



PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *PLECTRANTHUS AMBOINICUS*

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Abstract:

Medicinal plants have evolved over the centuries as essential parts of African civilization and are widely recognized today as it representing its rich cultural and scientific heritage. The increasing demand for medicinal plant products has renewed interest in the pharmaceutical industry in the production of herbal healthcare simulations, herbal-based cosmetic products, and herbal nutritional supplements. In the present study, reveals the importance of *Plectranthus amboinicus*. The aqueous stem and leaf extracts of *Plectranthus amboinicus* were subjected to phytochemical screening analysis. The findings of phytochemical screening analysis were analysed. The antioxidant activity of aqueous stem and leaf extracts of *Plectranthus amboinicus* was examined using DPPH Radical Scavenging Assay. The results showed reveals that the electron gets paired off, in presence of free radical scavengers the absorption fades and the resulting decolourization is in line with the number of electrons taken up. The findings showed that there is a significant antioxidant activity in a dose dependent manner in the samples subjected to Radical Scavenging activity.

Key words: civilization, screening, cosmetic, absorption, phytochemical

Introduction:

A medicinal plant is that species of the plant kingdom, whose parts (flowers, leaves, roots, stem, fruits, or seeds) are directly used or used in some preparation as a medicine to treat a condition or disease, stress, anxiety and anger, improve socialization, and practice goal setting for recovery ^[1]. Medicinal plants may provide three main kinds of benefit: health benefits to the people who consume them as medicines; financial benefits to people who harvest, process, and distribute them for sale; and society-wide benefits, such as job opportunities, taxation income, and a healthier labour force^[2]. Traditional medicinal plants are a major source of bioactive natural products that produce new chemical entities with potential therapeutic applications. Traditional knowledge leads to the discovery of new bioactive products that are achieved through the bioassay-guided screening of extracts through in-vitro, in-vivo, and in - silicon assays^[3]. Phytochemicals are naturally present in the plants and shows biologically significance by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms^[4]. Natural antioxidants are widely distributed in food and medicinal plants. These natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, including anti-inflammatory, anti-aging, anti- atherosclerosis and anti-cancer^[5].

Description of *Plectranthus amboinicus*:

Plectranthus amboinicus (Lamiaceae) and plants belonging to the genus Mexican mint are common in the Indian Ayurveda system of medicine. It has long been used as traditional medicine in rural and tribal areas in many countries ^[6].



Fig 1.1 Represents *Plectranthus amboinicus*

Medicinal uses of *Plectranthus amboinicus*:

It is widely used in folk medicine to treat conditions like cold, asthma, constipation, headache, cough, fever and skin diseases. The leaves of the plant are often eaten raw or used as flavouring agents or incorporated as ingredients in the preparation of traditional food. It has been found to be effective against respiratory, cardiovascular, oral, skin, digestive and urinary diseases. *Plectranthus amboinicus* is very commonly used in Unani, Ayurveda, Siddha, Folk and other traditional practices of healthcare management^[7]. *Plectranthus amboinicus* have analgesic, anti-inflammatory, antibacterial, and anti-ulcer properties. Different parts of *plectranthus* species (stems, leaves, roots, and tubers) are used to treat various ailments^[8].

Experimental methods:

Extraction of extracts from different solvents:

Decoction:

The dried stem and leaves of *Plectranthus amboinicus* was boiled with 300 ml distilled water in a 500 ml beaker for 45 minutes. The volume is then brought down to one-fourth of its original volume by boiling during the extraction procedure. Then the concentrated extract is filtered and used for further process^[9].

Preliminary phytochemical screening analysis:

a) Determination of Alkaloids:

About 3 ml of concentrated extract was taken in a test tube and 1 ml of HCl was added to the mixture which is heated gently for 20 minutes and is then cooled and filtered. The filtrate was used for the following tests.

i) Wagner's test:

The filtrate was treated with Wagner's reagent (solution of iodine in potassium iodide). Formation of a brown reddish precipitate indicates the presence of the alkaloids^[10].

b) Test for Saponins:

i) Froth test:

Exactly 0.5 g of the extract was dissolved in 2ml distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the saponins^[11].

ii) Foam test:

5 ml of the extracts were mixed with 20 ml of distilled water and then agitated in a graduated cylinder for about 15 minutes. Formation of foam indicates the presence of saponins.

c) Test for phenols:

Ferric chloride test:

Extracts were treated with 4 drops of alcoholic FeCl₃ solution. Formation of bluish black colour indicates the presence of phenols^[12].

d) Test for Proteins:

Xanthoprotein test:

The extracts were treated with few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins^[13].

e) Test for Glycosides:

i) Glycoside test:

0.5 mg of the extract was dissolved in 1 ml of water and then the aqueous NaOH solution was added to the extract. Formation of yellow colour indicates the presence of glycosides^[14].

ii) Concentrate H₂SO₄ test:

To 5ml of the extract, add 2ml of glacial acetic acid, one drop of 5% FeCl₃ and conc. H₂SO₄. Appearance of a brown ring indicates the presence of glycosides^[15].

f)Test for Tannins:**Lead acetate test:**

To the extracts few drops of 10% lead acetate solution were added. Formation of precipitate indicates the presence of tannins ^[16].

g)Test for coumarins:

10% NaOH (1ml) was added to 1 ml of the plant extracts. Formation of yellow colour indicates the presence of coumarins ^[17].

h)Test for Flavonoids:**i)Pew's test:**

To two ml of the extract zinc powder was added in a test tube, followed by drop wise addition of concentrated HCl. Formation of purple red or cherry colour indicates the presence of flavonoids.

ii) NaOH test:

To two ml of the extract sodium hydroxide were added in a test tube. Formation of intense yellow colour that becomes colourless on addition of few drops of dilute HCl indicates the presence of flavonoids ^[18].

i)Test for carbohydrates:**Molisch's test:**

To two or three ml of the aqueous extract two drops of alpha naphthol solution in alcohol is added and shaken well. Then add conc. sulphuric acid from the sides of the test tube. Violet ring formation indicates the presence of carbohydrates ^[19].

j)Detection of resins:**Acetone - water Test:**

Extracts were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins^[20].

k)Test for β-cyanin:

To 1 ml of the extract add 1 ml of 2N NaOH. The solution mixture is heated for 1 minute in 100°C. The formation of yellow colour indicates the presence of β-cyanin^[21].

Antioxidant activity of *Plectranthus amboinicus*:

The antioxidant activity of aqueous stem and leaf extracts of *Plectranthus amboinicus* was examined using DPPH Radical Scavenging Assay ^[22].

Radical scavenging activity of plant extracts against stable 2, 2, diphenyl 2- picrylhydrazyl hydrate (DPPH) was determined by the slightly modified method. DPPH reacts with an antioxidant compound which can donate hydrogen and reduce DPPH. The change in color (from deep violet to light yellow) was measured at 515nm on a UV visible light spectrophotometer. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the concentration (1μg/ 1000μl). The solution of DPPH in methanol 60μM was prepared fresh daily before UV measurements. This solution (3.9 ml) was mixed with 100 μl of test solution at concentrations (25, 50,100 and 200 μg). The samples were kept in dark for 15 minutes at room temperature and the decrease in absorbance was measured. The experiment was carried out in triplicate, Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank. Radical scavenging activity was calculated by the following formula

% Inhibition= (Absorbance of Control at 0 minute – Absorbance of Test)/ Absorbance of control at 15 minutes × 100

Where, C- absorption of control sample (t=0 min), C= absorption of control (t=15 min), T- absorption of test solution.

Table 1.1 Preliminary phytochemical screening Analysis of *Plectranthus amboinicus* Leaf:

Name of the phytochemical	Name of the test	A
Alkaloids	Wagner's test	+
Saponins	Froth test	+
	Foam test	+
Proteins	Xanthoprotein test	+
Glycosides	Glycoside test	+
	Concentrate sulphuric acid test	+
Tannins	Lead acetate test	+
Coumarins	10%NaOH+1ml plant extract	+
Flavonoids	Pew's test	-
	NaOH test	-
Carbohydrates	Molisch's test	-
Phenol	Ferric chloride test	+
Resins	Acetone - water Test	+
β - Cyanin	1 ml of the extract + 1 ml of 2N NaOH	+

Where, A- Aqueous extract, + indicates present, - indicates absent

Table 1.1 Represents the phytochemicals present in aqueous extract of *Plectranthus amboinicus* leaf. The phytochemical analysed for flavonoids and Carbohydrates show negative results.

Table 1.2 Preliminary phytochemical screening Analysis of *Plectranthus amboinicus* Stem:

Name of the phytochemical	Name of the test	A
Alkaloids	Wagner's test	=
Saponins	Froth test	+
	Foam test	+
Proteins	Xanthoprotein test	+
Glycosides	Glycoside test	+
	Concentrate sulphuric acid test	+
Tannins	Lead acetate test	+
Coumarins	10% NaOH+1ml plant extract	+
Flavonoids	Pew's test	+
	NaOH test	+
Carbohydrates	Molisch's test	+
Phenol	Ferric chloride test	+
Resins	Acetone - water Test	+
β - Cyanin	1 ml of the extract + 1 ml of 2N NaOH	+

Where, A- Aqueous extract, + indicates present, - indicates absent

Table 1.2 represents the phytochemicals present in aqueous stem extract of *Plectranthus amboinicus*. The phytochemical alkaloid was absent in aqueous stem extract of *Plectranthus amboinicus*. From the above results, the maximum numbers of phytochemicals are present in aqueous stem extract of *Plectranthus amboinicus*.

Antioxidant activity of *Plectranthus amboinicus*:

The antioxidant activity of *Plectranthus amboinicus* was examined by DPPH radical scavenging assay. The results are discussed in the table.

Table 1.3. DPPH radical scavenging assay of Aqueous extract of *Plectranthus amboinicus*

Sl. No	Sample Concentration (µg/mL)	% Inhibition	
		Stem	Leaf
1	25	21.559	24.061
2	50	36.669	31.857
3	100	49.734	49.74
4	200	54.12	62.714
	IC ₅₀	100.54 µg/mL	100.52 µg/mL

The Table 1.3 and Fig: represents the DPPH radical scavenging assay of Aqueous extract of leaf and stem extract of *Plectranthus amboinicus*. The samples were tested with DPPH, a free radical which is capable of producing violet colour. It reduces in presence of an antioxidant molecule giving rise to no colour, which is used to analyse the presence of antioxidant activity in extract. The polyphenols from the given samples exhibited a significant antioxidant activity in a dose dependent manner. The experimental results were very stable and produced reliable results in repeated tests, as the DPPH possess an odd electron giving away a strong absorption at 515 nm. As the electron gets paired off, in presence of free radical scavengers the absorption fades and the resulting decolourization is in line with the number of electrons taken up.



Fig 1.2 DPPH radical scavenging activity of Aqueous stem and leaf extract of *Plectranthus amboinicus*



Fig 1.3 Absorption fades in presence of free radical scavengers

Conclusion:

The Preliminary phytochemical screening was performed on aqueous of stem and leaf extract of *Plectranthus amboinicus*. The maximum numbers of phytochemicals were present in aqueous stem extract. Antioxidant activity was performed on aqueous extract of *Plectranthus amboinicus* using DPPH radical scavenging activity. The IC_{50} values of aqueous stem and leaf extract show a significant radical scavenging activity. Phenolic compounds present in the aqueous plant extract contribute significantly to their antioxidant potential because of their unique structure. There is a significant antioxidant activity in a dose dependent manner in the samples subjected to Radical Scavenging activity. From the above studies, it is found that the bioactive components are present more in aqueous stem extract of *plectranthus amboinicus*. The antioxidant study showed remarkable activity in dose dependent manner. Further studies should be extended for analysing the bioactive components, characterization of new separated compounds. This will be helpful for the pharmacological studies which result in the preparation of new herbal medicines.

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