Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0503.57**

PHYTOCHEMICAL INVESTIGATION OF *BARLERIA LONGIFLORA* LINN.F. IN WESTERN GHATS MADURAI

M. JothiMuniyandi[1], A. Jayachitra[2]*

1. Research and Development Centre, Bharathiyar University, Coimbatore.

2. Department of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai.

ABSTRACT: Objective: The present study was done to investigatethe phytochemical in the different extracts of *Barleria longiflora* leaves by qualitative screening and quantitative methods. Methods: Qualitative and quantitative screening in *Barlaria longiflora* was performed to identify the some primary and secondary phytochemicals by standard methods**.** Results: Ethanolic leaves extract of *Barleria longiflora* was found to contain more phytochemicals except Saponin and phlobotannin under study. Phenol, Terpenoid, Carbohydrates, Proteins and Vitamins were found in all six extracts. Quantitative estimation has shown that *Barlaria longiflora* leaf powder contained Carotenoids: 0.79±0.17 mg/g, chlorophylls: 1.33±0.10 mg/g, carbohydrates: 15.2±0.13 mg/g, proteins: 3.73±0.15 mg/g; alkaloid: 11.6%, Flavonoids: 5.6%, Terpenoids: 2.4% and saponin: 3%. Conclusion:These studies provide the preliminary scientific evidence for ethanobotanicaluses of *B.longiflora.*

KEYWORDS: *Barleria longiflora,* phytochemical, primary & secondary metabolites, qualitative & quantitative screening.

Corresponding Author: Dr. A. Jayachitra* Ph.D.

Department of Plant Biotechnology, School of Biotechnology,

Madurai Kamaraj University, Madurai. Email id: jchitralab@gmaill.com

1. INTRODUCTION

Plants are used as a medicine from ancient years because of their very less harmful effects. Western Ghats is extremely rich natural resources of more than 700 plant species found around the forest and hills of this region [1]. Plants are vital source of alkaloids, terpenoids, flavanoids, saponins, phenols, quinones, tannins, steroids, amines, coumarins and other metabolites [2]. Most of the research

Peer review under responsibility of Life Science Informatics Publications

reports of secondary metabolites have revealed that they have antioxidant, antibacterial, anticarcinogeneic, anti-inflammatory, antitumor, antileprosy, antiviral and many other properties [3-4].*B.longiflora* belongs to the Kingdom: Plantae, Class: Magnoliopsida, Order: Lamiales, Family: Acanthaceae, Geneus: Barleria, Species: *B.longiflora.B.longiflora* is a small flowering shrub 1-2 meter tall. Branches are covered with glandular hairs and oppositely arranged ovate, long leaves, densely hairy on both sides. Petals at the top are elliptic, pointed, 2 cm long. Capsules are 2cm long. This plant can reduce crop damage as well as pest population and help the development of new botanical insecticidal formulation and whole plants have the potential to treat the poisonous bites [5]. The present studies were carried out to qualitatively analyze the different solvent systems and to determinethe quantitative analysis of some primary and secondary phytochemical on the leaves of *Barleria longiflora.*

2. MATERIALS AND METHODS

2.1 Plant collection

Plants were collected from Western Ghats, Madurai. The taxonomic identities of these plants were confirmed by Taxonomist Rev.Fr.Dr.JohnBritto M.Sc., M.Phil., Ph.D. Rapinart herbarium, St.Joseph's College, Trichy.

2.2 Preparation of plant extracts

After the collection of plants, the leaves were air dried under shade, crushed in electric grinder and powdered. Out of this powder, 50g was suspended in 300ml of Petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water and the mixture was filtered and air dried by low pressure using (Sohuxlet apparatus).The residues were collected and dissolved in respective solvents and used for following activities.

2.3 Qualitative analysis of phytochemicals

The plant extracts of *Barleria longiflora* was screened for presence of phytochemicals such as alkaloids, flavonoid, saponin, phenol, glycosides, tannin, steroid, terpenoid, phlobotannin, coumarin, carbohydrate, protein and vitamin C [6-10].

2.3.1 Test for Alkaloids: Hager's test -2ml of test solution was treated with few drops of Hager's reagent (1% picric acid.) Formation of yellow precipitate would show a positive result for the presence of alkaloids.

2.3.2 Test for Saponin: Foam test- Add two ml extract with five milliliter distilled water and shake vigorously for 2min. If the appearance of foam persists for at least 15min, it confirms the presence of saponin.

2.3.3 Test for Total phenol: Ferric chloride test -Add two ml extract with five milliliter distilled water and few drops of 5% ferric chloride were added. Bluish black indicated the presence of phenolic compounds.

2.3.4 Test for Glycosides: Liebermann's test-2ml of test solution was mixed with 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully Conc. H_2SO_4 was added. A colour changed from violet to blue to green indicated presence of glycosides.

2.3.5 Test for tannin: Lead acetate test -Add 2ml extract with few drops of 1% lead acetate. If yellowish precipitate appears, then it contains tannin.

2.3.6 Test for flavonoid: Alkaline reagent test – 2ml of extract when treated with few drops sodium hydroxide solution, shows increase in the intensity of yellow colour which would become colorless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

2.3.7 Test for steroid: Salkowski test - Add 2ml of extract in a test tube with 2ml acetic anhydride acid, 1ml chloroform followed by 0.5 ml sulphuric acid. If the test solution shows violet to blue to green formation, it denotes the presence of steroids.

2.3.8 Test for terpenoid: Add 2ml of extract with 2ml of acetic acid followed by 1ml sulphuric acid might result in blue green ring formation shows the presence of terpenoid.

2.3.9 Test Phlobotannins: Precipitate test - The formation of red precipitate deposition by boiling 2ml of extract with 2ml 1% hydrochloric acid shows the presence of phlobatanins.

2.3.10 Test for coumarin: NaOH test - Add 2ml of extract with10% of 3ml sodium hydroxide in a test tube. If the solution turns to yellow colour, then it contains coumarin.

2.3.11 Test for Quinone: Add of 2ml extract with 5ml hydrochloric acid result in yellow colored precipitate denoting the presence of quinone.

2.3.12 Test for carbohydrate: Molisch's test-2ml of test solution in a tube and add 2drops of an ethanolic solution of α-naphthol (5%) Carefully run down the slides of the tube, bout 1ml of Conc.H_2SO_4 without mixing. A violet coloured ring at the junction of the two liquid in the positive test.

2.3.13 Test for free amino acids: Ninhydrin test-2ml of the test solution when boiled with 2ml of 0.2% of Ninhydrin solution, violet colour appeared suggesting the presence of amino acids.

2.3.14 Test for proteins: Biuret test-2ml of the test solution was treated with 10% of sodium hydroxide solution hydroxide solution and few drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour.

2.3.15 Test for vitamin C: DHPH test - 2ml of the test solution was treated with Dinitrophenyl hydrazine dissolved in Conc. H_2SO_4. The formation of yellow precipitate would suggest the presence of Vitamin C.

2.4 Quantitative analysis of primary metabolites

2.4.1 Estimation of Chlorophyll

One gram of *Barlerialongiflora* leaves were extracted with 20ml of 80% acetone, centrifuged at 5000rpm for 5 minutes and the supernatant was transferred to a 100ml volumetric flask. This procedure was repeated until the residue was colourless. The supernatant was made upto 100ml with

80% acetone. The absorbance of the solution was read at 645nm and 663nm against 80% acetone blank. The amount of chlorophyll present in the extract was calculated using the following formula:

$$\text{Total chlorophyll} = 20.2(A_{645}) + 8.02(A_{663}) \times V/1000xW$$

Where V was the final volume of the extract and W was the fresh weight of the leaves taken for extraction. The results are expressed as mg chlorophyll/g leaf [11].

2.4.2 Estimation of total carotenoids

0.5g of *Barleria longiflora* leaves was homogenized and saponified for about 30min in a shaking water bath at 37°Cwith a specific volume of 12% alcoholic KOH. The saponified extract was transferred into a separating funnel containing 10 to 15ml of petroleum ether (60-80°C) and mixed well. The lower aqueous phase was transferred to another separating funnel and petroleum ether containing the carotenoid pigment was collected. The extraction was repeated until the aqueous phase was colourless. To the petroleum ether extract, a small quantity of anhydrous sodium sulphate was added to remove turbidity. The absorbance of the extract at 450nm was noted in a spectrophotometer using petroleum ether as blank [12]. The amount of total carotenoid was calculating using the formula,

$$\text{Total carotenoids} = A_{450} \times \text{Volume of the sample} \times 100 \times 4 / \text{Weight of the sample}$$

2.4.3 Estimation of Carbohydrates

100 mg of sample was hydrolyzed in a boiling tube with 5ml of 2.5N HCl in a boiling water bath for a period of 3hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100ml using distilled water. From this 0.2ml of sample was pipetted out and made upto the volume to 1ml with distilled water. Then 1ml of phenol reagent was added, followed by 5ml of sulphuric acid. The tubes were kept at 25-30°C for 20min. The absorbance was read at 490nm [13].

2.4.4 Estimation of Proteins

One gram of leaves was extracted by stirring with 50ml of 50% methanol at 25°C for 24h and centrifuged at 7,000rpm for 10min. 0.2ml of extract was pipetted out and the volume was made to 1ml with distilled water. 5ml of alkaline copper reagent was added to all the tubes and allowed to stand for 10min. Then, 0.5ml 0f Folin'sCiocalteau reagent was added and incubated in dark for 30 min. The intensity of the colour developed was read at 660nm [14].

2.5 Quantitative analysis of secondary metabolites:

Secondary metabolites are derived from the plants to analyze the properties and to evaluate their possible use in the pharma industries.Alkaloid was determined by using alkaline precipitation gravimetric method described by Harbone (1973) [15].Flavonoid was determined by the method of Bohm and Kocipai-Abyazan (1974) [16]. Estimation of terpenoids [17] and saponin [18, 19] were done according to standard procedures.

3. RESULTS AND DISCUSSION

The plants have been occupying a distinct place in the human life right from ancient times. Various parts of plants such as root, stem, bark, leaves, flowers, fruits, seeds and whole plants also were used as a medicine for different kinds of diseases. In this present studies different solvents of leaves extract of *B.longiflora* were qualitatively and quantitatively analyzed.

Table 1 shows the crude extract value of leaves, according to the percentage yield; ethanol extract gives more percentage (5.6%) where as distilled water extract gives less percentage (2.4%) while petroleum either, chloroform, ethyl acetate and methanol gives 4.1%, 4.0%, 4.4%, and 5.2% respectively.

Table 1: Yield of various leaves extracts of *Barlerialongiflora* L.f.

Solvents	**Petroleum either**	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Methanol**	**Water**
Yield %)	4.1	4.0	4.4	5.6	5.2	2.4

Table 2 represents the phytochemical screening for various six solvents of leaves of *Barlerialongiflora.* The leaves extract of *Barlerialongiflora* when treated with petroleum ether revealed the presence of phenols, tannins, terpenoids, quinone, carbohydrates, protein and vitamins. The presence of phenols, terpenoids, quinone, carbohydrates, protein and vitamins was also confirmed by Chloroform extract. Further, chloroform extract indicated the presence of flavonoids, steroids and amino acids. Ethylacetate extract indicated the presence of alkaloids, saponin, phenol glycosides, tannins, steroids, terpenoids, coumarins, carbohydrates, proteins and vitamins. Except saponin and phlobotannin, the ethanol leaf extracts indicated the presence of all other phytochemical under study. Methanol extract also contained alkaloids, phenol, glycosides, steroids, terpenoids, coumarins, carbohydrates, proteins and vitamins.

Table 2: Phytochemicals analysis of various leaves extract of *Barleria longiflora* L.f.

Phytochemicals	Petroleum either	Chloroform	Ethyl acetate	Ethanol	Methanol	Water
Alkaloids	-	-	+	+	+	-
Saponin	-	-	+	-	-	-
Phenol	+	+	+	+	+	+
Glycosides	-	-	+	+	+	+
Tannin	+	-	+	+	-	+
Flavonoid	-	+	-	+	-	+
Steroid	-	+	+	+	+	+
Terpenoid	+	+	+	+	+	+
Phlobotanins	-	-	-	-	-	-
Coumarin	-	-	+	+	+	+
Quinone	+	+	-	+	-	-
Carbohydrates	+	+	+	+	+	+
Aminoacids	-	+	-	+	-	+
Proteins	+	+	+	+	+	+
Vitamins	+	+	+	+	+	+

+ Present, - Absent

Comparative quantitative estimation of primary phytochemicals present in the leaves of *Barleria longiflora* was found to contain Chlorophyll: 1.33±0.10, Carotenoids: 0.79±0.17, Carbohydrates: 15.2±0.13andproteins: 3.73±0.15 which were shown in table 3.

Table 3: Quantitative estimation of some major primary phytochemicals of *Barleria longiflora*L.f

Phytochemicals	Carotenoids	Chlorophylls	Carbohydrates	Proteins
Yields (mg/g)	0.79±0.17	1.33±0.10	15.2±0.13	3.73±0.15

Table 4 reported the quantities of secondary phytochemical found in the leaves of *Barlerialongiflora.* In the present investigation, secondary metabolites viz. alkaloid, flavanoid, terpeniod and saponin were quantitatively analyzed.

Table 4: Quantitative estimation of some major secondary phytochemicals of *Barleria longiflora* L.f

Phytochemicals	Alkaloids	Flavonoids	Terpenoids	Saponin
Yield (%)	11.6	5.6	2.4	3.0

DISCUSSION

Herbal plants are vital role in primary health care of village people. For example, Azadiracta indica, Ocimum sanctum and Acalypha indica are used as remedies against a stomach pain, poisonous bites, cough and skin diseases etc [25]. *Barleria* species have potntial effects of analgestic, antitumor, antihyperglycemic, antiamoebic, antiinflamatory, antidiabetic, antimicrobial, hepatoprotective and nephroprotective [28]. In this study, leaves of *B. longiflora* were used for screening phytochemicals. Six different solvents were chosen for extracting bioactive substance from B.longiflora leaf powder (Table 1) and phytochemical examinations were carried out for all the solvent extracts as per the standard methods. During extraction, the selected solvents diffuse into the solid plant material and solubilize compounds with similar polarity [22]. Phytochemical screening of *B.longiflora* revealed the possible presence of phenols, terpenoids, alkaloids, flavonoids and glycosides. Among the phytochemicals, phenols, terpenoids, carbohydrates, proteins and vitamins were found to be abundant in *B.longiflora* leaves and were found in all six solvent extracts. Least number of phytochemicals was present in petroleum ether leaves extract and showed very weak results (Table 2). Chloroform and methanol extract have shown a moderate number of phytochemicals from selected plant part of *B. longiflora* (Table 2). Ethyl acetate and aqueous extracts showed modest number of metabolites (Table 2). Ethanol is a good solvent system for extraction of secondary metabolites, the present result has also proved that the presence of metabolites such as alkaloids, flavonoid, saponin, phenol, glycosides, tannin, steroid, terpenoid, phlobotannin, coumarin, carbohydrate, protein and vitamin C in ethanol extracts. Phenol, Terpenoids, Carbohydrates, Proteins and Vitamins were found in all the six extracts (Table 2). Primary metabolites are precursors of pharmacologically active metabolites in pharmaceutical molecules [19]. The higher amounts of primary metabolites of carbohydrates are found to be present in the B.longiflora leaves followed by proteins, chlorophyll and carotenoinds (Table 3).Carbohydrates and proteins are macromolecules, they gives energy for physical and metabolic activities. Carotenoids have a wide range of biological effects, such as decrease the risk of cancer, age related macular degeneration, cataracts, cardio vascular diseases and sunburn skin damage [20]. Secondary metabolites are vital roles of protective agents. The considerable amount of alkaloids, flavonids, saponin and terpenoids were found in the leaves of *B.longiflora*(Table 4).These secondary metabolites like alkaloids, flavonids, saponin and terpenoids have great potential effects against pathogenic microorganisms [21, 22]. Alkaloids have numerous pharmacological activities such as emetic, anticholinergic, antitumor, diuretic, sympathomimetic, antiviral, antihypertensive, anticancer, analgesic, antidepressant, muscle relaxant, anti-inflammatory, antimicrobial, and antiulcer [29,30 &31]. Flavonoids a vital role in inhibition of key enzymes in mitochondrial respiration, protection against coronary heart disease antitumor, anti-inflammatory, and antimicrobial activities [23]. Terpenoids were used for therapy of several diseases, like antibacterial, antiviral, immunomodulatory activity

antifungal, antihyperglycemic, anticancer, anti-inflammatory and insecticidal properties [24]. Saponins are unique property of precipitating and coagulation of red blood cells [26 & 27]. The plant species studied here can be used as a potential source of useful drugs. It also justifies the folklore medicinal use and the claims about the therapeutic values of this plant as a curative agent.

4. CONCLUSION

To conclude *B.longiflora* ethanolic extract has been found to contain more photochemical except saponin and phlobotannin under study. Phenol, Terpenoids, Carbohydrates, Proteins and Vitamins were found in all six extracts. These photochemical may prove themselves as potential drug to cure various diseases. This study has also led the way to estimatethe antioxidant activities in all the six extracts of *B. longiflora* leaves by various spectroscopic methods.

ACKNOWLEDGEMENT

The authors declare no acknowledgement of interest.

CONFLICT OF INTEREST

The authors have not declared any conflicts of interest.

REFERENCES

1. Padmavathy S and Mekala V. Preliminary Phytochemical Investigation of some medicinal plants Western Ghats, the Nilgiris, Int. J. pharm. biomed. res. 2013; 4(1): 1-5.
2. Jayanthi P and Lalitha P. Reducing power of the solvent extracts of Eichhorniacrassipes [Mart.] Solms. Int J Pharm Pharm Sci; 2011; 3(3): 126-128.
3. Jothi Muniyandi M and Lakshman K. Preliminary Studies of Phytochemical Investigation on Coastal Medicinal Plants of Boloor, Mangalore, Indo Am. J. P. Sci, 2018; 05(02): 1309-1315.
4. Negi JS, Singh P, Rawat b. Chemical constituents and biological importance of Swertia: a review. Curr Res Chem. 2011; 3:1-15.
5. Chennaiyan V, Sivakami R and Jeyasankar, A.Evaluating Ecofriendly Botanicals of BarlerialongifloraLinn. F. (Acanthaceae) against Armyworm SpodopteralituraFab. and Cotton Bollworm HelicoverpaarmigeraHübner (Lepidoptera: Noctuidae). Annu Res Rev Bio. 2016; 10(3): 1-9.
6. Kokate C K, practical Pharmacognosy, VallabhPrakashan, Delhi, 2000; P.107-111.
7. Harbone J B, Phytochemicals methods, Chapman & Hall, London, 1999; P.60-66.
8. Brinda P, Sasikala B and Purushothaman KK. Pharmacogenostic studies on Merugankizhangu. Bull.Med.Eth.Bott.Res. 1981; 3: 84-96.
9. Sofowara A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan: Nigeria. 1993; 289-300.
10. S Sadasivam and A Manickam, New age international (P) limited, publishers, Newdelhi. 1992; 193-198.

11. Zakaria H, Simpson K, Brown PR, Krotulovic A. Use of reversed phase HPLC analysis for the determination of provitaminA carotenes in tomatoes. J.Chrom. 1979; 176:109-117.
12. Witham FH, Blaydes BF, Devlin RM. Experiments in plant physiology. Van.Nos.N.Y. 1971; 245.
13. Hedge JE, Hofreiter BT, Whistler RL, Be Miller JN. In: Carbohydrate Chemistry. 17th Ed. Academic Press: New York; 1962.
14. Lowery OH, Rosenberg NJ, Farr AL, Randal RJ. Protein measurement with the folin-phenol reagent. J Biol Chem. 1951; 193:265-75.
15. Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd.1973; 49-188.
16. Bohan BA and Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian Vacciniumvaticulatum and V.calycinium. Pacific Science. 1974; 48: 458-463.
17. Ferguson NM. A text book of pharmacognsosy. Mac Milan Company, New Delhi. 1956; 191.
18. Obadoni BO and Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global J Pure Appl Sci. 2001; 8: 203-208.
19. Schanderl S. In: Methods in food analysis. Acedemic Press: in New York; 1970; p. 70.
20. Harborne, J.B., & Williams, C.A. Advances in flavanoid research since 1992. Phytochemistry, 2000; 55: 481-504.
21. Ghosh P, Mandal A, Chakraborty P, Rasul MG, Chakraborty MandSahaA. Triterpenoids from Psidium guava with Biocidal Activity. INDIAN J PHARM SCI, 2010; 72 (4): 504–507.
22. Chung PY, NavaratnamP, and Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against Staphylococcus aureus strains. Ann Clin Microbiol Antimicrob. 2011; 10:25. doi: 10.1186/1476-0711-10-25.
23. Aust O, Sies H, Stahl W, & Polidori M.C. Analysis of lipophilic antioxidants in human serum and tissues: Tocopherols and carotenoinds. J Chromatogr A. 2001; 936, 83-96.
24. Roslin J Thoppil and AupamBishayee. Terponids as potential chemopreventive and therapeutic agents in liver cancer. World J Hepatol. 2011; 3(9): 228-249.
25. Ramadevi K and JothiMuniyandi M. Ethanobotanical study of medicinal plants in Devankurunchi hills in Madurai District, Tamilnadu. J.Nat.Prod.Plant Resour. 2015; 5(6):1-8.
26. Okwu DE. Phytochemicals and vitamin content of indigenous spices of southeastern Nigeria. J Sustain Agric Environ 2004; 6:30-37.
27. Sodipo OA, Akiniyi JA, Ogunbamosu JU. Studies on certain characteristics of extracts of bark of Pansinystalia macruceras (Kschemp) Pierre Exbeille. Global J Pure Appl Sci 2000; 6:83-87.
28. Manjula MS and Saravana Ganthi A. Nephroprotective activity of *Barleria longiflora* L. (Acanthacea) against gentamicin induced nephrotoxicity in male albino wister rats. J Pharmacogn Phytochem. 2018; 7(2):2835-2837.

29. Jin-Jian Lu, Jiao-Lin Bao, Xiu-Ping Chen, Min Huang, and Yi-Tao Wang. Alkaloids Isolated from Natural Herbs as the Anticancer Agents. Evid Based Complement Alternat Med. 2012; 1-12.
30. Henriques AT, Lopes SO, Paranhos JT, Gregianini TS, Von Poser GL, Fett-Neto AG, Schripsema J. N,β-D-glucopyranosyl vincosamide, a light regulated indole alkaloid from the shoots of Psychotria leiocarpa. Phytochemistry. 2004; 65(4): 449–454.
31. De Sousa Falcao H, Leite J.A, Barbosa-Filho JM, De Athayde-Filho PF, De Oliveira Chaves M.C, Moura MD, Ferreira AL, De Almeida ABA, Souza-Brito ARM, De Fátima Formiga Melo Diniz M, Batista LM. Gastric and Duodenal Antiulcer Activity of Alkaloids: A Review. Molecules. 2008; 13: 3198-3223.