PHYTOCHEMICAL PROFILING, FREE-RADICAL SCAVENGING AND ANTI-INFLAMMATORY ACTIVITIES OF MELIOSMA SIMPLICIFOLIA (L.)

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Received: 16 Apr 2020, Revised and Accepted: 19 Jun 2020

ABSTRACT

Objective: In the current research, to determine the stem extract of Meliosma simplicifolia (L.) for total phenol, tannin, total flavonoid, antioxidant activity, and identify the phytoconstituents utilizing GC-MS and FT-IR.

Methods: The ability of the plant extract to act as hydrogen/electrons donor or scavenger of radicals was determined by in vitro antioxidant assays using 2,2-diphenyl-1-picryl-hydrazyl free radical (DPPH) scavenging, reducing power assay, superoxide radical (O²⁻) scavenging activity. The anti-inflammatory activity was evaluated using carrageenan and formalin-induced paw edema models using Wistar albino rats. The GC-MS and FT-IR analysis of the methanolic stem extract of M. simplicifolia was revealed the presence of phytochemicals.

Results: Quantitative studies of estimated phenol, flavonoid and tannin, as for the methanol extract of stem showed the highest content of phenolic compounds (39.83±3.62%AE mg/g). Antioxidant activities were concluded the estimation M. simplicifolia stem for as followed the studies. In stem the methanol extract showed the highest DPPH scavenging activity (124.39±g/ml). The anti-inflammatory activity has shown in high doses of methanolic extract 250 mg/kg of significant value (p<0.05) inhibition of paw edema, on 6th hour, respectively. The FT-IR analysis has confirmed their characteristic peak values and functional groups.

Conclusion: M. simplicifolia has an effective of anti-inflammatory activity and constitutes a potential source for the development of new treatments.

Keywords: M. simplicifolia, Phytochemicals, Antioxidant, GC-MS, FT-IR, Carrageen an induced acute paw edema

INTRODUCTION

The medicinal plants are treating diseases with natural medicaments is turn out to be more important in developing countries. The therapeutically value of plants has some chemical substances that produce a definite physiological action on the human body. The medicinal plants are beneficial for curing human diseases because of the presence of phytochemical constituents. Several important medicinal components are derived from plants like alkaloids, flavonoids tannins, terpenoids, steroids etc [1]. The concept of ‘integrative′ medicine has come up several times earlier and is not new. This however, is not a call for Siddha alone, but it is all about the direction of change. World Health Organization (WHO) has announced desirable doctor–population ratio as 1:1,000. In India as per the current population, it gives a doctor (modern medicine) and a population ratio of 0.77:1000 [2]. Among the numerous naturally occurring antioxidants, ascorbic acid, carotenoids and phenolic compounds are more effective [3]. The presence of this type of phytochemical compound in the screened medical plants has a wide range of applications and could be certainly used for a variety of applications. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources [4]. Oxidative stress refers to an imbalance between the production of free radicals and the antioxidant defense system. Reactive oxygen species (ROS) are various forms of activated oxygen, which causes lipid peroxidation and as a consequence of that inflammation may develop [5]. These results suggest that tannins, which are found in many plant-based foods and beverages, are potentially very important biological antioxidants [6].

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [7]. These phytochemicals are responsible for the magnitude of biological effects, including antioxidant, antimicrobial and anti-cancer activities. Extraction is an important rate-limiting step to attain the optimum yield of any compound. The antioxidant activity of extracts was evaluated by the green phosphomolybdenum complex formation, according to the previously described method of [8]. Two major parameters affecting the content yield of analyses, includes the method of extraction and time required for it. There are a number of methods identified for extraction of plant-based activities and metabolites Among plant constituents, secondary metabolites including, phenolic compounds such as simple phenolics, flavonoids, phenolic acids and anthocyanins are of special interest due to their pronounced health-related properties such as antioxidant potential, anti-inflammatory, antibacterial and anti-diabetic properties [9]. Therefore underscores the need to try as much solvents as possible in screening plant parts for phytochemicals. This research work is an attempt to identify naturally occurring chemo-protective agents, particularly those present in medicinal plants, by evaluating the antioxidative activity of the methanol extract of the Meliosma and examining its anticancer effects on various cell lines, particularly in cancerous cells. The research on the medicinal plants should be extended with the identification of the active principles in the plants. Two major parameters affecting content yield of analytes, includes method of extraction and time required for it. There are a number of methods identified for the extraction of plant-based activities and metabolites [10]. Phytochemicals are naturally occurring in the plant parts stem, leaves, flower, fruits, seed and roots that have been believed to possess anti-inflammatory, anti-cancer, anti-allergic, anti-viral, anti-bacterial activities and anti-proliferative activities. These antioxidants may help to relieve oxidative stress, i.e. preventing free radicals from damaging biomolecules such as proteins, DNA, and lipids. The present study was aimed to examine M. simplicifolia stem extract phytochemicals, antioxidant and anti-inflammatory properties.

MATERIALS AND METHODS

Materials

Sodium phosphate, ammonium molybdate, potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picrylhydrazyl(DPPH), potassium,
was calculated as rutin equivalents. The amount of flavonoids added to all the test tubes incubated for AlCl$_3$ was evaluated as it goes about as a noteworthy antioxidant in plants. The flavonoid content was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical 1,1-diphenyl-2-picrylhydrazyl DPPH according to the method of [13]. Plants extracts at various concentrations (20-100 μg) was added to 5 ml of 0.1 m Methonol solution of DPPH and allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm. Methanol was chosen as blank and solution without extract is considered as control. The mixture of methanol, DPPH and standard (ascorbic acid) served as positive control. Radical scavenging activity was delivered as the inhibition percentage of free radical by the sample. More significantly the IC50 of the extracts were also calculated.

In vivo studies: Evaluation of Anti-inflammatory activity

Animals

Albino Wistar rats of either sex weighing 100–150 g were obtained from Indian Institute of Integrated Medicines, Jammu. All animals were housed in polypropylene cages (6 in each cage) at an ambient temperature; 25±2 °C, relative humidity; 55–65%, and were maintained under a 12 h light/dark cycle each in the animal house of KMCH College of Pharmacy, Coimbatore. Ethical clearance for this experimental protocol was obtained from the Institutional Animal Ethics Committee (Reg. No.688/PO/Re/S/02/CP/CEA). The animals were fed with standard diet and water ad libitum and were deprived of food overnight prior to the experiment.

Drugs and chemicals

Carrageenan was procured from Sigma Chemical Co. (St Louis, MO, USA), diclofenac injection (Voveran) from Novartis India Ltd., Bombay and formalin from Ranbaxy (Rankem), Vernier calipers purchased from Percision India Ltd. and standard chow diet from Ashirwad Industries, Ropar (Punjab) were used in the study.

Carrageenan induced acute paw edema in rats

To study the anti-inflammatory property of M. simplicifolia stem methanol extract against Carrageenan induced acute paw edema in rats methodology by [14] was followed. Wistar albino rats (100-150g body weight) were selected and housed in four groups with six rats in each group. They were fasted overnight but had free access to water. A mark was made on the left hind paw just beyond tibiotarsal junction. The initial paw thickness of each rats were noted by digital Vernier caliper.

The animal were divided in to four groups each comprising six rats namely;

- Group I: control (0.6 cm)
- Group II: Oral feeding of indomethacin (10 mg/kg)
- Group III: Oral feeding of plant extract: low dose (100 mg/kg)
- Group IV: Oral feeding of plant extract: high dose (200 mg/kg)

After one hour 0.1 ml of 1% (w/v) Carrageenan (suspended in normal saline) was injected in the sub-planta region of the left paw of control as well as plant extract treated group. Paw thickness of left leg of control and plant extract treated rats were noted at every hour after the administration carrageenan. The reading was taken for total of 4 h the average (mean) edema was assessed by measuring with digital vernier caliper.

The percentage (%) inhibition of edema is calculated using the formula

$$\% \text{Inhibition} = \frac{T_s - T_r}{T_s} \times 100$$

Where T is the thickness of paw of rats given test extract at corresponding time and T0 the paw thickness of rats of control group at the same time.

RESULTS

Quantification of total phenolics, tannins and flavonoid

In the Soxhlet method for extraction, the bound phenol compounds from plant materials can be released using heat treatment [15]. With

Collection of plant material

Melosoma simplicifolia plant parts were collected from Nilgiris, Western Ghats region of Tamil Nadu, India. The plant material was identified as M. simplicifolia and authenticated by Botanical Survey of India, Southern circle, Coimbatore and the voucher specimen has been deposited in the University, Coimbatore (No. BSI/SRC/5/23/2018/Tech/2910). The freshly collected plant materials were cleaned to remove dust and impurities using distilled water. Plant materials were washed with distilled water and dried at room temperature. The dried rhizomes were manually ground to a fine powder and the samples were used for further studies.

Plant extracts preparation

The powdered plant material was extracted in Soxhlet extractor successively with petroleum ether, chloroform, acetone, methanol and hot water. Each time before extracting with the next solvent, the thimble was dried in hot air oven below 40 °C. Rotary vacuum evaporator was used to concentrate different solvent extracts and then air-dried. The dried extract obtained with each solvent was weighed. The percentage yield of air-dried weight of plant material was expressed in terms.

Quantification of total phenolics and tannins

The total phenol content was determined according to the method described 100 μl aliquots for plants extracts (5 mg/ml) were taken in the test tubes and made up to 1 ml with distilled water. Then 0.5 ml of Folin-Cioceltau reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. After vortexing the mixture, the test tubes were kept in the dark for 40 min and the absorbance was taken at 725 nm against blank. Blank was a reaction mixture without plant extract. The analysis was performed in triplicate and the results were expressed as Gallic acid equivalents.

Using the same extract, the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP) 1 mg of PVPP was weighed into a 2 ml eppendorf tube and to this 900 μl distilled water and then 750 μl of the sample extracts were added. The content was vortexed and kept in the test tube at 4 °C for 4 h. Then the sample was centrifuged at 4000 rpm for 10 min at room temperature and the supernatant was collected. This supernatant has only simple phenolics other than the tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was calculated and articulated as the content of non-tannin phenolics. From the above results, the content of tannin in the sample was calculated formula as,

$$\% \text{Tannin} = \frac{SP-NP}{SP} \times 100$$

Where SP is the Sum of phenolics percentage and NP is Non tannin phenolics percentage

Quantification of total flavonoids

The flavonoid content of the considerable number of concentrates was evaluated as it goes about as a noteworthy antioxidant in plants diminished oxidative anxiety. Assessed according to portrayed by [11]. Initially, 150 μl of all the plant extracts were taken in different test tubes. To each extracts 2 ml of distilled water was added. Then 150 μl of NaNO$_2$ was added to all the test tubes followed by incubation at room temperature for 6 min. After incubation 150 μl of AlCl$_3$ (10%) was added to all the test tubes. The test tubes were incubated for 6 min at room temperature. Then 2 ml of NaOH was added to all the test tubes, which were made up to 5 ml using distilled water. The contents in all the test tubes were vortexes well and they were allowed to stand for 15 min at room temperature. The presence of flavonoid was confirmed by the development of pink colour in spectrophotometer at 510 nm. The amount of flavonoids was calculated as rutin equivalents.

Quantitative measurements of radical scavenging assay were carried out according to the method described by [12]. The DPPH radical scavenging abilities of different extracts of stem M. simplicifolia was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical 1,1-diphenyl-2-picrylhydrazyl DPPH according to the method of [13]. Plants extracts at various concentrations (20-100 μg) was added to 5 ml of 0.1 m Methonol solution of DPPH and allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm. Methanol was chosen as blank and solution without extract is considered as control. The mixture of methanol, DPPH and standard (ascorbic acid) served as positive control. Radical scavenging activity was delivered as the inhibition percentage of free radical by the sample. More significantly the IC50 of the extracts were also calculated.

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persulfate, polyvinyl polypyrrolidone (PVPP), sodium nitrite, trichloroacetic acid, ethylenediaminetetraacetic acid (EDTA), aluminium chloride, ferrous sulphate, gallic acid, rutin, quercetin, safranin and fast green were purchased from sigma-Aldrich. Solvents used are purchased from Himedia and all reagents were used without further purification.
powerful antiradical activity. The results of total phenolics tannin and flavonoid contents are presented in Table 1. Methanol extracts of M. simplicifolia stem revealed the presence of the highest phenolic compounds (39.3±3.62 g GAE/100 g). In Table 1. In this estimation of the methanol extract of M. simplicifolia stem revealed the maximum amount of flavonoid content (21.78±0.14g/100g extract). The plant extracts rich in phenolic compounds are often correlated with an abundant range of therapeutic and physiological benefits other than antioxidant properties [16]. The results were justifying the presence of more biological active phenolics, tannins and flavonoids.

Table 1: Total phenolics, flavonoid and tannin contents of M. simplicifolia

| Extract       | Total phenolic content (gGAE/100 g extract) | Flavonoid content (gGRE/100g extract) | Tannin content (g GAE/100 g extract) |
|---------------|---------------------------------------------|--------------------------------------|--------------------------------------|
| Pet ether     | 7.0±0.17*                                  | 11.2±0.62*                           | 1.9±0.05*                            |
| Chloroform    | 8.9±0.07*                                  | 11.95±0.34*                          | 1.55±0.37*                           |
| Acetone       | 22.49±0.85*                                | 19.47±0.59*                          | 2.77±0.46*                           |
| Methanol      | 39.83±1.62*                                | 21.78±0.14*                          | 4.47±2.49*                           |
| Water         | 18.92±1.03*                                | 17.82±0.43*                          | 2.49±0.04*                           |

*Values are mean of replicate determination (n=3)±standard deviation. GAE-Gallic acid equivalence, RU-Rutin equivalence. Statically significant at (P<0.05 where a>b>c)

Fig. 1: DPPH radical scavenging activity of M. simplicifolia stem, values are mean of three replicate determinations (n= 3)±standard deviation. Bars having different letters are significantly different (P<0.05)

Antioxidant activity

DPPH radical scavenging activity

In stem, petroleum ether and chloroform extracts show the higher inhibitor (1243 μg/ml), whereas acetone (2296 μg/ml) and methanol (1267 μg/ml) show the minimum inhibitory concentration activity.

Inflammation is a protective process that is essential for the preservation of the integrity of the organism in the event of physical, chemical, and infectious damages. Often, the inflammatory response to severe lesions, erroneously damages normal tissue. 

Leukocytes from the intravascular space into the tissues [17]. The injection of carrageenan into the hind paw of rats elicits an acute inflammatory response characterized by the accumulation of fluid (edema). The M. simplicifolia stem extracts tested orally for anti-inflammatory activity at a dose of 250 mg/kg showed a decrease in the paw edema after 6th hour. M. simplicifolia stem methanolic extract were showed decreases significance activities (p<0.05) maximum inhibition in paw edema low dosage (5.16±0.98) and high dosage level significance activities (p>0.05) (3.63±0.04) (table 1). The inhibition in the paw edema of the standard group observed was (5.16±0.98). The Inflammation activity of plant M. simplicifolia stem methanolic extract and standard drug were compared the control group. During acute inflammation, serum proteins and leukocytes migrate to areas of tissue injury. Recruitment of cells to inflammatory sites is dependent on the release of vasoactive and chemotactic factors that increase regional blood flow and microvascular permeability and promote the migration of leukocytes from the intravascular space into the tissues [18].

Table 2: Effect of Meliosma simplicifolia stem on carrageenan-induced paw edema in rats

| Group          | Mean paw volume before carrageenan injection | Paw volume after induction with carrageen in increase in paw volume (mm) after carrageenan injection |
|----------------|---------------------------------------------|---------------------------------------------------------------------------------|
|                | 0 min                                       | 30 min | 1h | 2h | 3h | 4h | 5h | 6h |
| Only Carragean | 3.5±0.98                                   | 6.5±0.57 | 7.3±0.69 | 7.7±0.86 | 8.1±0.68 | 8.3±0.45 | 8.1±0.58 | 7.9±0.96 |
| Carragean+Standard | 3.66±0.08                             | 5.86±1.67 | 6.37±1.75 * | 6.19±1.78 | 6.05±1.28 | 5.91±1.85 * | 5.64±1.12 * | 5.16±0.98 * |
| Carragean+LD    | 3.87±0.98                                 | 6.77±0.17 | 7.27±0.08 | 7.17±0.07 | 7.1±0.99 | 6.83±0.09 | 6.42±0.06 | 5.94±0.05 |
| Carragean+HD    | 3.33±0.68                                 | 6.89±0.08 | 6.98±0.07 | 7.85±0.04 | 6.49±0.09 | 5.47±0.08 | 4.43±0.07 | 3.63±0.04 |

Values are expressed as the mean±SD Statistical significance (p) calculated by one way ANOVA followed by dunnett’s. ns-not significant *P<0.05 calculated by comparing the treated group with control group.
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(GC-MS)-Gas chromatography-mass spectrometry of *M. simplicifolia* methanol stem extract

The GC-MS study of the methanolic extract of the stem *M. simplicifolia* had shown the presence of many phytochemicals which might contribute to the medicinal activity of that plant. The comparison of the GC with the database gave more than 90% match as well as confirmatory compound structure match. These compounds are having diverse medicinal purposes like anti-inflammatory, antibacterial, antifungal, acute toxicity, anticancer, skin conditioning properties have been identified. Based on this result, the present study analysed antioxidant properties of methanolic extract of the stem *M. simplicifolia* plant.

| Peak# | R. time | Area% | Height | Height% | Name |
|-------|---------|-------|--------|---------|------|
| 1     | 19.332  | 53911 | 1.37   | 1.17    | penol,3,5-bis(1,1-dimethylethyl)- | 191.15 |
| 2     | 22.096  | 105859| 2.70   | 3.09    | 3,4-dihydroxymandelic acid-tetraetrams | 73.05 |
| 3     | 25.464  | 309807| 7.90   | 9.32    | silicate anion tetramer | 73.10 |
| 4     | 28.453  | 375617| 9.58   | 11.58   | 3,4-dihydroxyphenyl)tetrasiloxane | 74.05 |
| 5     | 28.576  | 290124| 7.40   | 3.05    | heptadecanolcacld, methylester | 73.10 |
| 6     | 31.184  | 397314| 10.13  | 11.57   | 3,4-dihydroxyphenyl)tetrasiloxane | 74.05 |
| 7     | 32.390  | 157425| 10.13  | 11.57   | heptadecanolcacld, methylester | 73.10 |
| 8     | 33.663  | 267749| 6.83   | 6.02    | hexadecanol, hexadecamethyl | 73.10 |
| 9     | 35.957  | 425650| 10.34  | 9.46    | octas(octane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15- | 73.10 |
| 10    | 38.116  | 398760| 10.17  | 11.15   | heptadecanolcacld, methylester | 73.05 |
| 11    | 40.149  | 405650| 10.34  | 9.46    | cyclononasiloxane, actadecamethyl- | 73.10 |
| 12    | 42.828  | 267749| 6.83   | 6.02    | silicone oil | 73.05 |
| 13    | 43.828  | 267749| 6.83   | 6.02    | 1h-purin-6-amine,[(2-fluorophenyl)methyl]- | 73.10 |

**Fig. 2:** GC-MS of the plant *M. simplicifolia* stem of methanolic extract

**FTIR-Transform infrared spectroscopy spectral data interpretation**

In addition to the GC-MS results, the FT-IR spectrum was recorded to identify the functional groups present in the extract. The results of FT-IR analysis confirmed the presence of C-Br, C-O, C=O, C-H, N=C, C-H and O-H functional groups as presented in table 1. These functional groups confirm the presence of biologically active phytochemicals such as flavonoids, phenols and tannins etc.

**Fig. 3:** FTIR of the plant *M. simplicifolia* stem of methanolic extract
Free radicals are closely associated with oxidative damage and chemicals that have protected humans from numerous sicknesses. Medicinal pastime of plant life analgesic and can be attributed to their excessive steroids, tannin, sulphur as SO$_4$$^{2-}$ viz. sellers. While compared to the commonly used artificial chemotherapeutic are that they're greater powerful with little or no aspects results maximum critical belongings medicinal cost of these plants lies in a few chemical materials that tremendous function inside the identification of crude drugs. The are the basic source for the established order of several medicinal plants and many of them act as antioxidants. Therefore, the present study was undertaken to study the free-radical scavenging ability of $M.$ simplicifolia in vitro. Quantitative studies of estimated phenol, flavonoid and tannin, as for the acetone extract of stem showed the highest content of phenolic compounds (39.83±3.62GAE mg/100). The methanolic extract of stem showed highest amount of tannin content (44.7±2.9GAE mg/100).

The flavonoid content are presented of methanolic extract of $M.$ simplicifolia stem revealed the maximum amount of flavonoid content (21.78±0.14RE/100g). The stem of $M.$ simplicifolia was collected, separated and then the powdered material was extracted with various solvents using such as petroleum ether, chloroform, acetone and methanol by using maceration method. Extract yield percentage of methanol shows most recovery percentage (Stem 1:1,000) compared with other solvents. Antioxidant activities were concluded the estimation $M.$ simplicifolia stem for as followed the studies in stem the methanol extract showed the highest DPPH scavenging activity (124.3µg/ml). FT-IR technique is very useful to reveal different types of organic and inorganic compounds present in plants. In this present study, the analysis was carried out with dried stem sample of $M.$ simplicifolia. From the FT-IR spectra we can see clearly that each band represent characteristic absorption peaks of functional groups present in the sample. The results of FT-IR analysis confirmed the presence of C-Br, C-O, C=O, C-H, N-C, C-H and O-H functional groups as presented. Thus presence of phenolics in any plant sample indicates it as a potent source of therapeutic agents for different human disorders. The antioxidant properties of phenolics in Solanaceae plants has already been proved in earlier studies.

Table 4: FTIR of the plant $M.$ simplicifolia stem of methanolic extract

| Extracts                        | Peak values | Functional groups       |
|---------------------------------|-------------|-------------------------|
| Stem methanol extracts          | 666.72      | C=Br Streching          |
|                                 | 1021.72     | C=O Streching           |
|                                 | 1113.08     | C=O Streching           |
|                                 | 1450.12     | C=H Streching           |
|                                 | 2045.02     | N=C Streching           |
|                                 | 2941.65     | C=H Streching           |
|                                 | 3327.48     | O-H Streching           |

![Fig. 3: Effect of Meliosma simplicifolia (L.) Stem on carrageenin-induced paw edema in rats](image)

DISCUSSION

The concept of ‘integrative’ medicine has come up several times earlier and is not new. This, however, is not a call for Siddha alone, but it is all about the direction of change. World Health Organization (WHO) has announced desirable doctor–population ratio as 1:1,000. In India as per the current population, it gives a doctor (modern medicine) and a population ratio of 0.77:1,000 he concept of ‘integrative’ medicine has come up several times earlier and is not new. This, however, is not a call for Siddha alone, but it is all about the direction of change. World Health Organization (WHO) has announced desirable doctor–population ratio as 1:1,000.

In India as per current population, it gives a doctor (modern medicine) and a population ratio of 0.77:1,000 Phytochemical parts are the basic source for the established order of several pharmaceutical industries; the constituents is gambling a tremendous function inside the identification of crude drugs. The medicinal cost of these plants lies in a few chemical materials that produce an exact physiological action at the human frame. The maximum critical belongings of these bioactive elements of flowers are that they’re greater powerful with little or no aspects results while compared to the commonly used artificial chemotherapeutic sellers. The samples were processed for physicochemical analysis viz. pH, electrical conductivity, moisture contents, organic carbon, available form of nitrogen, potash as K2O, phosphorus as P2O5 and sulphur as SO4$^{2-}$[19]. The anti-inflammatory, antispasmodic, anti-nalgesic and can be attributed to their excessive steroids, tannin, terpenoids, and saponins. Phytochemicals are responsible for the medicinal pastime of plant life [20]. Those are non-nutritive chemicals that have protected human from numerous sicknesses. Free radicals are closely associated with oxidative damage and antioxidant are reducing agents, which limit oxidative damage to biological structures by donating electrons to free radicals and passivating them [21]. These free radicals interact with the antioxidants, which can eventually neutralize them before damages are initiated. Plants synthesize several compounds as secondary metabolites and many of them act as antioxidants. Therefore, the present study was undertaken to study the free-radical scavenging ability of $M.$ simplicifolia in vitro. Quantitative studies of estimated phenol, flavonoid and tannin, as for the acetone extract of stem showed the highest content of phenolic compounds (39.83±3.62GAE mg/100). The methanolic extract of stem showed highest amount of tannin content (44.7±2.9GAE mg/100).

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The biochemical or metabolic fingerprint of the stem extract of *M. simplicifolia* is generated by FT-IR technique, which is very unique and, therefore, useful as a standard in quality control of the plant drug in its crude form. By obtaining IR spectra from plant samples, it might be possible to detect the minor changes of primary and secondary metabolites. The identified compounds occupy many biological properties. GC-MS analysis of phytoconstituents in plants gives a clear picture of the pharmacological value of that plant. Thus, this type of GC-MS analysis is the first step towards understanding the nature of medicinal properties in this medicinal plant and this type of study will be helpful for further detailed study. Further investigation is needed to identify the pharmacological importance and phytochemistry of *M. simplicifolia*. Potential antioxidant and Carrageenan induced acute paw edema in rats were found in these compounds. Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of infectious agents [24]. Carrageenan-induced rat paw edema is used widely as a working model of inflammation in the search for new anti-inflammatory drug Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the antiedematous effect of the drug. Carrageenan is a strong chemical use for the release of inflammatory and pro-inflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF-α, etc) [25].

The course of acute inflammation is biphasic. First phase starts with the release of histamine, serotonin, and kinins after the injection of phlogistic agent in the first. Knowledge on the immune-mediated mechanisms in metabolic scenario has markedly increased in the recent past, evidencing the role that dietary components may have to modulate immunity by enhancing or suppressing the immune response. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema, leukocyte infiltration, and granuloma formation represent such components of inflammation. Though, it is a defense mechanism. The complex events and mediators involved in the inflammatory reaction can induce or aggravate many reactions [26].

CONCLUSION
Phenolic compounds, tannins and flavonoids have been reported to have multiple biological effects, including antioxidant and anti-inflammatory properties. The presence of phytochemicals such as phenols, flavonoids and tannins in the plant can act as valuable natural antioxidant due to. Various assays were used to screen these particular compounds and they were further confirmed by GC-MS and FT-IR. The extract was tested for their antioxidant activity and anti-inflammatory assays. The results show that the plant has potential antioxidant activity against number of free radicals which are really harmful.

ACKNOWLEDGMENT
The authors are grateful to Professor and Head, faculty, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu for providing necessary facilities during the study.

FUNDING
Nil

AUTHORS CONTRIBUTIONS
Pavithra designed the experiments performed in laboratory analysis, experiments, data analysis and participated in the writing of the manuscript. Dr. T. Sekar helped in paper writing and correction. Authors discussed the results and commented on the manuscript.

CONFLICT OF INTERESTS
Declared none

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