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Evaluation of antifungal activity of *Emblica officinalis*, *Aloe vera* and *Vitex negundo* extracts

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Abstract: *Emblica officinalis* Gaertn., *Aloe vera* L. and *Vitex negundo* L. are medicinal plants commonly found throughout India and used in traditional medicine. The present study was undertaken to screen potential antifungal activity of extracts of *Emblica officinalis* Gaertn. fruits, *Aloe vera* L. leaves and *Vitex negundo* L. leaves. The plant extracts were prepared by sequential cold maceration method using hexane, ethyl acetate, methanol and distilled water as a solvent. Extracts were evaluated for their antifungal activity against *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Penicillium chrysogenum* and *Trichoderma viridae* by using agar well diffusion method. All the plants showed maximum antifungal activity against *Trichoderma viridae.* While *Penicillium chrysogenum* was most resistant fungal strain against plant extracts used in the study. Aqueous extracts of all the plants showed maximum inhibitory action as compared to other extracts. Presence of various phytochemicals in the extract will lead to contribution in the antifungal activity. The knowledge of extent and mode of action for antifungal activity of specific compounds, present in the plant extracts, may lead to the successful utilization of such natural compounds for treatment of infections caused by pathogenic fungi.

Keywords: Antifungal activity, Medicinal plants, Phytochemicals, *Emblica officinalis*, *Aloe vera* and *Vitex negundo*.

INTRODUCTION

Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to the report of World Health Organization, more than 80% of world's populations depend on traditional medicine derived from plants for their primary health care needs[1]. The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. At present, nearly 30% or more of the pharmacological drugs are derived directly or indirectly from plants and their extracts dominating in traditional medicine systems and a common element in Ayurveda, Homeopathic and Naturopathic etc.[2]. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment[3].

Emblica officinalis Gaertn. (Synonym *Phyllanthus emblica* Linn. commonly known as Amla or Indian gooseberry) commonly found throughout India is a small and medium sized deciduous tree[4,5]. Dried fruits of amla are used in the treatment of haemorrhage, diarrhoea and dysentery in Unani system of medicine[6]. *Vitex negundo* L. is most commonly distributed in India is traditionally known as Nagol or nirgundi[7]. The genus *Vitex* belongs to the family Verbenaceae and comprises of large shrubs or small trees. It is a very common aromatic plant and is used in medicine[8]. Though, almost all parts of *V. negundo* are used, the leaves and the barks are the most important in the field of medicine[9]. *Aloe vera* L. (family: Liliaceae) is a semi tropical plant that has been used by herbalists for the treatment of different human diseases[10]. The *A. vera* plant is made up of fibrous roots, short stem and a spiral greenish leaves. The leaf is made of a gel, which is colourless, viscous liquid consisting primarily of water and ploysaccharides and has a bitter taste.

Considering the vast potentiality of these three plants as sources of phytomedicines with regard to antimicrobial drugs, a systematic investigation was undertaken to screen the potential antifungal activity of fruits of *Emblica officinalis* Gaertn., leaves of *Aloe vera* L. and leaves of *Vitex negundo* L.

EXPERIMENTAL

Collection of the plant samples: Fresh stem of *Emblica officinalis*, *Aloe vera* and *Vitex negundo* were collected from botanical garden of G. J. Patel Institute of Ayurvedic Studies & Research, New Vallabh Vidyanagar, Anand, Gujarat, India. The taxonomic identification of plants was confirmed by the taxonomist. Fresh plant material were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles at 4°C.

Preparation of plant extracts: The plant extracts were prepared by proceeding with sequential cold maceration method using hexane, ethyl acetate, methanol and distilled water as a solvent[11]. 50 gm of dried powder of plant material was soaked in 250 ml hexane for 24 hr at room temperature under shaking condition at 100 rpm. This solution was filtered with the help of whatman No. 1 filter paper. The filtrate was collected in 15 cm petri dishes and evaporated the solvent at room temperature. The solid dried extract was stored in 2 ml eppendorf tube and powder was used for antimicrobial assays after dilution. The filter cake was dried at room temperature and stored separately. The dried powder of filter cake was sequentially resuspended in 250 ml ethyl acetate, methanol and distilled water to prepare dried extract in each solvent. After extraction in each solvent remained filter cake was dried and further used with next solvent for extraction. All the dried extracts were stored at 4°C.

Preparation of sample for antifungal activity: Samples for antifungal activity were prepared by dissolving 100 mg of each extracts in 1 ml of dimethyl sulphoxide (DMSO).

Microorganisms used in the study: All test microorganisms were obtained from Department of Microbiology, ARIBAS, New V. V. Nagar, Gujarat, India. Tested fungal strains including *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Penicillium chrysogenum* and *Trichoderma viridae* were maintained at 4°C on potato dextrose agar (PDA) slants.

Antifungal activity using agar well diffusion method: For the antifungal activity, the stock cultures of fungi were revived by inoculating in broth media and grown at 27°C for 72 hr. The agar plates of the Potato Dextrose Agar media were prepared. Each plate was inoculated with an aliquot (0.1 ml) of the fungal suspension (10^3 spores/ml) which was spread evenly on the plate. After 20 min, the wells with 6 mm diameter were made by using sterile cork borer and filled with test samples of 50 mg/ml and 100 mg/ml concentration. The positive and negative control plates with Fluconazole (10 mcg/disc) (standard drug) and DMSO respectively were also prepared. All the plates were incubated at 27°C for 5-7 days and then the diameter of zone of inhibition was noted. Triplicates were carried out for each extract against each of the test organism[12].

Qualitative phytochemical analysis of plant extracts: The extracts were tested for the presence of alkaloids, tannins, saponins, cardiac glycosides, steroids, phenols and flavonoids according to standard protocols for detecting the presence of different chemical constitutes in the plant extracts[13].

Statistical analysis: Mean value and standard deviation were calculated for each test bacteria, fungi and fungi. Data were analyzed by one-way ANOVA and p values were considered significant at $p > 0.005$[14].

RESULTS AND DISCUSSION

Antifungal activity of hexane, ethyl acetate, methanol and aqueous extracts of three medicinal plants viz., *Emblica officinalis, Aloe vera* and *Vitex negundo* was evaluated *in vitro* using agar well diffusion method. As result of antifungal activity is shown in table 1 indicated that all the plants showed maximum antifungal activity against *Trichoderma viridae.*

Results of the study showed that ethyl acetate extract of *Emblica officinalis* was able to give maximum zone of inhibition against *Trichoderma viridae* followed by methanol and aqueous extracts at 100 mg/ml. Hexane extract of *Emblica officinalis* slightly inhibited *A. niger* (ZOI = 9.5±0.5). Other extracts of *Emblica officinalis* were failed to show any inhibitory effect against any of fungi. As per previous study, the extract of *E. officinalis* fruits was proven to be having significant antifungal activity against *C. albicans* and *A. niger*[15].

In the present study, aqueous extract of *Aloe vera* showed inhibition against *Aspergillus niger* (ZOI = 9.6±0.57), *Aspergillus flavus* (ZOI = 8.5±0.5) and *Trichoderma viridae* (ZOI = 9.5±0.5). Methanol extract of *Aloe vera* was able to show antifungal activity only against *A. oryzae* (ZOI = 8.3±0.57). Other extracts of *Aloe vera* like hexane and ethyl acetate were failed to show inhibitory activity against any of the fungi used in the study. One of the previous studies indicated that ethanol extract of *A. vera* leaf have antifungal activity against *C. albicans*[16]. In another study it was proven that *A. vera* juice was effective agent to inhibit the growth of *C. albicans* in disc diffusion assay[17].

In case of *Vitex negundo,* maximum antifungal activity was observed by aqueous extract against *Trichoderma viridae* (ZOI = 19.6±1.52) by 100 mg/ml followed by *Aspergillus niger* (ZOI = 10.5±0.5), *Aspergillus flavus* (ZOI = 9.6±0.57) and *Aspergillus oryzae* (ZOI = 9.5±0.5) by the same extract. Hexane extract of *V. negundo* slightly inhibited *Trichoderma viridae* (ZOI = 7.6±0.57). All the extracts used in the study failed to inhibit growth of white rot fungus *Penicillium chrysogenum.* Most susceptible fungus was *Trichoderma viridae,* which was inhibited by various extracts of all three plants used in this

study. All the *Aspergillus* species used in the study were slightly inhibited by some extracts. In previous study, crude (EtOH) extract of *V. negundo* fruits showed excellent antifungal activity against *Fusarium solani* and moderate inhibitory activity against *Microsporum canis*. While the same extract was proven ineffective against *A. flavus* and *C. albicans*[18].

Table 1: Antifungal activity (zone of inhibition in mm) of different extracts of *E. officinalis* fruits, *Aloe vera* leaves and *Vitex negundo* leaves

Name of Plant	Plant part used	Extract	Concentration (mg/ml)	Zone of inhibition (mm) (mean ± SD)				
				Aspergillus niger	***Aspergillus flavus***	***Aspergillus oryzae***	***Penicillium chrysogenum***	***Trichoderma viridae***
Emblica officinalis	Fruits	H	100	9.5±0.5	-	-	-	-
			50	-	-	-	-	-
		EA	100	-	-	-	-	11.0±0.5
			50	-	-	-	-	8.6±0.57
		M	100	-	-	-	-	9.6±0.57
			50	-	-	-	-	7.5±0.5
		DW	100	-	-	-	-	8.1±0.28
			50	-	-	-	-	-
Aloe vera	Leaves	H	100	-	-	-	-	-
			50	-	-	-	-	-
		EA	100	-	-	-	-	-
			50	-	-	-	-	-
		M	100	-	-	8.3±0.57	-	-
			50	-	-	-	-	-
		DW	100	9.6±0.57	8.5±0.5	-	-	9.5±0.5
			50	7.8±0.28	-	-	-	-
Vitex negundo	Leaves	H	100	-	-	-	-	7.6±0.57
			50	-	-	-	-	-
		EA	100	-	-	-	-	-
			50	-	-	-	-	-
		M	100	-	-	-	-	-
			50	-	-	-	-	-
		DW	100	10.5±0.5	9.6±0.57	9.5±0.5	-	19.6±1.52
			50	-	-	-	-	13.6±0.57
DMSO (-ve control)			-	-	-	-	-	-
Fluconazole (+ve control)			10 mcg/disc	15.33±1.15	20.0±1.0	16.3±0.57	10.7±0.57	21.0±1.0
H = Hexane, EA = Ethyl Acetate, M = Methanol, DW = Distilled water, (-) = No zone of inhibition								

The results of qualitative analysis of different phytochemicals in the various extracts used in the present study are given in table 2. Some work regarding qualitative analysis of phytochemicals of *Emblica officinalis*, *Aloe vera* and *Vitex negundo* was done in the recent past[8,19,20]. Presence of various phytochemicals in the extract will lead to contribution in the antifungal activity. Higher the amounts of phytochemicals present in the extract, higher the possibility of inhibitory action of the extract. There is need of further quantitative analysis of phytochemicals for the accurate measurement of the phytochemicals.

Table 2: Qualitative analysis of phytochemicals present in different extracts of *Emblica officinalis* fruits, *Aloe vera* leaves and *Vitex negundo* leaves

Plant	Extract	Name of Phytochemical						
		Alkaloids	**Saponins**	**Tannins**	**Sterols**	**Cardiac Glycoside**	**Flavanoids**	**Phenol**
Emblica officinalis	H	-	+	-	-	-	-	+
	EA	+	+	+	+	-	-	+
	M	+	+	+	+	+	+	+
	DW	+	+	+	+	-	+	+
Aloe vera	H	-	+	-	+	+	-	+
	EA	-	+	-	+	-	-	+
	M	+	+	-	+	-	-	+
	DW	+	+	+	-	+	+	+
Vitex negundo	H	+	-	-	-	-	+	+
	EA	+	+	-	-	-	+	+
	M	+	+	+	+	+	+	+
	DW	+	+	-	+	+	+	+
H = Hexane, EA = Ethyl Acetate, M = Methanol, DW = Distilled water, (+) = Presence, (−) = Absent								

The knowledge of extent and mode of action for antifungal activity of specific compounds, present in the plant extracts, may lead to the successful utilization of such natural compounds for treatment of infections caused by pathogenic fungi. For that there is need to identify such natural compounds from wide range of medicinal plants and to know the mode of action of such chemical constituents. The present status of work related to antifungal activity of *Emblica officinalis*, *Aloe vera* and *Vitex negundo* is very limited therefore this investigation may contribute in this field as a preliminary base. There is need of further work on the antifungal activity of these plants using different pathogenic fungi for the appropriate interpretation. Further identification and purification of active chemical constituents from the crude extracts of such medicinal plants will be helpful to develop drug against pathogenic fungi.

CONCLUSION

The present study was undertaken to screen the potential antifungal activity of extracts of *Emblica officinalis* Gaertn. fruits, *Aloe vera* L. leaves and *Vitex negundo* L. leaves. All the plants showed maximum antifungal activity against *Trichoderma viridae* and no inhibitory activity against *Penicillium chrysogenum*. Aqueous extracts of all the plants showed maximum inhibitory action as compared to

other extracts indicated that maximum phytochemicals contributing to antifungal activity were extracted in water. Some extracts could inhibit the growth of *Aspergillus* species which can be further utilized to develop drug against these pathogenic fungi. The knowledge of extent and mode of action for antifungal activity of specific compounds present in the plant extracts, may lead to the successful utilization of such natural compounds for treatment of fungal infections by developing antifungal drugs.

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