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Research Article

ANTIMICROBIAL ACTIVITY OF PLANT *MUKIA MADERASATANA*

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ABSTRACT

The main objective of the study was to find the antimicrobial activity of the plant mukia maderaspatana .(family;cucurbitacae).The antimicrobial activity was determined by the agar disc diffusion method. The phytochemicals were also determined. The antioxidant activity was performed by reducing power method using ascorbic acid as the standard. The antimicrobial activity was used against two gram positive *Bacillus subtilis*, *Staphylococcus aureus* and two gram negative bacterial strains *Escherichia coli* and *Klebsiella pneumonia.* Out of these *Klebsiella pneumonia* was found to be the most sensitive. The presence of alkaloids, saponins, flavonoids and Phenolics was detected. Of the entire extracts methanol showed maximum antioxidant activity.the results of both the antimicrobial activity and antioxidant activity indicates the usage of this plant as a medicinal herb.

Keywords: Phytochemical analysis, Agar well diffusion method, Reducing power, *Mukia Maderaspatana.*

INTRODUCTION

There is approximately 250,000 to 500,000 species of plants on earth. some of them are used as food by human and animal species. Even larger proportion of the plants are used for their medicinal properties. The antimicrobial property of the plant extract is extremely valuable tool for the clinical microbiologists .Clinical microbiologists have shown their deep interest in the antimicrobial properties of the plants mainly due to two reasons. Firstly due to the fact that the phytochemicals could be useful for prescribing antimicrobial drugs by the physicians and a large proportion of these are already practiced on humans. The public is becoming increasingly aware with the misuse of traditional antibiotics. A large proportion of the of the plant based compounds are nowadays available from the herbal suppliers and the natural food stores and self medication with them is possible. The use of plant based drugs has enjoyed great popularity in the late 90s.The use of botanical medicines increased 37% in1996 compared to 1995[1].

In a discipline known as ethnobotany the goal is to utilise knowledge gathered by significant people about the plant and animal based products they have used to maintain health of humans. Approximately three quarters of the world population is dependent on the plant extracts for their health benefits. As a rough estimate the market for the plant derived drugs is approximately for Rs 2, 00,000 crores. The Contribution of the plant based drugs in the US is 25% of the total drugs while in china it is 80%.Thus it can be concluded that the contribution of medicinal plants is much more to india than other countries in the world. Out of the total 250,000 plant species on earth approximately 80,000 are medicinal. India can be regarded as the 12 biodiversity centres where over 45000 species are available. From them 15000-20000 plants have good medicinal value . In india herbal drugs have been used in systems of medicine like omani and Ayurveda. In Ayurveda 700 species of plants are used. The drugs can be derived from whole plant or different parts like stem , leaf , root , bark, flower and seed. It can also be prepared from excretory plant products like gums, resins and latex [2].

Even the allopathic system of medicine has adopted some plant derived drugs which forms a part of the pharmacopoeia. The high population rise, the increased cost of the drugs, the side effects of allopathic drugs has led to the increased use of the plant derived drugs. As an effort to reduce the financial burden the use of the plant based drugs is on the rise in the future. The plant mukia maderaspatana is from the family cucurbitacae and the kingdom plantae. It is an annual herb which is densely covered with white hairs .It ascends up to 1800m in the hills in india. A decoction of the shoots and leaves can be used as asperiants for children. The shoots and leaves can also be used for vertigo and biliousness[3].The leaf sap can be used as a wound dressing the sap can also be given to children for amoebiasis dried powdered leaves can be dusted over scabies. The plant can be regarded as a remedy for oka a disease for childrens head. Perspiration can be cured when the seeds are chewed or taken as a decoction .The fruit is used as a vermifuge. The root can be chewed for relieving facial neuralgia, toothache, etc, and, in decoction, for flatulence[4]. The fruit can be used as poison antidotes. The leaves can be used for treating mental troubles. The fruits have been used for the treatment of dysuria, piles, polyuria and tuberculosis. The root and leaf have been used for treating fever, dysphoea, abdominal disorders, cough and vomiting.the plant extract have shown to posses hepatoprotective and immunomodulatory , antihypertensive and antilipidemic activities.[5].In this study the hexane, ethyl acetate, methanol and water extracts of the plant mukia maderaspatana was taken and the extracts were subjected to phytochemical analysis antimicrobial analysis by agar disc diffusion method and antioxidant activity was also analysed.

MATERIALS AND METHODS

Collection of samples

Mukia maderaspatana was collected

Preparation of extracts

For the preparation of the extracts the full plants were taken and shade dried for two weeks. Then the plant was powdered in a crusher and 100gms of the powder was extracted with four solvents hexane, ethyl acetate ,methanol and water based on polarity using soxhlet extraction. The extracts were dried and stored for further use. The extraction was done for six hours.

Phytochemical Analysis

The plant extracts with the four solvents hexane, ethyl acetate, methanol and water were subjected to phytochemical analysis for the presence of alkaloids, flavonoids, carbohydrates, proteins, oils, saponin, tannins and phenolics by **simple qualitative and quantitative methods of Trease and Evans (1989) and Sofowora (1993).**

Antimicrobial activities

Strains of human pathogen microorganisms tested in this study were as follows two gram negative bacteria *Escherichia coli*, *Klebsiella pneumonia* and two gram positive bacteria *bacillus subtilis* and *staphylococcus aureus*. The microorganisms were obtained from MTCC Chandigarh.

Antimicrobial testing

Antimicrobial activity of the four extracts of hexane, ethyl acetate, methanol and water obtained from *Mukia maderaspatana* was

determined by the agar well diffusion method. Bacterial strains were cultured overnight at 37°C in nutrient broth before use.

Agar well diffusion method

Suspension of microorganisms 5 ml was used. The antimicrobial spectrum of the extracts was determined qualitatively for the bacterial species in terms of zones of inhibition sizes around wells cut in plates of Mueller Hinton agar. The agar was sterilized and poured in to sterile Petri dishes and then allowed to solidify. After solidification the microorganism cultures were swabbed in to the agar surface under aseptic conditions. All these experiments were performed in duplicates. The plates were incubated at 37 degree Celsius for 24 hrs. The strains were designated as sensitive or resistant and the zones were measured at the end of incubation time. An inhibition zone of 10mm or greater was considered to indicate good antibacterial activities.

Reducing power test for the antioxidant

In this method there is an increase in the absorbance of the reaction mixture .Increase in absorbance indicates increase in the antioxidant activity. 500 micro-liter of the reaction mixture was taken .To this 1.5ml of phosphate buffer was added. And 1.5ml of potassium ferricyanide was added in five falcon tubes containing concentrations from 20microgram to 100 microgram. The tubes were incubated at 50 degree Celsius for 20 minutes). To this 2.5ml of TCA was added and the tubes were centrifuged at 6000 rpm for 5 minutes. From this 1.5ml of supernatant was taken. To that added 1.5ml of De-ionized water and 200 micro-liter of $FeCl_3$ The absorbance value was noted at 700 nm The same protocol was repeated for all the extracts. The experiments were done in triplicates.

RESULTS AND DISCUSSION

Phytochemical analysis

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, saponins etc. The results obtained from this work revealed that the plant contains bioactive compounds which are connected with antimicrobial activity of plants. The successive extracts of the plant with hexane, ethyl acetate and methanol have revealed the presence of alkaloids, flavonoids, saponins, tannins and phenolics.

Alkaloid **Flavonoid** **Phenolics**

Fig. 1: Phytochemical analysis of *Mukia maderaspatana*

Table 1: Phytochemical analysis

	Hexane	Ethyl acetate	Methanol	Water
Carbohydrates	-	-	-	-
Proteins	-	-	-	-
Sapponins	-	+	+	+
Phenolics	-	++	+++	++
Tannins	-	+	++	+
Oils	-	-	-	-
Flavonoids	-	++	++	+
Alkaloids	-	++	++	+

(- = absent, + = weakly present, + + = moderately present, + + + = abundant)

Antimicrobial activity

Plant extracts have been used for many thousands of years as pharmaceutical, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Extracts are potential sources of antimicrobial compounds especially against bacterial pathogens. These studies showed that plant extracts inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many medicinal plant extracts have been previously reviewed and classified as strong, medium or weak

The maximum inhibition for all the three extracts ethyl acetate, methanol and water was against *Klebsiella pneumonia*. This was followed by *Staphylococcus aureus* and *Escherichia coli*. The least inhibition was found for *Bacillus subtilis*. The methanol extract showed maximum antimicrobial activity with zones of inhibition 25mm for *Klebsiella pneumoniae*.16mm for *Escherichia coli* and 20 mm for *Staphylococcus aureus*. This was followed by water extract which had zones of inhibition as follows 22 mm for *Klebsiella pneumonia* for *Escherichia coli* and *Staphylococcus aureus* it was almost the same which is 20mm and for *Bacillus subtilis* it is 12mm. Finally the ethyl acetate shows a zone of inhibition around 20mm for *Klebsiella pneumonia* 19mm for *Staphylococcus aureus* and 15mm for *Escherichia coli*. The hexane extract did not show any antimicrobial activity. Along with these the control plates were taken which showed negative results.

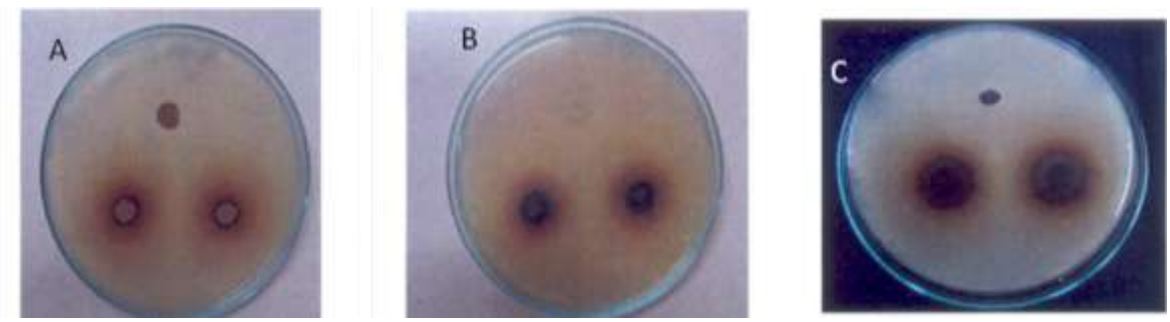

Fig. 2: Antibacterial activity of methanol extract of mukia maderaspatana A) *Escherichia coli* B) *Staphylococcus aureus C))Klebsiella pneumonia*

Hence we conclude that this plant extracts has great posses antimicrobial compounds .and it is most effective against *Klebsiella pneumonia*. It can be used in the treatment of diseases caused by this pathogen by the discovery of drugs against this pathogen.

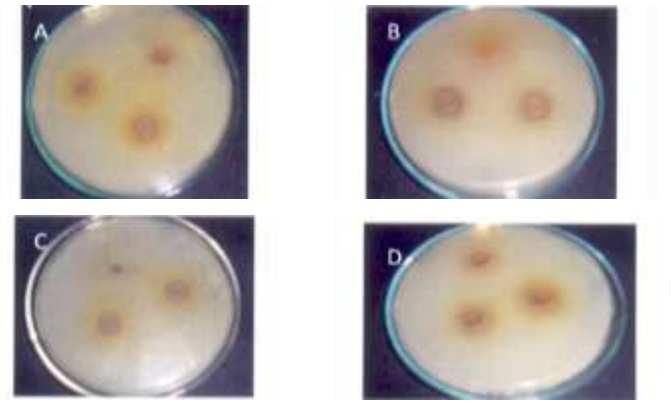

Fig. 3: Antibacterial Activity of Water extract of *Mukia maderaspatana A*) *Escherichia coli* B) *Staphylococcus aureus* C) *Bacillus subtilis* D) *Klebsiella Pneumonia*

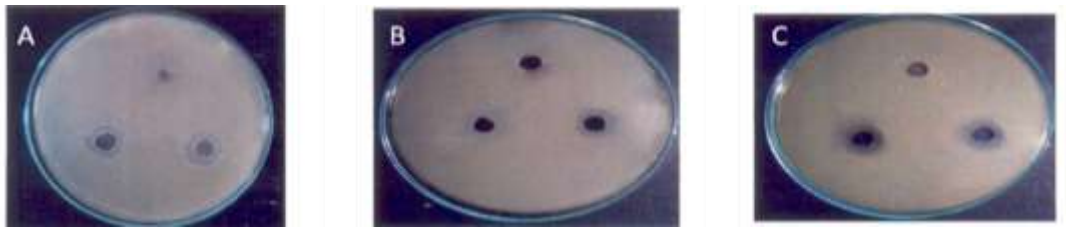

Fig. 4: Antibacterial activity of Ethyl Acetate extracts of *Mukia maderaspatana* maderaspatana A) *Escherichia coli* B) *Staphylococcus aureus C))Klebsiella pneumonia*

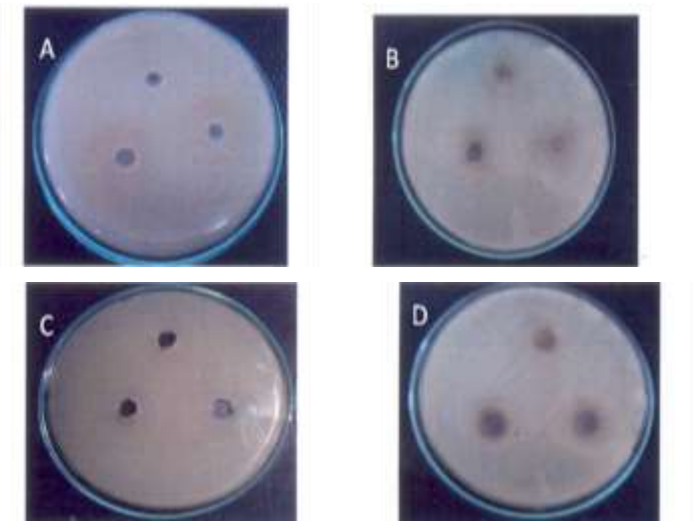

Fig. 5: Control Plates for four microorganisms without zone of inhibition *A*) *Escherichia coli* B) *Staphylococcus aureus* C) *Bacillus subtilis* D) *Klebsiella Pneumonia*

Antioxidant Activity

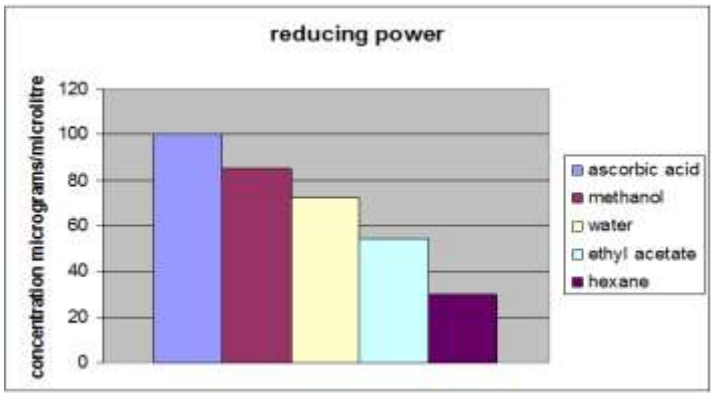

Fig. 6: Reducing Power Assay of *Mukia maderaspatana*

From the values it can be concluded that the ascorbic acid equivalent concentration of the plant extracts were maximum for methanol this is followed by water, ethyl acetate and finally hexane. The absorbance was found to increase with increasing concentration. The ascorbic acid concentration equivalents were as follows 85µg for methanol 72µg for water 54µg for ethyl acetate and 30µg for hexane. Hence the maximum reducing power is for methanol followed by water, ethyl acetate and the least is for hexane

CONCLUSION

Results from this study showed that the extracts of the medicinal plant *Mukia maderaspatana* contains phytochemicals such as flavonoids, alkaloid, phenolics and saponin which are responsible for the antimicrobial properties of the plant. The methanolic extract of the plant showed maximum antimicrobial activity against gram negative *Klebsiella* pneumonia, *Escherichia coli* and gram positive *Staphylococcus aureus* followed by water and ethyl acetate extract. The hexane extract showed no antimicrobial activity. *Klebsiella pneumonia* was found to be the most sensitive to all the extracts. *Escherichia coli* and *Staphylococcus aureus* showed moderate sensitivity. The sensitivity found for Bacillus subtilis was the least.The reducing power of the extracts was also determined. Methanol extract had maximum reducing power followed by water and ethyl acetate and hexane extract showed least reducing power. It can we concluded that the methanol extract is a good source of antioxidant.

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