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PHYTOCHEMICAL INVESTIGATION AND IN VITRO EVALUATION OF *LANTANA CAMARA* SEED EXTRACTS ON SELECTED HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Lantana camara Linn is an important weed with a vast array of medicinal uses. In this study, antimicrobial activities of methanol, chloroform, acetone, petroleum ether and hexane extracts of *L. camara* seed was investigated by using Agar Well Diffusion Method. This activity was tested against four human pathogenic bacteria *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli*. The extracts were used in three different concentrations (75,150 and 250 ug/ml). Among the extracts, methanolic extract of *Lantana camara* seed showed maximum inhibition against *S. aureus, P.aeruginosa* and *E. coli* and no inhibitory against *P. vulgaris*. Similarly the acetone extracts of *L.camara* seed also found to have enormous inhibition against *S.aureus, P. vulgaris* and lesser activity *E. coli*.. On the other hand, the other extract of chloroform proved to have no antibacterial activity against the bacterial strains used in this study. The result of phytochemical analysis exposes the presence of saponin, reducing sugar, steroids and flavonoids in the seed extract of *Lantana camara*.

KEY WORDS: ***Lantana camara*, antimicrobial activity-Agar Well Diffusion Method, Phytochemical analysis, Human pathogenic bacteria.**

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INTRODUCTION

Lantana camara L. (Verbanaceace), commonly known as wild or red sage, is the most widespread species of this genus and it is regarded both as a notorious weed and a popular ornamental garden plant[1]. However, it is listed as one of the most important medicinal plants of the world [2]. While *L. camara* contains lantadenes, the pentacyclic triterpenes possess a number of useful biological activities such as antifungal [3,4], antiproliferative [5,6] and antimicrobial activities [5,7-9].The expanding bacterial resistance to antibiotics has become a growing concern worldwide[10]and prompting a resurgence in research of the antimicrobial role of herbs against resistant strains [11,12].Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone and methanol are often used to extract bioactive compounds [13].Several tri-terpenoids, flavonoids, alkaloids and glycosides isolated from this plant are known to exert diverse biological activities.[14] In recent years, secondary metabolites (phytochemicals) that comprise of unknown pharmacological activities have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections[15].*Staphylococcus aureus* is an important pathogen in human infections from mild skin infections to more serious and invasive infections such as septicemia, pneumonia, endocarditis and cause food poisoning and toxic shock syndrome. *Escherichia coli,* a gram negative bacteria cause urinary tract infection and diarrhea in young children [16]. *Pseudomonas aeruginosa* is a gram negative motile rod which causes oligouria, leukocytosis and leucopenia disseminated intramuscular coagulation and adult respiratory distress syndrome[17]. *Proteus vulgaris* is an important nosocomial pathogen[17].In this work, the phytochemical analysis and the antibacterial activity of methanol, chloroform, petroleum ether, hexane and acetone extracts and that of *Lantana camara* seed against the human pathogenic bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli* were studied.

MATERIALS AND METHODS

Preparation of Seed extracts

Fresh seeds of Unni (*Lantana camara*) were collected, washed thoroughly and then air-dried under shade and finally milled into a coarse powder. The powdered material through Soxhlet was extracted after 48 hours using different solvents such as methanol, chloroform, acetone, petroleum ether and hexane. All the extracts were evaporated in vacuum under reduced pressure. They were stored in sterile glass bottles at room temperature until screened.

Preparation of micro organisms

The test organisms used in this study were the human pathogenic bacterial strains namely *Pseudomonas aeruginosa* (MTTC 741), *Escherichia coli* (MTTC 728), *Staphylococcus aureus* (MTTC 3160) and *Proteus vulgaris* (MTCC 7299) were bought from IMTECH, Chandigarh. The strains were maintained on nutrient agar slants at 4^{o}C. A loopful of each bacterial strain was added to 50 mL sterile nutrient broth in a 100 ml conical flask. The flasks were then incubated on a rotary shaker for 24 h to activate the strain.

Agar Well Diffusion Method

The antibacterial activities of the extracts of the *Lantana camara* seed obtained by using five different solvents, methanol, chloroform, acetone, petroleum ether and hexane were evaluated by the Agar Well Diffusion Method[18]. The strains that had been incubated for 24 h were used for the assay. A sterile cotton swab was dipped into the bacterial suspension. It was evenly spread over the entire surface of a sterile Muller Hinton agar plate to obtain a uniform inoculum. Wells were punched on the seeded plates using a sterile borer (8mm) and the plates were allowed to dry for 5 min. Methanol, chloroform, acetone, petroleum ether and hexane extracts (75, 150, 250

µg/ml) were dispensed into each well using a sterile micropipette. Control was used containing inoculums without seed extract. The plates were incubated overnight at 37^{o}C and the antibacterial activity was determined by measuring the diameter of zone of inhibition (mm).

Phytochemical analysis

Specific qualitative tests were performed for the detection of common secondary metabolites in the seed extracts. To reveal the presence of tannins 0.5 g of the dried powder of the seeds was boiled and filtered. Then, ferric chloride was added to the filtrate and kept undisturbed for the observation. Foam and heamolytic tests were used for saponins. Molisch test for carbohydrates was done[19]. Alkaloids were identified by Mayer's test while the Libermann test was performed for phytosterols. The presence of sterols was confirmed by the addition of 2 ml of acetic anhydride to 0.5g of dried methanol extract with 2 ml of concentrated sulphuric acid. Schinoda test was done for flavonoids.[20]

RESULTS AND DISCUSSION

In the present investigation, the inhibitory effect of different extracts (*viz.* methanol, chloroform, acetone, petroleum ether and hexane) of seeds from *Lantana camara* was evaluated against the specific bacterial strains. The antimicrobial activity was determined using Agar Well Diffusion Method is summarized in table 1. Among the extractions assayed, the methanol extracts of *L. camara* seed proved maximum inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*. Eloff (1998) reported that Methanol was the most effective solvent for plant extraction than ethanol, n–hexane and water. The methanol extracts of *Allium vineale* showed the higher antibacterial activity as compared with *Chaerophyllum macropodium* and *P. fexulacea* [22]. The methanol extracts of *Murraya koenigii* revealed less activity against *P. aeruginosa* and *E. coli*, whereas aqueous extract gave maximum zone of inhibition in the same pathogen but Vlientinck *et al.,* (1995) reported that water extracts of plants do not have much activity against bacteria. Methanolic extract of *L. camara* seed showed prominent inhibition against *S. aereus*. When the concentration increases, the inhibition also increases. The methanolic extract of *L.camara* seed did not exhibit any inhibitory activity against *Proteus vulgaris*. Maximum inhibition was observed in the acetone extract of *L. camara* seed against *S. aureus* and *E. coli* at all the concentrations. In the same way, the acetone extract on *L. camara* seed showed significant inhibition against *P. vulgaris* only at higher concentrations and no inhibition was found at the lower concentration. But the acetone extract of *L. camara* seed proved no inhibitory effect against the *P.aeruginosa*. It is reported that 20 µl acetone extract of other plants like *Glycrhiza globra, Cinnamomum cassia* and *Juniperus oxyudrus* exhibited maximum antibacterial activity against *Bacillus sp., P. aeruginosa* and *S. aureus*[24]. The present results proved that maximum antibacterial activity was noticed against *S. aureus* whereas no activity was found against *P.aeruginosa.*

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Table 1
Antibacterial Activity of Unni (Lantana camara seed) against selected pathogenic bacteria

Name of the bacterial strain	Different extract	Zone of inhibition (mm)		
		Extract concentration		
		75 µg/ml	150 µg/ml	250µg /ml
Staphylococcus aureus	Methanol	5.3±1.1	8±0	10.3±0.57
	Chloroform	0±0.0	0±0.0	0±0.0
	Acetone	7±0.0	10±1	12.1±0
	Petroleum ether	0±0.0	0±0.0	5±0.0
	Hexane	0±0.0	0±0.0	0±0.0
Pseudomonas aeruginosa	Methanol	5.3±1.1	9±0	13±1.0
	Chloroform	0±0.0	0±0.0	0±0.0
	Acetone	0±0.0	0±0.0	0±0.0
	Petroleum ether	0±0.0	0±0.0	0±0.0
	Hexane	0±0.0	0±0.0	0±0.0
Proteius vulgaris	Methanol	0±0.0	0±0.0	0±0.0
	Chloroform	0±0.0	0±0.0	0±0.0
	Acetone	0±0.0	5±0.5	7.6±0.5
	Petroleum ether	0±0.0	0±0.0	0±0.0
	Hexane	0±0.0	0±0.0	0±0.0
Escherichia coli	Methanol	7.5±0.5	9.4±0.3	12.2±0.4
	Chloroform	0±0.0	0±0.0	0±0.0
	Acetone	7.2±0.2	10.1±0.2	12.3±0.3
	Petroleum ether	0±0.0	0±0.0	5.3±0.4
	Hexane	0±0.0	0±0.0	8.2±0.2

When the choloform extract of *L. camara* seed was treated against *S .aureus, P. aeruginosa, P.vulgaris* and *E. coli* no inhibitory activity was recorded at all concentrations. Similarly, the hexane extract also exhibited no antibacterial activity against *S. aureus, P. aeruginosa* and *P. vulgaris* at all concentrations. But the same extract proved to have some antibacterial effect against *E. coli* only at the higher concentration. Milin *et al.,* (2012) proved that hexane and petroleum ether extracts of *L. camara* leaves exposed antibacterial activities against *S .aureus, E.coli* and *P. aeruginosa* at various concentrations (80,40,20,10 mg/ml). When the petroleum ether extract of *L. camara* seed was treated against *S.aureus* and *E. coli* slight inhibition was noted only at the higher concentration (250 µg/ ml). But the same extract displayed no activity against *P.aeruginosa* and *P. vulgaris* at all concentrations. The result revealed that *S. aureus* and *E. coli* were the most susceptible species to the different concentrations of methanol, acetone and petroleum ether extract of *L. camara* seed. *P. aeruginosa* was susceptible only to the extract of methanol and *P. vulgaris* was sensitive only to the extract of acetone.Bhakta and Ganjewala (2009) reported that the antibacterial properties of the *L. camara* were due to the presence of phenolics, anthocyanins and proanthocyanidins in their leaves. Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extracts and the one that predominates over the others[27]. The presence of phytochemical compounds in *L. camara* has been attributed to most of its biological and antimicrobial activities [28].Phytochemical screening of *L. camara* seed extracts (Table 2) revealed the presence of saponin, reducing sugar, steroids and flavonoids as major active secondary metabolites while tannins, alkaloids and terpenoids were absent. This is similar to phytochemical study of aerial parts of *L. camara* carried out by Naeem *et al.,* (2009). Lantadenes present in all *L. camara* is believed to be responsible for all the biological activities[30]. In addition, other secondary metabolites such as steroids, saponin, terpenoids and flavonoids could be held partially responsible for some of these biological activities [31].

Table-2
Phytochemical analysis of Lantana camara seed

Samples	Test							
	Tannin	Phytotanin	Saponin	Reducing sugar	Alkaloids	Steroids	Terpenoids	Flavonoids
Lantana camara	-	-	+	+	-	+	-	+

(+) : Present
(-) : Absent

The result of present investigation clearly indicates that the antibacterial activity varies with the species of the plants and plant material used such as seed. It is known that a lot of factors affect antibacterial activity. So it is considered that the bacterial inhibition can vary with the plant extract, the solvent used for extraction and the organism tested.

CONCLUSION

The present study reports the successful extracts of bioactive metabolites from *Lantana camara* seed. In this study, these extracts marked better control of these pathogens used. Thus, it is concluded that the seeds of *L. camara* is a potential source for antibacterial activity and provide some idea about phytochemical evaluation. Further studies should be undertaken to elucidate the exact mechanism of action by which the extracts exert their antibacterial effect.

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