INTRODUCTION

Nature has provided a complete storehouse of remedies to cure all ailments of humankind [1]. Traditional medicine use is a common practice in developed and developing countries at the primary healthcare level [2]. Herbal medicines are prepared from various plant parts like leaves, stem, roots, barks and seeds, which usually contain many bioactive compounds and used primarily for treating mild or chronic ailments. Due to the increasing demand in the field of herbal medicines, it has become necessary and pertinent to probe into the area of systematic knowledge about herbal drugs. There is a need for the application of this knowledge in authentication, detailed study and practical utilization of crude drugs [3].

Bauhinia acuminata L., belongs to the family Fabaceae is a species of flowering shrub native to tropical southeastern Asia. In Malaysia and Indonesia the plant is used in the treatment of common cold and cough [4]. While in India the leaves and bark of this plant are used for treating asthma [5]. Moreover, the leaf of B. acuminata is used to treat bladder stone, venereal diseases, leprosy, asthma and digestive diseases. Different part of this plant such as bark, leaves, stem, flowers and roots have been used in traditional medicine [6]. The plant was also used as fèribuf, vemífuf, anticonvulsant and against chicken pox, guinea worm and black quarter [7-8]. Though different parts of this plant were reported to possess good medicinal properties [9], there is no published study particularly on the phytochemical and antioxidant activity of B. acuminata flower. Hence, the focus of this study was to investigate qualitative and quantitative phytochemical analysis and antioxidant activities in flower extracts of B. acuminata.

MATERIALS AND METHODS

Collection and identification of plant materials

The plant B. acuminata were collected from Edapal, Malappuram District of Kerala, India. Taxonomic identification with Flora of Presidency of Madras by JS Gamble [10].

Preparation of extracts

Flower of the plant was shade dried for several days. The dried plant material was ground to a coarse powder and 50 gm of the powdered material was soaked in solvents of increasing polarity starting petroleum ether, chloroform, ethyl acetate, ethanol and distilled water (1:5) for 72 h [11]. The solvent was then removed by rotary evaporation. Each residue was weighed and the yield percentage was determined. The dried extract was stored in the refrigerator for further studies.

Qualitative phytochemical analysis

The phytochemical screening of the plant extract in various solvents was performed by using standard protocol given by Harbone [13].

Quantitative phytochemical analysis

Determination of total phenolic content

Total phenolic content analysis was performed by the method of Malick et al. [14]. Dissolve the test extract in the concentration of 1 mg/1 ml and make up this test solution up to 3 ml with distilled water. 0.5 ml Folin-Ciocalteau reagent and 2 ml of 20% Na2CO3 were respectively added. The samples were thereafter incubated in boiling water bath exactly for one minute. The absorbance was measured by using spectrophotometer at 765 nm against reagent as blank.

A standard calibration plot was generated at 650 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as catechol equivalent of mg/ g of sample. Each extract was assayed in triplicates.

Determination of total alkaloid content [15].

The plant extract (1 mg/1 ml) was dissolved in 2N HCl and then filtered. 1 ml of this solution was transferred to a separating test
The optical density recorded and percentage of inhibition calculated using Cecil taken in a test tube and mixed well. These solution mixtures were diluted test solutions picrylhydrazyl (DPPH), by a method given by Braca taken as control and methanol as blank.

Antioxidant property screening

DPPH radical scavenging assay

The free radical scavenging activity of the plant extracts assessed based on the radical scavenging effect of the stable 1,1-diphenyl-2-picylhydrazyl (DPPH), by a method given by Braca et al. [16]. The diluted test solutions (10 μg/ml-1000 μg/ml concentration) and 6.34 μM solution of DPPH were prepared in methanol and 100 μl test, along with 100 μl DPPH solution and 800 μl of methanol was taken in a test tube and mixed well. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (900 μl) with DPPH solution (6.34μM, 100μl) taken as control and methanol as blank. The optical density recorded and percentage of inhibition calculated using the formula given below [17]. Percentage (%) inhibition of DPPH activity = A-B/A x 100

Yield of extract

Comparatively, flower ethanol extract exhibited higher extraction yield. The extraction ability of different solvents for recovering extractable components from flower followed the order: ethanol (2.00g)>chloroform (1.26g)>petroleum ether (1.10g)>ethyl acetate (1.17g)>distilled water (1.02g). The variation in yield may be due to the polarity of the solvents used in the extraction process.

Preliminary phytochemical screening

The phytochemical analysis is of paramount importance in identifying a new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated [18]. The preliminary Phytochemical screening showed the presence of primary and secondary metabolites like sugar, carbohydrate, amino acid, fat, quinone, steroids, phenol, saponin, alkaloids, and acid content (table 1).

Quantitative estimation of phenol and alkaloid compounds

Secondary metabolites analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. Calibration curve of catechol and atropine is given in fig. 1 and fig. 2. Phenol and alkaloid are present in all the samples extracted by five different solvents. The total phenol content was highest with (1.26±0.01) catechol/g of extract followed by (0.72±0.03) catechol/g of ethyl acetate extract. Phenols are antioxidant and reduce inflammation when taken internally. These bioactive agents have an irritant effect when applied to the skin. Above of all, phenols have a high affinity to chelate metals and scavenge the free radicals in cells [19]. Polyphenols act as antioxidants, which protect cells and body chemicals against damage, caused by free radicals and reactive atoms that contribute to tissue damage in the body. It has been reported that these compounds deactivate the substances that promote the growth of tumours [20]. Consumption of diets rich in plant polyphenols offers protection against the development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.

Alkaloids protect against chronic diseases [21] and earlier recorded that bitter leaf contains an alkaloid that is capable of reducing headaches associated with hypertension. Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase [22]. Alkaloid constituents in flower showed that the chloroform extract had higher amounts (0.72±0.03) atropine/g of the extract followed by (0.66±0.02) atropine/g of the ethyl acetate extract. The results are tabulated in table 2.

Antioxidant property of flower

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [23]. Therefore, the importance of the search for natural antioxidants has increased in the recent years so many researchers focused the same [24].

Table 1: Phytochemical screening of flower of B. acuminata in different solvents

| Primary and secondary metabolites | Test/Reagent       | Petroleum ether | chloroform | Ethyl acetate | Ethanol | Distilled water |
|----------------------------------|-------------------|-----------------|------------|---------------|---------|-----------------|
| Carbohydrate                     | Molisch's reagent | +               | +          | +             | +       | +               |
| Starch                           | iodine test       | -               | -          | -             | -       | -               |
| Sugar                            | Benedict's test   | -               | +          | +             | +       | +               |
| Ketose                           | Sellwans's test   | -               | -          | -             | -       | -               |
| Proteins                         | Biuret test       | -               | -          | -             | -       | -               |
| Amino acid                       | Ninhydrin test    | +               | -          | -             | -       | -               |
| Fat                              | Filter paper test | +               | +          | +             | +       | +               |
| Quinone                          | H2SO4 test        | -               | -          | -             | -       | +               |
| Cardiac glycoside                | Kelkar-killani    | -               | -          | -             | -       | -               |
| Steroids                         | Salkowski test    | -               | +          | -             | -       | -               |
| Flavonoids                       | Fluorescent test  | -               | -          | -             | -       | -               |
| Phenols                          | Folin test        | +               | +          | +             | +       | +               |
| Saponins                         | FoCl3 test        | -               | -          | -             | -       | -               |
| Alkaloids                        | Dragendorff's reagent | +       | +          | +             | +       | +               |
| Tannin                           | FeCl3 test        | -               | -          | -             | -       | -               |
| Terpenoids                       | Salkowski test    | -               | -          | -             | -       | -               |
| Acid                             | NaHCO3 test       | +               | -          | +             | +       | +               |

+ indicates the presence of metabolite, -indicate the absence of metabolite.
Table 2: Quantitative estimation of total phenol and alkaloid content of different extracts

| Extracts       | Phenol (Catechol/gm) | Alkaloid (Atropine/gm) |
|----------------|----------------------|------------------------|
| Petroleum ether| 0.13±0.03            | 0.35±0.01              |
| Chloroform     | 1.05±0.01            | 0.72±0.03              |
| Ethyl acetate  | 1.17±0.02            | 0.66±0.02              |
| Ethanol        | 1.26±0.01            | 0.41±0.03              |
| Distilled water| 0.92±0.01            | 0.37±0.02              |

Values are means of three independent determinations±standard deviations (SD).

DPPH radical scavenging assay

Among the five different extracts, ethanol and chloroform extracts showed higher phenol and alkaloid contents respectively. Therefore the free radical scavenging potential of these two extracts tested by DPPH assay is given in the table 3. Reduction of the DPPH radicals was observed by a decrease in absorbance where a change in the color to yellow denotes quenching of the free radicals by the plant extracts. The analysis of the radical scavenging activity of the extracts increases with increase in concentration. Ethanol extract of the flower was found to have the most potent antioxidant property with IC_{50} value of 24.44±1.201µg/ml than chloroform extract with 196.68±0.456 µg/ml. The high activity of ethanol extract of the flower is generally attributed to the presence of alkaloids and phenols, as the majority of active antioxidant compounds are observed in these classes of phytochemical compounds. The secondary metabolites phenol and alkaloids which carry a major importance to increase an antioxidant potential. Information obtained from these studies can be used as markers in the identification and standardization of this plant as an herbal remedy and also towards monograph development of the plant.

Table 3: DPPH scavenging activities of extracts of flower (values represent mean±SD, n=3)

| S. No. | Concentration (µg/ml) | Percentage of inhibition |
|--------|-----------------------|--------------------------|
|        | Ethanol               | Chloroform               |
| 1      | 10                    | 45.20±0.644              | 15.52±0.998               |
| 2      | 15                    | 48.32±0.597              | 17.24±0.941              |
| 3      | 25                    | 55.42±1.325              | 25.59±0.716              |
| 4      | 50                    | 63.57±0.580              | 29.70±1.290              |
| 5      | 75                    | 76.68±1.154              | 31.56±1.087              |
| 6      | 100                   | 87.13±1.038              | 38.37±1.402              |
| 7      | 250                   | 95.28±0.689              | 59.17±0.691              |
| 8      | 500                   | 96.78±0.935              | 74.21±0.907              |
| 9      | 750                   | 97.79±0.358              | 81.35±1.206              |
| 10     | 1000                  | 98.97±0.740              | 85.67±0.544              |
| IC 50  | 24.44±1.201           | 196.68±0.456             |
CONCLUSION
Phytochemical studies portray the presence of several biologically active secondary metabolites such as phenol and alkaloids in the bark of *B. acuminata* for the first time. The antioxidant efficacy of ethyl acetate extract is very high with IC$_{50}$ value of 42.62±0.859 µg/ml, indicates that this plant can have great scope for isolation and identification of important antioxidant molecules which can be formulated to make antioxidant dosage forms. Significant correlations were found between the antioxidant capacities and phenolic contents indicating that phenolic compounds are the major contributors to antioxidant capacity. On top of that, these natural antioxidants can have potential advantages among various diseases with oxidative stress. So, further study is necessary to get maximum benefit from this plant.

CONFLICT OF INTERESTS
Declared none

REFERENCES
1. Kokate CB, Purohit AP, Gokhale SB. Pharmacognosy. 18th edition. Pune India: Nirali Publication; 2002.
2. Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. J Ethnopharmacol 2000;70:343-9.
3. Kirikar KR, Basu BD. Indian medicinal plants. Vol. 1. Dehar Dun, India: International books Distributers; 1980.
4. Timothy Johnson. CRC ethnobotany desk reference, CRC Press: LLC Boca Taton; 1999. p. 110.
5. Khare CP. Indian medicinal plants: an illustrated dictionary. Springer-Verlag Berlin; 2007. p. 85.
6. Reyad–Ul–Ferdous M, Azam MG, Hussain MD. Phytochemical screening in vitro membrane stabilizing activities and thrombolytic activities of Lophopetalum javanicum. Int J Pharm Sci Res 2014;5:753–76.
7. Singh S, Singh SK, Yadav AA. Review on cassia species: pharmacological, traditional and medicinal aspects in various countries. Am J Phytomed Clin Ther 2013;1:291–312.
8. Mohammad FK, Rabeya IS, Ridwan BR, Mohammad AR. Evaluation of cytotoxicity of *beta vulgaris* and membrane stabilizing activities. Biomed Pharmacol J 2014;17:99-101.
9. Gamble JS. Flora of presidency of Madras. Alldard and son publishing company Ltd; 2004.
10. Taleb-Contini SH, Salvador MJ, Balanco JMF, Albuquerque S, De Oliveira DCR. Antiproteozaal effect of crude extracts and flavonoids isolated from *Chromolaena hirsute* (Asteraceae). Phyto Res 2004;18:250–4.
11. Patil UH, Gaiwad DK. Phytochemical evaluation and bactericidal potential of *Terminaliaarjuna* stem bark. Int J Pharm SciRes 2010;2:614–9.
12. Harborne JB. Phytochemical methods, a guide to modern techniques of plant analysis. India: Springer prio Ed; 1998.
13. Malick EP, Singh MB. Plant enzymology and Histoenzymology, Kalyani Publishers, New Delhi; 1980.
14. Fadhil S, Monsef H. Spectrophotometric determination of total alkaloids in *Peganum harmala* L using Bromocresol green. Res J Phytochem 2007;1:79-82.
15. Braca A, Sorinto C, Polit C. Anti-oxidant activity of flavonoids from *Licaniaconiflora*, J Ethnopharmacol 2002;79:379–81.
16. Bors W, Saran M, Elstner EF. Screening for plant anti-oxidants. In: Linskens HF, Jackson JF. eds. Modern Methods of Plant Analysis-Plant Toxin Analysis-New Series, Berlin: Springer; 1992; 13:77-95.
17. Ambasta SP, Ramachandran K, Kashyapa K, Chaud R. Useful plants of India. Publication and information directorate, Council of Scientific and Industrial Research, New Delhi; 1986, p. 4337.
18. Michalak A. Phenolic compounds and their antioxidant activity in plant growing under heavy metal stress. Polish J Environ Studies 2006;15:523-30.
19. Rahaman Onike. Phytochemical Screening Tests and Medicinal Values of Plants Active Properties; 2010.
20. Ajitey Smith E, Addae Mensah I. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. W Afr J Pharmacol Drug Res 1977;4:7-8.
21. Bonjean K, De Pauw-Gillet MC, Defresne MP, Colson P, Houssier C, Dassonneville L, et al. The DNA intercalating alkaloid cryptolepine interferes with topoisomerase II and inhibits primarily DNA synthesis in B16 melanoma cells. J Ethnopharmacol 1998;69:241-6.
22. Andlauer W, Furst P. Antioxidative power of phytochemicals with special reference to cereals. Cereal Foods World 1998;43:356-9.
23. Jayaprakasha GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grape (*Vitisvinifera*) seed extract. Food Res Int 2003;36:17-22.