



Phytochemical screening and comparative analysis of *in vivo* and *in vitro* derived plant parts of *Plectranthus amboinicus* (Lour.) Spreng.

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Abstract

Phytochemical screening or characterization is an important biochemical process for identifying the major phytoconstituents present in medicinal plants. *Plectranthus amboinicus* (Indian borage) is an important perennial herb that has been valued for its medicinal properties since ancient times. The goal of the current study was to evaluate the initial phytochemical makeup of different plant sections (leaves, roots, and stems) grown under *in vivo* (naturally grown) and *in vitro* (laboratory-grown) conditions. For this purpose, different solvents with varying polarities, namely water, methanol, acetone, petroleum ether, chloroform, and hexane were used. Phytochemical screening revealed that tannins, steroids, terpenoids, and glycosides were present in most of the extracts and across all plant parts, whereas carbohydrates, proteins, flavonoids, and phenols were predominantly detected in polar solvents. Alkaloids and ascorbic acid were found to be absent in all plant parts and across all extracts. A comparison between *in vivo* and *in vitro* plant parts revealed that *in vivo* plant parts contained a higher diversity and abundance of phytochemicals than *in vitro* plant parts and callus of *Plectranthus amboinicus*. The present study suggests that *P. amboinicus* is a rich source of phytoconstituents, particularly secondary metabolites, which may be exploited for future pharmacological and industrial applications. Although the study was primarily qualitative and intended for preliminary screening, it provides valuable insight for the targeted extraction of bioactive compounds and the medicinal utilization of this plant. Furthermore, the findings establish an important basis for future investigations aimed at the isolation and characterization of phytoconstituents from *P. amboinicus* for the development of novel therapeutic agents.

Keywords: *Plectranthus amboinicus*, Phytochemical screening, Secondary metabolite, Medicinal plants

Introduction

Preliminary phytochemical screening or characterization of medicinal plants may prove helpful in identifying bioactive compounds, and in the discovery of novel drugs. These investigations were conducted to determine the presence of various chemical groups in the extracts. *Plectranthus amboinicus* is a perennial herb belonging to the family Lamiaceae. It is commonly employed in traditional medicine to cure a variety of illnesses, and is grown as a decorative plant (Lukhoba *et al.*, 2006) [23]. This herb possesses numerous therapeutic qualities as a result of phytoconstituents that are valuable to the herbal industry (Sahu *et al.*, 2022) [35].

Previous ethnobotanical literature indicates that the plant possesses numerous pharmacological properties, including anticancer, antibacterial, antimicrobial, anti-inflammatory, antioxidant etc., and several other biological aspects (Arumugum *et al.*, 2016) [2]. Finding the chemical components of therapeutic plants requires preliminary phytochemical screening and provides a foundation for subsequent quantitative analyses. The bioactive potential and therapeutic properties of plants are closely associated with important metabolites. Universally, all plant species contain primary metabolites, which are necessary for plant growth, and development. These metabolites serve as building blocks for the production of a variety of secondary metabolites that have a range of pharmacological effects (Chen *et al.*, 2022) [9].

Secondary metabolites perform specialized functions in plants and play a crucial role in mediating interactions with other organisms, particularly through aromatic compounds involved in plant defense mechanisms (Kirk *et al.*, 2012) [20].

The phytochemicals, mainly secondary metabolites, include tannins, phenols, alkaloids, saponins, terpenoids, flavonoids, and other related compounds (Nonita and Mylene, 2010) [29]. However, the presence, concentration, and biological activity of plant bioactive constituents vary among plant parts and are influenced by factors such as climate, geographical location, and development (Swamy *et al.*, 2015) [42]. Systematic morphological overview of *in vitro* and *in vivo* plant parts of *Plectranthus amboinicus* (Lour.) Spreng has shown in figure-1. Phytochemical profiling of plant parts under the semi-arid conditions of Rajasthan remains limited; therefore, preliminary screening of phytochemicals under *in vivo* and *in vitro* conditions using different solvents is essential to support future drug discovery efforts.

Materials and methods

a. Estimation of extractive values

Percentage yield determination was carried out following the method described by Regina (2003) [33]. Different plant parts, including leaves, roots, and stems from both *in vivo* and *in vitro* materials, as well as callus of *Plectranthus amboinicus*, was used for the present investigation. *In vivo* plants were harvested at 3.5 months of age and separated into roots, leaves, and stems. *In vitro* plant parts (leaves, roots, and stems) were similarly cultured and harvested at 3.5 months of age. Callus tissue was collected at 8 weeks of age. All plant materials were placed on blotting paper, air-dried at room temperature (RT), and finely powdered. The powdered samples were then properly labeled, and stored under refrigerated conditions for further analysis. A known quantity of plant material (25 g) was weighed, and

extraction was performed using a Soxhlet apparatus for 36 hours with various solvents, including water, methanol, acetone, petroleum ether, chloroform, and hexane (250 mL). The solvents were selected based on their polarity. The obtained extracts were filtered to remove debris and collected in pre-weighed flasks. The solvents were then completely evaporated, and each flask's ultimate weight was noted. The percentage yields were calculated accordingly

$$\text{Extractive value (EV)} = \frac{\text{Extract weight}}{\text{Dried sample weight}} \times 100$$

A qualitative preliminary phytochemical screening was carried out on *Plectranthus amboinicus* using various solvent extracts prepared from both *in vivo* and *in vitro* plant materials. Extracts were obtained using H₂O, CH₃OH, CH₃COCH₃, CHCl₃, n-C₆H₆ and petroleum ether to ensure broad-spectrum extraction of phytoconstituents. The presence of bioactive substances including carbohydrates, starch, proteins, tannins, glycosides, steroids, terpenoids,

phenols, saponins, vitamin C (ascorbic acid), and flavonoids in the extracts was qualitatively examined. The study highlights the phytochemical diversity of *P. amboinicus* and provides a comparative overview of bioactive constituents present in *in vivo* and *in vitro* plant parts.

b. Extraction procedure

For extraction, *in vitro* and *in vivo* plant materials (leaves, stems, and roots) and callus tissues of *Plectranthus amboinicus* was shade-dried at RT. The *in vitro* and *in vivo* plants were 3.5 months old, while 8-week-old mature callus tissues were selected. Dried samples were powdered, and 5 g of each sample was extracted with 25 millilitres of various solvents (CH₃OH, CHCl₃, n-C₆H₆, CH₃COCH₃, petroleum ether and water) were used to extract 5 grammes of each sample in 1:5 ratio at RT(room temperature), following method of Savitharamma *et al.* (2011)^[37]. The extracts were filtered using Whatman No. 1 paper after being centrifuged for 15 minutes at 5000 rpm, and stored under refrigeration for subsequent qualitative phytochemical screening using procedures indicated in Table 1.



Fig 1: Comparison of *in vitro* and *in vivo* plant parts of *Plectranthus amboinicus* (Lour.) Spreng

(A) *In vitro*-derived dried leaves; (B) *In vivo*-derived dried leaves; (C) *In vitro*-derived dried roots; (D) *In vivo*-derived dried roots; (E) *In vitro*-derived dried stem; (F) *In vivo*-derived dried stem; (G) Callus tissue; (H) Four-month-old *in vitro*-grown *P. amboinicus* plant supplemented with the phytohormone 6-benzylaminopurine (BAP, 500 µg/L); (I) Field-grown plant

Table 1: Phytochemicals screening test procedure

S. No	Test	Procedure	Observations indicating positive test	References
Test for Carbohydrates				
1.	Fehling test	Equal amounts of Fehling's solutions A (0.5 ml) and B (0.5 ml) were added to 1 ml of the sample aliquot. After that, the mixture was heated to 60 °C in a water bath until a color shift was noticed.	Red or brick-red precipitate. Indicated reducing sugars	(Thusa and Mulami, 2017) [45].
	Benedict's test	To 1 ml of the test sample, 2 ml of Benedict's solution was added and the mixture was heated in a water bath for a few minutes. After cooling, the resulting color seen	Green, yellow, orange, or red. Indicated reducing sugars	(Khandelwal <i>et al.</i> , 2006) [19].
Test for Proteins				
2	Biuret test	An equivalent volume of 20% KOH solution was added to 1 ml of the aliquot and carefully mixed. One millilitre of a 0.5% copper sulphate solution was then gradually added.	Formation of blue/purple colour	(Siddiqui <i>et al.</i> , 2008) [40].
	Ninhydrin test	A few drops of 0.1% ninhydrin reagent (made by dissolving 0.1 g in 100 ml of acetone) and two to three drops of pyridine were added to one millilitre of the test solution. When the mixture was heated, the presence of proteins observed.	Development of blue color	(Siddiqui <i>et al.</i> , 2008) [40].
Test for Lipids				
3.	Dichromate test	Three millilitres of concentrated HNO ₃ were added after a few drops of a 5% K ₂ Cr ₂ O ₄ (potassium dichromate) solution were added to two millilitres of the test extract. The presence of glycerol in the test sample was confirmed	Blue colour	(Folch <i>et al.</i> , 1957) [11]
Test for alkaloids				
4.	Dragendorff's reagent	Dragendorff's reagent was added to a test tube containing one millilitre of the test solution.	An orange-red precipitate	(Kokate <i>et al.</i> , 2008) [21].
	Mayer's test	To 1 ml of the test solution, 1 ml of Mayer's reagent, a potassium iodide solution, was added.	Creamy white precipitate	(Talukdar and choudhary, 2010) [43].
Test for steroids				
5.	Libermann-Burchard test	One millilitre of the test solution was mixed with a few drops of acetic acid. Next, 1 millilitre of concentrated H ₂ SO ₄ was carefully added along the wall of the test tube.	formation of a brown ring or a green upper layer	(Kokate <i>et al.</i> , 2008) [21].
Test for Tannins				
6.	FeCl ₃ test	Two millilitres of the test solution were mixed with a few drops of a 5% FeCl ₃ solution for this test. The presence of tannins was detected by a colour shift	For tannins, gallotannins, and pseudotannins presence, colour shifts are observed from blue to olive green, green and brown colour respectively	(Talukdar and choudhary, 2010) [43].
	Lead acetate test	A few drops of a 10% lead acetate solution were added to two millilitres of the extract solution. A crimson or yellow precipitate developed when the sample included tannins.	The presence of tannins-crimson or yellow precipitate	(Khandelwal <i>et al.</i> , 2006) [19].
Test for glycosides				
7.	Molisch's test	1 ml of the test solution was mixed with two to three drops of Molisch's reagent, and then 2 ml of concentrated H ₂ SO ₄ was carefully added along the test tube's side.	Reddish-violet ring	(Molisch, 1896) [26].
Test for phenols				
8.	FeCl ₃ test	A small quantity of the test sample (0.5 ml) was taken in water, and 5% ferric chloride solution was added.	A violet color	(Kokate <i>et al.</i> , 2008) [21].
Test for saponins				
9.	Foam test	One millilitre of the test sample was placed in a test tube for the foam test, water was added, and the mixture was agitated vigorously for five minutes.	Persistent foam that formed after a few minutes	(Kokate <i>et al.</i> , 2008) [21].
Test for terpenoids				
10.	Salkowaski test	In a test tube, 0.5 ml of the aliquot and 2 ml of chloroform were combined for the terpenoids test. Next, gently add 3 millilitres of concentrated sulphuric acid along the test tube's side.	Reddish-brown layer formed at the contact	(Kokate <i>et al.</i> , 2008) [21].
Test for flavonoids				
11.	Lead-acetate test	One millilitre of lead acetate solution (made by dissolving five grammes of lead acetate in fifty millilitres of water) was added to the test solution.	Yellow precipitate	(Khandelwal <i>et al.</i> , 2006) [19].
	Alkaline reagent test	Drop by drop, 2% aqueous NaOH was added to 1 millilitre of the test solution.	Yellow hue	(Khandelwal <i>et al.</i> , 2006) [19].
12.	Test for vitamin C	Distilled water, 0.02 g of ferrous sulphate, and 0.1 g of sodium bicarbonate were added to the test solution in order for the ascorbic acid test. After giving the mixture a good shake, it was left to stand.	Violet colour	Sadasivam and Manickam (2008) [34]

Results

In the current research, the highest percentage yield was seen in the *in vivo* leaf extract (27.32%), followed by the *in vitro* leaf extract (19.74%). The lowest extractive value was observed in the *in vitro* stem extract (0.13%),

followed by the callus extract (0.22%) (Fig.2). By comparing the solvents, water and methanol showed higher extractive values in the leaf, root, and stem parts compared to the other solvents. Only the callus exhibited the highest extractive value (EV) with acetone (Fig.2D).

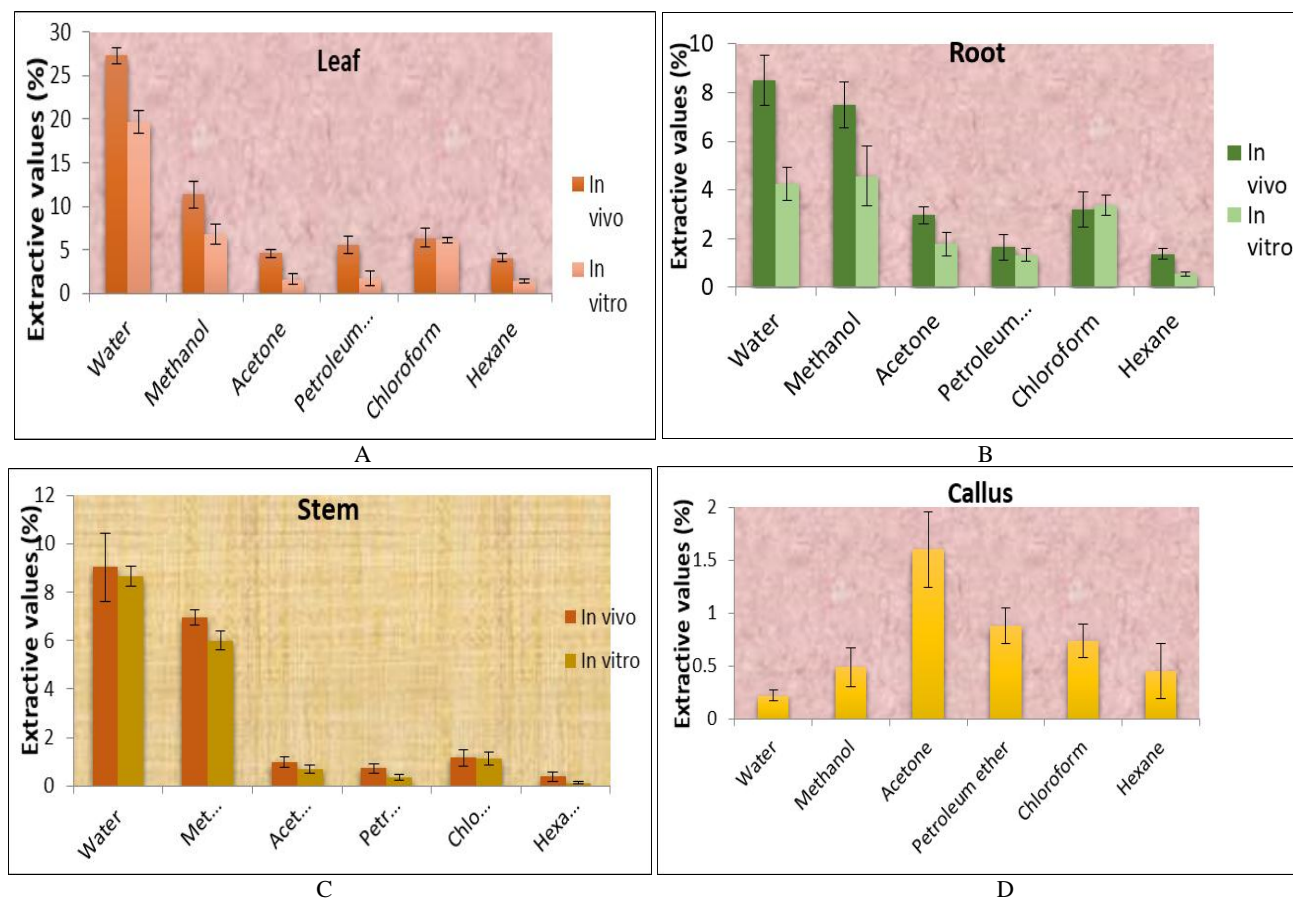


Fig 2: Extractive values of different *in vivo* and *in vitro* plant parts of *Plectranthus amboinicus* plant (A-D)

The preliminary screening or characterization of many phytochemicals was performed in the root, stem, and leaf (under *in vivo* and *in vitro* conditions), including callus (Tables 2–5).

1. Leaf

In the *in vivo* leaf sample, tannins and glycosides were predominantly found in all six extracts, whereas terpenoids and steroids were present in five extracts (except the water). Flavonoids were detected in four extracts (C_6H_6 , CH_3COCH_3 , H_2O and CH_3OH). Carbohydrates were found in the water, methanol, and chloroform extracts, while proteins and phenols were existing in the water and methanol extracts. H_2O and acetone extracts contained lipids, meanwhile saponins were found only in the chloroform extract (Table 2). Alkaloids and vitamin C were not

detected in any of the solvents in the *in vivo* leaf decoction. The *in vitro* leaf extract showed that glycosides were predominantly present in all six extracts, whereas steroids and terpenoids were present in five extracts (except the water extract). Tannins were found in four extracts (except PE and hexane). Carbohydrates were present in the water, methanol, and chloroform extracts, and flavonoids were detected in the water, methanol, and acetone extracts. Lipids were detected in methanol (CH_3OH), and chloroform ($CHCl_3$) extracts, while proteins and saponins were each detected in only one extract, i.e., water and chloroform, respectively. Alkaloids, phenols, and vitamin C were not detected in any of the solvents in the *in vitro* leaf samples (Table 2). From the overall observations of the leaf extracts, it was found that the *in vitro* and *in vivo* leaf extracts contained similar amounts of phytochemicals across all solvents.

Table 2: Qualitative preliminary phytochemical screening of leaf extracts of *Plectranthus amboinicus*

Phytochemicals	Testname	Solvents											
		Water		Methanol		Acetone		Petroleum ether		Chloroform		Hexane	
		<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>
Carbohydrates	Fehling's test	-	++	+	+	-	-	-	-	++	++	-	-
	Benedict test	+	+	+	+	-	-	-	-	+	+	-	-
Proteins	Biuret test	+	+	+	-	-	-	-	-	-	-	-	-
	Ninhydrin test	+	++	++	-	-	-	-	-	-	-	-	-
Lipids	Dichromate test	+	-	-	+	+	-	-	-	-	+	-	-
Alkaloids	Dragondrof test	-	-	-	-	-	-	-	-	-	-	-	-
	Mayer's test	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	Lead acetate test	+	++	++	+	+	+	+	-	+	+	+	-
Steroids	Libermann-Burchard test	+	-	++	+	++	++	+	+	+	+	++	+
Terpenoids	Salkowski test	-	-	++	++	+	+	+	+	+	+	++	+

Flavanoids	Alkaline reagent test	+	+	+	-	-	+	-	-	-	-	-	-
	Lead acetate test	++	+	+	+	+	+	-	-	-	-	+	-
Glycosides	Molish test	+	+	+	+	+	+	++	++	++	++	++	++
Phenols	FeCl ₃ test	+	-	+	-	-	-	-	-	-	-	-	-
Ascorbic acid	Ferrous sulphate test	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-	-	-	-	+	+	-	-

(- indicates absent; + indicates presence; ++ indicates remarkable amount)

2. Root

The phytochemical screening of *in vivo* root extract revealed that steroids, tannins, and glycosides were present in all six extracts, whereas terpenoids were detected in five extracts (except water) and flavonoids were found in the H₂O and CH₃OH extracts. Lipids were found in the water and acetone extracts, while there were carbohydrates in the methanol and chloroform decoctions. Saponins were only found in the chloroform (CHCl₃) decoctions, phenols and proteins were found in both aqueous and methanolic extracts. Alkaloids as well as vitamin C were not detected in any of the solvents in the *in vivo* root extract. The

phytochemical screening of the *in vitro* root extract showed that glycosides were present in all six extracts, whereas terpenoids and steroids were detected in five extracts (except water), and tannins were found in four extracts (except PE and hexane). Carbohydrates were present in the water, methanol, and CHCl₃ decoctions, lipids in methanol and chloroform, and flavonoids in water and acetone. Saponins were detected only in the chloroform extract. Alkaloids, phenols, and vitamin C were not detected in any of the solvents in the *in vitro* root extract (Table 3). Overall, the observations revealed that the *in vivo* root contained a higher number of phytochemicals compared to the *in vitro* root of *Plectranthus amboinicus* (Table 3).

Table 3: Qualitative preliminary phytochemical screening of root extracts of *Plectranthus amboinicus*

Phytochemicals	Testname	Solvents											
		Water		Methanol		Acetone		Petroleumether		Chloroform		Hexane	
		<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>
Carbohydrates	Fehling's test	-	++	+	+	-	-	-	-	++	++	-	-
	Benedict test	+	+	+	+	-	-	-	-	+	+	-	-
Proteins	Biuret test	+	+	+	-	-	-	-	-	-	-	-	-
	Ninhydrin test	+	++	++	-	-	-	-	-	-	-	-	-
Lipids	Dichromate test	+	-	-	+	+	-	-	-	-	+	-	-
Alkaloids	Dragondrof test	-	-	-	-	-	-	-	-	-	-	-	-
	Mayer's test	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	Lead acetate test	+	++	++	+	+	+	+	-	+	+	+	-
Steroids	Liebermann-Burchard test	+	-	++	+	++	++	+	+	+	+	++	+
Terpenoids	Salkowaski test	-	-	++	++	+	+	+	+	+	+	++	+
Flavanoids	Alkaline reagent test	+	+	+	-	-	+	-	-	-	-	-	-
	Lead acetate test	++	+	+	+	+	+	-	-	-	-	+	-
Glycosides	Molish test	+	+	+	+	+	+	++	++	++	++	++	++
Phenols	FeCl ₃ test	+	-	+	-	-	-	-	-	-	-	-	-
Ascorbic acid	Ferrous sulphate test	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-	-	-	-	+	+	-	-

(- indicates absent; + indicates presence; ++ indicates remarkable amount)

3. Stem

The phytochemical screening of the *in vivo* stem revealed that glycosides, tannins, and steroids were present in all six extracts, whereas terpenoids were detected in five extracts (except water), and flavonoids were found in the water and methanol extracts. Proteins and phenols were both detected in the H₂O and CH₃OH decoctions, while carbohydrates were present in the water, methanol, and CHCl₃ extracts. Lipids and saponins were detected in only one extracts each, i.e., water and chloroform, respectively. Alkaloids and vitamin C were not detected in any of the solvents in the *in vivo* stem extract, similar to the leaf and root extracts. The phytochemical screening of the *in vitro* stem extract showed

That glycosides were present in all six extracts, whereas terpenoids and steroids were detected in five extracts (except water). Tannins were found in the water, methanol, acetone, and chloroform extracts, and flavonoids were present in three decoctions i.e., CH₃OH, H₂O and CH₃COCH₃. Carbohydrates were also detected in three extracts, i.e., water, methanol, and chloroform. Proteins were present only in the H₂O extract, while lipids and saponins were detected only in the chloroform extract. Alkaloids, phenols, and vitamin C were not detected in any of the solvents in the *in vitro* stem extract (Table 4). Overall, the investigation revealed that *in vivo* stem parts contained a higher amount of phytochemicals compared to the *in vitro* plant parts.

Table 4: Qualitative preliminary phytochemical screening of stem extracts of *Plectranthus amboinicus*

Phytochemicals	Testname	Solvents											
		Water		Methanol		Acetone		Petroleumether		Chloroform		Hexane	
		<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>
Carbohydrates	Fehling's test	-	++	+	+	-	-	-	-	++	++	-	-
	Benedict test	+	+	+	+	-	-	-	-	+	+	-	-

Proteins	Biuret test	+	+	+	-	-	-	-	-	-	-	-	-
	Ninhydrin test	+	++	++	-	-	-	-	-	-	-	-	-
Lipids	Dichromate test	+	-	-	+	+	-	-	-	-	+	-	-
Alkaloids	Dragondrof test	-	-	-	-	-	-	-	-	-	-	-	-
	Mayer's test	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	Lead acetate test	+	++	++	+	+	+	+	-	+	+	+	-
Steroids	Libermann-Burchard test	+	-	++	+	++	++	+	+	+	+	++	+
Terpenoids	Salkowaski test	-	-	++	++	+	+	+	+	+	+	++	+
Flavonoids	Alkaline reagent test	+	+	+	-	-	+	-	-	-	-	-	-
	Lead acetate test	++	+	+	+	+	+	-	-	-	-	+	-
Glycosides	Molish test	+	+	+	+	+	+	++	++	++	++	++	++
Phenols	Fecl3 test	+	-	+	-	-	-	-	-	-	-	-	-
Ascorbic acid	Ferrous sulphate test	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-	-	-	-	+	+	-	-

(- indicates absent; + indicates presence; ++ indicates remarkable amount)

4. Callus

The phytochemical screening of callus demonstrated that terpenoids were present in the highest number of extracts, being detected in five extracts (except water), followed by steroids in four extracts (except water and hexane). Flavonoids were also found in four extracts, namely water, methanol, acetone, and PE, while glycosides were detected in four extracts: acetone, PE, chloroform, and hexane.

Carbohydrates were present in the water, methanol, and chloroform extracts, and tannins were detected in three extracts (water, methanol, and acetone). Lipids were present in two extracts, namely methanol and chloroform, and proteins were found only in the water extract. Alkaloids, phenols, vitamin C, and saponins were absent in all callus extracts (Table 5).

Table 5: Qualitative preliminary phytochemical screening of callus extracts of *Plectranthus amboinicus*

Phytochemicals	Testname	Solvents					
		Water	Methanol	Acetone	Petroleumether	Chloroform	Hexane
Carbohydrates	Fehling's test	+	++	-	-	++	-
	Benedict test	+	+	-	-	+	-
Proteins	Biuret test	+	-	-	-	-	-
	Ninhydrin test	+	-	-	-	-	-
Lipids	Dichromate test	-	+	-	-	+	-
Alkaloids	Dragondrof test	-	-	-	-	-	-
	Mayer's test	-	-	-	-	-	-
Tannins	Lead acetate test	+	+	+	-	-	-
Steroids	Libermann-Burchard test	-	++	++	+	+	-
Terpenoids	Salkowaski test	-	++	++	+	+	+
Flavonoids	Alkaline reagent test	+	-	-	-	-	-
	Lead acetate test	+	+	+	+	-	-
Glycosides	Molish test	-	-	+	+	+	+
Phenols	Fecl3 test	-	-	-	-	-	-
Ascorbic acid	Ferrous sulphate test	-	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-	-

(- indicates absent; + indicates presence; ++ indicates remarkable amount)

Discussion

Phytochemical screening or characterization is an important biochemical process for identifying the major phytoconstituents present in plant extracts. Qualitative tests or phytochemical screening is a scientific process in which analysis, examination, extraction, and experimentation are performed to identify different classes of phytochemicals. This process aids in the discovery of new drugs and active components that can be further investigated for their potential against various ailments or diseases, according to Sharma *et al.* (2020) [38]. Based on the metabolic activity of plants, phytochemicals are generally divided into two groups. Primary metabolites include sugars, amino acids, lipids, and proteins, that aid in the reproduction, growth and plant development. In present investigation, both aqueous and methanol extracts showed higher extractive values than the other solvents. The decoctions contain more polar phytochemicals like glycosides, phenols, and tannins, as

shown by the high extractive values of water and methanol (Baravalia *et al.*, 2011) [6].

In our study, carbohydrates and proteins were mainly found in the aqueous and methanolic extracts, indicating that polar solvents extracted more polar chemicals than non-polar solvents (Gupta *et al.*, 2012) [14]. These results are in agreement with Rajput *et al.* (2022) [32] on *Urtica dioica* L. Lipids were extracted in acetone and chloroform in our study. The chloroform and acetone extracts, which have intermediate polarity among the solvents used, yielded higher extraction amounts, reflecting the high presence of these components (Wang and Weller, 2006) [46]. In current research, many decoctions (like H₂O, CH₃OH, CH₃COCH₃, CHCl₃, n-C₆H₆ and petroleum ether) of *in vivo* as well as *in vitro* leaf, root, stem, and callus parts of *Plectranthus amboinicus* were screened to check the presence of different primary bioactive compounds- carbohydrates, proteins, lipids and secondary metabolites like terpenoids, glycosides,

flavonoids, alkaloids, phenols, ascorbic acid, saponins, steroids and tannins. Qualitative preliminary screening using these solvent extracts revealed that the methanolic extract possesses the maximum number of phytochemicals, including carbohydrates, proteins, lipids, terpenoids, flavonoids, tannins, steroids, and glycosides. Similar findings were reported by Mustafa *et al.* (2010) [27], Mathewos *et al.* (2015) [24], Shubhreet *et al.* (2019) [39], and Hanna *et al.* (2017) [15], who observed that methanolic extracts were more effective in extracting bioactive compounds compared to other solvents.

However, hexane was found to be less effective for the extraction of bioactive compounds from *Plectranthus amboinicus*. Similarly, Ketama *et al.* (2019) [18] and Aung *et al.* (2020) [3] reported comparable results in their phytochemical analyses of *Ruta chalepensis* and *Syzygium* species, respectively. They observed that methanol extracts were more efficient than other solvents, while hexane was less effective in extracting phytochemical compounds. Furthermore, a comparison between *in vivo* and *in vitro* plant parts revealed that *in vivo* parts contained higher levels of phytochemicals than *in vitro* parts. Comparable findings were reported by Joshi (2018) [16] in *Merremia* species. Secondary metabolites mainly consist of alkaloids, tannins, phenols, saponins, flavonoids, steroids, etc. (Krishnaiah *et al.*, 2007) [22]. Analysis of various plant extracts has shown the presence of bioactive substances with remarkable properties, and *Plectranthus amboinicus* has been identified as a rich reservoir of these compounds. The bioactive compounds present in this plant include phenols, tannins, saponins, steroids, flavonoids, and terpenoids. Among plant metabolites, phenolic compounds are the largest and most common class (Singh *et al.*, 2007) [41]. In our investigation, phenols were detected only in extracts obtained using methanol and acetone as solvents. According to previous literature, some medicinal plants show different dose dependent responses of the solvent used (Agidew, 2022) [1]. They have observed that methanol (a polar solvent) was the most effective for extracting phenolic compounds and confirmed that most plant parts could serve as antioxidants. Their findings are consistent with our results, where phenols were primarily extractable using methanol, followed by aqueous solvents (Ghasemzadeh *et al.*, 2011) [13]. Flavonoids are polyphenolic compounds that possess a benzoyl- γ -pyrone structure and are synthesized via the phenylpropanoid pathway. They have been identified as antimicrobial agents effective against a wide variety of pathogens and are commonly found in plants. Additionally, plants respond to microbial infection by producing flavonoids. Numerous studies have reported that flavonoids exhibit anti-cancer and antioxidant properties (Salah *et al.*, 1995; Okwu, 2004) [36, 31]. In the present investigation, flavonoids were detected in all plant parts in appreciable amounts and were extractable in most solvents. Furthermore, flavonoids are known to help prevent cardiovascular diseases and cancer and possess antibacterial activity (Ballard and Marosticka, 2019) [5]. Tannins are complex polyphenolic compounds frequently employed in the tanning process and as remedies for various ailments, including gonorrhoea, inflammation, and burns (Boroushaki *et al.*, 2016) [8]. They also possess anticancer properties (Mazni *et al.*, 2016) [25]. In our study, tannins were found in

significant amounts, similar to flavonoids. The absence of tannins in the hexane extracts of leaves, stems, and roots, and their presence in the methanol extracts, aligns with previous findings by Thavamoney *et al.* (2018) [44].

Terpenoids are the most widespread group of plant metabolites. They exhibit significant properties, including antibacterial, antiviral, antimalarial, anti-inflammatory, and anticancer activities (Boroushaki *et al.*, 2016) [8]. Phenolic compounds, tannins, terpenoids, and flavonoids possess antihelminthic properties, and therefore, they could be used to treat stomach problems (Nath and Yadav, 2016) [28]. Flavonoids, terpenoids, and quinine are plant-derived constituents that enhance anti-inflammatory, anti-carcinogenic, anti-mutagenic, and antioxidant activities (Bathia *et al.*, 2020) [7]. Nyarko and Addy (1990) [30] reported that glycosides have the ability to lower blood pressure in humans. Saponins are widespread phytochemicals found in vegetables, herbs, and beans (Francis *et al.*, 2002) [12]. In our results, the chloroform extract showed that saponins were present in all plant parts. According to Just *et al.* (1998) [17], saponins exhibit anti-inflammatory properties. Another report indicated that saponins possess cholesterol-lowering, cytotoxic, antibacterial, and antiviral properties (Bailly and Vergoten, 2020) [4]. Citrus fruits containing phenols, flavonoids, saponins, steroids, and reducing sugars have significant therapeutic value (Ehigbai *et al.*, 2016) [10]. Hence, this study revealed that *Plectranthus amboinicus* is a good source of bioactive compounds, primarily rich in secondary metabolites. Further quantification of these phytochemicals would be beneficial to better understand the plant's biochemical properties.

Conclusion

From our investigation, it was demonstrated that *Plectranthus amboinicus* contains valuable phytochemicals in all plant parts, including roots, leaves, and stems. These compounds possess diverse pharmacological and therapeutic properties, as reported in previous studies. Solvent-based extraction effectively targeted these phytochemicals, highlighting the potential for discovering new drugs from this plant. Comparative analysis between *in vivo* and *in vitro* plant parts revealed that *in vivo* plants contain higher levels of phytoconstituents than *in vitro* plants. Future studies could focus on enhancing phytoconstituents production under *in vitro* conditions, which could be applied in the pharmaceutical industry.

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