Investigation on Pharmacognostic Parameters of *Sirunagapoo* (*Mesua ferrea* L): A Traditional Indian Herbal Drug

Perumal Rajalakshmi*, Vellingiri Vadivel, Natesan Ravichandran, Pemaiah Brindha

ABSTRACT

Introduction: Flower buds of Sirunagapoo (Mesua ferrea) are used in Siddha system of medicine as carminative, astringent and anti-vatha. It is traditionally used to treat various diseases like cough, venerial, white discharge, diarrhea, over-bleeding and peripheral neuritis. It is one of the major ingredients in Amukkara choornam, Inji choornam and Elathi choornam which are used for indigestion, loss of appetite and gastritis. Methods: Pharmacognostic characters of M. ferrea flower bud were studied through powder microscopy. Both ethanol and aqueous extracts were investigated for phytochemical screening, total phenolic content, in vitro antioxidant and anti-inflammatory properties and the ethanolic extract was subjected to GC-MS analysis. Results: Powder microscopy of flower buds of M. ferrea revealed the presence of brachysclereids, macrosclereids, starch grain, crystals and parenchyma cells. The powdered material exhibited 6.07% of loss on drying, 2.93% of total ash, 11.34% of water-soluble extractive and pH value 5.35. Data showed the presence of sterols only in ethanol extract and phenols, flavanoids, saponins and coumarins in both ethanol and aqueous extracts. Ethanolic extract was found to contain higher concentration of total phenols (1030 mg GAE/L) when compared to aqueous extract. Totally forty compounds were detected in GC-MS analysis and the major compound is eugenol (61%) and cinnamaldehyde (15%). In vitro studies revealed antioxidant in terms of DPPH free radical scavenging property (IC-50 = 229.7 mg/ml) remarkable anti-inflammatory activity using RBC membrane stabilization assay (70.27%) were noted. Conclusion: This study provides the pharmacognostic standards, phytochemical profile, major volatile compounds and in vitro properties of Mesua ferrea flower bud. Key words: Mesua ferrea, Pharmacognosy, Phytochemicals, GC-MS, In vitro studies.

INTRODUCTION

Mesua ferrea L belongs to the Calophyllaceae family and it is widely distributed in tropical countries like India, Burma, Thailand, China and New Guinea.1 In India, it is growing in the mountains of Eastern Himalaya and East Bengal, Assam, Burma, Andaman, evergreen rain forests of North Canara and South Konkan, the Forests of Western Ghats from South Canara to Travancore.² It is a medium to large sized tree that grows up to a height between 18 and 30 m, with reddish-brown to grey colored bark that peels off in thin flakes and the wood is extremely hard. The leaves are simple, lanceolate, acute and leathery, covered in a waxy bloom below. Immature leaves are red in colour, oppositely arranged, 7 to 13 cm long and 2 to 4 cm wide. The flowers are white with a floral fragrance, has diameter up to 7.5 cm, with numerous golden-colored stamens, which are shorter than the length of the petals. The style is twice as long as the stamens, borne single or in pairs, axillary or terminal. The fruits are ovoid with a conical point, 2.5 to 5 cm long with a woody pericarp that contains one to four seeds.³

In *Siddha* system of medicine flower bud of *M. ferrea* is used as a carminative and astringent.⁴ It is tradition-

ally used for curing different aliments like cough, venerial diseases, white discharge, diarrhea, overbleeding, vatha diseases and peripheral neuritis.5 M. ferrea flower bud is found as an ingredient in many Siddha formulations like Amukkara choornam, Elathi choornam, Inji choornam, Ilagu santhanathi thylam, Kanthaga rasayanam, Parangipattai rasayanam, Narathai legium, Karisalai legium and Thalisathi vadagam etc.6 The flower buds of this plant have different uses in Ayurveda against fever, sweats, foul breath, scabies, skin eruption, itching, small tumours, head ache, blood related troubles, heart problems, sore throat, cough, cough, vomiting, thirst and dysentery.^{7,8} The flower buds have been reported to exhibit antioxidant, hepato-protective, analgesic, anti-spasmodic, anti-inflamatory, anti-ulcer, antiarthritic, anti-microbial, anti-venom, anticancer, immune-modulatory and central nervous system depressant activities.² It is already reported that the flower buds contain a yellow colouring material and two bitter poisonous substances. Further, a reddishbrown volatile oil with the characteristic aroma of the flowers is extracted by Chaubey Suresh et al.9

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Perumal Rajalakshmi*, Vellingiri Vadivel, Natesan Ravichandran, Pemaiah Brindha

Research carried out at Centre for Advanced Research in Indian System of Medicine, Shanmugha Arts, Science, Technology and Research Academy (SASTRA) Deemed University, Thanjavur, Tamil Nadu, INDIA.

Correspondence

Dr. Perumal Rajalakshmi

Centre for Advanced Research in Indian System of Medicine, School of Chemical and Biotechnology, Shanmugha Arts, Science, Technology and Research Academy (SASTRA) Deemed University, Thanjavur, Tamil Nadu, INDIA.

Phone no : +09442785007

E-mail: rajalakshmi@carism.sastra.edu

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Previous article reported that the bud and flower oils contained alphacopaene (28.7% and 20.2%) and germacrene D (19.0% and 16.1%).¹⁰

Mesua ferrea is called in Siddha medicine as Sirunagapoo or Nagakesaram. Commonly known as Nagpushpa in Sanskrit, Cobra's saffron or Ceylon ironwood or Indian rose chestnut or iron wood tree in English, Champey or Nagkesa or Nahar in Hindi, Nagesar in Assam and Bengali, Nagchampe in Maharastra and Kongani, Nagashappu or Gaja pushpam in Telungu, Nagachampakam in Malayalam, Nagesuri in Nepal, Narmishka in Urdu and Narae kaisar in Arabic.11 There is a controversial in identifying the M. ferrea bud because of presence of its adulterants (Cinnamomum iners, C. tamala, Calophyllum inophyllum, C. elatum, C. apetalum, Ochrocarpus longifolius, Nelumbo nucifera and Dillenia pentagyna), which are very common in Indian market.^{12,7,13} Though the reliable plant is available in abundance throughout the Western Ghats and parts of Himalayas, suppliers are unaware of it. There may also be some restrictions in forest collection and due to these reasons M. ferrea is being sold with adulterants in India. Till date, there is no report regarding the pharmacognostic information of M. ferrea flower buds and hence the present study was carried out to evaluate the pharmacognostic characters, pytochemical profile, antioxidant and anti-inflammatory properties of aqueous and ethanolic extracts of Mesua ferrea flower buds.

MATERIALS AND METHODS

Collection of Sample

Sirunagapoo (*Mesua ferrea* flower bud) was purchased from herbal market, Thanjavur, Tamil Nadu, India. The drug was cleaned and powdered using a lab mill and sieved (particle size 1 mm) and used for further studies.

Microscopic Studies

The powder microscopic characters of M. ferrea flower bud powder also studied according to the method of WHO (1998).¹⁴ Presence of calcium carbonate crystals was observed by taking a pinch powdered material and treated with acetic acid (60 g/L) and the preparation was mounted and observed under microscope. Presence of fats and fatty oils was analyzed by taking one pinch of powdered material with 1-2 drops of sudan red solution, heated gently and the preparation was irrigated with ethanol (750 g/L) and the slides were mounted and observed under microscope. Mucilage was studied by taking a pinch of powdered material and treated with Chinese ink (1:10 with water) and the slides were mounted and examined under the microscope. For Starch test, a pinch of powdered material was treated with Iodine (0.02 M) solution and the slides were mounted and observed under microscope. Presence of tannins was evaluated by treating a pinch of powdered material with 1-2 drops of ferric chloride (50 g/L) and the slide was mounted and examined under microscope.

Chemical Standardization

The pH of the aqueous solution of *M. ferrea* flower bud powder material (1%, W/V) was calculated using the pH meter at 24.4°C. The determination of the total ash content of *M. ferrea* flower bud powder was done by the method of Ayurvedic pharmacopeia of India, 1999,¹⁵ Joshi and Aeri.¹⁶ Powder (1.0896 g) is added to a pre-weighed silica crucible and heated in the muffle furnace at 400°C for about 3 h. Then the crucible was safely located in the dessicator and permitted to cool to room temperature and the weight is finally measured. The percentage weight of the ash is calculated using the formula (Weight of the ash / Weight of the drug x 100). The percentage of acid insoluble ash is calculated using the formula, Weight of the residue Weight of the powder x 100, where the weight of the residue is the net weight of ash.

The Loss on drying (LOD) was estimated by taking 1.0605 g of powder in a pre-weighed dish and kept in the hot air oven at 105°C and the LOD

was calculated by using the formula (Weight of the dish before LOD -Weight of the dish after LOD / Weight of the sample x 100). The water soluble extractives of the powder were analyzed according to the methodology written by Joshi and Aeri.¹⁶ Dry powder (1.0034 g) was taken in a beaker 50 ml of water was added and shaken well manually. The beakers were kept aside for 24 h and there after 10 ml of the solution was taken and kept in hot air oven at 105°C. Finally the percentage weight of the extract is calculated using the formula (Weight of residue / Weight of the drug x 100).

Extract Preparation

For preparing extract, 10 g of dry powdered material was taken with 100 ml ethanol and distilled water in a conical flask separately. The mixer was kept for 24 h at room temperature (37°C). Then the contents were filtered through a filter paper placed on the funnel and the volume of the extract was noted and the extract thus obtained was evaporated in rotovapor. Dry ethanol and aqueous extracts were re-dissolved in respective solvents at 10 mg / ml ration.

Pytochemical Screening

The presence of phenolic compounds was identified by taking 1 ml of extract with 5 ml alcohol and a pinch of ferric chloride. The presence of alkaloids was detected by using Dragendorff's test, in which, 0.5 ml of extract was taken with 0.2 ml of acetic acid and 1 ml of Dragendorrf's reagent and shaken well. The presence of flavonoids was detected by adding 2 ml of extract with 1 ml of Hydrochloric acid and a pinch of Magnesium turnings and boiled for few mins. The terpenoids were detected by taking 0.5 ml of extract with tin pellet and 0.2 ml of thionyl chloride and heated gently. The extract (0.5 ml) was mixed with 0.1 ml of lead acetate and observed for tannins. To identify the presence of saponins, 0.5 ml of extract was mixed with 5 ml of distilled water and shaken vigorously. For confirming the presence of steroids, the extract (0.5 ml) and 0.5 ml of acetic anhydride were taken and few drops of concentrated sulphuric acid were added. To know the presence of guinones, 0.5 ml of extract was added with 0.1 ml of sulphuric acid. For coumarins test, 0.5 ml of extract was mixed with 0.2 ml of sodium hydroxide. The extract (0.5 ml) was mixed with Fehling's (A and B) to reveal the presence of sugars.

Total Phenol Content

The total phenolic content of extract was estimated according to the method of Singleton *et al.*¹⁷ Sample (100 μ l) was taken with 250 μ l of Folin's-Ciocalteu reagent and 1000 μ l of 5% of Na₂CO₃ were added and incubated for 30 min in dark place. Then the absorbance was measured at 720 nm. A calibration curve was prepared using standard gallic acid (16 – 100 mg/L; y = 0.0094x – 0.0585; R2 = 0.9939) and used to express the results as gallic acid equivalents (GAE).

Antioxidant Activity

The DPPH radical scavenging assay was used to analyze the antioxidant property of ethanolic and aqueous extracts of the sample by following Sanchez-Moreno *et al.* method.¹⁸ Different concentrations (10, 5, 2.5, 1.25, 0.63, 0.31, 0.16, 0.08, 0.04 and 0.02 mg/ml) of extract (100 μ l) were added to 0.9 ml of methanolic solution of DPPH (2.5 mg/100 ml) and the reactants were incubated at room temperature for 30 min in dark. Different concentrations of Butylated hydroxyanisol (BHA) were used as a standard and the solvent (distilled water) was used instead of extract in control. After 30 min, the absorbance was measured at 515 nm using a spectrophotometer and the radical scavenging activity of the extract was calculated and expressed on percentage basis.

Anti-inflammatory Activity

Anti-inflammatory activity of ethanolic and aqueous extracts of sample was evaluated using RBC membrane stabilization assay.¹⁹ Human blood (2 ml) was drawn from volunteer in a heparinised tube and centrifuged at 2000 rpm for 10 min. The pellet (RBC cells) was washed twice with PBS (9 ml) and finally the pellet was re-suspended in 10 ml of PBS. Different concentrations (10, 5, 2.5, 1.25 and 0.63 mg/ml) of extract (500 μ l) were added to 1 PBS, 1 ml of 3% H₂O₂ and incubated for 30 min. In normal control, 1 ml PBS was added instead of extract and in negative control only H₂O₂ was added. After incubation, the contents were centrifuged at 2000 rpm for 10 min and the supernatant was used to measure the absorbance at 520 nm. Based on the absorbance, the percentage of RBC membrane damage and inhibition of membrane damage were calculated.

GC-MS Analysis

Ethanol extract was analyzed using Gas Chromatographic system coupled with Mass Spectrometry (Perkin Elmer, Model: Clarus-500). Silica capillary column (30 m x 0.25 mm, 0.25 µm film thicknesses, Elite-5 MS non-polar fused) was used. Oven temperature was programmed with an increase of 6°C/min to 150°C; injector temperature was 280°C; carrier gas was helium with the flow rate of 1 ml/min. Sample (1.4 μ l) was injected with split ratio of 1:10. Ionization energy 70 ev was used in the electron ionization mode; ion source temperature was set at 160-200°C, mass was scanned in the range of 40-450 amu. The resulted mass spectrum was compared with inbuilt NIST library database and fragments of various compounds present in the extracts were identified.

RESULTS AND DISCUSSION

Powder Microscopic Studies (Figure 1)

The powder microscopic studies of *Sirunagapoo* (flower bud of *Mesua ferrea*) revealed the presence of round to elongated brachysclereids with wide and narrow lumen, with simple and branched pits were found (Figure 1 A-C) and macroslereids with wide lumen were noted (Figure 1 D-E). Compound and simple spherical and ovoid starch grains with closely arranged striations were noted (Figure 1 F-G). Golden yellow coloured round shaped pollen grains with 1-3 protuberances and distinct exines were observed (Figure 1 H). Prismatic calcium oxalate crystals were also found (Figure 1 I). Lipid containing cells (Figure 1 J), polygonal parenchyma cells with starch grains (Figure 1 K.) and cells with brown to red contents (Figure 1 L-M) were also found.

Chemical Standardization (Table 1)

Loss on drying (LOD) test procedure indicated the amount of volatile matter (i.e. water drying off from the drug). The LOD of *M. ferrea* flower bud powder was 6.07% (Table 1). It point out the low level of moisture content in the drug, which will be useful to avoid the microbial spoilage of herbal drugs. Total ash was recorded as 2.93%, while acid insoluble ash was not detected. Water soluble extractive value was found to be 11.34% and pH value of aqueous solution of *M. ferrea* was noted to be slightly acidic (5.35). The chemical characterization results of *M. ferrea* fall within the range denoted by Ayurvedic Pharmacopoeia of India (1999).

Phytochemical Screening (Table 2)

Phytochemical screening of ethanol extracts showed the presence of sterols, phenols, flavanoids, saponins and coumarins and the aqueous extract has phenols, flavonoids, saponins and coumarins (Table 2). In this test sterols are presented only in ethanol extract. Generally sterols are found considerably high amount as phytosterols in wheat germ, nuts, pulses and grain products. Phytosterols has hypocholesterolemic effect, anticancer activity, anti inflamattoric effect and anti-accident properties.²⁰



A-C Brachysclereids, D& E - Macrosclereids, F - Simple starch grain, G - Compound starch grain, H - Pollen grain, I - Calcium oxalate crystal, J - Lipids, K - Parenchyma cells, L - Parenchyma cell containing brown content, M - Brown content

Figure 1: Powder microscopic images of Mesua ferrea flower bud.

Table 1: Chemical standardization of Mesua ferrea flower bud powder.

S. No.	Parameters	Results
1	Loss on drying (LOD)	6.07 %
2	Total ash	2.93 %
3	Acid insoluble ash	Nil
4	Water soluble extractive	11.34 %
5	pH	5.35

Table 2: Phytochemical profile of Mesua ferrea flower bud.

S. No.	Compounds	Ethanol extract	Aqueous extract
1	Sterols	+	-
2	Terpenes	-	-
3	Alkaloids	-	-
4	Phenols	+	+
5	Flavonoids	+	+
6	Tannins	-	-
7	Saponins	+	+
8	Quinones	-	-
9	Coumarins	+	+

Phenolics are widespread groups of substances in flowering plants, occurring in all vegetative organs, as well as in flowers and fruits, vegetables, cereals, grains, seeds and drinks. It has inhibition of pathogens and decay microorganisms, anti-deposition of triglycerides, reduce the incidence of non-communicable diseases such as cardiovascular diseases, diabetes, cancer and stroke, anti-inflammation and anti-allergic effect through processes involving reactive oxygen species.²¹ Presents of flavo-noids concluded that it has antioxidants activity, anticancer effect and act as a detoxifing agent.²² Saponins have antimicrobial activities, membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties, antifungal and antiviral effects.²² Coumarins are used as an anticoagulant, anti-cancer, chronic infections, inflammation, edema and skin diseases.



Figure 2: Total phenolic concentration of aqueous and ethanolic extracts of *Mesua ferrea* flower bud.

Total Phenolic Content (Figure 2)

Total phenol content was shown in Figure 2. This result denotes ethanolic extract of flower bud having very high level (1030 mg GAE/100 g) than aquous extract (933.39 mg GAE/100 g).

Antioxidant Properties (Figure 3)

Anti-inflammatory activities in terms of DPPH free radical scavenging property has revealed. High antioxidant power of ethanolic extract is 229.7 mg/ml and aquas extract value is 220 mg/ml. In addition to this, the year 2007 Mohan SK and Venkataramana G conducted a clinical trial about the relationships between oxygen-free radical production and osteoarthritis. In that he mentioned that oxygen - free radical creation and reduced catalase activity, supporting the higher oxidative stress hypothesis in osteoarthritis. The improved activities of antioxidant enzymes may be a compensatory regulation in response to improved oxidative stress. The results suggest the necessity for therapeutic codirection of antioxidants with conventional drugs to such patients. Therefore, treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidativedamage and deterioration of the musculoskeletal tissues in osteoarthritis.²³

Anti-inflammatory Activity (Figure 4)

The high anti-inflammatory activity was noted in aqueous extract 70.27% compared to aquas extract that was 58.64%. Previously this flower bud's anti-inflammatory potencial was explained by Tiwari and Nandy, 2012 in their animal study.²⁴ Also Gopalakrishnan C *et al.* explained the same result in his animal work.²⁵

GC-MS Analysis (Figure 5)

The results of the GC-MS analysis of ethanolic extract of *M. ferrea* flower bud was shown in Figure 5 and the major compounds were listed in Table 3. A total of 40 compounds were identified, among which eugenol is a phenolic compound and it recorded 61.49% peak area and 14.46 retention time. Other major compound is Cinnamaldehyde 15.1517 % peak area and retention time is 12.48.







Figure 4: Anti-inflammatory activity of aqueous and ethanolic extracts of *Mesua ferrea* flower bud.



Figure 5: GC-MS profile of ethanolic extract of *M. ferrea*.

Eugenol is a phenolic compound and major constituent of essential oils.²⁶ Clove is the major source of eugenol and it has a physical nature of volatile oil.²⁷ The irritant nature of the clove oil can be attributed to the eugenol content. Eugenol is also stated to have sensitising properties. The accepted daily intake of eugenol is up to 2.5 mg/kg. Other names are eugenic acid and caryophyllic acid. Eugenol has been identified in several aromatic plants. Previously it was noted in *Hibiscus sabdariffa* petals.²⁸ *Cinnamomum zeylanicum* leaf oil contains much higher concentrations of eugenol, from 80 to 96% depending on the species.²⁹ Clove

Table 3: Major compounds detected in ethanolic extract of *M. ferrea* through GC-MS.

S. No.	Peak Name	Retention time	%Peak area
	Cinnamaldehyde C ₉ H ₈ O. MW: 132	12.48	15.1517
	Terpinyl acetate $C_{12}H_{20}O_2$. MW: 196	13.98	7.9771
	Eugenol C ₁₀ H ₁₂ O ₂ . MW: 164	14.46	61.4908
	2H-1-Benzopyran C ₉ H ₆ O ₂ . MW: 146	16.34	1.9177
	Caryophyllene C ₁₅ H ₂₄ . MW: 204	16.43	1.0688
	2-Methoxycinnamaldehyde. $C_{10}H_{10}O_2$. MW: 162	18.74	2.5775
	n-Hexadecanoic acid C ₁₆ H ₃₂ O ₂ . MW: 256	30.29	2.3776

bud oil contains eugenol quantity is 80–90%, Stem oil contains eugenol is 90–95%, Leaf oil is 82–88. 30

Antiseptic and anaesthetic properties have been documented for Eugenol.^{27,31} There is limited evidence from *in vitro* investigations that eugenol inhibits prostaglandin synthesis.³² In addition, antimicrobial, anti-oxidant, anti-inflammatory, anticonvulsant, anticarcinogenic, antimutagenic, anti-plasmodial, anti-viral, repellant and anti-fumigant activities of eugenol were reported.33 The same paper explained about therapeutic uses of eugenol such as anti-ulcerogenic potential, effect on osteoporosis and its achievement on the central nervous system.33 Fernando J. Sutili et al. explained about potential of eugenol as anthelmintic activity.³⁴ It plays a prominent role in dental and oral hygiene preparations. Eugenol possesses anti-inflammatory effect on the dental pulp.35 It showed strong lipoxygenase inhibitory effects.³⁶ Hema et al. denoted that eugenol can be toxic in relatively small quantities.³⁷ It can promote percutaneous absorption and treat angiocardiopathy. Eugenol has a certain effect in reproductive regulation and immune-regulation. Eugenol also has obvious killing or repellent action on worldwide agricultural insects, such as red flour beetle and citrus fruit fly males.³⁸ Anuj and Sanjay (2010) explained that eugenol has neuroprotective effect.³⁹

Next high quantity compound - 2-Propenal, 3-phenyl- (C_9H_8O) is also called as Cinnamaldehyde, Benzylideneacet aldehyde, Cassia aldehyde and Cinnamal. It has good anti-inflammatory, antioxidant, anti-ulcer, anti-microbial, hypoglycemic and hypolipidemic potential effects.⁴⁰ Third one is à,à,4-trimethyl-, acetate formula is $C_{12}H_{20}O_2$. It is otherwise called as Terpinyl acetate and Cyclohexene-1-methanol. Next one is Benzopyran, it is used as an antifungal, anticoagulant, antibacterial and insecticidal.⁴¹

CONCLUSION

Present study provided the pharmacognostic properties of *M. ferrea* flower buds such as powder microscopy and physico-chemical parameters. Between the investigated extracts, ethanol extract showed presence of sterols in addition to phenols, flavonoids, saponins and coumarins and also exhibited better antioxidant properties, whereas aqueous extract showed good anti-inflammatory activity. GC-MS analysis of ethanol extract revealed the presence of eugenol and cinnamaldehyde as the manor volatile phytochemicals, which could be responsible for the medicinal effects shown by the *M. ferrea* flower buds.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

GC-MS: Gas chromatography-Mass spectrometer; **GAE:** Gallic Acid Equivalent; **DPPH:** 1 1-diphenyl-2-picrylhydrazyl; **RBC:** Red blood cells **WHO:** World health organization; **LOD:** Loss on drying; Na₂CO₃: Sodium carbonate; **PBS:** Phosphate buffered saline; H_2O_2 : Hydrogen peroxide; **NIST:** National Institute of Standards and Technology.

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GRAPHICAL ABSTRACT



SUMMARY

 In the present study, pharmacognostic characters, pytochemical profile, antioxidant and anti-inflammatory properties of aqueous and ethanolic extracts of *Mesua ferrea* flower buds were investigated. Powder microscopy of revealed the presence of brachysclereids, macrosclereids, starch grain, crystals and paren-chyma cells. Loss on drying (6.07%), total ash (2.93%), water-soluble extractive (11.34%) were recoded in *M. ferrea* buds. GC-MS analysis revealed the presence of eugenol (61%) and cinnamaldehyde (15%) as major compounds. High antioxidant power (229.7 mg/ml) and anti-inflammatory activity (70.27%) were noted.

ABOUT AUTHORS



Perumal Rajalakshmi: is an Assistant Professor with specialization in Siddha system of medicine.



Natesan Ravichandran: is a Research Scientist, working on pharmacognosy.



Vellingiri Vadivel: is an Assistant Professor, working on bio-assay guided fractionation of phyto-constituents.



Pemaiah Brindha: is an Associate Dean and Co-ordinator of Centre for Advanced Research in Indian system of Medicine

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