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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY ON *EMBLICA OFFICINALIS* SEED EXTRACT

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ABSTRACT
The present study was carried out to evaluate *in vitro* antibacterial and free radical scavenging activity of methanolic extract of *Emblica officinalis* seed. The antimicrobial activity was assessed against gram positive and gram negative bacteria namely *E.coli, P.aeruginosa, K.pneumoniae, S. aureus, Enterococcus* by using agar well diffusion method. The antioxidant activity of seed extract was evaluated by using the free radical scavenging activity assay i.e DPPH method, hydrogen peroxide and reducing potential method. The extract showed maximum zone of inhibition against *S. aureus* (21mm) whereas lowest against *P.aeruginosa* (17mm). MIC values of extract against *E.coli, S.aureus, K.pneumonia, P.aeruginosa and Enterococcus* were 50, 50, 50, 25 and 50 mg/ml respectively. Gentamicin was used as a standard drug. Herbal extract showed maximum relative percentage inhibition against *S. aureus* (91.11 %) and lowest relative percentage inhibition against *Enterococcus* (59.17%). Ascorbic acid was used as the standard. The extract showed good radical scavenging activity. IC_{50} values for methanolic extract of *Emblica officinalis* for DPPH and H_2O_2 were found to be 15ug/ml and 32ug/ml and for ascorbic acid was found to be 12ug/ml for both DPPH and hydrogen peroxide method respectively. The result of present study conclude that seeds of *E. officinalis* contain high antibacterial and antioxidant property and can be further explored for the isolation of its bioactive compound.
Keywords: Antibacterial, Antioxidant, *Emblica officinalis* seed.

INTRODUCTION

Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential[1-3]. Pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate use of antimicrobial drugs to treat the infectious diseases and also more toxic for human being during long term therapy[4,5]. So there is a need for less toxic, more potent and non anti-infectives antibiotics[6]. Therefore the use of plant extracts and phytochemicals with known antibacterial properties may be of immense importance in therapeutic treatments[7].

About 5% or more of the inhaled oxygen (O_2) is converted to reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and OH by univalent reduction of oxygen[8,9]. However, over-production of the ROS arising from either mitochondrial electron transport chain, excessive stimulation of NAD(P)H, or exposure to environmental pollutants, cigarette smoke, ultraviolet rays, some parasitic infections, radiation and toxic chemicals results in oxidative stress. Equilibrium status of pro-oxidant/antioxidants reactions is disturbed during oxidative stress in living systems, which mediates damage to cell structures, including lipids and membranes, proteins, and DNA[10]. So there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical- induced tissue injury. Thus, compounds or antioxidants that can scavenge free radicals have a vital role in the improvement of many diseased conditions[11].

Emblica officinalis (amla) belongs to family Euphorbiaceae. It is one of the most often used herb in Ayurveda. It has a reputation as a powerful rejuvenating herb. Amla is a medium-sized deciduous tree with gray bark and reddish wood. It is native to tropical southeastern Asia, particularly in central and southern India, Pakistan, Bangladesh and Sri Lanka. It is commonly cultivated in gardens throughout India and grown commercially as a medicinal fruit. Amla fruit is reputed to have the highest content of vitamin C. The major chemical constituents of amla are phyllemblin, ascorbic acid (vitamin c), gallic acid, tannins, pectin etc[12]. It is also a very important ingredient in the famous chyavanaprash, and a constituent of triphala (three fruits) powder. It is an ingredient of many Ayurvedic medicines and tonics, as it removes excessive salivation, nausea, vomiting, giddiness, spermatorrhoea, internal body heat and menstrual disorder and is an excellent liver tonic. It is useful in treatment of diabetes, asthma, bronchitis, jaundice, ulcerative stomatitis, diarrhoea, cough, leucorrhoea, inflammations, hepatomegaly, skin disease, greying of hair and also has as an antisenescent activity[13,14].

MATERIAL AND METHOD

Chemicals

Agar, 1,1-Diphenyl-2-picryl-hydrazil (DPPH), Hydrogen peroxide, phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, methanol, ascorbic acid.

Test organisms

Clinical isolated of pathogenic microbial strains gram negative and gram positive namely *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus* were collected from the Department of Microbiology MMISMR, Maharishi Markandeshwar

University, Mullana, Haryana, India. All these cultures were maintained on nutrient agar plates at 4°C.

Preparation *Emblica officinalis* Seed extract

The fruits of *Emblica officinalis* were purchased from local market, Ambala. The fruits were boiled at 70°C and pulps were separated to take out the hard seeds. The seeds were shade dried (2.5 kg) and were mechanically crushed and extracted with different solvents i.e. petroleum ether, chloroform, methanol and distilled water (65°C) using Soxhlet up to 72 h. The extract was filtered and concentrated in rotatory evaporator at 45-50°C under reduced pressure. The semisolid material, which was then lyophilized to get a powder (yield 12.3% w/w)[15,16].

Evaluation of Antimicrobial Activity

Agar well diffusion

The antimicrobial activity was tested against methanolic seed extract of *Emblica officinalis*. The inoculation of microorganism was prepared from bacterial culture. About 15 to 20 ml of Muller-Hinton agar medium was poured in the sterilized petri dishes and allowed to solidify. Bacterial strains were spread over the medium by a rod. Wells of 7 mm in diameter and about 2 cm apart were punctured in the culture medium using sterile cork borers. One ml extract of different concentrations i.e 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml of the *E.officinalis* seed extract were added to the wells. Plates were incubated in autoclave at 37°C for 24 h. Antimicrobial activities were evaluated by measuring the inhibition zone diameters. The inhibition zones with diameter less than 5 mm were considered as having no antibacterial activity. Gentamicin (10 µg/ml) was used as standard drug[17].

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentrations (MIC) was determined, using serial dilution method. Serial dilutions of various concentrations extract was individually placed in plates. Extract concentrations of 6.25mg/ml, 12.5mg/ml, 25mg/ml, 50mg/ml were used in the exercise. The lowest concentration of extract in each treatment, showing zero growth of bacteria after 24hrs were recorded as the MIC[18-20]. The lowest concentration of the extract that completely inhibited bacterial growth in comparison to control was regarded as MIC.

Determination of Minimum bactericidal concentration (MBC)

The concentration of plant extract that completely killed the organism was taken as MBC. Samples were taken and subcultured on freshly prepared nutrient agar plates, and then were incubated at 37°C for 48 h for bacteria. The MBC was taken as the concentration of the extract that did not show any colony formation or growth on a new set of agar plates[21].

Determination of Relative Percentage Inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula[22,23].

$$\frac{100 \times (x - y)}{(z - y)}$$

Where,

x: Total area of inhibition of the test extract

y: Total area of inhibition of the solvent

z: Total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2; where, r = radius of zone of inhibition.

Evaluation of free radical scavenging activity

DPPH Radical Scavenging Activity

The free radical scavenging activity was determined according to the method of Shimada *et al*. The extract was dissolved with methanol to prepare various sample solutions at 10, 20, 40, 60, 80 and 100ug/mL. 2 mL of extract solution was mixed with 1 mL of 0.2 mM DPPH in methanol. The mixture was shaken vigorously and maintained for 30 min in the dark. The absorbance was measured using at 517nm[24]. The % reduction in absorbance was calculated from the control and sample absorbance at each level by using the following formula;

% Reduction = (Ac - As)/Ac ×100

Ac = Control Absorbance

As = Sample Absorbance

Hydroxyl Radical Scavenging Activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. Solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (10-100 µg/ml) in distilled water were added to solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide[25]. The percentage of hydrogen peroxide scavenging by extracts and standard compounds was calculated as follows:

% Scavenged $[H_2O_2] = [(Ao - A1)/Ao] \times 100$

where Ao = absorbance of the control and

A1 = absorbance in the presence of the sample of extract and standard

Reducing power ability

The reducing power of the extract was determined according to the Vani *et al*[26]. Various concentrations of the extract (10-100 µg/ml) in 1.0 ml of demonized water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared $fecl_3$ solution (0.5 ml, 1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power[26].

Table 1: Sensitivity test for the bacterial species on the methanolic seed extract of *Emblica officinalis*

Test organisms	zone of inhibition (mm)				
mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	Standard (10ug/ml)
E.coli	12	17	18	19	21
S.aureus	12.5	15	18.5	21	22
K.pneumoniae	13.5	16	17	19	20
P.aeruginosa	14	15	16	17	18
Enterococcus	11	16	18	20	26

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the methanolic seed extract of *Emblica officinalis*

Bacterial species	*E.coli*	*S.aureus*	*K.pneumoniae*	*P.aeruginosa*	*Enterococcus*
Concentration for MIC (ug/ml)	50	50	50	25	50
Concentration for MBC (ug/ml)	200	100	Resistance on all doses + standard	200	150

Table 3: Relative percentage inhibition of *Emblica officinalis* seed extract

Test organism	Relative % inhibition
E.coli	81.8
S.aureus	91.11
K.pneumoniae	90.25
P.aeruginosa	89.19
Enterococcus	59.17

Table 4: Percentage reductions in absorbance of DPPH at 517 nm by methanolic seed extract of *Emblica officinalis* [MSEEO] and Ascorbic acid

Concentration (µg/ml)	% Reduction of MSEEO	% Reduction of ascorbic acid
10	47.12	48.46
20	53.14	58.50
40	61.12	64.12
60	71.75	73.36
80	76.97	81.12
100	85.94	88.62
IC_{50} (ug/ml)	15	12

Table 5: Percentage reductions of H_2O_2 by adding methanolic seed extract of *Emblica officinalis* [MSEEO] and Ascorbic acid

Concentration (µg/ml)	% Reduction of MSEEO	% Reduction of ascorbic acid
10	19.78	48.15
20	42.43	58.13
40	54.63	64.57
60	76.74	77.60
80	88.01	89.62
100	93.98	94.34
IC_{50} (ug/ml)	32	12

Table 6: Percentage increase in absorbance of reducing power method at 700 nm by adding methanolic seed extract of *Emblica officinalis* [MSEEO] and Ascorbic acid

Concentration (µg/ml)	Absorbance of MSEEO	Absorbance of ascorbic acid
10	1.755	1.815
20	1.867	1.890
40	1.917	1.956
60	1.978	1.984
80	2.010	2.029
100	2.040	2.091

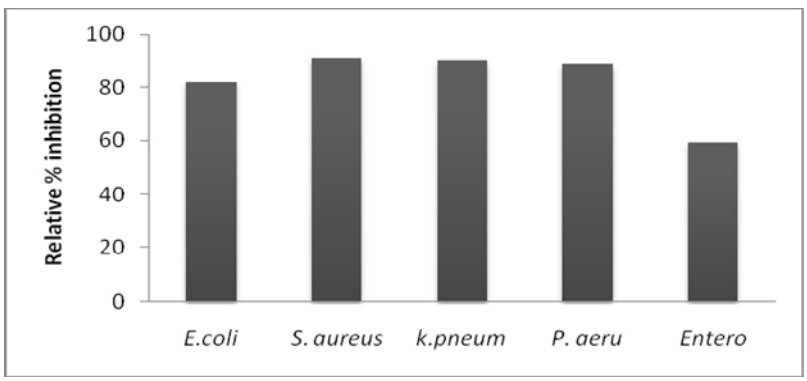

Figure 1: Relative percentage inhibiton of *Emblica officinalis*

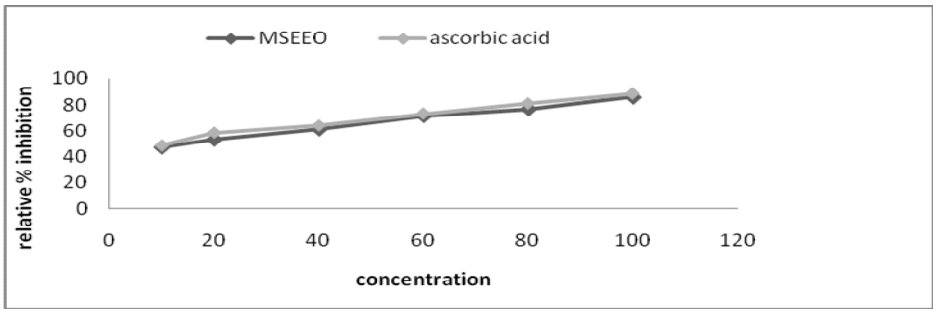

Figure 2: Percentage Inhibition of MSEEO and ascorbic acid at 517nm

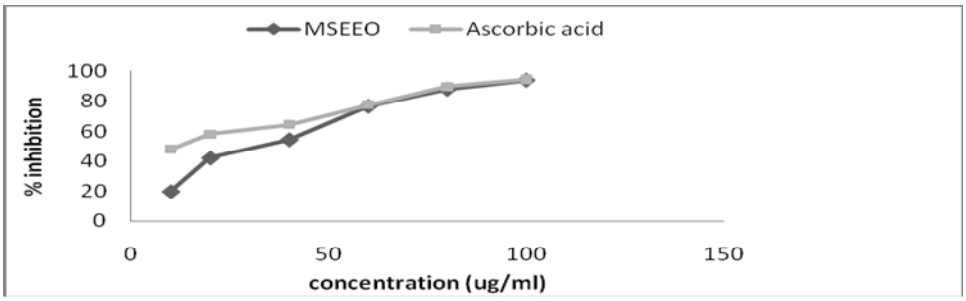

Figure 3: Percentage inhibition of MSEEO and ascorbic acid

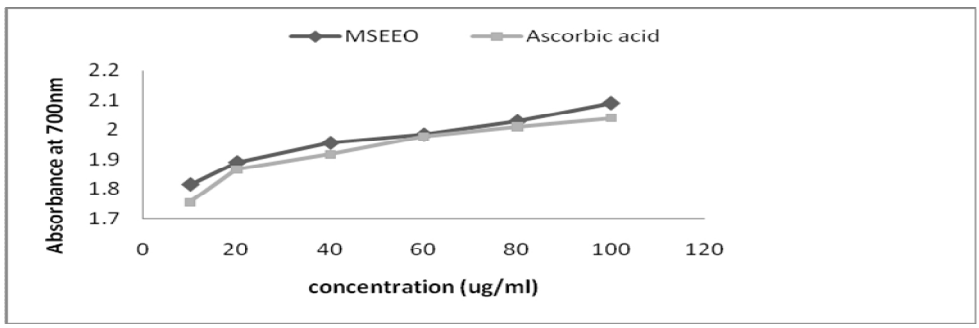

Figure 4: Absorbance of MSEEO and ascorbic acid by reduction potential at 700nm

RESULTS

Antimicrobial Activity

The methanolic extract of *Emblica officinalis* seed exhibited the antibacterial activity against five clinical isolates of bacteria namely *E.coli, P.aeruginosa, K.pneumoniae, S. aureus, Enterococcus*. The extract showed maximum zone of inhibition against *S. aureus* (21mm), whereas, lowest against *P. aeruginosa* (17mm) [Table 1]. Results of MIC and MBC are reported in table-2. MIC values of extract *against E. coli, S. aureus,K. pneumonia, P.aeruginosa* and *Enterococcus* were 50, 50, 50, 25 and 50 mg/ml and for MBC are 200, 100, resistant for *K.pneumoniae* , 200 and 150 mg/ml respectively [Table 2].

The results of antimicrobial activity of methanolic seed extract of *Emblica officinalis* was compared with the standard drug gentamicin. Methanolic seed extract of *Emblica officinalis* extract showed maximum relative percentage inhibition against *S. aureus* (91.11 %) and lowest relative percentage inhibition against *Enterococcus* (59.17 %) [Table 3 and Figure1]

Antioxidant activity

DPPH Radical Scavenging Activity

The methanolic seed extract of *Emblica officinalis* [MSEEO] showed promising free radical scavenging effect of DPPH in a concentration dependant manner. The reference standard ascorbic acid also demonstrated a significant radical scavenging potential. The IC_{50} values for MSEEO is 15ug/ml and 12ug/ml for ascorbic acid [Table 4]. The percentage reduction values for MSEEO and ascorbic acid are plotted in figure 2 respectively.

Scavenging of Hydrogen Peroxide

The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. The IC_{50} values for methanolic seed extract of *E.officinalis* and ascorbic acid were 32ug/ml and 12 ug/ml respectively [Table 5].The percentage reduction values for MSEEO and ascorbic acid are plotted in figure 3 respectively.

Reducing Power

The absorbance of reducing potential of methanolic seed extract of *Emblica officinalis* (sample) and ascorbic acid increases with increasing concentration. The results are shown in Table 6 and Figure 4.

DISCUSSION

Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development[27]. Approximately 80% of the word inhabitants rely on traditional medicine for their primary health care and play an important role in the health care system of the remaining 20% of the population[28]. The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health programs. Even though, pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganism has increased [29]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents[30].The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in future is still uncertain. Therefore actions must be taken to reduce this problem.

In the present study, in DPPH method, a freshly prepared DPPH solution is used that exhibits a deep purple colour with absorption maximum at 517nm. The purple colour generally fades or disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them to a colorless (i.e., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract[31,32]. Scavenging of hydrogen peroxide by extracts may be attributed to their phenolic compounds, which can donate electrons to hydrogen peroxide thus neutralizing it to water[33]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with the presence of reductones, which breaks the free radical chain by donating a hydrogen atom. The extract had reductive ability which increased with increasing concentrations of the extract[34].

CONCLUSION

The present study concludes that seeds of *Emblica officinalis* contain high antibacterial and antioxidant property. The presence of phytochemicals like flavonoids, terpenes, tannins, saponin glycosides, pregnanes and phenolic compounds are major constituents in *Emblica officinalis* which may acknowledge the medicinal as well as antioxidant property of this plant.[35] This implies that the plant extract may indeed be effective in the management of diseases caused by these organisms, supporting its ethnomedicinal uses; thus the plant may be presented as potential source of novel antimicrobial drugs. There is need for further investigation of this plant in order to identify and isolate its active antibacterial and antioxidant principle(s).

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