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in vitro Evaluation of Leaves and Fruits of *Elaeagnus latifolia* L. for Antioxidant and Antimicrobial Activities

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In this work, we have studied the antioxidant and antimicrobial potential of leaves and fruits of *Elaeagnus latifolia* L. Antioxidant activities were conducted by using hydrogen peroxide radical scavenging and DPPH method. Total phenolic and flavonoid content was measured by using gallic acid and quercetin equivalent. *in vitro* Antibacterial activity of these plants was examined against five pathogenic bacteria. Highest DPPH scavenging activity was shown by methanolic extract of the flower of *Elaeagnus latifolia* with an IC_{50} value of 144.64 ± 0.25 μg mL^{-1} in comparision to standard ascorbic acid with an IC_{50} value of 29.39 ± 7.11 μg mL^{-1}. Highest hydrogen peroxide radical scavenging activity was shown by the leaves of *Elaeagnus latifolia* with an IC_{50} value of 444.59 ± 3.77 μg mL^{-1}, whereas IC_{50} value of standard ascorbic acid is 279.89 ± 1.81 μg mL^{-1}. The leaves of *Elaeagnus latifolia* showed highest total phenolic and flavonoid content with 61.15 ± 1.23 μg mL^{-1} and 15.12 ± 0.125 μg mL^{-1}, respectively. The result of antimicrobial study showed that the plant is potent against the tested organism which is comparable to standard drug ofloxacin. From the findings, it can be interpreted that the plant could be utilized as natural source of antioxidant in food processing or in medicine industry.

Keywords: *Elaeagnus latifolia* L., Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Antioxidants are the substances which delay, prevent or even inhibit the oxidation of oxidizable substrate/system in which they are present, by inhibiting the propagation or initiation of oxidative chain reactions [1]. They reduce themselves, thus acts as an antioxidant. Oxidizable substrate means very nearly everything found in the living cells counting proteins, lipids, DNA and carbohydrates [2]. Antioxidants possess free radical chain reaction breaking properties thus defend living cells against oxidative damage. They help in reducing and quenching of singlet oxygen formation and function as radical scanvengers [3-5]. Antioxidant activity is indispensable for life to neutralize the strongly oxidizing environment present inside our body. The most interesting point about free radical caused cell injury is that we can combat against this physiological pathway, by doing some modifications in our food habits. Generally antioxidants mainly from fruits and vegetables are regarded as a richest reservoir of natural antioxidant compounds [6-10].

Due to extensive misuse of antimicrobial drugs, microbial resistance problem is gradually growing over the decades. As a result the antibiotics are becoming less effective which cause havoc in global healthcare worldwide. Therefore, proper research should be done to minimize the spreading and development of resistance and to create newer options for treatment of microorganisms. From historic times herbs have been a valuable source of therapeutically potent natural molecules for maintaining human health. Even the pharmaceutical companies focused on drug discovery from natural sources. A large number of medicinal plants are claimed to be useful in bacterial diseases in all traditional system of medicine and folklore [11-13]. Therefore, a comprehensive study of such plants should be investigated to produce a drug lead. As a part of search for new biological activity of a plant extract, preliminary bioscreening were performed to study the antimicrobial activity of methanolic extracts of leaf and fruit of *Elaeagnus latifolia* L.

The plant *Elaeagnus latifolia* L. is a thorny scandent shrub. Shoots are reddish brown colored with scales. Spines are

present in stems and branches. Leaves are 3-9 cm long and 2-5 cm broad, elliptic to obovate or elliptic-lanceolate, acute or rounded at base. The leaves are green coloured and characteristic silvery-white at the beneath while the flowers are silver coloured. The fruits are green at early stage and dark pink/orange colour at ripening. Seeds are 1.8 cm long, erect, hard and shining. It flowers during September-December and the light pink coloured fruits are harvested during March-April month. They are swamps and grow at the elevations of 1500 metres in the Himalayas. In North East India, it is found in Naga hills (Nagaland state), Sibsagar, Lakhimpur (Assam state); Khasi, Garo and Jaintia hills of Meghalaya state upto elevation of 1500 m altitude [11-13].

The fruit is acidic in nature and eaten as raw or cooked. They contain vitamins and minerals, flavonoids and a good source of essential fatty acids while the flowers are used as astringent. The plant is investigated as a good source of food plant inhibiting growth of cancer cells [14,15]. This current research communication deals with the study of antimicrobial and antioxidant activity of leaves and fruits of *Elaeagnus latifolia* L.

EXPERIMENTAL

The plant *Elaeagnus latifolia* L. was collected from Moidamia village, North Lakhimpur, India in the month of February, 2017. The plant was identified by Botanical survey of India, Eastern Regional Circle, Shillong. A herbarium specimen No. ID-HS-BT-PS-PL-E was sent to BSI eastern regional Centre, Shillong for identification of the plant species. After collecting the fruits and leaves, these were allowed to dry for 10-15 days in shady area under cool condition preventing from sunlight.

Three Gram-positive and two Gram-negative strains of bacteria were selected for *in vitro* antibacterial screening. These strains were obtained from Department of Sciences, Dibrugarh University, Dibrugarh, India.

Preparation of methanolic extract: The leaves and fruit of *Elaeagnus latifolia* L. were dried, grounded in a grinder to coarse powder and stored in airtight containers to prevent moisture. The methanolic extract of leaves of *Elaeagnus latifolia* (MELEL) and methanolic extract of fruits of *Elaeagnus latifolia* (MEFEL) were done by Soxhelation with methanol after pre-treatment with petroleum ether. The concentrated extract was dried under vacuum and stored.

Antioxidant activity

DPPH radical scavenging activity: DPPH radical scavenging activity of methanolic leaves and fruit extract of *Elaeagnus latifolia* was measured with UV at 517 nm. All determinations were performed in triplicate [16]. The following formula was used to measure capability of extract to scavenge the DPPH radical:

$$\text{Inhibition of DPPH scavenging activity (\%)} = \frac{(A_o - A_t)}{A_o} \times 100 \quad (1)$$

where, A_o was the absorbance of the control and A_t was the absorbance of test/standard.

Hydrogen peroxide radical scavenging activity: The hydrogen peroxide radical scavenging activity of the extract was determined as the percentage inhibition of hydrogen peroxide radical by the extract. The UV absorbance was measured at 230 nm [17]. All determinations were performed in triplicate. Hydrogen peroxide radical scavenging activity was calculated using the following formula:

$$\text{Inhibition of } H_2O_2 \text{ free radical scavenging activity (\%)} = \frac{(A_o - A_t)}{A_o} \times 100 \quad (2)$$

where, A_o was the absorbance of control and A_t was the absorbance of test/ standard. Antioxidant activity of extract was expressed as IC_{50} value.

Total phenolic content: In this experiment, gallic acid is used as a standard phenolic compound. From the standard curve of gallic acid, phenolic content was measured and expressed in gallic acid equivalents (GAE) [18]:

$$T = C \times VM \quad (3)$$

where, T= total phenolic contents (mg g^{-1}) plant extract in gallic acid equivalent (GAE), C = concentration (mg g^{-1}) of gallic acid obtained from calibration curve, V = volume of extract (mL), M = weight (mg) of methanolic plant extract.

Total flavonoid content: In this experiment, quercetin is used as a standard flavanoid compound. From the standard curve of quercetin, flavonid content was measured and expressed in quercetin equivalent by using the standard quercetin graph [18] and using the formula

$$T = C \times VM \quad (4)$$

where, T = total flavonoid content (mg g^{-1}) plant extract in quercetin equivalent (QE), C = concentration (mg g^{-1}) of quercetin obtained from calibration curve, V = volume of extract (mL), M = weight (mg) of methanolic plant extract.

Antimicrobial activity: The leaves and fruits extracts were evaluated against 3 Gram-positive and 2 Gram-negative microorganism by the determination of the zone of inhibition. The zone of inhibition was determined by disk diffusion method [13,19] using Muller-Hinton agar, procured from Hi-media Laboratories, Mumbai, India.

Determination of zone of inhibition: The antibacterial sensitivity tests were performed by Kirby-Bauer disc diffusion method.

Preparation of seed organisms: Nutrient broth medium was used to seed the microorganisms. Medium (5 mL) was filled in the test tubes were capped with cotton plugs and sterilization was done by autoclaving. Inoculation was done with a loop of microorganisms into the liquid broth and incubated at 37 ± 1 ºC and used within 12-18 h.

Preparation of sensitivity plate: The Mueller-Hinton Agar medium was used to prepare the plates. The media was autoclaved at 15 psig pressure (121 ºC) for 15 min and it was poured into glass, flat-bottomed sterilized Petri dishes uniformly.

Preparation of discs containing extracts: The paper discs of 6 mm diameter were sterilized by autoclaving at 25 psi g pressure (121 ºC) for 10 min. The extracts were dissolved into DMSO and in this way that each disc contains 250, 500 and 1000 µg of methanolic extracts of leaf and fruit of *Elaeagnus latifolia*. For reference, market supplied standard disk of ofloxacin (Hi-media), 5 µg per disk was used.

Procedure: Various microorganisms of 0.2 mL were aseptically put over the solid agar medium, which was previously

TABLE-1
DPPH FREE RADICAL SCAVENGING ACTIVITY OF MELEL AND MEFEL

Concentration (µg/mL)	Percentage inhibition (Mean ± S.E.M)		IC_{50} (µg mL^{-1})	
	MELEL	MEFEL	MELEL	MEFEL
100	36.69 ± 0.18	39.04 ± 0.05	200.39 ± 5.44	144.64 ± 0.25
150	41.78 ± 0.27	50.696 ± 0.273		
200	50.6 ± 2.71	60.46 ± 0.22		
250	56.35 ± 2.18	74.2 ± 0.97		
300	64.36 ± 0.005	84.03 ± 0.40		

prepared. After some time, previously prepared extracts discs (individually contain 250-1000 µg), solvent controlled discs and standard ofloxacin discs were placed aseptically on sensitivity plates. The plates were then put for 24 h incubation period at 37 ± 1 ºC. The clear zone of inhibition on agar plate was measured and calculated.

RESULTS AND DISCUSSION

Methanolic extract of the plants showed strong antioxidant activity by inhibiting DPPH and hydrogen peroxide when compared with standard ascorbic acid. Antioxidant activity of extract was expressed as IC_{50} value. The IC_{50} values were calculated by linear regression of plots, where the abscissa represents the concentration of the tested plant extracts and the ordinate represents the average percent of scavenging capacity.

The methanolic extract of fruits of *Elaeagnus latifolia* (MEFEL) exhibits the strongest DPPH scavenging activity with an IC_{50} value of 144.64 ± 0.25 µg mL^{-1} which is likely to be comparable with standard ascorbic acid with an IC_{50} value of 29.39 ± 7.11 µg mL^{-1} (Tables 1 and 2). In the assay against H_2O_2, methanolic extract of leaves of *Elaeagnus latifolia* (MELEL) found to be the strongest in hydrogen peroxide scavenging assay with an IC_{50} value of 444.59 ± 3.77 µg mL^{-1} as compared to standard ascorbic acid with an IC_{50} value of 279.89 ± 1.81 µg mL^{-1}, respectively (Tables 3 and 4).

TABLE-2
DPPH FREE RADICAL SCAVENGING ACTIVITY OF STANDARD (ASCORBIC ACID)

Concentration (µg/mL)	Percentage inhibition (Mean ± S.E.M)	IC_{50} (µg mL^{-1})
	Ascorbic acid	29.39 ± 7.11
20	59.11 ± 0.18	
40	89.16 ± 0.27	
60	91.41 ± 2.71	
80	92.83 ± 2.18	
100	96.17 ± 0.005	

TABLE-3
HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF MELEL AND MEFEL

Conc. (µg/mL)	Percentage inhibition (Mean ± S.E.M)		IC_{50} (µg mL^{-1})	
	MELEL	MEFEL	MELEL	MEFEL
100	5.83±1.1	2.642±1.3	444.59±3.77	487.31±6.17
200	20.59±1.0	13.28±1.5		
300	31.36±0.63	26.63±1.7		
400	46.21±0.61	40.32±1.6		
500	56.37±0.60	51.05±0.5		

TABLE-4
HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF STANDARD (ASCORBIC ACID)

Concentration (µg/mL)	Percentage inhibition (Mean ± S.E.M)	IC_{50} (µg mL^{-1})
	Ascorbic acid	279.89 ± 1.81
100	23.33 ± 0.2	
200	39.26 ± 0.28	
300	53.49 ± 0.56	
400	66.16 ± 0.49	
500	82.33 ± 0.47	

A significant amount of phenolic and flavonoids compounds plays a major role in controlling oxidative damages in human cells. Leaves were a good source of phenols with 61.15 µg mL^{-1} measured in gallic acid equivalent (GAE) (Table-5). Flavonoid content was found significantly in the methanolic extract of leaves which was calculated to be 15.12 µg mL^{-1} of quercetin equivalents (QE)/mg of extract (Table-6).

TABLE-5
DETERMINATION OF TOTAL PHENOLIC CONTENT

Test	In gallic acid equivalent (GAE) (Mean ± S.D.)	
	MELEL (mg g^{-1})	MEFEL (µg mL^{-1})
Total phenolic content	61.15 ± 1.23	8.18 ± 0.182

TABLE-6
DETERMINATION OF TOTAL FLAVONOID CONTENT

Test	In quercetin equivalent (GAE) (Mean ± S.D)*	
	MELEL (µg mL^{-1})	MEFEL (µg mL^{-1})
Total flavonoid content	15.12 ± 0.125	6.375 ± 0.128

The results of zone of inhibition of MELEL and MEFEL along with standard ofloxacin are presented in Table-5. The result showed that depending upon concentration the methanolic extract showed varied range of antimicrobial activity against the tested organism which is comparable to standard drug ofloxacin (Table-7).

Conclusion

Natural antioxidants and antimicrobial agent of plants origin have greater utilitization as nutraceuticals and phytopharmaceuticals as they have significant impact on human health and prevention of diseases. The present study scientifically validates the use of these plant extracts in traditional health practice. The extract showed significant antioxidant and antimicrobial activity. The result of antioxidant activity is comparable to standard ascorbic acid. Also a noticable amount of phenolic and flavonoid content is found in the extracts. In the

TABLE-7
RESULT OF THE ANTIMICROBIAL ACTIVITY

Extracts and standard drug	Zone of inhibition (mm) (Mean ± SD)				
	Gram-positive organisms			Gram-negative organisms	
	B. subtilis	*S. aureus*	*S. faecalis*	*E. coli*	*P. mirabilis*
MELEL (250 µg)	10 ± 1.00	12 ± 1.52	11 ± 1.60	–	10 ± 2.08
MELEL (500 µg)	12 ± 1.00	11 ± 2.10	14 ± 1.52	–	13 ± 1.51
MELEL (1000 µg)	15 ± 1.52	17 ± 2.51	19 ± 1.52	–	18 ± 1.52
MEFEL (250 µg)	10 ± 1.00	12 ± 1.52	13 ± 1.52	–	–
MEFEL (500 µg)	13 ± 1.10	15 ± 2.51	16 ± 2.51	–	–
MELFL (1000 µg)	18 ± 1.00	17 ± 1.52	17 ± 2.10	–	–
Ofloxacin (standard)	28 ± 1.57	35 ± 2.00	27 ± 1.52	29 ± 2.51	25 ± 1.0
DMSO	–	–	–	–	–

present study, screening of antimicrobial effects of *Elaeagnus latifolia* L. was done using selected pathogenic microbes. The result focussed on the significant antimicrobial activity against the tested organism which is comparable to standard ofloxacin effect. The antioxidant and antibacterial activity of methanol extract may be due to various types of phytochemicals constituents present in the extract. Thus, it is suggested that this plant provides health benefits to humans and may be employed in food processing or in medicine industry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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