QUALITATIVE ANALYSIS OF INORGANIC ACID, BASIC RADICALS, AND ESTIMATION OF BIOMOLECULES IN THE FLOWER EXTRACT OF BUTEA SUPERBA ROXB.

SEETHALAKSHMI B1*, KAVITHA K2

1 Assistant Professor, Department of Humanities and Science, Mugavari Eye Hospital and Research Institute, Medavakkam, Chennai - 600 100, Tamil Nadu, India. 2Research Scholar, Department of Zoology, Guru Nanak College, Velacheri, Chennai - 600 042, Tamil Nadu, India. Email: lakshmiseetha9895@gmail.com

Received: 03 May 2018, Revised and Accepted: 12 June 2018

INTRODUCTION

Nature has been the biggest storehouse of medicinal agents for 1000 years, and a majestic number of synthetic drugs have been isolated from natural resources, many of which is based on their use in traditional medicine [1]. Medicinal plants include different types of plants used in herbalism, and some of these plants have medicinal values and considered as a rich source of organic compounds and used for traditional medicinal system of drugs, food supplements, nutraceuticals, and medicines of modern era, folk medicines, pharmaceuticals, intermediates, and chemicals used for synthetic drug [2]. Besides that herbal plants play an important role in the progress of human cultures in worldwide [3].

Ancient people depend on herbal plant extracts for the remedial source of many diseases [4]. Medicinal plants play a vital role in the primary health care of traditional medicinal systems. The major merits of herbal medicines are their potential, easy availability, less expensive, and ecofriendly and have lesser side effects [5]. In India, almost 95% of the prescriptions have been reported to be plant based in the traditional systems of Unani, Ayurveda, Homeopathy, and Siddha [6,7]. In ancient time, Ayurveda is considering as one of the traditional Indian medicinal practices which use medicinal plants as curative agent [8].

The plant parts also contain both organic and inorganic substances. Nutritional and mineral components are consider as important factor to determine the quality of herbs. Inorganic compounds play a dynamic role against a variety of degenerative diseases. Inorganic constituents such as phosphorous, potassium, iron, zinc, calcium, and sodium are essential to lead a healthy life [9-11].

The trace amounts of inorganic compounds are essential into play an important role in nutrition, enzyme reaction and also in the metabolic processes [12]. Metals and trace elements have a key role to play in the pathophysiology of human diseases [13]. In biological system, metals and minerals play an important role in the metabolism [14].

Medicinal plants produce primary and secondary metabolites with different functions [15]. The secondary metabolites which include organic substances such as alkaloids, terpenoids, and phenolic compounds are known to be responsible for the therapeutic potential of the herbal plants [16]. Herbal plants can produce different kind of secondary metabolites also known as natural products as they provoke effects on other organisms [17]. The most important phytochemicals are saponins, flavonoids, alkaloids, phenolic groups, tannins, and terpenoids [18].

Saponin is one of the chemical compounds in the class of amphipathic glycosides due to formation of soap-like foam in aqueous solution [19]. The word saponin was derived from the plant “soapwort” and its roots were used for foaming. Most of these compounds have detergent properties and give the foams in water. It has colloidal solution form in water and if the mixture is shaken, it form foamy like soap [20]. Saponin can be found on the roots and leaves of plants [21]. Saponin compounds which are present in medicinal plants have profitable effects. Among the different biological effects of saponins are antibacterial and antipROTOzoal [22-24] and anticancer activities [25].

Saponin has association with sex hormone-like oxytocin. Oxytocin is a sex hormone involved into control the onset of labor in women and the succeeding release of milk [26]. Saponins are also used in veterinary industry. Some of the saponins act as adjuvant and it can be added to vaccines (e.g. foot-and-mouth disease vaccines) and help to improve immune response.

Terpenoids are the group of compounds, predominantly which occur in the plant kingdom, and few terpenoids have been obtained from other
sources. These terpenoid compounds are effectively used in disease therapy and prevention of many human diseases. Terpenoid compounds have been used to treat inflammation, malaria, cancer, and a variety of infectious disease which is caused by bacteria and virus [27].

Alkaloids are distinct group of nitrogen-containing, low-molecular-weight compounds which occur in plant kingdom. Approximately 12,000 different types of alkaloids have been well known to occur in all plants as well as more than 150 families, such as Fabaceae, Papaveraceae, Apocynaceae, Rubiaceae, and Solanaceae. Alkaloids are accumulating huge amounts in specific parts of plants such as roots, stem barks, and seeds [28]. Alkaloids have many biological activities. The alkaloids and their derivatives are used as antispasmodic, analgesic, and antibacterialidal drugs [29]. Morphine, quinine, ephedrine, nicotine, and strychnine are consider as major type of alkaloids, and some of these are used in narcotic analgesics as well as anti-tissue agents [30,31].

*Butea superba* Roxb. is a plant in the family Fabaceae and commonly known as “Red Kwao Krua” [32] in Tamil it is known as Kodimurukkan. The flowers are yellowish orange color, about 4–5 cm in length that blossom when the plant sheds its leaves. *B. superba* was traditionally reported to possess antioxidant activities, aphrodisiac, estrogenic, and antibacterial properties. Hence, the present study was undertaken for the analysis of inorganic acid, basic radicals, and estimation of total saponins in the flower extract of *B. superba*.

**MATERIALS AND METHODS**

**Collection site of plant materials**
The dried flower samples were purchased from the Herbal Health Research Consortium Pvt., Ltd., Amritsar, India.

**Authentication**
The flowers of *B. superba* (Family: Papilionaceae (Fabaceae) were authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre, Chennai. The specimen no is PARC/2016/3280.

**Scientific classification of *B. superba* Roxb.**

| Kingdom      | Plantae                        |
|--------------|--------------------------------|
| Division     | Magnoliophyta                  |
| Class        | Magnoliopsida                  |
| Order        | Fabales                        |
| Family       | Fabaceae                       |
| Genus        | Butea                          |
| Species      | *Superba*                      |
| Common name  | Red Kwao Krua                  |
| Tamil name   | Kodimurukkan                   |

**Preparation of plant extract**
The dried flowers were brought to the laboratory and kept at room temperature (30°C±2°C) under aseptic condition to prevent microbial contamination. After that, the flowers were ground into powder form using a mixer grinder. The powder was kept in the airtight polythene bags and stored at dry place (Fig. 1).

The plant extract was prepared using a standard method followed by [33,34]. 1 g of the powder was taken and mixed with 20 ml of acetone using an ULTRA-TURAX mixer (13,000 rpm) and left overnight at room temperature. The sample was then filtered using Whatman no.1 paper with an ULTRA-TURAX mixer (13,000 rpm) and left overnight at room temperature. The sample was then filtered using Whatman no.1 paper and stored at dry place.

**Qualitative analysis of mineral content in the flower extract of *B. superba***
The mineral content of *B. superba* flower extract was analyzed using the standard methods [35].
Analysis of inorganic basic radicals

Test for lead
1 ml of the flower extract was taken in a test tube and added 2 ml of potassium chromate solution. Formation of yellow precipitate indicated the presence of lead.

Test for arsenic
3 ml of the flower extract was taken and add 2 ml of 10% (2N) sodium hydroxide (NaOH) solution. Formation of brownish red precipitate indicated the presence of phosphates.

Test for mercury
3 ml of the flower extract was taken and add 2 ml of 10% (2N) NaOH solution. Appearance of yellow precipitate indicated the presence of mercury.

Test for copper
1 ml of the flower extract was taken in a test tube and added 1 ml of NH₄OH solution. Then, light blue precipitate appears. Add excess NH₄OH.

Initially, light blue precipitate formed after that it will become dark blue.

Test for ferric
2 ml of potassium ferrocyanide was taken in a test tube and add 1 ml of the flower extract. Formation of blue precipitate showed the presence of ferric.

Test for ferrous
2 ml of potassium ferricyanide was taken in a test tube and add 1 ml of the flower extract. Appearance of blue precipitate indicated the presence of ferrous.

Test for zinc
1 ml of the flower extract was taken in a test tube and added 1 ml of NaOH solution. White precipitate was observed indicated the presence of zinc.

Test for silver
1 ml of the flower extract was taken in a test tube and added 1 ml of conc. HCL after that curdy white precipitate appeared. Boil the precipitate to settle, and then, the precipitates were washed with dilute NH₄OH solution. Formation of curdy white precipitate indicated the presence of silver.

Estimation of total saponins
The estimation of total saponins was done by slight modification. 1 g of the powdered flower sample was weighed and it was dispersed in 100 ml of 20% ethanol. The suspension was heated over a water bath for 4 h with continuous stirring at about 55°C. The filtrate and the residue were re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40% over water bath at about 90°C. The concentrate was transferred into separator funnel, and 20 ml diethyl ether was added and shaken vigorously then extracted twice with 20 ml diethyl ether. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30 ml n-butanol was added. The n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven at 40°C to a constant weight. The saponin content was calculated using the following formula:

Calculation
Total saponin content (%) = (FW/IW) × 100

Where,
FW=Final weight of sample (g)
IW=Initial weight of extracts (g).

Estimation of total terpenoids
The terpenoid content was estimated using Ferguson’s method [37].

Procedure
1 g of the flower powder was taken and soaked in ethanol for 24 h. The extract was filtered, and the filtrate was extracted with petroleum ether using separating funnel. The ether extract was treated as total terpenoids. The obtained residue was dried and weighed. The total terpenoid was calculated using the following formula:

Calculation
Total terpenoid (%) = \frac{FW-IW}{SW} \times 100

Where,
FW=Final weight of petriplate and sample (g)
IW=Initial weight of petriplate (g)
SW=Weight of sample (g).

Analysis of inorganic acid radicals

Test for lead
1 ml of the flower extract was taken in a test tube and added 2 ml of 10% (2N) NaOH solution. Formation of yellow precipitate indicated the presence of lead.

Test for copper
1 ml of the flower extract was taken in a test tube and added 1 ml of NH₄OH solution. Then, light blue precipitate appears. Add excess NH₄OH.

Initially, light blue precipitate formed after that it will become dark blue.

Test for arsenic
3 ml of the flower extract was taken and add 2 ml of 10% (2N) sodium hydroxide (NaOH) solution. Formation of brownish red precipitate indicated the presence of phosphates.

Test for mercury
3 ml of the flower extract was taken and add 2 ml of 10% (2N) NaOH solution. Appearance of yellow precipitate indicated the presence of mercury.

Analysis of inorganic basic radicals

Test for lead
1 ml of the flower extract was taken in a test tube and added 2 ml of potassium chromate solution. Formation of yellow precipitate indicated the presence of lead.

Test for copper
1 ml of the flower extract was taken in a test tube and added 1 ml of NH₄OH solution. Then, light blue precipitate appears. Add excess NH₄OH.

Initially, light blue precipitate formed after that it will become dark blue.

Analysis of inorganic acid radicals

Test for lead
1 ml of the flower extract was taken in a test tube and added 2 ml of 10% (2N) NaOH solution. Formation of yellow precipitate indicated the presence of lead.

Test for copper
1 ml of the flower extract was taken in a test tube and added 1 ml of NH₄OH solution. Then, light blue precipitate appears. Add excess NH₄OH.

Initially, light blue precipitate formed after that it will become dark blue.

Analysis of inorganic basic radicals

Test for lead
1 ml of the flower extract was taken in a test tube and added 2 ml of potassium chromate solution. Formation of yellow precipitate indicated the presence of lead.
The inorganic components are obtainable only in trace amounts in plants, which may impact numerous functions. These components are widely used in chemotherapy and are essential in human and animal health [42-44].

In various parts of human body, pH and acid balance are regulated by the carbonate, as bicarbonate ions [45]. In plants, the prevalence of sulfur is blend in the reduced form [46]. Sulfur is an important element of vitamins, biotin, and coenzyme [47-49]. Chlorides play an important role for regulation of water balance, osmotic pressure, as well as acid-base equilibrium [47,49]. Nitrogen is essential for the digestion of food and growth [50]. Fluoride plays an important role to prevent the dental caries in human beings [51]. In plants cells, the basic component is phosphorous, as phosphate ions which maintain blood sugar level, normal heart contraction [52], bone growth, and kidney function when consumed by human beings [53].

Qualitative analysis of basic radicals results is shown in Table 2.

Current report shows that the basic radical such as lead, arsenic, and ferrous was present in the flower extract of B. superba. The role of the inorganic basic radicals in animals and plants is different. Lead is a trace metal which is not essential for either human beings or plants. The impact of lead in plants varies because plants differ in the uptake from soil and sensitivity to lead [54].

In the formation of hemoglobin, iron plays a vital role for normal functioning of the central nervous system [55,56] and transfer of oxygen from the lungs to the tissue [56,57]. Zinc is an essential trace element for plant growth and also plays an important role in various cell processes [58] and also zinc is important for the production of insulin hormone and carbonic anhydrase in the body [59]. Zinc helps in regulating immune function, sperm production, and fetus development [60]. Mercury is a toxic metal, which is not reported in plants [61]. In carbohydrate, metabolism chromium plays an important role. It also functions in protein and cholesterol synthesis. It is an essential component which is required for the maintenance of normal glucose metabolism. The function of chromium is directly related to the function of insulin, which plays a vital role in diabetes mellitus. Chromium is found in the pancreas, which produces insulin [61].

In the present study, estimation of total saponins, terpenoids, and alkaloids content is shown in the Table 3. Comparing the percentage of the secondary metabolites of the flower extract of B. superba, it was observed in the following order, saponins>terpenoids>alkaloids as 4.18±0.017%<79.833±0.351%<107.823±0.105%, respectively.

Saponins have antifungal properties [62,63]. Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory, and weight loss [64,65]. Saponins give foams in water; it has detergent properties and hemolytic activity [66]. The total saponin content was recorded in the following medicinal plants such as Ocimum sanctum, Mentha spicata, Trigonella foenum graecum, and Spinacia oleracea [67].

Terpenoid compound has been used to treat cancer, malaria, inflammation, and a variety of infectious diseases which are caused by virus and bacteria. The most famous anticancer drug taxol and the antimalarial drug artesiminin are terpene-based drugs [68].

At present, many plant-derived alkaloids are used in clinics, for example, vinblastine and taxol (anticancer agents), morphine and codeine (analgesics), (C)-tubocurarine (muscle relaxant), colchicine (gout suppressant), sanguinarine (antibiotic), and scopalamine (sedative). The important plant origin alkaloids are caffeine, cocaine, nicotine, and suppressant, sanguinarine (antibiotic), and scopolamine (sedative). (analgesics), (C)-tubocurarine (muscle relaxant), colchicine (gout suppressant), sanguinarine (antibiotic), and scopolamine (sedative).

In various parts of human body, pH and acid balance are regulated by the carbonate, as bicarbonate ions [45]. In plants, the prevalence of sulfur is blend in the reduced form [46]. Sulfur is an important element of vitamins, biotin, and coenzyme [47-49]. Chlorides play an important role for regulation of water balance, osmotic pressure, as well as acid-base equilibrium [47,49]. Nitrogen is essential for the digestion of food and growth [50]. Fluoride plays an important role to prevent the dental caries in human beings [51]. In plants cells, the basic component is phosphorous, as phosphate ions which maintain blood sugar level, normal heart contraction [52], bone growth, and kidney function when consumed by human beings [53].

Qualitative analysis of basic radicals results is shown in Table 2.

Current report shows that the basic radical such as lead, arsenic, and ferrous was present in the flower extract of B. superba. The role of the inorganic basic radicals in animals and plants is different. Lead is a trace metal which is not essential for either human beings or plants. The impact of lead in plants varies because plants differ in the uptake from soil and sensitivity to lead [54].

In the formation of hemoglobin, iron plays a vital role for normal functioning of the central nervous system [55,56] and transfer of oxygen from the lungs to the tissue [56,57]. Zinc is an essential trace element for plant growth and also plays an important role in various cell processes [58] and also zinc is important for the production of insulin hormone and carbonic anhydrase in the body [59]. Zinc helps in regulating immune function, sperm production, and fetus development [60]. Mercury is a toxic metal, which is not reported in plants [61]. In carbohydrate, metabolism chromium plays an important role. It also functions in protein and cholesterol synthesis. It is an essential component which is required for the maintenance of normal glucose metabolism. The function of chromium is directly related to the function of insulin, which plays a vital role in diabetes mellitus. Chromium is found in the pancreas, which produces insulin [61].

In the present study, estimation of total saponins, terpenoids, and alkaloids content is shown in the Table 3. Comparing the percentage of the secondary metabolites of the flower extract of B. superba, it was observed in the following order, saponins>terpenoids>alkaloids as 4.18±0.017%<79.833±0.351%<107.823±0.105%, respectively.

Saponins have antifungal properties [62,63]. Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory, and weight loss [64,65]. Saponins give foams in water; it has detergent properties and hemolytic activity [66]. The total saponin content was recorded in the following medicinal plants such as Ocimum sanctum, Mentha spicata, Trigonella foenum graecum, and Spinacia oleracea [67].

Terpenoid compound has been used to treat cancer, malaria, inflammation, and a variety of infectious diseases which are caused by virus and bacteria. The most famous anticancer drug taxol and the antimalarial drug artesiminin are terpene-based drugs [68].

At present, many plant-derived alkaloids are used in clinics, for example, vinblastine and taxol (anticancer agents), morphine and codeine (analgesics), (C)-tubocurarine (muscle relaxant), colchicine (gout suppressant), sanguinarine (antibiotic), and scopalamine (sedative). The important plant origin alkaloids are caffeine, cocaine, nicotine, and suppressant, sanguinarine (antibiotic), and scopolamine (sedative). (analgesics), (C)-tubocurarine (muscle relaxant), colchicine (gout suppressant), sanguinarine (antibiotic), and scopolamine (sedative).

CONCLUSION
It can be summarized that the flower was selected for the current study having importance in traditional medicine and it can be considered as a source for the analysis of inorganic elements, estimation of total saponins, terpenoids, and alkaloids. Hence, the present study may be useful for the quality and purity of the plant material in future studies.

ACKNOWLEDGMENT
None.

AUTHORS’ CONTRIBUTION
All authors contributed equally to this manuscript.

CONFLICTS OF INTEREST
Identification of bioactive compounds and drug designing.

REFERENCES
1. Florence AR, Joselin J, Brintha TS, Sukumaran S, Jeeva S. Preliminary phytochemical studies of select members of the family Annonaceae for bioactive constituents. Biosci Discovery 2014;5:85-96.
2. Hammer K, Carson C, Riley T. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999;86:985-90.
3. Hassan BA. Medicinal Plants (Importance and Uses). Open Access J Biosci Discovery 2014;5:85-96.
4. Anjoo K, Ajay KS. Isolation of stigmastanol and β-sitosterol from petroleum ether extract of aerial parts of Ageratum conyzaeoides. Int J Pharm Pharm Sci 2010;3:94-6.

Table 1: Analysis of inorganic acid radicals in the flower extract of B. superba

| S. No | Acid radicals | Inference |
|------|---------------|-----------|
| 1.   | Carbonate     | -         |
| 2.   | Chloride      | -         |
| 3.   | Chromate      | +         |
| 4.   | Nitrate       | +         |
| 5.   | Phosphate     | +         |
| 6.   | Sulfate       | +         |
| 7.   | Sulfide       | -         |

Key: (+): Present; (-): Absent. B. superba: Butea superba

Table 2: Analysis of inorganic basic radicals in the flower extract of B. superba

| S. No | Basic radicals | Inference |
|------|----------------|-----------|
| 1.   | Lead           | +         |
| 2.   | Arsenic        | +         |
| 3.   | Mercury        | -         |
| 4.   | Copper         | -         |
| 5.   | Ferric         | -         |
| 6.   | Ferrous        | +         |
| 7.   | Zinc           | -         |
| 8.   | Silver         | -         |

Key: (+): Present; (-): Absent. B. superba: Butea superba

Table 3: Estimation of total terpenoids, saponins, and alkaloids in the flower extract of B. superba

| S. No | Secondary metabolites | % in B. superba flower extract |
|------|-----------------------|-------------------------------|
| 1.   | Saponins              | 4.181±0.017                   |
| 2.   | Terpenoids            | 79.833±0.351                  |
| 3.   | Alkaloids             | *107.823±0.105                |

Each value is mean±standard deviation of 5 samples expressed as percentage.

*p=0.05 level. (saponins versus terpenoids versus alkaloids). B. superba: Butea superba
62. Zetlic VG, Tomas VS, Grba S, Lutilsky L, Kozlek D. Chromium uptake by Saccharomyces cerevisiae and isolation of glucose tolerance factor from yeast biomass. J Biosci 2001;26:217-23.
63. Karumari RJ. Studies on the Efficacy of Ocimum sanctum (Linnaeus, 1767) and its Antifertility Effect with Reference to Male Rattus norvegicus (Berkenhout, 1769). Ph.D., Thesis, University of Madras, Chennai; 2013.
64. Aboada OO, Efuwape BM. Antibacterial properties of some Nigerian species. Biol Res Comm 2001;13:183-8.
65. Mohanta TK, Patra JK, Rath SK, Pal DK, Thatoi HN. Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of Semicarpusana cardium (L.). Sci Res Essay 2007;2:486-90.
66. Mandal P, Sinhababu SP, Mandal NC. Antimicrobial activity of saponins from Acacia auriculiformis. Fitoterapia 2005;76:462-5.
67. Manjunatha BK. Antibacterial activity of Pterocarpus santalinus. Ind J Pharm Sci 2006;68:115-6.
68. Sezgin AE, Artik N. Determination of saponin content in Turkish tahini halvah by using HPLC. Adv J Food Sci Technol 2010;2:109-15.
69. Facchini PJ. Annual revised plant physiology. Plant Mol Biol 2001;52:29-66.
70. Guangyi W, Weiping T, Bidigare RR. Terpenoids as therapeutic drugs and pharmaceutical agents. Nat Prod 2005;2:197-227.
71. Anjali S, Sheetal S. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. J Pharm Phytochem 2013;2:22-9.
72. Anita SW, Santosh RB. Plant profile, phytochemistry and pharmacology of Spathodea campanulata P. Beauvais (African tulip tree): A review. Indian J Pharm Pharm Sci 2018;10:1-6.