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Evaluation of Antioxidant and Antibacterial Activities of *Emblica officinalis* (Amla)

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A B S T R A C T

E. officinalis (Amla) enjoys a holy position in Ayurveda-an Indian indigenous system of medicine. Hence, in the current study we aimed for determination of antioxidant and antibacterial potential of *Emblica officinalis* (Amla). Leaves of *E. officinalis* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with double distilled water. Antioxidant assay was carried out by *in-vitro* model using DPPH free radical scavenging activity. *In-vitro* antibacterial activity of aqueous (Aq.) extract of leaves of *E. officinalis* (Amla) was assessed using agar well diffusion method with Chloramphenicol as positive control and Dimethyl sulfoxide (DMSO) as negative control. The zones of inhibition after 48 hours of was measured in millimeters (mm). Results delineated that Aq. extract of leaves of *E. officinalis* (Amla) showed a dose dependent DPPH scavenging activity. Furthermore, the DPPH free radical scavenging activity of Aq. extract of leaves of *E. officinalis* (Amla) were comparable with that of standard ascorbic acid. In addition, Aq. extract of leaves of *E. officinalis* (Amla) possess potential antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsella pneumoniae*, and methicillin resistant *Staphylococcus aureus* (MRSA). In conclusion, *E. officinalis* could be used alternative to synthetic antioxidants and antimicrobials since Aq. extract of leaves of *E. officinalis* mimic the biological activities of synthetic antioxidant and antimicrobials.

Introduction

Plants have provided mankind with herbal remedies for many diseases for many centuries and even today. They continue to play a major role in primary healthcare as therapeutic remedies in developing countries. In India, herbal medicines have been the basis of treatment and cure for various diseases in traditional methods practiced such as

Ayurveda, Unani and Sidha (Ahmad *et al.,* 1998). Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect the plant from various diseases. Many plants species are reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes which is therefore, should be utilized to combat the disease-causing pathogens (Kamali and Amir, 2010; Lalitha *et al.,* 2012; Hussain *et al.,* 2011). Medicinal plants are being used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world.

Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs (Pavithra *et al.,* 2010; Zgoda and Porter, 2001).

Free radicals form in our body as a result of biological oxidation. Oxidation is a natural process in organisms to produce energy to fuel biological cycles. Oxidation by-products of normal metabolism cause extensive damage to DNA, protein, and lipids, constituting a major contribution to ageing and to degenerative disease. Oxidative damage is associated with chronic degenerative diseases, including cancer, coronary artery disease, hypertension, and diabetes (Upadhyay *et al.,* 2013). An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism (Miller *et al.,* 2000). Antioxidants occur naturally in many fruits and are able to neutralize free radicals by donating an electron and convert them into harmless molecules (Makris *et al.,* 2007).

Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from the synthetic products (Sharma, 2011). With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs (Preethi *et al.,* 2010). This is because of the emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics (Enne *et al.,* 2001; Westh *et al.,* 2004). Pharmacological industries have shaped several new antibiotics and in the last three decades resistance to these drugs by microorganisms has increased. In general, bacteria are the microorganisms which have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen *et al.,* 1992). Antibiotic resistance has increased substantially in the recent years and is posing an ever-increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants (Kim *et al.,* 1995; Alagesaboopathi *et al.,* 2011).

Emblica officinalis (Amla) enjoys a hallowed position in Ayurveda- an Indian indigenous system of medicine. According to believe in ancient Indian mythology, it is the first tree to be created in the universe (Ahmad *et al.,* 1998). *E. officinalis* (Amla) belongs to family Euphorbiaceae. It is also named as *Phyllanthus Emblica* or Indian gooseberry, Amla, Nelli, Amalaki.

The species is native to India and also grows in tropical and subtropical regions including Srilanka, South East Asia, China, Pakistan, Uzbekistan, and Malaysia. It is a deciduous tree, 8-15 m tall, with alternate, subsessile

leaves and greenish to creamy-yellow, unisexual, actinomorphic, trimerous flowers (Figure 1) (Khan *et al.,* 2009; Warrier *et al.,* 1995). Extracts of various plant parts of *E. officinalis* such as leaves, stem, root, seeds and fruits have been widely used in treatment of various diseases (Varghese *et al.,* 2013). *E. officinalis* plant extracts revealed antibacterial / antifungal (Hossain *et al.,* 2012), antioxidant (Golechha *et al.,* 2012), and cardioprotective properties (Bhattacharya *et al.,* 2002). With this scenario, we designed the current with the objectives of evaluation of antioxidant and antibacterial potential of *E. officinalis* (Amla).

Materials and Methods

Collection of plant material

The leaves of *E. officinalis* (Amla) were collected from Amla plant located around Chikkaballapura, Karnataka, India and washed with tap water for several times and then once with double distilled water. After washing, leaves were shade dried at room temperature and then grounded to fine powder. Powdered sample was stored for further use.

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *E. officinalis* (Amla) were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of double distilled water. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator and dried at 40ºC. The extracts were preserved in airtight containers and stored at room temperature until further use.

Antioxidant Assay

The modified literature protocol of Blois was used for antioxidant assay (Blois, 1958; Uddin *et al.,* 2012). Briefly 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-100ug) in methanol. The control was also run which contains only methanol. The hydrogen atom or electron donation abilities of extract and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated by using the formula; DPPH% = (Control abs – Extract abs / Control) × 100. The IC_{50} value was determined by using linear regression equation *i.e.,* Y = Mx + C; Here, Y = 50, M and C values were derived from the linear graph trendline.

Evaluation of Antibacterial Activity

Pathogenic Microorganisms

The multiple antibiotic-resistant isolates *viz. Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae,* and methicillin resistant *Staphylococcus aureus* (MRSA) were isolated from clinical samples of local hospital in and around Chikkaballapura and confirmed by various microscopic evaluation like Gram's staining (Gram, 1884). Motility, capsule and spore formation was confirmed as per the procedure prescribed by Collins and Lyne (1970). All the bacterial pathogens were further confirmed by suitable biochemical tests, (Barrow and Feltham, 1993) and used for antimicrobial activity studies.

The direct colony suspension method is the most convenient method for inoculum preparation. The inoculum was prepared by making a direct broth or saline suspension of

isolated colonies selected from an agar plate. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 × 10^8 colony-forming units (CFU)/mL. To perform this step accurately, used adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, the pathogenic bacteria culture was inoculated into culture plates to screen for antibacterial properties.

Determination of Antibacterial Activities

Antibacterial activities of plant extracts were tested by agar well diffusion method.(Khyade and Vaikos, 2009) The culture plates were prepared by pouring 20 ml of sterile Muller Hinton agar (MHA). 1 ml inoculums suspension was spread uniformly over the agar medium using a sterile glass rod to get uniform distribution of bacteria. A sterile cork borer (6 mm) was used to make wells in each plate for extracts. These plates were labeled and each plant extracts (at a concentration of 12.5, 25, 50, and 100 mg/ml) were added aseptically into the well. Also, 5% DMSO and chloramphenicol (10 µg) were used as negative and positive control respectively. Plates containing drug were left for one hour in order to diffuse properly in media and to get dry.

Then the plates were incubated for 24h at 37°C during which the activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of the diameter of the inhibition zones (mm) produced by the plant extracts when compared to the controls.

Results and Discussion

Antioxidant Activities

DPPH is often used to determine free radical scavenging activity of natural compounds due to its stability as a radical.(Abbes *et al.*, 2013) The presence of unpaired electron imparts a strong absorbance at 517 nm, giving the radical a purple color. With the exposure to antioxidants, it undergoes reduction, decreasing absorbance due to the formation of yellow colored anti-radical diphenyl picryl hydrazine (DPPH). The degree of colour change from purple to yellow is a measure of scavenging potential of the antioxidants in the extracts in terms of hydrogen donating ability.(Sre *et al.*, 1696-1700) The Aq. extract of leaves of *E. officinalis* (Amla) showed scavenging activity against the free radicals. The Aq. extract of leaves of *E. officinalis* (Amla) showed a dose dependent DPPH scavenging activity. It showed the highest scavenging activity (47.60%) at 10 mg/mL and lowest (15.31%) at 2 mg/mL. Furthermore, the DPPH free radical scavenging activity of Aq. extract of leaves of *E. officinalis* (Amla) were comparable with that of standard ascorbic acid (Table 1).

The results antioxidant activities of Aq. extract of leaves of *E. officinalis* (Amla) observed in our study were comparable with the various other studies reported in the literature. Sumalatha (2013) has observed that *E. officinalis* extract showed high scavenging activity with 71.75% inhibition in comparison to the standard ascorbic acid (Sumalatha, 2013). Furthermore, in another study carried out by Gulcin (2006) reported dose dependent antioxidant activities in the extracts of *Bacopa monnieri* and *Centella asiatica* at different concentrations (Gulçin, 2006). Scavenging of DPPH by Aq. extract of leaves of *E. officinalis* (Amla) may be accredited to their phenolic compounds (Nabavi *et al.*, 2008). 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 Aq. extract of leaves of *E. officinalis* (Amla) had reductive ability which increased with increasing concentrations of the extract (Orech *et al.,* 2005).

Antibacterial Activities

The enormous heritage of vast natural, time-tested medicinal resources are worth exploring the possibility of developing efficient, economically viable, and clinically acceptable antimicrobials for human application. *E. officinalis* (Amla) is one among them enjoys a hallowed position in Ayurveda-an Indian indigenous system of medicine. According to believe in ancient Indian mythology, it is the first tree to be created in the universe (Ahmad *et al.,* 1998). The antimicrobial activity of Aq. extract of leaves of *E. officinalis* (Amla) is tested against different bacterial strain *viz.* E. coli, *P. aeruginosa, S. aureus, K. pneumoniae,* and methicillin resistant *Staphylococcus aureus* (MRSA). All the bacterial strain were treated with different concentration of Aq. extract of leaves of *E. officinalis* (12.5 mg/mL, 25 mg/ mL, 50 mg/ mL, and 100 mg/ mL) and 5% DMSO as negative control and 10 microgram chloramphenicol as positive control. The results of antibacterial activities of Aq. extract of leaves of *E. officinalis* (Amla) was tabulated in Table 2. Results depicted that highest zone of inhibition (12.5 mm) was observed against *S. aureus* and lowest zone of inhibition (8.5 mm) was observed against MRSA at 100 mg/mL.

At 50 mg/mL the highest zone of inhibition (9.5 mm) was observed against *S. aureus* and lowest zone of inhibition (6.5 mm) was observed against MRSA. Whereas the highest zone of inhibition (8.0 mm) was seen against *S. aureus* and lowest zone of inhibition (5.0 mm) against MRSA at 25 mg/mL. At 12.5 mg/mL the highest zone of inhibition (6.0 mm) was observed against *S. aureus* and lowest zone of inhibition (3.0 mm) was observed against *MRSA.* The reference standard Chloramphenicol showed highest zone of inhibition (23.5 mm) against MRSA and the lowest zone of inhibition (17.5 mm) against at *K. pneumoniae.* These findings depicted that the tested Aq. extract of leaves of *E. officinalis* (Amla) possess potential antibacterial activity against *E. coli, P. aeruginosa, S. aureus, K. pneumoniae,* and MRSA.

The results of antibacterial activities exhibited in our study findings by Aq. extract of leaves of *E. officinalis* (Amla) were comparable with literature findings reported by various other research investigators. Javale and Sabnis (2010) reported that *Emblica officinalis* possess potent antibacterial activity against *E. coli, K. ozaenae, K. pneumoniae, Proteus mirabilis, P. aeruginosa, S. paratyphi A, S. paratyphi B* and *Serratia marcescens* (Javale P, Sabnis, 2010). Varghese *et al.,* (2013) reported that fruit, seed, stem and leaf extracts of *E. officinalis* is known to possess varying antimicrobial activity against different pathogens (Varghese *et al.,* 2013). According to Biswas *et al.,* (2011) the bactericidal activity of *E. officinalis* could be ascribed to the phytochemical components present in it namely flavonoids, ascorbic acid, gallic acid, alkaloids, and hydrolysable tannins (Biswas *et al.,* 2011).

Antibiotic resistance among bacteria caused by resistant strains is increasingly posing a threat to public health on a global scale. It is crucial to conduct research to examine the biological effects of medicinal plants against different pathogenic organisms and to discover novel antimicrobial compounds.

Table.1 Antioxidant activity of Aq. extract of leaves of *E. officinalis* (Amla)

Concentration of extract (mg/mL)	DPPH Scavenging Activity (%)	Concentration of Ascorbic Acid (mg/mL)	DPPH Scavenging Activity (%)
2	15.31	2	**28.23**
4	26.11	4	**49.21**
6	31.01	6	**56.18**
8	39.69	8	**71.91**
10	**47.60**	**10**	**83.23**

Table.2 Antibacterial activities of Aq. extract of leaves of *E. officinalis* (Amla)

Pathogenic Bacterial Strains	Zone of inhibition(mm)					
			Aq. extract of leaves of *E. officinalis* (Amla)			
	Negative Control	Positive Control	12.5 mg/mL	25 mg/mL	50 mg/mL	**100 mg/mL**
E. coli	-	18.0	4.0	7.5	8.0	**12.0**
P. aeruginosa	-	19.5	5.5	6.5	8.5	**10.5**
S. aureus	-	21.5	6.0	8.0	9.5	**12.5**
K.	-	17.5	4.5	7.0	9.0	**11.5**
MRSA	-	**23.5**	**3.0**	**5.0**	**6.5**	**8.5**

Fig.1 Showing *E. officinalis* (Amla) plant

The results of the current study made it clear that *E. officinalis* has strong antimicrobial effects against pathogens as well as antioxidant properties. Therefore, this preliminary study supplies as evidence-based study for *E. officinalis* could be used alternative to synthetic antioxidants and antimicrobials since Aq. extract of leaves of *E. officinalis* mimic the biological activities of synthetic antioxidant and antimicrobials.

However, dosage and safety & toxicity studies are recommended to carry out *in-vivo* for successful therapeutic modality.

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