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Phytochemical and Antimicrobial Screening of Mollugo Cerviana (L). Ser. In Methanolic Extract.

Sherin Rebecca F* and R. Sharmila
P.G & Research Department of Biotechnology, Bishop Heber College, Trichy-620017 Tamil Nadu, India.

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ABSTRACT:
Mollugo cerviana (L.) Ser. (Molluginaceae), a pot herb, enhances eyesight, reduces body odour, acts as a good antiseptic and is used in the treatment of cough. This study involves the investigation of the phytochemical screening and antimicrobial activity of the methanolic extract against some common gram positive and gram negative bacteria. Phytochemical screening was done by using the standard methods given by Harbone. Disc diffusion method was adopted for the antimicrobial screening. Minimum inhibitory concentration (MIC) was measured by using agar streak dilution method. The phytochemical screening in the four extracts namely hexane, chloroform, ethyl acetate and methanol revealed the presence of triterpenes, flavanoids, alkaloids, saponnins, phenolic groups and tannins. The methanolic extract was active against gram positive, gram negative bacteria. The extract shows maximum inhibition with 33.45 mm of Inhibition zone diameter (IZD) in Aspergillus niger and it has less activity against Candida albicans with IZD of 24.51 mm. The MIC values obtained ranges from 9.5 µg/ml - 16 µg/ml.
The results of our study showed that the extracts of M.cerviana were rich in secondary metabolites and also it posses significant antimicrobial activity.
KEY WORDS: Mollugo cerviana, Antimicrobial activity, Phytochemical screening

I. INTRODUCTION

For a long period of time, plants have been valuable sources of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies[1].The plant kingdom is a treasure house of potential drugs and there has been an increasing awareness about their importance of medicinal plants[2]. They are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites. Different plants have been used as a source of inspiration in the development of novel drug[3]. Plants derived medicines are widely used because they are relatively safer than the synthetic alternatives, they are easily available and cheaper[4]. Many plants species have been evaluated for their antimicrobial activity in the past twenty year[5]. The efficacy of many medicinal plants in the treatment of many diseases has been put to test in many laboratories[6] and the researchers are increasingly turning their attention to natural products looking for a new leads to develop better drugs against cancer, as well as viral and microbial infections[7-9].

Recently the World Health Organization (WHO) has recommended the use of Arthemisinin derivatives derived from Artemisia annua (Composite), a Chinese herb, as a first line drug in the treatment of malaria[10, 11] and this is as a result of WHO's recognition that 80 % of world population use herbal medicine for Primary Health Care[12]. Since the last decade, the rise in the failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity[4, 13].

M. cerviana (L.) Ser. (Molluginaceae) has been widely used as a pot herb, enhances eyesight, reduces body odour, acts as a good antiseptic and is used in the treatment of cough[14]. It is used as a blood purifier, for fever, post partum discharges[15], used as antiseptic, stomachic, febrifuge, gout and in rheumatic complaints[16] and it has been documented for its anti-inflammatory activity[17]. The aim of the present study is to assess the phytochemical content and to screen its antimicrobial activities in the extracts. The findings from this work may add to the overall value of the medicinal potential of the herb.

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II. MATERIALS AND METHODS

A. Preparation of plant extract

The aerial plant of Mollugo cerviana was collected from Thoothukudi, Tamil Nadu, India. The plant was botanically identified and authenticated by Dr.V.Chelladurai, Research Officer (Retired), Department of Botany, Central Siddha Research Unit, Tirunelveli. The aerial parts of plants were air dried and powdered mechanically. The plant extracts were made by successive extraction method using solvents of increasing polarity starting from hexane, chloroform, ethyl acetate and methanol and the extracts obtained were filtered and evaporated by using vacuum distillation method. The extract was used for the further studies.

B. Preliminary phytochemical analysis

A 200 mg extracts were dissolved in 20 ml of its mother solvents and the dissolved extracts were subjected to preliminary phytochemical tests as described in Harbone[18]. The tests were based on the visual observation of colour change or formation of a precipitate after the addition of specific reagents.

C. Antimicrobial activity

1. Microorganism used

The in vitro antibacterial strain like Staphylococcus aureus ATCC 6538p, Streptococcus pyogens ATCC 14289, Proteus vulgans ATCC 6380, Staphylococcus mutans ATCC 25175, Escherichia coli ATCC 8739, Salmonella typhi ATCC 6539, Shigella flexneri ATCC 29508 and Pseudomonas aeneginosa ATCC 9027 were used for present studies and the microbes were obtained from microbial type collection center, Chandigarh, India.

2. Paper disc diffusion method

The sterilized (autoclaved at 121 °C for 15 min) medium (40-50 °C) was inoculated (1 ml/100 ml of medium) with the suspension (10^5 cfu/ml) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the methanolic extract (25, 50 and 100 µg/ml in dimethyl formamide) was placed on the solidified medium. The plates were preincubated for 1 hr at room temperature for antibacterial and incubated at 37 °C for 28 hrs. Ciprofloxacin (50 µg/disc) was used as standard for antibacterial activities[19].

3. Minimum inhibitory concentration (MIC)

MIC of the extract was determined by agar streak dilution method. A stock solution of the extract (25, 50 and 100 µg/ml) in dimethyl formamide was prepared and graded quantities of the extract were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial activity). A specified quantity of the medium (40-50 °C) containing the extract was poured into a petridis to give a depth of 3-4 mm and allowed to solidify. Suspension of the microorganisms were prepared to contain approximately 10^5 cfu/ml and applied to plates with serially diluted extract in dimethyl formamide and incubated at 37 °C for 24 hrs. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate[20].

III. RESULTS

A. Phytochemical screening

With the increase in the incidence of resistance to antibiotics, alternative natural products of plants could be of interest. Some plant extracts and phytochemicals are known to have antimicrobial properties, which could be of great importance in the therapeutic treatments. In the last years, various studies have been conducted in different countries, demonstrating the efficacy of this type of treatment[21,22]. The phytochemical screening of nine different chemical compounds like steroids, triterpenoids, flavanoids, alkaloids, glycosides, phenolic groups, saponins, tannins and amino acids were tested in four different extracts. Thus out of 4x9=36 tests for the presence or absence of the above compounds, only 13 gave positive results and the remaining 23 gave negative results.

The 13 positive results show the presence of saponins, triterpenes, flavanoids, alkaloids, phenolic group and tannins (Table 1). The test for steroids and amino acids did not show any positive result for their presence in any of the four extracts. Through phytochemical prospecting of the extracts, it was possible to determine the presence of diverse classes of secondary metabolites that show a wide variety of biological activities such as antimicrobial[23,24], antioxidant[25], antitumor and antiophidic[26].

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Table 1: Phytochemical screening of M. cerviana in different solvent extracts

Type of compounds	Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	+	+	-	-
Flavanoids	-	-	-	+
Phenolic groups	+	+	-	+
Tannins	+	+	-	-
Saponnins	-	-	-	+
Triterpenoids	+	-	-	+
Steroids	-	-	-	-
Glycosides	+	+	-	-
Amino acids	-	-	-	-

(+) and (-) indicates the presence and the absence of the phytochemicals

B. Antibacterial activity

Depending on the measured values of the complete inhibition diameter of the zone including the well in millimeter, the antibacterial activity can be classified into the following types, such as > 12mm zone of inhibition – high sensitivity, 9-12mm zone of inhibition – moderate sensitivity, 6-9 mm zone of inhibition – less sensitivity and < 6mm zone of inhibition – resistant[27].

Escherichia coli was found to be the most inhibited pathogen by the methanolic extract with a diameter of zone of inhibition 32.62 mm and lowest inhibited pathogen was Staphylococcus aureus with a diameter of zone of inhibition 28.16 mm. The methanolic extract of M. cerviana showed considerable antibacterial activity against four gram positive and four gram negative bacteria. For bacteria strains, the IZD ranges between 15.61 mm to 32.62 mm.

The potency of the crude extract was comparable to those of antibiotics which are pure substances. Ciprofloxacin was used as standard against the bacterial strains; it shows the IZD ranging from 36.23 mm to 37.83 mm.

The result of MIC determination indicates the values against all the tested strains ranges from 9.5 µg/ml - 16 µg/ml. The lowest MIC value, 9.5 µg/ml was obtained on Shigella flexneri. The highest MIC value, 16 µg/ml was recorded for Staphylococcus aureus (Table 2, Figure 1 and Figure 2).

The zone of inhibition of methanol extracts against gram positive organisms were in the order: Staphylococcus mutans > Proteus vulgans > Streptococcus pyogens > Staphylococcus aureus, while the zone of inhibition against gram negative organisms were in the order: Escherichia coli > Shigella flexneri > Salmonella typhi > Pseudomonas aeneginosa.

Table 2: Antimicrobial activities of M. cerviana in methanolic extract at different concentrations

S.No	Micro organisms	Zone of inhibition (mm)				
		Standards[a]	25µg/Disc	50µg/Disc	100 µg/Disc	MIC µg/ml
Bacterial strains						
1	Staphylococcus aureus	36.26±0.15	15.61±0.24	20.31±0.27	28.16±0.11	16
2	Streptococcus pyogens	37.43±0.21	18.65±0.22	24.42±0.36	29.45±0.26	13.5
3	Proteus vulgans	36.62±0.17	20.76±0.15	22.56±0.15	30.13±0.35	10
4	Staphylococcus mutans	36.23±0.15	19.54±0.11	24.53±0.24	31.52±0.26	11.5
5	Escherichia coli	37.16±0.43	18.23±0.31	24.63±0.26	32.62±0.31	12.5
6	Salmonella typhi	37.83±0.11	19.43±0.15	23.47±0.25	30.41±0.26	10
7	Shigella flexneri	37.60±0.32	22.75±0.23	25.60±0.23	31.03±0.23	9.5
8	Pseudomonas aeneginosa	37.41±0.41	19.21±0.11	22.96±0.36	29.36±0.25	11

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[a]Standards (Ciprofloxacin for bacteria)
Data's are expressed as means ± standard deviation (SD).

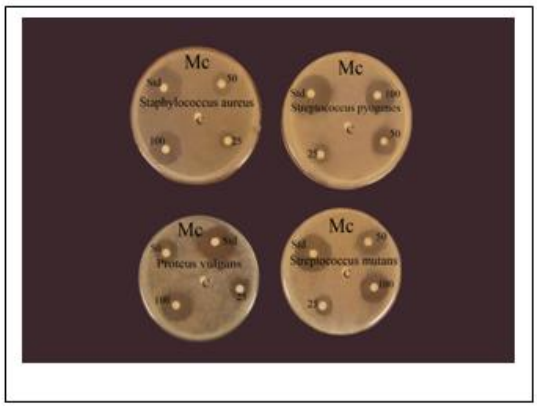

Figure 1:

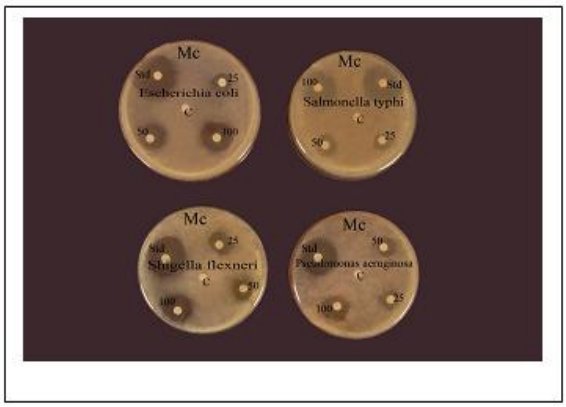

Figure 2:

Antibacterial activities of M. cerviana against gram positive bacteria at different concentrations

IV. CONCLUSION

Phytochemical investigation of the methanolic extracts of M. cerviana revealed the presence of various phytochemicals such as steroids, triterpenes, flavanoids, alkaloids, phenolic group and tannins. As phytochemicals often play an important role in plant defence against prey, microorganism, stress as well as interspecies protections. These plant components have been used as drugs for millennia. Hence, phytochemicals screening serves as the initial step in predicting the types of potential active compounds from plants[28].

Plants are an important source of potential useful bioactive compounds for the development of new therapeutic agents. There are many reports available on the antibacterial, antiviral, antifungal properties of plants[29-31]. Thus, these observations have helped in developing new drugs for the therapeutic use in human beings. However, not many studies are available on the antibacterial property of M. cerviana. In the present study, we have investigated the potential of the of methanolic extract of M. cerviana as a source for antimicrobial agents. The results in this study revealed that the methanolic extract of M. cerviana possesses great in vitro potential for antimicrobial activity to varying degrees against all microorganisms tested. Also revealed that, it was possessed all the necessary phytochemical constituents.

The demonstration of antimicrobial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds[9]. The highest MIC values of bacterial strains is an indication of that either the plant extract is less effective on some bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC values for bacteria is an indication of the efficacy of the plant extract.

M. cerviana exhibit some degree of antibacterial activity towards Staphylococcus mutans, Proteus vulgan, Escherichia coli and Shigella flexneri. Thus, it shows that this medicinal plant used in traditional medicine are potentially effective antimicrobial agents. The result of the above research shows that the methanolic extract of M. cerviana is a broad-spectrum agent which can be used against the diseases caused by gram positive and gram negative bacteria. The resulting information will contribute to a better understanding of the antimicrobial activity of the plant. This investigation has opened the possibility of the use of the plant in drug development for human consumption possibly for the treatment of wound infections and malarial fever. Isolation, identification and purification of these phyto-constituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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