacterial and antifungal influence of a melanin producer *Pseudonadsoniella brunnea* culture fluid

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## ABSTRACT

Fungicidal and bactericidal effects of melanin producer, black yeast *Pseudonadsoniella brunnea* (*Meripilaceae, Agaricomycotina*) culture fluid on test cultures of pathogenic fungi *Gibberella baccata* (anamorph: *Fusarium lateritium*), *Gibberella intricans* (anamorph: *Fusarium gibbosum*), *Gibberella pulicaris* (anamorph: *Fusarium sambucinum*), as well as *Fusarium incarnatum*, *Fusarium solani*, *Fusarium poae*, *Rectifusarium ventricosum* and phytopathogenic bacteria *Agrobacterium tumefaciens, Clavibacter michiganensis* subsp. *michiganensis, Pectobacterium carotovorum* subsp. *carotovorum, Pseudomonas syringae* pv. *syringae, Xanthomonas campestris* pv. *campestris* is for the first time established within this study.

**Keywords:** Pathogenic fungi; Phytopathogenic bacteria; Bioactive compounds; Antagonism; Biocontrol.

## 1. INTRODUCTION

The development of biotechnology, which is based on the potential of microorganisms

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in obtaining biologically active substances (BAS), which can be widely used in various fields of human activity, is one of the main areas of modern world science.

The fungi of the genus *Fusarium* Link are the most common pathogens and harmful fungal diseases of crops. Today, many species of the genus *Fuzarium* considered to be the members of the genus *Gibberella* Sacc. after the teleomorphic (sexual) stage. So *Fusarium verticilloides* (Sacc.) Nirenberg. (synonym *Fusarium moniliforme* J. Sheld.), now considered to be synonym to *Gibberella fujikuroi* (Sawada) Wollenw. complex. Fungi of the genus *Gibberella* are often pathogen of corn cobs disease and other crops (rice, corn, sugar reed, soybeans etc.) in the world, especially in areas with high humidity and in years with high rainfall (rain) (to more than 60% of corn harvest may be damaged by *Gibberella* in these areas) [1-6]. For most *Fusarium* species, the sexual cycle does not predominate in the field [7]. Fungi of the genus *Gibberella* (anamorphs of the genus *Fusarium*) can produce mycotoxins (the fumonisins), which cause a carcinogenic effect on animals and humans. The presence of these toxins in plants is subjected to strict control [3, 5, 8]. The bacteria of the genera *Pseudomonas* [9], *Pectobacterium* [10], *Xanthomonas* [11], *Clavibacter* [12], and *Agrobacterium* [13] are the most common pathogens and harmful diseases of crops too. Species of bacteria *Pseudomonas syringae* is actually represented by over 50 different pathovar strains, which is a set of bacterial strains with similar characteristics differentiated by their distinctive pathogenicity toward one or more plant hosts [9, 14]. The pathogen *Pseudomonas syringae pv. syringae* takes its name from the host from which it was first isolated, but strains that have been proved to be pathogenic to lilac also infect more than 44 plant species and there are strains with the same determinative characteristics that do not attack lilac [15]. In a comparative analysis of rpoD sequences from a comprehensive range of strains from the *P. syringae* complex, found that strains from hosts associated with particular pathovars are distributed widely in the *P. syringae* complex [16]. *Pseudomonas syringae* pv. *panici* is a phytopathogenic bacterium causing brown stripe disease in economically important crops worldwide [17]. The strains of *Pseudomonas syringae* that are pathogenic causing disease on their hosts through the release of toxins and cell wall degrading enzymes [18].

Bacteria belonging to *Pectobacterium carotovorum* cause soft rot disease in diverse vegetables and crops worldwide. These bacteria produce several different plant cell-wall degrading enzymes such as pectinase (PCWDEs), polygalacturonase and cellulase. Since *Pectobacterium* species can produce PCWDEs, soft rot disease can occur in the field as well as during transportation and storage. *P. carotovorum* subsp. *carotovorum* has a wide host range including potato, carrot, lettuce, onion and Chinese cabbage [19-21]. In Morocco,

approximately 95% of the *P. carotovorum* isolates from potato plants with tuber soft rot are *P. carotovorum* subsp. *carotovorum* [22]. The plant pathogen *Clavibacter michiganensis* subsp. *michiganensis* is a gram-positive bacterium responsible for wilt and canker disease of tomato [12]. *Agrobacterium tumefaciens* causes crown gall disease on various plant species by introducing its T-DNA into the genome. Therefore, *Agrobacterium* has been extensively studied both as a pathogen [13].

Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Conventional biogicides are widely used to manage fungal and disease of plants, with two major consequences. On the one hand, fungicide overuse threatens the human health and causes ecological concerns. On the other hand, this practice has led to the emergence of pesticide-resistant microorganisms in the environment [23-26].

Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market. Additionally, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Among these alternatives are those referred to as biological controls [23].

The microorganisms of different taxonomic and physiological groups and their metabolic products and the drugs created on the base on these biological substances are now widely used in many countries to protect crops from pathogenic microorganisms. This can significantly reduce the negative impact on the environment, including agrocoenosis which inflicted by chemicals (pesticides). The use of biological agents against pathogenic fungi and bacteria not only provides plant protection from diseases, but may stimulate their growth and development, enhance seed germination, increased productivity. The most common method of biocontrol of phytopathogenic microorganisms is search of antagonist microorganisms. The methods of biocontrol of phytopathogenic microorganisms are environmentally safe, cost-effective and do not disturb ecological [27-40]. For example, *Pectobacterium carotovorum* and *Pectobacterium atrosepticum* are dreadful causal agents of potato soft rot. Actually, there are no efficient bactericides used to protect potato against *Pectobacterium* spp.

Biological control using actinobacteria could be an interesting approach to manage this disease [41].

Thus, research aimed at finding BAS microorganisms to create on their basis of biological agents that protect plants from pathogens, is relevant direction. Special attention of researchers deserves microorganisms producers of BAS habitats of which are associated with extreme conditions. The stringent physical and chemical factors causing significant adaptive changes in microorganisms, accompanied by increased synthesis of a number of metabolites (potential BAS). For example, the metabolites of such Antarctic microscopic fungi found to be potent sources of antimicrobial and antifungal activity [42].

Microscopic fungi and bacteria with distinct physiological and biochemical characteristics, pointing the adaptation to adverse environmental conditions (synthesis and accumulation of lipids, expression of antagonistic properties in relation to other microorganisms) were found during a preliminary study of samples of moss, soil, lichen obtained from Ukrainian Antarctic expeditions [43]. The Antarctic black yeast-like fungus *Pseudonadsoniella brunnea* T.O. Kondratyuk et S.Y. Kondr. (Meripilaceae, Agaricomycotina) [44] synthesizes and excretes into a culture fluid dark pigment melanin. The first data on antioxidant, antibacterial, fungistatic wound healing properties of the gel containing 0.05% melanin ("Melanin-gel"), which was synthesized by *P. brunnea* are obtained. Application of the "Melanin-gel" on wound area enhanced wound cleaning from dead tissue and reduced eschar, stimulated the early growth of granulation tissue, and improved epithelialization of the wound [57].

The high fungicidal effect of melanin producer *Pseudonadsoniella brunnea* culture fluid on test cultures of pathogenic fungi *Fusarium oxysporum* Scherht. em. Snyder & Hansen and *Gibberella fujikuroi* (anamorph: *Fusarium verticilloides*) for the first time found in our previous studies [45, 46].

The purpose of this study was to find out the nature of influence of the culture fluid of *Pseudonadsoniella brunnea* on pathogenic fungi and bacteria.

## 2. MATERIAL AND METHODS

The pure cultures of pathogenic fungus from the collection of microscopic fungi of Taras Shevchenko National University of Kiev (international acronym of collection – FCKU) [47], and the phytopathogenic bacteria, as well as the culture fluid of Antarctic black yeast-

like fungus, melanin producers *Pseudonadsoniella brunnea* 470 FCKU [44] were used for this study.

The following fungi, i.e.: *Gibberella baccata* (Wallr.) Sacc. (anamorph: *Fusarium lateritium* Nees) 332 FCKU, *Gibberella intricans* Wollenw. (anamorph: *Fusarium gibbosum* Appel & Wollenw.) 147 FCKU, *Gibberella pulicaris* (Kunze) Sacc. (anamorph: *Fusarium sambucinum* Fuckel) 336 FCKU, *Fusarium incarnatum* (Desm.) Sacc. 330 FCKU, *Fusarium solani* (Mart.) Sacc. 329 FCKU, *F. solani* 331 FCKU, *F. solani* 334 FCKU, *Fusarium poae* (Peck) Wollenw. 339 FCKU, *F. poae* 340 FCKU, and *Rectifusarium ventricosum* (Appel & Wollenw.) L. Lombard & Crous 333 FCKU were used as test-cultures during our study. The fungi *Gibberella baccata* 332 FCKU, *Gibberella intricans* 147 FCKU, *Gibberella pulicaris* 336 FCKU were studied in anamorph stage. So hereafter in the discussion we will use names of anamorph stage of fungi mentioned.

The following phytopathogenic bacteria, i.e.: *Pseudomonas syringae* van Hall 590 FCKU, *Pectobacterium carotovorum* subsp. *carotovorum* 591 FCKU, *Xanthomonas campestris* 592 FCKU, *Clavibacter michiganensis* subsp. *michiganensis* 593 FCKU, *Agrobacterium tumefaciens* 594 FCKU were used as test-cultures during our study too. Thesebacteria were obtained from the D.K. Zabolotniy Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv and were grown on potato-dextrose agar (PDA) medium. Mentioned above bacterium cultures and some other bacterial isolates are included to the FCKU collection [47].

The standard potato-dextrose agar (PDA) culture medium was used for cultivation of phytopathogenic fungus. In the experimental process the zones of inhibition of fungal and bacterial pathogens (in mm) were assessed using the methods of well diffusion assay (the method of diffusion in agar) [48]. In the method of the well diffusion assay after stabilization of the PDA nutrient agar the medium surface was coated with 0.5 ml of spore suspension of each of the ten fungal pathogens and 0.5 ml of cell suspension of each of the five bacterial pathogens. Spores were washed-off from the initial medium with sterile water and placed in a sterile glass test tube. The suspension of test culture of fungi with density $1 \times 10^6$ conidia/ml was used. The density of the bacterial cell suspension ($5 \times 10^5$ cell/ml) - 0.5 per McF was determined using a densitometer (microbiological analyzer) Vitek-2 (Bio Merieux, France). Then, a small well (10 mm in diameter) in the center of agar in petri dish was created. This well was then poured with 100 μl of each examined of *Pseudonadsoniella brunnea* culture fluid treatment.

The zone of inhibition around the area of the well was measured after incubation at 28°C. Term of cultivation was 30 days at a temperature of 28°C for the fungal test-cultures and three days for bacterial test-cultures. The number of the experiments per treatment was 3 and the experiment was repeated twice.

Performance of *Pseudonadsoniella brunnea* culture fluid on test culture of fungus and bacteria was compared with the effect of the biocide of known class of quaternary ammonium compounds benzalkonium chloride (trade name Katamin AB) at a concentration of 3% for the active substance. The cultivation of *Pseudonadsoniella brunnea* was performed on a standard liquid culture medium the Maltese-extract broth (MEB, HiMedia Laboratories, India). The impact of *Pseudonadsoniella brunnea* culture fluid and benzalkonium chloride on fungal and bacterial cultures was determined by the diameter of the zones with absence of growth of test cultures of fungi that formed around the hole, where the test compound was added in culture. Cultures of phytopathogenic microorganisms without additions of *Pseudonadsoniella brunnea* culture fluid and biocide mentioned were served as control cultures.

Trinocular microscope Primo Star of Carl Zeiss and related morphometric computer program AxioVision 4.8 (Carl Zeiss) were used for study of morphological features of studied fungi. Calculation of arithmetic mean and standard deviation using Statistica 12 was used for analyzing morphometric parameters of *Gibberella fujikuroi*, and the diameter of the zones of growth absence.

## 3. RESULTS AND DISCUSSION

### 3.1. Influence of *Pseudonadsoniella brunnea* culture fluid on test culture of fungi

Within our study the *Pseudonadsoniella brunnea* culture fluid found to have fungicidal effect on the studied test cultures of pathogenic fungi *Gibberella baccata* (anamorph: *Fusarium lateritium* 332 FCKU), *Gibberella intricans* (anamorph: *Fusarium gibbosum* 147 FCKU), *Gibberella pulicaris* (anamorph: *Fusarium sambucinum* 336 FCKU), *Fusarium incarnatum* 330 FCKU, *Fusarium solani* (strains 329 FCKU, 331 FCKU and 334 FCKU), *Fusarium poae* (strains 339 FCKU and 340 FCKU), and *Rectifusarium ventricosum* 335 FCKU. Zones of growth inhibition of test cultures under the influence of the *Pseudonadsoniella brunnea* culture fluid found to be stable and did not change throughout the duration of the experiment. It was also established that the diameter of the zones without

growth of the test cultures of pathogenic fungi, exposed to fungicidal activity of *Pseudonadsoniella brunnea* culture fluid, is similar to the diameter of the sterile zones formed under the influence of biocide benzalkonium chloride (Table 1, Fig. 1).

**Table 1.** Antifungal effect of *Pseudonadsoniella brunnea* culture fluid and benzalkonium chloride on the test cultures of pathogenic fungi

| **The test cultures of pathogenic fungi** | **The diameter (mm) of growth inhibition zone (including well diameter)*** | |
|---|---|---|
| | ***Pseudonadsoniella brunnea* culture fluid** | **Benzalkonium chloride, 3% for the active substance** |
| *Fusarium lateritium* 332 FCKU | 59.8±0.2 | 65.1±0.1 |
| *Fusarium gibbosum* 147 FCKU | 45.6±0.25 | 48.2±0.15 |
| *Fusarium sambucinum* 336 FCKU | 48.5±0.3 | 49.7±0.2 |
| *Fusarium incarnatum* 330 FCKU | 49.8±0.25 | 55.6±0.2 |
| *Fusarium solani* 329 FCKU | 39.5±0.5 | 57.8±0.25 |
| *Fusarium solani* 331FCKU | 56.3±0.25 | 57.1±0.1 |
| *Fusarium solani* 334 FCKU | 46.7±0.2 | 43.9±0.1 |
| *Fusarium poae* 339 FCKU | 29.1± 0.15 | 45.6±0.2 |
| *Fusarium poae* 340 FCKU | 31.2±0.2 | 46.8±0.1 |
| *Rectifusarium ventricosum* 335 FCKU | 51.4±0.3 | 63.3±0.15 |

Note: *– diameter of well is 10 mm; ± Standard error of mean

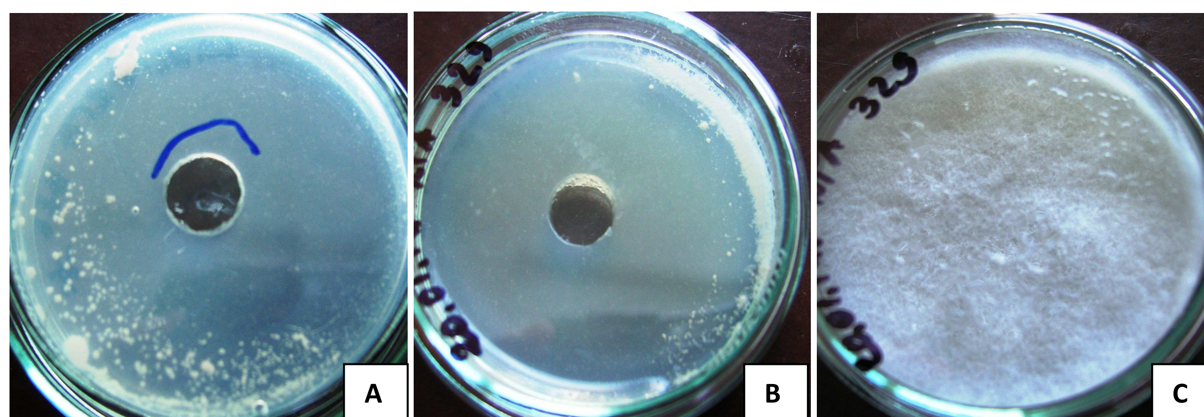


**Figure 1.** Zones of absence of *Fusarium solani* 329 FCKU growth under influence of *Pseudonadsoniella brunnea* culture fluid (A) and benzalkonium chloride (B). C = control culture. A – the reverse side of the Petri dish

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Thus all test cultures of the pathogenic fungi investigated appeared to show high sensitivity to influence of *Pseudonadsoniella brunnea* culture fluid and biocide benzalkonium chloride. The growth of pathogenic fungal cultures studied found to be often characterized mainly by individual colonies of different diameters under influence of tested substances (Fig. 2).

The color of the mycelium of *Fusarium poae* 339 FCKU, *F. poae* 340 FCKU cultures found to be also changed in comparison with mycelium color in control cultures of strains studied. That is especially well distinct at the reverse side of the Petri dish (Figs. 1 and 2).

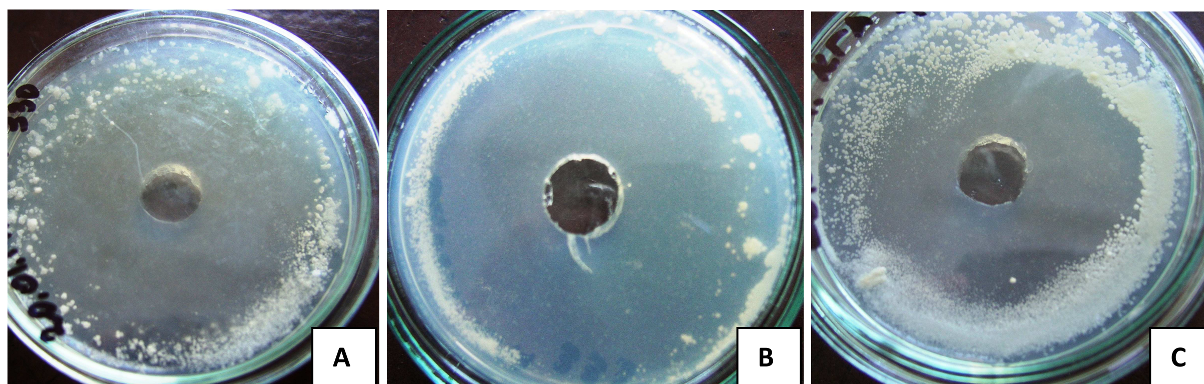


**Figure 2.** Effect of *Pseudonadsoniella brunnea* cultures fluid on the test culture: the absence of growth zone around the hole, where the *Pseudonadsoniella brunnea* culture fluid was added, growth by individual colonies. *Fusarium incarnatum* 330 FCKU (A), *Rectifusarium ventricosum* 335 FCKU (B), *Fusarium poae* 340 FCKU (C). B – the reverse side of the Petri dish

### 3.1. Influence of *Pseudonadsoniella brunnea* culture fluid on test culture of phytopathogenic bacteria

Within our study the *Pseudonadsoniella brunnea* culture fluid found to have biocidal effect on the test cultures of the phytopathogenic bacteria studied, except *Pectobacterium carotovorum* subsp. *carotovorum.* The impact of *Pseudonadsoniella brunnea* culture fluid on *Pectobacterium carotovorum* subsp. *carotovorum* 591 FCKU was evaluated as bacteriostatic: diameter of the growth inhibition zone after one day was similar to the zone of growth inhibition of the other bacterial test cultures (it was 39.3 ± 0.15). However zone of the growth inhibition of *Pectobacterium carotovorum* subsp. *carotovorum* decreased to 15.7 ± 0.1 at the third day (Table 2).

**Table 2.** Antifungal effect of *Pseudonadsoniella brunnea* culture fluid and benzalkonium chloride on the test cultures of phytopathogenic bacteria

| The test cultures of phytopathogenic bacteria | The diameter (mm) of growth inhibition zone (including well diameter)* | |
|---|---|---|
| | ***Pseudonadsoniella brunnea* culture fluid** | **Benzalkonium chloride, 3% for the active substance** |
| *Agrobacterium tumefaciens* 594 FCKU | 39.3±0.5 | 41.5±0.2 |
| *Clavibacter michiganensis* subsp. *michiganensis* 593 FCKU | 38.8±0.4 | 40.2±0.1 |
| *Pectobacterium carotovorum* subsp. *carotovorum* 591 FCKU | 15.7±0.1 | 39.7±0.15 |
| *Pseudomonas syringae* pv. *syringae* 590 FCKU | 38.5±0.5 | 39.9±0.2 |
| *Xanthomonas campestris* pv. *campestris* 592 FCKU | Growth absent | 41.1±0.3 |

Note: *– diameter of well is 10 mm; ± Standard error of mean

The test cultures of phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* 592 FCKU found to be the most sensitive to influence of *Pseudonadsoniella brunnea* culture fluid. The growth of this bacterium on the Petri dishes was not observed within our study (Fig. 3).

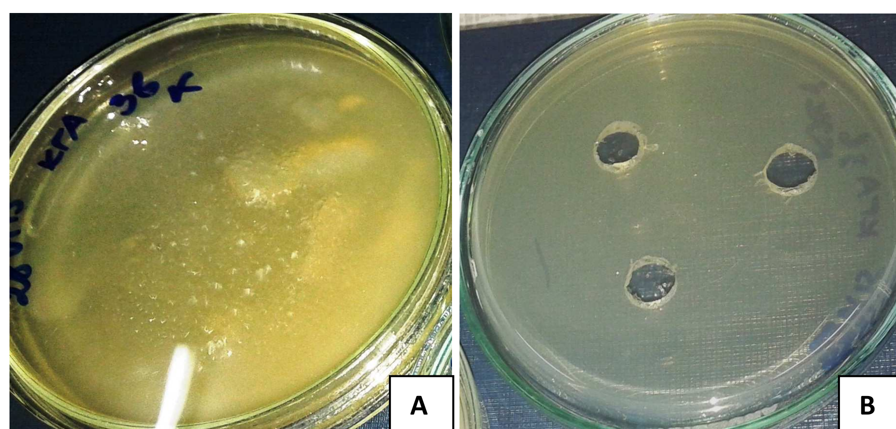


**Figure 3.** Effect of *Pseudonadsoniella brunnea* culture fluid on the test culture of phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* 592 FCKU. A – control, B – absence of growth (the reverse side of the Petri dish)

Thus, the different species and strains of test cultures of pathogenic fungi and phytopathogenic bacteria found to show different sensitivity to the effect of the compounds studied, as evidenced by data on the diameter of the zones of growth delay (Tables 1, 2) similarly to our previous study of the resistance of *Fusarium oxysporum* (strains 150 FCKU and 328 FCKU) [45] and *Gibberella fujikuroi* (anamorph: *Fusarium verticillioides*; strains 234 FCKU, 333 FCKU, 338 FCKU and 434 FCKU) to impact of compounds studied [46]. Our data on the diameter of growth inhibition zones of test cultures of pathogenic fungi were compared also with an antagonistic effect of fungi of the genus *Trichoderma* on pathogenic fungi of the genera *Gibberella* and *Fusarium* after data of other authors [32]. From this comparison the effect of *Pseudonadsoniella brunnea* culture fluid on pathogenic fungi of the genera *Gibberella, Fusarium* and *Rectifusarium ventricosum* can be assessed as highly active. It should be especially emphasized that increased synthesis of pigments by *Fusarium poae* 339 FCKU, *F. poae* 340 FCKU, similarly to those in previously investigated by us *Gibberella fujikuroi* strains 234 FCKU and 434 FCKU influenced by *Pseudonadsoniella brunnea* culture fluid was observed. It is known that under the influence of various factors, including stress, the fungi of the of genera *Gibberella* and *Fusarium* can synthesize important biologically-active pigments of interest in connection with a wide range of biological activity (antibacterial, antifungal, phytotoxic, insecticidal, and cytotoxic etc.) [49, 50].

According to our data obtaibed within this study the impact of *Pseudonadsoniella brunnea* culture fluid on the test cultures of the phytopathogenic bacteria is evaluated as antibacterial one.

Thus, fungicidal and bactericidal effects of melanin producer, black yeast *Pseudonadsoniella brunnea* culture fluid on test cultures of pathogenic fungi *Gibberella baccata* (anamorph: *Fusarium lateritium*), *Gibberella intricans* (anamorph: *Fusarium gibbosum*), *Gibberella pulicaris* (anamorph: *Fusarium sambucinum*), *Fusarium incarnatum*, *Fusarium solani*, *Fusarium poae*, *Rectifusarium ventricosum* and phytopathogenic bacteria *Agrobacterium tumefaciens, Clavibacter michiganensis* subsp. *michiganensis, Pectobacterium carotovorum* subsp. *carotovorum, Pseudomonas syringae* pv. *syringae, Xanthomonas campestris* pv. *campestris* is for the first time established within this study. This line of research is rather promising from our point of view.

Because one way of controlling phytopathogens is through the use of antagonistic microorganisms. The usage of metabolic substances of microorganisms which can inhibit the growth of other microorganisms (including pathogenic microscopic fungi and bacteria) as

biological methods of pest management of agricultural production will result in decreasing a negative impact on the environment that caused by biocidal chemicals.

The yeast-like fungus *Pseudonadsoniella brunnea* used by us within this study synthesizes and excretes into a culture fluid dark pigment melanin, which is abioactive compound. Earlier with the usage of the methods of the molecular phylogeny it was shown that *Pseudonadsoniella brunnea* shows the highest similarity to the members of the family *Meripilaceae* (*Poliporales, Basidiomycota*) [44]. The polyphenolcarbon complex of the *Pseudonadsoniella brunnea* culture fluid is a more than 90% of its.

It is also known that many polymeric and monomeric compounds, including polyphenols, tyrosine, indole, etc., play a significant role in the formation of melanin by various fungi [51-53]. It is known that polyphenolic compounds exhibit a strong biological effect, such as restorative, antioxidant, anti-inflammatory and wound healing. The effect of natural polyphenols derived from different parts of plant species on the growth of fungi and bacteria (especially on growth of bacteria of the *Pseudomonas syringae* complex) has been studied by many researchers [54, 55].

Our previous studies shown that the melanin, which is produced by Antarctic black yeast-like fungi *Pseudonadsoniella brunnea*, with oral administration has cyto-protective, stress-protective, radio-protective, antioxidant, anti-inflammatory, immunomodulatory and antitumor properties [56].

The first data on antioxidant, antibacterial, fungistatic wound healing properties of the gel containing 0.05% melanin ("Melanin-gel"), which was synthesized by *P. brunnea* are obtained by us earlier too. Biocidal effect of 0.05% "Melanin-gel" was revealed on test cultures of bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In a test culture of *Candida* yeasts melanin gel produced the fungistatic effect (there were areas of growth retardation, where a decrease in the intensity of yeast growth was observed) [57].

Thus, the use of the culture fluid of the yeast-like fungus *Pseudonadsoniella brunnea*, which synthesizes and excretes dark pigment melanin into a culture, against pathogenic fungi and bacteria is effective and represents promising results as for antifungal and antibacterial protection.

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## REFERENCES


1. Adamenko OP. Vydovyj sklad zbudnykov fuzariozu soyi ta yih patogennistj [in Ukrainian]. Visnyk Centru Naukovogo Zabezpechennya APV Kharkiv Oblastj. 2015; 18: 5-11.
2. Gamboa-Gaitán M. Colombian *Vanilla* and its microbiota. I First report of *Fusarium* taxa from both wild and cultivated species. Acta Botanica Hungarica. 2013; 55 (3-4): 6-10.
3. Görtz A, Oerke E-C, Steiner U, Waalwijk C, Vries PM, de Dehne H-W. Biodiversity of *Fusarium* species causing ear rot of maize in Germany. Cereal Research Communications. 2008; 36 (Suppl. 6): 617-622.
4. Hsuan HM, Salleh B, Zakaria L. Molecular identification of *Fusarium* species in *Gibberella fujikuroi* species complex from rice, sugarcane and maize from Peninsular Malaysia. International Journal of Molecular Sciences. 2011; 12(10): 6722-6732.
5. Leslie JF, Summerell BA. An overview of *Fusarium.* In: Brown DW, Proctor RH, editors. *Fusarium*: genomics, molecular and cellular biology. Norwich, United Kingdom: Horizon Scientific Press; 2013, pp. 1-10.
6. Saremi H, Ammarellou A, Marefat A, Okhovvat SM. Binam a rice cultivar, resistant for root rot disease on rice caused by *Fusarium moniliforme* in Northwest, Iran. International Journal of Botany. 2008; 4: 383-389.
7. Trail F. Sex and fruiting in *Fusarium*. In: Brown DW, Proctor RH, editors. *Fusarium*: genomics, molecular and cellular biology. Norwich, United Kingdom: Horizon Scientific Press; 2013, pp. 11-30.
8. Nishiuchi T. Plant responses to *Fusarium* metabolites. In: Brown DW, Proctor RH, editors. *Fusarium*: genomics, molecular and cellular biology. Norwich, United Kingdom: Horizon Scientific Press; 2013, pp. 165-178.
9. Hirano SS, Upper CD. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae* – a pathogen, ice nucleus, and epiphyte. Microbiology Molecular Biology Reviews. 2000; 64: 624-653.
10. Davidsson PR, Kariola T, Niemi O, Palva ET. Pathogenicity of and plant immunity to soft rot pectobacteria. Front Plant Sci. 2013; 4: 191.

11. Ryan RP, Vorhölter F-J, Potnis N, Jones JB, Van Sluys M-A, Bogdanove AJ, Dow JM. Pathogenomics of *Xanthomonas*: understanding bacterium–plant interactions. Nature Reviews Microbiology. 2011; 9: 344-355.
12. Savidor A, Chalupowicz L, Teper D, Gartemann KH, Eichenlaub R, Manulis-Sasson S, Barash I, Sessa G. *Clavibacter michiganensis subsp. michiganensis* Vatr1 and Vatr2 transcriptional regulators are required for virulence in tomato. Mol Plant Microbe Interact. 2014; 27(10): 1035-1047.
13. Gohlke J, Deeken R. Plant responses to *Agrobacterium tumefaciens* and crown gall development. Front Plant Sci. 2014; 5: 155.
14. Dvorak K, Sabluk V, Kalinichenko A, Butsenko L, Pasichnyk L, Patyka V. Biological properties of phytopathogenic bacteria *Pseudomonas syringae*, isolated from sugar beet. Journal Pure Applied Microbiology. 2014; 8(6): 4345-4349.
15. Young JM. Pathogenicity and identification of the lilac pathogen, *Pseudomonas syringae pv. syringae* van Hall 1902. Annals Applied Biology. 1991; 118: 283-298.
16. Young JM. Minireview taxonomy of *Pseudomonas syringae*. Journal Plant Pathology. 2010; 92(Suppl. 1): S1.5-S1.14.
17. Liu H, Qiu H, Zhao W, Cui Z, Ibrahim M, Jin G, Li B, Zhu B, Xie GL. Genome sequence of the plant pathogen *Pseudomonas syringae* pv. *panici* LMG 2367. Journal Bacteriology. 2012; 194(20): 5693-5694.
18. Arrebola E, Cazorla FM, Perez-Garc A, de Vicente A. Chemical and metabolic aspects of antimetabolite toxins produced by *Pseudomonas syringae* pathovars. Toxins. 2011; 3: 1089-1110.
19. Lee DH, Kim J-B, Lim J-A, Han S-W, Heu S. Genetic diversity of *Pectobacterium carotovorum* subsp. *brasiliensis* isolated in Korea. Plant Pathol J. 2014; 30(2): 117-124.
20. De Boer SH, Li X, Ward LJ. *Pectobacterium* spp. associated with bacterial stem rot syndrome of potato in Canada. Phytopathology. 2012; 102(10): 937-947.
21. Charkowski AO. The soft rot *Erwinia*. In: Gnanamanickam SS, editor. Plant-associated bacteria. Springer; Netherlands; 2006, pp. 423-505.
22. Kettani-Halabi M, Terta M, Amdan M, El Fahime el M, Bouteau F, Ennaji MM. An easy, simple inexpensive test for the specific detection of *Pectobacterium carotovorum* subsp. *carotovorum* based on sequence analysis of the pmrA gene. BMC Microbiol. 2013; 13: 176.
23. Pal KK, McSpadden Garneder B. Biological control of plant pathogens. Plant Health Instr. 2006: 1-25.

24. Bourguet D, Guillemaud T. The hidden and external costs of pesticide use. In: Lichtfouse E, editor. Sustainable Agriculture Reviews 1. Springer International Publishing, Switzerland, Cham. 2016; 19: 35-120.
25. Lamichhane JR, Dürr C, Schwanck AA, Robin M-H, Cellier V, Messéan A, Aubertot J-N. Integrated management of damping-off diseases. A review. Agronomy Sustainable Development. 2017; 37(2): 10.
26. Dias MC. Phytotoxicity: an overview of the physiological responses of plants exposed to fungicides. Journal Botany. 2012: 135479.
27. Alabouvette C, Olivain C, Steinberg C. Biological control of plant diseases: The European situation. European Journal Plant Pathology. 2006; 114 (3): 329-341.
28. Borrero C, Trillas M, Delgado A, Aviles M. Effect of ammonium/nitrate ratio in nutrient solutionon control of *Fusarium* wilt of tomato by *Trichoderma asperellum* T34. Plant Pathol. 2012; 61 (1): 132-139.
29. Castano R, Borrero C, Trillas MI, Aviles M. Selection of biological control agents against tomato *Fusarium* wilt and evaluation in green house conditions of two selected agents in three growing media. Biocontrol. 2013; 5 (1): 105-116.
30. Isaac G, Abu-Tahon M. In vitro antifugal activity of medicinal plant extract against *Fusarium oxysporum* f. sp. Acta Biol Hung. 2014; 65(1): 107-118.
31. Maslichenko LV, Kurilova DA, Manohin VL. Vliyanie mikrobiopreparatov na osnove perspektivnih schtamov antagonistov vozbuditelej fusariosa na kulturu soi [in Russian]. Maslichnyey Kuljtury. 2011; 2: 145-148.
32. Tkalenko GM, Borsih OI, Sergienko VG. Optimisaciya zahistu ovohevih kultur v Lisosstepu Ukraine [in Ukrainian]. Karantyn Zakhyst Roslyn. 2012; 3: 9-14.
33. Yang X, Chen L, Yong X, Shen Q. Formulation scan affect rhizosphere colonization and biocontrol efficiency of *Trichoderma harzianum* against *Fusarium* wilt of cucumbers. Biol Fert Soils. 2011; 47(3): 239-248.
34. Zaimenko N, Didik N, Ellanska N, Pavlyuchenko N, Yunosheva A, Zakrasov O, Rosicka N. Prospects of exometabolites of micromycetae and analcime application for cabbage plant protection from fusariose. Agroecological Journal. 2015; 3: 87-92.
35. Bardin SD, Huang HC, Liu L, Yanke LJ. Control, by microbial seed treatment, of damping off caused by *Pythium* sp. on canola, safflower, dry pea, and sugar beet. Can J Plant Pathol. 2003; 25: 268-275.

36. Bargabus RL, Zidack NK, Sherwood JE, Jacobsen BJ. Screening for the identification of potential biological control agents that induce systemic acquired resistance in sugar beet. Biol Control. 2004; 30: 342-350.
37. Gerbore J, Benhamou N, Vallance J, Le Floch G, Grizard D, Regnault-Roger C, Rey P. Biological control of plant pathogens: advantages and limitations seen through the case study of *Pythium oligandrum*. Environ Sci Pollut Res Int. 2014; 21(7): 4847-4860.
38. Jiang J-H, Tam S-L, Toda T, Chen L-C. Controlling *Rhizoctonia* damping-off of Chinese mustard by using endomycorrhizal *Rhizoctonia* spp. isolated from orchid mycorrhizae. Plant Dis. 2015; 100: 85-91.
39. Mastouri F, Björkman T, Harman GE. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology. 2010; 100: 1213-1221.
40. Yangui T, Rhouma A, Triki MA, Gargouri K, Bouzid J. Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. Crop Prot. 2008; 27: 189-197.
41. Baz M, Lahbabi D, Samri S, Val F, Hamelin G, Madore I, Bouarab K, Beaulieu C, Ennaji MM, Barakate M. Control of potato soft rot caused by *Pectobacterium carotovorum* and *Pectobacterium atrosepticum* by Moroccan actinobacteria isolates. World J Microbiol Biotechnol. 2012; 28(1): 303-311.
42. Svahn SK, Chryssanthou E, Olsen B, Bohlin L, Göransson U. *Penicillium nalgiovense* Laxa isolated from Antarctica is a new source of the antifungal metabolite amphotericin B. Fungal Biology Biotechnology. 2015; 1: 2-11.
43. Kondratiuk TO, Beregova TV, Ostapchenko LI. Diversity of Antarctic microorganisms – potential producers of biologically active substances. Ukrainian Antarctic Journal. 2016: 15: 153-159.
44. Kondratyuk TO, Kondratyuk SY, Morgaienko OO, Khimich MV, Beregova TV, Ostapchenko LI. *Pseudonadsoniella brunnea* (Meripilaceae, Agaricomycotina), a new brown yeast-like fungus producing melanin from the Antarctic; with notes on nomenclature and type confusion of *Nadsoniella nigra* Issatsch. Acta Botanica Hungarica. 2015; 57(3-4): 291-320.
45. Kondratiuk T, Beregova T, Ostapchenko L. Antifungal influence of culture fluid of melanin producer *Pseudonadsoniella brunnea*. Science Rise: Biological Science. 2016; 3(3): 74-78.

46. Kondratiuk T, Beregova T, Ostapchenko L. Antifungal influence of a melanin producer *Pseudonadsoniella brunnea* culture fluid on *Gibberella fujikuroi* (anamorph: *Fusarium verticilloides*). Acta Botanica Hungarica. 2017; 59(1-2): 63-69.
47. Kondratiuk T, Akulenko T, Beregova T, Ostapchenko L. Microorganisms, perspective for biotechnology, medicine, environmental technologies, in the collection of microscopic fungi ESC. Institute of biology and medicine, Taras Shevchenko national university of Kyiv. Bulletin Taras Shevchenko National University of Kyiv. Series: Biology. 2017; 73: 22-30.
48. Leontopoulos SV, Giavasis I, Petrotos K, Kokkora M, Makridis C. Effect of different formulations of polyphenolic compounds obtained from OMWW on the growth of several fungal plant and food borne pathogens. Studies in vitro and in vivo. Agriculture Agricultural Science Procedia. 2015; 4: 327-337.
49. Pradeep FS, Pradeep BV. Optimization of pigment and biomass production from *Fusarium moniliforme* under submerged fermentation conditions. Int J Pharm Pharm Sci. 2013; 5(Suppl. 3): 526-535.
50. Deshmukh SK, Verekar SA, Bhave SV. Endophytic fungi: a reservoir of antibacterials. Front Microbiol. 2015; 5: 715-724.
51. Permyakova N, Beregova T, Zheltonozhskaya T, Panyk O, Falalyeyeva T. Chemical structure, solubility, spectral and electrochemical properties of eumelanin. In: Biologically active substances and materials: Fundamental and Applied Problems. Proceedings of the Interdisciplinary Scientific Conference; 2013, May 27- June 1; Novy Svet, AR Crimea, Ukraine: Mavis Publisher; 2013. V1: 97-98.
52. Nosanchuk JD, Casadevall A. Impact of melanin on microbial virulence and clinical resistance to antimicrobial compounds. Minireview. Antimicrobial Agents Chemotherapy. 2006; 50(11): 3519-3528.
53. Dadachova E. Bryan RA, Huang X, Moadel T, Schweitzer AD, Aisen P, Nosanchuk JD, Casadevall A. Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. PLoS ONE. 2007; 2(5): e457.
54. Lattanzio V, Lattanzio TVM, Cardinali A. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperato F, Ed. Phytochemistry: Advances in Research, Research Signpost, Kerala; 2006. pp. 23-67.
55. Pani G, Dessì A, Dallocchio R, Scherm B, Azara E, Delogu G, Migheli Q. Natural phenolic inhibitors of trichothecene biosynthesis by the wheat fungal pathogen *Fusarium culmorum*: a computational insight into the structure-activity relationship. PLoS ONE. 2016; 11(6): e0157316.

56. Golyshkin DV, Falaleeva TM, Neporada KS, Beregova TV. Effect of melanin on the condition of gastric mucosa and reaction of the hypothalamic-pituitary-adrenal axis under acute stress. Physiological Journal. 2015; 61(2): 65-72.
57. Taburets OV, Morgaienko OO, Kondratiuk TO, Beregova TV, Ostapchenko LI. The effect of "Melanin-Gel" on the wound healing. J Pharm Chem Biol Sci. 2016; 7(3): 2031-2038.

# Antimicrobial activity of papain

# 2

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## ABSTRACT

Microbial contamination is one of the main problems that may affect the shelf life of food and may also cause consumer illness. Therefore, many chemicals are used as preservatives to increase the safety and shelf life of food products. Enzymes are biocatalysts that increase the rate of otherwise slow reactions by decreasing the activation energy, without undergoing any net change in their structures at the end of a reaction. Papain enzyme belongs to the papain superfamily, as a proteolytic enzyme. Papain is of crucial importance in many vital biological processes in all living organisms responsible for breaking down proteins. Besides, enzymes in the latex are also involved in protection of the plant against predator attack. The presence of bacteriolytic action in the lattices of *Carica papaya* confirms the fact that bacteriolytic and proteolytic enzymes act in unison to degrade undesirable proteins. It preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine. Papain is obtained by cutting the skin of the unripe papaya and then collecting and drying the latex which flows from the cut. The greener the fruit, more active is the papain. The unique structure of papain gives its functionality that helps to understand how this proteolytic enzyme works and it's useful for a variety of purposes.

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## 1. INTRODUCTION

Microbial contamination is one of the main problems that may affect the shelf life of food and may also cause consumer illness. Therefore, many chemicals are used as preservatives to increase the safety and shelf life of food products [1]. As a result of the increased awareness of the consumer about the deleterious effects of chemical preservatives and the increasing preference for natural components, researchers have focused on the generation of natural additives that demonstrate antimicrobial significance to be used in the food industry [2]. Enzymes are biocatalysts that increase the rate of otherwise slow reactions by decreasing the reactions activation energy, without undergoing any net change in their structures at the end of a reaction [3]. They are mostly protein in nature and mediate all synthesis and degradation reactions carried out by living organisms [3]. Papain is an endolytic plant cysteine protease enzyme which is isolated from papaya (*Carica papaya* L.) latex [4]. Papain enzyme belongs to the papain superfamily, as a proteolytic enzyme, papain is of crucial importance in many vital biological processes in all living organisms [5]. Papain shows extensive proteolytic activity towards proteins, short chain peptides, amino acid esters and amide links and is applied extensively in the fields of food and medicine [6]. Papain is also used in topical formulations as a proteolytic debriding agent for the treatment of open, extensive wounds and burnings. It is also employed as an enhancer for cutaneous permeation of active compounds, chemical peeling and as a progressive depilatory agent [7]. Gurudatta et al. [8] reported that papain preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine. The unique structure of papain gives its functionality that helps to understand how this proteolytic enzyme works and it's useful for a variety of purposes [9]. Gartika et al. [10] reported that papain is bactericidal, bacteriostatic, anti-inflammatory and debridement material and shows a broad proteolytic activity against the protein, short chain peptides, amino acid ester and amid. This chapter addresses mainly structural features of enzyme, the biological importance and anti-microbial action of papain.

## 2. PROPERTIES AND STRUCTURE

The globular protein, the papain is a single chain protein with molecular weight of 23,406 DA and consists of 212 amino acid with four disulfide bridges and catalytically important residues in the following positions Gln19, Cys25, His158 and His159 [11]. It is very stable even at elevated temperatures [6]. Papain is unusually defiant to high concentrations of denaturing agents, such as, 8M urea or organic solvent like 70% EtOH [5]. Amanu [12] reported the optimum pH for activity of papain is in the range of 3.0-9.0 which varies with different substrate. Edwin et al. [13] reported that cysteine proteases in papain superfamily are usually consisting of two well-defined domains which provide an excellent system for studies in understanding the folding-unfolding behavior of proteins. The protein is stabilized by three disulfide bridges in which the molecule is folded along these bridges creating a strong interaction among the side chains which contributes to the stability of the enzyme [11, 14]. Its three-dimensional structure consists of two distinct structural domains with a cleft between them. This cleft contains the active site, which contains a catalytic diad that has been similar to the catalytic triad of chymotrypsin [15]. Ezekiel and Florence [6] reported that Histidine-159. Aspartate-158 was thought to play a role analogous to the role of aspartate in the serine protease catalytic triad, but that has since then been disproved. Papain molecule has an all a domain and an anti parallel b-sheet domain [16]. Amanu [12] reported the enzymatic activity of papain may be influenced by environmental conditions such as temperature, light, oxygen, humidity and packing. This enzyme is more stable and active in pH 5.0-7.0. Atalla et al. [17] reported the stability of the enzyme has been investigated at different temperatures and results have confirmed the decrease in its activity with the temperature increase. Catalytic activity of papain involves hydrolysis of proteins with broad specificity for peptide bonds, but preference for an amino acid bearing a large hydrophobic side chain at the P2 position while does not accept Val in P1 [18]. The enzyme has been reported to be generally more stable in hydrophobic solvents and at lower water contents and can catalyze reactions under a variety of conditions in organic solvents with its substrate specificity little changed from that in aqueous media [19]. In general, native proteins have a hydrophobic core and charged and/or polar group on the surface. The hydrophobic core helps to stabilize the tertiary structure of the protein by hydrophobic interaction while the outer polar surfaces preferentially interact with the exterior aqueous medium [6, 20].

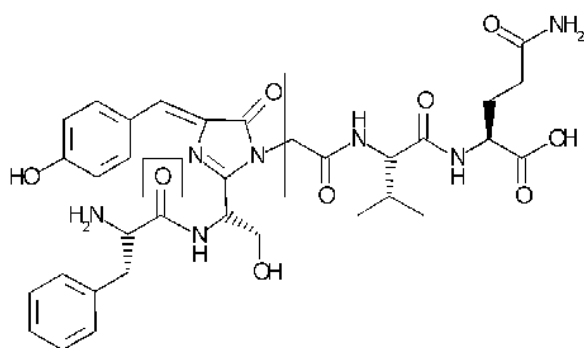

**Figure 1.** Structure of papain.

## 3. MODE OF ACTION

The mechanism in which the function of papain is made possible is through the cysteine-25 portion of the triad in the active site that attacks the carbonyl carbon in the backbone of the peptide chain freeing the amino terminal portion. As this occurs throughout the peptide chains of the protein, the protein breaks apart. The mechanism by which it breaks peptide bonds involves deprotonation of Cys-25 by His-159. Asparagine-175 helps to orient the imidazole ring of His-159 to allow this deprotonation to take place. Although far apart within the chain, these three amino acids are in close proximity due to the folding structure. It is though these three amino acids working together in the active site that provides this enzyme with its unique functions. Cys-25 then performs a nucleophilic attack on the carbonyl carbon of a peptide backbone [11, 21]. In the active site of papain, Cys -25 and His -159 are thought to be catalytically active as a thiolate-imidazolium ion pair. Papain can be efficiently inhibited by peptidyl or non-peptidyl N-nitrosoanilines [22]. The inactivation is due to the formation of a stable S-NO bond in the active site (*S* nitroso- Cys25) of papain [23]. Recently Srivastava and Singh [4] noted that feeding of bait formulation of papain with attractant (starch or serine) have sufficient molluscicidal activity against L. acuminata. It significantly reduced the fecundity of the snail and inhibited the AChE activity in the nervous tissue simultaneously. It seems that after sublethal treatment of papain caused the decrease in the level of serotonin and inhibits prostaglandins synthesis by inhibiting 5-lipoxygenase and leukotriene directly or indirectly CDCs. Possibly, the active molluscicidal component papain affect the CDCs and reduce the release of ovulation hormone, resulting a decrease in the fecundity of treated snail.

## 4. PAPAIN SUPER FAMILY

Cysteine proteases of the papain super family are widely distributed in nature [24]. They can be found in both prokaryotes and eukaryotes e.g. bacteria, parasites, plant, invertebrates and vertebrates [25]. The papain family contains peptidases with a wide variety of activities, including endopeptidases with broad specificity (such as papain), endopeptidases with very narrow specificity (such as glycyl endopeptidases), aminopeptidases, a dipeptidyl-peptidase, and peptidases with both endopeptidase and exopeptidase activities (such as cathepsins B and H) [26]. Dubey et al. [24] reported that enzymes of papain family are found in a wide variety of life forms: baculovirus, eubacteria like *Porphyromonas* and *Lactococcus*, yeast, and probably all protozoa, plants, and animals. Xiang et al. [27] reported that lysosomal cysteine proteases, also known as cysteine cathepsins (Cats), include Cat B, Cat H, Cat S, Cat K, Cat O/2, Cat F, Cat W and Cat U and also belong to the papain family sharing similar protein structure and mechanism of action. However, slight structural differences make these enzymes distinct with respect to their substrate specificity and regulation. Marksmann et al. [28] reported that cathepsins are synthesized as 30-50 kDa precursor proteins, which are glycosylated and phosphorylated in the Golgi apparatus. They are processed in the lysosomes to their active forms by one or more proteolytic cleavage. The optimum activity of cathepsins is pH 5.0-6.5, although they can hydrolyze large substrates also at neutral pH [29]. Dubay et al. [24] reported that the pH dependent activity of cathepsins is rather complex and depends not only on the microenvironment and the nature of the conformation of the substrate, but also on the presence or absence of stabilizing factors. Most of these papain-like enzymes are relatively small proteins with mass values in the range 20-35 kDa [30]. Disturbance of the normal balance of enzymatic activity of lysosomal cysteine proteases may lead to pathological conditions, and these proteases have been found to be involved in many such cases [31]. The participation of these enzymes in various diseases seems to be restricted to their proteolytic function outside the lysosomes, after secretion from lysosomes or after translocation into different intracellular granules [32]. The resulting uncontrolled proteolysis is a result of an imbalance between catalytically active proteases and their natural inhibitors, and can be observed in e.g. inflammation and tumor growth, although these processes are very complex [33]. Cysteine proteases of the papain family have been reported in bacteria as well [34]. How et al. [35] reported that proteolytic enzymes produced by *Porphyromonas gingivalis* are important virulence factors of this periodontopathogen. In the periodontal

disease proteolytic enzymes are produced in large quantities. It has been shown that these proteases can directly or indirectly degrade constituents of the periodontal tissues, destroy host defense elements, dysregulate coagulation and complement kallikerinkinin cascades [36, 37]. Recently, proteases belonging to two catalytic classes and produced by *P. gingivalis* have been identified. One enzyme is described as an Arg-X specific proteinase and another is Lys-X specific [38]. After transcription, the synthesis of the enzymes as inactive precursors, which are subject to several steps of post-translation modification, is the next regulatory mechanism for the papain like cysteine proteases [39]. Bravo et al. [40] reported that in the case of the lysosomal enzymes, the signal peptides are removed when the molecules pass into the lumen of the endoplasmic reticulum, and glycosylation, phosphorylation and formation of disulfides then take place in the golgi complex. He also reported that in the lysosomes, the proenzymes are dephosphorylated and converted to the active enzymes by limited hydrolysis. The N terminal proregions of the enzymes, which are removed during this final maturation step, act as a potent reversible inhibitor against the mature enzymes [41]. The propeptides of the plant enzymes act as their inhibitors [42]. The crystal structure procaricain have shown that the structure of the mature enzyme is already formed in its zymogens and the propeptides prevent the enzymatic activity by blocking the active site cleft using the inhibitory mechanism [24].

## 5. LOCALIZATION OF PLANT PAPAIN-LIKE CYSTEINE PROTEASES

Cornell and Stelmasiak [43] reported that in plants, they are primarily found in the latex and fruits of plants. In the latter, they are located in the vacuoles, which are plant counterpart of lysosomes, but are also extracellular as in the latices like papaya, figs and in arthopods such as lobsters. Dubey et al. [24] reported that cysteine proteases of plants play a major role in intracellular and extra cellular processes such as development and ripening of fruits, as nutritional reserve, degradation of storage protein in germinating seeds, activation of proenzymes and degradation of defective proteins. Otegui et al. [44] reported that cysteine proteases play an important role in seed germination and have been observed during maturation of storage proteins in *Cannavalia ensiformis*, *Riccinus communis*, *Glycine max*. Papain is an endolytic plant cysteine protease enzyme which is isolated from papaya (*Carica papaya* L.) latex. Papain is obtained by cutting the skin of the unripe papaya and then collecting and drying the latex which flows from the cut and the greener the fruit, more active

is the papain [6]. It preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine [45]. The unique structure of papain gives its functionality that helps to understand how this proteolytic enzyme works and it's useful for a variety of purposes [8].

## 6. ANTI-MICROBIAL ACTIVITY OF PAPAIN

Schmelcher et al. [46] reported that the lysis of micro-organisms by lysozyme and related enzymes and concluded that the lytic action of lysozyme on bacteria can be ascribed simply to the dissolution of the rigid cell-wall structures. When the degradation of the wall occurs in dilute media, the underlying structures of a lysozyme-sensitive bacterial cell will collapse and the liberation of the cytoplasmic components into the medium will result in the lysis of the bacterial suspension. Weibull [47] showed that dissolution of the wall of *Bacillus megaterium* in the presence of osmotically protective quantities of sucrose was accompanied by liberation of bacterial protoplasts and only partial optical clearing of the cell suspension. Although protoplast formation may account for incomplete lysis of bacterial suspensions when treated with lysozyme under appropriate conditions, it is evident that the walls of bacteria differ quantitatively in the 'amount' of lysozyme substrate present and such a factor as this may contribute to the partial optical clearing in dilute media. The cell-wall amino acids and sugars are not detectable as the free substances in the dialyzable fractions of the digests. The available evidence thus suggests that lysozyme is splitting the glycosidic bonds of the cell-wall amino sugars, liberating the disaccharide of acetylglucosamine and acetyl 'muramic acid' as the simplest product, together with more complex fragments which differ quantitatively rather than qualitatively in their chemical composition.

Gartika et al. [10] reported that the antibacterial activity of papain against *Streptococcus mutans* ATCC 25175 and concluded that this enzyme shows a broad proteolytic activity against the protein, short chain peptides, amino acid ester and amid, including bacterial cell wall. The purpose of this study is to produce a proper papain concentration to inhibit the growth of or kill *Streptococcus mutans*. The type of research is an experimental laboratory by determining the minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) with a dilution method, and measured using a microplate reader papain's minimum inhibitory concentration (MIC) papain against

*Streptococcus mutans* was 7.5% and the minimum bactericidal concentration (MBC) was 15%. Papain has antibacterial activity to *Streptococcus mutans.*

Bharwajd et al. [48] compared the antimicrobial activity of 2% chlorhexidine (100%), extract of *Morinda citrifolia* (86.02%), aloe vera gel (78.9%), papain gel (67.3%) and calcium hydroxide (64.3%) against *Enterococcus faecalis*. Phankhongsap et al. [49] compared the effectiveness of the antimicrobial between papain with mangosteen pericarp extract and papain with propolis extract against mixture *Streptococcus gordonii* and *Enterococcus faecalis* with the inhibiton zonw size11.25±0.66 and 10.42±0.72 mm, respectively. Minimum inhibitory concentration of the two materials were 25 mg/ml, while the MBC were 50 mg/ml.

Kumar et al. [50] tested papaya leaf extracts against human pathogenic microbes. Bacteria such as *Bacillus subtilis*, *Clostridium tetanus, Escherichia coli, Proteus vulgaris, Stapylococcus aureusand* fungi such as *Aspergillus conicus, spergillus flavus, Aspergillus niger, Aspergillus sulphureus* and *Rhizopus* by agar well diffusion method. All the leaf extracts of *Carica papaya* L. exhibited greater activity towards bacteria and fungi. The extract demonstrated higher activities against all the bacteria and fungi tested, with the highest activity (acetone extract of 13 mm zone of inhibition) demonstrated against *Staphylococcus aureus* and (ethanol extract of 18 mm zone of inhibition) demonstrated against *Aspergillus flavus*. *Carica papaya* may be used for the treatment of gastroenteritis, uretritis, otitis media, dengue fever, typhoid fever and wound infections.

Krishna et al. [51] reviewed on nutritional, medicinal and pharmacological properties of papaya and find that the seed of papaya has antimicrobial activity against *Trichomonas vaginalis* trophozoites. The report suggests the use of papaya seed in urinogenital disorder like trichomoniasis with care to avoid toxicity. The seed and pulp of papaya was shown to be bacteriostatic against several enteropathogens such as *Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa and Klebsiella pneumoniae* by the agar cup plate method 24. Purified extracts from ripe and unripe fruits also produces very significant antibacterial activity on *S. aureus, Bacillus cereus, E. coli, P. aeruginosa and Shigella flexneri*. The aqueous extract of fruit exhibited antimicrobial activity and promoted significant wound healing in diabetic rats. The seeds of irrespective stage of fruit maturity have bacteriostatic activity on Gram positive and Gram negative organisms, which could be useful in treating chronic skin ulcers. The papaya seed macerate has a clinical potential on conjugal R plasmid transfer from *Salmonella typhimurium* to *Escherichia coli*, on in vitro and in the digestive tract of genotobiotic mice. Herbal formulations containing papaya leaves and root or leaves alone as one of

the constituent has antibacterial activity against *Salmonella typhi, S. paratyphi* and *S. typhimurium*; however, water, acetone and ethanol extract of papaya leaves showed no microbicidal activity.

Anibijuwon and Udeze [52] reported the antimicrobial activity of *Carica papaya* (pawpaw leaf) on some pathogenic organisms of clinical origin from South-Western Nigeria and investigated for antibacterial activity against some human pathogenic bacteria using the agar diffusion method. The aqueous extracts of the root extracts did not show significant activity, but the organic extracts had significant activity with the methanol extracts demonstrating the highest activity against the test bacteria. The root extracts demonstrated higher activities against all the Gram-positive bacteria than the gram-negative bacteria tested, with the highest activity (14 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa* while the aqueous leaf extract showed pronounced inhibition demonstrating higher activities against the test bacteria than the organic solvents. The extracts demonstrated higher activities against all the Gram-positive bacteria than the Gram-negative bacteria tested, with the highest activity (4.2 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa.* Increase in temperature enhanced the activity of the extracts, while alkaline pH decreased the activity. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the root extracts ranged between 50-200 mg/ml. Preliminary phytochemical analyses showed that the extracts contain alkaloids, tannins, saponins, glycosides and phenols. *Carica papaya* may be used for the treatment of gastroenteritis, uretritis, otitis media and wound infections.

Kumar et al. [53] reported the antifungal medicinal properties of *Carica papaya*. The effects of different concentrations of alcoholic extract of *Carica papaya* (root, shoot and seed) on the radial growth of plant against the pathogenic fungi viz. *Aspergillus niger*, *Aspergillus flavus, Candida albicans* and *Microsporum fulvum*. That with the increase in concentrations the rate of growth inhibition also increases. Observation further shows that like root extract growth is also inhibited in the presence of shoot and seed alcoholic extract under culture medium. Further shows that the growth of these fungi inhibits more in presence of higher concentrations as compared to lower concentrations of extract.

Islam et al. [54] experimented the evaluation of antibacterial activities of latex of Caricaceae (*Carica papaya* L.) He has reported that latex was evaluated against one Gram-positive bacterium *Bacillus subtilis* and three Gram-negative pathogenic bacterial strains as *Escherichia coli, Agrobacterium sp* and *Rhizobium sp.* Ciprofloxacin was used as a control for investigating the bacterial species. Antibacterial activity was expressed in terms of the

radius of zone inhibition. Latex of this plant was tested in seven doses (1, 2, 5, 7, 10, 15 and 20 mg/disk) and it was found that the antibacterial activity was dose dependent and a significant difference was also observed in case of different bacterial stains. The results demonstrated noticeable inhibition of the bacterial growth against the tested organisms. In case of *Agrobacterium* sp. 20 mg of latex showed the average of 20.66 mm zone of inhibition and for *E. coli* this value was 16 mm for the same concentration of latex. The rest of the two bacterial species showed comparative resistance to papaya latex.

Coello et al. [55] assessed anti-protozoan activity of crude *Carica papaya* seed extract gainst *Trypanosoma cruzi* and in order to determine the *in vivo* activity against the protozoan *Trypanosoma cruzi*, two doses (50 and 75 mg/kg) of a chloroform extract of *Carica papaya* seeds were evaluated compared with a control group of allopurinol. A significant reduction ($p < 0.05$) in the number of blood trypomastigotes was observed in animals treated with the evaluated doses of the *C. papaya* extract in comparison with the positive control group (allopurinol 8.5 mg/kg). Parasitemia in animals treated with the fatty acids mixture was also significantly reduced ($p < 0.05$), compared to negative control animals. These results demonstrate that the fatty acids identified in the seed extracts of *C. papaya* (from ripe fruit) are able to reduce the number of parasites from both parasite stages, blood trypomastigote and amastigote (intracellular stage).

Coello et al. [56] evaluated the anti-protozoan activity of the chloroform extract of *Carica papaya* seeds during the sub-acute and chronic phase of infection of *Trypanosoma cruzi*, doses of 50 and 75 mg/kg, including a mixture of their main components (oleic, palmitic, and stearic acids). Subsequently, doses of 50 and 75 mg/kg in mice during the chronic phase of infection (100 dpi) were also evaluated. It was found that chloroform extract was able to reduce the amastigote nests numbers during the subacute phase in 55.5 and 69.7% ($p > 0.05$) as well as in 56.45% in animals treated with the mixture of fatty acids. Moreover, the experimental groups treated with 50 and 75 mg/kg during the chronic phase of the infection showed a significant reduction of 46.8 and 53.13% respectively ($p < 0.05$). It is recommended to carry out more studies to determine if higher doses of chloroformic extract or its administration in combination with other anti-chiasmic drugs allows a better response over the intracellular stage of *T. cruzi* in infected animal models and determine if the chloroform extract of *C. papaya* could be considered as an alternative for treatment during the indeterminate and chronic phase of the infection.

Kovendan et al. [57] reported the anti-malarial activity of the ethanol leaf extract of *Carica papaya*, blood stages of CQ-sensitive and CQ resistant strains against *Plasmodium*

*falciparum* as target species. The larvae and pupae values of 1st to 4th instars values of $LC_{50}$ = 3.65%, 4.28%, 5.41%, 6.70%, and 7.50%, respectively. The $LC_{90}$ to good 9.1%, 11.75%, 13.53%, 16.36%, and 16.92%, respectively. These four concentrations (25, 50,100 and 150 µg/ml) of ethanol leaf extracts exhibited promising inhibitory activity against the CQ sensitive strain with ($IC_{50}$) values 40.75%, 36.54%, 25.30%, and 18.0% and in CQ resistant 50.23%, 32.50%, 21.45%, and 23.12% against *P. falciparum*.

Quintal et al. [58] reported the antifungal activity in ethanolic extracts of *Carica papaya* L. cv. maradol leaves and seeds antifungal effectiveness was determined by challenging the extracts (LE, SRE, SUE) from the best extraction treatment against three phyto-pathogenic fungi: *Rhizopus stolonifer, Fusarium spp.* and *Colletotrichum gloeosporioides*. The leaf extract exhibited the broadest action spectrum. The MIC 50 for the leaf extract was 0.625 mg/ml for *Fusarium spp.* and 10 mg/ml for *C. gloeosporioides*, both equal to approximately 20% mycelial growth inhibition. Ethanolic extracts from *Carica papaya* L. cv. maradol leaves are a potential source of secondary metabolites with antifungal properties.

## 7. CONCLUSION

In conclusion, plant-based antimicrobials have enormous therapeutic and preferential potential. They can serve the desired purpose with lesser side effects that are often associated with synthetic antimicrobials. Papain is found naturally in papaya which is a versatile plant having number of uses, enzymatic properties and antimicrobial activity demonstrated in this chapter. Antimicrobial activity of papain is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents.

## REFERENCES

1. Hamid MA, Goda HA, Gobba CD, Jenssen H, Osman A. Antibacterial activity of papain hydrolysed camel whey and its Fractions. International Dairy Journal. 2016; 61: 91-98.
2. Ahmed N, Singh J, Mudasir, Malik A, Kour H, Gupta P, Chauhan H. Naturally occurring preservatives in food and their role in food preservation. International Journal of Pharmaceutical and Biological Archives. 2013; 4(1): 22-30.

3. Vishal T, Rathore RPS, Kamble PR, Manish G, Singh N. Pepsin, papain and hyaluronidase enzyme analysis: A review. International Journal of Research in Pharmacy and Science. 2013; 3(1): 01-18.
4. Srivastava AK, Singh VK. *Carica papaya* - a herbal medicine. International Journal of Research Studies in Biosciences. 2016; 4(11): 19-25.
5. Abidin MYBZ. Stability of papain in aqueous organic solvent by reverse phase liquid chromatography. Thesis submitted to Faculty of Chemical and Natural Resources Engineering Universiti Malaysia Pahang, 2013.
6. Ezekiel A, Florence M. Papain, a plant enzyme of biological importance: a review. American Journal of Biochemistry and Biotechnology. 2012; 8(2): 99-104.
7. Pinto CASO, Lopes PS, Sarruf FD, Polakiewicz B, Kaneko TM, Baby AR, Velasco MVR. Comparative study of the stability of free and modified papain incorporated in topical formulations. Brazilian Journal of Pharmaceutical Sciences. 2011; 47(4): 752-760.
8. Gurudatta M, Deshmukh YA, Naikwadi AA. Anticancer effects of *Carica papaya* in experimental induced mammary tumors in rats. International Journal of Medicinal Ressearch Health Sci. 2015; 4(3): 667-671.
9. Joslin D, Gowthami R, Jenitta EPE, Rama BP, Jayadev K, Shrinidhi S. Extraction, purification, characterization and metal ion concentration of papain from *Carica papay*a. World Journal of Pharmaceutical Research. 2015; 4(10): 2948-2954.
10. Gartika M, Sasmita IS, Satari MH, Chairulfattah A, Hilmanto D. Antibacterial activity of papain against *Streptococcus mutans* ATCC 25175. International Journal of Development Research.2014; 4(10): 2075-2077.
11. Tsuge H, Nishimura T, Tada Y, Asao T, Turk D. Inhibition mechanism of cathepsin L-specific inhibitors based on the crystal structure of papain-CLIK148 complex. Biochem. Biophys. Res. Commun. 1999; 266: 411-416.
12. Amanu M. Process optimization of milk coagulant extraction from latex of *Carica papaya* for production of pre ripened cheese. A thesis submitted to the school of graduate studies of Addis Ababa University, Institute of Technology, in partial fulfillment of the requirements for the degree of masters of science in School of Chemical and Bio-Engineering, Ethiopia, 2015.
13. Edwin F, Sharma, Jagannadham MV. Single disulfide bond reduced papain exists in a compact intermediate state. Biochem. Biophys. Res. Commun. 2002; 1479: 69-82.
14. Edwin F, Jagannadham MV. Single disulfide bond reduced papain exists in a compact intermediate state. Biochem. Biophys. Acta. 2000; 1479: 69-82.

15. Gutteridge A. Understanding the relationship between enzyme structure and catalysis. Dissertation is submitted for the degree of Doctor of Philosophy. Darwin College, Cambridge October 15, 2005.
16. Madej T, Addess KJ, Fong JH, Geer LY, Geer RC, et al. MMDB: 3D structures and macromolecular interactions. Nucleic Acids Res. 2012; 40: 461-464.
17. Atalla MM, Zeinabb HK, Emana RH, Amanib AY, Abeer AAEA. Characterization and kinetic properties of the purified *Trematosphaeria mangrovei* laccase enzyme. Saudi Journal of Biological Sciences. 2013; 20: 373-381.
18. Dalal S, Ragheb DRT, Klemba M. Engagement of the S1, S1' and S2' subsites drives efficient catalysis of peptide bond hydrolysis by the M1-family aminopeptidase from Plasmodium falciparum. Mol Biochem Parasitol. 2012; 183(1): 70-77.
19. Kumar A, Dhar K, Kanwar SS, Arora PK. Lipase catalysis in organic solvents: advantages and applications. Biological Procedures Online. 2016; 18(2): 3-11.
20. Wang LJ, Sun N, Terzyan S, Zhang XJ, Benson DR. A histidine/tryptophan π-stacking interaction stabilizes the heme-independent folding core of microsomal apocytochrome b5 relative to that of mitochondrial apocytochrome b5. Biochemistry. 2006; 45: 13750-13759.
21. Menard R, Khouri HE, Plouffe C, Dupras R, Ripoll D, et al. A protein engineering study of the role of aspartate 158 in the catalytic mechanism of papain. Biochemistry. 1990; 29: 6706-6713.
22. Guo Z, Ramirez J, Li J, Wang PG. Peptidyl N-nitrosoanilines: a novel class of cysteine protease inactivators. J. Am. Chem. Soc. 1998; 120: 3726-3734.
23. Xian M, Chen X, Liu Z, Wang K, Wang PG. Inhibition of papain by *s*-nitrosothiols. J. Biol. Chem. 2000; 275: 20467-20473.
24. Dubey VK, Pande P, Singh BK, Jagannadham MV. Papain-like proteases: applications of their inhibitors. African Journal of Biotechnology. 2007; 6(9): 1077-1086.
25. Portaro FCV, Santos ABF, Cezari MHS, Juliano MA, Juliano L, Carmona E. Probing the specificity of cysteine proteinases at subsites remote from the active site: analysis of P4, P3, P4 and P3 variations in extended substrates. Biochem. J. 2000; 347: 123-129.
26. Macedo MLR, Freire MDGM. Insect digestive enzymes as a target for pest control. ISJ. 2011; 8: 190-198.
27. Xiang Li, Liu Z, Cheng Z, Cheng X. Cysteinyl cathepsins: multifunctional enzymes in cardiovascular disease. Chonnam Med. 2012; 48: 77-85.
28. Markmann S, Thelen M, Cornils K, Schweizer M, Ahmadinejad NB, Willnow T, Heeren J, Gieselmann V, Braulke T, Kollmann K.Lrp1/LDL receptor play critical roles in

mannose 6-phosphate-independent lysosomal enzyme targeting. Traffic. 2016; 16(7): 743-759.

29. Guha S, Padh H. Cathepsins: fundamental effectors of endolysosomal proteolysis. Indian J of Biochemistry and Physic. 2008; 45: 75-90.
30. Paireder M, Mehofer U, Tholen S, Porodko A, Schahs P, Maresch D, Biniossek ML, Hoorn RAL, Lenarcic B, Novinec M, Schilling O, Mach L. The death enzyme CP14 is a unique papain-like cysteine proteinase with a pronounced S2 subsite selectivity. Archives of Biochemistry and Biophysics. 2016; 603: 110-117.
31. Syed R, Farukh R, Shaista R, Akbar M, Shajrul A. Role of proteases in cancer: a review. Biotechnology and Molecular Biology Review. 2012; 7(4): 90-101.
32. Stokaa V, Turka V, Turk B. Lysosomal cathepsins and their regulation in aging and neurodegeneration. Ageing Research Reviews. 2016; 32: 22-37.
33. Sevenich L, Joyce JA. Pericellular proteolysis in cancer. Genes Dev. 2014; 28(21): 2331-2347.
34. Peng SQ, Zhu JH, Li HL, Tian WM. Cloning and characterization of a novel cysteine protease gene (*HbCP1*) from *Hevea brasiliensis*. J. Biosci. 2008; 33(5): 681-690.
35. How KY, Song KP, Chan KG. Porphyromonas gingivalis: an overview of periodontopathic pathogen below the gum line. Front. Microbiol. 2016; 7: 53.
36. Guo Y, Nguyen KA, Potempa J. Dichotomy of gingipains action as virulence factors: from cleaving substrates with the precision of a surgeon's knife to a meat chopper-like brutal degradation of proteins. Periodontol 2000. 2010; 54(1): 15-44.
37. Carroll IM, Maharshak N. Enteric bacterial proteases in inflammatory bowel diseasepathophysiology and clinical implications. World J Gastroenterol. 2013; 19(43): 7531-7543.
38. Takayuki KN, Yuko ON. Exopeptidases and gingipains in *Porphyromonas gingivalis* as prerequisites for its amino acid metabolism. Japanese Dental Science Review. 2016; 52: 22-29.
39. Dubey VK, Pande M, Singh BK, Jagannadham MV. Papain-like proteases: applications of their inhibitors. International Research Journal of Genetic Engineering. 2013; 1(3): 42-50.
40. Bravo R, Parra V, Gatica D, Rodriguez AE , Torrealba N, Paredes F, Wang ZV, Zorzano A, Hill JA, Jaimovich E, Quest AFG, Lavandero S. Endoplasmic reticulum and the unfolded protein response: dynamics and metabolic integration. Int Rev Cell Mol Biol. 2013; 301: 215-290.

41. Chang AK, Park JW, Lee EH, Lee JS. The N-terminal propeptide of *Vibrio vulnificus* extracellular metalloprotease is both an inhibitor of and a substrate for the enzyme. Journal of Bacteriology, 2007; 189(19): 6832-6838.
42. Santamaria ME, Arnaiz A, Mendoza MD, Martinez M, Diaz I. Inhibitory properties of cysteine protease pro-peptides from barley confers resistance to spider mite feeding. PLoS ONE. 2015; 10(6): 128323.
43. Cornell HJ, Stelmasiak T. Caricain: a basis for enzyme therapy for coeliac disease. South African Journal of Science. 2011; 107(9/10): 529.
44. Otegui MS, Herder R, Schultze J, Jung R, Staehlin A. The proteolytic processing of seed storage proteins in Arabidopsis thaliana embryo cells starts in the multivesicular bodies. The Plant Cell. 2006; 18: 2567-2581.
45. Fauziya S, Krishnamurthy R. Papaya (*Carica papaya*): source material for anticancer. CIBTech Journal of Pharmaceutical Sciences. 2013; 2(1): 25-34.
46. Schmelcher M, Donovan DM, Loessner JM. Bacteriophage endolysins as novel antimicrobials. Future Microbiol. 2012; 7(10): 1147-1171.
47. Weibull C. The isolation of protoplasts from *Bacillus meguterium* by controlled treatment with lysozyme. J. Bact. 1953; 66: 688.
48. Bhardwaj A, Ballal S, and Velmurugan N. Comparative evaluation of the antimicrobial activity of natural extracts of *Morinda citrifolia*, papain and aloe vera (all in gel formulation), 2% chlorhexidine gel and calcium hydroxide, against *Enterococcus faecalis*: an *in vitro* study. J Conserv Dent. 2012; 15(3): 293-297.
49. Phankhongsap A, Chailertvanitkul P, Juntavee A, Peerapattana J. Antimicrobial effectiveness of root canal irritants from mangosteen pericarp extract with papain and propolis extract with papain on mixture of *Streptococcus gardinii* and *Entarococcus faecalis.* 1st Mae Fah Luang University International Conference, 2012.
50. Vijayakumar M, Bharathidasan R, Prince L. Antimicrobial activity of *Carica papaya* L. International Journal of Arts and Science Research. 2015; 2(2): 37-43.
51. .Krishna KL, Paridhavi M, Patel JA. Review on nutritional, medicinal and pharmacological properties of papaya (*Carica papaya*). Natural Product Radiance. 2008; 7(4): 364-373.
52. Anibijuwon II, Udeze OA. Antimicrobial activity of *Carica papaya* (pawpaw leaf) on some pathogenic organisms of clinical origin from south-western Nigeria. Ethno-botanical Leaflets. 2009; 13: 850-864.
53. Kumar M, Faheem M, Singh S, Shahzad A, Bhargava AK. Antifungal activity of the *Carica papaya* important food and drug plant. Asian Journal of Plant Science and Research 2013; 3(1): 83-86.

54. Islam AA, Al-Mamun MA, Parvin S, Sarker M, Zaman MK, Farhana P, Shahriar Z, Salah, Uddin M. Evaluation of antibacterial activities of latex of Caricaceae (*Carica papaya* l.). Asian J Pharm Clin Res. 2015; 8(1): 308-311.
55. Coello MJ, Marín EG, Pacheco AO, Gutiérrez SP, Viana KYA. Assessment of the anti-protozoal activity of crude *Carica papaya* seed extract against *Trypanosoma cruzi.* Molecules. 2013; 18: 12621-12632.
56. Coello MJ, Viana KYA, Pacheco AO, Gutierrez SP, Marin EG. *In vivo* antiprotozoal activity of the chloroforme extract from *Carica papaya* seeds against amastigote stage of *Trypanosoma cruzi* during indeterminate and chronic phase of infection. Evidence-Based Complementary and Alternative Medicine. 2014: article ID 458263.
57. Kovendan K, Murugan K, Panneerselvam C, Aarthi N, Kumar PM, Subramaniam J, Amerasan D, Kalimuthu K, Vincent S. Antimalarial activity of *Carica papaya* (Family: Caricaceae) leaf extract against Plasmodium falciparum. Asian Pacific Journal of Tropical Disease. 2012: S306-S311.
58. Quintal PC, Flores TG, Buenfil IR, Tintore SG. Antifungal activity in ethanolic extracts of *Carica papaya* L. cv. maradol leaves and seeds. Indian J Microbiol. 2011; 51(1): 54-60.

# Continuous research for natural drugs with potential non-resistance antimicrobial activity and reduced adverse effects

# 3

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## ABSTRACT

Resistant-microbes are found in people, animals, food, and the environment (in water, soil and air). Poor infection control, inadequate sanitary conditions and inappropriate food-handling encourage the spread of antimicrobial resistance also Antibiotic resistance is present worldwide so Patients with infections caused by drug-resistant bacteria are at increased risk of worse clinical outcomes causing death, in addition, this consumes more health-care resources than patients infected with non-resistant strains of the same bacteria. Nature is a generous source of a number of compounds with potential application for the treatment of several diseases including the infectious diseases. The presently investigated natural products derived from local botanical are promising candidates that could be used against multi drug resistant pathogens with high potency and less side effects.

**Keywords:** Resistant-microbes; Natural products; Structure-activity relationship.

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# 1. INTRODUCTION

Antimicrobial resistance has been attributed as a challenging problem worldwide. It happens when microorganisms (such as bacteria, fungi, viruses, and parasites) change when they are exposed to antimicrobial drugs (such as antibiotics, antifungals, antivirals, antimalarials, and anthelmintics). Microorganisms that develop antimicrobial resistance are sometimes referred to as “superbugs". As a result, the medicines become ineffective and infections persist in the body, increasing the risk of spread to others [1].

New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability, and death. Also, Antimicrobial resistance increases the cost of health care with lengthier stays in hospitals and more intensive care required. In addition, antimicrobial resistance is putting the gains of the Millennium Development Goals at risk and endangers achievement of the Sustainable Development Goals [1].

Antimicrobial resistance occurs naturally over time, usually through genetic changes. However, the misuse and overuse of antimicrobials is accelerating this process. In many places, antibiotics are overused and misused in people and animals, and often given without professional oversight. Antimicrobial resistant-microbes are found in people, animals, food, and the environment (in water, soil and air). They can spread between people and animals, and from person to person. Poor infection control, inadequate sanitary conditions and inappropriate food-handling encourage the spread of antimicrobial resistance [1].

# 2. RESISTANCE AMONG DIFFERENT PATHOGENS

## 2.1. Resistance in *Klebsiella pneumoniae*

The common intestinal bacteria that can cause life-threatening infections – to a last resort treatment (carbapenem antibiotics) have spread to all regions of the world. *K. pneumoniae* is a major cause of hospital-acquired infections such as pneumonia, bloodstream infections, and infections in newborns and intensive-care unit patients. In some countries, because of resistance, carbapenem antibiotics do not work in more than half of people treated for *K. pneumoniae* infections [1].

## 2.2. Resistance in *Escherichia coli*

Resistance in *E. coli* to one of the most widely used medicines for the treatment of urinary tract infections (fluoroquinolone antibiotics) is very widespread. There are countries in many parts of the world where this treatment is now ineffective in more than half of patients. Colistin is the last resort treatment for life-threatening infections caused by Enterobacteriaceae which are resistant to carbapenems. Resistance to colistin has recently been detected in several countries and regions, making infections caused by such bacteria untreatable [1].

## 2.3. Resistance in tuberculosis (TB)

WHO estimates that, in 2014, there were about 480 000 new cases of multidrug-resistant tuberculosis (MDR-TB), a form of tuberculosis that is resistant to the 2 most powerful anti-TB drugs. Only about a quarter of these (123 000 cases) were detected and reported. MDR-TB requires treatment courses that are much longer and less effective than those for non-resistant TB. Globally, only half of MDR-TB patients were successfully treated in 2014.

Among new TB cases in 2014, an estimated 3.3% were multidrug-resistant. The proportion is higher among people previously treated for TB, at 20%. Extensively drug-resistant tuberculosis (XDR-TB), a form of tuberculosis that is resistant to at least 4 of the core anti-TB drugs, has been identified in 105 countries. An estimated 9.7% of people with MDR-TB have XDR-TB [1].

## 2.4. Resistance in malaria

As of July 2016, resistance to the first-line treatment for *P. falciparum* malaria (artemisinin-based combination therapies, also known as ACTs) has been confirmed in 5 countries of the Greater Mekong subregion (Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand and Viet Nam). In most places, patients with artemisinin-resistant infections recover fully after treatment, provided that they are treated with an ACT containing an effective partner drug. However, along the Cambodia-Thailand border, *P. falciparum* has become resistant to almost all available antimalarial medicines, making treatment more challenging and requiring close monitoring. There is a real risk that multidrug

resistance will soon emerge in other parts of the subregion as well.The spread of resistant strains to other parts of the world could pose a major public health challenge and jeopardize important recent gains in malaria control [1].

### 2.5. Resistance in human immunodeficiency virus (HIV)

In 2010, an estimated 7% of people starting antiretroviral therapy (ART) in developing countries had drug-resistant HIV. In developed countries, the same figure was 10–20%. Some countries have recently reported levels at or above 15% amongst those starting HIV treatment, and up to 40% among people re-starting treatment. This requires urgent attention.

Increasing levels of resistance have important economic implications as second and third-line regimens are 3 times and 18 times more expensive, respectively, than first-line drugs.

Since September 2015, WHO has recommended that everyone living with HIV start on antiretroviral treatment. Greater use of ART is expected to further increase ART resistance in all regions of the world. To maximize the long-term effectiveness of first-line ART regimens, and to ensure that people are taking the most effective regimen, it is essential to continue monitoring resistance and to minimize its further emergence and spread [1].

### 2.6. Resistance in influenza

Antiviral drugs are important for treatment of epidemic and pandemic influenza. So far, virtually all influenza A viruses circulating in humans were resistant to one category of antiviral drugs – M2 Inhibitors (amantadine and rimantadine). However, the frequency of resistance to the neuraminidase inhibitor oseltamivir remains low (1-2%). Antiviral susceptibility is constantly monitored through the WHO Global Influenza Surveillance and Response System [1].

## 3. MAJOR BACTERIAL RESISTANCE STRATEGIES

There are four major bacterial resistance strategies:

### 3.1. Prevention of the antimicrobial from reaching its target by reducing its ability to penetrate into the cell

Antimicrobial compounds almost always require access into the bacterial cell to reach their target site where they can interfere with the normal function of the bacterial organism. Porin channels are the passageways by which these antibiotics would normally cross the bacterial outer membrane. Some bacteria protect themselves by prohibiting these antimicrobial compounds from entering past their cell walls. For example, a variety of Gram-negative bacteria reduce the uptake of certain antibiotics, such as aminoglycosides and beta lactams, by modifying the cell membrane porin channel frequency, size, and selectivity. Prohibiting entry in this manner will prevent these antimicrobials from reaching their intended targets that, for aminoglycosides and beta lactams, are the ribosomes and the penicillin-binding proteins (PBPs), respectively [2].

This strategy has been observed in:

- *Pseudomonas aeruginosa* e.g. against imipenem (a beta-lactam antibiotic);
- *Enterobacter aerogenes* and *Klebsiella pneumoniae* against imipenem;
- Glycopeptide intermediate-resistant *Staphylococcus aureus* so-called “GISA” strains with thickened cell wall trapping vancomycin/teicoplanin;
- Many Gram-negative bacteria against aminoglycosides;
- Many Gram-negative bacteria against quinolones.

### 3.2. Inactivation of antimicrobial agents via modification or degradation

Another means by which bacteria preserve themselves is by destroying the active component of the antimicrobial agent. A classic example is the hydrolytic deactivation of the beta-lactam ring in penicillins and cephalosporins by the bacterial enzyme called beta lactamase. The inactivated penicilloic acid will then be ineffective in binding to PBPs (penicillin binding proteins), thereby protecting the process of cell wall synthesis. This strategy has also been observed in:

- *Enterobacteriaceae* against chloramphenicol (acetylation)
- Gram negative and Gram positive bacteria against aminoglycosides (phosphorylation, adenylation, and acetylation) [3].

### 3.3. Expulsion of the antimicrobial agents from the cell via general or specific efflux pumps

To be effective, antimicrobial agents must also be present at a sufficiently high concentration within the bacterial cell. Some bacteria possess membrane proteins that act as an export or efflux pump for certain antimicrobials, extruding the antibiotic out of the cell as fast as it can enter. This results in low intracellular concentrations that are insufficient to elicit an effect. Some efflux pumps selectively extrude specific antibiotics such as macrolides, lincosamides, streptogramins and tetracyclines, whereas others (referred to as multiple drug resistance pumps) expel a variety of structurally diverse anti-infectives with different modes of action e.g. the *qac* genes which pump out chlorhexidine, propamidine and quaternary ammonium agents [4]. This strategy has also been observed in:

- *E. coli* and other *Enterobacteriaceae* against tetracyclines;
- Various members of the *Enterobacteriaceae* against chloramphenicol;
- *Staphylococci* against macrolides and streptogramins;
- *Staphylococcus aureus* and *Streptococcus pneumoniae* against fluoroquinolones.

### 3.4. Modification of the antimicrobial target within the bacteria

Some resistant bacteria evade antimicrobials by reprogramming or camouflaging critical target sites to avoid recognition. Therefore, in spite of the presence of an intact and active antimicrobial compound, no subsequent binding or inhibition will take place. This strategy has been observed in:

- *Staphylococci* against methicillin and other beta-lactams (changes or acquisition of different PBPs that do not sufficiently bind beta-lactams to inhibit cell wall synthesis);
- Gram-positive cocci: erythromycin-resistant methylase is encoded by erm genes and causes structural changes to rRNA which prevent macrolide binding and allow synthesis of bacterial proteins to continue;
- *Enterococci* against vancomycin (alteration in cell wall precursor components to decrease binding of vancomycin);
- *Mycobacterium* spp. against streptomycin (modification of ribosomal proteins or of 16s rRNA);
- Various microbes which develop mutations in RNA polymerase resulting in resistance to the rifamycins e.g. *Staphylococcus* spp;

- members of *Enterobacteriaceae* with mutations in DNA gyrase resulting in resistance to quinolones [5].

## 4. MECHANISMS OF RESISTANCE AGAINST DIFFERENT ANTIMICROBIAL CLASSES

**Table 1.** Mechanisms of resistance against different antimicrobial classes [6]

| Antimicrobial class | Mechanism of resistance | Specific means to achieve resistance | Examples |
|---|---|---|---|
| Beta-lactams Examples: penicillin, ampicillin, mezlocillin, peperacillin, cefazolin, cefotaxime, ceftazidime, aztreonam, imipenem | Enzymatic destruction | Destruction of beta-lactam rings by beta-lactamase enzymes. With the beta-lactam ring destroyed, the antibiotic will no longer have the ability to bind to PBP (Penicillin-binding protein), and interfere with cell wall synthesis | Resistance of staphylococci to penicillin; Resistance of *Enterobacteriaceae* to penicllins, cephalosporins, and aztreonam |
| | Altered target | Changes in penicillin binding proteins. Mutational changes in original PBPs or acquisition of different PBPs will lead to inability of the antibiotic to bind to the PBP and inhibit cell wall synthesis | Resistance of staphylococci to methicillin and oxacillin |
| | Decreased uptake | Porin channel formation is decreased. Since this is where beta-lactams cross the outer membrane to reach the PBP of Gram-negative bacteria, a change in the number or character of these channels can reduce betalactam uptake | Resistance of *Enterobacter aerogenes, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* to imipenem |
| Glycopeptides Example: vancomycin | Altered target | Alteration in the molecular structure of cell wall precursor components decreases binding of vancomycin so that cell wall synthesis is able to continue | Resistance of enterococci to vancomycin |

| Antimicrobial class | Mechanism of resistance | Specific means to achieve resistance | Examples |
| --- | --- | --- | --- |
| Aminoglyosides Examples: gentamicin, tobramycin, amikacin, netilmicin, streptomycin, kanamycin | Enzymatic modification | Modifying enzymes alter various sites on the aminoglycoside molecule so that the ability of this drug to bind the ribosome and halt protein synthesis is greatly diminished or lost entirely | Resistance of many Gram-positive and Gram negative bacteria to aminoglycosides |
| | Decreased uptake | Change in number or character of porin channels (through which aminoglycosides cross the outer membrane to reach the ribosomes of gram-negative bacteria) so that aminoglycoside uptake is diminished | Resistance of a variety of Gram-negative bacteria to aminoglycosides |
| | Altered target | Modification of ribosomal proteins or of 16s rRNA. This reduces the ability of aminoglycoside to successfully bind and inhibit protein synthesis | Resistance of *Mycobacterium* spp. to streptomycin |
| Quinolones Examples: ciprofloxacin, levofloxacin, norfloxacin, lomefloxacin | Decreased uptake | Alterations in the outer membrane diminishes uptake of drug and/or activation of an "efflux" pump that removes quinolones before intracellular concentration is sufficient for inhibiting DNA metabolism | Resistance of Gram negative and staphylococci (efflux mechanism only) to various quinolones |
| | Altered target | Changes in DNA gyrase subunits decrease the ability of quinolones to bind this enzyme and interfere with DNA processes | Gram negative and Gram positive resistance to various quinolones |

## 5. TEST METHODS TO DETECT ANTIMICROBIAL RESISTANCE

There are several antimicrobial susceptibility testing methods available today, and each one has their respective advantages and disadvantages. They all have one and the same goal, which is to provide a reliable prediction of whether an infection caused by a bacterial isolate will respond therapeutically to a particular antibiotict reatment. Some examples of antibiotic sensitivity tesing methods are:

### 5.1. Dilution methods

The Broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. Microdilution testing uses about 0.05 to 0.1 ml total broth volume and can be conveniently performed in a microtiter format. Macrodilution testing uses broth volumes at about 1.0 ml in standard test tubes. For both of these broth dilution methods, the lowest concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the minimal inhibitory concentration or MIC. The MIC is thus the minumum concentration of the antibiotic that will inhibit this particular isolate. The test is only valid if the positive control shows growth and the negative control shows no growth. A procedure similar to broth dilution is agar dilution. Agar dilution method follows the principle of establishing the lowest concentration of the serially diluted antibiotic concentration at which bacterial growth is still inhibited [7].

### 5.2. Disk diffusion method

Because of convenience, efficiency and cost, the disk diffusion method is probably the most widely used method for determining antimicrobial resistance in private veterinary clinics. A growth medium, usually Mueller-Hinton agar, is first evenly seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration (approximately 1 to 2 x $10^8$ colony forming units per ml). Commercially prepared disks, each of which are pre-impregnated with a standard concentration of a particular antibiotic, are then evenly dispensed and lightly pressed onto the agar surface. The test antibiotic immediately begins to diffuse outward from the disks, creating a gradient of antibiotic concentration in the

agar such that the highest concentration is found close to the disk with decreasing concentrations further away from the disk. After an overnight incubation, the bacterial growth around each disc is observed. If the test isolate is susceptible to a particular antibiotic, a clear area of "no growth" will be observed around that particular disk.

The zone around an antibiotic disk that has no growth is referred to as the zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. This zone is then measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant. MIC measurement cannot be determined from this qualitative test, which simply classifies the isolate as susceptible, intermediate or resistant [8].

### 5.3. E-test

E-test (AB Biodisk, Solna, Sweden) is a commercially available test that utilizes a plastic test strip impregnated with a gradually decreasing concentration of a particular antibiotic. The strip also displays a numerical scale that corresponds to the antibiotic concentration contained therein. This method provides for a convenient quantitative test of antibiotic resistance of a clinical isolate. However, a separate strip is needed for each antibiotic, and therefore the cost of this method can be high [9].

### 5.4. Automated antimicrobial susceptibility testing systems

Several commercial systems have been developed that provide conveniently prepared and formatted microdilution panels as well as instrumentation and automated reading of plates. These methods are intended to reduce technical errors and lengthy preparation times. Most automated antimicrobial susceptibility testing systems provide automated inoculation, reading and interpretation. These systems have the advantage of being rapid (some results can be generated within hours) and convenient, but one major limitation for most laboratories is the cost entailed in initial purchase, operation and maintenance of the machinery. Some examples of these include: Vitek System (bioMerieux, France), Walk-Away System (Dade International, Sacramento, Calif.), Sensititre ARIS (Trek Diagnostic Systems, East Grinstead, UK), Avantage Test System (Abbott Laboratories, Irving, Texas), Micronaut (Merlin, Bornheim-Hesel, Germany), Phoenix (BD Biosciences, Maryland) [10].

## 5.5. Mechanism-specific tests

Resistance may also be established through tests that directly detect the presence of a particular resistance mechanism. For example, beta lactamase detection can be accomplished using an assay such as the chromogenic cephalosporinase test (Cefinase disk by BD Microbiology Systems, Cockeysville, MD and BBL DrySlide Nitrocefin, Becton Dickinson, Sparks, MD) and detection for chloramphenicol modifying enzyme chloramphenicol acetyltransferase (CAT) may utilize commercial colorimetric assays such as a CAT reagent kit (Remel, Lenexa, Kansas) [11].

## 5.6. Genotypic methods

Since resistance traits are genetically encoded, we can sometimes test for the specific genes that confer antibiotic resistance. However, although nucleic acid-based detections systems are generally rapid and sensitive, it is important to remember that the presence of a resistance gene does not necessarily equate to treatment failure, because resistance is also dependent on the mode and level of expression of these genes. Some of the most common molecular techniques utilized for antimicrobial resistance detection are as follows:

### *5.6.1. Polymerase chain reaction (PCR)*

This is one of the most commonly used molecular techniques for detecting certain DNA sequences of interest. This involves several cycles of denaturation of sample DNA, annealing of specific primers to the target sequence (if present), and the extension of this sequence as facilitated by a thermostable polymerase leading to replication of a duplicate DNA sequence, in an exponential manner, to a point which will be visibly detectable by gel electrophoresis with the aid of a DNA-intercalating chemical which fluoresces under UV light [12].

### *5.6.2. DNA hybridization*

This is based on the fact that the DNA pyrimidines (cytosine and thymidine) specifically pair up with purines (guanine and adenine; or uracil for RNA). Therefore, a labeled probe with a known specific sequence can pair up with opened or denatured DNA

from the test sample, as long as their sequences complement each other. If this "hybridization" occurs, the probe labels this with a detectable radioactive isotope, antigenic substrate, enzyme or chemiluminescent compound. Whereas if no target sequence is present or the isolate does not have the specific gene of interest, no attachment of probes will occur [13].

#### *5.6.3. Modifications of PCR and DNA hybridization*

With these basic principles, several modifications have been introduced which further improvement of the sensitivity and specificity of these standard procedures. Examples of such development were the use of 5'-fluorescence-labeled oligonucleotides, the development of molecular beacons, development of DNA arrays and DNA chips, among many others [14].

## 6. NATURAL PRODUCTS ACTING AS ANTIMICROBIALS

Nature is a generous source of a number of compounds with potential application for the treatment of several diseases including the infectious diseases. The presently investigated natural products derived from local botanical are promising candidates that could be used against MDR pathogens. Nevertheless, there is still a vast flora that once systemically explored could provide additional antimicrobial leads and drugs.

The mechanisms of action of the natural products include the degradation of the cell wall [16], damaging the cytoplasmic membrane, cytoplasm coagulation [17], damaging the membrane proteins, increased permeability leading to leakage of the cell contents, reducing the proton motive force [18], reducing the intracellular ATP pool via decreased ATP synthesis and augmented hydrolysis that is separate from the increased membrane permeability and reducing the membrane potential via increased membrane permeability [19].

Herein we will discuss the following antimicrobials:

- Antibacterial
- Antifungal
- Antiviral
- Antiprotozoal

## 6.1. Antibacterial agents

The cinnamon, clove, pimento, thyme, oregano, and rosemary plants had strong inhibitory effect against several bacterial pathogens. It has been also reported that essential oils extracted from some medicinal plants had the antibacterial effects against all the five tested food borne pathogens due to presence of phenolic compounds such as carvacrol, eugenol and thymol [20]. However found that benzoic acids, benzaldehydes and cinnamic acid were able to inhibit the growth of *Listeria monocytogenes* [21].

The antimicrobial activity of garlic, ginger, clove, black pepper and green chilli analyzed on the human pathogenic bacteria viz. *Bacillus sphaericus, Enterobacter aerogenes*, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *S. typhi* and *Shigella flexneri* and stated that amongst all the tested spices, aqueous garlic extracts was sensitive against all the bacterial pathogens [22]. Similarly, effect of clove extracts on the production of verotoxin by enterohemorrhagic *Escherichia coli* O157:H7 was investigated [23]. Furthermore it was evident from the study that the verotoxin production was inhibited by clove extract. However the effectiveness of cardamom, anise, basil, coriander, rosemary, parsley, dill and angelica essential oil for controlling the growth and survival of pathogenic and saprophytic microorganisms. The results of their study showed that essential oils extracted oregano, basil and coriander plants have inhibitory effect against *Pseudomonas aeruginosa, S. aureus* and *Yersinia enterocolitica* [24].

The effect of oregano essential oils on the behavior of *Salmonella typhimurium* in sterile and naturally contaminated beef fillets stored under aerobic and modified atmospheres also they have concluded that the addition of oregano essential oils checked the reduction in initial population of the tested bacterial pathogens [25]. However the bacterial growth may be inhibited by the ample application of essential oils or their use at high concentrations and their mode of action results in decline of the bacterial cells [26].

The antibacterial activity of essential oils extracted from thyme and mint leaves against the *Staphylococcus aureus, Salmonella typhimurium* and *Vibrio parahaemolyticus* and the result showed that all the plants have antibacterial activity against the tested pathogens but the effect of thyme leaves extract was more pronounced compared to other plants [27]. Moreover cinnamon, oregano, clove, pomegranate peel, and grape seed were found effective against *S. enterica* at room temperature, but the clove extracts possess highest antibacterial activity, thyme, sage, myrtle, laurel, and orange essential oils have a potential to inhibit and inactivate four microorganisms in agar and milk medium at different concentrations also the

inhibitory effects of essential oils increased with increasing concentration so it is suggested to investigate higher essential oils concentrations than were those used in research, and to study the effects over a longer time period in milk and other available milk products to access the potential of plant species essential oils as preservatives [28].

### *6.1.1. Main antibacterial phytochemicals*

Plant-derived compounds of therapeutic value are mostly secondary plant metabolites traditionally used for medicinal purposes. They have a wide activity range, according to the species, the topography and climate of the country of origin, and may contain different categories of active principles [29]. Variations in the chemical composition modify their antimicrobial activity. Some main categories of phytochemicals extracted from medicinal plants are examined to evaluate their pharmacological activity.

#### *6.1.1.1. Flavonoids*

Flavonoids, previously called bioflavonoids and included in aromatic compounds, are phenolic structures ubiquitous in photosynthesizing cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. The basic structural feature of flavonoid compounds is the 2-phenyl-benzopyrane or flavane nucleus, consisting of two benzene rings linked through a heterocyclic pyrane ring.

In total, there are 14 classes of flavonoids, differentiated on the basis of the chemical nature and position of substituents on the different rings. The antibacterial properties of flavonoids are thought to come from the ability to form complexes with both extracellular and soluble proteins, as well as with bacterial membrane [30].

Kuete demonstrated that among the flavonoids hydroxylating the prenyl groups of stipulin, the compounds obtained, angusticornin B and bartericin A, had a superior antimicrobial activity [31]. Thus, the prenyl group plays an important role in the activity of chalcones. Recently two flavonoids (6-hydroxy-7-methoxyluteolin and the xanthone 8-carboxymethyl-1,5,6-trihydroxy-3-methoxyxanthone) extracted from the leaves of *Leiothrix spiralis*, a South American plant belonging to the Eriocaulaceae family, showed a promising activity on *Escherichia coli* and *Pseudomonas aeruginosa* [32]. Some flavonoids also revealed activity against *M. tuberculosis* [33].

A synergy has been demonstrated between active flavonoids as well as between flavonoids and existing chemotherapeutics, even if the reports of activity in the field of antibacterial flavonoid research are widely conflicting, probably owing to inter- and intra-assay variation in susceptibility testing [34]. Future optimization of these compounds through structural alteration may allow the development of a pharmacologically acceptable antimicrobial agent or group of agents. Existing structure-activity data suggest that it might be possible, for example, to prepare a potent antibacterial flavanone by synthesizing a compound with halogenation of the B ring as well as lavandulyl or geranyl substitution of the A ring. Also, it is worth noting that by elucidating flavonoid biosynthetic pathways it would be possible to produce structural analogs of active flavonoids through genetic manipulation. Numerous research groups have sought to elucidate the antibacterial mechanisms of action of selected flavonoids; the activity of quercetin has been at least partially attributed to the inhibition of DNA gyrase, whereas sophoraflavone G and (-)-epigallocatechin gallate inhibit cytoplasmic membrane function, and licochalcones A and C inhibit energy metabolism.

#### *6.1.1.2. Alkaloids*

Alkaloids are heterocyclic nitrogen compounds characterized by different antimicrobial activities. The analysis of the leaf extracts of *Gymnema montanum* and of ethanol extract of *Tabernaemontana catharinensis* root bark revealed an antimicrobial activity [35] in the first case due to an activity depending upon the chemical composition of the extracts and membrane permeability of the microbes, and in the second case linked to indole alkaloids responsible for the observed antibacterial and antidermatophytic activity. Diterpene alkaloids, commonly isolated from the plants of the Ranunculaceae group, had antimicrobial properties [36]. Berberine, an isoquinoline alkaloid, present in roots and stem-bark of *Berberis* species, is a hydrophobic cation widely used in traditional medicine owing to its activity against bacteria, fungi, protozoa and viruses [37]. It accumulates in cells driven by the membrane potential and is an excellent DNA intercalator active on several microorganisms with a target on RNA polymerase, gyrase and topoisomerase IV and on nucleic acid [38].

#### *6.1.1.3. Terpenes*

Terpenes compounds are also referred to as isoprenoids and their derivatives containing additional elements, usually oxygen, are called terpenoids. The antibacterial activity of some monoterpenes (C10), diterpenoids, sesquiterpenes (C15), triterpenoids and their derivatives was recently reviewed. The results obtained illustrate the strong structure-

function influence of the antibacterial potential of terpenes. Diterpenoids, such as sesquiterpenes, isolated from different plants exhibited bactericidal activity against Gram-positive bacteria and inhibited the growth of *M. tuberculosis* [39]. The mechanism of action of terpenoids is not fully understood, but is speculated to involve membrane disruption by the lipophilic compounds [40].

#### *6.1.1.4. Phenolics & polyphenols*

Phenolic compounds are widely distributed in plants, where they protect the plants from microbial infections. They have potential anti-oxidative properties but are also potent anti-infectives. They are a large group of aromatic compounds, consisting of flavones, flavanoids and flavanols containing one carbonyl group, quinones with two carbonyl groups, tannins, polymeric phenolic substances, and coumarins, phenolic compounds with fused benzene and pyrone groups [41].

#### *6.1.1.5. Flavones and their derivatives*

Flavones and their derivatives represent an antibacterial therapeutic possibility to disrupt bacterial envelopes. The catechins are included among the flavan-3-ols or flavanols, present in different plants, particularly in tea-plant *Camelia sinensis*, where they form complexes with the bacterial cell wall and are active on intestinal microorganisms [42]. Biological assays indicated the inactivation of specific bacterial enzymes by several of these compounds. Moreover significant synergy was also observed between theaflavin and epicatechin against important nosocomial Gram-negative pathogens [43].

#### *6.1.1.6. Quinones*

Quinones (aromatic rings with two ketone substitutions), ubiquitous in nature, are another significant group of secondary metabolites with potential antimicrobial properties. They provide a source of stable free radicals and irreversibly complex with nucleophilic amino acids in microbial proteins determining loss of their function. Anthraquinones in particular had a large spectrum of antibacterial (also antimycobacterial) activity, based on inactivation and loss of function of bacterial proteins, such as adhesins, cell wall polypeptides and membrane-bound enzymes, consequently leading to the death of the pathogens [41].

#### *6.1.1.7. Tannins*

Tannins are a group of polymeric phenolic substances found in almost every plant part

characterized by antibacterial activity owing to inactivation of bacterial adhesins, enzymes, cell envelope and transport proteins. Recently, gallotannin-rich plant extracts demonstrated inhibitory activities on different bacteria attributable to their strong affinity for iron and to the inactivation of membrane-bound proteins.

Hydrolysable and condensed tannins, derived from flavanols, and called proanthocyanidins, exert antimicrobial activity by antiperoxidation properties inhibiting in particular the growth of uropathogenic *E. coli*. Anthocyanidin synthesis occurs in plants on the cytoplasmic leaflet of the endoplasmic reticulum and then accumulates in the large central vacuole; in many plants, anthocyanidins might occur in oligomeric form and in this case they are called proanthocyanidins. Depending on the type of bond between the oligomer-forming anthocyanidin molecules, two general types (A and B) of proanthocyanidins are distinguished. In less common A-type proanthocyanidins, two bonds are formed between 2β-7 and 4β-8 carbon of oligomer-forming molecules; in B-type, only one 4β-8 bond is formed. The beneficial effects of anthocyanins on human health have been known at least from the 16th century, when blackberry juice was used in the treatment of mouth and eye infections. However, only few studies have focused on the antimicrobial activity of these compounds. Recently, Cisowska et al. described the anthocyanin profile of action of different fruits, mainly berries, but also red grapes and, by consequence, red wine, also containing stilbenoid resveratrol, indicating a superior activity against Gram-positive bacteria [44].

#### *6.1.1.8. Coumarins*

One known coumarin, scopoletin, and two chalcones were isolated as antitubercular constituents of the whole plant *Fatoua pilosa*.

Also, spices and aromatic plants have an antimicrobial effectiveness that depends on the kind of plant, its composition and concentration of essential oils, often rich in monoterpens and sesquiterpenes. Studies analyzing the antimicrobial activity of essential oil of *Allium sphaerocephalon* inflorescenses revealed the accordance with the popular use of plants belonging to the *Allium* genus in traditional medicine, indicating the importance of aroma precursors (cysteine sulfoxides) for a potent biologic activity [45].

### *6.1.2. Plant extracts with efflux pump inhibitory activity*

Multidrug resistance due to the expression by bacteria of an efflux pump is an increasing clinical problem. Therefore an interesting approach to the therapy of many

infections would be one based on the identification of molecules interfering with the process of efflux.

In 1998, it was shown that plant-derived compounds are active against Gram-positive bacteria, in particular *Staphylococcus aureus*; successively numerous phytochemicals were shown to act as potential efflux pump inhibitors (EPIs) with antimicrobials for Gram-positive bacteria [46]. Gram-negative bacteria have innate multidrug resistance to many antimicrobial compounds owing to the presence of efflux pumps, in particular, the AcrAB-TolC efflux system, and some authors suggested that plants may not produce molecules active on these organisms [47].

However, the chemical diversity between plants and microorganisms represents an ecological possibility to identify EPIs from natural sources. Reviewing the literature concerning bacterial resistance modulators from natural plants, Stavri et al. described different bacterial EPIs, such as the plant alkaloid reserpine, berberine and methoxylated flavones and isoflavones, that revealed putative interfering activity on efflux [48]. Moreover, the level of accumulation of berberine in the cells was increased in presence of 5'-methoxyhydnocarpin, a multidrug pump inhibitor, reported as a minor component of chaulmoogra oil, used in traditional therapy for leprosy [49]. Recent data indicate that the AcrAB-TolC (in Enterobacteriaceae) and MexAB-OprM (in *P. aeruginosa*) efflux pumps are involved in the resistance of Gram-negative bacteria to most of the natural products [50]. In the presence of the EPI phenylalanine arginine β-naphthylamide (PAβN), the activities of some natural products belonging to the phenolics, in particular to the naphthoquinones (plumbagin), and flavonoids (4-hydroxyloncharpin), showed a significant increase in activity, whereas terpenoids are not active, probably due to difficulty in passing through the bacterial membrane barrier. The natural products exhibiting the best antibacterial activities have the same pharmacophore; plumbagin, which revealed significant antibacterial activity in the absence of an EPI, is the minimal scaffold required for activity. The other functional groups may modulate the susceptibility of the molecule to bacterial resistance mechanisms. Moreover, extracts from plants, and in particular an extract of an essential oil from a Corsican plant, *Helicrysum italicum*, containing geraniol, was able to synergize with chloramphenicol against different Gram-negative bacteria [51]. Garvey et al. indicated that extracts of different plants that are used as herbal medicinal products contain inhibitors of efflux in Gram-negative bacteria [52]. The most active compound, falcarindiol, extracted from *Levisticum officinale*, revealed a synergistic activity with ciprofloxacin. By adding EPI PAβN to *Dichrostachys glomerata* extracts, an increase of the activity on *E. coli*, *Klebsiella pneumoniae* and

*Providencia stuartii* resistant strains was shown; moreover, a synergistic effect was noted by associating *D. glomerata* extracts with some antibiotics.

Since the antimicrobial effectiveness of flavonoids comes from the ability to form complexes with both extracellular and soluble proteins and bacterial membranes, penetration of, and maintaining its position in, a microorganism is a critical point. Thus, the presence of EPIs is essential for flavonoid antimicrobial activity. Recently Fowler et al. used the natural flavonoid scaffold to synthesize non-natural flavanone molecules with functional groups responsible for activity against bacteria and fungi with minimal toxicity to human cells [53].

### *6.1.3. Plant extracts with bacterial quorum sensing inhibitory activity*

It is now well recognized that populations of bacteria from many Gram-positive and Gram-negative species cooperate and communicate to perform diverse social behaviors, including swarming, toxin production and biofilm formation. Communication among bacterial cells involves the production and detection of diffusible signal molecules and has become commonly known as quorum sensing (QS), a density-dependent system that regulates the bacterial expression of specific genes, whose products modify the local host environment favoring the invasion and persistence of the pathogen [54]. The discovery that many pathogenic bacteria employ QS to regulate their virulence makes this system interesting as a target for antimicrobial therapy. Therefore, the ability to interfere with QS interrupting bacterial communication opens new therapeutic prospects. The ideal QS inhibitor (QSI) would be a low-molecular-mass molecule able to reduce the expression of QS-controlled genes; in order to avoid toxic side effects; the inhibitor should exhibit a high degree of specificity for the target QS-related molecule. Finally, the QSI agent should be chemically stable and resistant to the metabolic and disposal processes of the host organism. The study of a strategy to interfere with bacterial QS is the classical pharmacological approach to receptor antagonism. In particular, halogenated furanones, a class of natural products isolated from the marine red algae *Delisea pulchra*, have an effect on bacterial QS. Zang *et al.* showed that the mechanism of action is the modification and inactivation of LuxS (S-ribosylhomocysteine lyase), the enzyme which produces autoinducer-2, that mediates interspecies QS among many bacteria, but is absent in humans [55]. Moreover, a number of plant extracts and natural compounds inhibiting *P. aeruginosa* QS have been identified by Rasmussen *et al.*, including bean sprout, chamomile, carrot, garlic, habanero (*Capsicum chinensis*), propolis, water lily, yellow pepper, and two products of *Penicillium* fungi, patulin and penicillic acid [56]. The

authors further investigated the effects of garlic extract, which contains at least three different QS inhibitors and was able to inhibit QS in a concentration-dependent manner and with a structure–activity relationship hypothesizing competitive binding. In fact, GeneChip® analysis revealed that garlic extract had a profound effect on QS-regulated virulence genes, significantly reduced *P. aeruginosa* biofilm tolerance to tobramycin and lowered the pathogenicity of *P. aeruginosa* in a *Caenorhabditis elegans* nematode model. The phytoalexin resveratrol (3,5,4'-trihydroxystilbene), an antifungal agent found in grapes and other plants, has direct antibacterial activity against *Neisseria gonorrhoeae* and *Neisseria meningitides*. However, we observed that resveratrol can inhibit *P. aeruginosa* QS *in vitro*. Also, solenopsin A, a venom alkaloid from the fire ant *Solenopsis invicta*, has been shown to be able to interfere with

*P. aeruginosa* QS, probably by targeting the $C_4$-HSL-dependent *rhl* system [57]. Solenopsin A reduced biofilm production in *P. aeruginosa* in a dose-dependent manner, indicating a QS signaling suppression mechanism. An inhibition of QS-controlled virulence factors, such as *LasA* protease, *LasB* elastase, pyoverdin and biofilm production, in the same microorganism by extracts from different south Florida (USA) plants was also reported. Recently, some traditional Chinese medicine herbs, in particular *Areca catechu*, are a rich source of compounds which exhibit anti-QS properties. Several QSI of natural origin, in particular the isothiocyanate iberin from horseradish, and ajoene, a sulfur rich molecule from garlic that inhibits *P. aeruginosa* genes controlled by QS, were identified [58]. Both ajoene and horseradish juice extract, in combination with tobramycin, have a synergistic antibacterial efficacy. A natural nonpeptide compound isolated from the bark of *Hamamelis virginiana*, hamamelitannin (2',5-di-*O*-galloyl-D-hamamelose), was found to inhibit QS in *S. aureus* and *S. epidermidis*, inhibiting the production of RNAIII and δ hemolysin *in vitro* [59].

#### ***6.1.4. Plant extracts with biofilm inhibitory activity***

Biofilms are the default mode-of-life for many bacterial species and biofilm-based infections cause harm to millions of humans annually. The difficulty of eradicating biofilm bacteria with classic systemic antibiotic treatments is a prime concern of medicine. In particular, the ability of staphylococci to adhere on both eukaryotic cells and abiotic surfaces and to form biofilm are important virulence factors in chronic infections associated with implanted biomaterials, which are particularly difficult to eradicate. Recently, Artini et al., assessing four compounds (derived from aerial and root parts of *Krameria lappacea*, *Aesculus*

*hippocastanum*, *Chelidonium majus* and *Macleya cordata*) that contained several alkaloids and flavonoids, revealed a potentially interesting activity on staphylococci, clinically significant microorganisms also for the emergence of methicillin-resistant variants [60]. Two compounds in particular, proAc (proanthocyanidin A2-phosphatidylcholine) isolated from *A. hippocastanum* and CH (chelerythrine) purified from *Macleya cordata*, exhibited an inhibition of '*de novo*' biofilm formation without bactericidal activity. The treatment of bacteria with these alkaloids downregulates some important proteins belonging to different pathways. In particular, proAc acts on the iron-binding protein (determining the impairment of the uptake of iron, an essential micronutrient for microorganisms), blocking the switch process from planktonic to sessile state of bacteria and ablating autolysin (penicillin-binding protein), thus inhibiting biofilm formation. The treatment with sanguinarine and CH acts on some bacterial proteins involved in heat shock response, surface exposed lipids and methoxy–mycolic acid synthase, until protein synthesis disappearance. Both sanguinarine and CH also act on some elements of the bacterial cytoskeleton, structural compart recognized as a potential target for antimicrobial therapy; therefore, inhibitors of cytoskeletal proteins may function as lead compounds for the development of novel antimicrobials. Hamamelitannin, a polyphenol extracted from the bark of *Hamamelis virginiana* belonging to the family of tannins, significantly reduces biofilm metabolic activity of different microorganisms [61].

Carvacrol, a monoterpenic phenol natural biocide, had an effect on dual-species biofilms formed by *S. aureus* and *Salmonella enterica* serovar *typhimurium* [62]. Nonbiocidal concentrations of this molecule disrupted normal development of biofilm, preventing the build up of protein mass and arresting at the microcolony stage. This component, together with thymol, is the principal phenolic component that determined the antimicrobial activity of oregano oil on staphylococci. These molecules, characterized by a hydrophobic nature, interact with the lipid bilayer of cytoplasmic membranes causing considerable effects on its structural and functional properties and loss of integrity of bacterial cell. Moreover, these compounds may diffuse through the polysaccharide matrix of the biofilm thus destabilizing it. A compound (1-deoxynoijirimycin) purified from *Morus alba* inhibited biofilm formation of *S. mutans*, a major causal organism of dental caries, reducing bacterial extracellular polysaccharide secretion [63]. Similarly a new naphthalene compound from *Trachyspermum ammi* seeds exhibited the same effect indicating great potential as a therapeutic agent against caries [64]. Moreover, another novel strategy to reduce development of dental caries may be the use of plant lectins, proteins that recognize the glycoconjugates present on the surface of *S. mutans*; in particular glucose/mannose-specific lectin altered the adhesion of bacteria on

saliva-coated surfaces [65]. Also, *Propionibacterium acnes*, microorganism responsible for acne vulgaris and able to form biofilm, resulted susceptible to plant extracts containing icariin, resveratrol and salidroside, compounds able to reduce biofilm formation [66].

For the treatment of urinary tract infections, *Melia dubia*, a plant from Meliaceae family present in the Indian subcontinent, has been used in folk medicine. Ravichandiran et al., examining the antivirulent potentiality of this plant, evaluated the principles antagonizing the quorum sensing systems of uropathogenic *E. coli* [67]. They found few compounds which can curtail the bacterial biofilm formation and virulence factor by controlling their quorum sensing

### 6.2. Antifungal agents

Human fungal infections, particularly in immunocompromised persons (AIDS, cancer and transplant patients), are a very challenging problem because the therapeutic options are hampered by serious drawbacks, such as the development of drug resistance and toxic side effects [68]. Thus, there is a clear demand for new therapeutic approaches based on molecules found in plants that may be used directly or considered as a model for developing better molecules. Before 2009, more than 600 plants have been reported for their antifungal properties, but few were examined for the active molecules [69]. Recently the use of the natural product tea tree oil in antifungal therapy has been proposed. This compound appears to be effective *in vitro* against multidrug-resistant *Candida* and *in vivo* against mucosal candidiasis [70]. Moreover, it has also been documented that terpinen-4-ol rather than 1,8-cineole is the most likely mediator of tea tree oil activity or, at least, a main contributor to anti-*Candida* activity. The genus *Paeonia* is one of the most important sources of drugs in traditional Chinese medicine. Picerno et al. observed that its extracts and some of their compounds inhibited *C. albicans* growth [71]. The antifungal properties of essential oils obtained from different aromatic plants, in particular from *Mentha suaveolens*, whose main microbicidal components were pulegone and piperitone oxide [72]. A strong antifungal activity of essential oils obtained from other plants was demonstrated [73]. In particular, in *Bidens tripartite* L. roots, the main components are α-pinene, β-bisabolene, p-cymene, hexanal and linalool; in *Coriandrum sativum* extracts, the effect is fungicidal and responsible for a marked reduction of germ tube formation [74]. From several parts (flower, leaf and stem containing different compounds) of *Aloysia triphylla*, *Gypsophila bicolor*, *Lavandula viridis*, *Erigeron acris* and *annuus*, and also from star anise (*Illicium verum*) an activity, linked to

trans-anethole, was observed [75]. Coumarin and phytoalexins, which are hydroxylated derivatives of coumarins, revealed a certain antifungal activity. The antifungal activity of dill (*Anethum graveolens*) oil results from its ability to disrupt the permeability barrier of the plasma membrane and from mitochondrial dysfunction-induced reactive oxygen species accumulation in *Aspergillus flavus* [76].

Promising activity against *C. albicans* biofilm formation was displayed by eugenol and cinnamaldehyde, molecules belonging to the phenolic group of essential oil compounds, which also showed synergy with fluconazole *in vitro* [77].

The essential oils of different *Curcuma* spp., containing caryophyllene as major compound, displayed varying degrees of antimicrobial activity, in particular against *Cryptococcus neoformans*. A protective effect of an oral natural phytonutrient was observed in recurrent vulvovaginal candidiasis, and promising alternatives were revealed by several terpenic derivatives for the topic treatment of oral candidiasis and denture stomatitis [78]. Some antidermatophitic compounds that have been long used as Chinese medicines to treat various ailments such as dermatomycosis, were obtained from extracts of *Fructus psoraleae* and *Folium eucalypti globuli* and also from *Achillea millefolium* extracts. Moreover, flavonoids isolated from mango (*Mangifera indica*) leaves revealed antifungal activity on different species, in particular *Aspergillus* sp., and schinol and a new biphenyl compound were active on *Paracoccidioides brasiliensis*. Moreover, metronidazole showed a potentiation of its antifungal effect when combined with plant extracts, as did fluconazole with other phytocomponents. Using genetic and biochemical approaches, Xu et al. showed the antifungal activity of a plant-derived acetylenic acid by interfering with the fatty acid homeostasis pathway [79].

### 6.3. Antiviral agents

#### ***6.3.1. Oregon grape*** *(Mahonia aquifollium/nervosa)*

This antiviral herb's active ingredient is an alkaloid called berberine, a bright yellow substance that's most prevalent in its roots. The roots are best harvested during the fall and winter months when the plant is in a state of dormancy. At this time, all of the plant's energy goes into the roots making the potency of the medicine at its highest. It's also starting to be used as an alternative to goldenseal (*Hydrastis canadensis*), which is one of the antiviral herbs that has been over harvested and has similar medicinal properties. Once you've harvested the

roots, remove the brown outer bark and keep the yellow inner bark and wood. Scrape the yellow inner bark and wood into shavings and make a tincture with the shavings using grain alcohol. Oregon grape tincture can be used to address the following viruses: cytomegalovirus (CMV), human papillomavirus (HPV), influenza, common cold (rhinovirus) [80].

### *6.3.2.* ***St. John's Wort*** *(Hypericum perforatum)*

Hypericin is the active ingredient in St. John's Wort which is most prevalent in its flowers. The flowers bloom during mid to late summer and that's when it is the best time to harvest. People that infuse it in olive oil as well. While it has a multitude of medicinal properties, it's an incredibly strong antiviral which shouldn't be underestimated. St. John's Wort can b used to address the following viruses: HSV 1 and 2, HIV, Hepatitis C, MCMV, Sindbus virus [81].

### ***6.3.3. Ginger*** *(Zingiber officinale)*

Ginger root, like garlic, is best taken raw and it's typically my second "go to" herb when either a cold or flu sneaks up on me. While it's often used for upset stomachs and motion sickness, its 12 antiviral compounds make it incredibly effective against viruses as well. Oftentimes I will shave portions of the root off onto my vegetables or just eat the shavings raw. The root is best harvested during the fall and winter months when it's most potent. Ginger can be used to address the following viruses: HRSV, common cold, flu and most viral infections of the lungs [82].

### ***6.3.4. Astragalus root*** (*Astragalus membranaceus*)

This is of the most popular antiviral herb in Chinese medicine because of it's ability to strengthen the immune system. It's a popular treatment for people who have undergone chemotherapy because of its ability to help the body quickly recover from such a severe amount of stress. Roots are best harvested in the fall and winter months and you should make it into a tincture similar to the method used for Oregon grape. Astragulus roots can be used to address the following viruses: HIV, influenza, common cold and most viruses in general because of how strong it makes your immune system [83].

### *6.3.5. Oregano* (*Origanum vulgare*)

Oregano is a member of the mint family which is high in volatile oils. The active ingredient in these volatile oils is called carvatrol and its best extracted using an olive oil infusion (let the oregano infuse for 6 weeks submerged in the olive oil). Oregano has also been vastly underrated as a medicine since store-bought oregano oil is typically less than half as potent as the oil you make yourself with wildcrafted oregano. The wildcrafted variety is considered by many to be the best treatment for both strep throat and the flu. While the oil has many benefits, it also kills beneficial bacteria, especially in your digestive system. Either don't use for a prolonged period of time or supplement dosages with foods that contain probiotics such as yogurt or sauerkraut. It's best to harvest when it's about 4 inches high before it goes to seed. Oregano can be used to address the following viruses: HSV 1, shingles, influenza, strep throat [84].

### *6.3.6. Usnea* (*Usnea australis*)

While it's actually a lichen and not an herbaceous plant, I decided to include it on my list of antiviral herbs because it's one of my cure-all favorites. Also known as Old Man's Beard, it can often be found on fallen branches of deciduous trees here in the Pacific Northwest. I often gather my usnea after wind storms. Its active ingredient is usnic acid and its best consumed in tincture form. People with autoimmune disorders should avoid usnea since it will encourage the immune system to attack healthy cells. Usnea can be used to address the following viruses: influenza, common cold, HSV 1 and 2, Epstein-Barr, Junin virus, Tacaribe virus and polyomavirus [85].

### *6.3.7. Lemon balm* (*Melissa officinalis*)

Lemon balm is another member of the mint family whose volatile oils contain antiviral compounds. It's best to harvest it between summer and mid fall since that's when the oils are most abundant. You can make it into a tincture as an antiviral herb but I personally prefer it as a tea or an infusion. It loses its volatile oils quickly so it's best to steep the leaves fresh rather than dry them out. It's also a great herb for calming the central nervous system. Lemon balm can be used to address the following viruses: HSV 1and 2, influenza, common cold [86].

### *6.3.8.* ***Lomatium*** (*Lomatium dissectum*)

Lomatium is also known as desert parsley and is largely an underrated antiviral herb. The root was used by Native Americans to treat a host of different viruses but was mainly used for upper respiratory infections. It actually started to be written about in great detail around 1918 after the outbreak of the Spanish flu where it saved a great deal of lives. Today it's used for almost all types of bad respiratory infections, bad fevers and pneumonia. It's active ingredient is an oleoresin stored in it's roots. It's a big and powerful medicine that is best taken in tincture form. Take in small dosages since some people develop rashes when taken in large dosages. Harvest the root either in the fall/winter when the plant is dormant or in the early spring before the plant goes to flower. The root should be harvested when the plant is between 4-10 years of age because that's when they have their highest medicinal concentrations. It's considered to be one of the better influenza herbs because not only is it antiviral, it's also respiratory clearing, meaning it prevents secondary infections which are common occurrences with the flu virus. Lomatium can be used to address the following viruses: cytomegalovirus, HIV, Epstein-Barr, influenza, common cold and practically any respiratory virus [87].

### *6.3.9.* ***White sage*** (*Salvia apiana*)

White sage is yet another mint family member. Its active ingredients are the volatile oils camphor and eucalyptol. The best time to harvest sage is in the mid to late summer once the flowers have gone to seed. It is best used in tincture form as an antiviral herb since many of the active ingredients aren't water soluble. White sage can be used to address the following viruses: common cold, influenza [88].

## 6.4. Antiprotozoal agents

Several natural compounds have been identified for the treatment of leishmaniasis and research on plants and their metabolites can contribute to overcoming the drug resistance of *Leishmania* parasites. Among the plant species evaluated here, *N. falcifolia* presented the best results regarding anti-leishmanial activity, with the ethanolic leaf extract displaying an $LD_{50}$ of 138.5 μg/ml and 65.6 ± 5.4% growth inhibition of the promastigote forms of *L.* (*V.*) *braziliensis* at the highest concentration tested, 320 μg/ml. Extracts of *H. gardneriana* (aerial

parts) and *C. podantha* (leaves), which also demonstrated reasonable potency, presented an $LD_{50}$ of 237 and 271 µg/ml, respectively. No growth inhibition was obtained at drug concentrations lower that 40 µg/ml. The medium containing DMSO did not affect the growth of the protozoa [89].

Ethanolic extracts of *C. podantha* and *M. arenosa* (aerial parts) inhibited the growth of epimastigote forms of *T. cruzi* even at very low concentrations (10 µg/ml), presenting 90.4 ± 11.52 and 88.9 ± 2.20% growth inhibition of this protozoan, respectively. On the other hand, extracts of *H. gardneriana*, *N. falcifolia*, and *P. elegans* (leaves) showed similar activities only when a concentration of 1000 µg/ml was used. The medium containing 1.0% DMSO did not affect the growth of the protozoa. Benznidazole used as the positive control against *T. cruzi* at 10 µg/ml, showed 80% growth inhibitions [90].

The best results in terms of molluscicidal activity were obtained with the ethanolic extract of *M. arenosa*, which induced 100 and 60% snail mortality at concentrations of 200 and 150 µg/ml, respectively, with an $LD_{50}$ of 143 µg/ml. The *N. falcifolia* extract was 100% lethal to the snails at the concentration of 200 µg/ml, but mortality was not obtained at lower concentrations. Control assays with DMSO showed no effect on the snails. Niclosamide at 5 µg/ml was used as positive control against *B. glabrata* and showed 100% lethality [90].

Although the literature indicates that ideal concentrations of plant extracts are below 100 µg/ml for molluscicidal activity, the results obtained for *M. arenosa*, $LD_{50}$ of 143 µg/ml, justify the continuation of its study. This plant is native to the area and the extract was obtained from regenerating parts of the plant, factors that can be considered of importance [91].

The genus *Nectandra* is well represented in the Brazilian flora, with several species presenting many benefits to man. They have been used in popular medicine for the relief of pain, arthritis, rheumatism and diarrhea, and also as antifungals. Pharmacological studies have demonstrated the antitumoral activity of *N. rigida* Nees, the antimalarial activity of *N. cuspidata* Nees and the vascular and antimalarial activities of *N. salicifolia* Nees. In our study, *N. falcifolia* leaves presented good results regarding their antiprotozoal activity against promastigote forms of *L.* (*V.*) *braziliensis* [91].

Some species of the genus *Helicteres* have been used in folk medicine, such as *H. isora* L. (as an expectorant, demulcent, astringent, antigalactagogue, and for the relief of the flu, against empyema, stomach affections, and diabetes), *H. angustifolia* (analgesic, anti-inflammatory and anti-bacterial effects), *H. ovata* Lam. (depurative, emollient and antisyphilitic effects), and *H. sacarolha* Juss. (depurative and in syphilitic inflammations).

Pharmacological studies have demonstrated the antidiabetic and hypolipidemic activities of *H. isora* L. Also *H. gardneriana* (aerial parts) also displayed good antiprotozoal activity against promastigote forms of *L.* (*V.*) *braziliensis* [92].

Among the species of the genus *Cayaponia* that have been used popularly, *C. tayuya* (Vell.) Cogn. and *C. espelina* Cogn. (anti-snake venom, tonic, diuretic, anti-asthmatic, antisyphilitic, and purgative effects, and to combat epilepsy, diarrhea and bronchitis), *C. cabocla* M. (purgative and depurative effects in cutaneous diseases and as an emmenagogue) and *C. pilosa* Cogn. (emmenagogue, antisyphilitic and purgative effects). Our data demonstrated that *C. podantha* (leaves) presents important antiprotozoal activity against epimastigote forms of *T. cruzi* and promastigote forms of *L.* (*V.*) *braziliensis* [93].

Some species of the genus *Melochia* have been used in folk medicine, such as *M. corchorifolia* L. (dysentery, abdominal swellings and water-snake bites), *M. umbellata* (Houtt.) Stapf (deobstruent) and *M. pyramidata* L. (bronchitis and cough). The extract obtained from the aerial parts of *M. arenosa* demonstrated molluscicidal effects and activity against *T. cruzi* epimastigotes [94].

## REFERENCES


1. http://www.WHO.int/mediacentre/factsheets/fs194/en
2. Aeschlimann JR. The role of multidrug efflux pumps in the antibiotic resistance of *Pseudomonas aeruginosa* and other Gram negative bacteria. Pharmacotherapy. 2003; 22(7): 916-924.
3. Alekshun MN, Levy S. Molecular mechanisms of antibacterial multidrug resistance. Cell. 2007; 128: 1037-1050.
4. Baquero F, Blázquez J. Evolution of antibiotic resistance. Trends in Ecology and Evolution. 1997; 12(12): 482-487.
5. Depardieu F, Podglajen, Leclercq R, Collatz E and Courvalin P. Modes and modulations of antibiotic resistance gene expression. Clinical Microbiology Reviews. 2007; 20(1): 79-114.
6. Fàbrega A, Sánchez-Céspedes J, Soto S, Vila J. Quinolone resistance in the food chain. International Journal of Antimicrobial Agents. 2008; 31: 307-315.
7. Traczewski MM, Deane J, Sahm D, Brown SD, Chesnel L. Impact of variations in test method parameters on in vitro activity of surotomycin against *Clostridium difficile* and surotomycin quality control limits for broth microdilution and agar dilution susceptibility testing. Journal of Clinical Microbiology. 2016; 54(3): 749-753.

8. Gefen O, Chekol B, Strahilevitz J, Balaban NQ. TDtest: easy detection of bacterial tolerance and persistence in clinical isolates by a modified disk-diffusion assay. Scientific Reports. 2017; 7: 41284.

9. Meletiadis J, Geertsen E, Curfs-Breuker I, Meis JF, Mouton JW. In vitro activity of micafungin against common and rare *Candida* species with the EUCAST, CLSI and E-test method: intra-and inter-laboratory agreement. Antimicrobial Agents and Chemotherapy. 2016; 60(10): AAC.01027-16.

10. Hazelton B, Thomas LC, Olma T, Kok J, O'Sullivan M, Chen SC, Iredell JR. Rapid and accurate direct antibiotic susceptibility testing of blood culture broths using MALDI Sepsityper combined with the BD Phoenix automated system. Journal of Medical Microbiology. 2014; 63(12): 1590-1594.

11. 11-Johann S, Seiler TB, Tiso T, Bluhm K, Blank LM, Hollert H. Mechanism-specific and whole-organism ecotoxicity of mono-rhamnolipids. Science of the Total Environment. 2016; 548: 155-163.

12. Wakhle L, Saigal SR. Rapid and specific diagnosis of group B streptococcal infection by the polymerase chain reaction (PCR). Advances in Experimental Medicine and Biology. 1997; 418: 347-349.

13. Jiang X, Shao N, Jing W, Tao S, Liu S, Sui G. Microfluidic chip integrating high throughput continuous-flow PCR and DNA hybridization for bacteria analysis. Talanta. 2014; 122: 246-250.

14. Plongthongkum N, Diep DH, Zhang K. Advances in the profiling of DNA modifications: cytosine methylation and beyond. Nature Reviews Genetics. 2014; 15(10): 647-661.

15. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evidence-Based Complementary and Alternative Medicine. 2011; 2011: 680354.

16. Gill AO, Holley RA. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. International Journal of Food Microbiology. 2006; 108(1): 1-9.

17. Hafedh H, Fethi BA, Mejdi S, Emira N, Amina B. Effect of *Mentha longifolia* L. ssp longifolia essential oil on the morphology of four pathogenic bacteria visualized by atomic force microscopy. African Journal of Microbiology Research. 2010; 4(11): 1122-1127.

18. Ultee A, Smid EJ. Influence of carvacrol on growth and toxin production by *Bacillus cereus*. International Journal of Food Microbiology. 2001; 64(3): 373-378.

19. Burt S. Essential oils: their antibacterial properties and potential applications in foods - a review. International Journal of Food Microbiology. 2004; 94(3): 223-253.

20. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, Bakhrouf A. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. Phytotherapy Research. 2007; 21(6): 501-506.

21. Ramos-Nino ME, Clifford MN, Adams MR. Quantitative structure activity relationship for the effect of benzoic acids, cinnamic acids and benzaldehydes on *Listeria monocytogenes*. Journal of Applied Bacteriology. 1996; 80(3): 303-310.

22. Akthar MS, Degaga B, Azam T. Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: a review. Issues in Biological Sciences and Pharmaceutical Research. 2014; 2(1): 1-7.

23. Sakagami Y, Kaikoh S, Kajimura K, Yokoyama H. Inhibitory effect of clove extract on vero-toxin production by enterohemorrhagic *Escherichia coli* O157: H7. Biocontrol Science. 2000; 5(1): 47-49.

24. Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. Journal of Food Protection. 2001; 64(7): 1019-1024.

25. Bozin B, Mimica-Dukic N, Simin N, Anackov G. Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. Journal of Agricultural and Food Chemistry. 2006; 54(5): 1822-1828.

26. Lv F, Liang H, Yuan Q, Li C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Research International. 2011; 44(9): 3057-3064.

27. Marino M, Bersani C, Comi G. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. International Journal of Food Microbiology. 2001; 67(3): 187-195.

28. Shan B, Cai YZ, Brooks JD, Corke H. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. Journal of the Science of Food and Agriculture. 2009; 89(11): 1879-1885.

29. Assob JC, Kamga HL, Nsagha DS, Njunda AL, Nde PF, Asongalem EA, Njouendou AJ, Sandjon B, Penlap VB. Antimicrobial and toxicological activities of five medicinal plant species from Cameroon traditional medicine. BMC Complementary and Alternative Medicine. 2011; 11: 70-81.

30. Fowler ZL, Baron CM, Panepinto JC, Koffas MA. Melanization of flavonoids by fungal and bacterial laccases. Yeast. 2011; 28: 181-188.
31. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Medica. 2010; 76: 1479-1491.
32. 32- Araújo MG, Hilário F, Nogueira LG, Vilegas W, Santos LC, Bauab TM. Chemical constituents of the methanolic extract of leaves of *Leiothrix spiralis* Ruhland and their antimicrobial activity. Molecules. 2011; 16: 10479-10490.
33. Garcia A, Bocanegra-Garcia V, Palma-Nicolas JP, Rivera G. Recent advances in antitubercular natural products. European Journal of Medicinal Chemistry. 2012; 49: 1-23.
34. Cushnie TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. International Journal of Antimicrobial Agents. 2011; 38: 99-107.
35. Medeiros MR, Prado LA, Fernandes VC, Figueiredo SS, Coppede J, Martins J, Fiori GM, Martinez-Rossi NM, Beleboni RO, Contini SH, Pereira PS, Fachin AL. Antimicrobial activities of indole alkaloids from *Tabarnaemontana catharinensis*. Natural Product Communications. 2011; 6: 193-196.
36. Atta-ur-Rahman, Choudhary MI. Diterpenoid and steroidal alkaloids. Natural Product Reports. 1995; 12: 361-379.
37. Kim SH, Lee SJ, Lee JH, Sun WS, Kim JH. Antimicrobial activity of 9-O-acyl and 9-O-alkylberberrubine derivatives. Planta Medica. 2002; 68: 277-281.
38. Iwasa K, Moriyasu M, Yamori T, Turuo T, Lee D, Wiegrebe V. In vitro cytotoxicity of the protoberberine-type alkaloids. Journal of Natural Products. 2001; 64: 896-898.
39. Kurek A, Grudniak AM, Kraczkiewicz-Dowjat A, Wolska KI. New antibacterial therapeutics and strategies. Polish Journal of Microbiology. 2011; 60: 3-12.
40. Termentzi A, Fokialakis N, Skaltsounis AL.Natural resins and bioactive natural products thereof as potential antimicrobial agents. Current Pharmaceutical Design. 2011; 17: 1267-1290.
41. Saleem M, Nazir M, Ali MS, Hussain H, Lee YS, Riaz N, Jabbar A. Antimicrobial natural products: an update on future antibiotic drug candidates. Natural Product Report. 2010; 27: 238-254.
42. Friedman M, Henika PR, Levin CE, Mandrell RE, Kozukue N. Antimicrobial activities of tea catechins and theaflavins and tea extracts against *Bacillus cereus*. Journal of Food Protection. 2006; 69: 354-361.
43. Betts JW, Kelly SM, Haswell SJ. Antibacterial effects of theaflavin and synergy with epicatechin against clinical isolates of *Acinetobacter baumannii* and *Stenotrophomonas*

*maltophilia*. International Journal of Antimicrobial Agents. 2011; 38: 421-425.

44. Cisowska A, Wojnicz D, Hendrich AB. Anthocyanins as antimicrobial agents of natural plant origin. Natural Product Communications. 2011; 6: 149-156.

45. Lazarevič JS, Dordevic AS, Zlatkovič BK, Radulovič NS, Palič RM. Chemical composition and antioxidant and antimicrobial activities of essential oil of *Allium sphaerocephalon* L. subsp. sphaerocephalon (Liliaceae) inflorescences. Journal of the Science of Food and Agriculture. 2011; 91: 322-329.

46. Holler JG, Christensen SB, Slotved HC, Rasmussen HB, Gúzman A, Olsen CE, Petersen B, Mølgaard P. Novel inhibitory activity of the *Staphylococcus aureus* NorA efflux pump by a kaempferol rhamnoside isolated from *Persea lingue* needs. Journal of Antimicrobial Chemotherapy. 2012; 67: 1138-1144.

47. Tegos G, Stermitz FR, Lemovskaya O, Lewis K. Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrobial Agents and Chemotherapy. 2002; 10: 3133-3141.

48. Stavri M, Piddock LJV, Gibbons S. Bacterial efflux pump inhibitors from natural sources. Journal of Antimicrobial Agents. 2007; 59: 1247-1260.

49. Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. Proceedings of the National Academy of Sciences. 2000; 96: 1433-1437.

50. Kuete V, Alibert-Franco S, Eyong KO, Ngameni B, Folefoc GN, Nguemeving JR, Tangmouo JG, Fotso GW, Komguem J, Ouahouo BM, Bolla JM, Chevalier J, Ngadjui BT, Nkengfack AE, Pagès JM. Antibacterial activity against bacteria expressing a multidrug-resistant phenotype. International Journal of Antimicrobial Agents. 2011; 37: 156-161.

51. Lorenzi V, Muselli A, Bernardini AF, Berti L, Pagès JM, Amaral L, Bolla JM. Geraniol restores antibiotic activities against multidrug-resistant isolates from Gram-negative species. Antimicrobial Agents Chemotherapy. 2009; 53: 2209-2211.

52. Garvey MI, Rahman MM, Gibbons S, Piddock LJV. Medicinal plant extracts with efflux inhibitory activity against Gram-negative bacteria. International Journal of Antimicrobial Agents. 2010; 37: 145-151.

53. Fankam AG, Kuete V, Voukeng IK, Kuiate JR, Pages J-M. Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes. BMC Complementary and Alternative Medicine. 2011; 11: 104-114.

54. Zucca M, Crivellaro S, Savoia D. New trends in the inhibition of Pseudomonas

aeruginosa quorum sensing activity. In: Cystic fibrosis: etiology, diagnosis and treatments. Leatte PN, ed. Nova Publishing, NY, USA, 2009: 1-9.

55. Zang T, Lee BWK, Cannon LM et al. A naturally occurring brominated furanone covalently modifies and inactivates LuxS. Bioorganic & Medicinal Chemistry Letters. 2009; 19: 6200-6204.

56. Rasmussen TB, Bjarnsholt T, Skindersoe ME et al. Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. Journal of Bacteriology. 2005; 187: 1799-1814.

57. Park J, Kaufmann GF, Bowen JP, Arbiser JL, Janda KD. Solenopsin A, a venom alkaloid from the fire ant *Solenopsis invicta*, inhibits quorum-sensing signaling in *Pseudomonas aeruginosa*. Journal of Infectious Diseases. 2008; 198: 1198-1201.

58. Jakobsen TH, van Gennip M, Phipps RK et al. Ajoene, a sulfur rich molecule from garlic, inhibits genes controlled by quorum sensing. Antimicrobial Agents Chemotherapy. 2012; 56: 2314-2325.

59. Pan J, Ren D. Quorum sensing inhibitors: a patent overview. Expert Opinion on Therapeutic Patents. 2009; 19: 1581-1601.

60. Artini M, Papa R, Barbato G, Scoarughi GL, Cellini A, Morazzoni P, Bombardelli E, Selan L. Bacterial biofilm formation inhibitory activity revealed for plant derived natural compounds. Bioorganic & Medicinal Chemistry. 2012; 20: 920-926.

61. Cobrado L, Azevedo MM, Silva-Dias A, Ramos JP, Pina-Vaz C, Rodrigues AG. Cerium, chitosan and hamamelitannin as novel biofilm inhibitors. Journal of Antimicrobial Chemotherapy. 2012; 67: 1159-1162.

62. Knowles JR, Roller S, Murray DB, Naidu AS. Antimicrobial action of carvacrol at different stages of dual-species biofilm development by *Staphylococcus aureus* and *Salmonella enterica* serovar Typhimurium. Applied and Environment Microbiology. 2005; 71: 797-803.

63. Islam B, Khan SN, Haque I, Alam M, Mushfiq M, Khan AU. Novel anti-adherence activity of mulberry leaves: inhibition of *Streptococcus mutans* biofilm by 1-deoxynojirimycin isolated from *Morus alba*. Journal of Antimicrobial Chemotherapy. 2008; 62: 751-757.

64. Khan R, Zakir M, Khanam Z, Shakil S, Khan AU. Novel compound from *Trachyspermum ammi* (Ajowan caraway) seeds with antibiofilm and antiadherence activities against *Streptococcus mutans*: a potential chemotherapeutic agent against dental caries. Journal of Applied Microbiology. 2010; 109: 2151-2159.

65. Islam B, Khan SN, Naeem A, Sharma V, Khan AU. Novel effect of plant lectins on the

inhibition of *Streptococcus mutans* biofilm formation on saliva-coated surface. Journal of Applied Microbiology. 2009; 106: 1682-1689.

66. Coenye T, Brackman G, Rigole P et al. Eradication of *Propionibacterium acnes* biofilms by plant extracts and putative identification of icariin, resveratrol and salidroside as active compounds. Phytomedicine. 2012; 19: 409-412.
67. Ravichandiran V, Shanmugam K, Anupama K, Thomas S, Princy A. Structure-based virtual screening for plant-derived SdiA-selective ligands as potential antivirulent agents against uropathogenis *Escherichia coli*. European Journal of Medicinal Chemistry. 2012; 48: 200-205.
68. Pitman SK, Drew RH, Perfect JR. Addressing current medical needs in invasive fungal infection prevention and treatment with new antifungal agents, strategies and formulations. Expert Opinion on Emerging Drugs. 2011; 16: 559-586.
69. Arif T, Bhosale JD, Kumar N et al. Natural products – antifungal agents derived from plants. Journal of Asian Natural Products Research. 2009; 11: 621-638.
70. Mondello F, De Bernardis F, Girolamo A, Salvatore G, Cassone A. In vitro and in vivo activity of tea tree oil against azole-susceptible and -resistant human pathogenic yeasts. Journal of Antimicrobial Chemotherapy. 2003; 51: 1223-1229.
71. Picerno P, Mencherini T, Sansone F, et al. Screening of a polar extract of *Paeonia rockii*: composition and antioxidant and antifungal activities. Journal of Ethnopharmacology. 2011; 138: 705-712.
72. Pietrella D, Angiolella L, Vavala E, et al. Beneficial effect of Mentha suaveolens essential oil in the treatment of vaginal candidiasis assessed by real-time monitoring of infection. BMC Complementary and Alternative Medicine. 2011; 11: 18.
73. Agarwal V, Lal P, Pruthi V. Prevention of *Candida albicans* biofilm by plant oils. Mycopathologia. 2008; 165: 13-19.
74. Silva F, Ferreira S, Duarte A, Mendonca DI, Domingues FC. Antifungal activity of *Coriandrum sativum* essential oil, its mode of action against *Candida* species and potential synergism with amphotericin A. Phytomedicine. 2011; 19: 42-47.
75. Huang Y, Zhao J, Zhou L, et al. Antifungal activity of the essential oil of *Illicium verum* fruit and its main component trans-anethole. Molecules. 2010; 15: 7558-7569.
76. Tian J, Ban X, Zeng H, et al. The mechanism of antifungal action of essential oil from dill (*Anethum graveolens*) on *Aspergillus flavus*. PLOS One. 2012; 7: e30147.
77. Khan MS, Ahmad I. Antibiofilm activity of certain phytocompounds and their synergy with fluconazole against *Candida albicans* biofilms. Journal of Antimicrobial Chemotherapy. 2012; 67: 618-621.

78. Marcos-Arias C, Eraso E, Madariaga L, Quindos G. In vitro activities of natural products against oral *Candida* isolates from denture wearers. BMC Complementary and Alternative Medicine. 2011; 11: 119.

79. Xu T, Tripathi S, Feng, et al. A potent plant-derived antifungal acetylenic acid mediates its activity by interfering with fatty acid homeostasis. Antimicrobial Agents Chemotherapy. 2012; 56: 2894-2907.

80. Perumal Samy R, Gopalakrishnakone P. Therapeutic potential of plants as anti-microbials for drug discovery. Evidence-Based Complementary and Alternative Medicine. 2010; 7(3): 283-294.

81. Jacobson JM, Feinman L, Liebes L, Ostrow N, Koslowski V, Tobia A, Cabana BE, Lee DH, Spritzler J, Prince AM. Pharmacokinetics, safety, and antiviral effects of hypericin, a derivative of St. John's wort plant, in patients with chronic hepatitis C virus infection. Antimicrobial Agents and Chemotherapy. 2001; 45(2): 517-524.

82. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. New England Journal of Medicine. 2005; 352(17): 1749-1759.

83. Roxas M, Jurenka J. Colds and influenza: a review of diagnosis and conventional, botanical, and nutritional considerations. Alternative Medicine Review. 2007; 12(1): 25-49.

84. Stengler M. Nature's Virus Killers. M. Evans; 2001.

85. Levi JR, Brody RM, McKee-Cole K, Pribitkin E, O'Reilly R. Complementary and alternative medicine for pediatric otitis media. International Journal of Pediatric Otorhinolaryngology. 2013; 77(6): 926-931.

86. Nimitz JS, inventor. Antiviral supplement compositions and methods of use. United States patent application US 14/333,645, 2014.

87. Shock MP, Shock CC, Feibert EB, Shaw NL, Saunders LD, Sampangi RK. Cultivation and irrigation of fernleaf biscuitroot (*Lomatium dissectum*) for seed production. Hort Science. 2012; 47(10): 1525-1528.

88. Cohen S, Tyrrell DA, Smith AP. Psychological stress and susceptibility to the common cold. New England Journal of Medicine. 1991; 325(9): 606-612.

89. Suffredini IB, Sader HS, Gonçalves AG, Reis AO, Gales AC, Varella AD and Younes RN. Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic Forest. Brazilian Journal of Medical and Biological Research. 2004; 37: 379-384.

90. Marston A, Hostettmann K. Plant molluscicides. Phytochemistry. 1985; 24: 639-652.

91. Muñoz V, Sauvain M, Bourdy G, Callapa J, Bergeron S, Rojas I, Bravo JA, Balderrama L, Ortiz B, Gimenez A, Deharo E. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. Journal of Ethnopharmacology. 2000; 69: 127-137.
92. Chang YS, Ku YR, Lin JH, Lu KL, Ho LK. Analysis of three lupine type triterpenoids in Helicteres angustifolia by high-performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis. 2001; 26: 849-855.
93. Lorenzi H. Plantas Medicinais no Brasil: Nativas e Exóticas Cultivadas. Instituto Plantarum, Nova Odessa, SP, Brazil, 2002.
94. Lorenzi H. Plantas Daninhas do Brasil: Terrestres, Aquáticas, Parasitas, Tóxicas e Medicinais. Instituto Plantarum, Nova Odessa, SP, Brazil, 1991.

# Antimicrobial activity of certain secondary metabolites derived from family Scrophulariaceae

4

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## ABSTRACT

The figwort family (Scrophulariaceae) is a big family comprising about 87 genera with nearly 4800 species. Members of this family are highly popular for their folk medicinal uses in addition to their phytotherapeutic importance. Scrophulariaceae represents an appealing, unique and diverse resource that furnishes a vast array of bioactive secondary metabolites including alkaloids, phenylpropanoids, iridoid glucosides, and terpenoids. Additionally, various species belonging to the Scrophulariaceae contain substantial amounts of flavolignans, polyphenols and phenolic acids. Undoubtedly, these phytoconstituents are responsible for their wide medicinal values as anti-inflammatory, antimicrobial, antinociceptive, antioxidant and cytotoxic properties. *Buddleja, Eremophila, Leucophyllum, Myoporum, Scrophularia* and *Verbascum* are among the highly valuable medicinal genera in the figwort family. In this chapter we are shading light on the antimicrobial potency of various species belonging to the aforementioned genera with special emphasis on the effects of their derived secondary metabolites and interpretation of their previously reported mode of action. Moreover, in *silico* molecular modeling study of the major constituents isolated from these genera of interest on various key enzymes responsible for the incidence, resistance and progression of infectious diseases will be carried out in an attempt to verify the probable

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mechanism of action for the previously reported antimicrobial members belonging to Scrophulariaceae.

**Keywords**: Antimicrobial activity; *Buddleja; Eremophila; Leucophyllum*; *Verbascum*; *Myoporum*; *Scrophularia*; Molecular modeling; Phytoconstituents; Scrophulariaceae.

## 1. INTRODUCTION TO MICROBIAL INFECTIONS AND ITS HAZARDOUS EFFECT

Infection can be defined as an invasive attack of the living organism by disease producing agents as well as their multiplication within the organism's body with the subsequent interaction of the immune system of the host organism towards them and their toxins. Infectious diseases, also known as transmissible or communicable diseases, can be classified anatomically into skin, odontogenic, respiratory tract, urinary tract infections as well as vaginal and intra amniotic infections. The main symptoms of infectious diseases comprise aches, fatigue and appetite loss with concomitant loss of weight as well as fever, chills and night sweets. Additionally, other symptoms which are greatly specific to the affected organ including cough, runny nose and skin rashes may appear in addition to the existence of asymptomatic infections [1].

Despite of the enormous progress in the medicinal strategies for the curing of many health problems, infectious diseases due to bacteria, fungi and viruses still constitute a major impendence to public health in the 21$^{st}$ century. This is ultimately obvious in developing countries attributing to the lack of medicine in addition to the appearance of many resistant strains to commonly used antibiotics. Accordingly, novel classes of antibiotics are constantly required to overcome the disturbing side effects of synthetic antimicrobial agents. Hence, attention has been given to the beneficial therapeutic potential of herbal medicine setting an example of cheap, substantially safe remedy offering a mine that could be used as antimicrobial candidates [2].

The development of bacterial resistance combating the commonly used antibiotics has been seriously reducing the cure rate. The probable mechanisms of bacterial resistance that were previously reported include the inactivation of the antimicrobial agent directly through the changing of the important functional groups in the drug as acetylation, methylation and the opening of the beta-lactam ring of penicillin. Moreover, the modification of the targeted

site of action, the variation in the metabolic pathways that the drug prohibited, inhibition of drug uptake by the affected tissues and reduction of the intracellular amount of the drug *via* its exportation out of the infected cell by ABC transporters are also among the prominent mechanisms of bacterial resistance [3].

## 2. PREVIOUSLY REPORTED MECHANISMS OF ACTIONS FOR POPULAR ANTIMICROBIALS

The usage of antimicrobial agent is widely accepted to prohibit the spread of infection. It can be classified according to their purpose into antiseptics that are effective in the removal of microorganisms from living tissue and/or skin. However, disinfectants are used for the destruction of the microorganisms existing on non-living things in addition to the antibiotics that are given as a prophylactic rather than as a cure. Besides, hand washing, wearing gowns together with face masks, the adherence to healthy lifestyle with a balanced diet and regular exercise may help to reduce the risk of incidence of bacterial infections. It is worthy to mention that the prolonged utilization of these agents causes bacterial mutations with consequent appearance of bacterial resistance [4].

There are a lot of mechanisms by which the antimicrobial agents exert their effects; this includes inhibition of protein, nucleic acids as well as cell wall synthesis, prohibition of the function of cell membrane, in addition to interfering with other metabolic processes (Fig. 1) [5]. The existence of cell wall in the bacterial cells that is crucial to their survival make them more susceptible for being attacked by the antimicrobial agents that targeted cell wall synthesis inhibition as penicillins and cephalosporins comparable to human and animal cells that lack cell wall. On the contrary, polymixin B and colistin that affect the cell membrane structure causing its damage and the leakage of vital substances necessary for the cell's survival is nonselective and also adversely affect human and animal cells. However, antimicrobial agents that inhibit protein are designed to selectively attack certain bacterial enzymes and structures that are required for their multiplication and growth with concomitant interference with their metabolism as 30S or 50S subunits of the intracellular ribosomes as aminoglycosides chloramphenicol and tetracyclines. Meanwhile, it goes without saying that nucleic acids, DNA and RNA, are of great necessity to the bacterial division and survival so selective binding with the bacterial nucleic acids could effectively lead to their death as quinolones. Additionally, many antibiotics hits certain metabolic paths that are essential for

the bacterial survival as inhibition of dihydrofolate reductase and dihydropteroate synthase enzymes resulting in disruption of folic acid synthesis with consequence destruction of DNA synthesis as sulfonamides and trimethoprim [6].

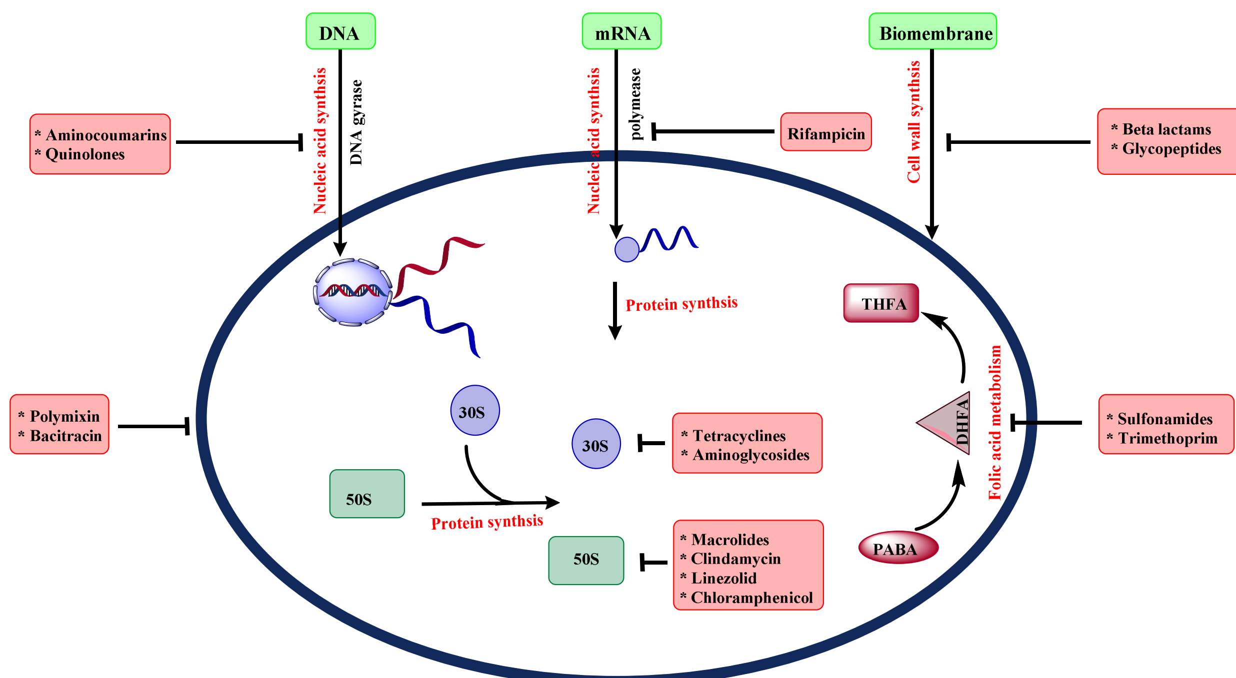


**Figure 1.** Major microbial targets for antimicrobial agents

Apart from the well-defined targets of the commonly used antimicrobial agents, antimicrobial enzymes that constitute a prominent part from the host immune system implicated in its defense against pathogenic microbes are nowadays adopted as an advanced method for combating infectious disease. These enzymes include hydrolyzing ones that prohibit bacterial growth either by invading the crucial components of the cell wall or by destroying the substances that adheres the cells together and to surrounding surfaces. Besides, proteolytic enzymes exemplified by subtilisins and lysostaphin are highly effective in hydrolyzing the adhesive proteins that are mandatory in the formation of bacterial biofilms. Polysaccharide-hydrolyzing enzymes comprising α-amylase, dispersin B, chitinases, *β*-glucanases and alginate lyases are found to be effective in both inhibiting biofilm formation as well as destroying the formed films in a vast array of microorganisms *via* cleavage of various glycoside linkages. Additionally, oxidative enzymes mainly cellobiose dehydrogenase, glucose oxidase and superoxide dismutase are known to elicit an anti-infective

manner *via* the liberation of a huge amount of the destructive hydrogen peroxide that causes cytotoxicity in the infectious agent. Among the interesting host enzymes that exhibited potent antifungal properties are quorum-quenching and urease enzymes. The formers include AHL-lactonase, AHL-acylase and paraoxonases that showed a great interference to bacterial cell-to-cell communication (quorum sensing) that undoubtedly decrease the ability of the organisms to release virulence components causing eradication of the organisms. However the latter is highly efficacious in case of urinary tract infections inhibiting the formation of hard coatings around the bacteria that protects it [7-9].

## 3. REPORTED ANTIMICROBIAL ACTIVITY OF SOME IMPORTANT GENERA BELONGING TO FAMILY SCROPHULARIACEAE AND THEIR ISOLATED PHYTOCONSTITUENTS

### 3.1. *Buddleja*

Genus *Buddleia (Buddleja)* belonging to the family Scrophulariaceae comprises about 100 species that are native to Africa, Asia, North and South America [10]. Traditionally, *Buddleia* species were highly recommended to be used as a topical antiseptic as well as a diuretic owing to the different classes of compounds predominating in the genus [11]. Recently many of the *Buddleia* species have been investigated for their antimicrobial properties and many of which are found to be highly effective either in the form of crude extract or isolated compounds.

The stems and the leaves of *B. saligna* were evaluated for their antimicrobial activity using isolates of 10 bacteria species comprising five Gram-positive and five Gram-negative strains. The tested samples showed substantial activity against the Gram-positive and some Gram-negative strains that further consolidates the fact that Gram-negative bacteria are more resistant relative to the Gram positive ones. The methanol extract of its leaves that showed higher antibacterial potency comparable to the stems was found to exert its antimicrobial effect against all the tested bacterial strains except for *Serratia marcescens* and *Pseudomonas aeruginosa. Bacillus cereus*, *Streptococcus pyrogens* and *Pseudomonas aeruginosa* only were susceptible to the stem effect [12].

Additionally, the ethanol extracts of *B. globosa* leaves exhibited a potent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* mainly attributing to the presence

of verbascoside that showed a minimal inhibitory concentration of 1 mM [13]. Verbascoside **(1)** was also isolated from *B. cordata* leaves methanol extract and was monitored for its antibacterial property against *Staphylococcus aureus* utilizing killing kinetics together with incorporation of precursor methods. Results clarified the lethal effect of verbascoside on *S. aureus via* interfering with protein synthesis and inhibition of leucine incorporation [14]. Additionally, *B. globosa* stem bark showed antifungal activity against *Trichophyton rubrum*, *Trichophyton interdigitale*, and *Epidermophyton floccosum*. Buddlejone **(2)**, maytenone **(3)**, buddledin A **(4)** and buddledin B **(5)** and deoxybuddlejone **(6)** were found to be responsible for the antifungal activity with buddledins A and B showed the greatest activity, with MIC values of 43 µM and 51 µM, respectively [15].

Similarly, the antifungal activity of the chloroform extracts of *B. cordata* and *B. davidii* stem bark against the soil fungiwas explained owing to the predominance of the sesquiterpene buddledin A [16].

Moreover, the acetone/water (4:1) crude extracts of *B. saligna* leaves and stem showed pronounced activity by bioautography against *Escherichia coli*, *Staphylococcus aureus*, and *Mycobacterium aurum*. Oleanolic acid **(7)** isolated from the *n*-hexane soluble fraction of the crude extracts exhibited bactericidal activity against *Mycobacterium microti*, *Mycobacterium avium* and *Mycobacterium scrofulaceum* at loading doses of 2.5 µg/spot for the first and 1.25 µg/spot for the last two ones. Its mechanism of action could be explained in virtue of suppression of DNA polymerase [17]. For *B. brasiliensis* leaves methanol extract, it showed antibacterial properties against *Aeromonas hydrophila*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [18].

*B. perfoliata* Kunth, used traditionally for the alleviation of digestive disorder, exhibited *in vitro* anti-*H. pylori* activity with minimum inhibitory concentration (MIC) of aqueous and methanol extracts of its aerial parts equals to 500, and 62.5 µg/ml, respectively [19]. The crude extract of the stem bark of *B.cordata* exhibited a marked anti-mycobacterial activity owing to the presence of various phytoconstituents comprising 2[4'-hydroxyphenyl]-ethyl lignocerate **(8)** that revealed a substantial antibacterial activity against *Mycobacterium tuberculosis* with MIC value of 64 micrograms/ml [20].

The leaf extracts of *B. salviifolia* as well as its isolated compounds namely, 4'-hydroxyphenyl ethyl vanillate **(9)**, verbascoside **(1)** and quercetin **(10)** showed a broad spectrum of antibacterial activity. Its ethyl acetate fraction revealed a good activity against *Bacillus subtilis* and *Staphylococcus aureus* wheraeas, the hexane and dichloromethane fractions showed the highest activity towards *Candida albicans* [21]. Besides, the volatile oil

of *B. asiatica* leaves that was found to be enriched with β-caryophyllene oxide **(11)**, citronellol **(12)**, and β-caryophyllene **(13)** revealed a highly potent antifungal, antibacterial and anthelmintic activity [22].

### 3.2. *Eremophila*

Members of the genus *Eremophila,* commonly known as Fuchsia bush or Emu bush, represent perennial shrubs containing approximately 214 species with an endemic existence in the arid and semi-arid areas in Australia. They have been employed in the folk medicine for the cure of many health disorders including respiratory, gastro-intestinal tract and skin infections. Additionally many biological activities have been assigned to the genus anti-infective, immunomodulatory, and anti-inflammatory as well as antiproliferative activities. This could be due to its richness with flavonoids, lignans, phenylpropanoids and terpenoid [23]. Evaluation of the antimicrobial potency of various *Eremophila* extracts as well as their isolated compounds revealed their antimicrobial efficacy particularly against Gram-positive bacteria [24]. *E. duttonii* extract showed *a* high antimicrobial potency against Gram-positive bacteria exemplified by *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus* and *Streptococcus pyogenes* [25]. Additionally, *E. alternifolia* and *E. duttonii* ethanol extracts of inhibited clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) [26]. Moreover, various food-borne pathogensas *Clostridium* spp. namely, *C. perfringens*, *C. sporogenes* in addition to *Listeria monocytogenes* were greatly susceptible to the lethal effect *of E. duttonii* extract. This pronounced activity could be to a great extent relied upon the existence of various sterols and terpenes as revealed by bioautography [27]. Recently, extracts for both *E. alternifolia* and *E. duttonii* have shown inhibition zones of 8.8 mm and 9.6 mm, respectively for *Listeria monocytogenes* [28]. Serrulat-14-en-7,8,20-triol **(14)** and serrulat-14-en-3,7,8,20-tetraol **(15)** isolated from its *n*-hexane fraction revealed potency against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pneumonia* [29].

Regarding the diethyl ether extract of *E. neglecta,* 8,19-dihydroxyserrulat-14-ene **(16)** and 8-hydroxyserrulat-14-en-19-oic acid **(17)** were isolated and showed antimicrobial potency against *Staphylococcus aureus, Streptococcus pyogenes*, and *Streptococcus pneumoniae* in addition to exertion of activity against the Gram-negative bacteria *Moraxella catarrhalis* showing MIC values of 3.1, 6.2 μg/ml for the two compounds, respectively [30, 31]. *E. serrulata* leaves extracts offered O-naphthoquinone **(18)**, and 20-acetoxy-8-

hydroxyserrulat-14-en-19-oic acid **(19)** that showed activity towards *Streptococcus pyogenes*, and *Streptococcus pneumonia* [32]. However the ethyl acetate fraction obtained from *E. sturtii* leaves together with its isolated compounds, 3,8-dihydroxyserrulatic acid **(20)** and serrulatic acid **(21)** showed potency with the latter being most potent against *Staphylococcus aureus* [33].

A study carried out by Ndi et al., in 2007 evaluating the antibacterial activity of 72 different extracts of *Eremophila* towards 68 clinical isolates of multi-resistant methicillin-resistant *Staphylococcus aureus* (MRSA). Results shaded the light on the antimicrobial property of several tested *Eremophila* species particularly *E. virens* that prohibited the growth of all the examined isolates at a concentration of 31μg/ml [34]. *E. longifolia* ethanol extracts of the stem lethally affect *Streptococcus mutans* and *Streptococcus sobrinus* growth owing to the prevalence of phenolics [35]. *E. maculata* leaves ethanol extract was effectively potent against three Gram-positive bacteria [36]. Additionally, *E. longifolia* offered antimicrobial agents which are neryl ferulate **(22)** and neryl *p*-coumarate **(23)**. The former showed moderate effect against several various Gram-positive bacterial strains whereas the latter was effective only towards *Enterococcus faecium* only [37].

The antimicrobial efficacy of *E. microtheca* and its isolated compound jaceosidin **(24)** recorded relevant antibacterial activity against *Staphylococcus aureus* strains [38]. In a mechanistic study to trace the antimicrobial potency of 8-hydroxyserrulat-14-en-19-oic acid **(17)** isolated from *E. neglecta*, results revealed that its bactericidal effect can be interpreted by its effect on the logarithmic-phase, stationary-phase, and adherent *Staphylococcus epidermidis*, as well as against methicillin-susceptible and methicillin-resistant S. aureus making it unable to produce polysaccharide intercellular adhesion-mediated biofilm. Thus, this clarified its multi-target effect through its hydrolytic properties on the cell membrane together with the general inhibition of macromolecular biosynthesis [39]. The smoke extract obtained from *E. longifolia* exhibited potent antimicrobial properties against the Gram-positive species *Staphylococcus aureus*, *Bacillus subtilis* and the yeast *Candida albicans* owing to the presence of genifuranal that is formed as a result of rearrangement of geniposidic acid upon heating that does not exist naturally in the leaves [40].

Besides, the antimicrobial properties for a number of *Eremophila* species have been reported comprising *E. bignoniiflora* and *E. maculata.* The former with its major compounds fenchyl-acetate and bornyl-acetate successfully inhibited pathogenic *Trichophyton* species associated with dermatophytosis, however substantial activity was proven against *Candida albicans* and *Staphylococcus epidermidis* [41]. Meanwhile the volatile oil of the  of the leaves

and flowers latter species showed a relevant antimicrobial activity against a panel of Gram positive, Gram negative, MRSA and fungi [42].

### 3.3. *Leucophyllum*

*Leucophyllum* is a small genus comprising about 15 species of evergreen shrubs , native to Mexico as well as the southwestern United States [43]. The methanol extracts obtained from the roots and leaves of *L. frutescens* revealed a high antimicrobial potency against the drug-resistant strain of *Mycobacterium tuberculosis*; with MICs of 62.5 and 125 µg/ml, respectively [44]. Besides, the diterpene leubethanol **(25)** isolated from *L. frutescens* showed notable antibacterial potency towards multidrug-resistant strains as *Staphylococcus aureus*, and is of great interest for being applied in the strict control of bacterial biofilms formation [45]. Additionally, the furolignan 2',5''-dimethoxysesamin **(26)**, isolated from the its root bark, exhibited promising antituberculous activity with a MIC equals to 63 µg/ml with no observed cytotoxicity [46].

### 3.4. *Myoporum*

*Myoporum* is a genus including about 30 species of flowering plants and recently included in the family Scrophulariaceae. It is native to the Australian areas as well as Pacific islands and Indian oceans. *Myoporum* members are mainly shrubs or small trees mostly with white flowers. Many *Myoporum* species have shown a potent antimicrobial activity including *M. acuminatum* in which the essential oil of its fruits revealed a notable antimicrobial potency against a panel of bacteria as *Bacillis subtilis,Streptococcus pneumonia*, *Escherichia coli* as well as fungi comprising *Aspergillus fumigates*, *Geotricum candidum* and *Syncephalastrum racemosum*. This may be attributed to the synergistic action of all its components that are represented mainly by D-limonene **(27)** and (-) carvone **(28)** as monoterpenoids in addition to negaione **(29)** and myomontanone **(30)** as furanoid sesquiterpenes [47].

Moreover, the leaves of *M. montanum* was found to contain three toxic furanosesquiterpenes namely (±)-myoporone **(31)**, (-)-10,11-dehydromyoporone and 11-hydroxymyoporone that showed a prominent antibacterial activity towards *Enterococcus faecalis*, *Moraxella catarrhalis* and *Staphylococcus epidermidis* with immeasurable cytotoxicity against a number of cancer cell lines as well as the normal breast cells [48]. Also, the essential oil of its leaves and stems revealed a potent antimicrobial activity [49].

Meanwhile, the ethereal oil of *M. crassifolium* showed a considerable antimycobacterial activity against *Mycobacterium* bovis [50]. Regarding *M. bontioides*, it showed a relevant antibacterial activity particularly towards *Staphyloccocus aureus* and *Escherichia coli* with its constituent, 5,7-dihydroxyflavone **(32)** showing substantial of antibacterial property with MIC of 62.50 μg/ml [51]. Besides, their isolated compounds namely tangeretin **(33)**, sinensetin **(34)**, dihydrokaempferol **(35)**, luteolin **(36)** exhibited significant antifungal activity towards *Colletotrichum musae* [52].

Additionally, in a recent study that was done using *Chromobacterium violaceum* assay to evaluate the ability of the substances to hinder and disrupt the bacterial communication system expressed in terms of quorum sensing (QS). Leaves of *M. laetum* revealed an interesting anti-quorum sensing that undoubtedly reflected in its potent antimicrobial activity *via* prohibiting the secretion of virulence factors facilitating the eradication of the organisms [53]. Additionally, a significant antiviral, antibacterial as well as antifungal activity was shown by its essential oil that showed its richness by as ngaione **(29)**, myoporone **(31)**, and myomontanon **(30)** [47].

### 3.5. *Scrophularia*

*Scrophularia* with its 200 species represents a large genus of herbaceous plants that are widely distributed in Asia. Its name was derived from a form of tuberculosis termed scrofula as the majority of its species are highly popular as antituberculous agents. Traditionally, it was employed for the relief of many ailments owing to its richness with various secondary metabolites particularly iridoids [54, 55].

Additionally many species of *Scrophularia* have been used for the alleviation of infectious diseases as *S. buergeriana* that showed potent antimicrobial and anti-viral properties [56]. Moreover, the leaves extract of *S. ningpoensis* together with its isolated saponin glycoside, scrokoelziside A **(37)**, showed a great activity against beta-haemolytic streptococci using disc-diffusion as well as the micro-well dilution methods [57]. Recent antimicrobial investigations done on the highly reputable traditional antimicrobial herbaceous herb *S. striata* applying micro broth dilution assay towards a wide array of bacteria and fungi resulted in a potent antimicrobial properties. Actually, this activity was found to be directly related to the total phenolic content as reveled from the total phenolics and flavonoids assessments [58]. Moreover, *S. deserti* showed a relevant antimicrobial activity that is probably relied on the presence of 3(beta)-hydroxy-octadeca-4(E),6(Z)-dienoic acid,

ajugoside **(38)** and scropolioside B that exhibited substantial antimicrobial activity towards multidrug strains as well as methicillin-resistant *Staphylococcus aureus* (MRSA) and a panel of rapidly growing mycobacteria showing MICs values between 32 and 128 μg/ml [59]. However, the essential oil isolated from the aerial parts of *S. subaphylla* that is composed mainly of terpenoids and fatty acids namely, linalool, phytol, geraniol and palmitinic acid that showed mild antibacterial activity comparable to other *Scrophularia* members [60].

### 3.6. *Verbascum*

The genus *Verbascum* is commonly named by velvet plant belongs to the figwort family and comprises about 250 species. They mostly spread in Asia, Africa, and North America and Europe. Many beneficial internal and external effects have been attributed to leaves and flowers of many of its species. Traditionally, they were highly adopted to induce diuresis, expectoration as well as sedation [61].

Many *Verbascum* species as *V. bombyciferum, V. olympicum*, *V. thapsus* and *V. xanthophoeniceum* showed potent antimicrobial activity in many recent researches [62, 63]. Additionally, the ethanol/water (70:30 v/v) extract of *V. macrurum* leaves revealed potent antibacterial activity [64]. It is worthy to mention that the antimicrobial activity of *V. densiflorum* and *V. phlomoides* is greatly attributed to their richness in flavonoids and phenylethanoids. Diosmin **(39)** and tamarixetin 7-rutinoside **(40)** are highly prevalent in the flower *V. phlomoides* meanwhile verbascoside **(1)** and luteolin 7-glucoside **(41)** (were greatly abundant in *V. densiflorum* flower [65].

*V. bottae* was proved to be a highly efficacious antibacterial agent exhibiting its effect against a wide panel of Gram-positive bacteria including multi-resistant ones owing to the existence of various classes of compounds like flavonoids and terpenoids [66]. Moreover, the antimicrobial evaluation done on various extracts of *V. pinetorum* and *V. antiochium* revealed that its methanol and methanol/chloroform extracts are highly potent on a broad range of microorganisms particularly *Haemophilus influenzae,* whereas the acetone extract of the former was highly active against and *Candida albicans.* Undoubtedly the observed activity relied upon its phytoconstituents mainly iridoid glycosides, flavonoids, saponins and phenolic compounds [67-69].

Besides, *V. leptostychum* flower showed a notable antimicrobial activity against *Proteus sp., Pseudomonas aeruginosa, Shigella dysenteria, Salmonella enteritidis, Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis,* and *Candida albicans* [70].

Hydroalcoholic extracts of *V. sinaiticum* showed sustainable antimicrobial activity towards *Staphylococcus aureus* and *Trichophyton mentagrophytes* that could provide evidence about its popularity in the folk medicine as a cure for various skin disorders [71].

## 4. *IN SILICO* MOLECULAR MODELING STUDY OF THE MAJOR CONSTITUENTS ISOLATED FROM THESE GENERA

In this section, molecular modelling studies of the isolated phytoconstituents from the 6 major Scrophulariaceae genera that showed relevant antimicrobial activity were done on important target enzymes implicated in the occurrence and dissemination of infection. This was done in an effort to explore the exact mechanism of action of these naturally occurring entities in fighting bacterial infections. Noteworthy to mention that plethora of enzymes could be targeted to either treat bacterial infections or prohibit the development of bacterial drug resistance. Herein, six enzymes which are crucial in the survival and division of bacteria as well as the development of resistance were chosen in the molecular modelling studies using C-docker protocol [72, 73]. The enzymes were downloaded from the protein data bank and are as follows: DNA-gyrase (PDB ID 4Z2D; 3.38 A°); topoisomerase IV (PDB ID 4Z3O; 3.44 A°); dihydrofolate reductase (PDB ID 4KM2; 1.4 A°); transcriptional regulator TcaR (protein) (PDB ID 4EJV; 2.9 A°), *β*-lactamase (PDB ID 3NBL; 2.0 A°) and aminoglycoside nucleotidyl transferase (PDB ID 4WQL; 1.73 A°).

DNA-gyrase is a vital enzyme that regulates the supercoiling of DNA in addition to relieving the topological stress resulting from the translocation of transcription and replication complexes within DNA. However, topoisomerase IV is an enzyme responsible for the decatenation and separation of interlinked daughter chromosomes consequently after DNA replication [74]. Folic acid is of great necessity to the bacterial growth as well as multiplication [75]. Dihydrofolate reductase is responsible for the catalysis of the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate; consequent metabolites of tetrahydrofolate are required for incorporation of single carbon units into purines, pyrimidines and amino acids. Thus prohibition of dihydrofolate reductase resulted in a deficiency of the components of nucleic acids and proteins with concomitant inhibition of DNA synthesis and eventually cell death and can undoubtedly employed as antibacterial agents [76, 77].

Regarding bacterial resistance, *β*-lactamases constitute enzymes formed by the bacteria that are responsible for the development of multi-resistance to antibiotics containing

*β*-lactam ring as penicillins *via* cleavage of the *β*-lactam ring and thus destroying the activity of the antibiotics. Thus development of *β*-lactamases inhibitors is important for the prevention of bacterial resistance [78]. Additionally, aminoglycoside nucleotidylyltransferase is an enzyme involved in bacterial resistance to aminoglycoside antibiotics that changes the structure of the antibiotics through adenylylation and thus deactivating the drug causing it to be inactive towards bacteria [79].

Results of the molecular modelling of forty one (Fig. 2) previously reported antimicrobial secondary metabolites from selected species belonging to Family Scrophulariaceae in the active sites of six important enzymes implicated in the incidence of bacterial infections as well as development of bacterial resistance using molecular modeling experiments calculated in Kcal/mol were illustrated in Table 1 and Fig. 3 and 4. Results revealed that tamarixetin 7-rutinoside **(40)** showed the highest binding in the active sites of dihydrofolate reductase, β-lactamase and aminoglycoside nucleotidyl transferase as evidenced from its biding free energies meanwhile diosmin **(39)** showed the highest inhibition to topoisomerase IV. However, scrokoelziside A **(37)** exhibited the highest inhibition towards DNA-gyrase and transcriptional regulator protein TcaR.

## 5. CONCLUSIONS

Family Scrophulariaceae with most of its genera offers a hidden mine for diverse promising secondary metabolites that could be of high relevant antimicrobial activity. Nevertheless, more thorough phytochemical and biological studies should be done on many of its species to discover many antimicrobial leads that can overcome the resistance exerted by many microbial strains.

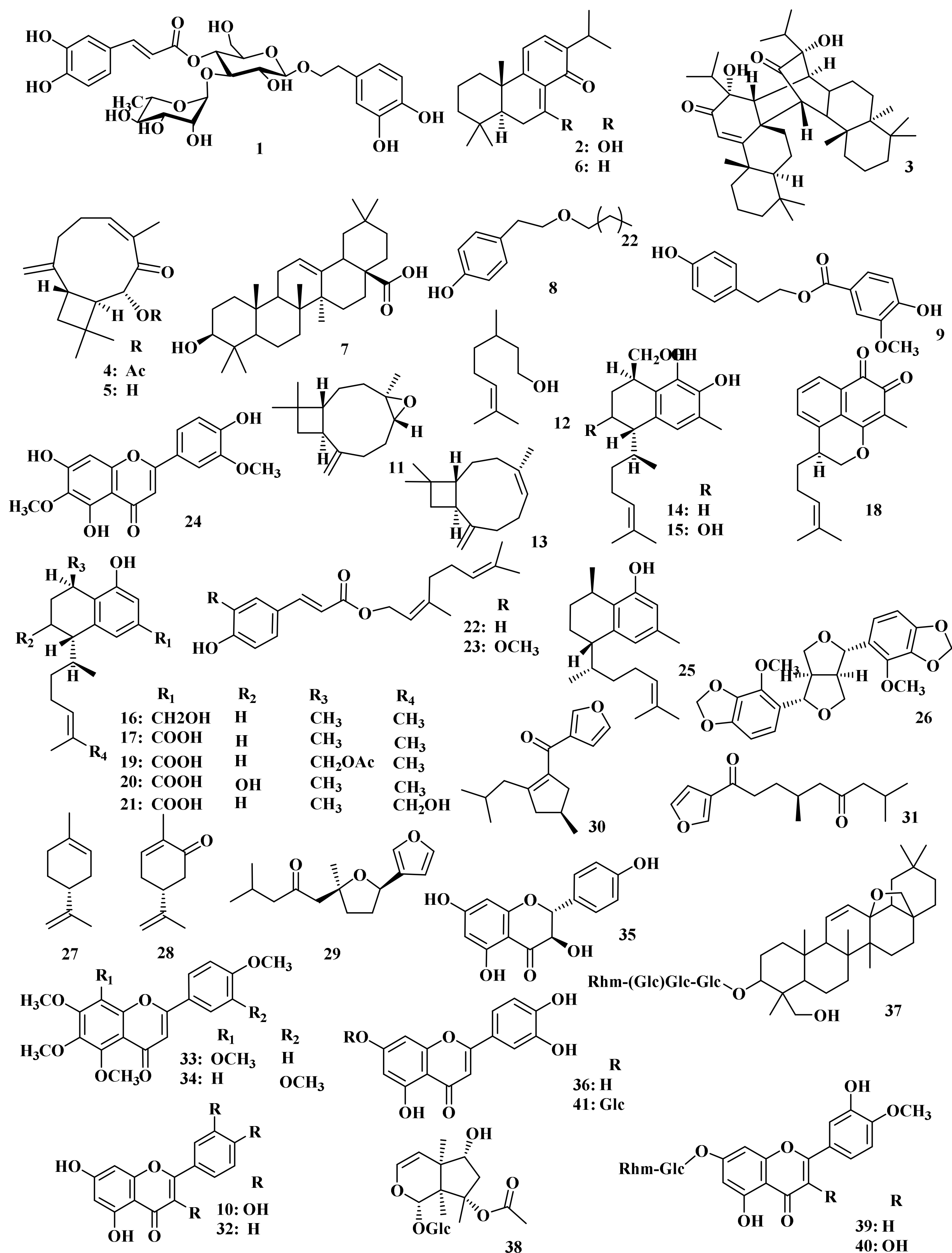

**Figure 2.** The structure of the secondary metabolites that showed antimicrobial activity from selected genera belonging to Schrophulariaceae

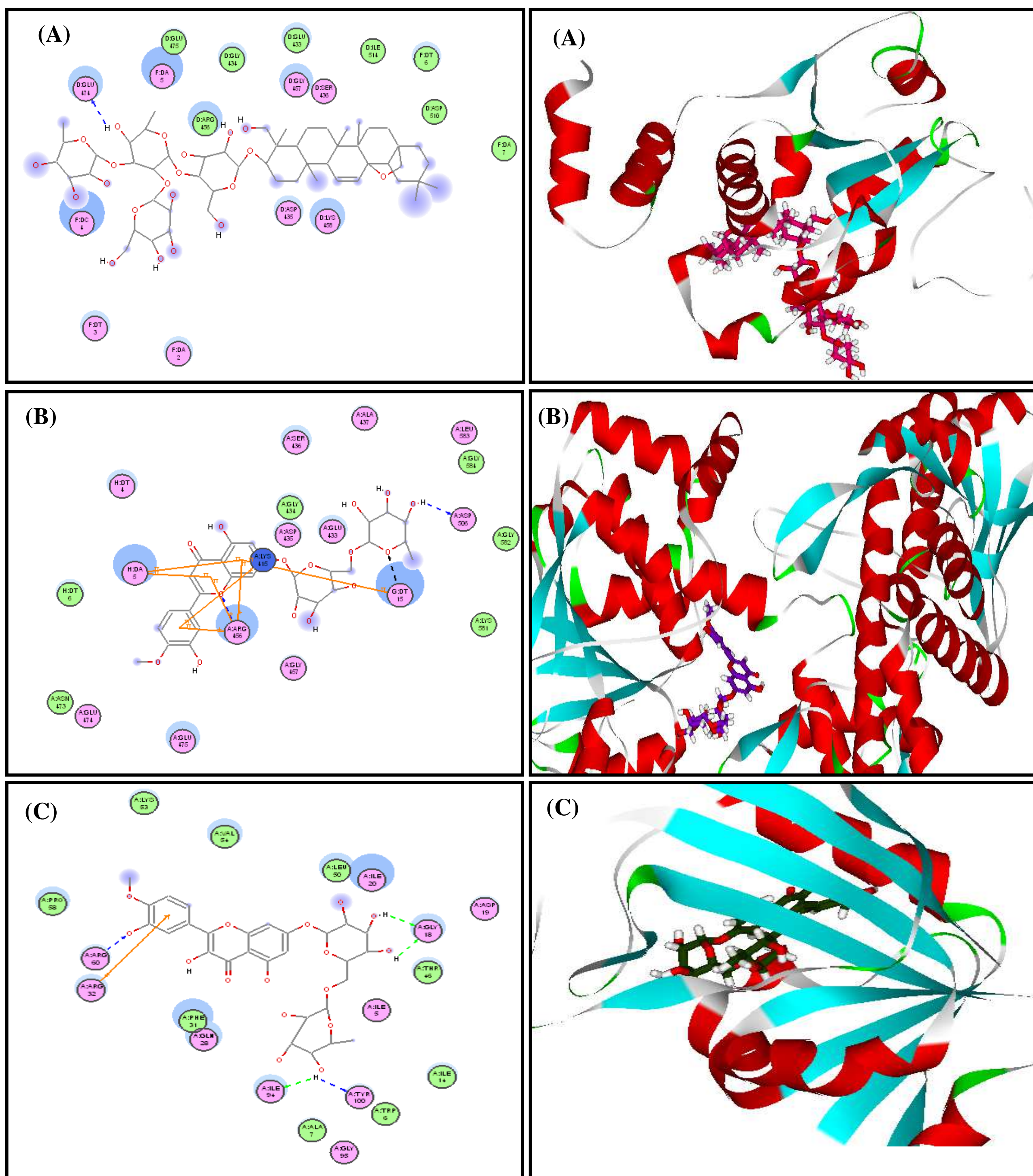


**Figure 3.** 2D and 3D binding modes of scrokoelziside A within the active sites of DNA-gyrase (A), 2D and 3D binding modes of diosmin within the active sites of topoisomerase IV (B) and 2D and 3D binding mode of tamarixetin 7-rutinoside within the active sites of dihydrofolate reductase (C)

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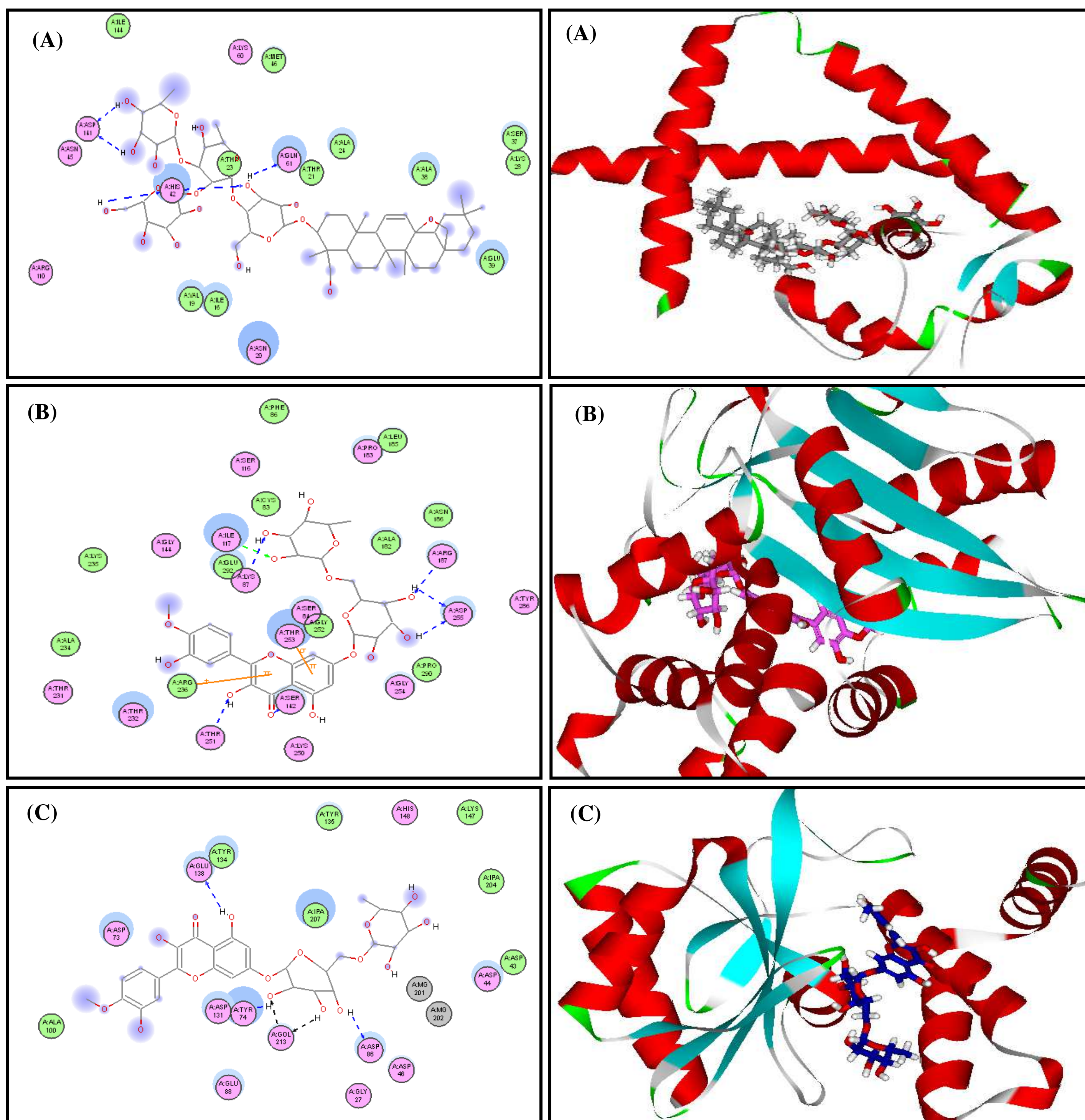


**Figure 4.** 2D and 3D binding mode of scrokoelziside A within the active sites of transcriptional regulator TcaRprotein (A), 2D and 3D binding mode of tamarixetin 7-rutinoside within the active site of $\beta$-lactamase (B) and 2D and 3D binding mode of tamarixetin 7-rutinoside within the active site of aminoglycoside nucleotidyl transferase (C).

**Table 1.** Free binding energies (ΔG) of some previously reported antimicrobial secondary metabolites from selected species belonging to family Scrophulariaceae in the active sites of six important enzymes implicated in the incidence of bacterial infections as well as development of bacterial resistance using molecular modeling experiments calculated in Kcal/mol

| Compounds | Binding energy (Kcal/mol) | | | | | |
|---|---|---|---|---|---|---|
| | **DNA-gyrase** | **Topoisomerase IV** | **Dihydrofolate reductase** | **Transcriptional regulator TcaR** | ***β*-lactamase** | **Aminoglycoside nucleotidyl transferase** |
| Verbascoside **(1)** | FD | FD | FD | -49.58 | FD | -76.83 |
| Buddlejone **(2)** | -25.73 | 31.20 | - 30.83 | -22.74 | FD | -51.02 |
| Maytenone **(3)** | -37.14 | FD | FD | -33.88 | FD | -33.75 |
| Buddledin A **(4)** | -23.99 | -26.78 | -29.53 | -26.22 | -33.26 | -44.33 |
| Buddledin B **(5)** | -21.86 | -24.57 | -29.63 | -21.25 | -29.07 | -32.70 |
| Deoxybuddlejone **(6)** | -28.84 | -27.70 | -30.16 | -25.77 | -32.76 | -39.66 |
| Oleanolic acid **(7)** | -33.01 | -33.28 | -42.22 | -31.24 | -42.80 | -43.21 |
| 2[4'-Hydroxyphenyl]-ethyl lignocerate **(8)** | -49.64 | FD | -48.91 | -42.39 | -57.09 | -65.69 |
| 4'-Hydroxyphenyl ethyl vanillate **(9)** | -32.84 | -43.32 | -39.88 | -27.22 | -45.32 | -47.07 |
| Quercetin **(10)** | -31.17 | -41.63 | -40.28 | -23.31 | -48.82 | -45.00 |
| Caryophyllene oxide **(11)** | -23.06 | -23.21 | -28.62 | -22.22 | -28.83 | -27.58 |
| Citronellol **(12)** | -23.61 | -24.34 | -25.84 | -24.64 | -29.62 | -32.05 |
| β-caryophyllene **(13)** | -21.32 | -21.41 | -22.80 | -20.32 | -28.84 | -25.47 |
| Serrulat-14-en-7,8,20-triol **(14)** | -32.59 | -39.46 | -41.26 | -28.76 | -44.20 | -46.57 |
| Serrulat-14-en-3,7,8,20-tetraol **(15)** | -37.05 | -40.00 | -44.69 | -28.01 | -40.94 | -58.80 |
| 8,19-Dihydroxyserrulat-14-ene **(16)** | -32.81 | -36.61 | -37.34 | -28.66 | -43.51 | -40.32 |
| 8-Hydroxyserrulat-14-en-19-oic acid **(17)** | -33.63 | -40.72 | -40.25 | -29.98 | -48.19 | -44.63 |
| *O*-naphthoquinone **(18)** | -29.32 | -33.18 | -34.71 | -27.14 | -36.66 | -47.44 |
| 20-Acetoxy-8-hydroxyserrulat-14-en-19-oic acid **(19)** | -40.95 | -43.44 | -45.86 | -34.20 | -49.20 | -60.25 |
| 3,8-Dihydroxyserrulatic acid **(20)** | -37.40 | -35.95 | -42.52 | -33.54 | -45.17 | -51.87 |
| Serrulatic acid **(21)** | -36.26 | -37.57 | -41.33 | -37.53 | -44.66 | -52.56 |
| Neryl ferulate **(22)** | -31.31 | -42.40 | -40.91 | -29.80 | -41.80 | -45.98 |
| Neryl p-coumarate **(23)** | -35.63 | -47.57 | -37.78 | -29.28 | -48.74 | -46.09 |
| Jaceosidin **(24)** | -33.18 | -41.79 | -40.13 | -27.25 | -41.39 | -48.96 |
| Leubethanol **(25)** | -31.30 | -36.26 | -33.68 | -28.17 | -38.94 | -41.86 |
| 2',5''-Dimethoxysesamin | -32.42 | FD | FD | FD | -46.28 | FD |

| Compounds | Binding energy (Kcal/mol) | | | | | |
|---|---|---|---|---|---|---|
| | DNA-gyrase | Topoisomerase IV | Dihydrofolate reductase | Transcriptional regulator TcaR | *β*-lactamase | Aminoglycoside nucleotidyl transferase |
| **(26)** | | | | | | |
| D-Limonene **(27)** | -18.69 | -21.12 | -16.59 | -14.13 | -19.74 | FD |
| (-) Carvone **(28)** | -20.43 | -23.00 | -21.81 | -18.53 | -26.21 | FD |
| Negaione **(29)** | -27.11 | -32.36 | -31.77 | -27.55 | -34.14 | -42.06 |
| Myomontanone **(30)** | -23.90 | -28.70 | -27.92 | -23.83 | -31.57 | -37.35 |
| (±)-Myoporone **(31)** | -27.99 | -34.66 | -33.07 | -24.85 | -42.89 | -43.16 |
| 5,7-Dihydroxyflavone **(32)** | -25.89 | -33.10 | -29.32 | -22.81 | -38.57 | -41.31 |
| Tangeretin **(33)** | -34.19 | -46.10 | -42.48 | -27.13 | -45.40 | -49.42 |
| Sinensetin **(34)** | -36.09 | -48.18 | -42.20 | -32.07 | -45.57 | -49.46 |
| Dihydrokaempferol **(35)** | -28.83 | -36.70 | -34.59 | -32.79 | -41.00 | -49.33 |
| Luteolin **(36)** | -32.86 | -40.12 | -39.00 | -26.62 | -38.15 | -45.04 |
| Scrokoelziside A **(37)** | **-62.28** | FD | FD | **-56.01** | FD | FD |
| Ajugoside **(38)** | -36.64 | -34.36 | -47.84 | -35.96 | -58.17 | -56.70 |
| Diosmin **(39)** | -48.97 | **-69.75** | -60.76 | -46.97 | -66.76 | -66.76 |
| Tamarixetin 7-rutinoside **(40)** | -46.12 | -64.60 | **-61.32** | -44.79 | **-67.11** | **-84.85** |
| Luteolin 7-glucoside **(41)** | -43.77 | -54.73 | -52.81 | -40.91 | -53.29 | -56.29 |
| Trimethoprim | ND | ND | -35.69 | ND | ND | ND |
| Chloramphenicol | ND | ND | ND | -29.02 | ND | ND |
| Cefuroxime | ND | ND | ND | ND | -70.87 | ND |
| Levofloxacin | -36.26 | ND | ND | ND | ND | ND |
| Moxifloxacin | ND | -53.10 | ND | ND | ND | ND |
| Kanamycin | ND | ND | ND | ND | ND | -73.94 |

ND: not done

FD: fail to dock

## REFERENCES


1. Ryan KJ, Ray CG. Medical microbiology. New York: McGraw Hill; 2004.
2. John JE. Natural products as lead-structures: a role for biotechnology, Elsevier Current Trends; 2010.
3. Wink M, Ashour ML, El-Readi MZ. Secondary metabolites from plants inhibiting ABC transporters and reversing resistance of cancer cells and microbes to cytotoxic and antimicrobial agents. Frontiers in Microbiology. 2012; 3: 130-145.

4. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clinical Microbiology Reviews. 1999; 12: 147-179.
5. Hooper DC. Mechanisms of action of antimicrobials: focus on fluoroquinolones. Clinical Infectious Diseases. 2001; 32: S9-S15.
6. Lewis K. Platforms for antibiotic discovery. Nature Reviews Drug Discovery. 2013; 12: 371-387.
7. Fuglsang CC, Johansen C, Christgau S, Adler-Nissen J. Antimicrobial enzymes: applications and future potential in the food industry. Trends in Food Science & Technology. 1995; 6: 390-396.
8. Thallinger B, Prasetyo EN, Nyanhongo GS, Guebitz GM. Antimicrobial enzymes: an emerging strategy to fight microbes and microbial biofilms. Biotechnology Journal. 2013; 8: 97-109.
9. Smith RS, Iglewski BH. *Pseudomonas aeruginosa* quorum sensing as a potential antimicrobial target. The Journal of Clinical Investigation. 2003; 112: 1460-1465.
10. Cortes AR, Delgadillo AJ, Hurtado M, Dominguez-Ramirez AM, Medina JR, Aoki K. The antispasmodic activity of *Buddleja scordioides* and *Buddleja perfoliata* on isolated intestinal preparations. Biological and Pharmaceutical Bulletin. 2006; 29: 1186-90.
11. Houghton P. Ethnopharmacology of some *Buddleja* species. Journal of Ethnopharmacology. 1984; 11: 293-308.
12. Adedapo A, Jimoh F, Koduru S, Masika P, Afolayan A. Assessment of the medicinal potentials of the methanol extracts of the leaves and stems of *Buddleja saligna.* BMC Complementary and Alternative Medicine. 2009; 9: 21.
13. Pardo F, Perich F, Villarroel L, Torres R. Isolation of verbascoside, an antimicrobial constituent of *Buddleja globosa* leaves. Journal of Ethnopharmacology. 1993; 39: 221-222.
14. Guillermo Avila J, de Liverant JG, Martínez A, Martínez G, Muñoz JL, Arciniegas A, Romo de Vivar A. Mode of action of *Buddleja cordata* verbascoside against *Staphylococcus aureus.* Journal of Ethnopharmacology. 1999; 66: 75-78.
15. Mensah AY, Houghton PJ, Bloomfield S, Vlietinck A, Vanden Berghe D. Known and novel terpenes from *Buddleja globosa* displaying selective antifungal activity against dermatophytes. Journal of Natural Products. 2000; 63: 1210-3.
16. Houghton PJ, Mensah AY, Iessa N, Yong Hong L. Terpenoids in *Buddleja*: relevance to chemosystematics, chemical ecology and biological activity. Phytochemistry. 2003; 64: 385-393.

17. Bamuamba K, Gammon DW, Meyers P, Dijoux-Franca M-G, Scott G. Anti-mycobacterial activity of five plant species used as traditional medicines in the Western Cape Province (South Africa). Journal of Ethnopharmacology. 2008; 117: 385-390.
18. Oliveira DF, Pereira AC, Figueiredo HCP, Carvalho DA, Silva G, Nunes AS, Alves DS, Carvalho HWP. Antibacterial activity of plant extracts from Brazilian southeast region. Fitoterapia. 2007; 78: 142-145.
19. Castillo-Juárez I, González V, Jaime-Aguilar H, Martínez G, Linares E, Bye R, Romero I. *Anti-Helicobacter pylori* activity of plants used in Mexican traditional medicine for gastrointestinal disorders. Journal of Ethnopharmacology. 2009; 122: 402-405.
20. Acevedo L, Martinez E, Castaneda P, Franzblau S, Timmermann BN, Linares E, Bye R, Mata R. New phenylethanoids from *Buddleja cordata* subsp. cordata. Planta Medica 2000; 66: 257-61.
21. Pendota SC, Aderogba MA, Ndhlala AR, Van Staden J. Antimicrobial and acetylcholinesterase inhibitory activities of *Buddleja salviifolia* (L.) Lam. leaf extracts and isolated compounds. Journal of Ethnopharmacology. 2013; 148: 515-20.
22. Garg SC, Dengre SL. Composition of the essential oil from the leaves of *Buddleia asiatica* lour. Flavour and Fragrance Journal. 1992; 7: 125-127.
23. Singab AN, Youssef FS, Ashour ML, Wink M. The genus *Eremophila* (Scrophulariaceae): an ethnobotanical, biological and phytochemical review. Journal of Pharmacy and Pharmacology. 2013; 65: 1239-1279.
24. Tomlinson S, Palombo EA. Characterisation of antibacterial Australian medicinal plant extracts by investigation of the mechanism of action and the effect of interfering substances. Journal of Basic Microbiology. 2005; 45: 363-70.
25. Palombo EA, Semple SJ. Antibacterial activity of traditional Australian medicinal plants. Journal of Ethnopharmacology. 2001; 77: 151-157.
26. Palombo EA, Semple SJ. Antibacterial activity of Australian plant extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Journal of Basic Microbiology. 2002; 42: 444-448.
27. Shah A CR, Palombo EA. Identification of the antibacterial component of an ethanolic extract of the Australian medicinal plant, *Eremophila duttonii.* Phytotherapy Research. 2004; 18: 615-618.
28. Owen RJ, Palombo EA. Anti-listerial activity of ethanolic extracts of medicinal plants, *Eremophila alternifolia* and *Eremophila duttonii*, in food homogenates and milk. Food Control. 2007; 18: 387-390.

29. Smith JE, Tucker D, Watson K, Jones GL. Identification of antibacterial constituents from the indigenous Australian medicinal plant *Eremophila duttonii* F. Muell. (Myoporaceae). Journal of Ethnopharmacology. 2007; 112: 386-393.
30. Ndi CP, Semple SJ, Griesser HJ, Pyke SM, Barton MD. Antimicrobial compounds from the Australian desert plant *Eremophila neglecta.* Journal of Natural Products. 2007; 70: 1439-1443.
31. Anakok OF, Ndi CP, Barton MD, Griesser HJ, Semple SJ. Antibacterial spectrum and cytotoxic activities of serrulatane compounds from the Australian medicinal plant *Eremophila neglecta.* Journal of Applied Microbiology. 2012; 112: 197-204.
32. Ndi CP, Semple SJ, Griesser HJ, Pyke SM, Barton MD. Antimicrobial compounds from *Eremophila serrulata.* Phytochemistry. 2007; 68: 2684-2690.
33. Liu Q, Harrington D, Kohen JL, Vemulpad S, Jamie JF. Bactericidal and cyclooxygenase inhibitory diterpenes from *Eremophila sturtii.* Phytochemistry. 2006; 67: 1256-1261.
34. Ndi CP, Semple SJ, Griesser HJ, Barton MD. Antimicrobial activity of some Australian plant species from the genus *Eremophila.* Journal of Basic Microbiology. 2007; 47: 158-164.
35. Hayhoe EJ, Palombo EA. Extracts of *Eremophila longifolia* inhibit the cariogenic activities of *Streptococcus mutans* and *Streptococcus sobrinus.* Journal of Medicinal Plants Research. 2011; 5: 2476-2482.
36. Pennacchio M, Kemp AS, Taylor RP, Wickens KM, Kienow L. Interesting biological activities from plants traditionally used by Native Australians. Journal of Ethnopharmacology. 2005; 96: 597-601.
37. Galappathie S, Edwards DJ, Elliott AG, Cooper MA, Palombo EA, Butler MS, Mahon PJ. Antibacterial nerol cinnamates from the Australian plant *Eremophila longifolia.* Journal ofNatural Products. 2017; 80: 1178-1181.
38. Kumar R, Lu Y, Elliott AG, Kavanagh AM, Cooper MA, Davis RA. Semi-synthesis and NMR spectral assignments of flavonoid and chalcone derivatives. Magnetic Resonance in Chemistry. 2016; 54: 880-886.
39. Nowakowska J, Griesser HJ, Textor M, Landmann R, Khanna N. Antimicrobial properties of 8-hydroxyserrulat-14-en-19-oic acid for treatment of implant-associated infections. Antimicrobial Agents Chemotherapy. 2013; 57: 333-342.
40. Sadgrove NJ, Jones GL, Greatrex BW. Isolation and characterisation of (-)-genifuranal: the principal antimicrobial component in traditional smoking applications of *Eremophila longifolia* (Scrophulariaceae) by Australian aboriginal peoples. Journal of Ethnopharmacology. 2014; 154: 758-66.

41. Sadgrove NJ, Hitchcock M, Watson K, Jones GL. Chemical and biological characterization of novel essential oils from *Eremophila bignoniiflora* (F. Muell) (Myoporaceae): a traditional Aboriginal Australian bush medicine. Phytotherapy Research. 2013; 27: 1508-16.
42. Youssef FS, Hamoud R, Ashour ML, Singab AN, Wink M. Volatile oils from the aerial parts of *Eremophila maculata* and their antimicrobial activity. Chemistry & Biodiversity. 2014; 11: 831-841.
43. Olmstead RG, DePamphilis CW, Wolfe AD, Young ND, Elisons WJ, Reeves PA. Disintegration of the Scrophulariaceae. American Journal of Botany. 2001; 88: 348-361.
44. Molina-Salinas G, Pérez-López A, Becerril-Montes P, Salazar-Aranda R, Said-Fernández S, de Torres NW. Evaluation of the flora of Northern Mexico for *in vitro* antimicrobial and antituberculosis activity. Journal of Ethnopharmacology. 2007; 109: 435-441.
45. Lu JM, Perkins MV, Griesser HJ. Total synthesis and structural confirmation of the antibacterial diterpene leubethanol. Tetrahedron. 2013; 69: 6468-6473.
46. Alanis-Garza B, Salazar-Aranda R, Ramírez-Durón R, Garza-González E, Waksman dTN. A new antimycobacterial furanolignan from *Leucophyllum frutescens.* Natural Product Communications. 2012; 7: 597.
47. Salama MTI. Antimicrobial activity of essential oil of *Myoporum acuminatum* R. Br fruits, cultivated in Libya. Journal of Essential Oil Bearing Plants. 2017; 20: 233-239.
48. Zaleta-Pinet D, McCluskey A, Hall S, Brophy J, Ashhurst-Smith C, Sakoff J, van Altena I. The use of the toxic plant *Myoporum montanum* in a traditional Australian aboriginal medicine. Australian Journal of Chemistry. 2016; 69: 161-168.
49. El-Hamouly M, Ammar H, Awaad A. Essential oil composition and antimicrobial activity of *Myoporum acuminatum* (G.) Foster, cultivated in Egypt; Az. Journal of Pharmaceutical Sciences. 2001; 28: 360.
50. Billo M, Cabalion P, Waikedre J, Fourneau C, Bouttier S, Hocquemiller R, Fournet A. Screening of some new Caledonian and Vanuatu medicinal plants for antimycobacterial activity. Journal of Ethnopharmacology. 2005; 96: 195-200.
51. Li X, Li C, Wu L, Yang F, Gu W. Chemical constituents from leaves of *Myoporum bontioides.* Chinese Traditional and Herbal Drugs. 2011; 42: 2204-2207.
52. Yecheng D, Zhen Y, Yanzhen Y, Xiulian B. Inhibitory activity against plant pathogenic fungi of extracts from *Myoporum bontioides* A. Gray and indentification of active ingredients. Pest Management Science. 2008; 64: 203-207.

53. Zaki AA, Shaaban MI, Hashish NE, Amer MA, Lahloub M-F. Assessment of anti-quorum sensing activity for some ornamental and medicinal plants native to Egypt. Scientia Pharmaceutica. 2012; 81: 251-258.
54. Culpeper N. Culpeper's complete herbal: a book of natural remedies for ancient ills. UK: Wordsworth Editions; 1995.
55. Garran TA. Western herbs according to traditional Chinese medicine: a practitioner's guide. San Deigo, California: Inner Traditions/Bear & Co.; 2008.
56. Kim SJ, Park JS, Myung NY, Moon PD, Choi IY, An HJ, Kim NH, Na HJ, Kim DH, Kim MC, An NH, Kim IK, Lee JY, Jeong HJ, Um JY, Kim HM, Hong SH. *Scrophularia buergeriana* regulates cytokine production in vitro. Immunopharmacology and Immunotoxicology. 2009; 31: 246-252.
57. Li J, Huang X, Du X, Sun W, Zhang Y. Study of chemical composition and antimicrobial activity of leaves and roots of *Scrophularia ningpoensis*. Natural Product Research. 2009; 23: 775-780.
58. Mahboubi M, Kazempour N, Boland Nazar AR. Total phenolic, total flavonoids, antioxidant and antimicrobial activities of *Scrophularia striata* boiss extracts. Jundishapur Journal of Natural Pharmceutical Products. 2013; 8: 15-19.
59. Stavri M, Mathew KT, Gibbons S. Antimicrobial constituents of *Scrophularia deserti*. Phytochemistry. 2006; 67: 1530-1533.
60. Asgharian P, Heshmati Afshar F, Asnaashari S, Bamdad Moghaddam S, Ebrahimi A, Delazar A. Characterization of terpenoids in the essential oil extracted from the aerial parts of *Scrophularia subaphylla* growing in Iran. Advanced Pharmceutical Bulletin. 2015; 5: 557-561.
61. Dulger B, Ugurlu E. Evaluation of antimicrobial activity of some endemic Scrophulariaceae members from Turkey. Pharmaceutical Biology. 2005; 43: 275-279.
62. Georgiev M, Alipieva K, Orhan I, Abrashev R, Denev P, Angelova M. Antioxidant and cholinesterases inhibitory activities of *Verbascum xanthophoeniceum* Griseb. and its phenylethanoid glycosides. Food Chemistry. 2011; 128: 100-105.
63. Escobar FM, Sabini MC, Zanon SM, Cariddi LN, Tonn CE, Sabini LI. Genotoxic evaluation of a methanolic extract of *Verbascum thapsus* using micronucleus test in mouse bone marrow. Natural Products Communication. 2011; 6: 989-991.
64. Guarino C. Antimicrobial activity of *Verbascum macrurum* Ten. (Scrophulariaceae). Bollettino Chimico Farmaceutico. 2002; 141: 238-242.

65. Klimek B, Olszewska MA, Tokar M. Simultaneous determination of flavonoids and phenylethanoids in the flowers of *Verbascum densiflorum* and *V. phlomoides* by high-performance liquid chromatography. Phytochemical Analysis. 2010; 21: 150-156.

66. Mothana RA, Abdo SA, Hasson S, Althawab FM, Alaghbari SA, Lindequist U. Antimicrobial, antioxidant and cytotoxic activities and phytochemical screening of some yemeni medicinal plants. Evidence Based Complementary and Alternative Medicine. 2010; 7: 323-330.

67. Ozcan B, Esen M, Caliskan M, Mothana RA, Cihan AC, Yolcu H. Antimicrobial and antioxidant activities of the various extracts of *Verbascum pinetorum* Boiss. O. Kuntze (Scrophulariaceae). European Review for Medical and Pharmacological Sciences. 2011; 15: 900-905.

68. Boga M, Ertas A, Yilmaz MA, Kizil M, Ceken B, Hasimi N, Ozden TY, Demirci S, Yener I, Deveci O. UHPLC-ESI-MS/MS and GC-MS analyses on phenolic, fatty acid and essential oil of *Verbascum pinetorum* with antioxidant, anticholinesterase, antimicrobial and DNA damage protection effects. Iranian Journal of Pharmceutical Research. 2016; 15: 393-405.

69. Ozcan B, Yilmaz M, Caliskan M. Antimicrobial and antioxidant activities of various extracts of *Verbascum antiochium* Boiss. (Scrophulariaceae). Journal of Medicinal Food. 2010; 13: 1147-1152.

70. Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN. Screening of selected indigenous plants of Lebanon for antimicrobial activity. Journal of Ethnopharmacology. 2004; 93: 1-7.

71. Tadeg H, Mohammed E, Asres K, Gebre-Mariam T. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. Journal of Ethnopharmacology. 2005; 100: 168-75.

72. Youssef FS, Ashour ML, Sobeh M, El-Beshbishy HA, Singab AN, Wink M. *Eremophila maculata*- Isolation of a rare naturally-occurring lignan glycoside and the hepatoprotective activity of the leaf extract. Phytomedicine. 2016; 23: 1484-1493.

73. Youssef FS, Ashour ML, Ebada SS, Sobeh M, El-Beshbishy HA, Singab AN, Wink M. Antihyperglycaemic activity of the methanol extract from leaves of *Eremophila maculata* (Scrophulariaceae) in streptozotocin-induced diabetic rats. Journal of Pharmacy and Pharmacology. 2017; 69: 733-742.

74. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiology and Molecular Biology Reviews. 1997; 61: 377-392.

75. Bailey SW, Ayling JE. The extremely slow and variable activity of dihydrofolate reductase in human liver and its implications for high folic acid intake. Proceedings of the National Academy of Sciences. 2009; 106: 15424-15429.

76. Onodera Y, Haag JR, Ream T, Nunes PC, Pontes O, Pikaard CS. Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. Cell. 2005; 120: 613-622.

77. Hawser S, Lociuro S, Islam K. Dihydrofolate reductase inhibitors as antibacterial agents. Biochemical Pharmacology. 2006; 71: 941-948.

78. Drawz SM, Bonomo RA. Three decades of *β*-lactamase inhibitors. Clinical Microbiology Reviews. 2010; 23: 160-201.

79. Stogios PJ, Evdokimova E, Morar M, Koteva K, Wright GD, Courvalin P, Savchenko A. Structural and functional plasticity of antibiotic resistance nucleotidylyltransferases revealed by molecular characterization of lincosamide nucleotidylyltransferases lnu (A) and lnu (D). Journal of Molecular Biology. 2015; 427: 2229-2243.

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