

Phytochemical analysis of leaf callus of *Bacopa Monnieri* L

Sunil Kumar Singh

Dept. of Biotechnology, Sathyabama University, Chennai - 600119, TN, India

Abstract- *Bacopa monnieri* L. (family Scrophulariaceae) is a traditional medicinal plant in India, commonly known as "brahmi". Used for different diseases such as- nervous disorder, respiratory problem, leprosy, splenomegaly, skin disease etc. In the present study, the ethanolic and aqueous extract of the leaf callus of *Bacopa monnieri* was investigated for its phytochemical analysis by modified Kirby-Bauer diffusion method. The finding of this study revealed that the extract of the leaf callus of *Bacopa monnieri* revealed the presence of Tannins, flavonoids, glycosides, terpenoids, saponins, and steroids and absence of Anthroquinone, Phenolic etc.

Index Terms- Phytochemical analysis, leaf callus extract, *Bacopa monnieri*

I. INTRODUCTION

Plant tissue culture is the process of small pieces of living tissues (explants) isolated from a plant and grown aseptically for indefinite periods on a semi defined or defined nutrient medium (Ignacimuthu, 1997). It is considered in wide sense which comprises the various culture methods of plant organs, tissues which facilitates experimental approach with a large objective of developmental biology and crop modification. It provides new possibilities for in vitro propagation and manipulation of plants and also recognized as an efficient tool for rapid clonal propagation (Negrutiu et al., 1984). Murashige and Skoog's medium is commonly used for plant tissue culture studies (Murashige & Skoog's, 1962). Hence, the present study is justifiably planned to propagate the valuable medicinal plant *Bacopa monnieri* L. in in vitro condition with various combinations/ concentrations of plant growth regulators, and transplant the plants from laboratory in to field condition.

According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab, Kamalnejad, Ghaderi and Vahidipour, 2003).

Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents (Iyengar, 1995).

Bacopa monnieri Linn. Is a prostrate, creeping, juicy, succulent, glabrous herb that branches profusely, found in wet places, damp or marshy areas near the border of the ponds, water canals, wells, irrigated fields etc. [Chopra, R N, Nayer 1992] The plant is reported to contain tetracyclic triterpenoids, saponins, bacosides A and B [Chatterjee, N, Rastogi, 1965], phytosterols, hirsaponin [Sastry MS, Dhalla N S and Malhotra 1959], flavonoids viz. luteolin-7-glucoside, glucuronyl-7-apigenin. It is a valuable nervine tonic for curing memory loss [Anonymous 1997,], mental stress [Handa, 1994], and anxiety [Singh R H Singh L 1980]. It is used for controlling asthma, rheumatism, hoarseness and fever. Also it is used in generalized weakness, lethargy, fatigue and exhaustion [Jayaram S 1993].

Literature survey showed that much work has not been reported from leaf callus. So here in the present study we have initiated and developed the callus on the leaf of plant *Bacopa monnieri* and then evaluated the phytochemical analysis of ethanolic and aqueous extract of leaf callus of *Bacopa monnieri* L.

II. MATERIALS AND METHODS

Bacopa monnieri L. plant was collected from nursery. The collected plant was kept under green house condition in Sathyabama University Chennai for further study. The leaf callus of *Bacopa monnieri* was initiated on M S medium supplemented with BAP(0.5)+NAA (1.0, 2.0mg/l), 2,4-D(2.0)+BAP(0.5, 1.0mg/l) Finally, leaf callus was successfully maintained on M S medium supplemented 2,4-D (1.0)+ BAP (0.5mg/l)

III. PREPARATION OF PLANT EXTRACT

The ethanolic and aqueous extract of the leaf callus was prepared by heating the samples (5g) in ethanol and (5g) in aqueous at 100°C on water bath. The extract was filtered and the filtrate was evaporated to dryness. The dried extract was stored till the time of the study.

One gram of sample was weighed and dissolved with 10ml various solvent (ethanol and aqueous). Then the sample was allowed to stay overnight for 24 hours. After overnight incubation the sample was filtered by Whatmann filter paper and the filtrate was centrifuged at 25,000 rpm for 10 mins and the supernatant was used for phytochemical screening.

IV. PHYTOCHEMICAL STUDY

Preliminary phytochemical screening was performed [Harborne1988]. The presence of phytoconstituents such as Tannins, Saponins, Phenolic, Terpenoids, Steroids, Phytosterol, Anthraquinone, Glycosides, Flavonoids were confirmed by the following procedure.

1. TEST FOR TANNINS

About 2ml of filtered extract was taken in a test tube and 2ml of ferric chloride added. The presence of blue-black coloured precipitate indicates the presence of tannins.

2. TEST FOR SAPONINS

To 0.5 ml of extract was added 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for stable persistent froth.

3. TEST FOR TERPENOIDS (salkowski test)

To 0.5 ml each of the extract was added 2ml of chloroform. Concentrated Sulphuric acid 3ml was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

4. TEST FOR CARDIAC GLYCOSIDES

To 2ml of extract 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1ml of sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

5. TEST FOR ANTHRAQUINONE

0.5ml of the extract was boiled with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette in to another test tube observed for colour changes.

6. TEST FOR FLAVONOIDS

Dilute ammonia 5ml was added to the extract. Concentrated sulphuric acid 1ml was added. A yellow colouration that disappears on standing indicates the presence.

7. TEST FOR STEROIDS

To 1ml extract 10ml of chloroform was added. 10ml of concentrated sulphuric acid was added carefully to form coloured layer. Upper layer turns red, sulphuric acid layer forms yellow with green fluorescence, indicates the presence of steroids.

8. TEST FOR PHYTOSTEROL

1ml of extract was dissolved in 10ml of chloroform and 10ml concentrated sulphuric acid along the side of the test tube. Brown ring indicates presence of phytosterol.

9. TEST FOR PHENOLIC

2ml of extract 1ml ferric chloride was added, a blue or green colour indicates presence of phenolic.

V. RESULT AND DISCUSSION

Bacopa monnieri L. (family Scrophulariaceae) is a traditional medicinal plant in India, commonly known as "brahmi". Used for different diseases such as- nervous disorder, respiratory problem, leprosy, splenomegaly, skin disease etc. The preliminary phytochemical studies received pronounced importance, because the crude drugs possess varied composition of secondary metabolites [11]. In the present study preliminary phytochemical tests on the leaf callus ethanolic and aqueous extract revealed the presence or absence of Tannins, Saponins, Phenolic, Terpenoids, Steroids, Phytosterol, Anthraquinone, Glycosides, Flavonoids (given tab-1)

Tab-1

Preliminary phytochemical screening of callus extraction of *Bacopa monnieri*.

Sl No.	Phytochemical (Test)	Ethanol	Aqueous
1.	Tannins	+	+
2.	Saponins	+	+
3.	Terpenoids	+	+
4.	Glycosides	-	+
5.	Anthraquinone	-	-
6.	Flavonoids	+	-
7.	Steroids	+	+
8.	Phytosterol	+	+
9.	Phenolic	-	-

+ve Presence of phytochemical.

-ve Absence of phytochemical.

VI. CONCLUSIONS

The ethanolic and aqueous extracts of the studied leaf callus of *Bacopa monnieri* L. contained many bioactive chemical constituents including Tannins, Saponins, Terpenoids,

Steroids, Phytosterol, Anthraquinone, Glycosides, Flavonoids etc.

ACKNOWLEDGEMENT

Grateful thanks goes to the Department of Biotechnology, Sathyabama University for providing the facilities for research work. I also thank Dr. Y. Justin Koilpillai, Ph.D. for providing the invaluable help during the research work.

REFERENCES

- [1] Anonymous, "Indian Medicinal Plants" (A compendium of 500 species) Vol-1, Orient Longman Ltd. 1997, p. 84
- [2] Chatterjee, N, Rastogi, R P and Dhar ML, Indian J. Chem., 1965, 3, 24.
- [3] Chopra, R N, Nayer, S L, and Chopra, I C, "Glossary of Indian Medicinal Plants", CSRI, New Delhi 1992, p.32.
- [4] Handa, SS Pharma Times, 1994, 26 (3), 17.
- [5] Harborne JB: Phytochemical methods, Third Edition, Chapman and Hall, London, 1988, p 117.
- [6] Ignacimuthu S (1997) Plant Biotechnology, Oxford and IBH publishing Co. Pvt.Ltd, p. 180.
- [7] Iyengar, M.A., 1995. Study of Crude Drugs. 8th ed., Manipal Power Press, Manipal, India. pp 2.

- [8] Jayaram S, Walwaikar, PP Rajadhyaksha, S.S., Indian Drugs 1993, 30 (10), 498.
- [9] Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15, 473-497
- [10] Mojab, F., Kamalinejad, M., Ghaderi, N., Vahidipour, H., 2003. Phytochemical Screening of Some Iranian Plants. *Iranian Journal of Pharmaceutical Research.* pp 77-82.
- [11] Negrutiu I, Jacobs N and Caboche M (1984) *Theor. Appl. Genet.* 67, 289-304.
- [12] Singh RH Singh L., *J. Res. Ayur. Siddha*, 1980, 1 (1), 133
- [13] Sastry MS, Dhalla N S and Malhotra CL, *Indian J. Pharma.*, 1959, 21, 303.
- [14] Wink, M. (eds.) 1999. Function of plant secondary metabolites and their exploitation in biotechnology. Sheffield Academic Press, Sheffield, England.

AUTHORS

First Author – Sunil Kumar Singh, Dept. of Biotechnology, Sathyabama University, Chennai - 600119, TN, India