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Antimicrobial Activity of *Emblica officinalis* Seeds on Human Pathogens

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The antimicrobial activity of aqueous and methanolic extracts of *Emblica officinalis* seeds were tested on different microbial pathogens.The zones of inhibition ranged between 6mm to 16mm for bacterial pathogens and 6mm to 24mm for fungal pathogens.The aqueous extract showed zones of inhibition only for *E.coli* and *Candida albicans*. The cold methanol extract showed zones of inhibition ranging from 0.0mm to 12mm, with maximum activity against *Geotrichum* and *Fusarium* and minimum activity against *Salmonella typhimurium*. The zones of inhibition to distillate and residual extract ranged between 0.0mm to 17mm and 0.0mm to 24mm respectively suggesting that the residual extract was more effective than aqueous, cold methanol and distillate extracts. The MICs ranged from 50 μg/ml to 800 μg/ml indicates that *A.flavus* was more sensitive as it showed less MIC. There results showed that seeds of *Emblica officinalis* extracts possess potent antimicrobial activity.

Key words: Antimicrobial activity, Pathogenic microorganisms, MIC's, *Emblica officinalis.*

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have lead to the screening of several medicinal plants for the potential antimicrobial activity[1-7] *Emblica officinalis* is commonly called as Indian gooseberry. It belongs to the family Euphorbiaceae. It is called as Amla in Hindi and Amalaki in Sanskrit. The fruit (pulp) is known to have antipyretic, spasmolytic, antifungal, antibacterial, antiviral, anticytotoxic, immunomodulatory, immunostimulatory activities[9,11]. However, no literature is available on the antimicrobial aspects of the seeds of *Emblica officinalis.* In this paper, the aqueous, cold methanol, distillate and residual extracts of the seeds were evaluated against various human pathogens.

MATERIAL AND METHODS

The seeds were collected from Rytu bazaar, Gopalapatnam, Visakhapatnam, Andrapradesh, India. The seeds were dried and

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ground to a fine powder and mixed in sterile distilled water to give a concentration of 100 mg / ml of stock solution, the extracts were stored in the refrigerator till further use. The residual methanol extract was prepared by mixing 100g of dried and ground powder in 1litre of methanol in aspirator bottle for 48 hours. Later, the solution was collected and subjected to 6 cycles of distillation until a thick brown coloured paste was obtained. 500mg of residual methanol extract was mixed in 5ml of methanol to give a concentration of 100 mg /ml *Emblica officinalis* seeds.

The microbial strains used in this study were obtained from MTCC Chandigarh, India. The strains used are *Salmonella typhi* (ATCC 10749), *Pseudomonas aeruginosa (*ATCC 25619), *Salmonella typhimurium* (ATCC 23564), *Yersinia enterocolitica* (ATCC 9610), *E.coli* (uropathogen), *Candida albicans* (ATCC 2091), *Rhizopus, Geotrichum, Fusarium* , and *A.flavus.*

Microbial culture conditions

The bacterial cultures were maintained on nutrient agar slants or plates (peptone 0.5%, beef extract 0.3%, NaCl 0.5%, agar 2.0%) and fungal cultures on sabourauds agar slants or plates (mycological peptone 1%, dextrose 2-4%, agar 2%). Over night cultures were used in all experiments by inoculating a simple colony of each type of culture in respective 5ml broth and incubating at 37°C for 18 to 24 hours (bacteria) or at room temperature for 48 hours (fungi). Antimicrobial reference substances used were ampicillin (10mg/disc) and nystatin (100units/disc) procured from Himedia, Mumbai, India. Nutrient agar plates and sabourauds agar plates were inoculated with 0.1ml of over night liquid culture of each type containing 1.0×10^7 cells and spread by L-shaped glass rod. Later 5 sterile filter paper discs (5mm) and reference antibiotic discs such as ampicillin (10mg/disc) and nystatin (100 units / disc) were placed in correspondinding plates on 4 sterile paper discs aqeous, cold methanol, distillate, residual extract were placed at 2mg concentration in 20 µl/disc.

On the 5th disc 20µl of absolute methanol was placed as control. The bacterial cultures were incubated at 37°C for 18 to 24 hours and fungal cultures at 28°C for 48 hours. Zones of inhibition were measured. The microbes were plated in duplicates and average zone diameter was noted.

The minimal inhibitory concentrations (MICs) were determined by the tube dilution techniques[8]. Different concentrations of residual extracts of *Emblica officinalis* seeds were serially diluted in duplicates. Control test tube did not receive any extract. Later 10^3cells of microorganisms were added into each test tube and incubated at 37°C or at room temperature for 18-24 hours. The lowest concentrations which inhibited the growth was considered as MIC.

RESULTS AND DISCUSSION

The aqueous extract showed 9mm zone of inhibition for *E.coli* and 8mm zone of inhibition for *Candida albicans*. All the microorganisms tested showed resistance in this extract. The cold methanol extract showed zones of inhibition ranging from 6mm to 12mm. *Geotrichum* and

Table 1. Antimicrobial activity of *Emblica officinalis (*Seed extract).
Zones of inhibition expressed in mm on certain human pathogens (Bacteria).

S. No.	Micro Orgamisms Tested	Aqueous Extract (2mg / disc)	Cold Methanol (20ml/disc)	Distillate (20ml/disc)	Residual (2mg / disc)	Methanol (20ml/disc)	Ampicillin (10ml/disc)
1	STATCC10749	R	R	R	12 mm	11 mm	6 mm
2	PAATCC25619	R	9 mm	14 mm	16 mm	13 mm	R
3.	STMATCC23564	R	6 mm	6 mm	14 mm	6 mm	13 mm
4	YEATCC9610	R	9 mm	15 mm	13 mm	17 mm	16 mm
5.	ECU	9 mm	10 mm	R	R	R	11 mm

R = Resistant; R = 0.0mm; ST = *Salmonella typhi*; PA = *Pseudomonas aeruginosa*;
STM = *Salmonella typhimurium*; YE = *Yersinia enterocolitica*; ECU = *E.coli uropathogen*.

Table 2. Antimicrobial activity of *Emblica officinalis* (seed extract)

S. No.	Micro Orgamisms Tested	Aqueous Extract (2mg / disc)	Cold Methanol (20µl/disc)	Distillate (20ml/disc)	Residual (2mg / disc)	Methanol control (20µl/disc)	Nystatin (10µl/disc)
1	*Rhizopus Sps*	R	8 mm	12 mm	13 mm	16 mm	29 mm
2	*C. albicans*	8 mm	10 mm	9 mm	24 mm	12 mm	27 mm
3	*Geotrichum Sps*	R	12 mm	17 mm	16 mm	12 mm	37 mm
4	*Fusarium Sps*	R	12 mm	11 mm	19 mm	19 mm	27 mm
5	*A.flavus*	R	8 mm	8 mm	15 mm	24 mm	20 mm

R - Resistant

Table 3. Zones of inhibition of concentrations of aqueous extract of *E. officinalis* of leaf and stem[10]

S. No	Microorganisms tested	Leaf	Stem
1	*S.aureus*	15 mm	11 mm
2	*B.subtilis*	10.2 mm	6.8 mm
3	*E.coli*	19 mm	19.2 mm
4	*P.vulgaris*	21.2 mm	14 mm

Table 4. Zones of inhibition of Alcoholic extract of *Emblica officinalis* of leaf and fruit[10]

S. No	Microorganisms tested	Leaf	Fruit
1	*Staphylococcus*	20 mm	25 mm
2	*E.coli*	20 mm	20 mm
3	*S.typhi*	R	R
4	*C.albicans*	25 mm	27 mm

R - Resistant

Table 5. Minimum inhibitory concentrations of residual extracts of *Emblica officinalis* seeds on Bacterial and Fungal pathogens (mg/ml)

S. No	Microorganism tested	MIC µg/ml
1	*Salmonella typhi* ATCC 10749	100
2	*Pseudomonas aeruginosa* ATCC 25619	100
3	*S.typhimurium* ATCC 23564	800
4	*Yersinia enterocolitica* ATCC 9610	800
5	*E.coli* (uropathogen)	200
6	*Rhizopus* (isolate)	800
7	*Candida albicans* ATCC 2091	200
8	*Geotrichum* (isolate)	400
9	*Fusarium* (isolate)	200
10	*A.flavus* (isolate)	50

Fusarium are sensitive showing 12mm zones of inhibition. The distillate showed zones of inhibition from 6mm to 17mm *Geotrichum* is sensitive and *S.typhimurium* is resistant. The residual extract showed zones of inhibition from 12mm to 24mm. *Candida albicans* showed maximum zone of inhibition and *S.typhi* showed least zone of inhibition. The results clearly indicate that the seeds possess potent antimicrobial compounds which can be used to kill the pathogens especially *Candida albicans* and *Pseudomonas aeruginosa* (which is commonly hospital acquired organism), the leaf and fruit showed maximum zone of inhibition against candida albicans 25mm and 27mm respectively.The active compounds have to be isolated and its efficacy has to be tested against other microbial pathogens.

CONCLUSIONS

Earlier studies showed that only the pulp, leaf and stem of the *Emblica officinalis* has been tested for its antimicrobial properties. From the present investigation it is clear that the seeds of this fruit also are active against different pathogenic microorganism which shows that the whole fruit can be used in isolating the bioactive compounds.

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REFERENCES

1. Ritch-kro, E.M, Turner, N.J and Towers, G.H., Carrier herbal medicine: an evaluation of the antimicrobial and anticancer activity in some frequently used remedies *J. Ethno Pharmacol.* 1996; **5**: 151-156.
2. Colombo, M.L., and Bosisio, E., Pharmacological activities of C*helidonium magus* L (Papaveraceae). *Pharmacol. Res*. 1996; **33**: 127-134.
3. Rastogi, Pand Mehrotra, B.N., Compendium of Indian medicinal plants, Drug research perspective, CDRI Lucknow and NISCOM, New Delhi, 1999; **2**: 1-859.
4. Chung, K.T. Wong, T.Y., Wei, C.I., Huang, Y.W. and Llin,Y., Tannins and human health: a review, *Crit. Rev. Food Science Nutr*. 1998**; 38**: 421-464.
5. Dr. M.R.Uniyal; Director (Drugs) Maharishi Ayurved products NEP2. Noida effective Ayurvedic Medicinal plants used in rasayan therapy.
6. T.Srinivasu, S.N.Pathan* and S.N.Pardfsh: Biodiversity of medicinal plants-Mumbai railway track-sides, INDIA. *Asian Jr. of microbial. Biotech. Env. Sc.* 2004; **6**(4): 625-633.
7. C..J.Khilare and S.E.Saindanshiv. Servey of medicinal plants and their conservation for sustainable health, Hygiene and Environment. *India Journal of Environ. & Ecoplan*, 2004; **8**(3): 737-740.
8. Cruck shank R, Dugrid IP, Marmoin BP and Swain RHA., Microbiologia Medica, vol-II, 4th ed. Fundacao Calouste Gulbenkian Lisboa, 1975; 393-403.
9. Elizabeth K.M., Antimicrobial activity of *Allium sativum* on some pathogenic bacteria. *Indian Journal of Microbiology.* 2001; **41**: 321-323.
10. Elizabeth K. M, Karthik K., The antimicrobial activity of *Emblica officinalis* on certain human pathogenic microorganisms. Paper presented in National symposium on Environmental strategies and action plane. Held in the Dept of Environmental sciences, Andhra University, Visakhapatnam, 2001; 10/13/2001.
11. Kumar G. P* and Chaturvedi A., Antimicrobial activity of some medicinal plants of Euphorbiaceae. *Indian drugs*, 2006; **43**(2): 156-157.