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IJPBS |Volume 3| Issue 1 |JAN-MAR |2013|41-45

Research Article
Pharmaceutical Sciences

EVALUATION OF ANTIMICROBIAL ACTIVITY OF PLANT LEAF ARGEMONE MEXICANA

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ABSTRACT

Increasing prevalence of multidrug resistance strains of micro-organism has initiated the exploration of alternate antimicrobial agent. Taking in account the medicinal important of Argemone mexicana (leaf) in this respect an attempt was made in the current study to investigate the antimicrobial potential of this plant. Authenticated fresh plant leaf were selected for determination of antimicrobial activity against eleven clinical isolates of Gram +ve (2), Gram –ve (4) and fungi (5).The methanol extracts of Argemone mexicana leaf were screened in vitro for antibacterial activity by well diffusion method it doesn't show any antibacterial action against bacteria, but it quite sensitive to fungi. It shows remarkable action against Candida albicans, Aspergilus niger 24 mm and 22 mm respectively which was moderately to flucanazole. The MIC value 3.12mg/ml. Thus the current investigation leads to fresh source of new antimicrobial in future. The results suggest that argemone mexicana is a potential candidate plant for future exploitation in medical microbiology.

KEY WORDS

Argemone Mexicana, Candida albicans, Aspergilus niger

INTRODUCTION

Argemone mexicana L (Papaveraceae) is an herb with branches, which has naturalized widely in many tropical and subtropical regions although it's a native of tropical American[1]. It grows commonly in abandoned and cultivated fields of South-West, Nigeria where it is renowned for its high medicinal properties. *A.Mexicana L.* is known by many names in Nigeria, it is called "Kaju" in Yoruba, "Ahon ekun" in Ijebu land, "Kadinnia" among the Hausas It is an herb with bright yellow flowers and yellow juice. *A. mexicana's* concoction from its ethnological survey in Nigeria is used in treatment of bacterial infection. It is widely believed that the latex from this plant cures cataract, reddening and itching in the eyes. Traditional healers in Mali use *A. mexicana* to treat Malaria[2] Ayurveda reported that the plant is purgative, diuretic and destroys worms. It cures skin-diseases, leprosy and inflammation bilious fevers. Roots are equally used to cure anthelmintic. Juice is used to cure opacity of cornea and ophthalmia. Seeds are purgative and sedative. In Mexico the seed is used as an antidote to snake poisoning and the fresh yellow milky seed extract contains protein-dissolving substances, effective in the treatment of warts, coldsores, cutaneous infections, skin diseases, itches and also dropsy and jaundice [3]. The present study was to screen the leaf of the plant, *A. mexicana*, against some selected bacteria and fungi often implicated in nosocomial and community infection [4, 5, 6].

Even today plants are the almost exclusive source of drugs for the majority of the world population. People in developing countries

utilize traditional medicine for their primary health care needs[7, 8]. The potential of higher plants as a source for new drugs is thus still largely unexplored[9]. This is also true in India and only a small percentage of plants of this region been evaluated for antibacterial activity against human pathogens [10, 11]. Thus considering the vast potentiality of plant as a source of new therapeutic agents, hence detail investigations were conducted to test the efficacy of some plant extract against important human pathogenic bacteria.

MATERIALS AND METHODS

Plant Materials:

Authenticated Fresh plant materials *Argemone mexicana* (Papaveraceae) (Leaf) free from disease were collected from Vidarbha region of Maharastra, washed thoroughly 2-3 times with running tap water and once with sterile water, shade-dried, powdered and used for extraction.

Preparation of Solvent Extract:

Sample (250 gm) of the shade-dried powder of *Argemone mexicana* was extracted in a Soxhlet extractor successively with 1 L Petroleum ether, Chloroform, and Methanol until colourless extract was obtained on the top of the extractor. Each of the solvent extract was concentrated separately under reduced pressure. After complete solvent evaporation, each of these solvent extracts was weighed and subjected to antimicrobial activity assay. For only methanol extract, which recorded highest antibacterial activity, the minimal inhibitory concentration (MIC) was determined[12, 13.]

Antimicrobial assays

The methods of Hufford et al. (1975) were used with some modification. Agar-well diffusion assay was used to evaluate the antimicrobial activities of the leaf extract. Mueller-Hinton agar (Scharlau Chemie) was used for the culturing of bacteria while Sabouraud Dextrose agar (Difco) was used for the fungi. Twenty milliliters of the specified molten agar (45°C) was aseptically mixed with 1 ml of bacterial suspension (3 × 108 CFU/ml) and poured into sterile Petri dishes. Once the agar has hardened, 6mm wells were bored using a sterile cork borer. From the various concentrations of 500, 1000 and 1500 µl/ml of the Leaf extracts, which was prepared using methanol as diluents, 0.1 ml of the extract was separately placed into each well. The plates were incubated for 24 h at 37°C for the bacteria and 24 - 72 h at room temperature for the moulds. Ampicillin (10 µg) serves as positive control for the bacteria species while Fluconazole serves as positive control for the Candida species. The antimicrobial activity was measured as the diameter (mm) of clear zone of growth inhibition. Methanol was included in every experiment as negative controls [14, 15].

Antibacterial Activity Assay:

Antibacterial activity was determined by cup diffusion method on MHA medium the sterile medium (20ml) was poured into a 9 cm petriplates. The medium was allowed to cool in a sterile condition and plates were then inoculated with cultures of test bacteria. Agar cup of 5 mm diameter were made in the plates with the help of sterile borers. The desired different concentrations of the extracts, fractions and pure compounds were prepared by first reconstituting in methanol then diluting in sterile distilled water. A 100µl volume of each dilution was introduced in triplicate wells into MHA plates already seeded with the standardized inoculums of the test bacterial cells. All test plates were incubated at 37°C for 24h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC. Negative controls were prepared using the same solvent employed to dissolve the extracts. Gentamicin and Streptomycin were used as

positive reference to determine the sensitivity of each bacterial species tested [16].

Antifungal Activity Assay:

To evaluate the antifungal activity, sterile agar plates were used according to the disc diffusion assay. Activated cultures of fungal strains in Sabouraud's broth.100 µl of the inocullum was introduced to molten Sabouraud dextrose agar and poured in the sterile Petri plates. Sterile filter paper discs (7.0 mm diameter) were impregnated with 500 µg/disc, 250 µg/disc and 125 µg/disc of the plants extracts dissolved in 100% DMSO (dimethylsulphoxide) and dried. The discs were placed on fungal seeded plates incubated at 28°C for 48hrs. Disc impregnated with only 100% DMSO served as the negative control. As a positive control, Flucanazole.The (10 µg/disc) was used. Following an incubation period of 48hrs, plates were removed from the incubator and antifungal activity was evaluated by measuring zones of inhibition of fungal growth.

RESULT AND DISCUSSION

In the initial stages the Methanolic extracts of plant *Argemone mexicana* leaf was evaluated by antibacterial activity against Gram positive such as *Bacillus subtilis, Staphylococus aureus* and negative bacteria such as *E.coli, Pseudomonas aereaginosa, Salmonella typhi, Proteus vulgaris* but it doesn't shown inhibitory action. This study revealed that the extract of plant Argemone mexicana leaf has a poor antibacterial action. And then this extract evaluated by antifungal activity against human pathogenic yeast strain of *Candida albicans, Candida tropicalis, Aspergilus niger, Aspergilus flavus, Aspergilus candidus.* The antifungal activity of Methanolic extracts of the plant *Argemone mexicana* leaf against fungal strain was shown on **Table No.01** from this table it is revealed that the methanol extracts of plant leaf *Argemone mexicana* having the more potent activity against Candida albicans as compared to other yeast strain but it is moderate to flucanazole.The. And the Minimum inhibitory concentrations of plant leaf extract were shown on the **Table No.02**. From this table it was found that the lowest MIC value 3.12mg/ml for methanol extract against the Candida albicans as compared to other fungal strain.

Table No.1: Antifungal activity of Methanolic extracts against different fungal strains

S.No	Organisms	Methanol extracts	Fluconazole
01	*Candida albicans*	24 mm	26 mm
02	*Candida tropicalis*	20 mm	23 mm
03	*Aspergilus niger*	22 mm	24 mm
04	*Aspergilus flavus*	18 mm	24 mm
05	*Aspergilus candidus*	18 mm	24 mm

Table No.2: Minimum inhibitory concentration of extracts against different fungal strain

Solvent Extract	Candida albicans	Candida tropicalis	Aspergilus niger	Aspergilus flavus	Aspergilus candidus
Methanol	3.12mg/ml	6.25mg/ml	6.25mg/ml	6.25mg/ml	12.5mg/ml

CONCLUSION

In vitro evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing eco-friendly management of infectious diseases of humans by search for new bio-molecules of plant origin. Considering these, plant Argemone mexicana, screened in vitro for antibacterial as well as antifungal activity against eleven human pathogenic bacteria and yeast strain known to cause diseases in humans. The plant was selected based on traditional medicine knowledge. On the basis of zone of inhibition, the result of the present investigation revealed that the leaf of the plant Argemone mexicana leaf has a potential source of antifungal action and its activity against various clinical isolates may be sufficient to perform further studies for isolation and identification for active principles.

ACKNOWLEDGEMENTS

The authors are thankful to head Manoharbhai Patel Institute of Pharmacy Gondia M.S.India for providing laboratory facilities.

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