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RESEARCH ARTICLE

Invitro Antioxidant Activity of *Embelia basaal* Burm.f. (Myrsinaceae)

S. Vennila, M. Saradha*

PG & Research Department of Botany, Nirmala College for Women, Coimbatore, Tamil Nadu.

Abstract

This study evaluated the phytochemical screening, secondary metabolite content of different extracts and antioxidant activity of acetone extracts of *Embelia basaal* leaf and bark. Using Soxhlet extraction the different solvents such as petroleum ether, chloroform, acetone and ethanol were used for determining the qualitative phytochemical analysis and it confirmed the presence of primary and secondary metabolites. The quantification of total phenolic, tannin and flavonoid contents were found to be higher in the acetone extract of *E.basaal* bark. Subsequently the acetone extracts were subjected to antioxidant assays (DPPH, ABTS, nitric oxide, ferric reducing, superoxide, lipid peroxidation, and reducing power) and the results revealed that the acetone extract of *E.bassal* bark demonstrated superior free radical scavenging activity compared to the leaf. Thus, the findings highlight *E.basaal* has potential for developing health promoting drugs.

Keywords: *Embelia basaal*, *In-vitro* Antioxidant, Secondary metabolites, Phytochemical, Polar solvents.

1. Introduction

The genus *Embelia* is a member of the Primulaceae family, with over 130 species, (Angiosperm Phylogeny Group IV 2016). *Embelia ribes* Burm. f. is a shrubby creeper with woody climbers that belongs to the Primulaceae family and is found in semi-evergreen and deciduous forests in India. It is widely referred to as "Vidanga," one of the oldest traditional medicinal species in India, and is mostly used in the ayurvedic medical system. in various forms like churna, asava, aristha, lauha and taila.

E. basal is a highly valued material in many formulations and has strong anthelmintic and anti-oxidant effects in Ayurvedic medicine[1]. The bigger elliptical leaves of the plants are used with ginger, as a gargle for sore throats, the dried root bark is used as a toothache cure, and the finely ground berries are used as an ointment to treat pleuritis [2]. Fruits are carminative, astringent, light, arousing, stimulant, anthelmintic, and alternative. It is renowned for its ability to drive out tapeworms. A seed decoction helps with skin conditions, fevers, and chest issues [3].

In traditional medicine, plants are the most precious source of medicines used by many diverse populations by various communities [4]. The World Health Organization (WHO) states that the greatest source of a wide range of medications is medicinal plants [5]. Approximately 80% of people in wealthy nations utilize traditional medicines [6], that contains phytochemical compounds, which are responsible for their potential to cure many diseases.

All the parts of medicinal plants naturally contain phytochemicals that act as defense mechanisms and offer protection against a variety of illnesses. Secondary metabolites are naturally occurring plant chemicals that are the byproducts of primary metabolites, which include phenolic compounds, alkaloids, steroids, flavonoids, terpenoids, glycosides, saponins, tannins, and others [7].

Secondary metabolites are natural plant chemicals. These compounds play a key role in many medications, helping to neutralize a harmful free radicals produced during metabolism [8]. Excess free radicals, caused by abnormal metabolism increase the risk of diseases [9]. Natura antioxidant have gained popularity because of synthetic ones, like BHA and BHT that are linked to toxicity, side effects and high production costs [10]. This study focuses on analyzing the phytochemicals and antioxidant properties of *E. basaal* to improve global health.

2.Materials and Methods

2.1. Collection and extraction of Plant material

The *E. basaal* leaves and bark, were gathered from Manjoor Village in the Nilgiri District and authenticated by the Botanical Survey of India (voucher specimen no: BSI/SR/5/23/2024/Tech –

*Correspondence: M. Saradha, PG & Research Department of Botany, Nirmala College for Women, Coimbatore, Tamil Nadu India. E.mail: *saradha.bio@gmail.com*

234.), the Southern Regional Center in Coimbatore, Tamil Nadu. After being cleaned with tap water, the plant material was shade-dried for 20 days, ground into a fine powder, packed in thimbles, and then extracted successively using a Soxhlet apparatus utilizing solvents such as petroleum ether, chloroform, acetone, and ethanol. The extract was placed in an airtight container for future studies.

2.2 Qualitative phytochemical analysis

Standard protocols were used to evaluate the extracted samples for the initial phytochemical screening in order to identify the presence of primary and secondary metabolites [11].

2.3 Quantitative analysis of Secondary metabolites

2.3.1 Determination of total phenolics

The method described by [12] was used to determine the total phenolic content. In the test tubes, fifty microliter triplicates of the extracts (20 mg/20 mL) were obtained, and distilled water was added to bring the volume up to one millilitre. Next, each tube was filled with 2.5 mL of sodium carbonate solution (20%) and 0.5 mL of Folin-Ciocalteu Phenol Reagent (1:1 with water). The absorbance at 725 nm was measured against the reagent blank shortly after the reaction mixture was vortexed and the test tubes were left in the dark for 40 minutes. Gallic acid equivalents were used to express the results of the triplicate analysis.

2.3.2 Determination of total Tannins

The tannins were quantified using the same extract after the treatment with polyvinyl polypyrrolidine (PVPP) following, [13] procedure. A 100 × 12 mm Eppendorf tube was filled with 100 mg of PVPP, 1 mL of distilled water, and 1 mL of the sample extracts. After vortexing the material, it was frozen for 15 minutes at 4°C. The sample was then centrifuged at 4000 rpm for 10 minutes at room temperature, and the supernatant was gathered. Apart from the tannins, which would have precipitated with the PVPP, this supernatant solely contains simple phenolics. Using the foregoing method, the phenolic content of the supernatant was determined and reported as the amount of non-tannin phenolics.

2.3.3 Determination of total flavonoids

The method by [14] was used to quantify the flavonoid levels of the plant extracts. Two milliliters of distilled water were added to each test tube containing about 100 microliters of the plant extracts. The blank was a test tube filled with 2.5 mL of distilled water. After adding 150 µL of 5% $NaNO_2$ to each test tube, the tubes were incubated for six minutes at room temperature. Following incubation, all test tubes, including the blank, received 150 µL of 10% $AlCl_3$. At room temperature, every test tube was incubated for six minutes. After adding 2 mL of 4% NaOH to each test tube, the volume was increased to 5 mL with distilled water. After thoroughly vortexing the contents in each test tube, they were let to stand at room temperature for fifteen minutes. At 510 nm, the pink hue that resulted from the presence of flavonoids was detected using spectrophotometry. The quercetin equivalent (mg/g) was represented as the standard during the absorbance.

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging activity

According to [11] method, the extract's antioxidant activity was assessed using the stable radical DPPH to measure its capacity to donate hydrogen or scavenge radicals. Different concentrations of sample extracts were obtained, and methanol was used to adjust the volume to 100 µL. approximately 5 mL of a 0.1 mM methanolic solution of DPPH was added Sample and standard aliquots (BHA, BHT, rutin, and quecertin) and shaken vigorously. 100 µL of methanol was added to 5 mL of 0.1 mM methanol solution DPPH as the negative control. At 27°C, the tubes were allowed to stand for 20 minutes. At 517 nm, the sample's absorbance was measured in comparison to the blank (methanol). The IC_{50}, or the concentration of the sample needed to inhibit 50% of the $DPPH^{\cdot}$ concentration, was used to express the samples' radical scavenging ability.

2.4.2 ABTS radical cation scavenging activity

Using the [1] methodology, the ABTS cation radical decolorization assay was used to measure the samples antioxidant activity. 7 mM ABTS aqueous solution and 2.4 mM potassium persulfate were mixed in the dark for 12 -16 hours at room temperature to yield ABTS•+. This solution was diluted in ethanol (about 1:89 v/v) and allowed to equilibrate at 30°C prior to the experiment, to exhibit an absorbance of 0.700 ± 0.02 at 734 nm. The sample extracts stock solution was diluted so that, when 10 µL aliquots were added to the experiment, the blank absorbance was inhibited by 20% to 80%. After the initial mixing of 10 µL of sample or Trolox (final concentration 0–15 µM) in ethanol with 1 mL of diluted ABTS solution, absorbance was measured at 30°C precisely 30 minutes. Each standard dilution was determined in triplicate, and the % inhibition was shown as a function of Trolox concentration after being

compared to the blank (ethanol) at 734 nm. The concentration of trolox with comparable antioxidant activity, given as μM/g sample extracts, is the unit of total antioxidant activity (TAA).

2.4.3 Phosphomolybdenum assay

The green phosphomolybdenum complex production, as per the [15] method, was used to assess the antioxidant activity of the samples. One milliliter of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added to a test tube containing 40 microliters of sample or ascorbic acid in 1 milliliter of dimethyl sulfoxide (standard) or distilled water (blank). After being wrapped in foil, the test tubes were placed in a water bath set at 95°C for 90 minutes. Following the samples' cooling to room temperature, the mixture's absorbance at 695 nm was measured in comparison to the reagent blank. Total antioxidant capacity data are mean values in milligrams of ascorbic acid equivalents per gram of extract, or mg AAE/g.

2.4.4 Ferric reducing antioxidant power (FRAP) assay

A method outlined by [16] was used to determine the antioxidant capabilities of various sample extracts. 30 μL of test sample or methanol (for the reagent blank) and 90 μL of distilled water were combined with 900 μL of freshly made FRAP reagent that had been incubated at 37°C. In a water bath, the test samples and reagent blank were incubated for 30 minutes at 37°C. The test sample was finally diluted to 1/34 in the reaction mixture. 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM FeCl3. 6H2O, and 25 mL of 0.3 M acetate buffer (pH-3.6) were combined to create the FRAP reagent. A spectrophotometer was used to acquire absorbance measurements at 593 nm against the reagent blank immediately as the incubation was completed. The calibration curve was prepared using methanolic solutions with known Fe (II) concentrations, ranging from 100 to 2000 μM (FeSO4.7H2O). The concentration of antioxidant with a ferric-TPTZ reducing capacity equal to 1 mM FeSO4.7H2O was defined as the Equivalent Concentration parameter. The antioxidant concentration that increased absorbance in the FRAP assay to the theoretical absorbance value of a 1 mM concentration of Fe (II) solution was determined to be the equivalent concentration.

2.4.5 Superoxide radical scavenging activity

The experiment was predicated on the ability of different extracts to scavenge the superoxide radicals produced in the riboflavin–light–NBT system, hence inhibiting the synthesis of formazan [17]. 50 mM sodium phosphate buffer (pH-7.6), 20 μg riboflavin, 12 mM EDTA, 0.1 mg NBT, and 40 μL of an aliquot of sample solution or BHA and BHT (standard) were all included in each 3 mL reaction mixture. The reaction was initiated by exposing the sample extract on the reaction mixture for ninety seconds. And the negative control, with identical tubes containing the reaction mixture were stored in the dark, the absorbance was measured immediately after exposure in comparison to the reagent blank (reaction mixture devoid of plant sample) at 590 nm, and the scavenging activity (%) was calculated.

2.4.6 Nitric oxide scavenging activity

This process is based on the approach [18] Sodium nitroprusside in aqueous solution at its natural pH spontaneously produces nitric oxide, which combines with oxygen to form nitrite ions that may be calculated using the Greiss reagent. Nitric oxide scavengers compete with oxygen, which lowers the amount of nitrite ions produced. For the experiment, different concentrations of sample solution of different extracts or BHT and rutin (standard) were combined with sodium nitroprusside (10 mM) in phosphate buffered saline (0.2 M, pH-7.4), and the mixture was incubated for 150 minutes at room temperature. The negative control was the same reaction mixture without the sample. 0.5 mL of Griess reagent (1% sulfanilamide, 2% H3PO4, and 0.1% N-1-naphthyl) ethylene diamine dihydrochloride) was added following the incubation period. At 546 nm, the chromophore's absorbance was measured in comparison to the blank (phosphate buffer) and the scavenging activity (%) was calculated.

2.4.7 Lipid peroxidation assay

Using egg yolk homogenates as lipid-rich media, the amount of lipid peroxide produced was measured using a modified thiobarbituric acid-reactive species (TBARS) assay [19]. malondialdehyde (MDA), a subsequent byproduct of the oxidation of polyunsaturated fatty acids reacts with two molecules of TBA yielding a pinkish red chromogen with a maximum absorbance at 532 nm. Egg homogenate (500 μl of 10%, v/v in phosphate-buffered saline pH 7.4) and 200 μl of sample were added to a test tube and made up to 1.0 ml with distilled water. Then, 50 μl of $FeSO_4$ (0.075 M) and 20 μl of L-ascorbic acid (0.1 M) were added and incubated for 1 h at 37˚C to induce lipid peroxidation. Thereafter, 0.2 ml of EDTA (0.1 M) and 1.5 ml of TBA reagent (3 g TBA, 120 g TCA and 10.4 ml 70% $HClO_4$

in 800 ml of distilled water) were added in each sample and heated for 15 min at 100˚C. After cooling, samples were centrifuged for 10 min at 3000 rpm and absorbance of supernatant was measured at 532 nm and inhibition (%) of lipid peroxidation was calculated.

2.4.8 Reducing power assay

The reducing power of different solvent extracts was determined by the method adopted by [20]. One milliliter of phosphate buffer (pH 6.6) was mixed with 50–250 µg of extract. After adding 5 mL of a 1% potassium ferricyanide solution, the mixture was incubated for 20 minutes at 58°C. Five milliliters of 10% TCA were added following the incubation. For five minutes, the material was centrifuged at 5000 rpm. 0.5 mL of 1% ferric chloride and 5 mL of distilled water were combined with the top layer of the supernatant (5 mL). Using spectrophotometry, the absorbance of the reaction mixture was determined at 700 nm. The following formula was used to determine the percentage increase in reducing power.

2. Results and Discussion

Table 1: Phytochemical screening of various leaf and bark extracts of *E.basaal*

Phytochemicals	Leaf				Bark			
	P.E	C.F	A	E	P.E	C.F	A	E
Carbohydrates	+	+++	++	+++	+	+++	++	+++
Proteins	_	+++	_	++	++	++	++	_
Alkaloids	+++	_	+	_	+++	+++	_	++
Saponins	_	_	_	_	_	_	_	_
Flavonol Glycosides	_	_	++	++	_	_	++	_
Glycosides	+++	+	++	_	++	+	+++	++
Cardiac glycosides	+++	_	++	_	+	+	+++	++
Phytosterols	++	+++	++	+	++	+	+++	++
Flavonoids	++	++	++	+	+	++	++	+
Phenolic compounds	++	++	+++	++	++	++	+++	++
Tannins	+++	+++	+++	++	+++	+++	+++	_
Steroids	+++	_	_	+	+++	_	_	++
Terpenoids	+	+	+++	+	+	+	+++	+

P.E - Petroleum ether, C.F. - Chloroform, A - Acetone, E - Ethanol

(+): Presence of chemical compound, (-): Absence of chemical compound

(+) < (++) < (+++): Based on the intensity of characteristic colour produced

Table 2: Total phenolic, tannin and flavonoid in various leaf and bark extracts of *E.basaal*

Extracts	Plant part	Total Phenolics (mg GAE/g extract)	Total Tannin (mg GAE/g extract)	Flavonoid (mg RE/g extract)
Petroleum ether	Leaf	88.79 ± 2.56	78.02 ± 2.85	34.6 ± 0.58
	Bark	71.42 ± 3.02	57.63 ± 2.84	24.52 ± 0.51
Chloroform	Leaf	44.53 ± 1.68	30.84 ± 1.71	11.76 ± 0.26
	Bark	134.73 ± 0.48	116.69 ± 0.73	47.56 ± 0.81
Acetone	Leaf	952.94 ± 2.11	610.15 ± 2.26	62.7 ± 0.51
	Bark	959.66 ± 0.84	800.31 ± 1.78	98.12 ± 0.19
Ethanol	Leaf	682.35 ± 0.84	497.24 ± 3.31	19.46 ± 0.33
	Bark	120.17 ± 3.85	119.09 ± 4.01	35.53 ± 0.12

GAE – Gallic Acid Equivalents; RE – Rutin Equivalents
Values are mean of triplicate determination (n=3) ± standard deviation.

***Invitro* antioxidant activity**

Table 3: *Invitro* antioxidant activity in various in leaf and bark extracts of *E.basaal*

Antioxidant assay	Solvent	Leaf	Bark	Standard	
DPPH IC_{50} (µg/mL)	Acetone	49.53	54.74	Rutin	5.56
				BHT	4.44
ABTS (µgTE/g extract)	Acetone	139444.4 ± 240.56	137500 ± 208.33	Rutin	144167 ± 416.6
				BHT	145347 ± 636.4
FRAP mM Fe (II)/mg extract)	Acetone	512.09 ± 1.13	225.92 ± 1.95	Rutin	522.83 ± 4.46
				BHT	566.91 ± 3.5
Nitric oxide (% of inhibition)	Acetone	42.3 ± 1.56	50.02 ± 1.61	Rutin	52.7 ± 0.25
				BHT	54.2 ± 0.1
Phosphomolybdenum assay mg AAE/g (extract)	Acetone	550.99 ± 0.65	1274.16 ± 0.79	AAE	
Superoxide radical (% of inhibition)	Acetone	26.47 ± 0.66	40.91 ± 0.21	Rutin	53.2 ± 0.1
				BHT	54.7 ± 0.25
Lipid peroxidation (% of inhibition)	Acetone	52.39 ± 0.13	54.65 ± 0.05	Rutin	73.4 ± 0.87
				BHT	74.2 ± 0.94

TE – Trolox Equivalents; Fe (II) - Ferric Equivalents; AAE- Ascorbic acid equivalent
Values are mean of triplicate determination (n=3) ± standard deviation

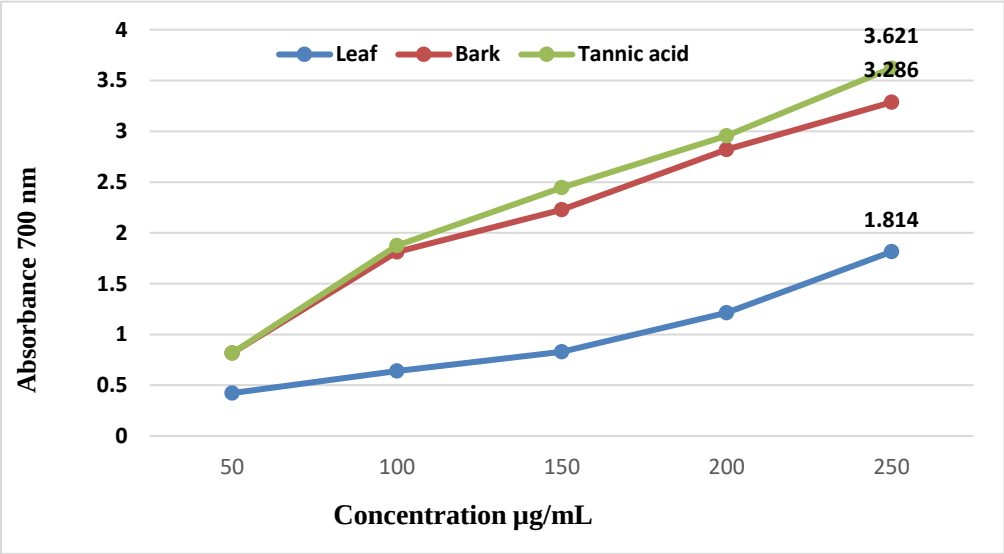

Figure. 1. Reducing power assay of acetone leaf and bark extract of *E.basaal*

3.1 Phytochemical analysis

3.1.1 Qualitative phytochemical screening

In general, the phytochemicals found in plants and plant-derived products are nontoxic and have therapeutic qualities [21] Table 1. shows the findings of the qualitative phytochemical analysis that was performed on the different extracts of *E.basaal* leaf and bark.

In all extracts, carbohydrates were found, but they were more prevalent in ethanol and chloroform. Proteins were found in chloroform and ethanol leaf extracts. Petroleum ether and chloroform bark extracts had high levels of alkaloids, while ethanol bark and petroleum ether leaf extracts had minimal quantities. Acetone and ethanol leaf extracts and acetone bark extracts consisted of flavonol glycosides, whereas petroleum ether and acetone bark extracts contained significant amounts of glycosides and cardiac glycosides. The findings of the phytochemical screening of *E. basaal* leaf and bark extracts are consistent with earlier research conducted by [2] in *E. ribes* leaf extracts, which found important bioactive substances like alkaloids, flavonoids, proteins, carbohydrates, and amino acids in various solvent extracts (acetone, ethanol, methanol, aqueous). In general, flavonoids and phenolic compounds were found, with acetone extracts having the highest concentration of phenolics. Tannins were not present in ethanol bark extracts, although they were present in petroleum ether, chloroform, and acetone extracts of bark and leaf of *E.basaal*. This study also supports the findings of [22] they observed similar compounds in the stem extracts, including alkaloids, carbohydrates, cardiac glycosides, quinones, phenols, and tannins, with saponins and starch being absent. Terpenoids and phytosterols were both highly present in the acetone and chloroform extracts, respectively. All extracts were devoid of saponins.

Overall, a notable phytochemical diversity was shown in the bark and leaves extracts of *E.basal*. However, the bark was the richer part in terms of phytochemical contents, as evidenced by the increased presence of important secondary metabolites such as alkaloids, glycosides, cardiac glycosides, and tannins in bark extracts. The high intensity of the colour showed a high concentration of specific secondary metabolites, denoted by the +++ sign and the lack of a chemical constituents is indicated by the - sign. The phytochemical richness of the plant and its potential for more pharmacological research are highlighted by these findings.

3.2 Quantitative analysis of secondary metabolites

3.2.1 Determination of total phenolic and tannin contents of *E.basaal*

Phenolic substances are comparatively persistent phenoxyl radicals that interfere with biological components' chain oxidation reactions [23]. These play a specific role in scavenging of free radicals [8]. The yields of the phenolic contents of *E. basaal* using various solvents varied. Acetone was the most efficient solvent, with the highest yields from both the leaf (952.94 ± 2.118 mg GAE/g) and the bark (959.66 ± 0.848 mg GAE/g). The results were

compared with previous reports, where [4] analyzed the total phenolic content of *E.bassal* dried ethanolic extract (in terms of gallic acid equivalents) and found that it was higher (5.8 mg GAE/g) than other extracts. However, ethanol yielded more phenols from the leaf (682.35 ± 0.848 mg GAE/g), while petroleum ether and chloroform yielded more phenols from the bark (134.73 ± 0.488 mg GAE/g), (71.42 ± 3.028 mg GAE/g) respectively.

The maximum tannin content was found in acetone (610.15 ± 2.26 mg GAE/g) leaf and (800.31 ± 1.78 mg GAE/g) and bark extracts of *E.basaal*. Followed by ethanol (497.24 ± 3.31 mg GAE/g) in the leaf and (119.09 ± 4.01 mg GAE/g) in the bark extract of *E.basaal*. Both leaf and bark yields lower tannin content in petroleum ether and chloroform extracts of *E.basaal*. These outcomes are consistent with those of Saraf *et al*., (2016), who found that flavonoids and tannins were the main phytochemicals present in fruit extracts from *E. ribes* and that they were soluble in polar solvents such as acetone. Similarly, Ananth and Anand Gideon, (2021) results depicted that tannins are a major secondary metabolite in the stem extracts of *E. ribes* are supported by the notable tannin concentration found in acetone and chloroform extracts.

3.2.2 Quantification of flavonoids

Table 2 displays the analysis and flavonoid content of *E.basaal.* Acetone produced the maximum flavonoid concentration in the leaf (62.7 ± 0.518 mg RE/g) and bark (98.12 ± 0.198 mg RE/g) of *E. basaal* when compared to other solvents. This demonstrates that acetone is the best solvent for extracting flavonoids from *E. basaal.* Acetone extracts high flavonoid content is in line with research by [2], which showed that *E. ribes* leaf extracts containing flavonoids were abundant when polar solvents were used.

3.3 Invitro Antioxidant Activity

3.3.1 DPPH Radical Scavenging activity

Table 3. displayed the extracts of *E.basaal* capacity to scavenge DPPH radicals. The strongest free radical scavenging activity was represented by the lowest IC_{50} values. The assay was contrasted with BHT and standard rutin. Acetone leaf extract has the strongest DPPH radical scavenging activity among the extracts tested, as evidenced by its higher IC_{50} values compared to *E.basaal* bark (54.74 µg/mL). The standard rutin and BHT were found to have radical scavenging activities of 5.56 µg/mL and 4.44 µg/mL, respectively. According to [24], the ethanolic extract of *E.ribes* had a superior DPPH value of 67.48±0.17 µg/mL, which is significantly greater than that of the *E.basaal* research sample.

3.3.2 ABTS radical cation scavenging activity

An oxidation process with potassium persultate produced the ABTS (2, 2-azinobis-3-ethylbenzothiazolino-6-sulfonic acid) radicals. Using ABTS, the total antioxidant capacity of *E.basaal* acetone extracts was calculated as indicated in Table 3. The acetone extract of the leaf has a slightly higher activity (139,444.4 µg TE/g) than the bark extract (137,500 µg TE/g) when compared to the synthetic antioxidant BHT (145,347 µg TE/g) and the natural standard antioxidant rutin (144,167 µg TE/g). In accordance with [25], *E. ribes* methanolic extracts have high ABTS scavenging activity because of their abundance of bioactive compounds, such as embelin, which produced results comparable to those of *E.basaal* and is essential for scavenging free radicals and shielding biological systems from oxidative stress.

3.3.3 Ferric Reducing antioxidant power assay

The ferrous complex, which has a strong blue colour and is detected at 593 nm, is produced when ferrictripridyltridyltriazine is reduced. Comparing the acetone extract from the leaf to the standard antioxidants rutin (522.83 mM Fe (II)/mg extract) and BHT (566.91 mM Fe (II)/mg extract), the FRAP results show that the leaf extract has the highest activity at 512.09 mM Fe (II)/mg extract, while the bark extract has a lower capacity at 225.92 mM Fe (II)/mg. Similarly, it was observed that *E.ribes* had the maximum FRAP activity in a berry methanolic extract, measuring 66.73 ± 0.60 mg Fe(II)/g [24]. It said that the species *Embelia* contains a wealth of phytocompounds that are excellent natural antioxidant sources.

3.3.4 Nitric oxide Scavenging activity

In comparison to the leaf extract (42.3%) bark has a higher percentage of inhibition (50.02%) in contrast to the common antioxidants, BHT and rutin. The substantial nitric oxide scavenging action of embelin-rich extracts of *E. ribes* is highlighted in a study by [26]. This property is attributed to the phenolic hydroxyl groups in the extracts, which efficiently donate electrons to neutralize NO radicals.

3.3.5 Phosphomolybdenum assay

The results demonstrate that the acetone extract of bark of *E.basaal* (1274.16 mg AAE/g) has a significantly greater capacity to reduce free radicals than the leaf extract (550.99 mg AAE/g), indicating superior antioxidant potential over the leaf extract

and being equivalent to the natural antioxidant ascorbic acid. Based on the findings of the earlier study by [27] the presence of polyphenols and flavonoids, which function as electron donors in lowering molybdenum ions to their lower oxidation states, was associated with the significant antioxidant activity that the methanolic extracts of *E. ribes* demonstrated in this assay.

3.3.6 Superoxide radical scavenging activity

Superoxide radicals are a precursor to additional reactive oxygen species, they are known to be harmful to biological components. However, the results, which are displayed in Table 3, depicts that the acetone extract of bark of *E.basaal* shows the highest percentage of inhibition at 40.91%, while the leaf extract shows the lowest percentage of inhibition at 26.47%. BHT (54.7%) and standard rutin (53.2%) showed better radical inhibition. Research shows that the presence of embelin, flavonoids, and phenolic acids in the methanolic and ethanolic extracts of *E. ribes* results in considerable superoxide radical scavenging action [28], emphasized the fact that *E. ribes* extracts scavenge superoxide radicals, which is consistent with their rich phytochemical profile, especially embelin. These bioactive components shield cells from harmful effect by stopping the cascade of oxidative stress brought on by superoxide.

3.3.7 Lipid peroxidation

The findings indicate that, in comparison to normal Rutin (73.4%) and BHT (74.2%), the acetone extract from the bark has an effective percentage of inhibition of 54.65%, while the leaf extract has an inhibition of 52.39%. Similarly, [29] found that *E. ribes* extracts antioxidant qualities considerably lower malondialdehyde (MDA), a biomarker of lipid peroxidation, indicating their potential to lessen damage brought on by oxidative stress.

3.3.8 Reducing power assay

An effective indicator of the plant's antioxidant activity is the reducing power assay. According to the graph, the reducing power of *E.basaal* acetone extracts showed a concentration-dependent activity between 50 and 250 µg/mL of samples and absorbance at 700 nm. The acetone extract of *E. bassal* exhibited a concentration-dependent increase in reducing power. As the absorbance of the leaf extract increased, it peaked at 250 µg/mL with a value of 1.814. The bark acetone extract, with the greatest absorbance of 3.286 at 250 µg/mL, showed superior reducing ability compared to the leaf. Significant reducing power has been demonstrated in studies on *E. ribes*, especially in extracts like methanol, ethanol, and acetone. Both methanolic and ethanolic extracts of *E. ribes* contain high number of phenolic compounds such as embelin, which is known to have electron-donating qualities[30].

4. Conclusion

E. basaal contains many phytochemicals, including alkaloids, flavonoids and tannins. The bark extract has been shown to contain the highest concentration of these compounds. Antioxidant studies reveal that both leaf and bark extracts possess strong free radical scavenging properties. With the bark extract demonstrating superior antioxidant capacity. These finding suggest the *E.basaal* has the potential to produce bioactive substances with significant medicinal value.

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