Pharmaceutical studies of leaf and bark extracts of *Mammea suriga* (Buch.-Ham. ex Roxb.) Kosterm

Abstract

Mammea suriga is a familiar endemic medicinal plant belonging to the family Guttiferae/Clusiaceae, grows abundantly in the Western Ghats of India. The present focuses on studying the phytoconstituents of bark and leaf extracts, to assess antioxidant and antimicrobial properties of methanol and aqueous extracts using both leaf and bark. Phytochemical screening showed presence of carbohydrates, phenols, flavonoids, alkaloids, cardiac glycosides, terpenoids, triterpenoids, steroids and saponins. The carbohydrate contents were highest in leaf methanol(140.90 ± 1.19 mg/g), phenol contents highest in leaf methanol (154.32 ± 1.80 mg/g), the highest flavonoid content were observed in bark aqueous(437.3878 ± 0.09mg/g). The antioxidant screening the highest inhibition concentration was found to be IC_{50} (53.33 ± 0.13) for leaf methanol for DPPH, highest ferric reducing activity was observed in both the bark extracts at 300µg/mL concentrations, antioxidant activity for phoshomolybdenum assay was observed highest in leaf methanol extract at 300µg/mL which was 75%. Antibacterial activity by agar well diffusion method was carried out using extracts against *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus* which showed diameter ranging from 12-22mm. Antifungal test by poison bait method against *Fusarium solani* and *Colletotrichum gloeosporioides* have shown good results.

Keywords: Leaf, bark, phytochemicals, antioxidants, antimicrobial activity

Introduction

"Medicinal plants are used with the intention of maintaining health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine. The Food and Agriculture Organization (FAO) estimated in 2002 that over 50,000 medicinal plants are used across the world. The Royal Botanic Gardens, Kew more conservatively estimated in 2016 that 17,810 plant species have a medicinal use, out of some 30,000 plants for which a use of any kind is documented" [1].

"In modern medicine, around a quarter of the drugs prescribed to patients are derived from medicinal plants, and they are rigorously tested. In other systems of medicine, medicinal plants may constitute the majority of what are often informal attempted treatments, not tested scientifically" **[2]**. The World Health Organization estimates, without reliable data, that some 80 per cent of the world's population depends mainly on traditional medicine. The use of plant-based materials including herbal or natural health products with supposed health benefits is increasing in developed countries.

“Medicinal plants may provide three main kinds of benefits: health benefits to the people who consume them as medicines; financial benefits to people who harvest, process, and distribute them for sale; and society-wide benefits, such as job opportunities, taxation income, and a healthier labour force. However, development of plants or extracts having potential medicinal uses is blunted by weak scientific evidence, poor practices in the process of drug development, and insufficient financing” [3].

“Researchers and pharmacists are replacing synthetic chemicals with natural resources for drug manufacturing purposes. Plants produce secondary metabolites which possess antibacterial, antifungal, anticancer, antimalarial, antiprotozoal and antioxidant properties. These metabolites are beneficial for plant growth and development by protecting them from harmful UV irradiation, insects and extreme temperatures. In general, medicinal plants harbour endophytes which are a prolific source of secondary metabolites and phytochemical production. Production of these metabolites by the endophytes from medicinal plants has shifted the focus from using chemicals to natural resources for drug discovery. The microbial biotransformation is an alternative method for the production of bioactive compounds. A single endophyte is responsible for various other secondary metabolites like terpenoids, steroids, alkaloids, flavonoids etc. Hence, phytochemicals with novel structures can be exploited in the field of medicine for new drug discovery” [4].

“Medicinal and aromatic plants (MAPs) are traded as such in bulk from many developing countries for further value addition in developed countries. The extracts of *Mammea suriga* revealed the presence of various bioactive phytocompounds in rich quantity. Several phytocompounds were described to be active antimicrobial constituents against diverse microorganisms. The mode of activity of these phytoconstituents may be due to their capability to complex with soluble and extracellular proteins. Our recent publication on *M. suriga* reports that its root bark extracts exhibit strong *in vitro* antimicrobial and antioxidant activities when compared to the standards” [5].

“Mammea suriga (Buch.-Ham. ex Roxb.) Kosterm., synonym *Mammea longifolia* (Wight) Planch. & Triana is a familiar endemic medicinal plant belonging to the family Guttiferae/Clusiaceae, which grows abundantly in the Western Ghats of India. Vern. Names: Surige (Kan.), Surangi (Konkani.) and Suranpunna (Mal.). The plant is well known for its diverse applications in folk medicine. It is a large tree, growing to a height of 12-18 m and its bud is used as a minor spice. Wood is good timber, used in making furniture, building construction, boats, also used in folk medicine. Flowers used in perfumes, seeds used for making viscid gum, fruits are edible and delicious. The flower buds possess mild stimulant, carminative and astringent properties and are used in the treatment of dyspepsia and haemorrhoid” [6]. Its root-paste is widely used as medicine to cure partial headache. However, various parts of the tree have been traditionally used in the treatment of many disorders, no any systematic documents to confirm its folklore uses. Besides, the plant has not yet undergone any

chemical or pharmacological investigation except for the antimicrobial activity of root extracts, stem bark extract and flower bud extract, which have been reported earlier.

"Mammea longifolia are shown to contain interesting molecules, *viz*., coumarins surangin A, surangin B and taraxerol. It was reported that the extract of stem bark of *M. longifolia* showed one more coumarin named surangin C" [7]. "The methanol extract of *M. longifolia* buds (nagkesar) was found to scavenge DPPH and superoxide radicals" [8]. "Indeed, *Mammea* coumarins have been investigated to possess a wide array of biological activities. These coumarins are good radical scavengers and are cytotoxic to human tumor cells that suppress tumor growth in animal models and also, they exhibit anti-HIV, antifungal and antibacterial activities. The essential oil of the flowers known as punnaga oil is being used in perfumery industry and silky red dye extracted from its dried flowers is used in textile industries. The flower buds have good medicinal value and are used against disorders of blood such as abscess, skin disorders, bleeding disorders such as menorrhagia, nasal bleeding hepatocyte diseases, cardiac problems and useful in pitta imbalance disorders such as gastritis, burning sensation, etc. and also useful in psychiatric disorders. A special collyrium prepared using the seed oil is used in ophthalmic disorders" [9].

Considering the studies, the present approach was done on phytochemical extracts of leaf and bark of *Mammea suriga* to evaluate the *in vitro* antioxidant and antimicrobial properties.

Materials and Methods

Collection of Samples:

The mature leaves and bark samples of *Mammea suriga* were collected from Karwar, Uttara Kannada District, Karnataka during the months of January-February 2023 (Plate 1, 2). The plant specimens were identified with the help of Dr. Rama Bhat P., H.O.D. of Biotechnology, Alva's College, Moodbidri. They were allowed to shade dry for about two weeks and crushed coarsely and stored in air tight container for further use.

Plate 1: Vegetative branch of *M. suriga*

Plate 2: Bark of *M. suriga*

Preparation of extracts:

1. Preparation of methanolic extract: 20g of bark and 15g of leaf sample were taken separately in an Erlenmeyer flask and mixed with 150mL methanol and placed on rotary shaker for 18h. The obtained extract was then filtered and the filtrate was placed in water bath in a China dish for evaporation. The weight of the dried extract was noted and was stored in cool temperature for further use.

2. Preparation of aqueous extract: 20g of bark and 15g of leaf sample were taken separately mixed with 150mL distilled water and kept in water bath at 80°C for 3h. The filtration, evaporation, storage process was same as followed for methanolic extract.

Extraction yield for both extracts were obtained using formula:

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

Phytochemical screening of plant extracts:

Using standard methods, preliminary extract analysis was performed to identify the presence of several phytoconstituents [10].

Test for carbohydrates

(a) Molisch test: Few drops of alcoholic a-naphthol solution were added to 2 mL of extract. Later, few drops of concentrated H_2SO_4 were added along the walls of test tube. At the junction of two liquids, a violet colour ring appeared, indicating that carbohydrates were present.

(b) Benedict's test: To 5 mL of Benedict's reagent, 8-10 drops extract were added, and then heated for five minutes; the resulting dark red precipitate indicated the presence of carbohydrates.

Tests for alkaloids

(a) Mayer's test: Few drops of Mayer's reagent were added to 1 mL of extract. A yellowish or white precipitate was formed, indicating the presence of alkaloids.

(b) Wagner's test: A few drops of Wagner's reagent were added to few mL of plant extract along the sides of test tube. A reddish-brown precipitate confirms the test as positive [11].

Test for phenolic compounds and tannins

Ferric chloride test: Two millilitres of 5% neutral ferric chloride solution were added to 1 mL of extract, the dark blue colouring indicating the presence of phenolic compounds and tannins.

Test for Terpenoids: "5mL plant extract was mixed with 2mL chloroform and 3mL sulphuric acid was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show presence of terpenoids" [12].

Tests for flavonoids

Alkaline reagent test: Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCl, indicating that flavonoids were present.

Tests for glycosides

Keller Killiani test: A solution of 0.5 mL, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 mL of extract. Later, 1 mL of concentrated H_2SO_4 was added along the walls of the test tube. The appearance of deep blue colour at the junction of two liquids indicated the presence of cardiac glycosides.

Test for steroids

Salkowski test: The test extract was shaken with chloroform and concentrated H_2SO_4 was added along the walls of a test tube; a red colour appeared, indicating the presence of steroids.

Test for triterpenoids

Horizon test: Two millilitres of trichloroacetic acid was added to 1 mL of extract. The presence of terpenoids was confirmed by the formation of a red precipitate.

Tests for saponins

A drop of Na_2CO_3 solution was added to 5 mL of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins.

Estimation of carbohydrate content

"The carbohydrate content was estimated using Phenol-sulfuric acid method. Phenol-sulfuric acid method is the most reliable method among all the quantitative assays for carbohydrate estimation. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a yellow-brown-coloured product with phenol and has absorption maximum at 490 nm UV spectroscopy". [13]

Dextrose was used as a standard (20-100μg/mL) and 1mL of test samples were used for estimation. 0.2, 0.4, 0.6, 0.8, and 1 mL of working standard into a series of test tubes and made up to 1 mL using distilled water. The blank was set initially with all reagents without sample. 1 mL of 5% phenol solution was added to each tube and shaken well. Then, 5 mL of 96% sulfuric acid was added and again shake well. Test tubes were incubated at lab temperature for 20 min. Absorbance was measured at 490 nm using spectrophotometer. The concentrations of plant samples were calculated. The samples were prepared in triplicate for each analysis. Carbohydrate content of the extracts was

expressed as mg dextrose equivalents (DE) per gram of sample in dry weight (mg/g). The carbohydrate content in all the samples were calculated by the using the formula:

$$C = X(V/M)$$

Where, C = Carbohydrate content mg DE/g dry extract, X = concentration of test obtained from calibration curve in µg/mL, V = volume of extract in mL, and M = mass of extract in gram.

Estimation of total phenol content

"Total phenolic contents (TPC) in the leaf and bark extracts were determined by Folin–Ciocalteu colorimetric method as described by Singleton et al. with some modifications. Standard gallic acid solution was prepared by dissolving 10 mg of it in 10 mL of methanol (1000µg/mL). Various concentrations of gallic acid solutions in methanol (200,400,600,800 and 1000 μg/mL) were prepared from the standard solution. To each concentration, 0.5 mL of 10% Folin–Ciocalteu reagent (FCR) and 2 mL of 7% Na_2CO_3 were added. Then, the absorbance was measured using spectrophotometer at 760 nm against blank". [14]

The FCR reagent oxidizes phenols in plant extracts and changes into the dark blue colour, which is then measured by UV-visible spectrophotometer. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve. Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic contents in all the samples were calculated by the using the formula:

$$C = X(V/M)$$

where C = total phenolic content mg GAE/g dry extract, X = concentration of test obtained from calibration curve in µg/mL, V = volume of extract in mL, and M = mass of extract in gram.

Estimation of total flavonoid content (TFC) [15]

TFC was determined according to the aluminum chloride colorimetric method; Rutin was used as standard (200-1000µg/mL) and 0.5mL (500µg/mL) of test samples were used for estimation. To 0.5 mL sample and standard (rutin), volume was made up to 1 mL with distilled water and 0.3 mL 5% $NaNO_2$ solutions were added. After 6 min, 0.3 mL 10% $AlCl_3$ solution was added and kept for another 6 min. To this reaction mixture, 2 mL 1M NaOH solution and 4.4 mL water was added to make up the final volume 8 mL. The reaction mixture was mixed well and allowed to stand for 15 min after which absorbance was recorded using spectrophotometer at 510 nm. All the experiments were carried out in triplicates. Total flavonoid content was expressed as mg rutin equivalent (RE)/g plant sample. The total flavonoid contents in all the samples were calculated by the using the formula:

$$C = X(V/M)$$

Where, C = total phenolic content mg RE/g dry extract, X = concentration of test obtained from calibration curve in µg/mL, V = volume of extract in mL, and M = mass of extract in gram.

Antioxidant assays

1. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

"In vitro antioxidant activities of the extracts were determined using the DPPH free radical scavenging assay method. DPPH in oxidized form gives a deep violet colour in methanol. An antioxidant compound donates the electron to DPPH, thus causing its reduction and in reduced form its colour changes from deep violet to yellow. DPPH solutions show a strong absorbance at 517 nm appearing as deep violet colour. Scavenging of DPPH free radical determines the free radical scavenging capacity or antioxidants potential of the test samples, which shows its effectiveness, prevention, interception, and repair mechanism against injury in a biological system". [16]

DPPH solution (0.1 mM) was prepared by dissolving 0.39 mg of DPPH in a volumetric flask, dissolved in methanol, and the final volume was made 100 mL. Thus, prepared purple-coloured DPPH free radical solution was stored in amber coloured flask at -20°C for further use.

Stock solution of different extracts of 1 mg/mL was prepared by dissolving required quantity of each extract in required volume of methanol. From the sample stock solution, 100,200 and 300 µg/mL solutions were used for the activity.

To the sample solutions of different concentration, 2 mL DPPH solution was added and incubated at room temperature for 30 min in dark. A control was prepared by mixing 1 mL methanol and 2 mL DPPH solution. Finally, the absorbance of the solutions was measured by using a spectrophotometer at 517 nm. Ascorbic acid was used as the standard. 50% inhibitory concentrations (IC_{50} values) of the extracts were calculated from graph as concentration versus percentage inhibition. Radical scavenging activity was expressed as percentage of inhibition. IC_{50} value is the concentration of sample required to scavenge 50% of DPPH free radical. Measurements were taken in duplicates. IC_{50} of the extracts indicates the corresponding concentration in which the radical scavenging potential is 50%. The IC_{50} of the extract and standards were determined graphically.

The percentage of inhibition was calculated by using the formula:

$$\text{Scavenging activity}(\%) = \left(\frac{\text{Absorbance}^{\text{control}} - \text{Absorbance}^{\text{sample}}}{\text{Absorbance}^{\text{control}}}\right) \text{x}100$$

2. Ferric Reducing power assay [17]

Methanol and aqueous extracts of *M. suriga* leaf and bark were used to determine the reducing power. Each extract were taken in different concentrations (100,200,300µg/mL) mixed with 2.5mL of phosphate buffer (0.2M, pH 6.6) and 2.5 mL of potassium ferricyanide. The mixtures were incubated at 50^{o}C for 20 minutes. 2.0 mL of TCA was added to the mixture and 0.5mL of ferric chloride solution and absorbance were made at 700nm in spectrophotometer. Ascorbic acid used as a standard reference and distilled water was used as a blank solution. Increase in the absorbance value signifies the increase in reducing power.

3. Phosphomolybdenum assay [18]

For the conduction of the phosphomolybdenum assay, the method of Prieto *et al*. (1998) was followed. An aliquot of 0.1 mL of sample solution of different concentrations (100-300 µg/mL)

treated with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C in a water bath for 90 min. The samples were cooled to room temperature and their absorbance was recorded at 700 nm. Ascorbic acid was used as the positive control. Antioxidant capacity was estimated by using following equation:

$$\text{Antioxidant activity (\%)} = \left(\frac{\text{Absorbance}^{\text{control}} - \text{Absorbance}^{\text{sample}}}{\text{Absorbance}^{\text{control}}}\right) \text{x100}$$

Antimicrobial assays

1. Agar diffusion well method for antibacterial test [19]

Pure cultures of bacteria were collected from Alva's College of Medical Laboratory Technology, Moodbidri, Karnataka. The bacterial cultures of *Escherichia coli* (Gram negative), *Klebsiella pneumoniae* (Gram negative), *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative) which were maintained in nutrient media.

Inoculum was prepared using 50mL nutrient broth and inoculating a loopful of culture in the broth which was then used for the test after 24h. About 100µl of inoculum was uniformly spread using glass slider on a sterile Petri dish nutrient agar. Wells of about 9mm diameter were made in the medium using sterile cork borer. Concentrations of 50,100 µg/mL of extracts were dispensed into the wells. Methanol and distilled water were used as negative control and streptomycin was used as positive control with same concentrations as extracts. The extracts were allowed to diffuse in the media for 15 min, and then kept for 24h for incubation. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm. Tests were performed in triplicates.

2. Poison bait method for antifungal test [20]

Fungal strains of C*olletotrichum gloeosporioides* and *Fusarium solani* were maintained in PDA media. The test was done by inoculating 8mm mycelial disc in 25mL potato dextrose broth with 1 mL extracts in 100 mL conical flask. A broth with methanol and only disc served as negative control and Bavistin was used as positive control. The flasks were incubated for 3-5 days and their dry weight was measured on Whatman No.1 filter paper.

Statistical analysis:

All the assays were performed in triplicates. The results were recorded and expressed in mean ± standard deviation

Results and discussion

Extraction yield:

In the present study, the aqueous extracts showed maximum yield of 21.1% (4.22g) for bark and 19.2% (2.89g) for leaf, compared to the methanol extracts the yield was 17% (3.4 g) and 14.6% (2.19g) respectively. The yield, color and consistency of different extracts of *Mammea suriga* are given in table 1. In a study conducted by Krishna *et al*. [21], obtained 2.17% (Brownish gum) yield of stem bark of *M. suriga* petroleum ether extract and 1.9% (yellow gum) flower bud extract. Likewise, Poojary *et al.* [5] reported that ethanol extraction produced maximum yield of 11.5% (Reddish semisolid), whereas petroleum ether extraction yielded only 7.4% (Yellowish gum), aqueous extracts of about 10.43% (reddish solid) in *M. suriga.*

Table 1: Yield, colour and consistency of leaf and bark extracts of *Mammea suriga*

Extracts	Extraction yield (%)	Colour and consistency
Bark methanol (BM)	3.4	Brownish gum
Leaf methanol (LM)	2.19	Dark green semisolid
Bark aqueous (BA)	4.22	Brownish solid
Leaf aqueous (LA)	2.89	Dark green semisolid

Phytochemical screening of plant extracts:

1. **Qualitative analysis:**

In the present study, the qualitative analysis of the phytochemicals of bark and leaf extracts of *M. suriga* indicated the presence of carbohydrates, phenols, glycosides, alkaloids, flavonoids and saponins (Table 2). Similarly, Shastri *et al.* [22] reported the presence of flavonoids, steroids, terpenoids and cardiac glycosides in the petroleum ether stem bark extract of *M. suriga*. The results indicated the presence of large amounts of flavonoids, cardiac glycosides and alkaloids in the root bark extracts of *M. suriga* as reported by Poojary *et al.* [5]. They also reported that in all the extracts, carbohydrates and reducing sugars were found to be absent. Phytochemical screening of *M. americana* seeds indicated

presence of alkaloids, coumarins, flavonoids, triterpenes and steroids in ethanolic, hexanoic, and chloroformic extracts; likewise, presence of saponins in ethanolic, ethyl acetate and methanolic extracts; and finally, presence of tannins in ethanolic, ethyl acetate and methanolic extracts [23].

Table 2: Preliminary phytochemical screening in leaf and bark extracts of *M. suriga*

Phytochemicals	Test	Bark Methanol	Leaf Methanol	Bark Aqueous	Leaf Aqueous
Carbohydrates	1.Benedict's test	+	+	-	+
	2.Molisch's test	+	+	+	+
Phenols	$FeCl_3$ test	+	+	+	+
Flavonoids	Alkaline reagent test	+	-	+	+
Alkaloids	1.Mayer's test	+	+	-	-
	2.Wagner's test	+	+	+	-
Cardiac glycosides	Keller Killiani test	+	-	+	+
Terpenoids		+	-	+	+
Triterpenoids	Horizon test	+	-	+	+
Steroids		+	+	-	+
Saponins		+	+	+	+

'+'present, '-' absent

2. Quantitative analysis:

Carbohydrate content:

In the present study carbohydrate content was estimated by phenol-sulfuric acid method with dextrose as standard. Varied quantities of total carbohydrate content were observed in the extracts. The leaf methanol extract was found to have maximum carbohydrate content 140.90 ± 1.19 mg/g whereas leaf aqueous extract had low amount of sugar content 20.67 ± 1.25 mg/g (Table 3). The present data corroborated with findings of earlier work [22].

Table 3: Carbohydrate content* in leaf and bark extracts of *M. suriga*

Extracts	Total carbohydrate content (mg/g)*
Bark methanol (BM)	63.63 ± 1.44
Leaf methanol (LM)	140.90 ± 1.19
Bark aqueous (BA)	41.41 ± 1.32
Leaf aqueous (LA)	20.67 ± 1.25

*Mean ± standard deviation, N=3

Total phenol content:

Total phenolic contents (TPC) in the leaf and bark extracts were determined using gallic acid as a standard. The highest phenol content was observed in leaf methanol extract with 154.32 ± 1.80(mg/g) and lowest was observed in bark aqueous extract which was 51.20 ± 0.37(mg/g) (Table 4). In a study,

Shastri *et al.* [22] reported the phenolic content of 20.65 µg/mL in stem bark petroleum ether extract of *M. suriga*. Krishna *et al.* [21] reported phenolic content as 74.97µg/mg in the flower bud aqueous extracts of *M. suriga*. Similarly, phenolic content in *Mammea longifolia* methanol and aqueous bud extracts the total phenolic content of the aqueous extract (250 ± 6.66 mg/g) was higher than that of methanol extracts (247 ± 6.66 mg/g) [24]. Similarly, in water and methanol extracts, the highest TPC was observed in stem of *Calophyllum apetalum* (17.48 mg/g) while the lowest TPC in leaves of *Garcinia* gummigutta (7.08 mg/g) on other hand, TFC was highest in *Mammea suriga* stem (8.46 mg/g) with lowest content in *Calophyllum inophyllum* stem (3.12 mg/g) [25] .

Table 4: Total phenolic content in leaf and bark extracts of *M. suriga*

Extracts	Phenol Content(mg/g) *
Bark Methanol (BM)	80.15079 ± 1.42
Leaf Methanol (LM)	154.324 ± 1.80
Bark Aqueous (BA)	51.20274 ± 0.37
Leaf Aqueous (LA)	93.03849 ± 0.34

'*' Mean ± standard deviation, N=3

Total flavonoid content:

TFC was determined according to the aluminium chloride colorimetric method; Rutin was used as standard (200-1000µg/mL) and 1mL of test samples were used for estimation. In the present study the highest flavonoid content was observed in bark aqueous 437.3878 ± 0.092169 (mg/g) and lowest was observed in leaf methanol 160.5845 ± 0.613694(mg/g) (Table 5). Shastri *et al.* [22] reported the flavonoid content of 71.5 µg/mL in stem bark petroleum ether extract of *M. suriga*, while it was 113µg/mg in the flower bud aqueous extracts as reported by Krishna *et al.* [21]. Rathee *et al.* [24] reported flavonoid content in *Mammea longifolia* methanol and aqueous bud extracts the flavonoid content in aqueous extract was found to be enriched with the flavonoids (ECE value 203 ± 3.18) compared with methanol extracts (ECE value 152 ± 2.29). leaves of all species contained higher amount of total phenolic content (TPC), total flavonoid content (TFC) [25]. The seed, leaf and bark extract of *G. farquhariana* exhibited the presence of good quantity of proteins, phenollcs and flavonoids [26].

Table 5: Total flavonoid content in leaf and bark extracts of *M. suriga*

Extracts	Flavonoid content (mg/g)
Bark methanol (BM)	404.75± 1.67
Leaf methanol (LM)	160.58 ± 0.61
Bark aqueous (BA)	437.38 ± 0.09
Leaf aqueous (LA)	326.72 ± 0.09

‘ ;*’ Mean ± standard deviation, N=3

Antioxidant assays

1. DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity

Krishna *et al.* [21] reported the radical scavenging activity of stem bark petroleum ether extract of *M. suriga* and observed highest per cent activity which was 85.09±0.20 at 300µg/mL. Similarly, Rathee *et al*. [24] studied *M. longifolia* bud methanolic and aqueous extracts and found methanolic extract was a better radical-scavenger (IC_{20} 2.75 ± 0.13 lg/mL, IC_{50} 8.33 ± 0.39l g/mL) than aqueous extracts (IC_{20} 3.49 ± 0.13 lg/mL, IC_{50} 9.82 ± 0.16 lg/mL). In the present study, the radical scavenging activity by DPPH method was calculated using formula and ascorbic acid was used as a standard. The activity of the extracts was higher at higher concentrations and overall the activity was less compared to standard ascorbic acid. The IC_{50} value was found to be lowest for leaf methanol extract which was 53.33 ± 0.13 µg/mL and highest was observed for bark aqueous extracts showing 333.331 ± 0.71 µg/mL (Table 6,7).

Table 6: DPPH Radical scavenging activity for leaf and bark extracts of *M. suriga*

DPPH radical scavenging activity (%) for leaf and bark extracts of *M. suriga*			
	100 µg/mL	200 µg/mL	300 µg/mL
BM	56 ± 0.2	64 ± 0.11	88 ± 0.23
LM	54 ± 0.30	66 ± 0.66	74 ± 0.56
BA	4 ± 0.11	10 ± 0.31	48 ± 0.3
LA	24 ± 0.5	50 ± 0.22	74 ± 0.36
CONTROL	84 ± 0.23	88 ± 0.5	92 ± 0.61

*Mean ± standard deviation, N=3

Table 7: IC_{50} value for DPPH radical scavenging activity of extracts of *M. suriga*

Standard/Extract	**IC_{50}**
BM	79.16 ± 0.91
LM	53.33 ± 0.13
BA	333.33 ± 0.71
LA	202.66 ± 0.33
Ascorbic acid	80.73 ± 0.47

Ferric Reducing power assay:

Umadevi and Kamalam [17] reported that the methanol extract exhibited higher reducing activity (1.737) than the aqueous extract (1.429) in the seed extracts of *M. longifolia*, when subjected to the reducing power assay. In the present study, highest absorbance value was found in standard ascorbic acid (1.832) than the plant material extracts where highest reducing activity was observed in both the bark samples at 300µg/mL concentrations and lowest was for leaf aqueous extracts which was at 300µg/mL (Fig. 1). Shastri et al. [22] found that the reducing power increased with the concentration of the sample. Reducing power at 100 µg/ml was evident in *M. suriga* extract.

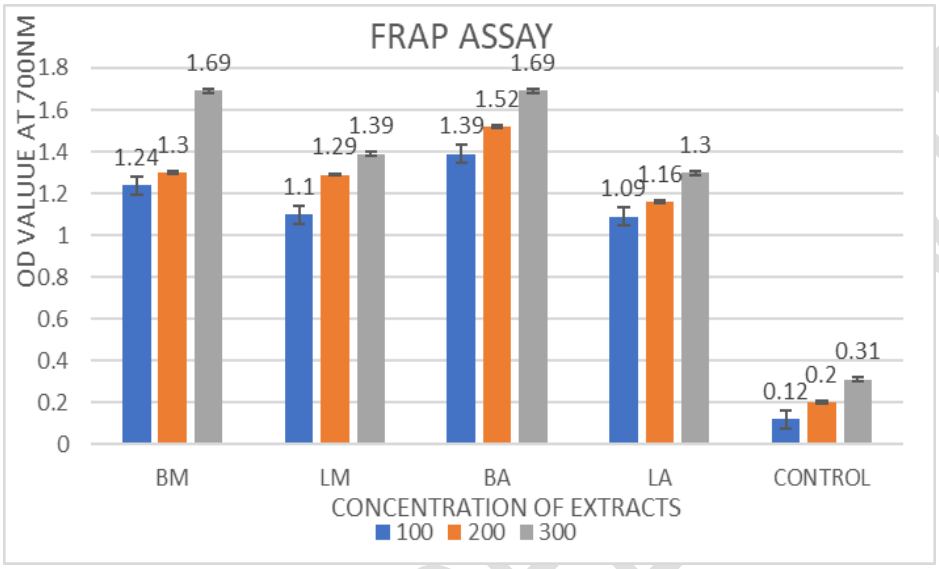

Fig. 1: Ferric reducing power activity for standard and leaf and bark extracts of *M. suriga*

Phosphomolybdenum assay: The antioxidant activity was observed highest in leaf methanol extract at 300µg/mL which was 75% and lowest was observed at 100µg/mL for bark aqueous extract which was 14% (Table 8). In Nitric oxide radical scavenging activity dose-dependent range was 48.71±0.29 to 66.64±0.39% and the IC50 values were 144.45 and 172 mg/ml [22].

Table 8: Antioxidant activity (%) for standard ascorbic acid and leaf and bark extracts of *M. suriga* by phosphomolybdenum assay

Antioxidant activity (%) for leaf and bark extracts of *M. suriga*			
	100 µg/mL	200 µg/mL	300 µg/mL
BM	19.74 ± 0.32	44.08 ± 0.36	54.61 ± 0.41
LM	52.63 ± 0.11	61.84 ± 0.61	75 ± 0.52
BA	14.47 ± 0.66	30.92 ± 0.65	46.05 ± 0.66
LA	44.08 ± 0.09	49.34 ± 0.55	67.11 ± 0.32
CONTROL	26.97 ± 0.61	47.37 ± 0.25	73.68 ± 0.61

* Mean ± standard deviation, N=3

Antimicrobial assays

1. Agar diffusion well method for antibacterial test:

Poojary *et al*. (2015) reported good antimicrobial activity which were (10±1.0mm, 33±1.4mm and 10±1.2mm) against pathogenic bacteria, viz., *E. coli, S. aureus* and *P. aeruginosa* by agar disc diffusion method with 1000 µg/disc on the aqueous root bark extracts of *M. suriga*. Similarly, Krishna *et al*. [21] reported that stem bark petroleum ether extracts inhibited P. *aeruginosa* at concentrations 20,40,80 (mg) which were 2.73±0.27, 4.00±0.00 and 5.33±0.33 mm respectively. In the present study agar well diffusion was done using streptomycin as a positive control the highest inhibition was observed in *E. coli* for leaf aqueous extract at 100µg/mL for *K. pneumoniae* at 50µg/mL for bark methanol extracts, for *P. aeruginosa* at 100µg/mL for bark methanol extract, for *S. aureus*100µg/mL for bark methanol extract (Table 9). Pájaro-González *et al*. [27] observed mammea A isolated from seeds of *Mammea americana* a potent inhibitor against methyciline resistant *S. aureaus*.

Table 9: *In vitro* antibacterial activity of leaf and bark extracts of *M. suriga*

Minimum inhibition in diameter (mm)*								
	E. coli		***K. pneumoniae***		***P. aeruginosa***		***S. aureus***	
	50µg/mL	100µg/mL	50µg/mL	100µg/mL	50µg/mL	100µg/mL	50µg/mL	100µg/mL
PC	29.56±1.95	36.67±1.15	38.11±0.84	43.22±1.02	31.00±0.67	33.00±1.20	31.78±1.39	34.44±1.50
NC	10.89±0.38	12.89±0.19	11.56±0.51	11.67±0.33	-	-	-	-
BM	12.89±1.50	14.44±0.96	20.11±0.51	18.89±0.51	13.78±0.38	17.78±1.84	21.67±1.45	22.78±0.69
LM	15.2±0.51	15.33±0.33	-	12.00±0.58	-	-	23.22±1.90	15.67±0.33
BA	14.67±0.33	13.00±0.58	14.00±1.15	13.33±1.86	12.67±0.58	14.33±0.88	13.89±1.95	12.89±0.69
LA	14.89±0.69	17.56±1.39	-	-	-	-	11.33±0.33	11.56±0.38

* Mean ± standard deviation, N=3

2. Poison bait method for antifungal test:

Poison bait method was used to test the antifungal activity. Bavistin was used as a positive control (1000µg/mL). For the test, the extracts were used at 1000µg/mL concentration. The inhibition growth was observed highest for bark methanol for *Fusarium solani* at 0.22±0.01g dry weight and for *Colletotricum gloeosporioides highest* activity was for bark aqueous extract 0.57±0.01g (Table 10). Chaithanneya and Bhat [28] studied the methanolic and aqueous extracts which showed varied levels of antifungal activity against two fungal species. *Candida albicans* and *A. niger* gave the highest zone of inhibition at the concentration 500 µg/mL. Gupta *et al*. [29] reported the antifungal activity was also found highest in acetone extract with *A. niger* (14.4 ± 0.37 mm).

Table 10: *In vitro* antifungal activity of leaf and bark extracts of *M. suriga*

	Dry mycelial weight (g)*	
Extracts/control	*Fusarium solani*	*Colletotrichum gloeosporioides*
PC	0.13±0.00	0.35±0.00
NC	0.29±0.00	0.78±0.00
BM	0.22±0.01	1.22±0.00
LM	0.23±0.00	0.71±0.00
BA	0.93±0.03	0.38±0.00
LA	1.34±0.04	0.57±0.01

* Mean ± standard deviation, N=3

Conclusion:

The present investigation was done on phytochemical extracts of leaf and bark of *Mammea suriga* to evaluate the *in vitro* antioxidant and antimicrobial properties.

- The extracts were prepared using methanol and water which indicated the presence of carbohydrates, phenols, glycosides, alkaloids, flavonoids and saponins.
- Quantitative analysis of carbohydrate, phenol, flavonoid revealed carbohydrate content to be maximum in leaf methanol extract whereas low amount for leaf aqueous extract
- The highest phenol content was observed in leaf methanol extract lowest was in bark aqueous extract highest flavonoid content was observed in bark aqueous lowest was observed in leaf methanol.
- The IC_{50} value was found to be lowest for leaf methanol extract and highest was observed for bark aqueous extracts.
- FRAP assay was done where the highest OD is considered to have highest activity hence highest absorbance value was found in standard ascorbic acid than the plant material extracts hence in the present study highest reducing activity was observed in both the bark samples at and lowest was for leaf aqueous extracts.
- Agar well diffusion was done using streptomycin as a positive control the highest inhibition was observed in *E. coli* for leaf aqueous extract for *K. pneumoniae* for bark methanol extracts, for *P. aeruginosa* for bark methanol extract and *S. aureus* for bark methanol extract.
- The fungal inhibition growth was observed highest for bark methanol for *Fusarium solani* at and for *Colletotricum gloeosporiodes* highest activity was for bark aqueous extract.
- Clusiaceae species including *Mammea suriga* with respect to phytochemicals and antioxidants study from stem and leaves by using water and methanol as solvents strengthens its medicinal use.

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