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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *EMBLICA OFFICINALS* SEED EXTRACT

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ABSTRCT

Amla has been a valuable source of natural rejuvnative herb for maintaining human health. The preliminary phytochemical and antimicrobial activity of amla leaf and bark were analyzed by researchers and its efficacy of amla fruit is widely proved. The use of amla seeds for oil extaction, to find out the biologically active compound and check its antimicrobial activity. The crude extract of amla seed showed maximum zone of inhibition in antibacterial and antifungal activity against standard drugs.

KEYWORDS: Amla, natural rejuvnative, oil extaction.

INTRODUCTION

Emblica officinals (Amla) belongs to the plant family phyllanthaceae. It is a good dietary source of vitamin C, minerals and amino acids.[1,2] Vitamin C is found to be highly stable due to the presence of tannins and polyphenols. This fruit can be used as major constituents in Ayurveda preparations.[3] The plant leaves have anti-platelet,anti-neurophilic, anti-viral, anti-mutagenic, anti-allergic and antibacterial activities.[4,5] Amla is widely used for treatment of diarrhoea , inflammatory disease, jaundice and act as glucose lowering agent in Type II diabetes. Thus all parts of plants including fruit,seed,leaves,root,bark and flowers are used in herbal preparation.[6] TE leaves of amla is also used as an anti-inflammatory and antipyretic activity, more common in Asian population. The nutritional benefits of amla can be used as beverage, candy powder,sauce etc. The present study was to extract the biological active compounds in different solvents from seeds of amla and check its antibacterial activity.

MATERIALS AND METHODS

The fruits of Emblica officinalis (amla) were purchased from local market. The fruits were cut to remove the pulp and seeds were collected. The seeds were shade dried and

mechanically crushed into pwder. The physical properties of amla seeds were analyzed in terms of pH and moisture content.

Total phenolic content

Total poly phenol was measured in seed samples by taking 250 mg of amla seeds in 10 ml of methanol and water (70:30) in a test tube and heated on a water bath at 70ºC for 10 minutes . The samples were cooled and centrifuged at 3500 rpm for 10 minutes.0.2 ml of supernatant was made up to 10ml with distilled water. 5ml of sample was mixed with 0.5 ml of saturated sodium carbonate and 0.2 ml of folin –ciocalteau reagent . The volume was made upto 10 ml with distilled water. The absorbance was read at 765nm after 1 hour by UV-visible spectrophotometer.[7]

Ascorbic Acid

Sample solution was prepared and its equivalent to 0.2 mg of standard ascorbic acid in water containing 3% metaphosphoric acid. It was titrated against standard 2,6 dichloroindophenol dye. The development of pink color was an end point. The titration was repeated to get concordant value.[8]

Phytochemical analysis

25 g of seed powder was soaked in different solvents namely benzene, methanol,ethylacetate and chloroform. This was kept for 48 hours incubation and filtered through whatman No 1 filterpaper. The seed extract was dissolved in 10ml of DMSO and stored for further use. The crude extract of different solvents were subjected to phytochemical procedures to identify the constituents as explained by Harborne.[9]

Antimicrobial activity

The crude extract of seed was tested for antibacterial and antifungal activity. Drugs like gentamycin (10µg) and DMSO used as control. Antibacterial activity of crude samples in different solvents were tested by disc diffusion technique against pathogenic organisms such as E.Coli,Staphylococus aureus, Pseudomonas aeroginosa and Klebsella pneumonia. The nutrient agar plates were inoculated with 0.1 ml of pathogenic microbes by spread plate method. The whatmann filter paper disc were sterilized and inoculated with the samples and DMSO was kept as negative control. All the plates were incubated at 30◦C for 24 hours to measure the zone of inhibition.

The crude extract of samples was also subjected to antifungal activity by similar disc diffusion method against pathogenic micro organisms like Aspergillus niger, Aspergillus fumigates and Candida tropicalis. The same procedure was followed and kept the petriplates at 37◦C for 48 hours the zone of inhibition was measured.

Minimum Bactericidal Concentration(MBC)

The concentration of seed extract that completely killed the organism was taken as MBC. Samples were taken, subcultured on freshly prepared nutrient agar plates and then were incubated at 37◦C for 48 hours .The MBC was taken as the concentration of the extract that did not show any colony formation.[10]

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration was determined by serial dilution method. Serial dilution of various concentration extract wasindividuall placed in plates. The lowest concentration of each extract in various solvents showing zero growth of bacteria after 24 hours were recorded as MIC.[11]

Relative percentage of inhibition

The relative percentage of inhibition of seed extract was compared with positive control was calculated by 100x((x-y)/(z-y)) where x=total area of inhibition of test extract; y= total area of inhibition of solvent; z= total area of inhibition of standard drug.

RESULTS AND DISCUSSION

The physical properties of pH and moisture content of amla were found to be slightly acidic and 65% .The phytochemical analysis of amla seed extract was summarized in Table 1. Amla seeds contain lot of nutritive and antinutritive compounds. Saponins, tannins,flavonoids ascorbic acid,cardiac glycosides and terpenoids were found to be higher in methanolic extract than other solvent extraction. The alkaloids, steroids,anthocyanins and anthroquinones were not present in the seed extract. The less amount of biologically active compound was extracted by benzene and chloroform(Table1).

The amount of total phenol was observed as 25.5 mg/100g of seeds. The total phenolic contents were found to be higher in juices and residues than seed.[12] The ascorbic acid in seeds also showed lower amount of 200g/100g when compared with juice(478 g/100 g. Poonam mishra reported that different processing of amla lead to change in ascorbic acid

content, 70% of antioxidant activities as percentage of inhibition of oxidation in amla fruits which correlated positively with total phenols.[6]

The different solvent extract of *Emblica officinalis* seed showed antibacterial activity against the clinical isolates of bacteria. The methanolic extract showed maximum zone of inhibition against *E.Coli* (18mm). The minimum zone of inhibition was observed in benzene extract of seed approximately 2-3mm (Table 2) . The high antifungal activity was found in ethyl actate extract especially against *Aspergillus niger.* The MIC activity of seed extract in different solvents were represented in (Fig1). MIC values of seed against all pathogenic bacteria were in the range of 25µg-50µg. The MBC activity was found to be high(250µg)in chloroform extract and resistance was observed in *K.Pneumoniae* . The maximum relative inhibition against against S.aureus(91.5%) and low(45%) in *E.Coli* All traditional plants and natural products make excellent activity for new drug development.[13]

Many pharma industries have produced a number of new antibiotics in last three decades resistance to these drugs by microorganism has increased.[14,15] These bacteria have its genetic ability to transmit and give resistance to drugs.

Table1: **Anti-Nutritional constituents of *Emblica officinals* seed extract.**

Sl.No	Phytochemicals	Benzene extract	Methanolic extract	Ethyl acetate extract	Chloroform extract
1.	Alkaloids	-	+	+	-
2	Saponins	+	++	+	+
3	Tannins	+	++	++	+
4	Steroids	-	-	-	-
5	Anthocyanines	-	-	-	-
6	Flavonoids	+	+++	+	+
7	Anthraquinones	-	-	-	-
8	Phenolic flavonoids	+	+++	++	
9	Ascorbic acid	+	+++	+	+
10	Cardiac glycosides	++	+	+	-
11	Tri-terpenoids	-	+++	++	+
12	Phlobatannins	-	-	-	-

Table2: Antibacterial activity of seed extract of *Emblica officinals* (Zone of inhibition in mm)

S.No.	Test organisms	Standard drug(10µg)	Benzene	Methanol	Ethylacetate	Chloroform
1	*E.Coli*	20	4	18	8	10
2	*S.aureus*	21	3.0	14	7.2	8
3	*K.pneumoniae*	19	3.2	17	7	7
4	*P.aeruginosa*	18	2.5	15	6	9

Table3: Antifungal activity of seed extract of *Emblica officinals* (Zone of inhibition in mm)

S.No.	Test organisms	Standard drug(10µg)	Benzene	Methanol	Ethylacetate	Chloroform
1	*Aspergillus niger*	28	6.4	20	8.5	10
2	*Aspergilus fumigates*	21	3.2	14	9	4
3	*Candida tropicalis*	19	3.0	15	10	6

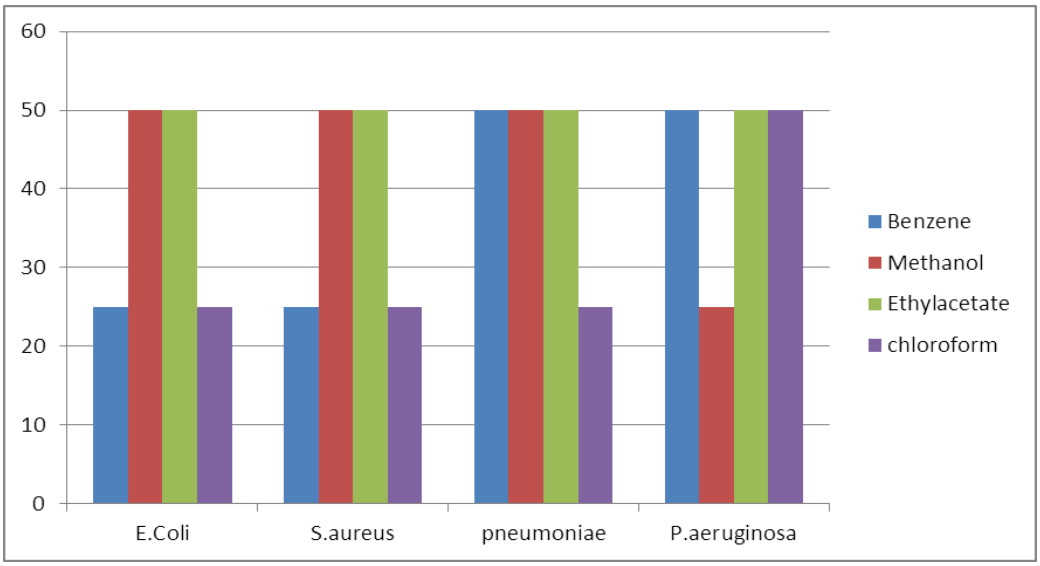

Figure1: MIC activity of seed extract of *Emblica officinals*

CONCLUSION

The present study deals that seeds of Indian gooseberry contain antibacterial and antifungal activity. The phytochemicals in amla seed were flavonoids, terpenes,tannins, and saponins. The bioactive compounds were extracted through different solvents. The polyphenols and ascorbic acids which lead to the medicinal and antioxidant activity. This therapeutic nature of seed also support the treatment of diseases and discovery of new drugs.

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