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PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL SCREENING AND ANTIHELMINTHIC PROPERTIES OF *Phyllanthus emblica*

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ABSTRACT

In our study, preliminary phytochemical analysis, antimicrobial and antihelminthic investigation on *Phyllanthus emblica* was done. *P.emblica* is a well known and an important medicinally valued plant. Although the efficacy of *P.emblica* fruit is widely proved, use of leaf and bark is less investigated. Present aim of our study is to find the biologically active compound present in this particular plant, check its antimicrobial and antihelminthic property. Different solvent extract of the leaf and bark were used to identify the bioactive compounds present and its antimicrobial activity was checked against different human pathogens(MTCC).Antihelminthic activty was checked against *Phertima posthuma*. The study shown it has promising antimicrobial and antihelminthic property. Methanol of the leaf sample showed highest zone of inhibition against *Enterobacter aerogens* and *Enterobacter feacalis*. Ethyl acetate of leaf and bark sample showed antifungal activity against *Rhizomucor* species.The pharmacological property of this medicinally important plant has to be further investigated.

KEYWORDS : ***Phyllanthus emblica*, anti microbial, MTCC, Anti helminthic, *Phertima posthuma***

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INTRODUCTION

Medicinal plants are rich sources of anti microbial agents and its therapeutic use is becoming popular because of its lesser side effects and resistance. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growing different parts of the country. Herbal medicine is still the mainstay of about 75 - 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [1]. The indigenous knowledge of medical practitioners has helped in the development of newer drugs[2]. Although antimicrobial potential of different medicinal plants are extensively studied all over the world, only few are carried out in a systematic way. In the absence of scientific validation, the medicinal use of pharmacologically active compound found in plants is questionable[3] *Phyllanthus emblica* is a medium-sized deciduous tree. It is also named *Emblica officinalis*. It belongs to the plant family Phyllanthaceae. It is also called Aonla, Aola, Amalaki, Dhatry and Indian Gooseberry. *P. emblica* is highly nutritious and is an important dietary source of vitamin C, minerals, and amino acids, such as calcium, phosphorus, iron, carotene, thiamine, riboflavin, and niacin[4,5]. In *P. emblica* fruit, vitamin C is considered to be highly stable due to the presence of tannin and polyphenols [6]. The important constituent of plant leaves have the anti-neutrophilic activity and anti-platelet properties *in vitro*. The extracts also posses several pharmacological properties like anti-viral (HIV, AIDS, Herpes Virus, CMV) antimutagenic, anti-allergic, anti-bacterial activities.[7,8].The study also showed that the plant leaves have antineutrophilc and antiplatelet properties *in vitro*. This agrees with the anti-inflammatory and antipyretic usage of this tree in traditional medicine by rural populations in Asia[9] . The leaf infusion with fenugreek seeds is given to treat chronic diarrhea. The pulp of the fruit is smeared on the head to dispel headache and dizziness [10]. The fresh or dry fruit is widely used for the treatment of diarrhea, jaundice and inflammatory disorder. It is also shown that the fruits of this particular plant can be used as a glucose lowering agent with minimal differentiation of pre-adipocytes and so can be used in treating Type II diabetes very effectively without the fear of obesity [11] Moreover, this plant is an essential ingredient of Ayurvedic preparation Triphala (*P. emblica*, *Terminalia chebula* Retz., *Terminalia bellerica* Roxb).The cheif ingredient of Chyawanprash, an ancient ayurvedic health tonic widely used in India as a rejuvnative health energizer and immunity booster, is *Phyllanthus emblica* (syn. *Emblica officinalis*), the Indian gooseberry [12] .The present objective of the study is to find out the different pharmacologically active compounds present in *P.emblica* ,its anti microbial and antihelminthic properties.

MATERIALS AND METHODS

(i) Collection extraction and sample preparation

The plant (*Phyllanthus emblica)* was identified and collected from its distribution university campus. The collected plant sample (Bark and Leaf) was washed thoroughly under running tap water to remove dust and sand particles. Then it was shade dried for 2-3 weeks, powdered and stored for further use.In order to extract the active compounds from plant parts such as leaves and bark, 25 g of each dried sample was soaked in 100 ml of solvents such as Benzene, Methanol, ethyl acetate and water. This was kept for 48 hours incubation and filtered using Whatmans No. 1 filter paper. The extract obtained was evaporated completely for dryness. The 2 g of dried sample obtained was dissolved in10 ml of DMSO and stored for futher use.

(ii) Phytochemical analysis

Chemical tests were carried out using the extracts obtained (methanol, butanol,ethyl acetate, aqueous) from plants using standard procedures to identify the constituents as described by Harborne [13] .To identify alkaloids wagners and molishs tests were

conducted.Keller killani test,sulphuric acid test,forthin test,Ferric chloride test and salkowski tests were conducted to identify cardiac glycosides,flavinoids,saponins,tanins and terpenoids respectively.

(iii) Anti microbial screening

The crude extract of the plant and drug were tested for antimicrobial activity (antibacterial and antifungal) against strains of pathogenic microbes (MTCC).Drug like ciprofloxacin(3 µg) and kanamycin(15µg)and DMSO were used as controls. Antibacterial activity of samples against human pathogens The crude extract of the plant and drug were tested for their antibacterial activity by disc diffusion method against pathogenic organism like *Pseudomonas aeroginosa, Proteus vulgaris, Klebsiella pneumonia, Enterobacter aerogens, Bacillus megaterium, Pseudomonas putida, Lactococcus lactis, Bacillus substilis, Enterobacter faecalis, Escherichia coli.* Prepared nutrient agar plates were inoculated with pathogenic organism(0.1ml) by spread plate method. Whatmans no.1 filter paper disc were sterilized and inoculated with the sample and DMSO is kept as negative control After incubatione at 30^{o}C for 24 hours zone of inhibition was measured. Antifungal activity of samples against human pathogens The crude extract of the plant and drug were tested for their antifungal activity by disc diffusion method against pathogenic organism like *Aspergillus fumigates, Aspergillus niger, Candida albicans, Candida glabrata, Candida tropicalis, Rhizomucor miehei.* Prepared Rose Bengal Agar plates were inoculated with pathogenic organism by spread plate method .Whatmans filter paper were sterilized and inoculated with sample and DMSO were kept as negative controls. After incubation at37 ^{o}C for two days the zone of inhibition was measured.

(iv) Antihelmintic activity

Anti helminthic activity was conducted using *Pheretima posthuma* (Earth worm) of nearly equal size (±8 cm) due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. 2-3 earth worm of nearly equal size were placed in each petridish containing 2 ml (0.4g)sample at room temperature. Observation was made for the time taken for paralysis when there was no movement of any kind except when shaken vigorously and are not revived in normal saline. Time for death were recorded when they lost their mobility even after vigorous movement and also by fading off their body colors.

RESULTS

1. Phytochemical analysis

Phytochemical analys showed the presence of alkaloids ,caridac glycosides ,saponins, tannins and terpenoids in methanol, butanol and ethylacetate extract of both bark and leaf samples as shown in Table1.

Table 1
Phytochemical analysis of Phyllanthus emblica leaf and bark

Sl NO	Compound	Methanol		Butanol		Ethyl acetate		Aqueous	
		leaf	Bark	Leaf	bark	Leaf	Bark	Leaf	bark
1	Alkaloid	+	+	+	+	+	+	_	_
2	Carbohydrate	+	+	_	+	+	+	_	_
3	Cardiac glycoside	+	_	+	+	+	+	+	+
4	Flavanoids	+	_	_	_	+	+	+	_
5	Saponin	+	+	+	+	+	+	+	+
6	Tannins	+	+	+	+	+	+	+	+
7	Terpenoids	+	+	_	+	+	+	+	+

(+ - present, - absent)

2. Antibacterial activity

Methanol extract of leaf sample showed the highest zone of inhibition against *Enterobacter aerogens* and *Enterobacter feacalis*(18 mm). Ethyl acetate extract also showed high zone of inhibition against *E.coli*. In Bark sample butanol extract showed an inhibiton zone of 18mm against *Klebsiella pneumoniae*.All other samples obtained almost equal range of inhibition zone(13mm-16mm).Results are shown in Table 2, Fig 1, and Fig 2

Table 2

Diameter of Zone of inhibition of Phyllanthus emblica Leaf and Bark sample

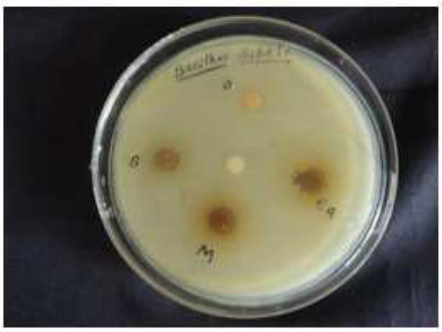
Sl NO	Bacteria	zone of inhibition(mm)							
		Methanol		Butanol		Ethyl acetate		Drugcontrol	
		Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
1	*Pseudomonas aeroginosa*	16	12	11	14	9	10	16	10
2	*Proteus vulgaris*	15	12	10	14	10	12	10	13
3	*Klebsiella pneumoniae*	11	14	13	18	9	11	11	12
4	*Enterobacter aerogens*	18	10	13	12	16	15	10	13
5	*Bacillus megaterium*	15	14	14	15	10	10	10	13
6	*Pseudomonas putida*	11	10	13	13	8	11	10	13
7	*Lactococcus lactis*	14	14	13	15	11	13	16	12
8	*Bacillus substilis*	12	14	11	11	14	15	10	11
9	*Enterobacter faecalis*	18	13	16	14	15	13	15	15
10	*E.coli*	12	10	10	12	18	15	15	11

Figure 1

Diameter of Zone of inhibition of Phyllanthus emblica leaf extract
B-Butanol M-Methanol E-Ethyl acetate D-Drug C-Control E.coli Lactobacillus lacti

Bacillus subtilis

Enterococcus feacalis



Proteus vulgaris

Bacillus megaterium

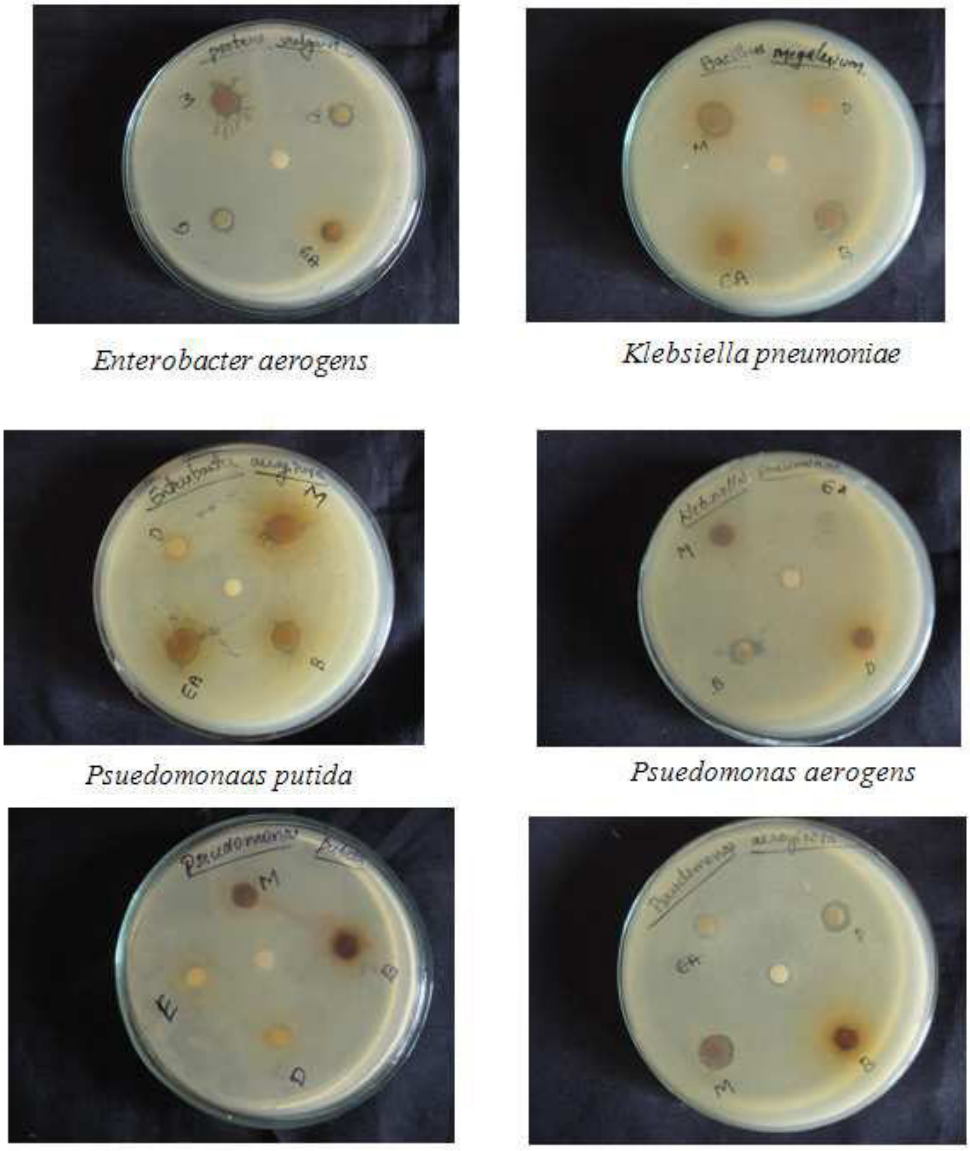
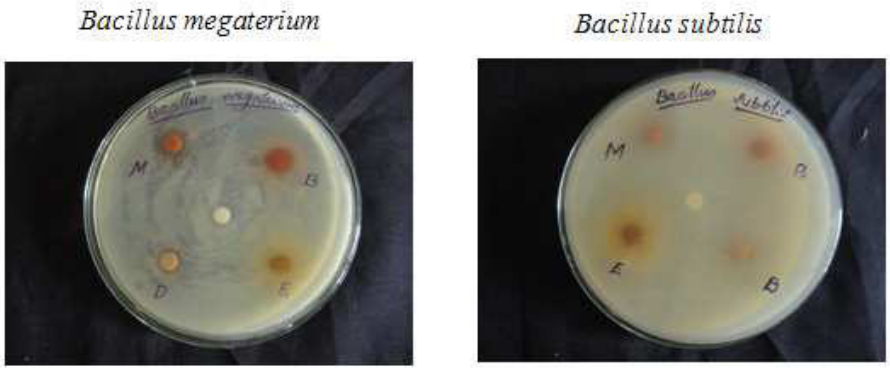
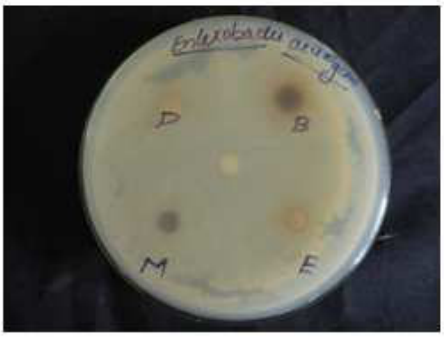


Figure 2
Diamater of Zone of inhibition of bark extract of Phyllanthus emblica
B-Butanol M-Methanol E-Ethyl acetate D-Drug C-Control

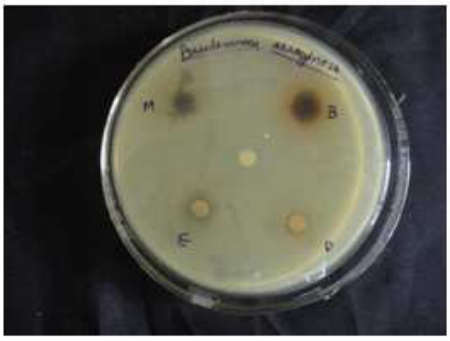


Enterococcus faecalis

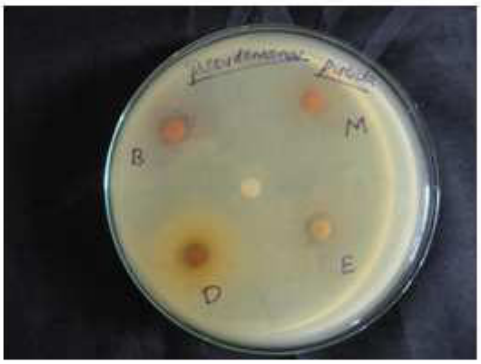


Psuedomonas aereginosa

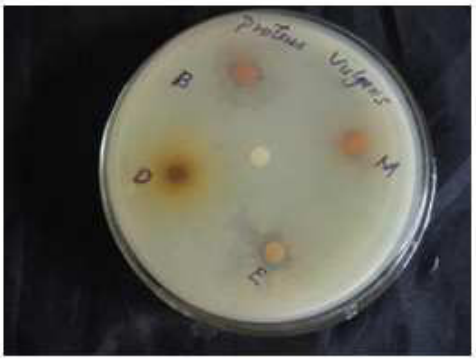
Pseudomonas putida



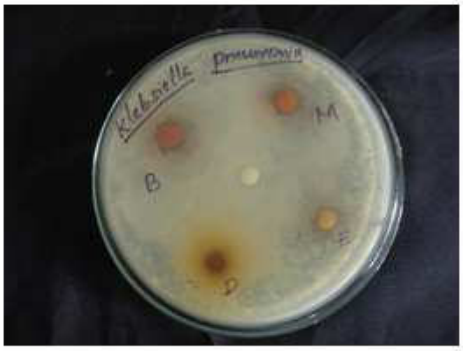
Proteus vulgaris



Lactococcus lactase



Klebsiella pneumoniae



3. Anti fungal activity

Ethyl acetate extract of both bark and leaf sample showed highest anti fungal activity against *Rhizomucor* species.(20mm and 19 mm respectively)all other samples showed almost same zone of inhibition(10mm- 16mm).Results are shown in Table 3, fig3 and Fig 4.

Table 3
Diameter of Zone of inhibition of Phyllanthus emblica leaf and bark

SI NO	Fungi	Diameter of zone of inhibition(mm)					
		Methanol		Butanol		Ethyl acetate	
		Leaf	Bark	Leaf	Bark	Leaf	Bark
1	*Aspergillus fumigatus*	13	12	15	9	11	10
2	*Aspergillus niger*	11	8	9	10	9	12
3	*Candida albicans*	9	16	15	15	10	11
4	*Candida glabrata*	10	19	14	10	11	15
5	*Candida tropicalis*	17	19	10	10	13	15
6	*Rhizomucor miehei*	13	16	11	14	19	20

Figure 3
Zone of inhibition of Phyllanthus emblica leaf extract
B-Butanol M-Methanol E-Ethyl acetate D-Drug C-Control

Aspergillus fumigatus

Aspergillus niger

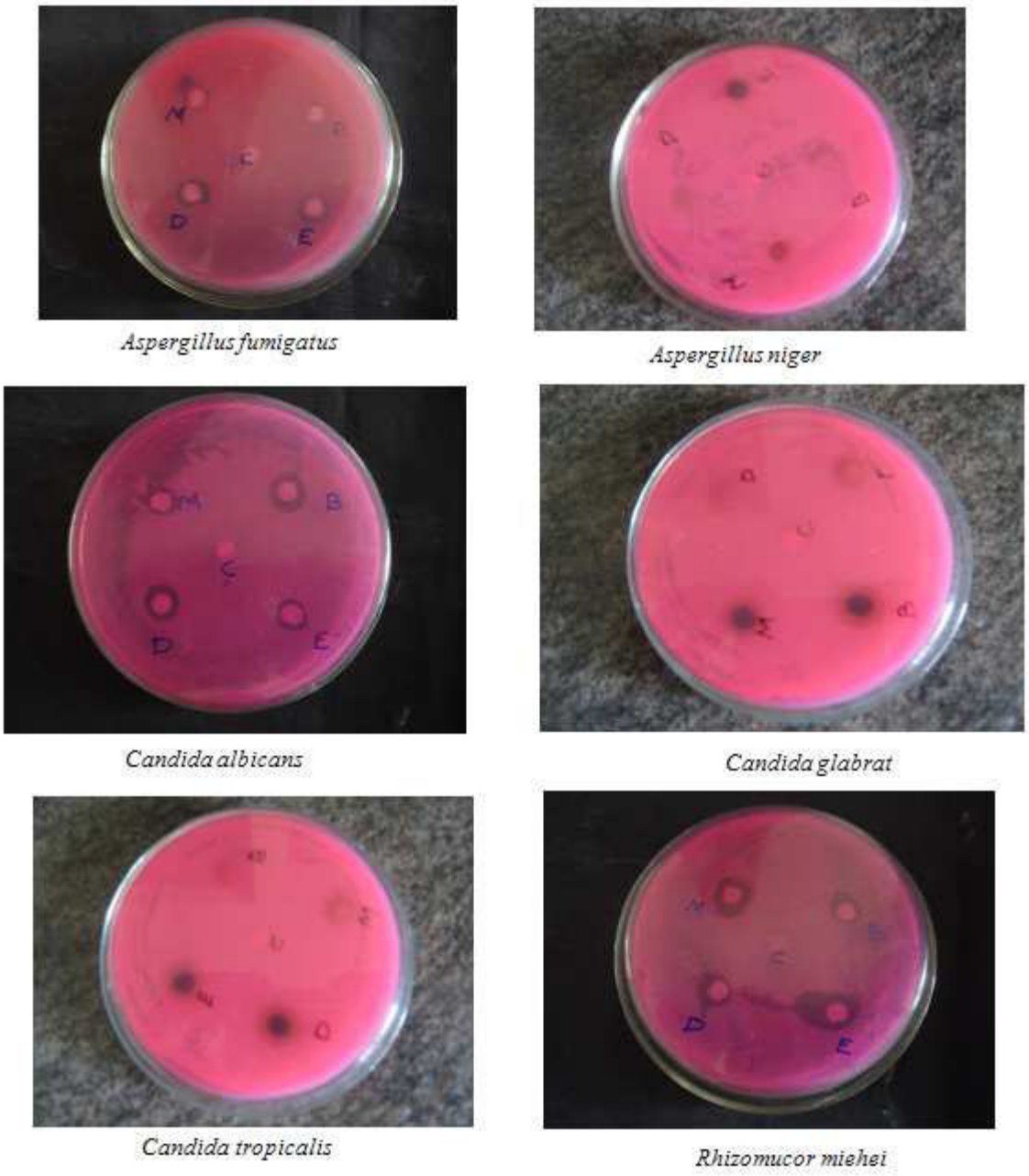
Candida albicans

Candida glabrata

Candida tropicalis

Rhizomucor miehei

Figure 4
Zone of inhibition of Phyllanthus emblica bark extract
B-Butanol M-Methanol E-Ethyl acetate D-Drug C-Control



4. Anti helminthic activity.

Butanol extract of leaf and bark exhibited less time for paralysis and death for *Pheretima posthuma.* Aqueous extract of leaf show the maximum time for paralysis and subsequent death. Results are shown in Table 4.

Table 4
Antihelminthic activity of Phyllanthus emblica leaf and bark

Sl NO	Name of earthworm	Extract	Time in minutes			
			For paralysis		For death	
			Leaf	Bark	Leaf	Bark
1	*Pheretima posthuma*	Methanol	14	17	25	50
2		Butanol	10	16	21	45
3		Ethylacetate	22	16	35	150
4		Aqueous	135	52	190	65

DISCUSSION

Here a preliminary phytochemical analysis of various solvent extract of *P. emblica* , its anti microbial and antihelminthic activity were conducted. Phytochemical analysis of the plant *emblica* showed the presence of many biologically active compounds, such as alkaloids, cardiac glycosides ,saponins, tannins terepenoids, flavinoids and carbohydrates .Different extracts showed promising anti bacterial activity against commonly found human pathogens. Butanol extract showed maximum zone of inhibition (18 mm) for two pathogens*,E.coli* and *Enterobacter aerogens* and also by ethyl acetate for *E.coli*. Antifungal activity was shown to be highest in ethyl acetate fraction of bark sample. As all the bioactive compounds were present in the butanol and ethyl acetate extract, except falvanoids in case of butanol and methanol bark extract, further studies to identify and isolate the active e compound has to be conducted. Although the fruit of *emblica* is widely used as an immunity booster ,lower blood cholestrol, enhances memory and intelligence, a natural source of vitamin c and iron,(14,15) the use of leaf and bark is less investigated. Further research has to be conducted to find out the possibility of this medicinally important plant as a potent anti microbial drug and for other pharmacological properties to develop as cost effective formulation.

CONCLUSION

To conclude, many of the phytochemicals found in this particular plant proved to have potential anti microbial and antihelminthic properties, which has to further investigated. Complete purification and identification of the biologically active compounds might help in understanding the mechanism of action and thereby to formulate a new potent drug

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