

## Phytochemical screening and *in vitro* antioxidant potentials of *Elaeagnus conferta* Roxb.

Sachin S Nayaka<sup>1</sup>, V Krishna\*<sup>1</sup>, J Narayana<sup>2</sup>, Ravi Kumar S<sup>1</sup>, Raagavalli K<sup>1</sup>, Shashi Kumar R<sup>1</sup>, Ullas prasanna S<sup>1</sup> and Vinay Kumar N M<sup>1</sup>.

<sup>1</sup>Department of Studies and Research in Biotechnology, Kuvempu University, Shankaraghatta.

<sup>2</sup>Department of Studies and Research in Environmental Science, Kuvempu University, Shankaraghatta.

Corresponding Author - [Krishnabiotech2003@gmail.com](mailto:Krishnabiotech2003@gmail.com).

### ABSTRACT:

Free radical reactions are recognized to have a significant role in the pathophysiology of many acute and chronic human diseases and the body's natural antioxidant capacity and ROS levels were out of balance, which led to the requirement of dietary and/or pharmacological supplementation. *Elaeagnus conferta* Roxb. is an endangered medicinal plant of the Western Ghats, chosen for this study to antioxidant potentials of the leaf, stem bark and fruits sequential soxhlet extracts. Qualitative and quantitative phytochemical screening of the revealed the presence of flavonoids and glycosides in the ethanol fraction of leaf, stem bark and fruits. The *in vitro* antioxidants assay results also indicated that LEE, SBCE, and SEE possess significant scavenge of super oxide radicals. This work validates the traditional claims that *E. conferta* extracts has the potential source of anti-oxidant medication.

**Key words:** *Elaeagnus conferta* Roxb., flavonoids, super oxide radicals, Antioxidant drug.

### 1. Introduction:

Plants continue to play a significant role in healthcare despite the significant advancements in modern medicine that have been seen in recent years. Herbal remedies have been utilized for treating disease from for a very long time [1]. However, the widespread use of medicinal plants in traditional medicine and their preventive capabilities, particularly in underdeveloped nations, have generated a great deal of interest in them. Numerous medicinal plants have had their antioxidant capacities studied. Natural antioxidants are particularly powerful at stopping the harmful effects of oxidative stress, either as their chemical constituents or as raw extracts [2]. It is generally agreed that medications made from plant products are safer than those made from synthetic materials,

even though the toxicity profile of the majority of medicinal plants has not been properly assessed [3][4]. Reactive oxygen species (ROS) and other oxidants have been linked to a number of ailments and diseases, according to a large body of research. The research has drawn scientists' attention to the value of antioxidants in the prevention and treatment of diseases as well as in maintaining human health [5].

Numerous biological processes, including the ant mutagenic, anti-carcinogenic, and anti-aging responses, arise from the human body's innate ant oxidative mechanism [6][7]. Free radicals are generally stabilized or inactivated by antioxidants before they may damage targets in biological cells [8]. The application of naturally occurring antioxidants in food, cosmetic, and pharmaceutical products have recently seen a significant increase in interest due to their versatility in terms of their range and intensity of activity as well as their vast potential for redressing imbalance [9] [10].

*Eleagnus conferta* Roxb. is an endemic and endangered medicinal plant of the Western Ghats belongs to family Elaeagnaceae. It is found to be distributed in Vietnam, Malaysia, India and South China. Fruit is sweetish sour in taste, edible and have been used in traditional Indian, Tibetan, Mongolian and Uygur medicine for the treatment of indigestion [11]. The native people in Yunnan province (South China) have been using its dried fruits to relieve the after effects of alcohol for hundreds of years. Effect of *Elaeagnus Conferta* Roxb. (Elaeagnaceae) dry fruit on the activities of hepatic alcohol dehydrogenase and aldehyde dehydrogenase activities. The traditional practitioners residing in the vicinity of Bhadra Wild Life Sanctuary of the Western Ghats, Karnataka, India are using the seed extract to cure diarrhea and liver cirrhosis leaf extract is used to control cough branchial disorders and the stem bark paste is used to cure diabetic pus wounds. Reports are very scare on the phytoconstituents and the antibacterial properties. So, present study reports the Phytochemical screening and in vitro antioxidant potentials of *Eleagnus conferta* Roxb.

## **2. Materials and Methods:**

### **2.1 Collection of plant material:**

Healthy leaves, seeds and stem bark materials of *Elaeagnus conferta* Roxb. were collected from the forests of Bhadra wild Life Sanctuary of the Western Ghats of Shivamogga District, Karnataka, India. The plant has been identified and authenticated by a taxonomist Dr. Y L Krishnamurthy, Professor, Post Graduate Studies and Research in Botany, Kuvempu University.

Thereafter, the plant materials were washed 2-3 times with running tap water followed by distilled water treatment and shade dried.

## 2.2 Extraction of crude drug:

The leaves, stem bark and fruit were cleaned with deionized water and were dried in shade and powdered mechanically using a blender. The powdered material (leaves and stem bark) of 1 kg each were refluxed successively with the solvents petroleum ether, chloroform, and ethanol in a Soxhlet extractor for 48 h. The extracts were filtered (Whatman No.1 filter paper), the filtrates were concentrated in a vacuum under reduced pressure on a rotary evaporator (Buchi, Switzerland). The solvent was removed carefully at reduced pressure and dried in a desiccator for further studies.

The extraction yield was expressed as,

$$\text{Percent yield (\%)} = \frac{\text{Weight of the dry extract (g)}}{\text{Weight of the sample used for the extraction (g)}} \times 100$$



## 2.3 Phytochemical screening:

### 2.3.1 Qualitative Analysis:

The petroleum ether, chloroform and ethanol extracts of leaves, seeds and stem bark of *Elaeagnus conferta* Roxb were subjected to qualitative phytochemical screening using standard methods [12]. Each extract was tested to detect the presence of alkaloids, flavonoids, terpenoids, saponins, tannins, phenolic compounds and glycosides using standard procedures [13] [14].

### 2.3.2 Quantitative Analysis:

### 2.3.3 Estimation of Alkaloid:

1 gm of sample was added to 40 ml of 10% acetic acid, covered and allowed to stand for 4 h. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was

complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed [15].

### **2.3.4 Estimation of Phenolic compounds:**

The amounts of total phenolic contents of extracts of *Elaeagnus conferta* Roxb were determined by the spectrophotometric method of Singleton [16]. 20 µl of extracts (5mg/ml) was mixed with 0.75ml of 20% sodium carbonate solution and 0.25 ml of Folin-Ciocalteu reagent. The reaction mixture was allowed to stand in light for 3 min and incubated for 2 h in dark. The absorbance was measured at 765 nm using UV-Visible Spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of known concentration of Gallic acid standard (0-100 µg/ ml). The concentrations were expressed as µg of Gallic acid equivalents per ml and all the determination were performed in triplicates.

### **2.4 In vitro antioxidant activity:**

The current study was planned to assess the antioxidant activity of leaf, stem bark and fruit extracts of *Elaeagnus conferta* Roxb.

#### **2.4.1 Determination of DPPH radical scavenging activity:**

DPPH radical scavenging activity of extracts was conducted by adding 1 ml of extract with three different concentrations (100,200 and 300 µg/ml) in to three test tubes of 3 ml of 0.004% DPPH in 95% ethanol was added to all test tubes and the mixture was kept in the dark for 30 min before and absorbance was read at 517 nm. DPPH radical scavenging activity was calculated using the following formula; Percentage of inhibition =  $[(A_{control} - A_{test})/A_{control}] \times 100$ , where  $A_{control}$  is the absorbance of the control reaction and  $A_{test}$  is the absorbance of the extract reaction.  $IC_{50}$  value was calculated using the formula  $IC_{50} = [(\Sigma C/\Sigma I) \times 50]$ , where  $\Sigma C$  is the sum of extracts concentrations used to test and  $\Sigma I$  is the sum of percentage of inhibition at different concentrations [17].

#### **2.4.2 Nitric oxide radical scavenging activity:**

Nitric oxide radical scavenging activity of extracts was conducted by adding 250µl extracts of different concentrations (100, 200 and 300 µg/ml) by adding into different test tubes along with sodium nitropruside (5 ml in phosphate buffer saline, pH 7.4). The mixture was incubated at room temperature under light source (24 W compact fluorescent light bulb). After 150 min, 0.6 mL of the mixture was transferred into a new tube containing 0.6 ml of Griess reagent (1%

sulphanilamide and 0.1% N-(1-naphthyl)-ethylene diamine dihydrochloride in 5% phosphoric acid). After incubating for 10 min in darkness, absorbance was recorded at 546 nm. Percentage of inhibition was calculated using formula; % Inhibition =  $[(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}] \times 100$ , where  $A_{\text{control}}$  is the absorbance of the control reaction and  $A_{\text{test}}$  is the absorbance of the extract reaction. IC<sub>50</sub> value was calculated using the formula  $IC_{50} = [(\Sigma C/\Sigma I) \times 50]$ , where  $\Sigma C$  is the sum of extracts concentrations used to test and  $\Sigma I$  is the sum of percentage of inhibition at different concentrations [18].

#### 2.4.3 Superoxide anion scavenging activity:

The scavenging activity against chemically generated superoxide radicals of the leaf, stem and fruit extracts was measured by means of spectrophotometric measurement of the product on reduction of nitro blue tetrazolium (NBT). Test samples were dissolved in DMSO and diluted in water to give a final concentration of 12% (v/v) for DMSO. Superoxide anions were generated in a non-enzymatic [phenazine methosulfate (PMS)/NADH] system. The reaction mixture contained 1 ml of test solution, 1.9 ml 0.1 M phosphate buffer, pH 7.4, 1 ml of 20mM PMS, 156mM NADH, and 25mM of NBT in phosphate buffer, pH 7.4. After 2 min of incubation at 25 °C, absorbance of the resulting solution was measured at 560 nm [19].

**The percentage of scavenging activities (%) was calculated as follows:**

Scavenging activities % (capacity to scavenge the superoxide radical) =  $[1 - (\text{absorbance of sample}) / (\text{absorbance of control})] \times 100$ .

### 3. Results:

#### 3.1 Phytochemical Screening:

Preliminary qualitative phytochemical screening of the crude extracts showed the presence of alkaloids, flavonoids, phenolic, terpenoids, glycosides, tannins and saponins compounds are in all the sequential extracts of leaf Ethanol extract, Flavonoids, Saponin and tanins were absent in LPE and LCE of leaf extract, SBPE and SBCE of stem bark extracts and SPE and SCE of fruit extracts as shown in the **Table 1**. The glycosides are absent in LPE and SBPE of *Elaeagnus conferta*

**Table 1: Qualitative phytochemical analysis of extracts of *Elaeagnus conferta* Roxb.**

Tests	LPE	LCE	LEE	SBPE	SBCE	SBEE	SPE	SCE	SEE
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoids	-	-	+	-	-	+	-	-	+
Phenolic	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+
Glycosides	-	+	+	-	+	+	+	+	+
Tannins	-	-	+	+	+	+	-	-	+
Saponins	-	-	+	-	-	+	-	-	+

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract. (+) indicate the presence and (-) the absence of the respective phytochemicals.

The quantitative estimation of total phenolics, alkaloids and terpenoids contents present in the extracts of *Elaeagnus conferta* have shown in the **Table 2**. The stem bark ethanolic extract has more phenolic content (24.02 µg/mg) followed by seed ethanol extract (14.87 µg/mg) whereas, alkaloid content is more (19.32 µg/mg) in stem bark ethanol extract. The leaves pet ether extract showed more terpenoid content (47.32 µg/mg) and in the chloroform extracts the concentrations of alkaloids, phenolics and terpenoids are very less.

**Table 2: Quantitative determination of Phenolics, Alkaloids and Terpenoids (µg/mg)**

Tests	LPE	LCE	LEE	SBPE	SBCE	SBEE	SPE	SCE	SEE
Phenolic	10.07	12.36	<b>19.92</b>	17.32	19.54	<b>24.02</b>	08.39	11.58	<b>14.87</b>
Alkaloids	11.87	13.84	<b>14.58</b>	12.11	9.25	<b>19.32</b>	15.21	18.74	<b>21.58</b>
Terpenoids	<b>47.32</b>	38.41	31.62	<b>27.94</b>	22.37	20.98	<b>32.54</b>	29.47	28.46

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

### 3.3 Antioxidant Activity:

#### 3.4 DPPH free radical scavenging activity:

Discoloration of the purple color of the DPPH radical on thin layer chromatographic (TLC) plates was assessed as a positive sign of antioxidant activity of LPE, LCE, LEE, SBPE, SBCE, SBEE, SPE, SCE, SEE. Among them The LCE showed DPPH free radical scavenging activity in dose dependent manner and its IC<sub>50</sub> values were: IC<sub>50</sub>; DPPH: 201.80 µg/ml respectively. Comparatively, the IC<sub>50</sub> values of SBPE was significantly IC<sub>50</sub>; DPPH: 189.08 µg/ml and The the

IC<sub>50</sub> values of SCE was significantly DPPH: 196.50 µg/ml, IC<sub>50</sub> value of synthetic antioxidant was tested as the positive control, and data are shown in **Table 3**.

**Table 3: DPPH radical scavenging assay of extracts of *Elaeagnus conferta* Roxb.**

Sl. No	Activity	Extracts	Concentration in µg/ml	% of Inhibition	Standard IC <sub>50</sub> µg/ml
01	DPPH free radical scavenging activity	LPE	100	38.67±0.33	169.89
			200	54.33±0.33	
			300	83.58±0.33	
02		LCE	100	27.67±0.33	<b>201.80</b>
			200	49.33±0.33	
			300	71.33±0.33	
03		LEE	100	48.33±0.33	141.07
			200	74.00±0.33	
			300	90.33±0.33	
04		SBPE	100	33.00±0.33	<b>189.08</b>
			200	51.33±0.33	
			300	74.33±0.33	
05	SBCE	100	39.67±0.33	161.57	
		200	58.67±0.33		
		300	87.33±0.33		
06	SBEE	100	38.00±0.33	181.09	
		200	55.33±0.33		
		300	72.33±0.33		
07	SPE	100	30.33±0.33	171.10	
		200	55.67±0.33		
		300	89.33±0.33		
08	SCE	100	29.67±0.33	<b>196.50</b>	
		200	47.67±0.33		
		300	75.33±0.33		
09	SEE	100	34.67±0.33	175.77	
		200	58.67±0.33		
		300	77.33±0.33		

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

### 3.5 Superoxide and nitric oxide activities:

LPE, LCE, LEE, SBPE, SBCE, SBEE, SPE, SCE, SEE. Were screened for superoxide radical scavenging activity by PMS–NADH–NBT system. The IC<sub>50</sub> values of quenching radicals were found to be 189.17 µg/ml, 190.28 µg/ml and 172.74 µg/ml for LPE, SBEE and SEE, The standard gallic acid, respectively. Similarly, Nitric oxide radical scavenging activity of LPE (IC<sub>50</sub>: 189.17 µg/ml), SBPE (IC<sub>50</sub>: 162.46 µg/ml) and SCE (IC<sub>50</sub>: 171.75 µg/ml) were more than to the value of standard ascorbic acid, the result of above activities clearly indicates strong concentration dependent activity and it is showed in **Table 4 and Table 5**.

**Table 4: Superoxide radical scavenging assay of extracts of *Elaeagnus conferta* Roxb.**

Sl. No	Activity	Extracts	Concentration in µg/ml	% of Inhibition	Standard IC <sub>50</sub> µg/ml
01	Superoxide radical scavenging activity	LPE	100	29.67±0.33	189.17
			200	54.33±0.33	
			300	74.58±0.33	
02		LCE	100	26.67±0.33	179.64
			200	53.33±0.33	
			300	87.33±0.33	
03		LEE	100	31.33±0.33	164.23
			200	60.00±0.33	
			300	91.33±0.33	
04	SBPE	100	28.00±0.33	166.05	
		200	66.33±0.33		
		300	86.33±0.33		
05	SBCE	100	31.67±0.33	169.80	
		200	63.67±0.33		
		300	81.33±0.33		
06	SBEE	100	35.00±0.33	190.28	
		200	49.33±0.33		
		300	73.33±0.33		
07	SPE	100	35.33±0.33	155.17	
		200	68.67±0.33		
		300	89.33±0.33		
08	SCE	100	29.67±0.33	162.45	
		200	66.67±0.33		
		300	88.33±0.33		
09	SEE	100	29.67±0.33	172.74	
		200	52.67±0.33		
		300	91.33±0.33		

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

**Table 5: Nitric oxide radical scavenging assay of extracts of *Elaeagnus conferta* Roxb.**

Sl. No	Activity	Extracts	Concentration in µg/ml	% of Inhibition	Standard IC <sub>50</sub> µg/ml
01	Superoxide radical scavenging activity	LPE	100	28.67±0.33	189.17
			200	52.33±0.33	
			300	77.58±0.33	
02		LCE	100	29.67±0.33	176.12
			200	51.33±0.33	
			300	89.33±0.33	
03		LEE	100	30.33±0.33	160.72
			200	66.00±0.33	
			300	90.33±0.33	
04	SBPE	100	26.00±0.33	162.46	
		200	69.33±0.33		
		300	89.33±0.33		



05	Nitric oxide radical scavenging activity	SBCE	100	30.67±0.33	156.25
			200	72.67±0.33	
			300	89.33±0.33	
06		SBEE	100	31.00±0.33	160.72
			200	66.33±0.33	
			300	89.33±0.33	
07		SPE	100	37.33±0.33	144.92
			200	78.67±0.33	
			300	91.33±0.33	
08		SCE	100	30.67±0.33	171.75
			200	56.67±0.33	
			300	87.33±0.33	
09		SEE	100	29.67±0.33	166.97
			200	59.67±0.33	
			300	90.33±0.33	

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

#### 4. Discussion:

The investigation phytochemical screening, *in vitro* antioxidant potentials of *Eleagnus conferta* Roxb. showed that the chemicals with low antioxidant activity would likely exhibit little action, a variety of approaches have been utilized to determine the antioxidant activity in order to allow quick screening of substances [8]. It is well recognized that free radicals play a significant part in a wide range of clinical symptoms. Antioxidants save us from numerous diseases by battling free radicals. They work either by removing reactive oxygen species from the environment or by defending the antioxidant defense systems [20]. The ability of natural products to donate electrons can be assessed by bleaching 2,20-diphenyl-1-picrylhydrazyl radical (DPPH)-colored solution [8]. The technique relies on the DPPH being scavenged by the addition of an antioxidant or radical species that makes the DPPH solution less colorful. The concentration and potency of the antioxidants are inversely correlated with the degree of color change. Considerable free radical scavenging activity of the tested chemical is shown by a significant drop in the absorbance of the reaction mixture [21].

In the current investigation, LPE, SBPE, SBEE, SCE and SEE demonstrated considerably greater inhibitory percentages among all tested extracts and had a positive correlation with total phenolic content. According to the study's findings, a plant extract contains phytochemical components that can donate hydrogen to a free radical in order to scavenge potential damage. One important biological source of reactive oxygen species is thought to be the superoxide radical [22].

Despite being a weak oxidant, superoxide ion produces singlet oxygen and potent and hazardous hydroxyl radicals, both of which contribute to oxidative stress [23].

## 5. Conclusion:

In traditional medicines the decoction of the stem bark and fruits have been used to relieve chest pain and cardiac related problems, in qualitative analysis study the fruit and stem bark extracts showed the presences of flavonoids and glycosides, *in vitro* antioxidants assay also LEE, SBCE and SEE showed potential scavenging of super oxide radicals, therefore this investigation authenticates the traditional claim of *E. conferta* extracts as the potential anti-oxidant drug.

## 6. Acknowledgments:

The authors are thankful to DBT, New Delhi, India for providing financial support through DBT-BUILDER program (Order No. BT /PR9128 /INF /22 /190 /2013, Dated: 30/06/2015) and the Kuvempu University administrative authority for offering the facility to carry out the work.

## 7. References:

1. Maqsood S, Singh P, Samoon MH, Balange AK: Effect of dietary chitosan on non-specific immune response and growth of *Cyprinus carpio* challenged with *Aeromonas hydrophila*. *Inter Aqua Res* 2010, 2:77–85.
2. Zengin G, Cakmak YS, Guler GO, Aktumsek A: Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz. *Rec Nat Prod* 2011, 5:123–132.
3. Vongtau HO, Abbah J, Chindo BA, Mosugu O, Salawu AO, Kwanashie HO, Gamaniel KS: Central inhibitory effects of the methanol extract of *Neorautanenia mitis* root in rats and mice. *J Pharm Biol* 2005, 43:113–120.
4. Oluyemi KA, Okwuonu UC, Baxter DG, Oyesola TO: Toxic effects of methanolic extract of *Aspilia africana* leaf on the estrous cycle and uterine tissues of Wistar rats. *Int J Morphol* 2007, 25:609–614.
5. Halliwell B, Gutteridge JMC: Formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. *FEBS Lett* 1981, 128:347–352.

6. Gulcin I: Antioxidant activity of food constituents: an overview. Arch Toxicol 2012, 86:345–391.
7. Gocer H, Gulcin I: Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. Int J Food Sci Nutr 2011, 62:821–825.
8. Nunes PX, Silva SF, Guedes RJ, Almeida S: Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health. 2012.
9. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N: Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem 2006, 97:654–660.
10. Wannas WA, Mhamdi B, Sriti J, Jemia MB, Ouchikh O, Hamdaoui G, Kchouk ME, Marzouk B: Antioxidant activities of the essential oil and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. Food Chem Toxicol 2010, 48:1362–1370.
11. Osawa T, Kavakishi S, Namiki M, Kuroda Y, Shankal DM, Waters MD: Antimutagenesis and anticarcinogenesis mechanisms II. New York: Plenum; 1990:139–153.
12. Arunodaya HS, Krishna V, Shashikumar R, Girish Kumar K. Antibacterial and antioxidant activities of stem bark essential oil constituents of *Litsea glutinosa* C. B. Rob. Int. J. Pharm. Pharm. Sci. 2016; 8:258–64.
13. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African J. Tradit. Complement. Altern. Med. 2011; 8:1–10.
14. Mostafa, R. M., & Essawy, H. S. (2021). Screening and Quantification of Bioactive Compounds and Antimicrobial Activities of Fresh Juice, Methanolic Peel and Pulp Extract of *Citrus sinensis* L. (Sweet Orange). Egyptian Academic Journal of Biological Sciences, G. Microbiology, 13(2), 1-10.
15. Sen A, Batra A. Evaluation of Antimicrobial Activity of Different Solvent Extracts of Medicinal Plant: *Melia Azedarach* L. Int. J. Curr. Pharm. Res. 2012; 4:67–73.
16. Murray PR, Baron EJ, American Society for Microbiology. Manual of clinical microbiology. 8th ed. Washington D. C: ASM Press, 2003.

17. Rajesh, K. P., Manjunatha, H., Krishna, V., & Swamy, B. K. (2013). Potential in vitro antioxidant and protective effects of *Mesua ferrea* Linn. bark extracts on induced oxidative damage. *Industrial Crops and Products*, 47, 186-198.
18. Kumaran, A., & Karunakaran, R. J. (2006). Nitric oxide radical scavenging active components from *Phyllanthus emblica* L. *Plant Foods for Human Nutrition*, 61(1), 1-5.
19. Fazilatun, N., Nornisah, M., & Zhari, I. (2004). Superoxide radical scavenging properties of extracts and flavonoids isolated from the leaves of *Blumea balsamifera*. *Pharmaceutical biology*, 42(6), 404-408.
20. Umamaheswari M, Chatterjee TK: In vitro antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. *Afr J Trad Compl Altern Med* 2008, 5:61–73.
21. Krishnaiah D, Sarbatly R, Nithyanandam RR: A review of the antioxidant potential of medicinal plant species. *Food Bioprod Process* 2011, 89:217–233.
22. Alves CQ, David JM, David JP, Bahia MV, Aguiar RM: Methods for determination of in vitro antioxidant activity for extracts and organic compounds. *Química Nova* 2010, 33:2202–2210.
23. Meyer AS, Isaksen A: Application of enzymes as food antioxidants. *Trends Food Sci Tech* 1995, 1995(6):300–304.