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ANTIMICROBIAL ACTIVITY OF PHYLLANTHUS EMBLICA – A MEDICINAL PLANT

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ABSTRACT: Objective: *Phyllanthus emblica* is an ethnomedicinal plant that has several medicinal claims and it hasn't been explored thoroughly. Various parts of the plant are used medicinally such as antioxidant, anti-inflammatory, analgesic and anti-pyretic etc. The study aims to explore the different qualitative, quantitative, and antifungal aspects of *Phyllanthus emblica.* Materials and methods: The present study was conducted to evaluate the anti-microbial activity of *Phyllanthus emblica* extracts against Gram-positive bacteria (Staphylococcus aureus), Gram-negative bacteria (E. coli), and Fungal (Candida albicans). The agar well diffusion method was used to test the antimicrobial activity. Result & Discussion: *Phyllanthus emblica* extracts exhibited potent antibacterial and antifungal against all the selected bacterial and fungal species. The extracts exhibited the growth inhibitory activity in a dose-dependent manner. Also, the study reveals *Phyllanthus emblica* shows good antimicrobial activity. Conclusion: The *Phyllanthus emblica* plant extracts could be used as an antimicrobial after comprehensive *in-vitro* biological studies.

KEYWORDS : *Phyllanthus emblica,* Anti-microbial, Staphylococcus aureus, E. coli, Candida albicans.

INTRODUCTION

Plants play a pivotal role in the survival and development of human civilization. The use of medicinal plants as a therapeutic aid for alleviating human ailments can be traced back to over five millennia. *Phyllanthus emblica* Linn., belonging to the family Euphorbiaceae, is widely distributed throughout most tropical and subtropical countries and is the greatest boon to humanity and one of the effective traditional herbal medicines, which had been used to

treat and manage diseases since ancient times. *Phyllanthus emblica* is a medium-sized deciduous tree growing up to the height of 10–18 m. *Phyllanthus emblica* contains vitamin C, minerals, amino acids, tannins, phyllembelic acid, phyllemblin, rutin, curcuminoids, emblicol, and some phenolic compounds[1,2]. Studies reported that *Phyllanthus emblica* has antimicrobial[3], antioxidant[4], anti-inflammatory,[5] analgesic and antipyretic[6], adaptogenic[7], hepatoprotective[8], antitumor[9], and antiulcerogenic[10] activities. Plants are also used to treat cough, bronchitis, skin disease, enlarged spleen and liver, jaundice, and fever. *Phyllanthus emblica* is one of the most used among Ayurvedic medicinal plants due to its varied pharmacological properties. The traditional use of *Phyllanthus emblica* enforces its effects on almost all human ailments, but very few of them have been validated through clinical research and still, the vast majority of these traditional uses are yet to be proved through systematic researches. So, the present study was aimed to evaluate the antimicrobial activity of *Phyllanthus emblica*, a medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms.

MATERIALS AND METHODS

Sample Collection: The leaves for the present study from the respective plant *Abutilon indicum* was collected from gujarat allowed to dry under the shade and made into a fine powder.

Preparation of plant extract: The powder (100grams) was Soxhlet extracted with methanol and dried under rota vapour at 40-50°C for 3hours. This measure was taken to evaluate the antimicrobial activity.

Physicochemical Screening

Assessment of the parameters such as foreign matter, moisture content, ash value, acid insoluble ash, pH, water-soluble extractive, and alcohol-soluble extractive was carried out by following standard procedures recommended by Ayurvedic Pharmacopoeia of India[11].

Preliminary phytochemical screening

About 5g of the test drug was macerated with methanol and water separately (100ml) in a closed flask for 24 hours where initial shaking frequently during the first 6hrs and kept it for 18 hrs. After 24 hours it was filtered. The filtrate was evaporated with the help of a water bath and the extract was collected in solid form. For qualitative analysis, the presence of various secondary metabolites was done as per reference[12].

Microorganisms: The bacterial and fungal cultures for the present investigation were obtained from Primer Biotech Research Center, Hyderabad. Two bacteria used for the present study were*; E. coli* and *Staphylococcus aureus and* the fungi used for the present study is *Candida albicans.*

Procedure

In-vitro antimicrobial activity

Culture Used: Two bacteria used for the present study were*; E. coli* and *Staphylococcus aureus.*

Media used: Muller Hinton broth

The reference standard used: commercially available gentamycin discs were used as a positive control in this study.

Testing procedure: The antimicrobial activity of the extract was evaluated by the agar well diffusion method. Bacteria were grown in Muller Hinton broth (HiMedia Laboratories Ltd., India) to match the turbidity of 0.5 McFarland standards to be inoculated on Muller-Hinton agar (HiMedia Laboratories Ltd., India. After inoculation, plates were dried for 15 min, and the wells were punched using sterile cork borers. Once wells were formed, they were filled with 100 µL of plant extracts and blanks. Plates were incubated for 24 h at 37 °C to allow leaf extracts to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different leaf extracts against different bacteria were measured in millimeters for further analysis. An agar well (6 mm) showing no zone of inhibition was considered as no antimicrobial activity. All experiments were done in triplicate and the average values were used for drawing bar diagrams[13].

In-vitro antifungal activity:

Culture used: Candida albicans (ATCC 10231)

Media used: Sabroud dextrose agar (SDA) Make: Hi-media

The reference standard used: Itraconazole capsule

Culture Preparation: Freshly prepared slants of C. albicans were used and washed the slant by using 10 mL of sterile Normal saline solution.

Media preparation: Sabroud Dextrose Agar was used for determining the activities of, Candida albicans. Media was prepared as per the Manufacturer's Instruction. The media was then autoclaved at 121˚C temp. & 15lbs pressure for 20 minutes.

Sample Preparation: Take approximately 100 mg of sample & dissolved it into a 1:1 ratio of Methanol: Dimethyl Sulfoxide. Dissolved the samples by cyclomixture. Filter the samples & use filtrate to evaluate antifungal activity.

Standard preparation of Itraconazole - Take the weight of the filled capsule. The active content of the capsule i.e. pellets was powdered into mortar-pestle. Took powder equivalent to one capsule weight into 100 ml volumetric flask and make up the volume 100 ml with Dichloromethane. The solution was sonicated and prepared 50 mcg/ml standards solution by dilution method.

Testing Procedure: Cooldown sterile media up to 55°C then measured 15 ml of SDA media by sterile measuring cylinder and transferred into sterile Petri plate. Likewise, prepared 3 plates for evaluation. The plates were allowed to solidify on the smooth surface. In the rest of the media add 5µl fungal culture and mix slowly. Then the media was poured on above SDA containing plates. The plates were solidified and then made required wells in SDA plates labelled as an std. & test, at a proper distance by the sterile borer. Add std. & test samples in respected labelled well. When samples were diffused completely in well, incubate SDA plates into Biological Oxygen Demand (BOD) incubator at 25°C for 72 hours and observe the zone of inhibition[14].

RESULT

Physico-chemical study

Leaves and Fruit powder of *Phyllanthus emblica* were subjected to physicochemical parameters like the loss on drying, total ash, acid insoluble ash, alcohol soluble extractive value, pH value, etc. Results are depicted in Table no. 1

Table no.2- Results of Physicochemical parameters

S.No.	Parameter	*Phyllanthus emblica*
1.	Loss on drying (%w/w)	13.75
2.	Ash value (%w/w)	2.51
3.	Acid insoluble ash (%w/w)	0.34
4.	Alcohol soluble extractive(%w/w)	17.94
5.	Water-soluble extractive (%w/w)	15.33
6.	pH (5% aq. Sol^n)	5.9

Preliminary phytochemical results showed the presence of Alkaloids, tannin, flavonoids, and carbohydrates in the extracts of *Phyllanthus emblica*. The results are depicted in Table no. 2

Table no.2- Results of phytochemical screening of the *Phyllanthus emblica*

S.No.	Phyto-constituents	Tests	*Phyllanthus emblica*	
			ME	**WE**
1.	Carbohydrates	Molisch's	+	+
2.	Reducing sugar	Fehling test	-	-
3.	Proteins	Biuret	+	+
4.	Amino acids	Ninhydrin	-	-
5.	Alkaloids	Wagnor	+	-
		Mayor	+	-
6.	Tannins	Lead Acetate	+	+
7.	Steroids	Salkowski	_	_
8.	Flavonoids	Shinoda test	+	+
9.	Saponin glycoside	Foam	_	_

The antimicrobial activity of the extract was examined against Gram-positive and Gram-negative bacteria and fungal strains by measuring the zone of inhibition. The antimicrobial activity was performed by Agar disc diffusion method at concentration level of 25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml respectively. Gentamycin (antibacterial), Itraconazole (antifungal) as standard drug. Antibacterial and antifungal potential of ethanolic extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antimicrobial activity and antifungal are shown in table no. 3.

Table No. 3- Anti-microbial and Antifungal Activity of Extracts of *Phyllanthus emblica*

	25mg/ml	**50mg/ml**	**75mg/ml**	**100mg/ml**
E.coli (mm)	9 ± 0.32	10 ± 0.49	12 ±0.56	13 ± 0.70
Staphylococcus aureus (mm)	19 ± 0.18	20 ± 0.42	21 ±0.45	22 ± 0.43
Candida albicans (mm)	5 ± 0.25	6 ± 0.32	7 ± 0.49	8 ± 0.35

DISCUSSION

Phyllanthus emblica is the most renowned plant in the Indian traditional system of unani and ayurvedic medicine. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries, low income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infections. Though all parts of the plant are used for medicinal purposes, the fruits especially are found in tremendous pharmacological applications. They are used both as a medicine and as a tonic to build up lost vitality and vigor. The present study was carried out to evaluate the antibacterial and antifungal activities of the *Phyllanthus emblica* against bacterial and fungal species.

Physicochemical parameters show that loss on drying is 13.75%, alcohol soluble extractive value is found more in comparison to water-soluble extractive value. Alcohol soluble value indicates the presence of phenols, alkaloids, glycosides, and flavonoids, etc. Phytochemical screening shows the presence of Alkaloids, tannin, flavonoids, and carbohydrate in the extracts of *Phyllanthus emblica*. Studies also reported that the fruits are rich in ascorbic acid i.e. Vitamin C. Also, they contain phenols, including ellagic acid, gallic acid, quercetin, kaempferol, corilagin, geraniin, furosin, gallotanins, emblicanins, flavonoids, glycosides, and proanthocyanidins[15-17].

Phyllanthus emblica extracts exhibited potent antibacterial and antifungal against all the selected bacterial and fungal species. The extracts exhibited the growth inhibitory activity in a dose-dependent manner. The results show that *Phyllanthus emblica* extracts were found to be more effective against all the microbes tested. The activity appears to be stronger against gram-positive bacteria, and only limited efficacy against fungi.

CONCLUSION

Phyllanthus emblica has been used as an herbal medicine for its healing properties since ancient times. The most prominent of the bioactive compounds are alkaloids, tannin, flavonoid, and phenolic compounds, etc which are responsible for unique medicinal properties for a specific plant. The present study aimed at assaying quantitatively the antibacterial activity of extracts of fruits of *Phyllanthus emblica* against Staphylococcus aureus and E. coli and thereby compares differentially the antibacterial action on gram-positive and gram-negative bacteria. Alcoholic and aqueous extracts of *Phyllanthus emblica* showed positive results against common human pathogens, including bacteria, and fungi. This could be due to the active chemicals which are present in the *Phyllanthus emblica* making it a potential antimicrobial activity.

REFERENCES

1. Zhang YJ, Tanaka T, Iwamoto Y, Yang CR, Kouno I. Novel norsesquiterpenoids from the roots of Phyllanthus emblica. J Nat Prod 2000;63:1507-1510.
2. Bhattacharya A, Ghosal S, Bhattacharya SK. Antioxidant activity of tannoid principles of Emblica offi cinalis (amla) in chronic stress induced changes in rat brain. Ind J Exp Biol 2000;38:877-880
3. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998;62:183-193.
4. Chatterjee UR, Bandyopadhyay SS, Ghosh D, Ghosal PK, Ray B. In vitro anti-oxidant activity, fl uorescence quenching study and structural features of carbohydrate polymers from Phyllanthus emblica. Int J Biol Macromol 2011;49:637-642.

5. Golechha M, Bhatia J, Ojha S, Arya DS. Hydroalcoholic Chin J Integr Med • 7 • extract of Emblica officinalis protects against kainic acid-induced status epilepticus in rats: evidence for an antioxidant, anti-inflammatory, and neuroprotective intervention. Pharm Biol 2011;49:1128-1136.
6. Perianayagam JB, Sharma SK, Joseph A, Christina AJ. Evaluation of anti-pyretic and analgesic activity of Emblica offi cinalis Gaertn. J Ethnopharmacol 2004;95:83-85.
7. Baliga MS, Meera S, Mathai B, Rai MP, Pawar V, Palatty PL. Scientifi c validation of the ethnomedicinal properties of the Ayurvedic drug Triphala: a review. Chin J Integr Med 2012;18:946-954.
8. Gulati RK, Agarwal S, Agrawal SS. Hepatoprotective studies on Phyllanthus emblica Linn. and quercetin. Ind J Exp Biol 1995; 33:261-268.
9. Suresh K, Vasudevan DM. Augmentation of murine natural killer cell and antibody dependent cellular cytotoxicity activities by Phyllanthus emblica, a new immunomodulator. J Ethnopharmacol 1994;44:55-60.
10. Sairam K, Rao Ch V, Babu MD, Kumar KV, Agrawal VK, RK KG. Antiulcerogenic effect of methanolic extract of Emblica officinalis: an experimental study. J Ethnopharmacol 2002;82:1-9.
11. Anonyms. The Ayurvedic Pharmacopoeia of India. PartII.1st ed., New Delhi: Government of India, Ministry of health and Family Welfare, Department of Ayush, 2008, 2.
12. Khandelwal KR. Practical Pharmacognosy. 19th ed. Nirali Prakashan, Pune, 2008.
13. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement, M100S21. New York: Clinical and Laboratory Standard Institute; 2011. [Online] Available from: http://www. techstreet.com/products/1760826 [Accessed on 10th Jan, 2021]
14. Anonymous. The ayurvedic pharmacopoeia of india, part-II, vol- II 1st ed. New delhi : Government Of India, Ministry Of Health And Family Welfare, Department Of Ayush, 2008, 2
15. Nisha P, Singhal RS, Pandit AB. A study on degradation kinetics of ascorbic acid in amla (Phyllanthus emblica L .) during cooking. Int J Food Sci Nutr, 2004; 55(5): 415-422.
16. Zhang YJ, Abe T, Tanaka T, Yang CR, Kouno I. Phyllanemblinins A-F, new ellagitannins from Phyllanthus emblica . J Nat Prod, 2001; 64(12): 1527-1532.
17. Kumaran A, Karunakaran RJ. Nitric oxide radical scavenging active components from Phyllanthus emblica L. Plant Foods Hum Nutr, 2006; 61(1): 1-5.