International Journal of
Recent Innovations in Academic Research
Open Access, Peer Reviewed, Abstracted and Indexed Journal

E-ISSN: 2635-3040; P-ISSN: 2659-1561
Homepage: https://www.ijriar.com/
Volume-5, Issue-12, Dec-2021: 71-75

Research Article

Phytochemical Studies in Leaf and Callus Extracts of *Hyptis suaveolens* L. a Medicinally Important Plant

Satyanarayana, B[*a]. and Subhashini Devi, P[b].

[a&b]Department of Biochemistry, Andhra University, Visakhapatnam–530 003
*Corresponding Author Email: satyabiochemau@gmail.com

Received: December 09, 2021 **Accepted:** December 21, 2021 **Published:** December 29, 2021

Abstract: The present study evaluates the qualitative and quantitative phytochemical analysis of secondary metabolites in leaf and callus extracts of *Hyptis suaveolens* L. Preliminary phytochemical screening showed the presence phenolics and flavonoids in leaf and callus extracts. Quantitative analysis of leaf and callus extract contains the phenolic compounds (92.77±2.04 and 72.31±1.16) and flavonoids (74.99±2.72; 62.36±3.64 mg/gram) respectively. Further studies are needed to isolate, characterize and test the bio-efficacy of the bioactive compounds from callus extract of *H. suaveolens*.
Keywords: *Hyptis suaveolens,* Callus extract, Phytochemical screening, Phenolics, Flavonoids.

Introduction

Medicinal plants have been used to treat many diseases since ancient time. Today in the modern world several synthetic drugs are available in the market and are highly effective in curing various diseases but people prefer the traditional folk medicine to avoid harmful side effects (Iqbal *et al.,* 2015). These plants are the rich sources of ingredients that can be used in the synthesis and production of drugs (Oladeji *et al.,* 2020). Plants consist of wide diversity of chemical constituents known as phytoconstituents or secondary metabolites such as flavonoids, phenols, phenolic glycosides, saponins, cyanogenic glycosides, tannins, nitrogen containing compounds (alkaloids, amines and betains) and terpenoids (Sharma *et al.,* 2020).

Phytoconstituents assist the plants by contributing secondary functions such as safeguarding the plants by activating defense mechanism (Sharma *et al*., 2020). Natural products exhibit minimal side effects and improved efficacy than the synthetic counterparts. These phytoconstituents (phenolics and flavonoids) exhibits the significant biological functions that enhance therapeutic potential such as anti-cancer, anti-inflammatory and antioxidant properties (Batiha *et al.,* 2020). Phytochemical screening is the schematic process for analysing, examining, extracting and identifying the various classes of phytoconstituents present in various parts of the plant for drug discovery.

Hyptis suaveolens (L.) Poit. is a medicinally important plant belongs to the family *Lamiaceae* and all parts of the plant are in use as traditional medicine (Jesus *et al*., 2013). It exhibits anti-rheumatic, anti-inflammatory, anti-fertility, cytotoxicity, insecticidal and larvicidal activities (Sumitha and Thoppil, 2016). *In vitro* regeneration of callus and shoot cultures from various explants using plant tissue culture methods is an alternative approach for the optimization of secondary compounds. The callus cultures of *H. suaveolens* are explored for the biosynthesis of AgNPs as it is rich in secondary metabolites. Earlier reports (Botcha and Prattipati, 2019 & 2020) indicates that *H. suaveolens* plant and callus extract has potential anticancer and antioxidant properties due to the presence of bioactive principles, which are actively involved in the bioreduction of Ag^{+} to AgNPs.

The objective of the study to carry out preliminary phytochemical screening and also to determine the total phenolic and flavonoid content in leaf and callus extracts of *H. suaveolens* L.

Materials and methods

Preparation of leaf extract

Fresh and healthy leaves of *H. suaveolens* were thoroughly rinsed under running tap water followed by double distilled water and were cut into small pieces, dried under shade at room temperature. Ten grams of dried chopped leaves were finely powdered and boiled for 30 minutes at 70^0C in 100 ml of double distilled water. The extract was allowed to cool, filtered using Whatmann No. 1 filter paper, labelled as aqueous leaf extract and was finally used for phytochemical analysis.

Preparation of callus extract

Fresh biomass of 45 days old callus was collected, washed thoroughly with sterile distilled water and dried in the hot oven at 40^0C for 24 hours and the dried callus was crushed into fine powder. About 10 grams of fine powder was taken in 250 ml conical flask and 100 ml of sterile double distilled water was added and boiled for 30 min at 80^0C. Then the callus extract was allowed to cool and then filtered using Whatman No. 1 filter paper. The filtrate (aqueous callus extract) was used for phytochemical analysis.

Phytochemical screening

Preliminary phytochemical analysis of aqueous leaf extract and leaf derived callus extract of *H. suaveolens* was carried out for the qualitative detection of different phytochemicals such as phenols, flavonoids, alkaloids, terpenoids, tannins, saponins and proteins. All phytochemical tests were performed according to the standard methods described by Sithara *et al*., (2016).

Total phenolic content

Total phenolic content of leaf and callus extract was determined spectrophotometrically by Sadasivam and Manickam (1996) and the amount of phenolic content in extract was expressed as gallic acid equivalent per gram extract. To 2ml of phenol extract 1.0 ml of Folin-Ciocalteu reagent was added. After 3 minutes, 13 ml of distilled water was added. Later 2 ml of sodium carbonate (7.5%) solution was added and the volume was adjusted to 20 ml. The above mixture was kept for one hour for colour development and absorbance was recorded at 630 nm. The concentration of total phenolic content in plant extracts was calculated from the calibration curve of gallic acid and it was expressed as gallic acid equivalents/gram fresh weight. Each experiment has three replicates and the experiment was repeated thrice.

Total flavonoid content

Total flavonoid content was measured by aluminum chloride colorimetric assay described by Marinova *et al.,* (2005) and the result was expressed as quercetin equivalent per gram extract. One ml of plant extract was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3ml of 10% $AlCl_3$ was added. At 6^{th} min, 2 ml of 1 M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. The total flavonoid content in plant extracts was calculated from the calibration curve of quercetin and it was expressed as quercetin equivalents/gram fresh weight. Each experiment has three replicates and the experiment was repeated thrice.

Protein content

Total protein was estimated by the method of Lowry *et al.,* (1951) with Bovine Serum Albumin as standard. One ml of 20% TCA was added to 1ml of extract. The pellet was washed twice with acetone and again centrifuged at 8000 rpm for 5 min and the pellet was dissolved in 5ml of 0.1 N NaOH. This was used for protein estimation. A standard graph was constructed by taking standard BSA (10µg-100µg/ml). To 1ml extract 5ml of alkaline copper sulphate was added, mixed thoroughly

and incubated for 30 min at room temperature. Then 0.5ml of Folin-Ciocalteu reagent was added. Contents were mixed and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 660 nm. The amount of protein in the extract was determined using standard graph. Each experiment has three replicates and the experiment was repeated thrice.

Statistical analysis

Each experiment has three replicates and the experiment was repeated thrice and the data was subjected to one way ANOVA using Minitab version 15. A significance level of 0.05 was used for all statistical tests.

Results and discussion

Phytochemical analysis

The phytochemical analysis of leaf and callus extracts of *H. suaveolens* showed the presence of phenolics, flavonoids and proteins (Table 1). Quantitative analysis of leaf extract of *H. suaveolens* contains the phenolic content (92.77±2.04 mg/gram), flavonoids (74.99±2.72 mg/gram) and protein content (56.18±2.18 mg/gram). Similarly, the callus extract contains phenolic content (72.31±1.16 mg/gram), flavonoids (62.36±3.64 mg/gram) and protein content (61.32±1.37 mg/gram) (Table 1).

Table 1. Qualitative and quantitative analysis of secondary metabolites in leaf and callus extracts of *H. suaveolens*

Name of the phytochemicals	Qualitative analysis		Quantitative analysis	
	Leaf extract	Callus extract	Leaf extract (mg/gram)	Callus extract (mg/gram)
Phenols	+ve	+ve	92.77±2.04*	72.31±1.16*
Flavonoids	+ve	+ve	74.99±2.72*	62.36±3.64*
Alkaloids	−ve	−ve	-	-
Terpenoids	−ve	−ve	-	-
Tannins	−ve	−ve	-	-
Saponins	−ve	−ve	-	-
Proteins	+ve	+ve	56.18±2.18*	61.32±1.37*
*Each value represents Mean ± SE of three independent experiments				

The results of phytochemical analysis of leaf and callus extract of *H. suaveolens* showed the presence of phenols, flavonoids and proteins. Phenolics are the abundant secondary metabolites synthesized by the plants under stress conditions in order to adopt and survive the environmental conditions (Dai and Mumper, 2010). Phytochemicals are the non-nutrient compounds produced by the plants in self-defence to protect them from biotic and abiotic stress. The pharmacological value of a plant is due to the presence of phytochemical present within this plant. These diverse secondary metabolites produced by the plants have ability to produce specific pharmacological responses making particular plant species a potential source of new medicine (Cragg and Newman, 2013).

Phenolics possess antioxidant property by acting as reducing agent, free radical scavenging activity, metal ion chelator, reduce lipid peroxidation, prevent DNA damage, and scavenging reactive oxygen species (Oscar *et al*., 2020). Lamiaceae family members contain diverse polyphenolic compounds such as rosmarinic acid, methyl rosmarinate, caffeic acid, vanillic acid, p-coumaric acid and ferulic acid (Mishra *et al*., 2021). Polyphenolics exhibit the strong antioxidative, hepatoprotective, cytoprotective and antimicrobial activity (Ghaffari *et al*., 2012; Pham *et al*., 2018).

Flavonoids (flavones, flavonol, isoflavone and chalcone anthocyanidins) are naturally occurring plant phenolics and contains phenyl-benzo-pyrone sytem (Evan, 2009). They are the important bioactive chemical constituents and exhibit the wide range of biological activities. It possesses anticancer activity by promoting apoptosis and thereby binds to the carcinogenic enzymes (Mishra *et*

al., 2021). They also exhibits the anti-inflammatory activity by modulating gene expression, thus produces inflammation inducers like cytokines, chemokines and eicosanoids (Choy *et al*., 2019). Flavonoids act as antioxidants by free radical scavenger and chelating metal ions by inhibiting the microsomal monoxygenase, glutathione-S-transferase and NADH oxidase (Wang *et al*., 2018). The flavonoids in *H. suaveolens* are gallic acid, ferulic acid, quercetin, chlorogenic acid, rutin and the content ranges from 10-13% (Asha *et al*., 2015).

Conclusion

The present study concludes that the leaf and callus extract of *H. suaveolens* contains the phenolic compounds and flavonoids. Presence of these two phytochemicals may be responsible for its therapeutic values. Further studies are needed to establish the therapeutic potential of phytochemicals from *H. suaveolens*.

Acknowledgments: The authors acknowledge the Head, Department of Biochemistry, Andhra University for providing the facilities to carry out the research work.

Conflict of interest: The authors have no conflict of interest.

References

1. Asha, D., Mathew, L. and Rishad, K.S. 2015. Evaluation of HPTLC fingerprints of flavonoids and antioxidant activity of selected medicinal plants of Lamiaceae Family. International Journal of Pharmacognosy and Phytochemical Research, 7(2): 240-245.
2. Batiha, G.E.S., Beshbishy, A.M., Alkazmi, L., Adeyemi, O.S., Nadwa, E., Rashwan, E. and Igarashi, I. 2020. Gas chromatography-mass spectrometry analysis, phytochemical screening and antiprotozoal effects of the methanolic *Viola tricolor* and acetonic *Laurus nobilis* extracts. Bmc Complementary Medicine and Therapies, 20: 87.
3. Botcha, S. and Prattipati, S.D. 2019. Green synthesis of silver nanoparticles using *Hyptis suaveolens* (L.) Poit leaf extracts, their characterization and cytotoxicity evaluation against PC-3 and MDA-MB 231 cells. Biologia, 74(7): 783-793.
4. Botcha, S. and Prattipati, S.D. 2020. Callus extract mediated green synthesis of silver nanoparticles, their characterization and cytotoxicity evaluation against MDA-MB-231 and PC-3 cells. BioNanoScience, 10: 11-22.
5. Choy, K.W., Murugan, D., Leong, X.F., Abas, R., Alias, A. and Mustafa, M.R. 2019. Flavonoids as natural anti-inflammatory agents targeting nuclear factor-kappa B (NFκB) signaling in cardiovascular diseases: a mini review. Frontiers in Pharmacology, 10: 1295.
6. Cragg, G.M. and Newman, D.J. 2013. Natural products: a continuing source of novel drug leads. Biochimica et Biophysica Acta (BBA)-General Subjects, 1830(6): 3670-3695.
7. Dai, J. and Mumper, R.J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15(10): 7313-7352.
8. Evans, W.C. 2009. Flavone and related flavonoid glycosides, Trease and Evans Pharmacognosy. 16th Edition. Saunders Elsevier Publication, International Edition (Edinburg, London, New York, Philadelphia, St. Louis, Sydney). pp. 252–259.
9. Ghaffari, H., Ghassam, B.J. and Prakash, H.S. 2012. Hepatoprotective and cytoprotective properties of *Hyptis suaveolens* against oxidative stress–induced damage by CCl4 and H2O2. Asian Pacific Journal of Tropical Medicine, 5(11): 868-874.
10. Iqbal, E., Salim, K.A. and Lim, L.B. 2015. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. Journal of King Saud University-Science, 27(3): 224-232.

11. Jesus, N.Z.T., Falcão, H.S., Lima, G.R.M., Caldas Filho, M.R.D., Sales, I.R.P., Gomes, I.F. and Batista, L.M. 2013. *Hyptis suaveolens* (L.) Poit (Lamiaceae), a medicinal plant protects the stomach against several gastric ulcer models. Journal of Ethnopharmacology, 150(3): 982-988.

12. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry, 193: 265-275.

13. Marinova, D., Ribarova, F. and Atanasoval, M. 2005. Total phenolics and flavonoids in Bulgarians vegetables. Journal of the University of Chemical Technology and Metallurgy, 40(3): 255-260.

14. Mishra, P., Sohrab, S. and Mishra, S.K. 2021. A review on the phytochemical and pharmacological properties of *Hyptis suaveolens* (L.) Poit. Future Journal of Pharmaceutical Sciences, 7: 65.

15. Oladeji, O.S., Odelade, K.A. and Oloke, J.K. 2020. Phytochemical screening and antimicrobial investigation of *Moringa oleifera* leaf extracts. African Journal of Science, Technology, Innovation and Development, 12(1): 79-84.

16. Oscar, S.A., Antonio, C.N., Marina, G.V., Elsa, R.S. and Gabriel, V.A. 2020. Phytochemical screening, antioxidant activity and in vitro biological evaluation of leave extracts of *Hyptis suaveolens* (L.) from south of Mexico. South African Journal of Botany, 128: 62-66.

17. Pham, H.N.T., Sakoff, J.A., Van Vuong, Q., Bowyer, M.C. and Scarlett, C.J. 2018. Screening phytochemical content, antioxidant, antimicrobial and cytotoxic activities of *Catharanthus roseus* (L.) G. Don stem extract and its fractions. Biocatalysis and Agricultural Biotechnology, 16: 405-411.

18. Sadasivam, S. and Manickam, A. 1996. Biochemical Methods, Second edition, New Age International (P) Ltd Publishers, New Delhi. pp: 108-110.

19. Sharma, T., Pandey, B., Shrestha, B.K., Koju, G.M., Thusa, R. and Karki, N. 2020. Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination. Tribhuvan University Journal, 35(2): 1-11.

20. Sithara, N.V., Komathi, S., Rajalakshmi, G., Queen, J. and Bharathi, D. 2016. Phytochemical analysis of *Andrographis paniculata* using different solvents. European Journal of Biotechnology and Bioscience, 4(8): 28-30.

21. Sumitha, K.V. and Thoppil, J.E. 2016. Genotoxicity assessment of two common curing weeds: *Hyptis suaveolens* (L.) Poir. and *Leucas indica* (L.) R. Br. Cytotechnology, 68: 1513-1527.

22. Wang, T., Qing, L. and Bi, K. 2018. Bioactive flavonoids in medicinal plants; Structure, activity and biological fate. Asian Journal of Pharmaceutical Sciences, 13(1): 12-23.

Citation: Satyanarayana, B. and Subhashini Devi, P. 2021. Phytochemical Studies in Leaf and Callus Extracts of *Hyptis suaveolens* L. a Medicinally Important Plant. International Journal of Recent Innovations in Academic Research, 5(12): 71-75.