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Phytochemical, antioxidant, antimicrobial and antiproliferative potential of *Elaeagnus indica*

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ABSTRACT

The present investigation was aimed to evaluate the phytochemical nature as well as antimicrobial, antioxidant and antiproliferative properties of *Eleaegnus indica*. Different solvent (hexane, chloroform, acetone, ethyl acetate, and methanol) leaf extracts of *E. indica* were tested for their antimicrobial, antioxidant, antiproliferative and phytochemical properties by adopting standard methods. The extracts of *E. indica* were found to be good sources of a variety of phytoconstituents *i.e.*, phenols (72.97 μg/mg GAE), flavonoids (92.07 μg/mg CE), tannins (62.97 μg/mg TAE), ascorbic acid (154.07 μg/mg AAE), proteins (115.46 μg/mg BSAE) and carbohydrates (193.77 GE). The acetone extract exhibited strong *in vitro* antioxidant potentials ($NO^{\bullet}$ IC_{50} = 39.43 μg/mL, $^{\bullet}OH$ IC_{50} = 43.91 μg/mL, $O_2^{\bullet-}$ IC_{50} = 48.30 μg/mL, $DPPH^{\bullet}$ IC_{50} = 70.32 μg/mL, and FRAP EC_{50} = 48.69 μg/mL and total antioxidants 425.92 μg/mg AAE of dry extract) in all the tested methods. All the extracts of *E. indica* expressed a wide range of antimicrobial potential (6–24 mm) against most of the pathogens used in this study. The methanol extract exhibited remarkably high antibacterial potential against *S. epidermidis* (24 mm) followed by *S. pneumoniae* (20 mm) in the well diffusion method. The results of the antiproliferative activity of acetone extract showed a concentration dependent activity on the tested cell lines (U-937 cell line IC_{50} = 46.75 μg/mL and HL-60 cell line IC_{50} = 59.46 μg/mL). The present investigation highlighted that the acetone extract exhibited significant antiproliferative, antimicrobial, and antioxidant capacities and has provided scientific evidence for the development of novel antimicrobial, antioxidant and antiproliferative agents from *E. indica*.

1. Introduction

Microbes (such as bacteria, fungi, and viruses) are the major causative agents of infectious diseases, which pose threats to public health (Majeed et al., 2019). Nowadays, several lifethreatening pathogens have developed resistance to the current antibiotics available in the market (Latifa et al., 2012). In addition to resistance, synthetic antibiotics are causing undesirable effects on host health such as allergic reactions, depletion of the beneficial gut and mucosal microorganisms, hypersensitivity, and immunosuppression. Moreover, the costs of synthetic antibiotics are high (Asha Shalini et al., 2019). Thus, there is a need to find out new, broad spectrum, high potential, and safer antimicrobial agents from natural sources (Doudach et al., 2012).

Free radicals are essential for various cellular processes (*viz.*, cell signalling and defense mechanisms) and metabolism of aerobic organisms. However, these radicals may be highly synthesized on an out of control manner in cells that generate an oxidative stress condition in cells leading to the damage of several cellular macromolecules, such as proteins, lipids, and DNA (Almulaiky et al., 2018). Several degenerative diseases such as anemia, arthritis, asthma, atherosclerosis, brain

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dysfunction, cancer, cataracts, diabetes mellitus, inflammation, liver diseases, malfunction of the immune system, myocardial infarction, and renal failure are principally associated with the oxidative stress induced by the excessive free radicals (Aseervatham et al., 2019).

Since ancient times, plants have played an important role in human civilization as constituents of food, cosmetics, clothing, dyes, fibers, flavors, fragrances, medicines, pesticides, fertilizers and herbicides, etc., (Seebaluck-Sandoram et al., 2019). Plants have been used as medicine for thousands of years to treat several human and animal diseases (Mahomoodally et al., 2018). Different parts such as flowers, fruits, leaves, roots, and stems of medicinal plants were utilized in traditional medicine. The extracts of several plants have been used as crude drugs as they harbor a variety of medicinal properties (Samrot et al., 2018).

Phytochemicals are responsible for the medicinal properties of plants. Plants are able to synthesize a vast number of secondary metabolites, of which only less than 10% have been isolated and their biological activities have been explored (Uysal et al., 2018). In drug discovery, phytochemicals act as crucial components for the development of potential and safer drugs. Nowadays, hundreds of plant- derived pharmaceutical products and synthetic analogs of phytochemicals are available in the drug market. Currently, allopathic therapy uses 120 plant-derived compounds obtained from 90 plant species (Natarajan et al., 2012). Recently, researchers are gaining interest in plant research, particularly, on herbs and herbal preparations, which have been used in traditional medicine to treat diabetes, cancer, hypertension, inflammation, microbial infections, degenerative diseases, etc., (Vijayaraghavan et al., 2018).

Elaeagnus indica Servett. belongs to the Elaeagnaceae family and grows above 1000 m MSL altitude in the Eastern Ghats of India. Based on the traditional knowledge, several tribal communities of Eastern Ghats of Tamil Nadu utilize this plant as medicine. A few bioactive compounds were identified from the extracts of *E. indica* (Srinivasan et al., 2014, 2015, 2016). Similarly, a limited number of scientific evidences is available on the biological activities of *E. indica* (Ramesh Kannan et al., 2013; Shivakumar et al., 2013; Srinivasan et al., 2015, 2016). The preliminary and essential stages of isolation and identification of therapeutic molecules from a plant is the screening of its extract for bioefficacy. To the best of the knowledge of the authors, *E. indica* has been least explored for its health benefits and biological activities. Hence, documentation of various biological activities of *E. indica* viz. antimicrobial, antioxidant and antiproliferative, is the need of the hour.

2. Materials and methods

2.1. Plant material

Aerial parts of *E. indica* were collected from the Shervarayan Hills (1300–1400 m MSL, latitude 11° 47′–12° 33′ N, longitude 77° 02′–78° 40′ E), Salem District, Tamil Nadu, India. The nomenclature of the collected plant material was authenticated by the Botanical Survey of India (reference number: BSI/SRC/5/23/2014–15/Tech/1942), Coimbatore, Tamil Nadu, India. The authenticated plant specimen (No. PU/BT/NDRL/2010/03) was deposited in the herbarium collection center at the Natural Drug Research Laboratory (NDRL), Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India. The leaves were detached from the twigs, cleaned with running water prior to sterile distilled water; air dried at room temperature for 3 weeks and then, pulverized.

2.2. Preparation of extracts

About 2 kg of the powdered plant material was successively extracted with different organic solvents such as hexane, chloroform, acetone, ethyl acetate, and methanol (in order of increasing polarity) in a Soxhlet apparatus until the efflux solvent turned colorless. All the extracts were filtered through Whatman filter paper and concentrated under vacuum at 40 °C, which yielded 1.67%, 3.48%, 5.33%, 4.68%, and 6.21% of hexane, chloroform, acetone, ethyl acetate, and methanol extracts, respectively.

2.3. Phytochemical analysis

2.3.1. Qualitative phytochemical analysis

Primary phytochemical examination of different solvent leaf extracts of *E. indica* was performed to identify the presence of alkaloids, fixed oils, flavonoids, glycosides, polyphenolics, saponins, steroids and tannins using the previous protocol described by Evans (2002).

2.3.2. Quantitative phytochemical analysis

2.3.2.1. Determination of total phenols. The polyphenol content of *E. indica* was assessed by the Folin–Ciocalteau method, as described by Barreira et al. (2008). Gallic acid (0–100 μg/mL) was used to construct the standard calibration curve ($y = 0.0087x + 0.0339$; $R^2 = 0.9987$). The results were represented as gallic acid equivalents (GAE) per mg of dry extracts.

2.3.2.2. Determination of total flavonoids. Total flavonoid content was measured according to the modified method of Kathirvel and Sujatha (2012). (±) Catechin (0–140 μg/mL) served as the standard for calibration curve ($y = 0.0044x - 0.0259$; $R^2 = 0.9971$). The detected flavonoid contents were presented as catechin equivalents (μg) per mg of dry extracts.

2.3.2.3. Determination of tannins. Tannin content was detected by the Folin-Denis protocol (Oyaizu, 1986). Tannic acid (0–120 μg/mL) was used to construct the standard curve ($y = 0.0153x - 0.0036$; $R^2 = 0.9995$). The findings were expressed as (μg) equivalents of tannic acid (TAE) per mg of dry extracts.

2.3.2.4. Determination of ascorbic acid. Determination of ascorbic acid content was done as per the method of Omaye and Reddy Cross (1962). A standard graph ($y = 0.0097x + 0.0304$; $R^2 = 0.9987$) was plotted using different concentrations (0–100 μg/mL) of ascorbic acid. The results were denoted as ascorbic acid equivalents (AAE) per mg of extracts.

2.3.2.5. Determination of proteins. Lowry's method (Lowry et al., 1952) was used to determine the protein contents of the extracts. Different concentrations (0–100 μg/mL) of bovine serum albumin (BSA) were used to construct the standard graph ($y = 0.0015x + 0.0039$; $R^2 = 0.9969$). Total protein contents were recorded as μg of BSA equivalents (BSAE) per mg of extracts.

2.3.2.6. Determination of carbohydrates. Estimation of carbohydrate content was performed by the anthrone method (Hedge et al., 1962). Glucose (0–100 μg/mL) was used for the preparation of the standard graph ($y = 0.0109x - 0.0054$; $R^2 = 0.9932$) and the values were presented as glucose equivalents per mg of extracts.

2.3.2.7. Total antioxidant capacity (TAC). Total antioxidant activities of crude *E. indica* extracts were determined by the phosphomolybdenum method as described by Prieto et al. (1999). Ascorbic acid (0–1000 μg/mL) was used to plot the linear curve ($y = 0.0026x + 0.2436$; $R^2 = 0.9995$). The total antioxidant potentials were denoted as mean values of triplicates of μg of ascorbic acid equivalents (AAE) per mg of extracts.

2.4. In vitro antioxidant studies

Antioxidant potentials of various leaf extracts of *E. indica* were

analyzed as per the previously described methods on various free radicals *i.e.*, DPPH (Chew et al., 2009), nitric oxide (Kathirvel and Sujatha, 2012), hydroxyl (Halliwell et al., 1992), superoxide (Liu et al., 1997), and ferric reducing antioxidant power assay (FRAP) was performed (Yen and Chen, 1995). Different concentrations (50–1000 μg/mL) of leaf extracts were examined for various types of radical scavenging potentials. Butylated hydroxyanisole (BHA) and ascorbic acid served as reference compounds for all *in vitro* antioxidant assays.

2.5. Antimicrobial activity

The antimicrobial efficacies of plant extracts were evaluated against four clinically isolated fungal pathogens (*Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, and *Mucor racemosus*) and twenty four bacterial pathogens along with nine reference strains viz., three Grampositive [*Bacillus subtilis* (MTCC 441), *Staphylococcus epidermidis* (MTCC 435), and *Streptococcus pneumoniae* (MTCC 655)] and six Gramnegative [*Escherichia coli* (MTCC 739), *Klebsiella pneumoniae* (MTCC 109), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 741), *Salmonella typhimurium* (MTCC 98), and *Shigella flexneri* (MTCC 1457)] strains. The MTCC cultures were procured from IMTECH, Chandigarh, India. Fifteen clinical isolates namely, *B. subtilis*, *Corynebacterium diphtheriae*, *E. coli*, *Enterococcus faecalis*, *K. pneumoniae*, *P. vulgaris*, *S. aureus*, *S. boydii*, *S. dysenteriae*, *S. flexneri*, *S. marcescens*, *S. sonnei*, *S. typhi*, *V. alginolyticus*, and *V. vulnificus* were collected from the microbial diagnostic centers, in and around Salem District, Tamil Nadu, India. The antimicrobial activities of various leaf extracts of *E. indica* were assessed using both agar well diffusion and disc diffusion method, as described by Srinivasan et al. (2014).

2.6. Cellular Studies

2.6.1. Cells and cell culture

The human leukemic monocyte lymphoma (U-937) and human acute promyelocytic leukemia (HT-60) cell lines were acquired from the National Institute of Cell Sciences, Pune, India and maintained in Minimal Essential Medium (MEM) supplemented with 3% L-glutamine, 10% (v/v) heat-inactivated Fetal Bovine Serum (FBS), 100 μg/mL streptomycin and 100 IU/mL penicillin G mix in a 5% CO_2 incubator at 37 °C. Owing to the high amount of antioxidants and other phytoconstituents, the crude acetone extract of *E. indica* was considered as the bioactive extract and subjected to antiproliferative activity analysis.

2.6.2. Antiproliferative activity

The antiproliferative potential of acetone extract of *E. indica* was detected by methyl thiazolyl diphenyl-tetrazolium bromide (MTT) method as stated by Mosmann (1983) on U-937 and HT-60 cell lines. The cultured cells were treated with various concentrations of acetone extract (10–500 μg/mL). The effect of extract on the proliferation of U-937 and HT-60 cells was recorded as cell viability percentage. The toxicity IC_{50} values were estimated graphically from the curve plotted for the concentration of the extract against the percentage of cell viability.

2.7. Statistical analysis

All the analyses stated in this investigation were done in triplicates. Data were noted as mean ± standard deviation of triplicates. The inhibitory concentration 50 (IC_{50}) were estimated graphically from the curve fitted method from the mean values of three quotients. The significant differences between the samples were determined by performing the analysis of variance (ANOVA) in a completely randomized design and Tukey's test (at $p < 0.05$) by adopting a statistical software (SPSS 25.0).

Table 1
Qualitative phytochemical analysis of different solvent leaf extracts of *E. indica*.

Phytochemical constituents	Extracts				
	Methanol	Acetone	Ethyl acetate	Chloroform	Hexane
Alkaloids	+	+	+ + +	–	–
Carbohydrates	+ +	+ + +	+ +	+	+
Fixed oils	–	–	+	+	+
Flavonoids	+ +	+ + +	+ +	+ +	+
Glycosides	+	+	+	+	–
Phenolics	+ +	+ + +	+ +	+ +	+
Proteins	+ +	+ +	+ +	+	+
Saponins	+	+	+	+	+ +
Steroids	–	–	–	+	+
Tannins	+ +	+ + +	+ +	+ +	+

+ + + = highly present; + + = moderately present; + = slightly present; – = absent.

3. Results and discussion

3.1. Phytochemical analysis

The phytochemical analysis of different solvent leaf extracts of *E. indica* revealed the occurrence of saponins, phenols, tannins, glycosides, flavonoids, steroids, alkaloids and oils in most of the tested extracts (Table 1). Various classes of phytoconstituents such as carbohydrates, flavonoids, phenolic compounds, proteins, tannins, and vitamin C were detected at different concentrations in all the tested extracts of *E. indica* (Table 2). However, maximum amounts of both the primary and secondary metabolites were observed in the acetone extract followed by the ethyl acetate extract. Acetone extract contained a significantly higher amount of primary metabolites such as carbohydrates (193.77 ± 8.03 GE), ascorbic acid (154.07 ± 2.78 μg/mg AAE) and proteins (115.46 ± 2.3 μg/mg BSAE) followed by secondary metabolites (flavonoids-92.07 ± 2.56 μg/mg CE, phenols-72.97 ± 1.40 μg/mg GAE and tannins-62.97 ± 0.16 μg/mg TAE). Moderate quantities of the tested phytoconstituents were observed in methanol and chloroform extracts. Hexane extract showed the lowest quantities of the tested phytochemicals.

The variations in the number of phytochemicals in the different solvent extracts of *E. indica* were based on the sample-to-solvent ratio, solvents, method, time and temperature employed for the extraction, and physicochemical characteristics of the plant sample (Cacace and Mazza, 2003; Pinelo et al., 2005). This is the first report on the estimation of primary and secondary metabolites of different solvent extracts of *E. indica*. However, the results of the present investigation would be supported by previous reports on the quantification of primary and secondary metabolites of *Elaeagnus* species namely, *E. umbellata* (Hussain, 2011; Khattak, 2012; Ahmad et al., 2005), *E. conferta* (Dandge et al., 2011), *E. kologa* (Merculieff et al., 2014), *E. latifoila*, and *E. pyriformis* (Prakash et al., 2012; Singh et al., 2014; Tapan, 2011).

3.2. In vitro antioxidant studies

All the extracts of *E. indica* exhibited varied levels of antioxidant capacities in all the tested methods (Fig. 1). Nonetheless, the superior antioxidant capacity was noticed for the acetone extract in all the evaluated antiradical analyses followed by the ethyl acetate extract. The acetone extract expressed the highest nitric oxide radical scavenging potential with the lowest IC_{50} value (39.43 μg/mL) followed by hydroxyl radical scavenging (IC_{50} value- 43.91 μg/mL). Similarly, the maximum total antioxidant capacity was detected for the acetone extract (425.92 μg AAE/mg extract) followed by the ethyl acetate extract (213.62 μg AAE/mg extract) (Table 2). The methanol extract exhibited considerable antiradical abilities in all the tested assays with the least

Table 2
Quantitative phytochemical analysis of different solvent leaf extracts of *E. indica*.

Phytoconstituents (μg/mg of dry extract)	Extracts*				
	Methanol	Acetone	Ethyl acetate	Chloroform	Hexane
Total antioxidant capacity (AAE)	194.58 ± 0.19^{c}	425.92 ± 0.38^{e}	213.62 ± 0.38^{d}	165.73 ± 7.50^{b}	141.31 ± 0.38^{a}
Total ascorbic acid content	125.84 ± 4.56^{c}	154.07 ± 2.78^{e}	138.51 ± 2.78^{d}	75.23 ± 2.83^{b}	38.12 ± 5.17^{a}
Total carbohydrate content	159.23 ± 2.29^{c}	193.77 ± 8.03^{e}	122.44 ± 1.74^{d}	77.81 ± 4.08^{b}	33.63 ± 4.68^{a}
Total flavonoid content (CE)	41.72 ± 4.77^{c}	92.07 ± 2.56^{e}	51.37 ± 5.12^{d}	31.02 ± 2.21^{b}	17.19 ± 4.19^{a}
Total phenolic content (GAE)	46.84 ± 2.58^{d}	72.97 ± 1.40^{e}	43.02 ± 1.01^{c}	34.15 ± 0.11^{b}	14.76 ± 1.07^{a}
Total protein content	83.42 ± 0.81^{c}	115.46 ± 2.30^{e}	97.54 ± 0.99^{d}	46.76 ± 0.81^{b}	17.43 ± 0.58^{a}
Total tannin content (TA)	39.50 ± 0.03^{c}	62.97 ± 0.16^{e}	58.75 ± 0.07^{d}	23.56 ± 0.26^{b}	13.75 ± 0.03^{a}

*-The values are mean of triplicates with (±) standard deviation (mean ± S.D; n = 3). Different superscript letters (a-e) in rows indicates the effectiveness of extracts (e > d > c > b > a) with significant differences (at $p < 0.05$) when subject to Tukey's multiple comparison test.

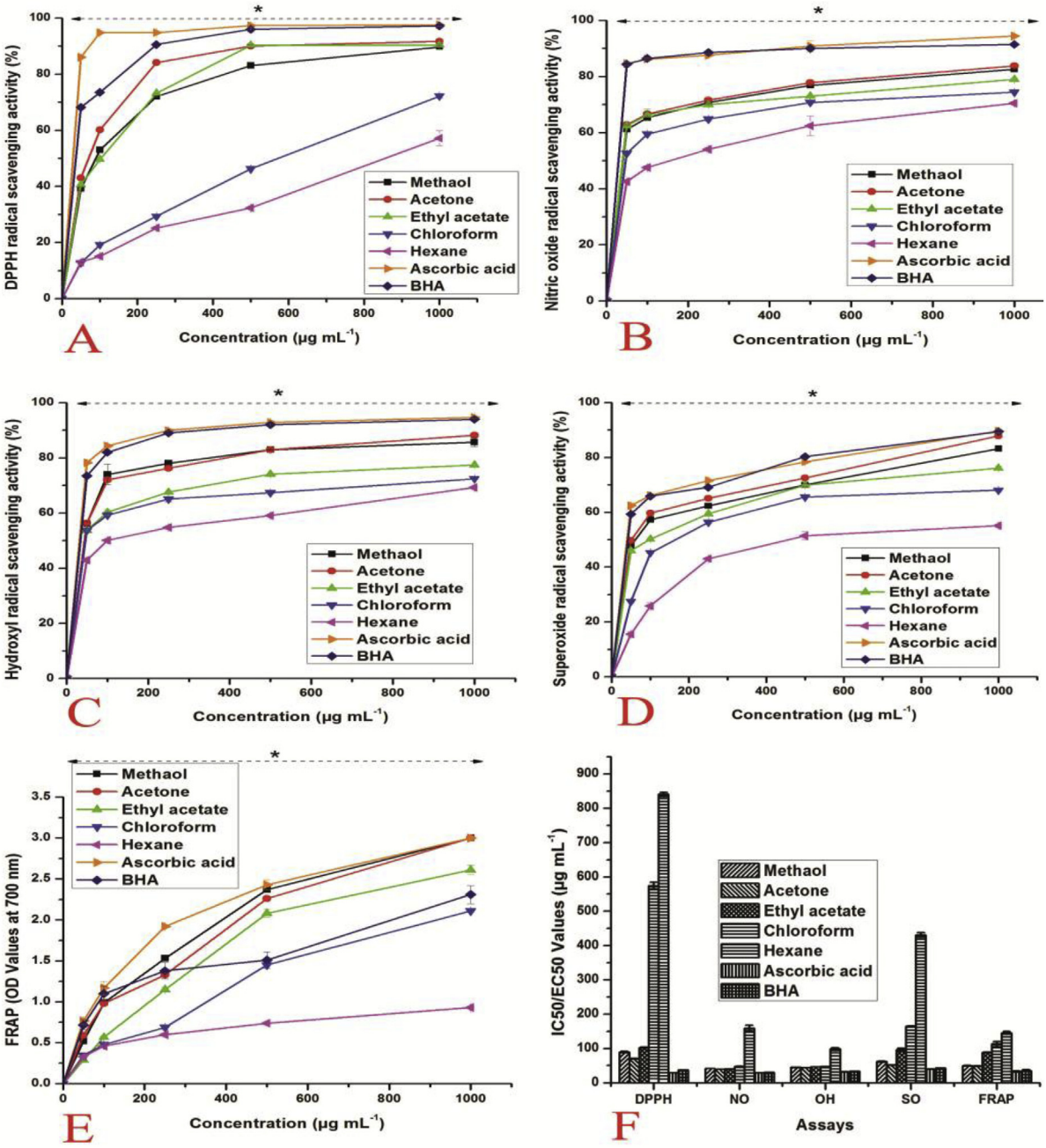

Fig. 1. Antioxidant activity of different solvents extracts of *E. indica*. A = DPPH, B = Nitric oxide, C = Hydroxyl, D = Superoxide radical scavenging activity, E = FRAP and F = IC_{50} values of all radical scavenging analysis.

Table 3
Correlation between phytoconstituents and antioxidant activities of different solvent leaf extracts of *E. indica*.

Assay	Phytoconstituents*			
	Phenol	Flavonoid	Tannin	Ascorbic acid
$NO^{\bullet}$	0.808	0.617	0.745	0.926
$^{\bullet}OH$	0.799	0.573	0.556	0.786
$O_2^{\bullet-}$	0.848	0.660	0.749	0.927
$DPPH^{\bullet}$	0.749	0.660	0.885	0.974
FRAP	0.746	0.534	0.617	0.843
TAC	0.836	0.952	0.604	0.542

* – values are R^2 of linear regression (equations are not given).

IC_{50} values. Moderate antiradical capacities were noticed for the chloroform extract with substantial IC_{50} values. The hexane extract showed the minimal antiradical potentials with high IC_{50} values.

Significant positive linear correlations (R^2 = 0.534 to 0.974) were noticed between the phytoconstituents and IC_{50} values of various antiradical activities of *E. indica* extracts (Table 3). The highest correlation was found between DPPH and total ascorbic acid assays (R^2 = 0.974). A positive correlation was observed between DPPH antioxidant capacity and ascorbic acid content. Moreover, the ascorbic acid content showed significant correlations with most of the tested antiradical activities. Similarly, phenolic, flavonoid and tannin contents of the extracts showed considerable positive correlations with most of the tested antioxidant activities. Previous investigations revealed the direct relationship between the antioxidant potential and phytochemicals of plant extracts (Kaur and Kapoor, 2002; Ivanova et al., 2005). Thus, it was speculated that the radical scavenging potentials of the extracts could be attributed to the existence of high levels of phenolic and flavonoid compounds, which are well known for antioxidant defense. No data are available on the antioxidant activity of *E. indica*. Previously, various reports demonstrated the antioxidant potential (DPPH radical scavenging activity) of some of the plants belonging to *Elaeagnus* genus, namely, *E. angustifolia* (Xu et al., 2009; Wang et al., 2012; Okmen and Turkcan, 2013), *E. macrophylla* (Moussa et al., 2011), *E. multiflora* (Lee et al., 2007), *E. kologa* (Merculieff et al., 2014; Vinayaka et al., 2009), *E. umbellata* (Khattak, 2012), *E. latifolia* and *E. pyriformis* (Prakash et al., 2012; Tapan, 2011) which would strengthen the findings of the present study.

3.3. Antimicrobial activity

The extracts of *E. indica* expressed a wide range of antimicrobial potential (6–24 mm) against most of the tested pathogens (Table 4). The agar well diffusion analysis revealed that the methanol extract had harbored significant antibacterial ability against *S. epidermidis* (24 mm) followed by *S. pneumoniae* (20 mm). The ethyl acetate extract exhibited the maximum growth inhibitory effect against *E. faecalis* (22 mm) in the disc diffusion method. Significant antibacterial activities were observed for the acetone and methanol extracts against most of the examined microorganisms. The Chloroform and hexane extracts expressed the least and/or nil inhibitory effects against most of the tested pathogens in all the evaluated methods.

The results of the antifungal analysis showed that most of the extracts of *E. indica* exhibited only minimal or nil activities against the examined fungal pathogens in all the tested methods (Table 4). The maximum antifungal activity was observed for the acetone and hexane extracts against *M. racemosus* (10 mm) in the agar well method. The standard antifungal agent used in this study also expressed nil growth inhibitory effects against all the fungal pathogens except *C. neoformans* in the disc diffusion method.

Several plant-derived products such as essential oils and extracts have been used as traditional antiseptics and have been reported to possess moderate to significant levels of antimicrobial properties (Cowann, 1999). Similarly, the present study has clearly demonstrated that the extracts of *E. indica* possess potent antimicrobials. The outcomes of the present study revealed that the rate of inhibition was varied for different extracts against the tested microbes. Most of the polar extracts showed good antimicrobial activities. The present results concluded that the polar solvents would be more beneficial in the extraction of antimicrobials from *E. indica*. Similar findings were reported by Ramesh Kannan et al. (2013) on the antimicrobial activities of *E. indica* extracts.

Our findings correlated well with the observations of Uddin and Rauf (2012), who reported that *Elaeagnus* genus extracts were found to be more active against Grampositive than Gramnegative bacteria. Likewise, Arias et al. (2002) demonstrated similar antimicrobial potentials of several plant extracts, which concorded with the present findings. Minhas et al. (2013) reported that the extracts of *E. umbellata* showed high growth inhibitory effects against bacteria than fungi, which was in good agreement with the outcomes of the present study.

The chloroform extract showed lower antimicrobial effects against most of the tested bacteria as compared to the previous report (Ramesh Kannan et al., 2013), which might have been due to the variations in the source material, cultivation climate, soil type and extraction method. The antimicrobial activities of some other species of *Elaeagnus* genus, namely, *E. angustifolia* (Khan et al., 2013; Okmen and Turkcan, 2013, 2014), *E. macrophylla* (Liu et al., 2011), *E. mollis* (Fenjuan et al., 2009; Zhang and Ming, 2010), *E. kologa* (Merculieff et al., 2014), *E. umbellata* (Arias et al., 2002), *E. maritime* and *E. submacrophylla* (Lee et al., 2004) have been documented, which would further substantiate the results of the present study.

3.4. Antiproliferative activity

The results of the antiproliferative effects of the acetone extract of *E. indica* on U-937 and HL-60 cell lines showed dose-dependent effects on cell viability (Fig. 2). The results revealed that the acetone extract exhibited a better growth inhibitory potential on U-937 cell line with a considerable IC_{50} value (46.75 µg/mL). A moderate cytotoxic activity was observed on the HL-60 cell line with the IC_{50} value of 59.46 µg/mL. No significant effect was observed in the normal cells after treatment (24 h) with the tested concentrations. Several studies have reported the relationship between the antioxidant and antiproliferative activities of the extracts from the plant sources (Meyers et al., 2003; Gul et al., 2011). Especially, phenolic compounds have been widely studied for their antioxidant and antiproliferative effects (Nagmoti et al., 2012). Thus, the acetone extract of *E. indica* has been chosen in the present study as it entailed high phenolic content and antioxidant property. The antiproliferative effects of the phenolic compounds are linked to their roles in the modifications of key proteins involved in many cellular pathways related to cell differentiation, cell death, cell expression, and cell proliferation (Lin, 2002; Filomeni et al., 2007).

The standards for cytotoxicity of crude extracts as set by the National Cancer Institute (NCI) reveal that the extracts having an IC_{50} value lower than 30 µg/mL are significantly effective (Suffness and Pezzuto, 1990). The present results revealed that the IC_{50} values of the acetone extract were considerably higher than the NCI recommended level (30 µg/mL). Ramesh Kannan et al. (2013) reported the cytotoxic potential of *E. indica* on EAC cell line but did not determine the IC_{50} value. Moreover, the concentration range used in the previous report (Ramesh Kannan et al., 2013) for the evaluation of cytotoxic capacity was much higher (250–1000 µg/mL) than the one used in the present investigation. A number of plants belonging to the *Elaeagnus* genus such as *E. angustifolia* (Dahab and Afifi, 2007; Badrhadad et al., 2012), *E. glabra* (Li et al., 2009), *E. oldhamii* and *E. multiflora* (Liao et al., 2014) have been reported to possess moderate antiproliferative activities on various cell lines, which back the present findings.

Table 4
Antimicrobial activity of different solvent leaf extracts of *E. indica*.

Organisms	Method	The diameter of zone of inhibition (in mm)*					
		Methanol	Acetone	Ethyl acetate	Chloroform	Hexane	Control[#]
B. subtilis	Well	08.06 ± 0.87[a]	08.00 ± 1.00[a]	09.19 ± 1.05[a]	07.99 ± 0.99[a]	08.00 ± 1.00[a]	19.67 ± 0.58[b]
	Disc	06.83 ± 1.04[a]	08.33 ± 1.53[a,b]	09.33 ± 1.53[a,b]	07.16 ± 0.77[a,b]	09.00 ± 1.00[a,b]	10.19 ± 1.05[a,b]
C. diphtheria	Well	00.00 ± 0.00[a]	09.33 ± 1.32[b]	09.00 ± 1.00[b]	09.00 ± 1.00[b]	08.29 ± 0.62[b]	23.51 ± 0.58[c]
	Disc	07.00 ± 1.00[a]	07.67 ± 0.57[a]	08.11 ± 0.85[a]	09.00 ± 0.87[a]	07.95 ± 0.34[a]	12.00 ± 1.00[b]
E. coli	Well	11.00 ± 1.00[b]	09.00 ± 1.00[b]	10.33 ± 1.53[b]	00.00 ± 0.00[a]	09.00 ± 1.00[b]	19.10 ± 1.02[c]
	Disc	06.33 ± 1.53[a]	09.00 ± 1.00[a]	09.33 ± 1.53[a]	08.00 ± 1.00[a]	09.67 ± 1.53[a]	13.18 ± 0.75[b]
E. faecalis	Well	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	28.93 ± 0.12[b]
	Disc	08.00 ± 1.00[a]	10.22 ± 0.70[b]	21.83 ± 0.30[d]	19.00 ± 1.00[c]	09.00 ± 1.00[a,b]	20.00 ± 1.00[c,d]
K. pneumoniae	Well	06.85 ± 1.03[a]	11.15 ± 1.03[b]	11.20 ± 0.73[b]	09.00 ± 1.00[a,b]	08.00 ± 1.00[a]	20.00 ± 2.00[c]
	Disc	07.67 ± 1.53[a]	09.00 ± 1.00[a]	08.72 ± 0.63[a]	08.00 ± 1.00[a]	08.00 ± 1.00[a]	10.33 ± 1.53[a]
P. vulgaris	Well	00.00 ± 0.00[a]	00.00 ± 0.00[a]	11.20 ± 1.06[b]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	21.00 ± 1.00[c]
	Disc	07.20 ± 1.31[a]	07.19 ± 1.00[a]	08.03 ± 1.00[a]	07.63 ± 1.81[a]	08.00 ± 1.00[a]	20.00 ± 1.53[b]
S. aureus	Well	00.00 ± 0.00[a]	00.00 ± 0.00[a]	09.28 ± 0.91[b]	07.97 ± 0.95[b]	07.67 ± 1.53[b]	21.30 ± 1.13[c]
	Disc	07.67 ± 1.53[a]	06.67 ± 2.08[a]	08.20 ± 1.06[a]	07.67 ± 1.50[a]	08.00 ± 1.00[a]	14.21 ± 0.70[b]
S. boydii	Well	00.00 ± 0.00[a]	00.00 ± 0.00[a]	08.67 ± 1.53[b]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	21.67 ± 1.53[c]
	Disc	06.50 ± 0.99[a]	08.43 ± 1.40[a]	07.83 ± 0.76[a]	07.31 ± 1.49[a]	08.29 ± 0.62[a]	19.33 ± 1.53[b]
S. dysenteriae	Well	00.00 ± 0.00[a]	00.00 ± 0.00[a]	10.00 ± 1.00[b]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	21.67 ± 1.53[c]
	Disc	08.00 ± 1.00[a]	09.00 ± 1.00[a]	08.67 ± 1.53[a]	08.00 ± 1.00[a]	08.00 ± 1.00[a]	13.56 ± 1.26[b]
S. flexneri	Well	00.00 ± 0.00[a]	09.00 ± 1.00[b]	09.67 ± 1.53[b]	00.00 ± 0.00[a]	08.00 ± 1.00[b]	21.00 ± 1.00[c]
	Disc	08.00 ± 1.00[a]	09.00 ± 1.00[a]	08.33 ± 1.53[a]	08.13 ± 0.58[a]	09.00 ± 1.00[a]	13.00 ± 1.00[b]
S. marcescens	Well	00.00 ± 0.00[a]	08.00 ± 1.00[b]	09.00 ± 1.00[b]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	20.56 ± 0.51[c]
	Disc	07.67 ± 1.53[b]	00.00 ± 0.00[a]	07.67 ± 0.58[b]	06.43 ± 0.51[b]	07.19 ± 0.73[b]	10.53 ± 0.50[c]
S. sonnei	Well	00.00 ± 0.00[a]	09.33 ± 0.58[b]	08.93 ± 0.64[b]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	20.67 ± 1.53[c]
	Disc	00.00 ± 0.00[a]	07.99 ± 0.99[b]	08.18 ± 0.75[b]	07.67 ± 1.53[b]	08.00 ± 1.00[b]	11.00 ± 1.00[c]
S. typhi	Well	07.67 ± 1.53[b]	09.33 ± 0.58[b]	09.00 ± 1.73[b]	08.00 ± 1.00[b]	00.00 ± 0.00[a]	19.67 ± 0.58[c]
	Disc	07.19 ± 1.05[b]	08.00 ± 1.00[b]	09.00 ± 1.00[b,c]	08.67 ± 1.53[b,c]	00.00 ± 0.00[a]	11.00 ± 1.00[c]
V. alginolyticus	Well	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	28.22 ± 2.08[b]
	Disc	08.60 ± 1.00[a]	07.33 ± 1.53[a]	07.00 ± 1.00[a]	07.67 ± 1.53[a]	08.00 ± 1.00[a]	14.22 ± 0.69[b]
V. vulnificus	Well	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	22.42 ± 0.91[b]
	Disc	07.53 ± 1.00[a]	08.62 ± 1.00[a]	08.00 ± 1.00[a]	08.33 ± 0.58[a]	09.00 ± 1.00[a]	13.40 ± 1.01[b]
B. subtilis (MTCC 441)	Well	00.00 ± 0.00[a]	10.00 ± 1.00[c]	09.52 ± 0.53[b,c]	08.00 ± 1.00[b]	00.00 ± 0.00[a]	27.65 ± 0.56[d]
	Disc	08.43 ± 0.68[b,c]	10.67 ± 1.53[c]	09.30 ± 0.71[b,c]	07.30 ± 1.13[b]	00.00 ± 0.00[a]	19.53 ± 0.50[d]
E. coli (MTCC 739)	Well	12.15 ± 1.03[d]	11.30 ± 0.61[c,d]	09.53 ± 0.50[b,c]	08.83 ± 0.70[b]	00.00 ± 0.00[a]	32.00 ± 1.00[e]
	Disc	09.67 ± 1.00[b,c]	10.67 ± 1.53[c]	09.18 ± 0.75[b,c]	07.31 ± 1.60[b]	10.38 ± 1.20[c]	00.00 ± 0.00[a]
K. pneumoniae (MTCC 109)	Well	10.52 ± 0.50[c]	09.50 ± 0.50[b,c]	09.53 ± 0.50[b,c]	08.33 ± 0.58[b]	00.00 ± 0.00[a]	29.53 ± 0.50[d]
	Disc	07.66 ± 0.57[b]	08.53 ± 0.50[b]	10.19 ± 0.74[c]	10.53 ± 0.50[c]	10.89 ± 0.20[c]	00.00 ± 0.00[a]
P. aeruginosa (MTCC 741)	Well	12.42 ± 0.68[c]	09.26 ± 0.93[b]	10.29 ± 0.62[b]	08.49 ± 1.22[b]	00.00 ± 0.00[a]	24.53 ± 0.50[d]
	Disc	08.48 ± 0.56[c]	09.33 ± 0.58[c,d]	11.00 ± 1.00[d]	06.99 ± 0.99[b]	08.67 ± 0.58[c]	00.00 ± 0.00[a]
P. vulgaris (MTCC 426)	Well	13.37 ± 0.74[c]	12.47 ± 0.55[c]	08.00 ± 1.00[b]	08.27 ± 1.18[b]	00.00 ± 0.00[a]	27.97 ± 0.35[d]
	Disc	08.67 ± 0.58[b]	07.99 ± 0.99[b]	09.26 ± 1.10[b]	09.33 ± 1.53[b]	08.23 ± 0.69[b]	00.00 ± 0.00[a]
S. epidermidis (MTCC 435)	Well	23.26 ± 0.65[d]	13.09 ± 1.01[c]	12.48 ± 0.55[b,c]	11.31 ± 0.70[a,b]	09.60 ± 0.53[a]	29.47 ± 0.50[e]
	Disc	15.36 ± 0.92[c]	14.80 ± 0.72[b,c]	12.71 ± 0.61[b]	13.37 ± 0.71[b,c]	09.22 ± 0.70[a]	18.64 ± 1.10[d]
S. flexneri (MTCC 1457)	Well	13.55 ± 0.51[c]	14.28 ± 0.86[c]	09.98 ± 0.91[b]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	13.59 ± 0.52[c]
	Disc	07.55 ± 0.51[b]	07.59 ± 0.53[b]	09.59 ± 0.44[c]	08.51 ± 0.50[b,c]	08.26 ± 0.65[b,c]	00.00 ± 0.00[a]
S. pneumoniae (MTCC 655)	Well	19.67 ± 1.53[c]	18.33 ± 1.53[c]	09.00 ± 1.00[b]	00.00 ± 0.00[a]	08.67 ± 2.08[b]	33.48 ± 1.50[d]
	Disc	11.53 ± 0.50[b]	12.33 ± 1.15[b]	10.63 ± 0.55[a,b]	09.31 ± 0.60[a]	10.26 ± 0.65[a,b]	21.00 ± 1.00[c]
S. typhimurium (MTCC 98)	Well	13.52 ± 0.50[d]	11.81 ± 0.32[c]	09.10 ± 1.01[b]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	32.22 ± 0.70[e]
	Disc	08.37 ± 0.58[b]	08.33 ± 0.60[b]	07.86 ± 0.32[b]	06.86 ± 0.25[b]	07.87 ± 0.82[b]	00.00 ± 0.00[a]
A. niger	Well	06.67 ± 0.58[b]	08.47 ± 0.50[d]	08.50 ± 0.50[d]	06.80 ± 0.35[b]	07.33 ± 0.58[c]	00.00 ± 0.00[a]
	Disc	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]
C. albicans	Well	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]
	Disc	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]
C. neoformans	Well	00.00 ± 0.00[a]	08.33 ± 1.53[c]	00.00 ± 0.00[a]	06.85 ± 0.25[b]	00.00 ± 0.00[a]	00.00 ± 0.00[a]
	Disc	06.75 ± 0.66[b]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	07.92 ± 0.89[c]	09.83 ± 0.29[d]
M. racemosus	Well	11.73 ± 0.46[c]	14.80 ± 0.35[d]	09.87 ± 0.23[b]	11.83 ± 0.15[c]	11.67 ± 0.58[c]	00.00 ± 0.00[a]
	Disc	09.67 ± 0.58[d]	12.50 ± 0.50[e]	07.67 ± 0.58[b]	09.47 ± 0.50[d]	08.60 ± 0.53[c]	00.00 ± 0.00[a]

– Standard antibiotics (For antibacterial assay: Ciprofloxacin (1 mg/mL) used for well diffusion method, Gentamicin, vancomycin and ampicillin (10 mcg/disc) were used for disc diffusion method); (For antifungal assay: Fluconazole (10 mg/mL) used for well diffusion method, fluconazole disc (10 mcg/disc) used for disc diffusion method); *- The values are mean of triplicates with (±) standard deviation (mean ± S.D; n = 3). Different superscript letters (a-e) in rows indicate significant differences (at $p < 0.05$) when subject to Tukey's multiple comparison tests.

4. Conclusion

The results of the present study revealed that *Elaeagnus indica* is a good source of various phytoconstituents and possess significant antimicrobial, antioxidant and antiproliferative effects. High amounts of phytochemicals such as carbohydrates, ascorbic acid, proteins, flavonoids, phenols, and tannins were found in the acetone extract. Similarly, the acetone extract showed the maximum antioxidant and antiproliferative potentials. Moreover, the methanol extract expressed remarkable antimicrobial property against most of the tested pathogens. The present investigation encourages the utilization of *E. indica* in the treatment of microbial infections, oxidative stress related diseases and identifying lead and novel bioactive molecules.

Conflicts of interest

The authors declare no conflict of interest.

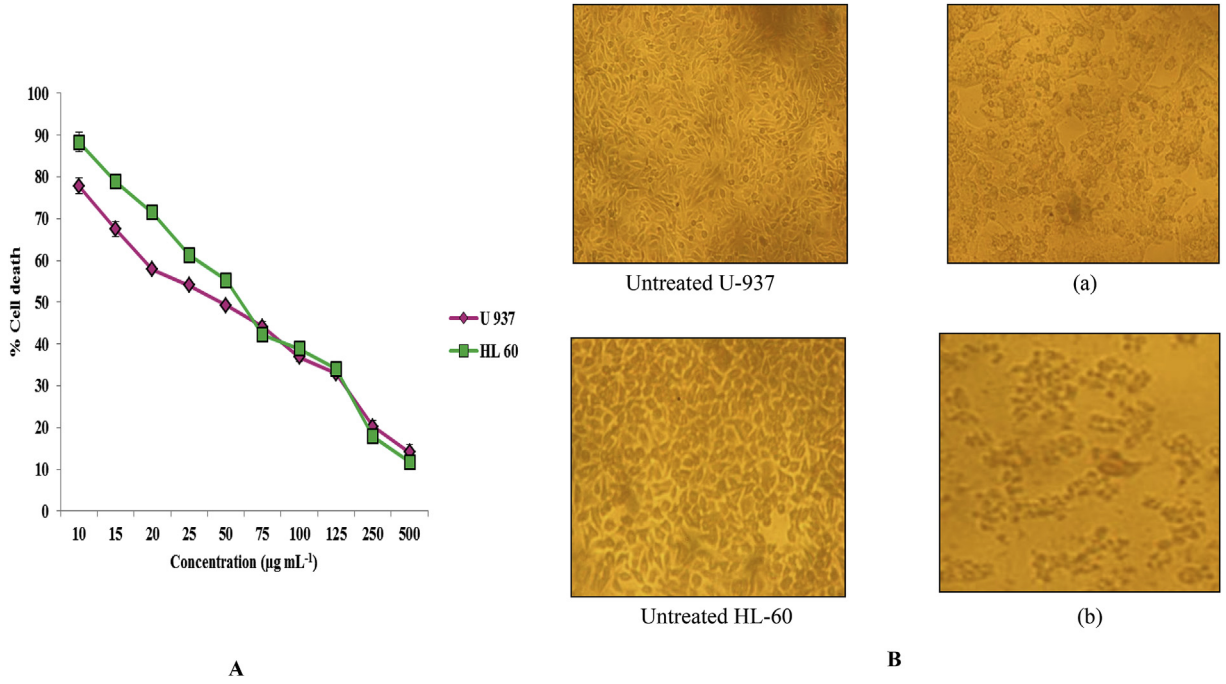

Fig. 2. A - Antiproliferative potential of acetone leaf extract of *E. indica* on U-937 and HL-60 cell lines. B - Acetone extract of *E. indica* induced morphological changes in U-937 (a) and HL-60 cells (b) at 500 µg/mL concentration (magnification 100×).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101265.

References

Ahmad, S.D., Sabir, M.S., Juma, M., Asad, H.S., 2005. Morphological and biochemical variations in *Elaeagnus umbellata* Thunb. from mountains of Pakistan. Acta Bot. Croat. 64, 121–128.

Almulaiky, Y., Zeyadi, M., Saleh, R., Baothman, O., Al-shawafi, W., Al-Talhi, H., 2018. Assessment of antioxidant and antibacterial properties in two types of Yemeni guava cultivars. Biocatalysis Agric. Biotechnol. 16, 90–97.

Arias, R.M., Prado, A., Hernandez-Perez, B.M., Sanchez Mateo, C.C., 2002. Antimicrobial studies on three species of *Hypericum* from the Canary Islands. J. Ethnopharmacol. 40, 287–292.

Aseervatham, G.S.B., Manthra, V., Ireen, C., Thilagameena, S., Akshaya, S., Clara Mary, A., Giriprashanthini, S., Sivasudha, T., 2019. Free radical scavenging potential and antihaemolytic activity of methanolic extract of *Pouteria campechiana* (Kunth) Baehni. and *Tricosanthes tricuspidata* Linn. Biocatalysis Agric. Biotechnol. 18.

Asha Shalini, A., Syed Ali, M., Anuradha, V., Yogananth, N., Bhuvana, P., 2019. GCMS analysis and *in vitro* antibacterial and anti-inflammatory study on methanolic extract of *Thalassiosira weissflogii*. Biocatalysis Agric. Biotechnol. 19, 101148.

Badrhadad, A., Piri, K.H., Mansouri, K., 2012. *In vitro* anti-angiogenic activity fractions from hydroalcoholic extract of *Elaeagnus angustifolia* L. flower and *Nepeta crispa* L. aerial part. J. Med. Plants Res. 6 (31), 4633–4639.

Barreira, C.M.J., Ferreira, C.F.R.I., Beatriz, M., Oliveira, P.P.J., Pereira, A., 2008. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food Chem. 107, 1106–1113.

Cacace, J.E., Mazza, G., 2003. Mass transfer process during extraction of phenolic compounds from milled berries. J. Food Eng. 59, 379–389.

Chew, Y.L., Goh, J.K., Lim, Y.Y., 2009. Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in peninsular Malaysia. Food Chem. 116, 13–18.

Cowann, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564–582.

Dahab, R.A., Afifi, F., 2007. Antiproliferative activity of selected medicinal plants of Jordan against a breast adenocarcinoma cell line (MCF7). Sci. Pharm. 75, 121–136.

Dandge, P.B., Kasabe, P.J., Patil, R.M., 2011. Evaluation of medicinal and nutritional components from the *Eleagnus conferta* fruit. Sci. Res. Repo. 1 (2), 56–60.

Doudach, L., Meddah, B., Alnamer, R., Chibani, F., Cherrah, Y., 2012. *In vitro* antibacterial activity of the methanolic and aqueous extracts of *Anacyclus pyrethrum* used in Moroccan traditional medicine. Inter. J. Pharm. Pharmaceut. Sci. 4 (3), 402–405.

Evans, W.C., 2002. Trease & Evans Pharmacognosy, fifteenth ed. WR sauders, London, pp. 137–140.

Fenjuan, S., Zhiyong, C., Zhifeng, Z., Ling, X., Guiqin, Y., 2009. Study on Antimicrobial Activity of the Alkaloids from *Elaeagnus Mollis*. Plant Protection 01.

Filomeni, G., Graziani, I., Rotilio, G., Ciriolo, M.R., 2007. Trans-resveratrol induces apoptosis in human breast cancer cells MCF-7 by the activation of MAP kinases pathways. Gene. Nutr. 2, 295–305.

Gul, M.Z., Bhakshu, L.M., Ahmad, F., Kondapi, A.K., Qureshi, I.A., Ghazi, I.A., 2011. Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using *in vitro* assays. BMC Complement Altern. Med. 11, 64.

Halliwell, B., Gutteridge, J.M.C., Cross, C.E., 1992. Free radicals, antioxidants and human disease: where are we now? J. Lab. Clin. Med. 119, 598–620.

Hedge, J.E., Hofreiter, B.T., Whistler, R.L., BeMiller, J.N., 1962. Methods in Carbohydrate Chemistry, vol. 17. Academic Press, New York, pp. 420.

Hussain, I., 2011. Physiochemical and sensory characteristics of *Elaeagnus umbellata* (Thunb) fruit from Rawalakot (Azad Kashmir) Pakistan. Afr. J. Food Sci. Tech 2 (7), 151–156.

Ivanova, D., Gerova, D., Chervenkov, T., Yankova, T., 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J. Ethnopharmacol. 96, 145–150.

Kathirvel, A., Sujatha, V., 2012. Phytochemical studies, antioxidant activities and identification of active compounds using GC-MS of *Dryopteris cochleata* leaves. Arabian. J. Chem. https://doi.org/10.1016/j.arabjc.2012.03.018.

Kaur, C., Kapoor, H.C., 2002. Antioxidant activity and total phenolic content of some Asian vegetables. J. Food Sci. Technol. 37, 153–161.

Khan, S.U., Khan, A.U., Ali Shah, A.U.H., Shah, S.M., Hussain, S., Ayaz, M., Ayaz, S., 2013. Heavy metals content, phytochemical composition, antimicrobial and insecticidal evaluation of E*laeagnus angustifolia*. Toxicol. Ind. Health 9, 92.

Khattak, K.F., 2012. Free radical scavenging activity, phytochemical composition and nutrient analysis of *Elaeagnus umbellata* berry. J. Med. Plants Res. 6 (39), 5196–5203.

Latifa, A., Wafaa Hassan, H.B., Hanan, M., 2012. Chemical composition and antimicrobial activity of the essential oil and lipid content of *Carduus pycnocephalus* L. growing in Saudi Arabia. J. Chem. Pharm. Res. 4 (2), 1281–1287.

Lee, H.B., Kim, C.S., Ahn, Y.J., 2004. Anti-helicobacter pylori activity of methanol extracts from Korean native plant species in Jeju Island. Agric. Chem. Biotechnol. 47 (2), 91–96.

Lee, Y.S., Chang, Z.Q., Chul Oh, B., Chun Park, S., Ryeul Shin, S., Woo Kim, N., 2007. Antioxidant activity, anti-inflammatory activity, and whitening effects of extracts of *Elaeagnus multiflora* Thunb. J. Med. Food 10 (1), 126–133.

Liao, C.R., Kuo, H.Y., Ho, Y.L., Wang, C.Y., Yang, C.S., Lin, C.W., Chang, Y.S., 2014. Studies on cytotoxic constituents from the leaves of *Elaeagnus oldhamii* Maxim. in non-small cell lung cancer A549 cells. Molecules 19, 9515–9534.

Li, Li-Hua, Baek, In Kyu, Kim, Jin Hee, Kang, Kyung Ho, Koh, Yang Seok, Jung, Young Do,

Cho, Chol Kyoon, Choi, Seok-Yong, Shin, Boo Ahn, 2009. Methanol extract of *Elaeagnus glabra*, a Korean medicinal plant, inhibits HT1080 tumor cell invasion. Oncol. Rep. 21 (2), 559–563.
Lin, J.K., 2002. Cancer chemoprevention by tea polyphenols through modulating signal transduction pathways. Arch Pharm. Res. (Seoul) 25, 561–571.
Liu, F., Ooi, V.E.C., Chang, S.T., 1997. Free radical scavenging activity of Mushroom polysaccharide extracts. Life Sci. 60, 763–771.
Liu, J.P., Chang, Y.B., Ya-ling, J., Xiang, Y.G., Chao, L., 2011. Study on the Antibacterial Activity of *Elaeagnus Macrophylla* Thunb Leaf Extract. Northern Horticulture 01.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1952. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Mahomoodally, M.F., Zengin, G., Aumeeruddy, M.Z., Sezgin, M., Aktumsek, A., 2018. Phytochemical profile and antioxidant properties of two *Brassicaceae* species: *Cardaria draba* subsp. draba and *Descurainia sophia*. Biocatalysis Agric. Biotechnol. 16, 453–458.
Majeed, F.A., Munir, H., Rashid, R., Zubair, M.T., 2019. Antimicrobial cytotoxicity mutagenicity and anti-epileptic potential of ethanol extracts of a multipurpose medicinal plant *Dalbergia sissoo*. Biocatalysis Agric. Biotechnol. 19, 101155.
Merculieff, Z., Ramnath, S., Sankoli, S.M., Venkataramegowda, S., Murthy, S.G., Ceballos, R.M., 2014. Phytochemical, antioxidant and antibacterial potential of *Elaeagnus kologa* (Schlecht.) leaf. Asian Pac. J. Trop. Biomed. 4 (9), 687–691.
Meyers, K.J., Watkins, C.B., Pritts, M.P., Liu, R.H., 2003. Antioxidant and antiproliferative activities of strawberries. J. Agric. Food Chem. 51, 6887–6892.
Minhas, F.A., Rehaman, H., Yasin, A., Awan, Z.I., Ahmed, N., 2013. Antimicrobial activities of the leaves and roots of *Elaeagnus umbellata* Thunb. Afr. J. Biotechnol. 12 (48), 6754–6760.
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.
Moussa, A.M., Emam, A.M., Diab, Y.M., Mahmoud, M.E., Mahmoud, A.S., 2011. Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of *Punica granatum* leaf extract on rats. Intern. Food. Res. J. 18, 535–542.
Nagmoti, D.M., Khatri, D.K., Juvekar, P.R., Juvekar, A.R., 2012. Antioxidant activity free radical scavenging potential of *Pithecellobium dulce* Benth seed extracts. Free Radic. Antioxidants 2, 37–43.
Natarajan, D., Srinivasan, R., Shivakumar, M.S., 2012. *Phyllanthus wightianus* Mull. Arg.: a potential source for antibacterial and phytochemical analysis. Int. J. Pharm. Pharm. Sci. 4 (5), 241–245.
Okmen, G., Turkcan, O., 2013. The antibacterial activity of *Elaeagnus angustifolia* L. against mastitis pathogens and antioxidant capacity of the leaf methanolic extracts. J. Anim. Vet. Adv. 12 (4), 491–496.
Okmen, G., Turkcan, O., 2014. A study on antimicrobial, antioxidant and antimutagenic activities of *Elaeagnus angustifolia* L. leaves. Afr. J. Tradit., Complementary Altern. Med. 11 (1), 116–120.
Omaye, K.A., Reddy Cross, C.E., 1962. Enhanced lung. Dystrophy in vitamin-E deficient rabbits. J. Biol. Chem. 237, 916–921.
Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucosamine. J. Nat. Prod. 44, 307–315.
Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., Nuñez, M.J., 2005. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. J. Agric. Food Chem. 53, 2111–2117.
Prakash, D., Upadhyay, G., Gupta, C., Pushpangadan, P., Singh, K.K., 2012. Antioxidant and free radical scavenging activities of some promising wild edible fruits. Inter. Food Res. J. 19 (3), 11091116.
Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341.
Ramesh Kannan, N., John Nayagam, Agnel Arul, Gurunagara, Sridharan, Muthukumar, B., Natarajan, Ekambaram, Manimaran, A., 2013. Photochemical screening from *Elaeagnus indica* activity against human pathogens and cancer cells. Adv. Biol. Res. 7 (3), 95–103.
Samrot, A.V., Raji, P., Jenifer Selvarani, A., Nishanthini, P., 2018. Antibacterial activity of some edible fruits and its green synthesized silver nanoparticles against uropathogen – *Pseudomonas aeruginosa* SU 18. Biocatalysis Agric. Biotechnol. 16, 253–270.
Seebaluck-Sandoram, R., Lall, N., Fibrich, B., Blom van Staden, A., Saleem, H., Mahomoodally, M.F., 2019. Antimicrobial antioxidant and cytotoxic evaluation of two underutilised food plants: *Averrhoa bilimbi* L. (Oxalidaceae) and *Phyllanthus acidus* L. Skeels (Phyllanthaceae). Biocatalysis Agric. Biotechnol. 18, 100998.
Shivakumar, M.S., Srinivasan, R., Natarajan, D., 2013. Larvicidal potential of some Indian medicinal plant extracts against Aedes aegypti (L.). Asian J. Pharmaceut. Clin. Res. 6 (3), 77–80.
Singh, K.K., Singh, M., Joshi, S.C., 2014. Phenolic content and antioxidant activity of some underutilized wild edible fruits of the Sikkim Himalaya. SMU Medi. J. 1 (2), 283–293.
Srinivasan, R., Natarajan, D., Shivakumar, M.S., Vinuchakkaravarthy, T., Velmurugan, D., 2015. Bioassay-guided isolation of mosquito larvicidal compound from acetone leaf extract of *Elaeagnus indica* Servett Bull and its in-silico study. Ind. Crops Prod. 76, 394–401.
Srinivasan, R., Natarajan, D., Shivakumar, M.S., 2014. Antimicrobial and Gc-Ms analysis of *Memecylon edule* leaf extracts. Inter. J. Curr. Pharmace. Revi. Res. 5 (1), 1–13.
Srinivasan, R., Natarajan, D., Shivakumar, M.S., Nagamurugan, N., 2016. Isolation of fisetin from *Elaeagnus indica* serv. Bull. (Elaeagnaceae) with antioxidant and antiproliferative activity. Free Radic. Antioxidants 6 (2), 145–150.
Suffness, M., Pezzuto, J.M., 1990. Assays related to cancer drug discovery. In: In: Hostettmann, K. (Ed.), Methods in Plant Biochemistry: Assays for Bioactivity, vol. 6. Academic Press; UK, London, pp. 71–133.
Tapan, S., 2011. Antioxidant activity of some wild edible fruits of Meghalaya state in India. C 5 (3), 155–160.
Uddin, G., Rauf, Abdur, 2012. Phytochemical screening and biological activity of the aerial parts of *Elaeagnus umbellate*. Sci. Res. Essays 7 (43), 3690–3694.
Uysal, S., Aumeeruddy-Elalfi, Z., Zengin, G., Aktumsek, A., Mašković PZ Vujić JM Mahomoodally, M.F., 2018. In vitro antioxidant cytotoxicity and chemical profile of different extracts from *Acanthus hirsutus* Boiss used in Anatolian folk medicine. Eur. J. Integr Med. 17, 135–140.
Vijayaraghavan, K., Rajkumar, J., Seyed, M.A., 2018. Phytochemical screening free radical scavenging and antimicrobial potential of *Chromolaena odorata* leaf extracts against pathogenic bacterium in wound infections–a multispectrum perspective. Biocatalysis Agric. Biotechnol. 15, 103–112.
Vinayaka, K.S., Prashith Kekuda, T.R., Nethravathi, H.R., Thippeswamy, N.B., Sudharshan, S.J., 2009. Free radical scavenging and insecticidal of *Elaeagnus kologa* Schldl. Drug Inveno Today 1 (1), 74.
Wang, Y., Guo, T., Li, J.Y., Zhou, S.Z., Zhao, P., 2012. Four flavonoid glycosides from the pulps of E*laeagnus angustifolia* and their antioxidant activities. In: Proceedings of the 2nd International Conference on Computer and Information Application (ICCIA 2012).
Xu, J., Wu, L., Hui, L.F.P., Liu, T., Liu, Z., 2009. Antioxidant flavonoids from bark of *Elaeagnus angustifolia*. Planta Med. 75, 943–944.
Yen, G.C., Chen, H.Y., 1995. Antioxidant activity of various tea extracts in relation to their Antimutagenicity. J. Agric. Food Chem. 43 (1), 27–32.
Zhang, Ming, J., 2010. Study on antibacterial activity of alkaloids from *Elaeagnus mollis* Diels. J. Anhui Agri. Sci. 17.