International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 9 | Special Issue 2| ICAM-2019 | 145-148
Proceedings of International Seminar on "Agricultural Microbiome"
Held at Hindustan College of Arts and Science, behind Nava India, Coimbatore, Tamil Nadu 641028, India, 22nd February 2019
| Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

Extraction of Phytochemical from the Leaf and Stem of *Plectranthus amboinicus* using Different Parameters and their Antioxidant Activity

Sruthi Radhakrishnan*[1], K. Divya[1], N. Karthik[1], A. Madhumitha[1], J. Meenambigai[1] and M. Santhoshkumar[1]
[1]PG and Research Department of Biotechnology, Hindusthan College of Arts and Science (Autonomous), Coimbatore.

Received: 25 Feb 2019 / Accepted: 20 Mar 2019 / Published online: 01 Apr 2019
Corresponding Author Email: radhakrishnan.sruthi4@gmail.com

Abstract
Ethanolic and aqueous extract of leaves and stems of *Plectranthus amboinicus* was prepared by extracting at four different time periods. The preliminary phytochemical analysis showed the presence of alkaloids and further quantitative estimation of alkaloids proved higher concentration of alkaloids in ethanolic extract. The antioxidant efficacy of the leaf an stem ethanolic extracts was calculated with equivalent to ascorbic acid and 16th hour leaf ethanolic extract showed highest percentage of DPPH scavenging activity.

Keywords
Plectranthus amboinicus, phytochemical, alkaloids, antioxidant, DPPH.

*****

INTRODUCTION:
Nowadays, there are revivals of interest with herbal-based medicine due to the increase realization of the health hazard associated with the in discriminate use of modern medicine. Medicinal plants are richest bio-resource of drugs of traditional systems of medicine, modern medicines, natural food supplements, nutraceuticals, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drug (Cube *et al*., 2008).Phytochemicals are non-nutritive plant chemicals that have protective properties (Nisha and Radhamany, 2010).Some of the phytochemicals produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al*., 2005).

The extraction method for various phytochemical compounds differs from plant to plant and an ideal extraction method for a particular phytoconstituent source has to be individually designed and optimized (Silva *et al*., 2007).The ever increasing commercial need for medicinal plant based products has led to overexploitation and unscientific extraction of medicinal plants causing their extinction (Pushpangadan ,2004).Therefore the study deals with developing an effective and cheaper extraction protocol for a specific medicinal herb species.

The evaluation of various plant products according to their therapeutic efficacy leads to the discovery of newer and recent drugs for treating various ailments. One of such plant of medicinal value is *Plectranthus amboinicus*, belonging to the family Lamiaceae, commonly 2 known as 'Country borage' (Banu *et al*., 2013).

The present study deals with extracting phytochemicals present in *Plectranthus amboinicus* leaf and stem using different parameters like solvents of varying polarity and four different time of extraction and also determining their antioxidant activity.

MATERIALS AND METHODS:

Sample collection

The leaf and stem of Plectranthus amboinicus were collected from Sungam, Coimbatore, TamilNadu, India situated between latitude 10.6328 and longitude 77. 1027.The plant material were cleaned and stored in tightly closed container till the extraction process.

Extraction preparation

The leaves and stems were trimmed and weighed. 1 gram of the plant material was extracted with water and ethanol at varying time period of 4,8,16 and 24 hours and kept in shaker. Filtered before further use and storage.

Preliminary analysis

01. Test for Alkaloids Wagner's test:0.5ml of leaf extract was treated with Wagner's test reagent (1.27g of Iodine and 2g of potassium iodide in 100ml of water) and the formation of reddish brown colour indicates the presence of alkaloids (Wagner, 1993).

02.Test for Flavonoids Sulphuric acid (H2SO4) test: A fraction of extract was treated with concentrated H2SO4 and the formation of orange colour indicates the presence of flavonoids (Khandelwal, 2000)

03.Test for Tannins Braymer's test:0.5ml of extract was treated with 10% alcoholic FeCl3solution and the formation of blue or greenish colour tannin the presence of tannins (Treare and Evans, 1985).

04.Test for Saponins Foam test:0.5ml of extract was shaken with water and the formation of Persistent foam indicates the presence of saponins (Kumar *et al*., 2009).

05. Test for Quinones 0.5ml of extract was treated with concentrated hydrochloric acid (HCL) and the formation of yellow colour precipitate indicates the presence of quinines (Khandelwal, 2000).

06. Test for Sterols Sulphuric acid (H2SO4) test:0.5ml of extract was treated with ethanol and sulphuric acid (H2SO4) and the formation of violet, blue or green colour indicates the presence of sterols (Finar, 1996).

07. Test for Phenols Ferric chloride test:0.5ml of extract was treated with 5% ferric chloride and the formation of deep blue or black colour indicates the presence of phenols (Mace, 1963).

08.Test for Anthocyanin Sodium hydroxide (NaOH) test:0.5ml of extract was treated with 2M NaOH and the formation of blue green colour indicates the presence of anthocyanins (Paris and Moyse, 1969).

09. Test for Proteins Ninhydrin (acetone) test:0.5ml of the extract was treated with ninhydrin solution (Ninhydrin dissolved in acetone) and the formation of purple colour indicates the presence of proteins (Yasuma and Ichikawa, 1953).

10. Test for carbohydrates Fehling's test: 0.5g of the extract was dissolved in distilled water and filtered. The filtrate was heated with 5ml of equal volumes of solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars (Ramakrishnan *etal*., 1994). Solution A: 0.1M Copper (II) sulphate penta hydrate Solution B: 0.1M Sodium potassium tartarate and 3 M NaOH.

11. Test for terpenoids 5ml of methanolic extract is mixed with 2ml of chloroform in a test tube. 3ml of concentrated sulphuric acid (H2SO4) is carefully added to the mixture to form a layer. An interface with a reddish brown colouration is formed (Ayoola *et al*., 2008).

12. Test for steroids 1ml of the extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turned sulphuric acid layer showed yellow with green fluorescence indicates the presence of steroids (Ayoola *et al*., 2008).

Quantitative estimation of alkaloids

A part of extract residue was dissolved in 2N HCL and then filtered.1ml of this solution was transferred to separatory funnel and washed with 10ml chloroform (3 times).The pH of this solution was adjusted to neutral with 0.1N NaoH. Then 5ml of BCG solution and 5ml of phosphate buffer were added to this and absorbance was read at 470nm. Concentration of alkaloids was determined as mg with atropine equivalent (mg AE).

Antioxidant activity- DPPH assay

The free radical scavenging activity of the plant sample was determined by DPPH assay using ascorbic acid as standard following the protocol from Bhatt and Negi, 2012, with slight modification. About 0.1mM solution of DPPH (1, 1-Dipheny- 2-Picrylhydrazyl) in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (20-100 mg /ml). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100mg/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation: DPPH Scavenged (%) = [(A control– A test) / A control] × 100

Where a control is the absorbance of the control reaction and a test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC50 and compared with standard.

RESULTS AND DISCUSSION:

1. Preliminary analysis

To determine the chemical constituents, qualitative phytochemical screening of the aqueous stem bark extract of Plectranthus amboinicus was carried out with various standard procedures routinely used in the laboratory (Tchamadeu et al., 2010).From the preliminary qualitative analysis of the ethanolic and aqueous extracts leaf and stem at varying extraction time of *Plectranthus amboinicus*, only alkaloids were found to present.

1.1 Table showing the results of Phytochemical analysis of the extracts prepared using ethanol and water at varying time period of four, eight, sixteen and twenty-four hours.

S.No	Phytochemicals	Ethanolic extract				Aqueous extract			
		4th hr	8th hr	16th hr	24th hr	4th hr	8th hr	16th hr	24th hr
1.	Alkaloids	+	+	+	+	+	+	+	+
2.	Flavonoid	-	-	-	-	-	-	-	-
3.	Tannins	-	-	-	-	-	-	-	-
4.	Saponins	-	-	-	-	-	-	-	-
5.	Quinines	-	-	-	-	-	-	-	-
6.	Sterols	-	-	-	-	-	-	-	-
7.	Phenols	-	-	-	-	-	-	-	-
8.	Proteins	-	-	-	-	-	-	-	-
9.	Carbohydrates	-	-	-	-	-	-	-	-
10.	Terpenoids	-	-	-	-	-	-	-	-
11.	Steroids	-	-	-	-	-	-	-	-
12.	Anthocyanin	-	-	-	-	-	-	-	-

Quantitative estimation of alkaloids:

Chemical investigation of Plectranthus sps started more than 100 years ago, yet new compounds are still being discovered. There are a variety of compounds with different carbon skeletons, some of which have been considered unique to the genus. A broad classification of these components is given below along with the special features of each group (Seshadri 1971).

The present study of P. amboinicus determined for the secondary metabolite, alkaloid which are present in the screening analysis and estimated with atropine standard. The ethanolic leaf and stem extract for all the extraction time period showed highest concentration of alkaloid compared to aqueous extracts.

1.2 Table showing the results of Quantitative Analysis

Each of this value were expressed in mg equivalent to standard atropine (mg AE)

Solvent system	4th hr		8th hr		16th hr		24th hr	
	L	S	L	S	L	S	L	S
Ethanolic Extract	51.5mg	48.3mg	50.1mg	52.7mg	59.4mg	50.2mg	51.1mg	43.4mg
Aqueous Extract	37.1mg	29.6mg	18.6mg	26.0mg	11.9mg	9.5mg	27.7mg	31.0mg

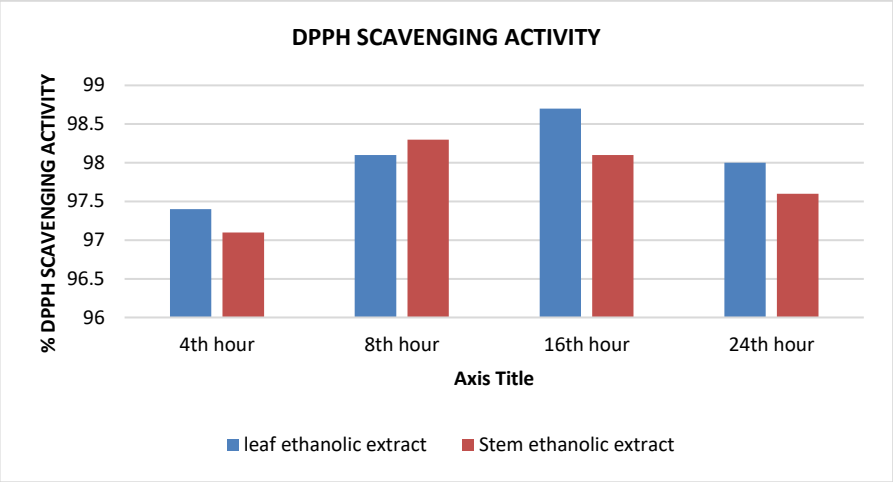
Antioxidant activity

Free radicals and oxidative stress involved in many disorders like neurodegenerative diseases, cancer and AIDS. They can be targeted for the destruction, regulate oxidative process involved in the signal transduction also can effect in the gene expression, the pathways of the cell proliferation and cell death. Antioxidants through their scavenging power are useful for the management of those diseases (Pratap et al., 2004). The present model of scavenging the stable DPPH radical is widely used method to evaluate the free radical scavenging ability of various samples. DPPH is stable nitrogen centred free radical where the colour changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers.

Antioxidant activity was determined for the ethanolic extracts and it was found that the 16hour extract showed highest percentage of DPPH scavenging

activity in the range 97-99% compared to aqueous extracts.

1.3 Bar Chart showing DPPH scavenging activity of the ethanolic extract



CONCLUSION:

This study aimed at identifying appropriate extraction parameters for optimized yield of phytochemicals present in the leaves, stem of *Plectranthus amboinicus*, a medicinally important herb. Solvents of varying polarities, time of extraction were the parameters selected for optimization. Ethanol and aqueous extracts were prepared, and the preliminary phytochemicals showed the presence of alkaloids. Further quantitative estimation for alkaloid proved that ethanolic extracts yielded higher concentration of alkaloids. The antioxidant efficacy of leaf and stem ethanolic extracts was calculated with equivalent to standard ascorbic acid and it was found that 16th hour leaf ethanolic extract showed highest % of DPPH scavenging activity, indicates that these natural antioxidants have potential advantages to control diseases with oxidative stress.

This study thus:

1. Enabled developing new extraction protocols for optimizing yield of phytochemical especially alkaloid from *Plectarnthus amboinicus*.
2. Confirmed the presence of antioxidant activity of this plant.

   This paves way for future studies on this plant for isolation and identification of phytochemicals with improved, advanced and economic extraction methods.

REFERENCES:

[1] Ayoola, G.A, Coker, H.A.B, Obaweya, K (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy. Trop journal of Pharm. Res., 7: 1019-1024.

[2] Edeoga, H.O., Okwu, D.E., (2005). Phytochemical constituents of some Nigerian Medicinal plants. Afr. J. Biotechnol, 4(7): 685-688.

[3] Finar, I.L. (1996). Stereo chemistry and the chemistry of natural product. Journal of Organc chemistry. 2:518.

[4] Khandelwal. (2000). Pharmacognosy: techniques and experiments Nirali Prakashan. Asian Journal of Pharmaceutical Research. 40: 146-161.

[5] Kumar, A., Ilavarasan, R., Jayachandran, T., Krishna. V. (2009). Phytochemical investigation on tropical plants. Pakistan Journal of Nutrition, 8: 83-85.

[6] Mace, M.D. (1963). Histochemical localization of phenols in healthy and diseased tomato roots. Phytopathology. 16: 915-925.

[7] Nisha Raj, R.S and Radhamany, P.M. (2010). Preliminary phytochemical and in vitro are antioxidant properties of brunfelsia Americana. Journal of Pharmacy Research, 3(11): 271292713.

[8] Ramakrishnan, S.K., Prasannan, G., and Rajan, R. (1994). Textbook of medical biochemistry. Grient longman, New delhi, India. 582.

[9] Yasuma, A., and Ichikawa, T. (1953). Ninhydrin-Schiff and alloxan- Schiff staining; a new hitochemical staining method for protein. J. Lab Clin Med. 41(2): 296-9