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Phytochemical Screening and *in vitro* Free Radical Scavenging Activity of Hydroalcohol Extract of *Mimusops elengi* L. Root

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Abstract

Background: Plants have been proven to be offering a wide number of medicinal uses. *Mimusops elengi* plant is rich in phytoconstituents, present almost in every part of the plant. Roots are the parts on which minimum work has been done.

Objective: The objective of the present study was a preliminary evaluation of physicochemical and free radical scavenging activity of *Mimusops elengi* L. root.

Methods: Many standardization parameters like extractive values, total ash value, water-soluble ash value and acid insoluble ash, moisture content, and loss on drying (LOD) of *Mimusops elengi* L. root were analyzed. The total phenolic content (TPC) and total flavonoid content (TFC) were measured. Free radical scavenging (FRS) activity was evaluated by assessing DPPH scavenging activity.

Results: The results of phytochemicals screening of hydroalcohol extract revealed the presence of various secondary metabolites, including alkaloids, flavonoids, saponins, sterols, and tannins. The amount TPC and TFC were found to be 21.63±0.0663 mg of gallic acid equivalent weight/g of extract and 159.0 ± 0.9 mg of quercetin equivalent weight/ g of extract respectively. The extract showed potent FRS activity (IC_{50} value of 96.13 µg/mL).

Conclusions: The present study revealed *Mimusops elengi* L. Root contains most potent antioxidant agents and has significant FRS activity.

Keywords: Antioxidant, Flavonoid, Phenolic, Hydroalcohol, *Mimusops elengi*

Introduction

Medicinal plants have been proved to be of great importance to the health of individuals and communities.[1] In recent years, scientific investigations of various plants used in traditional remedies for various diseases have lead to the development of alternative drug and therapeutic strategies. Since the consumption of medicinal plants is increasing, the use of these plants as a supplement in food also increased.[2-4]

This empirical knowledge comes from the plant defense system, which generates numerous compounds with diverse molecular structures far superior to those derived from synthetic products, so the great interest in the elucidation of new active principles.[5,6] The herbal treatment is accepted all across the world.[5] Free radicals or reactive oxygen species (ROS) generated results in oxidative stress. They are responsible for multiple chronic diseases including cardiovascular

and neurodegenerative diseases. Several studies have hypothesized that the plants secondary metabolites can scavenge free radicals naturally.[7] Phenolic and non-phenolic compounds have a core role in inhibiting oxidative stress and act as antioxidants. Antioxidants are chemically synthesized such as Butylatedhydroxytoluene (BHT), Butylatedhydroxyanisole (BHA), and Propyl gallate, that are known to cause several severe side effects. This resulted in a thirst for search of potential natural antioxidants and this was the main objective of the present study.

Hence, studying the phytochemical constituents helps to reveal the usage of plants.[8] *Mimusops elengi* known as Bakul or Spanish cherry tree belongs to the family Sapotaceae and is found all over India. It is cultivated in north and Andaman Island, Indomalaysia, India, and in some areas of Karnataka like Bangalore, Mysore, Shimoga, Hassan, Davanagere, and Dharwad.

Synonyms

Sanskrit: Bakula. Malayalum: ilanji, Kannada: pagadimara, Baaglumara. Tamil: ilanchi. Telugu: Parijathamu. Marathi: Bakula. English: Spanish cherry, Bakul Tree. A large glabrous evergreen tree 12-15 m high, with a compact leafy head and short erect trunk, bark smooth, scaly. Leaves 6.3-10 by 3.5-5 cm, elliptic, shortly acuminate, glabrous, and base acute or rounded. Petioles are 1.3-2.5 cm long. Flowers white, fragrant, nearly 2.5 cm across solitary or in fascicles of 2-6, buds ovoid, acute, pedicels 6-20 mm long, appressed young, often deflexed. Calyx 1 cm long fulvous.

Figure 1: Photographs of the plant, flowers and root of *Mimusops elengi* Linn.

Pubescent, segments eight, the four outer ovate-lanceolate, acute, the four inners narrower than the outer. Corolla longer than the calyx, tube 1.5 mm long, lobes 8 mm long, about 24, in two series, the inner series of eight, the outer of 16 lobes, linear-oblong, acute. Stamens eight, opposite the inner circle of lobes, filaments short, glabrous, anthers glabrous, slightly twisted, acuminate, alternate with the stamens, lanceolate, acuminate, and densely clothed on the back and margins with white hairs. Ovary silky pubescent, style grooved, slightly longer than the corolla. Berry about 2.5 cm, long, ovoid, yellow when ripe. Seed solitary, ovoid, compressed, shining, and brown. The bark is acrid and sweet, the root is sweet and sour, fruit and seeds are sweet and sour.

Therefore, the objective of this study was to determine the phytochemicals qualitatively and quantitatively. *Mimusops elengi* L. commonly known as "Spanish cherry tree" belongs to family Sapotaceae. It is well known in ayurvedic medicine. All parts of the plant have medicinal properties. Bark is acrid, sweet. It possesses cardiotonic, alexipharmic, astringent properties and used to cure biliousness, diseases of the gums and teeth. Unripe fruit is used as masticatory and helps to fix loose teeth. Ripe fruit pulp is useful in chronic dysentery. The bark and fruit of this plant are used in the treatment of diarrhoea and decoction of the bark is used as a gargle.[9-10] Charaka documented the root and bark extract with honey used in helminthiasis, neurotonic, and fevers. Sushruta gave flowers internally in coughs and bilious derangement, as an ingredient of medicinal liquor in diseases of the urinary tract. The bark powder is an ingredient in several commercial tooth powders, in folk medicine and is prescribed internally in diseases of the bladder and urethra.

Phytochemically, the plant showed the presence of active constituents like alkaloids, tannin, saponins, taraxerol, ursolic acid, betulinic acid, α-spinosterol, β-sitosterol mixture of triterpenoid, saponins, quercetin, lupeol, and steroidal in the bark *of Mimusops elengi*. Flowers, leaves, heartwood, and roots contain sterols, glucosides, hentriacontane, β-carotene, and lupeol. Fruits and seeds contain fatty oil, a pentacyclictriterpene, glucose, quercetin, dihydroquercetin, and β-sitosterol glycoside. The kernel contains sapogenin, triterpenoid, saponin-α-spinasterol, Mimusopside A and B, sterol-3-epi-chondrillasterol.[11-13] Studies on this proved that the bark, leaves, fruits, possess anti-anxiety, antihyperlipidemic, anti-ulcer, anticonvulsant, anti-inflammatory, analgesic, antipyretic, antioxidant, cytotoxic, antidiabetic, anti-tubercular, diuretic, and hypotensive activities.[14] This study aimed to evaluate physicochemical, phytochemical screening, and free radical scavenging activity of hydroalcohol root extract of *Mimusops elengi*.

Materials and Methods

Plant material

The plant was collected from the garden area of Davanagere, Karnataka. The *Mimusops elengi* was identified by botanist Prof. L C Kulkarni, Department of Botany, PC Jabin Science College, Hubli, Karnataka and authenticated by him. The specimen was stored in the department.

Preparation of extract

Mimusops elengi roots were cleaned and air-dried, then powdered and passed in sieve # 44. The sieved powder was first extracted with petroleum ether to defat and then 1000 g of defatted root powder was macerated in 6 L of hydroalcohol solvent [Water: Ethanol (7:3)] for about 48 hours. Macerate was decanted and filtered through cloth and then filter paper to obtain a clear extract. The filtrate was poured in trays to evaporate solvent at 30-35°C and completely dried by lyophilization. The dried powder was placed in an air-tight container under refrigeration. The obtained extract was utilized for qualitative and quantitative phytochemical analysis.

Physicochemical properties of the powdered root of *Mimusops elengi*

In the physicochemical evaluation, the total ash, acid insoluble ash and water-soluble ash, extractive values, and finally loss on drying (LOD) were determined.[15,16] The ash values indicate the presence of inorganic salts. Extractive values represent approximately the measures of chemical constituents they contain, the diversity and properties of extract content.

Determination of Total ash, Acid insoluble ash, and Water soluble ash value

Two grams of powder of *Mimusops elengi* root was taken in a silica crucible tared and incinerated until free from carbon at 450°C. The resultant ash was allowed to cool and weighed finally. The % of ash was calculated and the total ash obtained from 2 g of *Mimusops elengi* root powder was boiled with dilute HCL 25 mL and insoluble matter was collected on filter paper and cleaned with hot water, followed by ignited and weighed finally. The % of acid insoluble ash and water-soluble ash, extractive values were calculated.

Determination of extractive value

Accurately weighed powder 5 g of *Mimusops elengi* root was extracted by maceration with 95% alcohol 100 mL or chloroform 100 mL or water 100 mL for 24 h in an airtight container to determine alcohol soluble extractive, chloroform soluble extractive, and water-soluble extractive values, respectively. First six hours, the content was shaken frequently. After 24 h, the content was filtered and the filtrate was evaporated and dried at 105°C to get constant weight and the extractive value was calculated as % w/w.

Loss on drying

One and half grams of the powder was taken in a porcelain tared dish and dried at 105°C in the oven to obtain constant weight and was weighed finally. The weight difference and the % loss on drying were calculated.

Qualitative phytochemical analysis of hydroalcohol extract of *Mimusops elengi* (HEME)

The HEME root of 1 g was added in its mother solvents to get a stock solution 1% w/v. Preliminary phytochemicals of this solution were screened by standard procedure. The test for carbohydrates (Molash's test and Fehling's test), protein and amino acids (Biuret, Million's and Ninhydrin test), glycosides (Legal's, Borntrager's test and Balijet's test), alkaloids (Mayer's, Dragendroff's test and Hanger's test), phytosteroids (Salkowski and Libermann-Burchard test), flavonoids tannins and phenols (Shinoda, ferric chloride, lead acetate, and alkaline reagent test), Saponins (Foam test) were carried out.[15-17]

Quantitative phytochemical analysis of HEME root

i) Determination of total phenolic content (TPC)

TPC was estimated by Folin Ciocalteu's method. Briefly, aliquots 1 mL and gallic acid (GA) (6.25, 12.5, 25, 50, 100, and 200 µg/mL) were placed serially in tubes and distilled water 5 mL and Folin Ciocalteu's reagent 0.5 mL were added and shaken. After five minutes, sodium carbonate 1.5 mL, 20% was placed and volumes made with 10 mL of distilled water and incubated for 2 hours. Blue color intense was developed and absorbance was taken at 750 nm. GA was taken as standard anda calibration curve was prepared. TPC of the extract was expressed as GA mg equivalent weight GAE / 1 g of extract.[18,19]

ii) Determination of total flavonoid content (TFC)

TFC was obtained by aluminum chloride colorimetric assay. Aliquots of 1 mL or standard quercetin 1 ml solution (6.25, 12.5, 25, 50, 100, 200, 400 and 800 µg/mL) were added serially in tubes and for each tube, distilled water 4 mL and sodium nitrite solution 0.3 mL, 5% was placed and 5 minutes later, aluminum chloride 0.3 mL, 10 % was added. After six minutes, 2 ml of 1 M NaOH was added and mixed thoroughly. The final volume was made to 10 mL with distilled water. A orange yellowish color was developed and measured at 510 nm. The quercetin standard calibration curve was plotted and the data of TFC was expressed as mg of quercetin equivalents/ 1 g of extract.[20]

In-vitro antioxidant activity of HEME root

Antioxidants react with stable DPPH radical and convert it to 1,1-diphenyl-2-(2,4,6-trinitrophenyl) hydrazine. The discoloration degree indicates the antioxidants scavenging potency. DPPH radical once oxidized has an absorbance maximum centered at about 517 nm. The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. One ml of 0.1 mM DPPH in methanol was mixed with 3.0 mL of control (without the test compound, but an equivalent amount of methanol) and test solutions at different concentrations of HEME 3.94 to 500 µg/mL in methanol in different test tubes. After 30 minutes, the absorbance was taken at 517 nm. The maximum percentage inhibition of DPPH radical and IC_{50} values were determined. Absorbance was converted to the DPPH radical-scavenging rate according to the equation.[21]

DPPH radical scavenging activity (%) = $[(A_{control}-A_{sample})/A_{control}] \times 100$

Results

Physicochemical properties of *Mimusops elengi* root

Ash value percentage of 11.5 ± 0.03 are important quantitative measurements for the purity of plant drugs. The water-soluble ash value percentage of *Mimusops elengi* was 10.85 ± 0.12 which denotes that this plant powder has more solubility in water. The mean percentage of water-soluble, alcohol soluble and chloroform soluble extractive values were 22 ± 0.07, 24± 0.04, and 8.0 ± 0.11 respectively. The extractive value of drugs in a definite solvent is an index for checking the purity of a drug. Results are summarized in table 1.

Table 1:Physicochemical properties of *Mimusops elengi* root

Parameters	Values in %(w/w)
Ash Value	
Total ash value	11.5 ± 0.03
Acid insoluble ash value	4.5 ± 0.09
Water-soluble ash value	10.85 ± 0.12
Extractive value	
Alcohol soluble extractive value	24 ± 0.04
Water soluble extractive value	22 ± 0.07
Chloroform soluble extractive value	8 ± 0.11
Loss on drying	2.5 ±0.05

Preliminary phytochemical screening

The HEME root proved to contain carbohydrates, proteins, steroids, glycosides, amino acids, alkaloids, tannins, phenolic, and flavonoids compounds, and results are presented.

Phenolic and flavonoids contents

The TPC in the HEME was found to be 21.63±0.0663 mg of GAE weight/g extract. The concentration of flavonoids in plant HEME root was found 159.0 ± 0.900 mg equivalent to quercetin weight / g extract. Results are summarized in table 2. The activity is shown in figures 2 and 3.

Table 2: TPC and TFC of the root of HEME

Extract	Phenolic content (mg of Gallic acid equivalent weight/ g of extract)	Flavonoid content (mg of quercetin equivalent weight/ g of extract)
HEME	21.63±0.0663	159.0 ± 0.900

*All values are shown in Mean ± SEM.

HEME = Hydroalcoholic extract of *Mimusops elengi root*

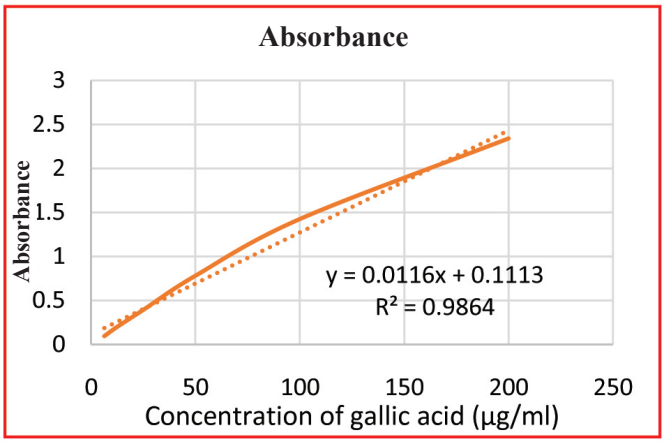

Figure 2: TPC for standard Gallic acid

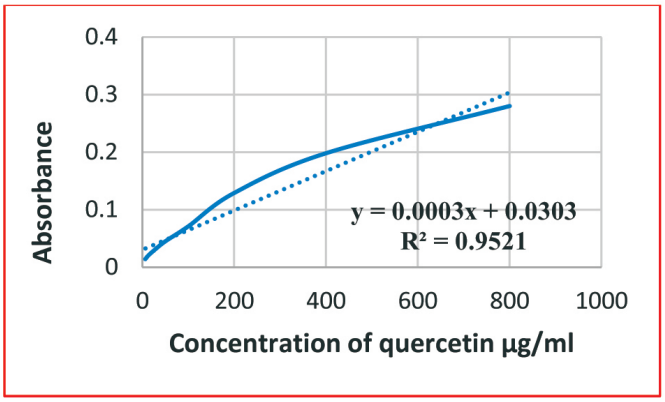

Figure 3: TFC for standard quercetin

Invitro antioxidant activity

HEME of concentrations ranging from 3.94 to 500 µg/mL was subjected for antioxidant activity. The maximum percentage inhibition free radical activity with different concentrations of the extract is given in Table 3. The activity is shown in figures 4 and 5 and Table 3. The

maximum activity of ascorbic acid as standard and HEME exerted an inhibition of 97.01% and 87.65% at 500 µg/mL and the IC_{50} of the ascorbic acid was 58.31 µg/mL and the extract was 96.13 µg/mL.

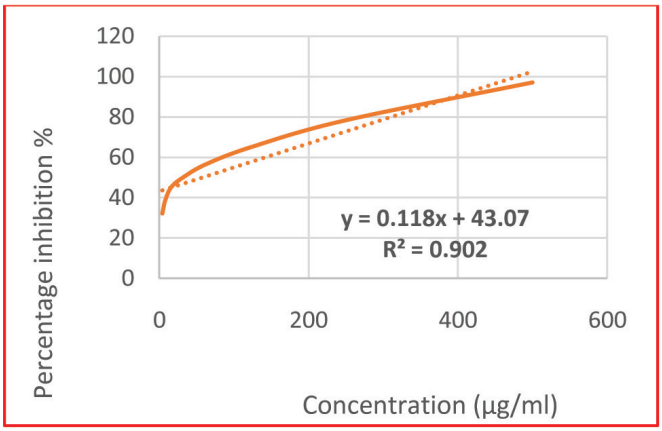

Figure 4: DPPH radical scavenging activity of ascorbic acid

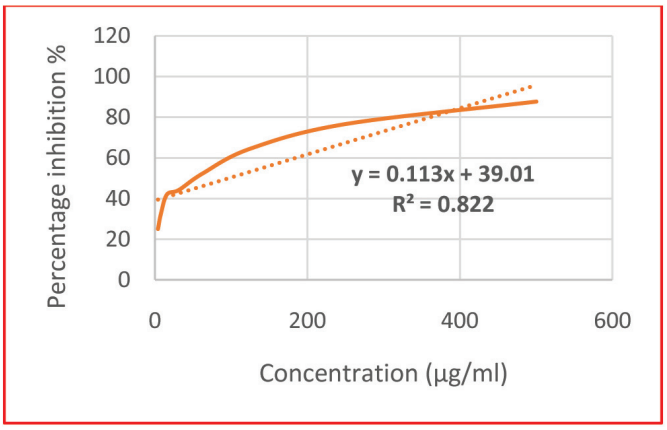

Figure 5: DPPH radical scavenging activity of HEME root

Table 3: DPPH radical scavenging activity

Concentration of solution (µg/mL)	Percentage (%)		IC_{50} Value (µg/mL)	
	Ascorbic acid	Hydroalcholic extract of *Mimusops elengi*	Ascorbic acid	Hydroalcholic extract of *Mimusops elengi*
3.94	32.14	25.12		
7.81	38.64	32.65		
15.65	45.06	42.03		
31.25	49.87	44.25		
62.5	56.54	52.36	58.31	96.13
125	65.25	64.54		
250	78.32	76.63		
500	97.01	87.65		

Discussion

Mimusops elengi L extract showed the presence of alkaloids, amino acids, carbohydrates, flavonoids, phenols, amino acids, carbohydrates, reducing sugar, glycosides, tannins, and saponins. High content of phenolic and flavonoids are present in this plant. These phenolic and flavonoids phytochemicals are responsible for antioxidant activity.[15]

The *Mimusops elengi* root showed a lesser amount of 2.5% moisture content which could be at a minimal level to discourage the growth of bacteria, yeast, or fungi during storage. This moisture content provides information to establish the plant material quality as prospects. Ash values are used to decide the quality and purity of plant extracts, it indicates the presence of various impurities like silicate, oxalate, and carbonate. The water-soluble ash indicated the number of inorganic compounds present in the extract. The insoluble acid ash helps to determine the amount of silica present in the material. The total water-soluble portion of the ash is considered water-soluble ash. Less amount of these three parameters indicate that the inorganic matter and silica were less in *Mimusops elengi* root.

These phytochemical constituents of the plant seems to be the source of potential drugs and the presence of many constituents which have a role in good health.[22] Qualitative phytochemical screening is an essential step towards the discovery of new entity as it gives the information regarding the primary and secondary metabolites in the plant extract.

Antioxidants are inhibiting the oxidation of other molecules by accepting electrons or free radicals produced during oxidation. Unless trapped by antioxidants, free radicals initiate chain reactions and lead to damage or death to the cell.[23] The antioxidant activity of the extract and its serially diluted solutions of the HEME root were subjected for free radical activity using DPPH radical scavenging assay.[24]

The flavonoids and phenols of the *Mimusops elengi* root extract act as antioxidants. FRS potency is promoted by their OH groups and the TPC acts as a base for screening of antioxidant activity. Plant flavonoids showed potential antioxidant activity in *in vitro* and *in vivo* systems.[25-27] As this is the first report on the antioxidant activity of HEME root, thorough phytochemical studies should be conducted to determine phenolic and flavonoid components. These phenolic and flavonoids are responsible for observed antioxidant activity.

Conclusion

The hydroalcohol extract of *Mimusops elengi* root contains a high level of flavonoids, phenolics, and tannins. Due to the presence of flavonoid and phenolic components in *Mimusops elengi* root, it possesses potential free radical scavenging activity against DPPH radical. From the results of the present study, it can be suggested that *Mimusops elengi* root is a source of

significant natural antioxidants and may be useful in protection against oxidative stress.

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Conflicts of Interest

None.

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