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Phytochemical Screening of Primary and Secondary Metabolites of Leaf and Root Extracts of *Baccaurea courtallensis* (Wight) Muell.-Arg., an Evergreen Tree.

Blessy Sharon Babu[1*] and K.Surekha[2]

[1]Research Scholar, Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore, Tamil nadu, India-641 029.

[2]Associate professor, Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore, Tamil nadu, India-641 029.

ABSTRACT

Several plants of high economic and medicinal value belong to Euphorbiaceae family such as *Hevea brasiliensis* (the main commercials source of rubber), *Embilica officinalis* (the source of Amla), *Manihot esculenta* (the source of tapioca). Evergreen tree *Baccaurea courtallensis* (Wight) Muell.-Arg. belongs to Euphorbiaceae family which is endemic and seen in Western Ghats of India is chosen for the present phytochemical screening. Using various organic and inorganic solvents, extraction of the leaves and roots of *Baccaurea courtallensis* (Wight) Muell.-Arg. was preliminary screened with the aim of assessing the availability of some biologically active compounds using standard methods. The primary and secondary phytochemicals screened from the leaf and the root extracts of *Baccaurea courtallensis* (Wight) Muell.-Arg. showed positive results for alkaloids, flavonoids, saponins, tannins, steroids and cardiac glycoside compounds. These compounds found in the leaf and root extracts of *Baccaurea courtallensis* (Wight) Muell.-Arg. have a wide range of biological activities which could be used for pharmaceutical significance.

KEYWORDS: *Baccaurea courtallensis* (Wight) Muell.-Arg, leaves, roots, phytochemicals.

***Corresponding Author**

Blessy Sharon Babu

PhD Scholar,

Department of Biochemistry,

Kongunadu Arts and Science College,

Coimbatore, Tamil nadu, India-641 029.

Email: sbblessy28@gmail.com

INTRODUCTION

Traditional medicines play an important role in health services around the globe. About three quarters of the World's population relies on plants and its extracts for health care. Tribal medicinal plants constitute an important natural value and play a significant role in providing primary health care services to rural people. They also serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine[1, 2]. Medicinal plants have invariably been a rich source of modern drug discoveries. Medicinal plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and or reduced toxicity [3]. Nowadays, the crude extracts samples from medicinal plants are used for the development and preparation of alternative traditional medicine. According to World Health Organization (WHO), 60% of the world's population depends on herbal medicine and 80% of the population in developing countries depends almost entirely on traditional herbal medicine practices, for their primary health care needs. As a result, there are increasing concerns about the safety, standardization, efficacy, quality, availability and preservation of herbal products by policy-makers, health professionals as well as the general public[4]. Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fibers to protect human against diseases. They are non-nutritive compounds (secondary metabolites) that contribute to flavor and color. Many phytochemicals have antioxidant activity and reduce the risk of many diseases, for example, alkyl sulfide (found in onions and garlic), carotenoids (from carrots), and flavonoids (present in fruits and vegetables)[5].

Baccaurea courtallensis (Wight) Muell.-Arg. of Euphorbiaceae family which is an evergreen flowering tree, that is very attractive when flowers are grown [6]. Leaves are alternate. Male and female flowers are borne on separate trees. Inflorescence bearing several flowers arises in tufts on tubercles on the stem. Fruits are crimson red in color and edible. Seeds are covered with a fleshy aril[7]. Evergreen flowering tree is mostly seen in the Western Ghats of India [8]. This is an endemic species [8]. Plant parts are used by paniya tribes of Wayanad for medicinal purpose and as food[9]. Leaf and root is used for digestive disorder and treatment of piles[10]. In the present study, an attempt was made to identify the major classes of phytochemicals present in *Baccaurea courtallensis* (Wight) Muell.-Arg. leaf and root extract using various solvents. The scientific classification is shown in table: 1.

Table:1 Scientific Classification of *Baccaurea courtallensis* (Wight) Muell.-Arg.

 **Baccaurea courtallensis**	
GBIF Taxonomy Hierarchy [admin]	
Scientific classification	
Kingdom:	Plantae
Phylum:	Magnoliophyta
Class:	Magnoliopsida
Common name (Malayalam):	Moottilthoori, Moottilpazham, Keranda.
Order:	Malpighiales Juss.
Family:	Euphorbiaceae
Genus:	Baccaurea
Species:	B. courtallensis
Binomial name	
Baccaurea courtallensis (Wight) Muell.-Arg.	

MATERIALS AND METHODS

Collection of Plant Material

Western ghats are the source of a large number of plants used for medicinal purposes by the tribes and ayurvedic practitioners. Plant parts (leaves and roots) of *Baccaurea courtallensis* (Wight) Muell.-Arg. were collected from Wayanad district, Kerala during the month of January-March.

Preparation of Plant Extracts

Plant parts collected were washed in sterile water and shade dried for three to four weeks and powdered. The powder was stored in air tight container.

Solvent extraction

About 10 g of the powdered samples were soaked in 100 ml of 8 different organic and inorganic solvents. The solvents used for extraction are Ethyl acetate, Iso-butanol, n-butanol, Cyclohexane, Acetone, Petroleum ether, Benzene using orbital shaker for 6-8 hours at 40^{O} Celsius and Aqueous extraction were done in water bath at 80^{O} Celsius. The extracts were filtered using Whatsmann No. 1 filter paper and evaporated to dryness which is then stored in sterile containers in the refrigerator till further analyses.

Qualitative Phytochemical Analysis

Phytochemical screenings of primary and secondary metabolites were carried out using standard methods.

1. Test for carbohydrate

Qualitative determination of carbohydrates was performed by

a)**Molisch's test:** 2 ml of filtrate were treated with 2 drops of alcoholic α-naphthol solution the mixture were mixed well and concentrated sulphuric acid were added slowly along sides of the tubes and allowed to stand. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) **Benedicts test:** 0.5 ml of the filtrate was taken in a test tube, 0.5ml Benedict's reagent was added. The mixture was heated in boiling water bath for 2 minutes. A characteristic red colored precipitate indicates the presence of sugar.

c) **Fehling's test:** Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

d) **Iodine test:** 2 drops of iodine were added to 1 ml of the test solution. Deep blue color indicates presence of polysaccharides.

e) **Barfoed's test:** 5 ml of test solution added to 5ml of Barfoed's reagent and heated to boiling. Brick red precipitate is obtained at the bottom of the test tube shows the presence of reducing monosaccharide in the test solution.

2. Detection of proteins and amino acids

a) **Biuret test:** 1 ml of the substance added to the test tube followed by few drops of biuret reagent. If purple color is formed presence of protein is confirmed.

b) **Ninhydrin test:** Taken 1 ml of the test solution added few drops of ninhydrin reagent and heated for 2 minutes. Presence of amino acid in protein is observed if a purple color is obtained.

c) **Xanthoprotic test**: Taken 1ml of test solution added few drops of conc. nitric acid and heated and cooled. Then few drops of 40% NaOH were added to the test tube. Here yellow color is formed after the addition of conc. Nitric acid and this turns red on the addition of NaOH, hence confirms the presence of aromatic amino acid in the protein[12].

3. Test for alkaloids

Extracts were dissolved individually in diluted Hydrochloric acid and filtered for further analysis. Standard procedure described by Evens (1997) and Wagner (1993 and 1996) were carried out.

a) **Mayer's test:** The filtered extracts were treated with a few drops of Mayer's reagent (Potassium Mercuric Iodide). The samples were then observed for the presence of turbidity or yellow precipitation to confirm the presence of alkaloids.

b) **Wagner's test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). That is, weighed 2g of Iodine and 6g of potassium iodide mixed in 100ml of distilled water. Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) **Hager's test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). That is, 1 or 2 ml of the reagent were added to the few ml of filtrate. Presence of alkaloids is confirmed by the formation of yellow colored precipitate.

4. Detection of flavonoids

a) **Alkaline Reagent Test**: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

b) **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids and orange to crimson color shows the presence of flavonones.

c) **Ferric chloride test:** A few drops of neutral ferric chloride solution are added to the extract, to form blackish red color, which indicates the presence of flavonoids.

d) **NaOH test**: To the extract, 2 ml of the 10% aqueous sodium hydroxide is added to produce a yellow coloration. A change in color from yellow to colorless, on addition of dilute hydrochloric acid was an indication for the presence of flavonoids. This method was described by Trease and Evans.

5. Detection of steroids

a) **Acetic anhydride test**: 2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml H_2S0_4. The change in coloration from violet to blue or green in samples indicates the presence of steroids.

b**) Copper acetate test:** Extracts were dissolved in water. This is treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

c) **Terpenoids:** 2 ml of extract was added to 2 ml of acetic anhydride and concentrated solution H_2SO_4. Formation of blue - green rings indicate the presence of terpenoids in the test solution.

d) **Liebermann-Burchard test**: Crude extracts were dissolved in 2 ml of chloroform, to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red color from blue to green indicates the presence of phytosterol.

6. Detection of glycosides:

Extracts were hydrolyzed with dilute Hydrochloric acid, and then subjected to test for glycosides.

a) **Modified Borntrager's test:** Extracts were treated with Ferric Chloride solution and then immersed in boiling water for about 5 minutes. The mixture was cooled down and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides in the test solution.

b) **Legal's test:** The extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides in the test tube.

7. Detection of phenols

a) **Ferric Chloride test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols. The extract was diluted to 5ml with distilled water and to this added a few drop of neutral 5% ferric chloride solution. The appearance of dark green color indicates the presence of phenolic compounds in the extract.

b) **Gelatin test:** About 1% solution of gelatin containing 10% NaCl is added to the ethanolic extract. White precipitation formation if observed at the bottom of the test tube it shows the presence of phenolic compounds.

c) **10% Lead acetate:** 50 mg of the extract is dissolved in distilled water and then added 3 ml of 10% lead acetate solution. A bulky white precipitate indicates the presence of phenolic compounds in the test tube.

8. Detection of saponin

a) **Froth test:** Extracts are diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) **Foam test:** 0.5 gm of the extracts was shaken with 2 ml of water. If foam persists for ten minutes it indicates the presence of saponins.

9. Detection of tannins

a) **1% Lead acetate:** The extracts are treated with few drops of 1% lead acetate solution. Yellow or red precipitate formation in the test tube shows the presence of tannins.

10. Detection of coumarin

a) **NaOH test**: 10% NaOH was added to the extract, and then added chloroform. Formation of yellow color shows the presence of coumarin.

11. Detection of emodins: 2 ml of NH_4 OH and 3 ml of Benzene was added to the extracts. Appearance of red color indicates the presence of emodins.

12. Detection of gum and mucilages: 1 ml of the extract is made up to 10 ml distilled water and then add 25 ml of alcohol and stir thoroughly. Cloudy precipitate shows the presence of gum and mucilages.

13. Detection of thiol's test: Taken 0.5 ml of the extract added 2-4 drops of 5 % of sodium nitroprusside. Then 1 or more drops of HNO_3 were added. Formation of magenta color shows the test is positive.

14. Detection of anthocyanins: 2 ml of the extracts were added to 2 ml of 2N Hcl and ammonia. The appearance of pink-red turn's blue-violet indicates the presence of anthocyanins.

15. Detection of leucoanthocyanins: To 5 ml of extracts added 5 ml of isoamyl alcohol. Upper layer appears red in color, which indicates the presence of leucoanthocyanins in the sample[13, 14, 15, 16, 17, 18, and 19].

Data Analysis

The change in the color of the sample when the respective reagents were added was observed for the phytochemical test, and recorded as '+' or '-' sign based on the results.

RESULTS AND DISCUSSION

The results of the qualitative phytochemical analysis of primary and secondary metabolites such as alkaloids, flavonoids, carbohydrates, saponin, proteins and amino acid, steroids, phenol, tannin, glycosides, emodin, coumarins, gum and mucilages, thiol's test, leucoanthocyanin and anthocyanin in the ethyl acetate, iso-butanol, n-butanol, cyclohexane, acetone, petroleum ether, benzene and aqueous leaf and root extracts of the plant species of *Baccaurea courtallensis* (Wight) Muell.-Arg. are shown in Table 2 and Table 3.

Phytochemical screening of leaf crude extracts of *Baccaurea courtallensis* (Wight) Muell.-Arg. revealed the presence of bioactive compounds is shown in detail in Table 2. In all leaf extracts presence of sugar and reducing sugar were seen. Polysaacharides are absent in ethyl acetate, acetone, aqueous but present in remaining solvents. Reducing monosaccharides are present in ethyl acetate, acetone, aqueous, and benzene. Carbohydrates are mostly present in all extracts. In eight extracts, the data reveals presence of aromatic amino acid in the protein. In leaf crude extracts the test for alkaloids, flavonoids, steroids, phenol, saponins, and glycosides shows positive results. In ethyl acetate crude extract presence of emodin and anthocyanin are found. Gum and mucilages are present

in n-butanol, cyclohexane, and aqueous extract of leaves. In iso-butanol crude extract coumarins and anthocyanin are seen. Thiol's test showed positive result only in acetone leaf extract. Leucoanthocynin were not present in the extracts. Tannin is present in acetone and aqueous extracts of leaf.

Table 2: Phytochemical constituents present in various solvents of leaf extracts of *Baccaurea courtallensis* (Wight) Muell.-Arg.

S/No	Test	EA	IB	nB	Cy	Ac	PE	Be	Aq
1	**Detection of carbohydrates**								
	a)Benedict's Test (Sugar)	+	+	+	+	+	+	+	+
	b) Fehling's Test (Reducing sugar)	+	+	+	+	+	+	+	+
	c)Molisch's test	+	+	+	+	+	-	+	+
	d)Iodine test	-	+	+	+	-	+	+	-
	e)Barfoed's test	+	-	-	-	+	-	+	+
2	**Detection of proteins and amino acid**								
	a)Xanthoproteic	+	+	+	+	+	+	+	+
	b)Ninhydrin	+	+	+	+	+	+	+	+
	c)Biuret test	+	+	+	+	+	+	+	+
3	**Detection of alkaloids**								
	a)Wagner's reagent	+	+	+	+	-	+	+	+
	b)Mayer's reagent	-	+	-	-	+	-	-	+
	c)Hager's reagent	+	+	+	+	+	+	+	-
4	**Detection of flavonoid**								
	a)Ferric chloride	+	+	+	+	+	+	+	+
	b)Lead acetate	+	+	+	+	+	+	+	+
	c)NaOH	+	+	+	+	+	+	+	+
	d)Alkaline reagent	+	-	-	+	+	-	-	+
5	**Detection of steroids**								
	a)Acetic anhydride	+	+	+	+	+	+	+	+
	b)Dipentitenes (copper acetate test)	-	+	+	+	+	+	+	+
	c)Terpenoids	+	+	+	+	+	+	+	+
	d)Libemann Burchard's Test (phytosterol)	+	+	+	+	+	+	+	+
6	**Detection for phenol**								
	a)Ferric chloride (Phenolic compound)	+	+	+	+	+	+	+	+
	b)Gelatin	-	-	-	-	+	-	-	+
	c)10% lead acetate	-	-	-	-	+	-	-	+
7	**Detection of saponins**								
	a)Saponin (Sodium bicarbonate)	+	+	+	+	+	+	+	+
	b)Froth test	+	+	+	+	+	+	+	+
8	**Detection of tannin**								
	1% lead acetate	-	-	-	-	+	-	-	+
9	**Detection of coumarins**	-	+	+	-	-	-	-	+
10	**Detection of emodin**	+	-	+	-	+	-	-	+
11	**Detection of glycosides**								
	a)Modified Bortrager (Antranol glycosides)	+	+	+	+	+	+	+	+
	b)Legal's test	+	+	+	+	+	+	+	+
12	**Gum and mucilages**	-	-	+	+	-	-	-	+

13	**Thiol's test**	-	-	-	-	+	-	-	-
14	**Anthocyanin**	+	+	-	-	-	-	-	-
15	**Leucoanthocyanin**	-	-	-	-	-	-	-	-

Table 2: '+' Positive; '-' Negative; EA - Ethyl Acetate, IB - Iso-butanol, nB - n-butanol, Cy – Cyclohexane, Ac – Acetone, PE - Petroleum ether, Be – Benzene, Aq- Aqueous.

Preliminary phytochemical screening of root crude extracts of *Baccaurea courtallensis* (Wight) Muell.-Arg. revealed the presence of bioactive compounds is shown in detail in Table 3. Presence of sugar was noted in acetone, petroleum ether and aqueous crude extracts of root. In all root extracts presence of reducing sugar were seen. Polysaacharides and reducing monosaccharides are present in crude extract of root but not in all extracts. Carbohydrates are mostly present in all extracts. In eight root crude extracts, the data reveals presence of aromatic amino acid in the protein. In root crude extracts the test for alkaloids, saponins and antranol glycosides shows positive results. In ethyl acetate and iso-butanol crude extracts flavonoids are absent. Terpenoids and phytosterols are present in root crude extracts. In iso-butanol, n-butanol, acetone and aqueous crude extracts phenolic compounds and tannin are present. In iso-butanol and acetone crude extracts coumarins are found. Emodin, free thiol's, anthocyanin and leucoanthocynin are absent in all crude extracts of root. Gum and mucilages are present in iso-butanol, acetone, benzene and aqueous extracts of root.

Table 3: '+' Positive; '-' Negative; EA - Ethyl Acetate, IB - Iso-butanol, nB - n-butanol, Cy – Cyclohexane, Ac – Acetone, PE - Petroleum ether, Be – Benzene, Aq- Aqueous.

Various phytochemicals have been used to treat cancer. Some of them are in the clinical trial studies. However, their mechanism of action is very different[20]. Phenol compounds such as flavonoids, tannins found in plants, all act as antioxidants[21]. Alkaloids are used for the treatment of psychiatric disorders, tumors, and diarrhea. It possesses anti-microbial activity and sedative effects. Flavonoids help to strengthen capillary walls and referred as phytoestrogens. Steroids are used to reduce stress, reduce cholesterol levels, activate immune system, and enhance memory and learning and to treat tumors. Saponins are used as anti-inflammatory, anti-hepatotonic, wound healing, anti-microbial and anti-viral[22].

Table 3: Results of phytochemical constituents present in various solvents of root extracts of *Baccaurea courtallensis*(Wight)Muell.-Arg

S/No	Test	EA	IB	nB	Cy	Ac	PE	Be	Aq
1	**Detection of carbohydrates**								
	a)Benedict's Test (sugar)	-	-	-	-	+	+	-	+
	b) Fehling's Test (Reducing sugar)	+	+	+	+	+	+	+	+
	c)Molisch's test	-	+	+	+	+	+	+	+
	d)Iodine test	+	+	-	-	+	-	-	+
	e)Barfoed's test	+	+	-	-	+	-	-	-
2	**Detection of proteins and amino acid**								
	a)Xanthoproteic	+	+	+	-	+	-	-	+
	b)Ninhydrin	-	-	+	-	-	-	-	+
	c)Biuret test	+	+	+	+	+	+	+	+
3	**Detection of alkaloids**								
	a)Wagner's reagent	+	+	+	+	-	+	+	+
	b)Mayer's reagent	-	-	+	-	+	-	-	+
	c)Hager's reagent	+	+	+	+	+	+	+	-
4	**Detection of flavonoid**								
	a)Ferric chloride	-	-	-	-	+	-	-	+
	b)Lead acetate	-	-	-	-	+	-	-	+
	c)NaOH	-	-	-	-	+	-	-	+
	d)Alkaline reagent	-	-	+	+	+	+	+	-
5	**Detection of steroids**								
	a)Acetic anhydride	+	+	+	+	+	-	-	+
	b)Dipentitenes (copper acetate test)	-	+	+	-	+	-	-	+
	c)Terpenoids	+	+	+	+	+	+	-	+
	d)Libemann Burchard's Test (phytosterol)	+	+	+	+	+	+	+	+
6	**Detection for phenol**								
	a)Ferric chloride (phenolic compound)	-	-	-	-	-	-	-	+
	b)Gelatin	-	+	-	-	-	-	-	-
	c)10% lead acetate	-	+	+	-	+	-	-	+
7	**Detection of saponins**								
	a)Saponin (Sodium bicarbonate)	+	+	+	+	+	+	+	+
	b)Froth test	+	+	+	+	+	+	+	+
8	**Detection of tannin**								
	1% lead acetate	-	+	+	-	+	-	-	+
9	**Detection of coumarins**	-	+	-	-	+	-	-	-
10	**Detection of emodin**	-	-	-	-	-	-	-	-
11	**Detection of glycosides**								
	a)Modified Bortrager (Antranol glycosides)	+	+	+	+	+	+	+	+
	b)Legal's test	+	-	-	-	+	+	-	+
12	**Gum and mucilages**	-	+	-	-	+	-	+	+
13	**Thiol's test**	-	-	-	-	-	-	-	-
14	**Anthocyanin**	-	-	-	-	-	-	-	-
15	**Leucoanthocyanin**	-	-	-	-	-	-	-	-

CONCLUSION

Qualitative phytochemical analysis of leaf and root extracts of *Baccaurea courtallensis* (Wight) Muell.-Arg. were investigated and showed the presence of biologically active compounds.

The phytochemical analysis revealed the presence of alkaloids, saponins, phenols, tannins, flavonoids, terpenoids, coumarins, cardiac glycosides, steroids group in varying concentrations. The present study provides evidence that extracts of *Baccaurea courtallensis* (Wight) Muell.-Arg. contains medicinally important antioxidant compounds and justifies the use of this plant species for treatment of various diseases.

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