**International Journal of Medicine Research**
**ISSN: 2455-7404**
**www.medicinesjournal.com**
**Volume 1; Issue 1; March 2016; Page No. 01-04**

# *In vitro* antioxidant, antibacterial activities and physicochemical evaluation of ethanolic extracts of three medicinal plant leaves

**P Selvakumar**

Department of Chemistry, Periyar University, Salem, Tamilnadu, India.

**Abstract**
**Aim:** The study was conducted to assess the antibacterial, antioxidant activity and physicochemical evaluation of ethanolic leaf extract of *Vitex negundo, Gaultheria fragrantissima* and *Camellia sinensis*.
**Methods:** Antioxidant property was tested by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity and ABTS•+ radical cation decolorization. Antibacterial property was tested using disc diffusion method. Physicochemical studies like total ash, acid insoluble ash, water insoluble ash, alcohol soluble extractive, hexane soluble extractive, water soluble extractive values and loss on drying at 105 °C were carried out as per the WHO guidelines for individual leaf extract.
**Results:** Results of the present study showed that ethanolic extract of *V. negundo* leaf exhibited significantly ($p$<0.05) higher antioxidant activity in terms of measurements of DPPH free radical ($IC_{50}$). Results showed that *vitex negundo, gaultheria fragrantissima* and *camellia sinensis* exhibit potent antioxidant activity with $IC_{50}$ values of 20.31±1.131 µg/mL, 25.91±1.243 µg/mL and 31.05±1.534 µg/mL respectively when the results were compared with standard ascorbic acid with an $IC_{50}$ value of 12.76±1.001 µg/mL. Total ash value of plant material indicated the amount of minerals and earthy materials present in the plant material. Analytical results showed the total ash higher value was 6.25% w/w in ethanolic extract of *vitex negundo*. The higher amount of acid-insoluble siliceous matter present in the *gaultheria fragrantissima* was 1.91% w/w. The water-soluble extractive value indicated the presence of sugar, acids, and inorganic compounds.
**Conclusions:** This study shows that ethanolic extract of leaves of *Vitex negundo, Gaultheria fragrantissima and Camellia sinensis* has bioactive compounds but further active compounds isolation is necessary to confirm the activities of individual compounds.

**Keywords:** *Gaultheria fragrantissima*, *Vitex negundo, Camellia sinensis*, Antioxidant activity, Antibacterial activity, Physicochemical Analysis

## 1. Introduction

*Camellia sinensis* belongs to the family theaceae, and also known as tea. Tea is native to mainland China, Southern Asia, but it is today cultivated across the world in tropical and sub-tropical regions. Leaves are 4–15 cm long and 2-5 cm broad [1]. Fresh leaves have 4% caffeine. Light green and young leaves are preferred for tea production. Leaves of *Camellia sinensis* contain flavonoids, their analysis and their functions [2] they inhibits the enzyme ribonuclease A [3], medicinal activity [4, 5], tea polyphenols used in the herbal infusions, protection against reactive oxygen species induced degradation of lipids, proteins and 2-deoxyribose by tea catechins [6], green tea polyphenols having anti-atherogenic properties [7]. *Vitex negundo* (Family: Verbenaceae) is a commonly used as a medicinal plant. It has a broad range of uses in folk medicine. The plant parts possess diverse pharmacological activities [8, 9]. Considering its importance in folk medicine, an *in vitro* method of callus induction for this plant was developed. The objective was to investigate the effects of phytohormones for callus induction in the species.

Drugs like tetracycline, erythromycin, minocycline and metronidazole are gaining more importance and are preferred over antibiotics because of their antioxidant effect. Hence antioxidants are extensively studied for their capacity to protect organism and cell from damage that are induced by oxidative stress. There are a number of synthetic antioxidants like butylated hydroxy anisole, butylated hydroxy toluene, propyl gallate and gallic acid esters which are available but are suspected to cause negative health effects and are also unstable at elevated temperatures. Hence, the objective of our research work was to investigate antibacterial and antioxidant potential of the following three medicinal plants viz, *vitex negundo* [10-13], *gaultheria fragrantissima* and *camellia sinensis* [14, 15]. *Camellia sinensis* [16] as an infusion of flavourful leaves has been consumed for centuries as a beverage and is valued for its medicinal properties.

## 2. Materials and methods

### 2.1. Collection of plant material

The leaves of the plants *vitex negundo, gaultheria fragrantissima* were collected from Coimbatore district and *camellia sinensis* leaves were collected from nilgiris district in the month of January, 2012. During the surveys, personal interviews were conducted with the village people to know their medicinal uses. The leaves were cleansed and shade dried for a week and ground into uniform powder.

### 2.2. Preparation of extracts, chemicals and reagents

The air dried leaves 100 g of plant material was added to 1000 ml of ethanol for 18 h at room temperature. The extracts were filtered extracts were evaporated under vacuum conditions using a rotary evaporator and stored at 4°C in air tight containers for further studies. The percentage yield was recorded. The extracts were used for the study of antibacterial,

antioxidant activity and Physico-chemical analysis. All chemicals used were of analytical grade and obtained from either Sigma-Aldrich or Merck.

### 2.3. Antibacterial screening of extracts

#### 2.3.1. Microorganisms and media

Aerobic bacteria: *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 2639) and anaerobic bacteria: *Propionibacterium acnes* (MTCC 1951) were obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology Chandigarh. Fresh cultures of the isolates of aerobic and anaerobic bacteria were suspended in nutrient broth and reinforced clostridium medium respectively. *S. aureus* and *S. epidermidis* cultures were incubated for 24 h at 37°C and 30°C, respectively. *P. acnes* culture was incubated in an anaerobic chamber at 37°C consisting of 10% $CO_2$, 10% $H_2$ and 80% $N_2$ for 48 h.

#### 2.3.2. Disc Diffusion method

Antibacterial activity of extracts was tested using agar disc diffusion method [17]. 100 µl of fresh culture suspension of test bacteria was evenly spread on nutrient agar and reinforced clostridial agar plates. The concentration of cultures was 5x10 [5] CFU/ml. For screening, 6 mm diameter filter paper disc, impregnated with 20 µl of extract solution equivalent to 0.2 mg of extract, was placed on the surface of inoculated media agar plates. Incubation was done at 37°C or 30°C for 24 h and 48 h depending upon the type of bacteria under optimum conditions. Clear zones of inhibition were measured in mm and Clindamycin [18, 19] (10 µg/disc) was used as positive control. The results are summarized in Table 1.

#### 2.3.3. Antioxidant capacity DPPH Assay

The free radical scavenging activity [20] was estimated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay using Blois method with some modifications. The reaction mixture contained 100 µl of test extracts (100-500 µg/mL) and 1 mL of m solution of 0.1 mM DPPH radical. The mixture was then vigorously shaken and incubated at 37ºC for 30 min. The absorbance was measured at 517 nm using ascorbic acid (100-500 µg/mL) as positive control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity which was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = 100 \times (A_o - A_1) / (A_o)$$

Where $A_o$ is the absorbance of the control reaction and $A_1$ is the absorbance of reaction mixture containing DPPH and extract at 517 nm. The antioxidant activity of the extract was expressed as $IC_{50}$ value which is defined as the concentration (µg/mL) of extract that inhibits the formation of DPPH radicals by 50%. This was obtained from linear regression analysis. The results are summarized in Table 2.

#### 2.3.4. ABTS•+ radical cation decolorization assay

ABTS•+ radical scavenging activity [21, 22] of the test samples was measured using Re *et al*. method with minor modifications. It measures the reduction of the ABTS radical cation by an antioxidant. ABTS radical cation (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate in 100 mM phosphate buffer solution (pH 7.4) and keeping the mixture in a dark place at room temperature for 12- 24 h before use. This solution was further diluted with 100 mM phosphate buffer solution (pH 7.4) to give an absorbance of 0.700±0.02 at 734 nm. For the study of radical scavenging activity, 900 µl of ABTS was added to 100 µl of various concentrations (100-500 µg/mL) of the extracts and ascorbic acid. The reaction mixture was then incubated for 20 min and the absorbance was measured at 734 nm using methanol as a blank. ABTS•+ radical scavenging activity was calculated using the same formula as mentioned in DPPH assay. The results are summarized in Table 3.

#### 2.3.5. Physicochemical studies

Physicochemical studies like water soluble extractive value, total ash, acid insoluble ash, water insoluble ash, alcohol soluble extractive, hexane soluble extractive and loss on drying at 105 °C were carried out as per the WHO guidelines for individual leaf extract. Physico-chemical properties of ethanolic extracts of *Vitex negundo, Gaultheria fragrantissima, Camellia sinensis* (Leaf) were determined by following WHO standards and the values are recorded in the Table 4.

## 3. Results

### 3.1. Antibacterial Screening

#### 3.1.1. Disc Diffusion method

*In vitro* antibacterial screening using Clindamycin phosphate as a positive control clearly indicated that ethanolic extract of vitex negundo; gaultheria fragrantissima and camellia sinensis show promising antimicrobial activity against all the three organisms. It was observed that ethanolic extracts of *vitex negundo* and *camellia sinensis* [23] show significant antimicrobial activity against test organisms. *Gaultheria fragrantissima* did not exhibit antimicrobial activity against *Staphylococcus epidermidis* but showed activity against *Staphylococcus aureus and Propionibacterium acnes.* Highest zone of inhibition, 18.1±0.13 mm, was observed in ethanolic extract of *camellia sinensis* against *Staphylococcus epidermidis* [24].

**Table 1:** Antimicrobial screening of plants against *S. aureus* (MTCC 96), *S. epidermidis* MTCC 2639) and *P. acnes* (MTCC1951) using disc diffusion method

| Entry | Zone of inhibition of extracts in mm | | |
|---|---|---|---|
| | Anaerobic bacteria | | |
| **Name of plants ethanolic leaf extracts** | ***Staphylococcus aureus*** | ***Staphylococcus epidermidis*** | ***Propionibacterium acnes*** |
| Vitex negundo | 7±0.15 | 12.1±0.11 | 8.2±0.12 |
| Gaultheria fragrantissima | 8.13±0.15 | NA | 14.2±0.10 |
| Camellia sinensis | 13.5±0.15 | 18.1±0.13 | 7.1±0.13 |
| Clindamycin phosphate | 15.22±0.10 | 19.12±0.21 | 18.21±0.12 |

### 3.1.2. Effect on DPPH radical

The *in vitro* antioxidant activity of test extracts was estimated using DPPH assay. All extracts exhibited potent antioxidant activity when DPPH radical was used as a substrate to evaluate the free radical scavenging activity. The antioxidants react with DPPH forming a purple colour stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The amount of DPPH reduced was estimated by measuring the decrease in absorbance at 517 nm. Lower $IC_{50}$ value indicated a greater antioxidant activity. Our experimental data indicated that though all the test extracts demonstrated H-donor activity, the highest DPPH radical scavenging activity was observed in *Camellia sinensis* ($IC_{50}$=43.04±1.652 µg/mL) followed by *gaultheria fragrantissima* ($IC_{50}$=42.07±2.050 µg/mL) and *vitex negundo* ($IC_{50}$=101.01±1.109 µg/mL).

**Table 2:** DPPH Scavenging effect of extracts of active medicinal plants and ascorbic acid

| Name of the plants | $IC_{50}$ Concentration (µg/mL) |
|---|---|
| Vitex negundo | 97.01±1.109 |
| Gaultheria fragrantissima | 42.07±2.050 |
| Camellia sinensis | 43.04±1.652 |
| Standard ascorbic acid | 114.04±1.025 |

### 3.1.3. ABTS radical scavenging activity

ABTS assay is an excellent tool to determine the antioxidant activity of hydrogen donating and chain breaking antioxidants. ABTS•+ is a blue chromophore produced by the reaction between ABTS and potassium persulfate. Addition of the plant extracts to this pre-formed radical cation reduced it to ABTS in a concentration-dependent manner. Reduction of free radicals by the test extracts using ABTS was measured at 734 nm. Results showed that Vitex negundo, Gaultheria fragrantissima and Camellia sinensis exhibit potent antioxidant activity with $IC_{50}$ values of 20.31±1.131 µg/mL, 25.91±1.243 µg/mL and 31.05±1.534 µg/mL respectively when the results were compared with standard ascorbic acid with an $IC_{50}$ value of 12.76±1.001 µg/mL.

**Table 3:** ABTS Scavenging effect of ethanolic extracts of active medicinal plants and ascorbic acid

| Name of the plants | Concentration (µg/mL) |
|---|---|
| Vitex negundo | 20.31±1.131 |
| Gaultheria fragrantissima | 25.91±1.243 |
| Camellia sinensis | 31.05±1.534 |
| Standard ascorbic acid | 12.76±1.001 |

### 3.2. Physico-chemical parameters

Results of quantitative analysis for total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive, hexane soluble extractive, loss on drying at 105ºC values are tabulated in Table 4. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. Percent weight loss on drying or moisture content was found for all three extracts. The less value of moisture content could prevent bacterial, fungal or yeast growth.

Total Ash value of plant material indicated the amount of minerals, and earthy materials present in the plant material. Analytical results showed the total ash higher value was 6.25% w/w in ethanolic extract of *Vitex negundo*. The higher amount of acid-insoluble siliceous matter present in the *Gaultheria fragrantissima* was 1.91% w/w. The water-soluble extractive value indicated the presence of sugar, acids, and inorganic compounds. All the three ethanolic extract have higher value of water soluble extractive values. The alcohol soluble extractive values indicated the presence of polar constituents.

**Table 4:** Physico-Chemical parameters of ethanolic extracts of *Vitex negundo, Gaultheria fragrantissima and Camellia sinensis*

| Test Parameters | Vitex Negundo | Gaultheria fragrantissima | Camellia sinensis |
|---|---|---|---|
| Odour | Pleasant | Pleasant | Pleasant |
| Foreign Matter | 0.95 | 0.72 | 0.82 |
| Ash Value (w/w %) | 6.25 | 3.31 | 4.59 |
| Acid insoluble ash (w/w %) | 0.71 | 1.91 | 0.19 |
| Water insoluble ash (w/w %) | 0.21 | 0.11 | 0.15 |
| Alcohol soluble extractive (w/w %) | 10.11 | 8.22 | 12.97 |
| Water soluble extractive (w/w %) | 35.71 | 24.81 | 35.82 |
| Loss on drying (w/w %) | 6.11 | 8.55 | 3.79 |
| Hexane soluble extractive (w/w %) | 13.55 | 20.11 | 18.17 |

## 4. Discussion

The results clearly indicate that ethanolic extracts of *gaultheria fragrantissima, vitex negundo and camellia sinensis* possess broad spectrum of antibacterial activity. The above three ethanolic leaf extract of *vitex negundo, gaultheria fragrantissima and camellia sinensis* showed very good antioxidant properties. Presence of higher concentration of phenolic compounds in these test extracts makes them a strong free radical scavenger, which further indicates that these plants can be a good source of natural antioxidants to prevent free radical mediated oxidative stress in acne. Therefore, further investigation is needed to explore the parameters essential for formulation so that antibacterial and antioxidant potential of these medicinal plants can be utilized to provide safe and effective topical herbal formulation for the treatment of acne. Physico-chemical analysis, water soluble extractive value indicated the presence of sugar, acids, and inorganic compounds. All the three ethanolic extracts show higher water soluble extractive values. This study shows that ethanolic extract of leaves of *Vitex negundo, Gaultheria fragrantissima and Camellia sinensis* has bioactive compounds but further active compounds isolation is necessary to confirm the activities of individual compounds.

**5. References**


1. Kiritikar KR, Basu BD. Indian Medicinal Plants, Dehradun, International Book Distributors (2nd ed) 1987; 2:1225, 2128 & 2327.
2. Hamilton JMT. Antimicrobial properties of tea (Camellia sinensis L.). Antimicrobial Agents and Chemotherapy 1995; 39:2375-2377.
3. Kalyan SG, Kanti TM, Dasgupta S. Green tea polyphenols as inhibitors of ribonuclease A. Biochemical and Biophysical Research Communications 2004; 325:807-811.
4. Chan EWC, Lim Y, Chew YL. Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. Food Chemistry 2007; 102:1214-1222.
5. Turkmen N, Sari F, Velioglu S. Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. Molecules 2007; 12:484-496.
6. Raza H, John A. In vitro protection of reactive oxygen species-induced degradation of lipids, proteins and 2-deoxyribose by tea catechins. Food and Chemical Toxicology 2007; 10:1814-1820.
7. Rosenblat M, Volkova N, Coleman R, Almago Y, Aviram M. Antiatherogenicity of extra virgin olive oil and its enrichment with green tea polyphenols in the atherosclerotic apolipoprotein-E-deficient mice enhanced macrophage cholesterol efflux. The Journal of Nutritional Biochemistry. 2008; 19:514-523.
8. Anonymous. The Wealth of India, Raw Materials. New Delhi: CSIR (Council of Scientific & Industrial Research) 2003; 10:158-160.
9. Ayurvedic Pharmacopoeia of India. New Delhi, Government of India Publication 2001; 3(1):142-144.
10. Das B, Das R. Medicinal properties and chemical constituents of Vitex negundo Linn. Indian Drugs 1994; 31:431-435.
11. Alam MI, Gomes A. Snake venom neutralization by Indian medicinal plants (Vitex negundo and Emblica officinalis) root extracts. J. Ethnopharmacol. 2003; 86:75-80.
12. Barry AL. Procedure for testing antimicrobial Agents in Agar media. In: Antibiotics in Laboratory Medicine. Lorin V (eds), Williams Wilkins Co. Baltimore: USA, 1980, pp.1-23.
13. Rusia K, Srivastava SK. Antimicrobial activity of some Indian medicinal plants. Ind. J. Pharm. Sci. 1998; 60:57-58.
14. Mbata TI, Debiao L, Saikia A. Antibacterial Activity of The Crude Extract of Chinese Green Tea (Camellia sinensis) On Listeria monocytogenes. The Internet Journal of Microbiology. 2006, 2(2).
15. Toda M, Okubo S, Hiyoshi R, Shimamura T. Antibacterial and bactericidal activities of Japanese green tea. Jpn. J. Bacteriol. 1989; 44(4):669-672.
16. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10:178-182.
17. Harborne JB. Phytochemcial methods. (London). Chapman and Hall, 1973.
18. Valsaraj R, Pushpangadan P, Smitt UW, Adersen A, Nyman U. Antimicrobial screening of selected medicinal plants from India. J. Ethnopharmacol. 1997; 58:75-83.
19. Vander-Berghe Da, Vlietinck N. Screening methods for antibacterial and antiviral agents from higher plants. In: Dey PM, Harborne JB (eds). Methods in plant biochemistry, London: Academic Press, 1991.
20. Miliauskas, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004; 85:231-237.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999; 26:1231-1237.
22. Makwana HG. Pharmacological and Biochemical studies on Vitex species with special references to their role in inflammatory disorders. Ph. D. Thesis submitted to Gujarat University, Ahmedabad, 1993.
23. SC Dutta. Medicinal plants, National Council for Education Research and Training, New Delhi, 1973.
24. Ahmad I, Mehamood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 1998; 62:183-193.