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Research Article

Evaluation of Antimicrobial Activity of the Whole Plant Extract of *Mollugo cerviana* (L.)Ser Against Human Pathogens

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Abstract

The evaluation of the spectrum of antimicrobial activity of a drug preparation or a plant extracts plays a significant role in therapeutics. The methanolic and ethanolic extracts of the whole plant of *Mollugo cerviana* were tested against common pathogens infecting wound by agar disc diffusion method. Sterile filter paper discs loaded with plant extract at a concentration of (10 mg/ml) tested against the pathogenic strains. Filter paper discs loaded with 5 µg of Tetracycline was used as positive control. Pure solvents serve as negative control. The study has revealed the significant antibacterial activity shown by the methanolic and ethanolic extracts of the whole plant against common pathogens infecting the wounds including *Staphylococcus aureus* and *Pseudomonas aeruginosa* besides other pathogens.

Keywords: antimicrobial activity, *Mollugo cerviana*, common pathogens on wound, disc diffusion method, zone of inhibition.

INTRODUCTION

The evaluation of the antimicrobial activity of a drug preparation or a plant extracts plays a significant role in therapeutics. The spectrum of activity against specific pathogens provides an insight to the pharmacologist towards the development of a drug to treat specific disease conditions. Crude extracts of plants were traditionally used to treat infections. The antimicrobial activity of plants had been received attention many years ago as one of the most effective mechanism for the control of microorganisms[1]. Plant based antimicrobials are being investigated for their ability to act against the pathogens in natural way with minimal side effects. Antimicrobial drugs may either kill microorganisms outright or simply prevent their growth. There are various ways in which these agents exhibit their antimicrobial activity[2]. The development of drug resistance strains has led to the necessity of identifying new drugs possessing broad spectrum of activity. There is increased prevalence of antibiotic resistant bacteria emerging from the extensive use of antibiotics which renders current antimicrobial agents insufficient to control at least some bacterial infections[3]. Biomolecules of plant origin appear to be one of the alternatives for the control

of these antibiotic resistant human pathogens[4].The secondary metabolites produced by the plants are responsible for their antimicrobial activity. Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve as a defence mechanism against prediction by many microorganisms, insects and other herbivores[5]. The ancient civilizations considered plants as the main source of new leads for antimicrobial remedies and pharmaceutical development[6]. In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is pertinent to thoroughly investigate their composition and activity and thus validate their use[7]. Antimicrobial susceptibility tests are normally performed to determine the efficacy of potential antimicrobial preparation from natural or plant origin against number of microorganisms. Antimicrobial assays provide an insight for the researcher to study the spectrum of antimicrobial activity exhibited by the plant and to proceed further with the plant towards characterisation and isolation of compounds present and development of naval drug preparations from the plant.

Materials and methods

Collection of plant material

The whole plants of *Mollugo cerviana* were collected from OrathanaduTaluk of Thanjavur district in the state of Tamilnadu , India and authenticated by John Britto Rapinat Herbarium, St.Joseph's college, Tiruchirapalli. Then examined carefully old, infected and fungus damaged portion of the plants were removed. Healthy plants were spread out and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder.

Preparation of extract

10 g of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4°C. In the same ratio ethanolic extract was also prepared.

Inoculum preparation

The microbial strains used in this study were collected from isolated clinical specimens and grown in the nutrient broth and maintained on nutrient agar slants at 4°C. Each bacterial strain was subcultured overnight at 35 °C in Mueller-Hilton agar slants. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10^7 CFU/ml using spectrophotometer. All the isolated colonies were further identified using Gram staining and biochemical studies for confirmation of pure culture.

Biochemical tests for identification of Microorganisms

Gram's staining

A preliminary Gram staining was performed to determine the likely organism present. A loopful of organism was taken from a nutrient agar plate and smear was made out of it on a glass slide. First crystal violet (primary stain) was added and left for one minute. After the stipulated time, the slide was washed with distilled water. Secondly, Gram's iodine a mordant stain was added and left for one minute. Again the slide was washed with distilled water and then decolourization with organic solvents like ethanol or acetone was added in drops to remove the primary stain. Again the slide was washed with distilled water. Finally, secondary stain like safranin was added. After washing and air drying of the slide it was microscopically observed for morphology of the organism.

Motility Test : A loopful of culture was kept on a cover slip and the hanging drop technique was performed for motility. Then the results were observed.

Indole test: About 2 to 3 ml of peptone water culture was taken in a test tube. Then 0.5 ml of Kovac's reagent was added into the tube.Then the results were observed for record.

Methyl red test: 48 hours of glucose phosphate culture was taken in a sterile test tube. Then one or two drops of methyl red alcoholic reagent were added. Then the results were observed for record.

Voges-Proskauer test: A loopful of culture was inoculated into glucose phosphate broth for 48 hrs. After incubation, added 1/5 volume of potassium hydroxide solution and 5% alpha naphthol solution in absolute ethanol. Kept in the incubator at 37 °C for two hours. Then the results were recorded.

Catalase test: Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and

water. The organism was taken in a clean, dry glass slide using a loop and a drop of 3% hydrogen peroxide solution was placed on to the slide and mixed. Observed for immediate bubbling and recorded the result.

Coagulase test: 1 in 10 dilution of the plasma in saline was prepared. 0.5 ml of the diluted plasma was transferred into the small test tubes. The diluted plasma was inoculated with 0.1 ml of loopful culture. The tubes were incubated at 37 °C preferably at water bath and examined after first, second, third and fourth hours.

Oxidase test: The enzyme oxidase when reacts with the reagent *N, N*-tetramethyl paraphenylenediamine dihydrochloride gives coloured product indophenol. The bacterial culture was rubbed over a reagent impregnated filter paper disc using glass rod. The colour change was observed within 10 seconds. The purple colour change of the disc indicates oxidase positive.

Urease test: Urea is a diamide of carbonic acid. Urease is the enzyme that hydrolyses urea and releases ammonia and carbon-dioxide. Ammonia reacts in solution to form ammonium carbonate which is alkaline leading to increase in the pH. If the phenol red which is incorporated in the medium, changes colour from yellow to red in alkaline pH shows urease activity. The urea agar slant was inoculated with a drop of culture and incubated at 37 °C for 24 hrs.

Nitrate reduction test : To determine organism's ability to reduce nitrates in to nitrites. The culture was inoculated in nitrate broth and incubated at 37 °C for 24 hrs. After incubation 0.5 ml of alpha naphthalene reagent and 0.5 ml of sulfanilic acid reagent was added. The red colour of the medium before and after addition of zinc indicates positive result.

Determination of antibacterial activity by Disc Diffusion Method[8]

The method is used to evaluate antimicrobial activity of the plant extract. The plant extract residues (50 mg) were re-dissolved in 5 ml of ethanol, sterilized through Millipore filter (0.22 µm) then loaded over sterile filter paper discs (8 mm in diameter) to obtain final concentration of 10 mg/ml. 10 ml of Mueller-Hilton agar medium was poured into sterile Petri dishes (as a basal layer) followed by 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 10^7 CFU) to attain 10^5 CFU/ml of medium. Sterile filter paper discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of Mueller-Hilton agar plates. Filter paper discs loaded with 5 µg of Tetracycline was used as positive control. The plates were kept in the fridge at 5 °C for 2 h. to permit plant extracts diffusion then incubated at 35 °C for 24 hours. The presence of inhibition zones were measured by Himedia zone scale, recorded and considered as indication for antibacterial activity. In similar manner the methanolic extract of the whole plant extract of *Mollugo cerviana* having the final concentration of (10 mg/ml) was separately prepared and subjected to the procedures described above to for assessing the activity against the selected strains and the respective zone of inhibition was measured and tabulated.

Results:

The biochemical identification tests for the standard microorganisms used in this study were done as per the standard procedures and the results were tabulated in Table-1. The antimicrobial activity of ethanolic and methanolic extracts of the whole plant extract of *Mollugo cerviana* against five selected human pathogen strains(*Escherichia coli, Staphylococus aureus, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia*) were determined using disc diffusion method and the results were tabulated in Table-2.

The results reveal the zone of inhibition in mm for the standard drug Tetracycline (positive control) and the tests comprising of methanolic and ethanolic extract of the whole plant of *Mollugo cerviana* against the selected pathogenic strains.

The methanolic extract has shown significant antibacterial activity against the selected strains when compared with the standard drug Tetracycline and the zone of inhibition measured for *Escherichia coli* is 16 mm, for *Staphylococcus aureus* 14 mm and for *Klebsiella pneumonia* 15 mm. The zone of inhibition noticed for *Pseudomonas aeruginosa* and *Salmonella typhi* is 11 mm. The ethanolic extract has shown a moderate activity against the above said organisms but is slightly less than the methanolic extract and

the zone of inhibition for *Escherichia coli* is 14 mm, for *Staphylococcus aureus*12 mm and for *Klebsiella pneumonia* it is 13 mm. *Pseudomonas aeruginosa* and *Salmonella typhi* has shown 9 mm and 10 mm as the zone of inhibition respectively.

Table.1 Biochemical tests for identification of Microorganisms

Name of the Test	Name of the organism				
	Escherichia coli	***S.aureus***	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Klebsiella Pneumonia***
Gram's staining	Negative (-)	Positive (+)	Negative (-)	Negative (-)	Negative(-)
Motility test	Motile	Non motile	Motile	Motile	Non motile
Indole test	Positive (+)	Negative (-)	Negative (-)	Negative (-)	Negative(-)
Methyl red test	Positive (+)	Positive (+)	Negative (-)	Positive (+)	Negative(-)
Voges-Proskauer test	Negative (-)	Positive (+)	Negative (-)	Negative (-)	Positive(+)
Catalase test	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive(+)
Coagulase test	_	Positive (+)	_	_	_
Oxidase test	Negative (-)	Negative (-)	Positive (+)	Negative (-)	Negative(-)
Urease test	Negative (-)	Positive (+)	Negative (-)	Negative (-)	Positive(+)
Nitrate reduction test	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive(+)

(- not applicable)

Table 2: Antimicrobial activity of methanolic and ethanolic extracts of *Mollugo cerviana*(in vitro) (Values are mean of three replicates)

S. No	Name of the Organism	Zone of inhibition (mm)		
		Standard	Methanolicextract	Ethanolic extract
1.	***Escherichia coli***	21	16	14
2.	***Staphylococcus aureus***	29	14	12
3.	***Pseudomonas aeruginosa***	25	11	9
4.	***Salmonella typhi***	25	11	10
5.	***Klebsiella pneumonia***	27	15	13

Discussion

The results of this study indicated the spectrum of antimicrobial activity exhibited by the plant *Mollugo cerviana* against the human pathogens. The presence of biologically active secondary metabolites in the plant is responsible for its activity. Antimicrobials are substances either natural, semisynthetic or of synthetic origin capable of killing or inhibiting the growth of microorganisms. Medicinal plants are rich in a numerous variety of secondary metabolites of antimicrobial properties such as saponins, tannins, alkaloids, phenols, glycoalkaloids, flavonoids, sesquiterpenes, lactones, terpenoids and phorbol esters[9]. In an antimicrobial study conducted on ethanolic extracts of 45 Indian medicinal plants traditionally used in medicine against certain drug-resistant bacteria and *Candida albicans*, the study reveals that 40 plant extracts showed varied levels of antimicrobial activity against one or more test bacteria and the phytochemical analysis demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as major active constituents[10].Antimicrobial study conducted on the ethanolic extracts of aerial shoots of *Mollugo cerviana* and its leaf derived callus against *Escherichia coli* (Gramnegative) and *Bacillus subtilis* (Gram positive) and two fungi namely *Aspergillus niger* and *Aspergillus flavus* revealed that the aerial shoot extract have shown slightly more antibiotic activity than leaf callus extract. The antifungal activity of both the extracts was almost nil[11]. In the study conducted on the crude methanol extract as well as

the ethyl acetate and n-butanol fractions from the whole plant of *Mollugo cerviana,* the fractions have shown strong antimicrobial activity against all the organisms tested and concluded that the methanol extract and n-butanol fraction from the plant of *Mollugo cerviana* have antimicrobial properties which explain the basis for its use in traditional medicine to treat infected wounds[12].The petroleum ether and methanol extracts of the whole part of *Mollugo oppositifolius* have got profound antimicrobial and moderate antioxidant effect[13]. In a reported study on the antimicrobial activity of *Mollugo pentaphylla* fruit extract the antimicrobial activity against both gram positive and gram negative bacteria has been revealed[14].An alcoholic extract of the plant *Mollugo cerviana* shows antibacterial activity against *Escherichia coli*[15]. In a reported study on *in vitro* antioxidant and antibacterial activity of aqueous and methanolic extract of *Mollugo nudicaulis* Lam. Leaves it was concluded that the ethanolic extract has shown higher in *in vitro* antioxidant and antimicrobial activity compared to aqueous extract[16].

Conclusion

The present study has revealed the significant antibacterial activity shown by the methanolic and ethanolic extracts of the whole plant of *Mollugo cerviana* against common pathogens infecting the wounds such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* besides other pathogens. The study correlates with the results of preliminary phytochemical analysis which revealed the presence of biologically active secondary metabolites including phenolic compounds, flavonoids and tannins in the plant extract. The results further substantiated the recommended use of *Mollugo cerviana* in treating wounds and infections associated with wounds as a wound healing agent.

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