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Research Article

EVALUATION OF ANTIFUNGAL ACTIVITY OF SELECTED MEDICINAL PLANTS

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ABSTRACT

Plants have been used from thousands of years to treat different health disease. The development of new antimicrobial agents against resistant pathogens is increasing interest. *Emblica officinalis* Gaertn., *Mentha piperita* L. and *Abrus precatorius* L. are traditionally used medicinal plants found in tropical and subtropical regions of India. Therefore, different extracts from leaf extracts of these medicinal plants used locally in folk medicine were evaluated for antifungal activity. In the present study, evaluation of antifungal activity of the leaf extracts of *Emblica officinalis* Gaertn., *Mentha piperita* L. and *Abrus precatorius* L. against the fungus *Fusarium oxysporum* and *Aspergillus niger*. The antifungal tests were performed using Agar well diffusion method. The organic (methanol, chloroform and acetone) extracts of the leaves were found to possess strong antifungal activity against two pathogenic fungi. Methanolic extracts shows maximum antifungal properties than chloroform and acetone extracts. This study is an indication that *Emblica officinalis* Gaertn., *Mentha piperita* L. and *Abrus precatorius* L. has the potential to be used as a source for new broad spectrum oral antibiotics.

Keywords: Antifungal, Medicinal plants, Leaf extracts, Agar well diffusion method.

INTRODUCTION

Medicinal plants are great source of antimicrobial agents and its uses are becoming popular due its lesser side effects and are easily available to mankind. Since primeval period, traditional herbs are used in the treatment of various diseases[1]. Large variety of secondary metabolites is unique feature of most of the higher plant kingdoms which possess great amount of antimicrobial properties for example tannins, alkaloids, terpenoids and flavonoids. Thus, plants provide natural remedies and offer new agents for antimicrobial usage[2]. The knowledge about the chemical constituents which plays the main role in treating many diseases is very important and helps to produce many important pharmaceutical products. Once the chemical components or the metabolites are separated or isolated, many tests are carried for their characterization like specific color tests, physical constants and hydrolysis of compounds[3]. Their structures are confirmed by spectroscopy methods, it is one of the helpful techniques to determine the structure of phytochemical compounds[4].Thus, these types of findings are necessary to boast up the use of medicinal plants, extracts or natural products, either used as alone, together or with any kind of antibiotics[5].

The aim and main focus of this study was to investigate the antifungal activities of the plant extracts of *Emblica officinalis* Gaertn., *Mentha piperita* L. and *Abrus precatorius* L. which possess high medicinal properties. A natural blue print can be created for the development of novel drugs or a development of the Phytomedicines which are used to treat various diseases. Two standard strains of fungi *Fusarium oxysporum* and *Aspergillus niger* with their respective clinical isolates were collected as test microorganisms for the evaluation of antimicrobial activity of the plant extracts. This study will serve as the pilot or basic experiment for large scale production of the secondary metabolite or extracts or to scale up the processes.

MATERIALS AND METHODS

Plant collection

Leaves of *Emblica officinalis* Gaertn., *Mentha piperita* L. and *Abrus precatorius* L. were collected from the different places of Ahmedabad, Gujarat, India. The Plant specimens were identified with help of flora[6]. The following microorganisms were used *Aspergillus niger* (locally isolated organism, obtained from the Department of Microbiology, USS, Gujarat University), *Fusarium oxysporum* (locally isolated organism, obtained from the Department of Microbiology, USS, Gujarat University).

Preparation of extract

Fresh and healthy leaves were collected and washed with tap water and then rinsed with distil water. Dried material was cut into the small pieces and grounded mechanically or pulverized in the electronic blender. Powdered plant materials were used for the preparation of solvent extracts. Dried powdered plant material was weighed 10g and extracted with 100ml of solvents i.e. Methanol, Acetone and Chloroform. They are kept in rotary shaker at 110rpm, for 24 hours. The extracts were filtered with Whatman No 1 filter paper and filtrates were concentrated. Concentrated filtrates are poured into the Petri dishes for evaporation and further uses[7].

Preparation of extracts at different concentrations

5mg of plant extracts were dissolved in 5ml of respective solvents. For different concentrations were prepared i.e. 5µg/ml,

10µg/ml, 15µg/ml and 20µg/ml. Extracts were prepared in highly aseptic conditions. 2ml of DMSO is used for the preparation of stock solutions for control.

Agar well assay method

The culture media used is for this study, is general purpose media known as potato dextrose agar. The media preparation was done according to the manufacturer's instruction. They are sterilized by using autoclave at 121°C at 15 psi for almost half an hour. Media was prepared using pure distil water. The media was immediately poured in the Petri dishes. After pouring, they are allowed to solidify for 5-6 hours. Care should be taken of proper homogenization. The experiment was done under strict aseptic conditions. The Fungus were spread around the whole Petri dish by swab or sterile glass rods. After that, the wells were prepared by using sterile cork borer (6mm). The stock solution which is prepared by the different extracts concentrations almost about 100µl were poured into wells. The plates were incubated under optimum growth conditions of various organisms. Inhibition zone were measured with zone scale of 1 mm or more was considered positive inhibition. All the samples are tested in triplicates[8].

Statistical analysis

The observation or results which are recorded from the study were subjected to the average and standard error. All experiments were repeated at least three times. Results are reported as Mean ± SEM (Standard Error of Mean).

Table: 1 Antifungal activity of *Mentha piperita* against *Fusarium oxysporum*

Extracts	Extract concentration (µg/ml)				Control (DMSO)
	5µg	10 µg	15 µg	20 µg	
Methanol	2 ± 0.288	2 ± 0.288	3.12 ± 0.167	4.83 ± 0.167	11.32
Acetone	1.5 ± 0	2.33 ±0.166	3.5 ± 0.44	3.56 ± 0.288	5.7
Chloroform	1.0 ± 0	1.84 ± 0.93	2 ± 0.5	3.66 ± 0.44	4.87

Note- Zone of Inhibition is expressed as mean ± Standard error of three replicates.

Table: 2 Antifungal activities of *Mentha piperita* against *Aspergillus niger*

Extracts	Extract concentration (µg/ml)				Control (DMSO)
	5µg	10 µg	15 µg	20 µg	
Methanol	1.5 ± 0.29	1.67 ± 0.45	3 ± 0.5	3.9 ± 0.34	9.2
Acetone	1.6 ± 0.17	1.6 ± 0.17	1.7 ± 0.45	3.17 ± 0.34	4.7
Chloroform	-	0.6 ± 0.67	0.84 ± 0.84	1 ± 0.16	4.37

Table: 3 Antifungal activities of *Abrus precatorius* against *Fusarium oxysporum*

Extracts	Extract concentration (µg/ml)				Control (DMSO)
	5µg	10 µg	15 µg	20 µg	
Methanol	2.33 ± 0.166	3.16 ± 0.166	4.34 ± 0.166	5 ± 0.29	7.65
Acetone	2.5 ± 0.5	4 ± 0.288	4.17 ± 0.17	4.27 ± 0.73	5.6
Chloroform	0	0.83 ±0.45	1.5± 0.77	2.84 ± 0.45	5.45

Table: 4 Antifungal activities of *Abrus precatorius* against *Aspergillus niger*

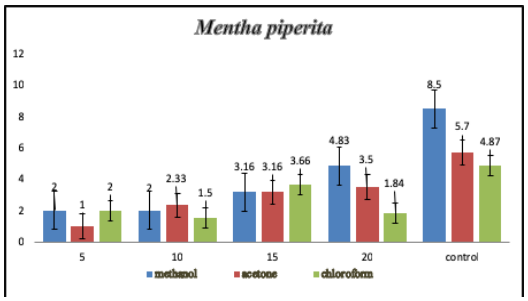
Extracts	Extract concentration (µg/ml)				Control (DMSO)
	5µg	10 µg	15 µg	20 µg	
Methanol	1.34 ± 1.7	2.5 ± 0.289	3.17 ± 0.34	3.9 ± 4.5	8.35
Acetone	1.5 ± 0.29	1.6 ± 0.17	2.17 ± 0.34	3.1 ± 1.74	4.7
Chloroform	0	0.3 ± 0.34	1.34 ± 0.17	1.9 ± 0.46	4.35

Table: 5 Antifungal activities of *Emblica officinalis* against *Fusarium oxysporum*

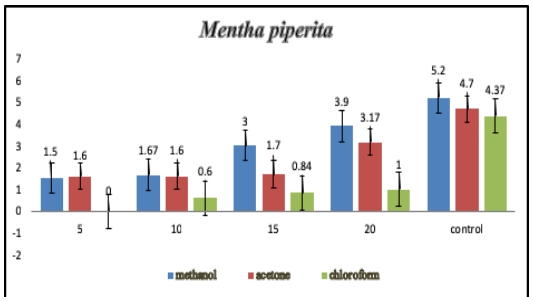
Extracts	Extract concentration (µg/ml)				Control (DMSO)
	5µg	10 µg	15 µg	20 µg	
Methanol	1.5 ± 0.5	2.5 ± 0.29	3.5 ± 0.29	4.7 ± 0.34	6.2
Acetone	1.4 ± 0.34	2.6 ± 0.34	2.5 ±0.298	2.9 ± 0.17	4.3
Chloroform	0	0.34 ± 0.35	1.5 ± 0.29	2.45± 0.0088	4.85

Table: 6 Antifungal activities of *Emblica officinalis* against *Aspergillus niger*

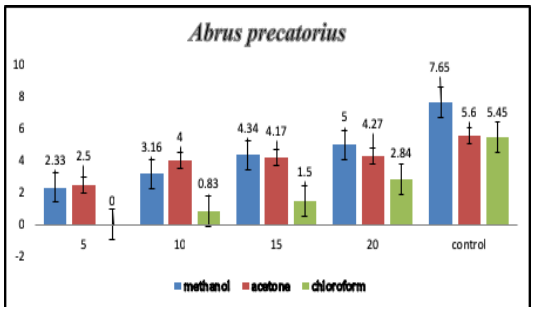
Extracts	Extract concentration (µg/ml)				Control (DMSO)
	5µg	10 µg	15 µg	20 µg	
Methanol	1.34 ± 0.17	2.5 ± 0.29	2.7 ± 3.4	3.7 ± 0.45	6.7
Acetone	1.2 ± 0.45	1.2 ± 0.17	2.5 ± 0.29	3.9 ± 0.34	4.9
Chloroform	-	1 ± 0	1.4 ± 0.17	2 ± 0.29	4.4



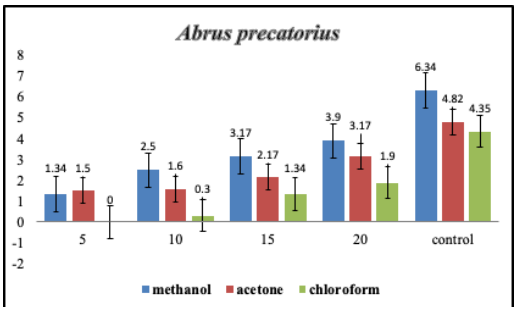
Graph: 1 Antifungal activities of *Mentha piperita* against *Fusarium oxysporum* expressed as fungal diameter in mm.



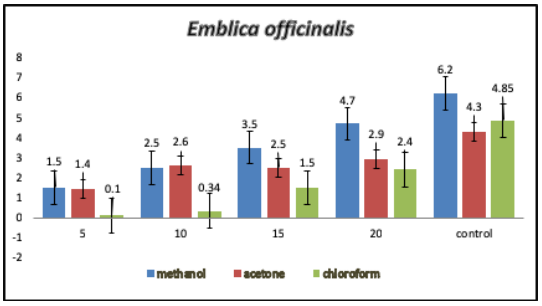
Graph: 2 Antifungal activity of *Mentha piperita* against *Aspergillus niger* expressed as fungal diameter in mm.



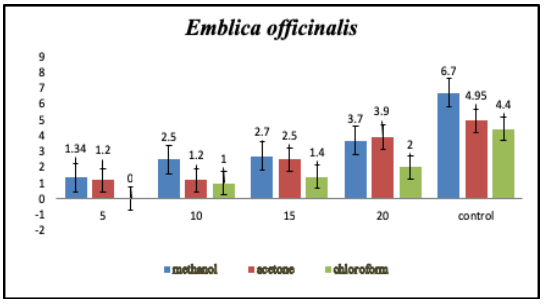
Graph: 3 Antifungal activities of *Abrus precatorius* against *Fusaraium oxysporum* expressed as fungal diameter in mm.



Graph: 4 Antifungal activities of *Abrus precatorius* against *Aspergillus niger* expressed as fungal diameter in mm.



Graph: 5 Antifungal activities of *Emblica officinalis* against *Fusarium oxysporum* expressed as fungal diameter in mm.



Graph: 6 Antifungal activities of *Emblica officinalis* against *Aspergillus niger* expressed as fungal diameter in mm.

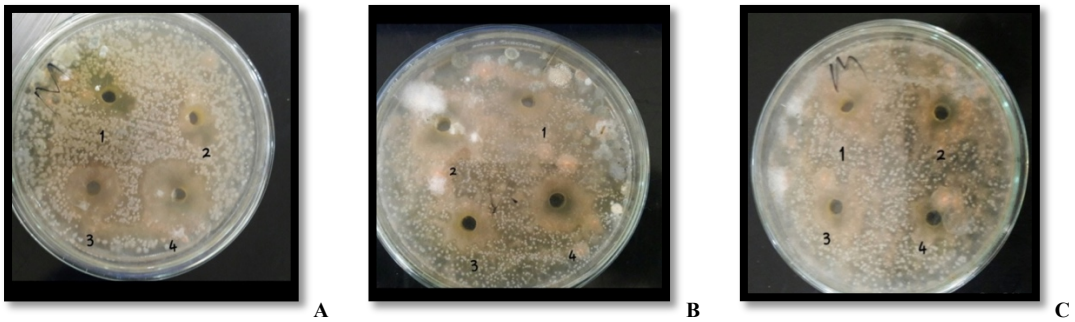


Figure 1: Zone of inhibition of *Fusarium oxysporum* (1)5µg (2)10µg (3)15µg (4)20µg/ml concentrations of *Mentha piperita* of different extracts i.e. (A) Methanol (B) Acetone (C) Chloroform

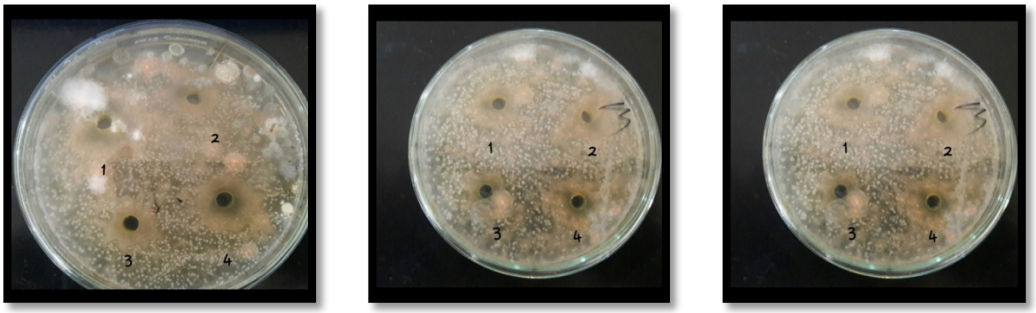


Figure 2: Zone of inhibition of *Aspergillus niger* (1)5µg (2)10µg (3)15µg (4)20µg/ml concentrations of *Mentha piperita* of different extracts i.e. (A) Methanol (B) Acetone (C) Chloroform

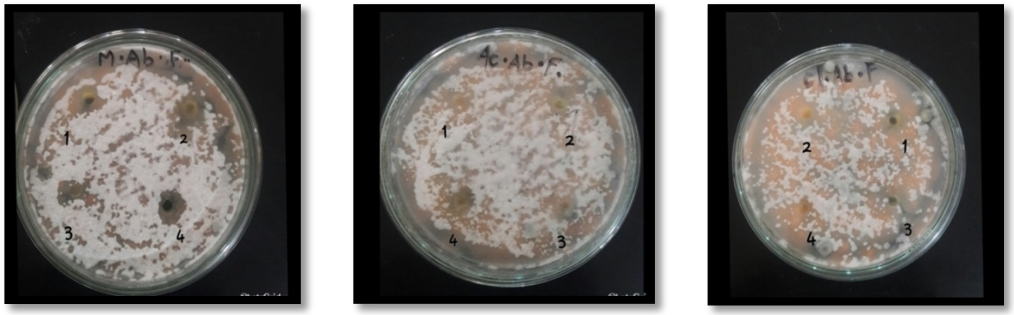


Figure 3: Zone of inhibition of *Fusarium oxysporum* (1)5µg (2)10µg (3)15µg (4)20µg/ml concentrations of *Abrus precatorius* of different extracts i.e. (A) Methanol (B) Acetone (C) Chloroform

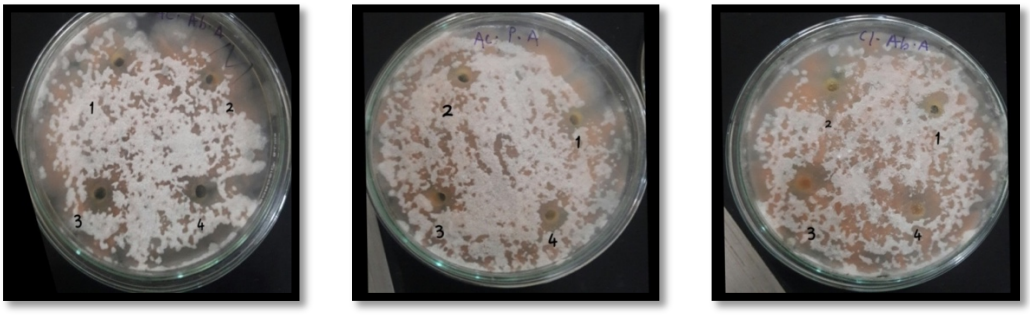


Figure 4: Zone of inhibition *Aspergillus niger* (1)5µg (2)10µg (3)15µg (4)20µg/ml concentrations of *Abrus precatorius* of different extracts i.e. (A) Methanol (B) Acetone (C) Chloroform

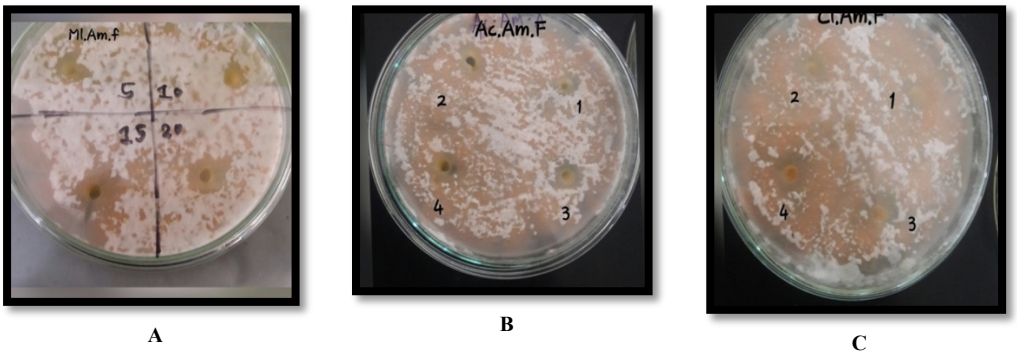
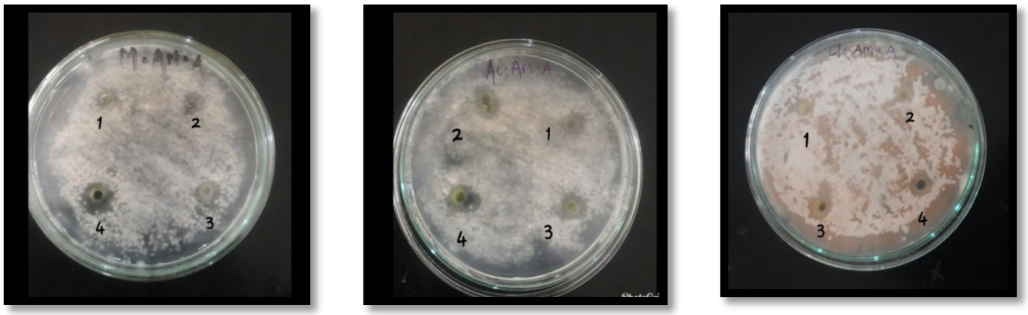


Figure 5: Zone of inhibition of*Fusarium oxysporum* (1)5µg (2) 10µg (3)15µg (4)20µg/ml concentrations of *Emblica officinalis* of different extracts i.e. (A) Methanol (B) Acetone (C) Chloroform



A B C

Figure 6:Zone of inhibition of *Aspergillus niger* (1)5µg (2)10µg (3)15µg (4)20µg/ml concentrations of *Emblica officinalis* of different extracts i.e. (A) Methanol (B) Acetone (C) Chloroform

RESULTS AND DISCUSSION

In the present study results showed, in *Mentha piperita* against *Fusarium oxysporum* zone of inhibition was seen maximum in 20µg (4.83 ± 0.167) in methanolic extract and minimum in 5µg (1.0 ± 0) in chloroform extract (Table 1, Graph 1 and Fig. 1). In *Mentha piperita* against *Aspergillus niger* the zone of inhibition maximum in 20µg (3.17 ± 0.34) in acetone extract and minimum in 10µg (0.6 ± 0.67) in chloroform extract (Table 2, Graph 2 and Fig. 2). In *Abrus precatorius* against *Fusarium oxysporum* zone of inhibition was seen maximum in 20µg (5 ± 0.29) in methanol extract and minimum in 10µg (0.83 ±0.45) in chloroform extract (Table 3, Graph 3 and Fig. 3). In *Abrus precatorius* against *Aspergillus niger* zone of inhibition was seen maximum in 20µg (3.9 ± 4.5) in methanol extract and minimum in 10µg (0.3 ± 0.34) in chloroform extract (Table 4, Graph 4 and Fig. 4). In *Emblica officinalis* against *Fusarium oxysporum* zone of inhibition was seen maximum in 20µg (4.7 ± 0.34) in methanol extract and minimum in 5µg (0.1 ± 0.17) in chloroform extract (Table 5, Graph 5 and Fig. 5) and in *Emblica officinalis* against *Aspergillus niger* zone of inhibition was seen maximum in 20µg (3.9 ± 0.34) in acetone extract and minimum in 10µg (1 ± 0) in chloroform extract (Table 6, Graph 6 and Fig. 6).

CONCLUSION

From the above study, the antifungal activity of the plant *Mentha piperita* with 4.83 ± 0.167 against fungus *Fusarium oxysporum* is the most effective. The extracts with solvent methanol showed antifungal activity better than chloroform and acetone. Chloroform showed the least inhibitory activities, which may be due to the poor solubility of the secondary metabolites in the solvent chloroform. Further isolation of compounds holds the great scope and future for the pharmacological industries.

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