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Antibacterial activity of ethanolic extracts of some arid zone plants

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Abstract: In the present investigation, the inhibitory effect of crude ethanolic extracts from five plants viz., *Artemisia scoparia*, *Allium sativum, Glycine max, Solanum dulcamara* and *Venidium decurrens* were evaluated against some pathogenic bacterial strains (gram positive and gram negative) using agar disc diffusion method. The ethanolic extracts of all the plants were found to be more or less active against almost all tested pathogenic strains. The inhibition zone and activity index varied in gram positive (IZ= 8mm- 30mm and AI= 0.57-2.5mm) and gram negative bacterial strains (IZ= 7mm- 20mm and AI= 0.46-1.36mm). *A.sativum* has the highest zone of clearing on *S.faecalis* (IZ= 30mm and AI= 2.5mm). *G.max* and *V.decurens* was found to be the most active plants that exhibited more or less similar activity against all the pathogens tested. The present investigations suggest that the plants studied could be useful for preparation of neutraceuticals as potent antibacterial agent to treat various human diseases and its complications.
Key words: Antibacterial activity, arid zone plants, ethanolic extracts.

Introduction:

With the advancement in science and technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products. This is because of the emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant misuse and abuse of antibiotics[1,2,3]. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs[4,5].

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. Plants are complex chemical storehouses of undiscovered biodynamic compounds, many of which serve as plant defense mechanisms against invasion by micro-organisms, insects and herbivores that can provide valuable sources of natural antibacterial agents[6,7]. The active principles isolated from plants appear to be one of the important alternatives when compared with many sub standard orthodox synthetic medicines because of their less or no side effect and better bioavailability. Plant extracts have been studied against pathogens for years for assays to detect new and previously undiscovered antimicrobials from plant sources[8,9]. Hence, in the present investigation, the antibaterial potential of ethanolic extracts of five plants namely, *Artemisia scoparia* Waldst et. Kit., *Allium sativum* Linn., *Glycine max* Linn., *Solanum dulcamara* Linn. and *Venidium decurrens* Less. from arid zone have been evaluated against common bacterial pathogens.

Material and methods

Plant material

The whole plants of *Artemisia scoparia*, *Allium sativum, Glycine max, Solanum dulcamara* and *Venidium decurrens* were collected from local farm in Jaipur, India. The plants were authenticated from National Institute of Ayurveda (NIA), Jaipur, Rajasthan and the voucher specimens were conserved

to the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India.

Extraction

The various plants viz., *A.scoparia*, *A.sativum, G.max, S.dulcamara* and *V.decurrens* were air dried and powdered using motor and pestle. The coarsely powdered materials were successively extracted with ethanol in Soxhlet apparatus for 24 hrs. The extracts were further evaluated for their antibacterial activity against selected bacterial strains.

Antibacterial Screening

Test microorganisms

In vitro antimicrobial activity was evaluated against ten common pathogenic bacteria, Gram positive – *Streptococcus pyogenes, Streptococcus viridans, Streptococcus faecalis, Staphylococcus aureus* (coo+), *Staphylococcus aureus* (coo^-) Gram negative- *Proteus vulgaris*, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella pseudomonas*. All the tested microorganisms were obtained from SMS (Sawai Mansingh) Hospital, Jaipur, Rajasthan. The bacterial cultures were grown and maintained on Nutrient Broth medium at 37^0C for 24h.

Antibacterial activity

Antibacterial assay of the ethanolic extracts were performed against ten tested pathogenic strains by agar disc diffusion method[10] using Gentamycin as standard antibiotic. The nutrient agar plates were seeded with suspension (10^6 CFU/ml) of the bacterial strains. The empty sterilized Whatmann No.1 filter paper disc (6mm) were impregnated with 10 µl of extract diluted with two volumes of DMSO, dried and placed aseptically on seeded plates with the help of a sterile forceps. Finally, the sensitivity discs were pressed with forceps to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 minutes (Pre diffusion time). The standard discs (6mm) impregnated with antibiotic gentamicin (2µg/ml) were used as positive control. The plates were incubated at 37^0C for 24 h. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

Results and discussion:

The search for antibacterials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antibacterial agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of micro-organism[11,12]. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds. Therefore, medicinal plants are finding their way into pharmaceuticals, neutralceuticals and food supplements[13,14,15,16].

In the present investigation, the inhibitory effect of crude ethanolic extracts from five plants viz., *A.scoparia*, *A.sativum, G.max, S.dulcamara* and *V.decurrens* were evaluated against some pathogenic bacterial strains (gram positive and gram negative). *In vitro* antibacterial activity was determined using agar disc diffusion method and summarized in Table 1 and 2. The activity was quantitatively assessed on the basis of inhibition zone and their activity index was also calculated. The ethanolic extracts of all the plants were found to be more or less active against almost all tested pathogenic strains as compared to standard antibiotic. The inhibition zone and activity index varied in gram positive (IZ= 8mm- 30mm and AI= 0.57-2.5mm) and gram negative bacteria (IZ= 7mm-20mm and AI= 0.46-1.36mm). The most susceptible bacteria was *S. faecalis* (IZ= 30mm and AI= 2.5mm) among gram positive strains and *P.vulgaris* (IZ= 20mm and AI= 1.0mm) among gram negative strains.

Table 1: Antibacterial activity ethanolic extracts against gram positive bacteria

Plants used	Plant parts used									
	S.pyogens		***S. viridans***		***S.faecalis***		***S.aureus*(coo$^+$)**		***S.aureus*(coo$^-$)**	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
A.scoparia	11	0.78	11	O.7	t	t	12	0.8	15	0.90
S.dulcamara	8	0.57	25	1.6	t	t	10	0.66	20	1.20
G.max	8	0.57	10	0.6	15	1.2	24	1.6	20	1.20
V.decurens	15	1.07	15	1.0	1.6	1.3	15	1.0	15	0.90
A.sativum	20	1.4	20	1.3	30	2.5	15	1.0	15	0.90
Gentamycin	14	-	15	-	12	-	15	-	16.6	-

IZ= Inhibition Zone, AI= Activity index, **t** = trace activity

Table 2: Antibacterial activity ethanolic extracts against gram positive bacteria

Plants used	Plant parts used									
	E.coli		***K.pneumoniae***		***P.vulgaris***		***P.aeruginosa***		***S.pseudomonas***	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
A.scoparia	14	0.74	13	1.36	20	1.0	7	0.46	t	t
S.dulcamara	19	1.0	12	1.26	12	0.6	10	0.66	10	0.66
G.max	18	0.96	9	0.95	10	0.5	9	0.6	t	t
V.decurens	9	0.45	10	1.65	20	1.0	t	t	16	1.06
A.sativum	20	1.06	9	1.02	10	0.5	12	0.8	12	0.8
Gentamycin	18.7	-	9.5	-	20	-	15	-	15	-

IZ= Inhibition Zone, AI= Activity index, **t** = trace activity

A.sativum exhibited highest zone of clearing on *S.faecalis* (IZ= 30mm and AI= 2.5mm). However, lowest inhibitory effect was shown by *A.scoparia* on *P.aeruginosa* (IZ= 7mm and AI= 0.46mm) and *S.pseudomonas* (t). *G.max* and *V.decurens* was found to be the most active plants that showed more or less similar activity against all the pathogens tested, while *S.dulcamara* had maximum effect on *S.viridans* (IZ= 7mm and AI= 0.46mm). Among bacterial pathogens, gram positive bacterial strains were found to be more susceptible than gram negative bacterial strains. This may be attributed to the fact that cell wall in gram positive bacteria consist of a single layer, whereas, gram negative cell wall is multilayered structure bounded by an outer cell membrane [17]. The inhibitory effect of the ethanolic extract may be attributed to the presence of bioactive metabolites like alkaloids, terpenoids and phenolic coumpounds. Researchers have documented the antimicrobial activities of the ethanolic extracts of other plants[18,19]. The findings of the present investigation suggest that the plants investigated are a potent source of biologically active compounds which may potentially prove to be efficient natural antibacterial agents. Further studies are required to identify the actual chemical constituents that are present in the ethanolic extracts of these plants which are responsible for antibacterial activity.

Conclusion

The present investigation revealed that the studied plants are potentially a good source of antimicrobial agents and validates their traditional use and as a source for natural antibacterial that can serve as an alternative to more expensive conventional medicines and further pharmacological evaluation.

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