Original Research Paper Science

Chemical and Biological Characteristics of Essential Oil Isolated From Fresh Flower of *Tripleurospermum Auriculatum*

Marzough A. Albalawi — Chemistry Department, University College of Alwajh, University of Tabuk, Saudi Arabia

Jayda, G. Eldiasty — Biology Department, Faculty of Science, University of Tabuk, Saudi Arabia

ABSTRACT

Eesential oil extracted by hydrodistillation from flower of Tripleurospermum auriculatum and characterized by GC/MS. The main compounds identified in the oil were α-pinene (23.27%), linalyl isobtyrate (17.92%), 4-Carene (14.17%), boreniol (11.90%), pinocarvone (9.07%), p-cineol (7.92%), α-Terpenen-4-ol (6.77%), Piperiton (5.71%). Antimicrobial activity of essential oil of Tripleurospermum auriculatum was investigated in this study. Testing was conducted against 8 pathogenic microorganisms, *Escherichia coli*, *Pseudomonas aeruginosa*, *Morganella sp*. and *Proteus sp*. (gram-negative bacteria), *Entrococcus Faecalis*, *Micrococcus sp*. and *Streptococcus agalactia* (gram-positive bacteria) in addition *Candida albicans* as yeast like fungi. Activity and minimum inhibitory concentration were determined using Agar well assay method. The gram -ve bacteria were more sensitive as compared to the gram +ve bacteria and essential oil of Tripleurospermum auriculatum had no effect on the growth of Candida albicans. Gentamycine and Ampicilline (10µg) were used as positive controls respectively.

KEYWORDS *Tripleurospermum auriculatum;* Eesential oil; GC/MS; antimicrobial activity.

Introduction

Medicinal plants have been used for centuries as traditional health remedies for human diseases because they contain chemical components of therapeutic value (1). Their usage is most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects (2, 3 and 4). Natural products are known to play an important role in both drug discovery and chemical biology. In fact, many of the recently available drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs (5). Natural antimicrobials can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. (6 and 7).

Essential oils are hydrophobic liquids containing volatile aroma compounds extracted from plants generally by distillation, mostly by using steam. It has been reported that many essential antimicrobial compounds like thymol, carvacrol, linalool, and eugenol are the integral constituents of some plants' essential oils (8) that impart focal access in pharmaceutical, food and cosmetics industries. Besides, with increasing legislation on utilization of chemical preservatives and methods to combat phytopathogen, there has been increasing interest amongst the scientists to explore the antimicrobial potential of plant essential oils against the destructive plant pathogens (9). Whereas, it has been recommended to explore antimicrobial activity of medicinally important plants owing to enrichment of novel antimicrobial compounds (10 and 11).

Antimicrobial activity of essential oils has been known for long time. This activity is being studied throughout the world as substitute for existing antibiotics that have adverse side effects. Several scientists (7, 11, 12, 13, 14 and 15) have reported antimicrobial activity of essential oils of different plants against various bacteria and fungi.

Tripleurospermum Sch. Bip . Is a member of the tribe Anthemideae of the Family Asteraceae with ca. 40 species, mainly distributed in Europe, temperate Asia, North America and North Africa (16). Chemistry of genus ***Tripleurospermum*** is mainly focused on its essential oil composition (17, 18 and 19). In general these plants are not rich aromatic terpenoid compounds, but certainly they reveal several beneficial medicinal properties, such as anti-inflammatory (20), antifungal (21), antibacterial (22) and antioxidant (23).

Materials and methods

1- Collection and Identification of Herb

Tripleurospermum auriculatum (Alguris) was collected from alkur village, Alwajh, Tabuk, KSA in 14/4/2014 (Fig. 3) and identified by dr. Amal fakri (Flora of KSA) at the botany department, University of Tabuk.

2- Extraction and Isolation of Essential oil

Essential oil extracted by hydrodistillation (Fig. 4) from 250g fresh flower of ***T. auriculatum*** and isolated with di ethyl ether.

3- **Gas Chromatography /Mass Spectrometry analysis of essential oil GC/MS (Made in Germany):** Varian 3700 Column: DB-1 WCOT Fused Silica (J & W Scientific), Long: 30 m, Diameter: 0.32, Film thickness: 0.1 µm.

Gas chromatography, Typ/Hersteller: GC 8130 (Factor Fisions Instrument), Column: FS DB-1, Long: 30 m, Diameter: 0.32, Film thickness: 0.1 µm.

4-Microbial strains

The bacterial and fungal strains tested were provided by Culture Collection of Antibiotic Resistant Microbes (CCARM)" Military Hospital Tabuk. They are: Gram-positive ***Enterococcus faecalis***, ***Streptococcus agalactia*** and ***Micrococcus sp***; Gram-negative ***Escherichia coli***, ***Pseudomonas aeruginosa***, ***Morganella sp*** and ***Proteus sp*** and yeast strain ***Candida albicans***. The bacterial strains were maintained and grown in a nutrient agar medium at 37 °C. Yeast strain was cultured at 27 °C on Sabouraud Dextrose Agar. Each of the microorganisms was freshly cultured prior to susceptibility testing.

5-Antimicrobial activity

Antibacterial and Antifungal activities of the plant essential oil were tested using Well diffusion method (24). The inoculum size of each group of bacteria and yeast were prepared by using nutrient broth to give a concentration of 1×10^8 bacteria and 1×10^6 yeast per milliter. 100 µl of standardized inoculum of each test bacterium and yeast were spread over a sterile Muller-Hinton Agar plate, to achieve confluent growth, and allowed to dry. Wells were made on the agar surface with 6mm in diameter and about 2 cm a part punctured in the culture medium using sterile cork borers. The plates were then turned upside down and the wells were labelled with a

marker. Dimethyl sulphoxide (DMSO) was added in pure essential oils (100%) in order to obtain 50, 25, and 10% dilutions. 60 µl of extract was placed into a well and the plate was held for 1 hr at room temperature for diffusion of extract into the agar. Subsequently, the plates containing bacteria and fungi were incubated at 37 °C for 24 h and 27 °C for 48 h, respectively. The diameter of zone of inhibition (DIZ) was measured in millimeters after incubation as an indication of activity and compared with the solvent (DMSO) as negative control. Gentamycin (10 µg), Ampicilline (10 µg) were used as positive control. All the tests were repeated in triplicate. The means and standard error (±SE) of (DIZ) were done. The minimal inhibitory concentration (MIC) values of essential oil against different microorganism were determined by disc diffusion method (25). The oil was serially diluted with 10% DMSO (to increase miscibility) which contains 60- 0.5 mg/ml of oil. MIC values were defined as the lowest concentrations of oil that inhibit bacteria and fungi after 24 and 48 h, respectively.

Results and Discussion

1- Physical properties of essential oil

Wight: 0.93mg, Color: yellow and Smell: Rose

2- Gas Chromatography /Mass Spectrometry analysis (GC/MS)

The main compounds identified in the oil were α- pinene (23.27%), linalyl isobtyrate (17.92%), 4-Carene (14.17%), boreniol (11.90%), pinocarvone (9.07%), *p*-cineol (7.92%), α-Terpenen-4-ol (6.77%), Piperiton (5.71%) by GC/MS analysis.

3-Antimicrobial activity

The antimicrobial activity of ***Tripleurospermum auriculatum*** essential oil assessed by determining the DIZ as given in table (1) and figure (1 & 2). Essential oil of ***T. auriculatum*** exhibited some antimicrobial activity against different microorganisms and varied according to the type of pathogen. Gram negative bacteria was more sensitive than Gram positive bacteria and fungi. The most sensitive strain is ***Escherichia coli*** with DIZ of (25 mm) at 100% and MIC value at 40 mg/ml. This finding is in agreement with the report of Simin ***et al***. (26) who observed that ***Pongamia pinnata*** oil also showed the maximum activity against ***S. aureus*** and ***P. aeruginosa*** at 100% which must be due to active chemicals like pongarotene and or karanjin. The DIZ values for ***Morganella sp*** and ***Proteus sp*** were between (14-22 mm) and (9-14 mm) at concentration (10-100 %) and MIC values at 15 and 30 mg/ml respectively. ***Enterococcus faecalis*** was sensitive among Gram positive bacteria, presented an inhibition diameter ranging between (11.3-20 mm) and MIC value at 35 mg/ml. These results are in concord with those of Banso et al. (27) who also observed that higher concentrations of antimicrobial substances showed more growth inhibition. ***T. auriculatum*** was inactive against the other tested bacterial strains and ***Candida albicans***. Tofighi ***et al***. (28) indicates that ***Tripleurospermum disciforme*** extract showed antimicrobial effects only against ***Staphylococcus aureus*** and ***Staphylococcus epidermidis*** with MICs 112 and 224 µg/mL, respectively.

Dorman and Deans (29) indicate that the antimicrobial activity depends, not only, on the chemical composition of the essential oil, but also on lipophilic properties and power of functional groups or aqueous solubility. The mixture of compounds with different biochemical properties can improve the effectiveness of essential oils.

It is reported that the essential oils can inhibit the growth and kill pathogenic bacteria like standard antibiotics in different ways (30 and 31). They can precipitate bacterial proteins, including RNA and DNA. They can also kill the bacterial cell by deformation of its morphological characteristics. Mostly of oils cause destruction of selective permeability properties of cell membrane. The process of respiration by absorption of nutrients and excretion of wastes are interrupted and the cell dies.

Table (1): Antimicrobial activity of Essential Oil of *Tripleurospermum auriculatum*. Each value is the mean of 3 replicates ± S.E.

Microorganism		Zone of inhibition (mm)						
		10%	25%	50%	100%	Gentamycine	Ampicilline	DMSO
G-ve	*Escherichia coli*	15 ± 0.00	18.3 ± 0.33	21.6 ± 0.33	25 ± 0.00	20.3 ± 0.33	16 ± 0.00	0.00
	Pseudomonas aeruginosa	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	20.6 ± 0.33	0 ± 0.00	0.00
	Morganella sp.	14 ± 0.00	15.6 ± 0.33	18.3 ± 0.33	22 ± 0.00	14 ± 0.00	0 ± 0.00	0.00
	Proteus sp.	9 ± 0.00	10.3 ± 0.33	12 ± 0.00	14 ± 0.57	0 ± 0.00	0 ± 0.00	0.00
G+ve	*Entrococcus Faecalis*	11.3 ± 0.33	14.3 ± 0.33	16 ± 0.57	20 ± 0.00	20 ± 0.00	15 ± 0.57	0.00
	Micrococcus sp.	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	24.3 ± 0.33	0 ± 0.00	0.00
	Streptococcus agalactia	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	14.3 ± 0.33	9.8 ± 0.16	0.00
Yeast	*Candida albicans*	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	6.8 ± 0.88	0 ± 0.00	0.00

Fig. (1): Effect of different concentrations of *Tripleurospermum auriculatum* oil on Gram negative bacteria.

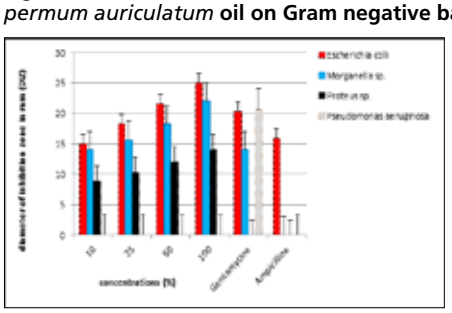

Fig. (2): Effect of different concentrations of *Tripleurospermum auriculatum* oil on Gram positive bacteria.

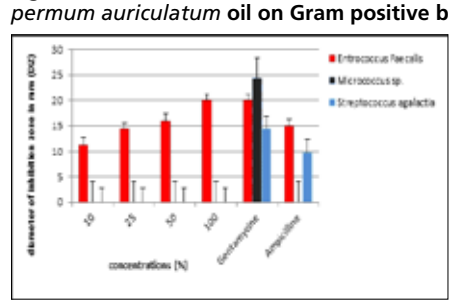

Fig. (3): Extraction of essential oils by hydrodistillation (Clevenger apparatus)

Fig. (4): The aerial part of *Tripleurospermum auriculatum*

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