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Histochemical and Phytochemical Analysis of Medicinally Important Plants

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was aimed to investigate the histochemical and phytochemical studies of four important medicinal plants. Leaf, stem and root sections were examined for alkaloid, protein and phenolic tests. Methanolic extracts of leaf, stem and root were analysed for the phytochemical screening of major bioactive constituents. Histochemical analysis reveals the presence of alkaloids, phenols and proteins in epidermis, cortex and vascular bundles of root, stem and leaf. Preliminary phytochemical analysis showed the presence of alkaloids, tannins, phenols, flavonoids, steroids, saponins, terpenoids, coumarins, quinone and proteins. The root extract of *Catharanthus roseus* recorded maximum content of phenols compared to other extracts. *Costus pictus* root and leaf extracts exhibited a significant amount of flavonoids and tannins respectively. Further studies were focused on isolation and characterization of each plant compounds.

Keywords: Medicinal plants; histochemical; quantitative; bioactive compounds.

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1. INTRODUCTION

India has a great wealth of medicinal and aromatic plants due to its rich plant diversity. The traditional systems of medicine, namely Ayurveda, Siddha and Unani used only a smaller amount number of plants or herbs for various herbal preparations for medicinal purposes [1]. Plants have substances that induce a great interest due to their versatile applications [2]. It is estimated that 14-18% of higher plants were used medicinally and related to 74% of pharmacologically active plants are discovered after following up on ethnomedicinal usage of the plants [3]. Phenolic compounds are important natural compounds which have been shown to have a range of bioactivities including antioxidant activity, anti-carcinogenic, anti-atherosclerotic, antibacterial, antiviral, anti-inflammatory activities, etc. [4,5]. Recently, the investigation of natural products has gained interest due to potential therapeutic effect against infectious diseases. Plant extracts and their components (alkaloids, tannins, flavonoids and phenolic compounds) have been known to exhibit antifungal and antibacterial properties. Therefore, the present study aims at investigating the potentials of four medicinal plants as a source of phytochemicals for nutritional and therapeutic purposes such as *Alpinia galanga, Costus pictus, Catharanthus roseus and Ruta graveolens*.

A. galanga is commonly known as greater galangal which belongs to the family Zingiberaceae. It is a perennial herb with rhizomatous rootstocks and tall leafy stem. The plant is distributed in Himalaya and Southern region of the Western Ghats in India [6]. The plant is reported to be rich in essential oils such as cineole, methyl cinnamate, methyl eugenol and also said to contain various flavones such as galanin, alpinin, and kaemferide and 3-dioxy-4-methoxy flavone. It is known to possess antimicrobial, antioxidant, antifungal, anticancer and gastro-protective activities (Janssen and Scheffer, 1985). *A. galanga* is used in medication culinary and cosmetics for centuries. It is used in dietary intake as well as in the traditional system of medicine i.e., in Ayurveda, Unani, Chinese and Thai folk medicine [7]. It is used to flavour foods throughout south and South-east Asia. Its aromatic characteristic is described as woody, minty and floral [8]. The essential oil from *galanga* was reported as a potential anti-carcinogen [9]. 1-acetoxychavicol acetate (ACA) and 1-acetoxynegend acetate from *galanga* were antitumor substances [10].

Costus pictus is also called as insulin plant (*C. pictus*) is one of the folk medicines used for the treatment for diabetes mellitus. This plant belongs to the Costaceae family which has been separated from Zingiberaceae based on the presence of spirally arranged leaves and rhizomes being free from aromatic essential oils. The plant commonly known as spiral ginger is originated in Mexico and is found to have anti-diabetic properties [11]. *Costus* is native to the Malaya peninsula of Southeast Asia. The species of *Costus* are widely distributed throughout the tropic regions of the world. In India, the plants occur in Sub-Himalayan tract, some parts of central India and the Western Ghats of Maharashtra, Karnataka and Kerala [12].

Catharanthus roseus (L) G. Don (*C. roseus*) is commonly called a Madagascar periwinkle. It is a perennial evergreen herb, cultivated in two common names, which is named based on their flower colours, Pink: Rosea, White: Alba [13]. *C. roseus* contains significant amounts of volatile and phenolic compounds including caffeoylquinic acids and flavonol glycosides which are known to antioxidant activity. Traditionally, leaves are used as medicine for the treatment of many diseases, such as Menorrhagia, Rheumatism, Dyspepsia, Indigestion, Dysmenorrheal, Diabetes, Hypertension, Cancer, Menstrual disorders, Skin diseases, Bleeding diarrhoea and has sedative and antiviral properties [14]. The flower's petals, seeds and other parts of *C. roseus* exhibit antioxidant properties. Besides antioxidant activity, these compounds exhibit antiallergic, anti-inflammatory, antimicrobial, anti-thrombotic, cardioprotective and vasodilatory effects [15].

Ruta graveolens (*R. graveolens)* is also known as "herb of grace" and "common rue" is a hardy, evergreen shrub of up to one-meter-tall with a characteristic greyish colour and a sharp unpleasant odour. *R. graveolens* (Rue) is native to Europe, especially the Mediterranean region but widely distributed into all the temperate and tropical regions. It is a very popular and attractive garden shrub in South America, where it is grown not only for ornamental and medicinal reasons but also because of the belief that it protects against evil [16].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The plants were collected from G K V K, Bengaluru. The plants were grown in pots

containing soil mixture and maintained in greenhouse conditions. The plant materials were authenticated at the Department of Botany, Bangalore University, Bengaluru and a voucher specimen is deposited in the herbarium number 2292, 2294, 2365 and 2346.

2.2 Histochemical Studies

The healthy leaf, stem and root materials were collected from the greenhouse. Freehand sections were taken and treated with a respective reagent to localize the components like phenols, alkaloids and proteins in the tissues. Fresh unstained sections were treated as the negative control. The positive control was showed as suggested by the respective authors of histochemical tests. Test for alkaloids, phenols and proteins were done according to the procedure of Kuster and Vale [17]. Temporary mount sections were observed under the compound microscope.

2.3 Preparation of Plant Extract

5 g of fresh leaf, stem and root were soaked in methanol for 3-4 days. The solvent extracts were filtered with Whatman filter paper No.1. The procedure was repeated for another two cycles to ensure complete extraction of phytochemical compounds and the filtrates were stored at 4ºC until the further analysis [18].

2.4 Qualitative Analysis

The lyophilized extracts were dissolved in respective solvents and were screened for the qualitative analysis for the presence of alkaloids, flavonoids, proteins, tannins, phenols, steroids, terpenoids and coumarins etc., by standard methods of Harbone [19].

2.5 Quantitative Estimation

The preliminary phytochemical analysis of the leaves, root and stem indicated the presence of primary and secondary metabolites. These plant fractions were isolated and assessed for their bio-activity.

2.5.1 Determination of total phenolics content (TPC)

The total phenolic content of different extract of *A. galanga*, *C. roseus*, *C. pictus* and *R. graveolens* was determined by the Folin-Ciocalteu method [20] with slight modification. According to this method, 0.5 ml of extracts were mixed with 0.5 ml of FC reagent and allowed to stand for 5 min at room temperature to allow complete reaction with FC reagent. 1 ml of 7% sodium carbonate was added and the final volume was made up to 5 ml using distilled water. The absorbance of the blue colour solution was measured at 750 nm using UV-visible spectrometer against the blank after the incubation of 90 min in dark. The total phenolic was expressed in mg/g of gallic acid equivalent, as standard. The estimation of phenolic content was performed in triplicates.

2.5.2 Determination of total flavonoids

Flavonoids content of different extracts of *A. galanga*, *C. roseus*, *C. pictus* and *R. graveolens* was measured based on methods by Embrahimzadeh et al. [21]. According to this method, 0.5 ml of extracts were mixed with 2 ml of 95% methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and final volume was made up to 5 ml by adding distilled water. Then the mixture was incubated at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with UV-visible spectrometer against the blank. The experiments were carried out in triplicates. The total flavonoid content was expressed in mg/g of quercetin equivalent, as standard. The estimation of flavonoid content was performed in triplicates.

2.5.3 Estimation of tannins

Total tannins were determined according to the procedure of Medini et al. [22]. Briefly, to 0.5 ml of sample, 3 ml of 4% vanillin solution in methanol and 1.0 ml of concentrated hydrochloric acid were added. The mixture was then shaken and incubated at RT for 15 min, the absorbance was measured at 500 nm against the blank. The experiments were carried out in triplicates. The final absorbance of each sample was compared with a standard curve plotted from catechin. The total tannin content was expressed in mg of catechin equivalents per gram of extract (mg CE/g).

3. RESULTS AND DISCUSSION

3.1 Histochemical Studies

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. This method has been developed for qualitative and quantitative

analysis for the cellular components, including proteins, carbohydrates, lipids, nucleic acids and the range of ionic elements occurring in the cells [23]. Through histochemical tests, it was possible to identify the region of synthesis and storage of metabolites in tissue level for pharmacological uses in leaves, stem and root of medicinal plants [17].

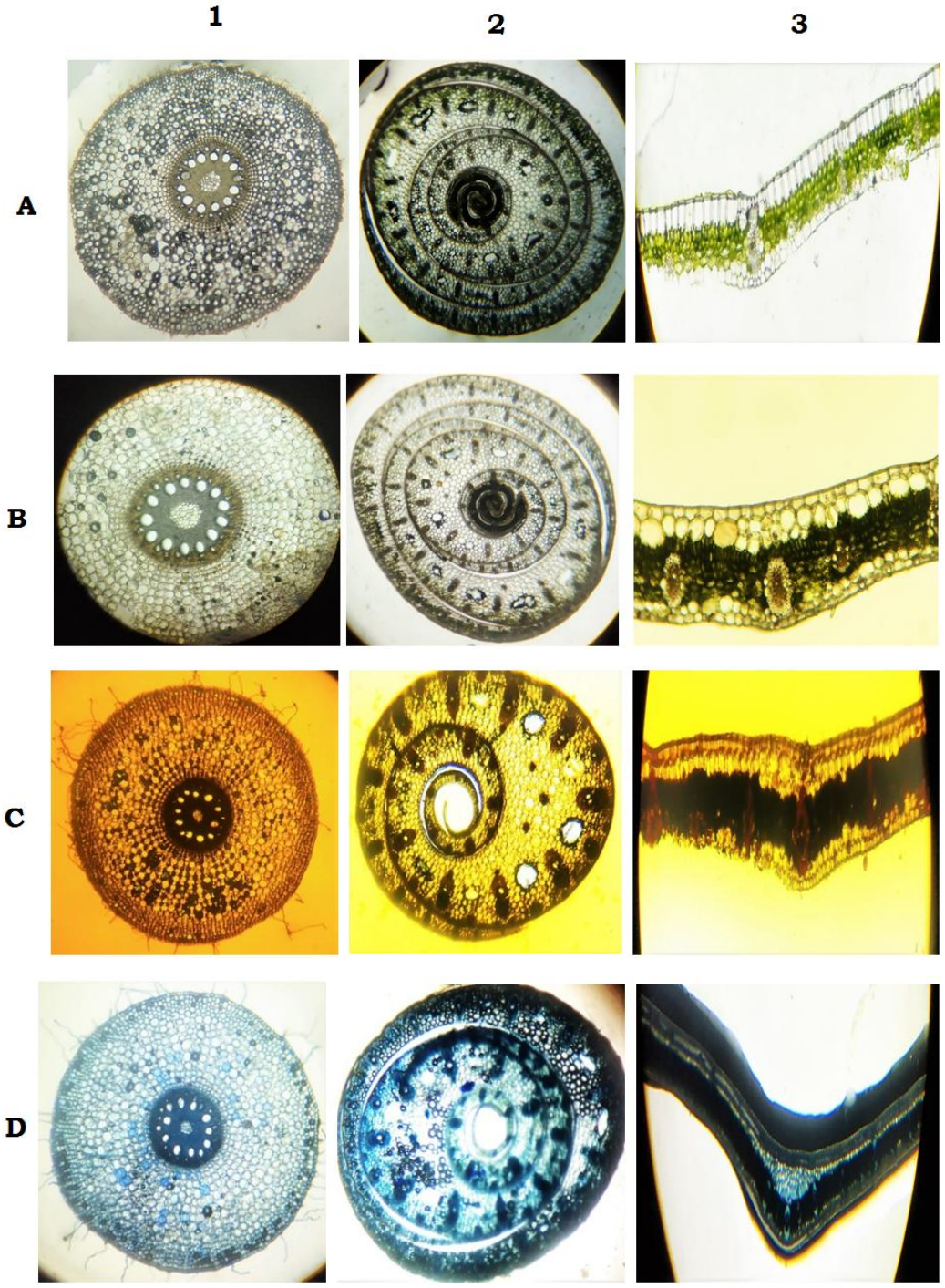

Fig. 1. Histochemical studies on 1) root, 2) stem and 3) leaf of *Alpinia galanga*
A - Unstained sections, B - Test for phenols, C - Test for alkaloids and D - Test for proteins

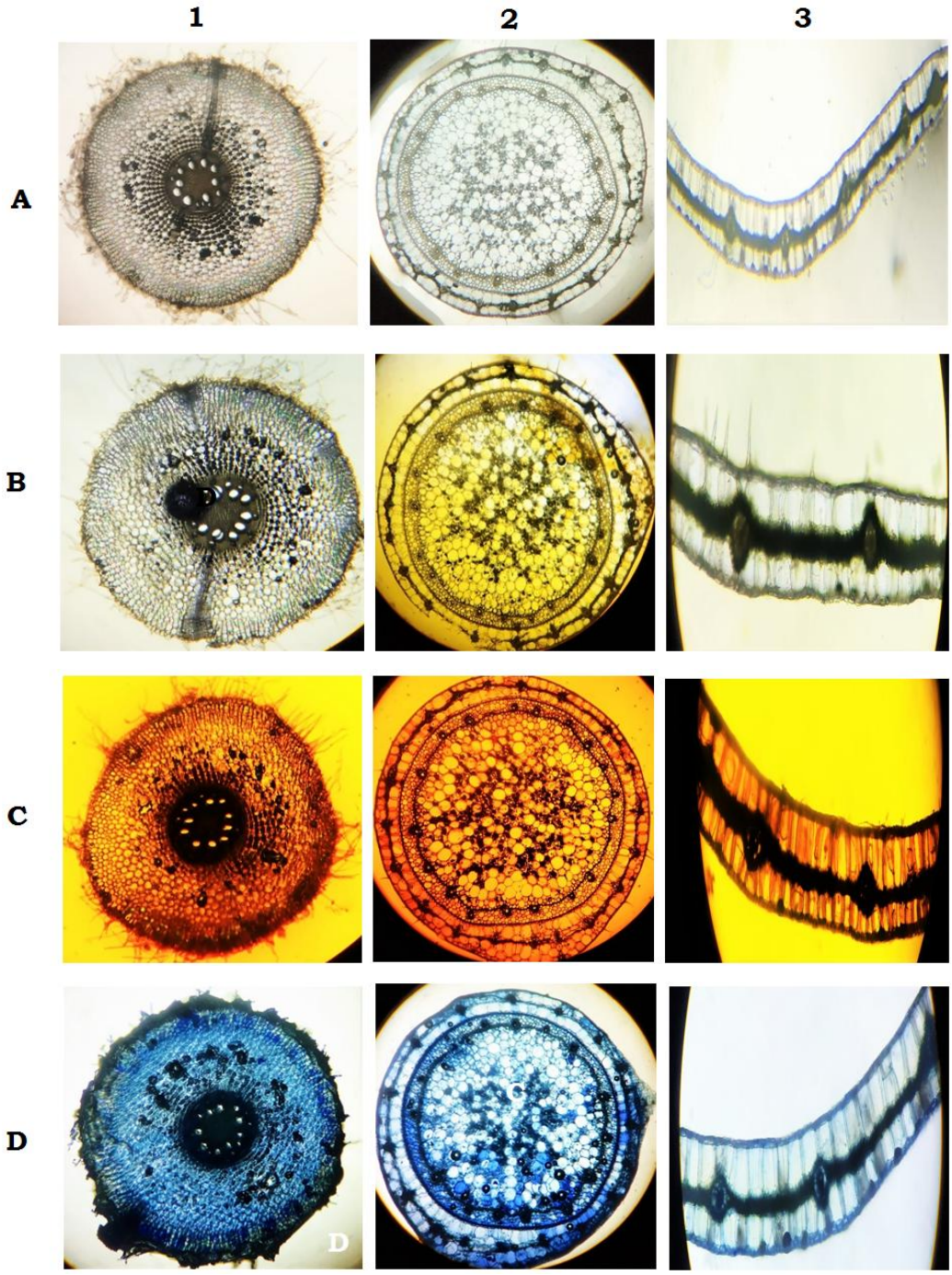

Fig. 2. Histochemical studies on 1) root, 2) stem and 3) leaf of *Costus pictus*
A - Unstained sections, B - Test for phenols, C - Test for alkaloids and D - Test for proteins

In our present study, epidermal tissue, cortex and vascular bundles of root, stem and leaf showed the phytochemical constituents such as phenols, alkaloids and proteins (Figs. 1-4). Alkaloids are a group of secondary metabolites and they are simple molecules present in plants. Many alkaloids are toxic, which are used for plants to protect themselves against the aggression from other organisms [24]. Dhale studied alkaloids in three species *Adhatoda zeylanica, Ruta graveolens* and *Vitex negundo*. It was shown that alkaloids existed in the

epidermis, mesophyll cells and parenchyma cells of the leaf veins. According to Kadam et al. [25] the presence of alkaloids reported in the mesophyll cells of leaves of *Mimusops elengi* and *Syzygium cumini*. Kuster and Vale [17] were identified the storage of metabolites in tissue level of *Byrsonima verbascifolia, Campomanesia adamantium* and *Roupala Montana* leaves. The same was not identified in the mature leaves of *S. lycocarpum.*

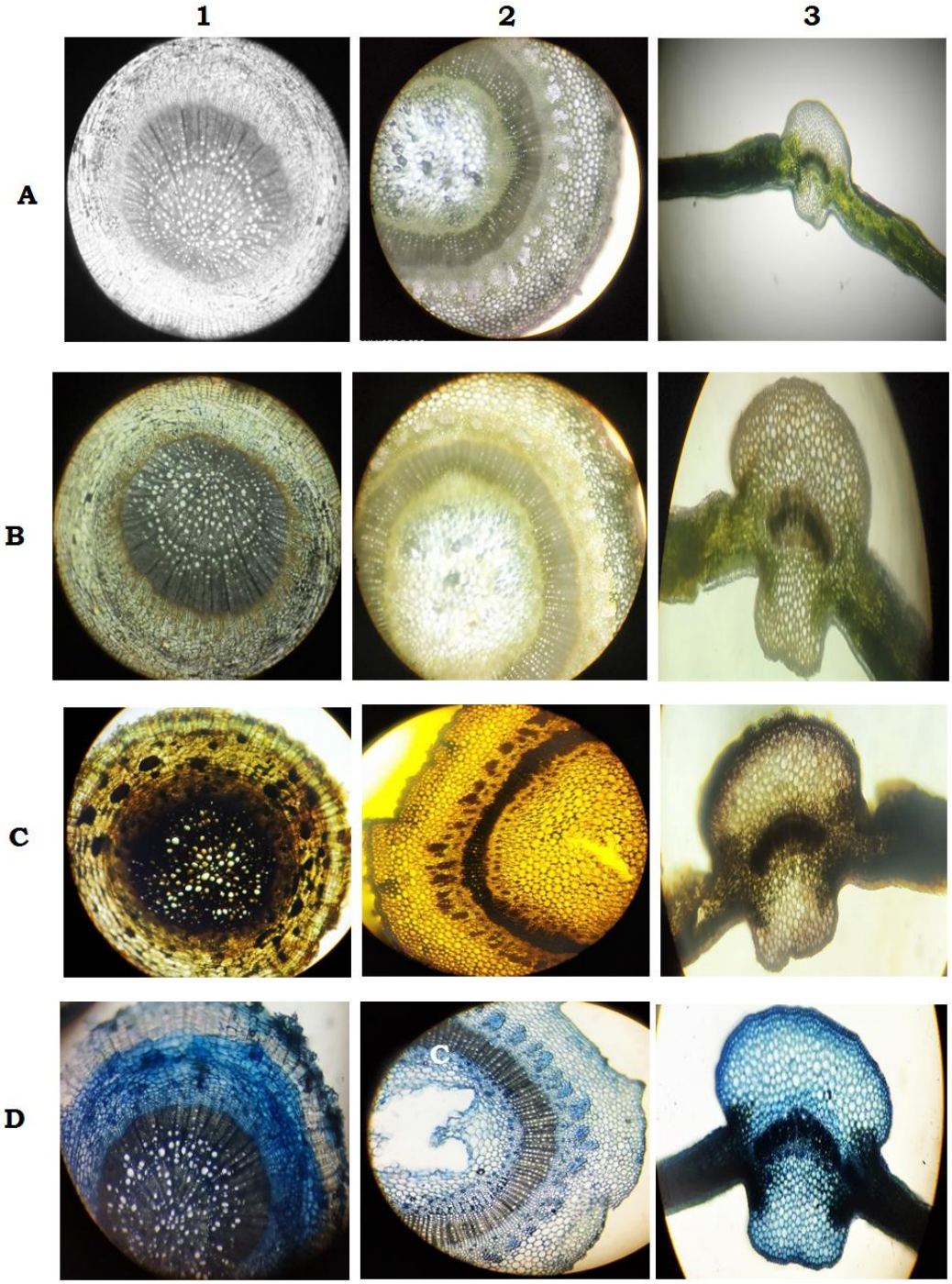

Fig. 3. Histochemical studies on 1) root, 2) stem and 3) leaf of *Catharanthus roseus*
A - Unstained sections, B - Test for phenols, C - Test for alkaloids and D - Test for proteins

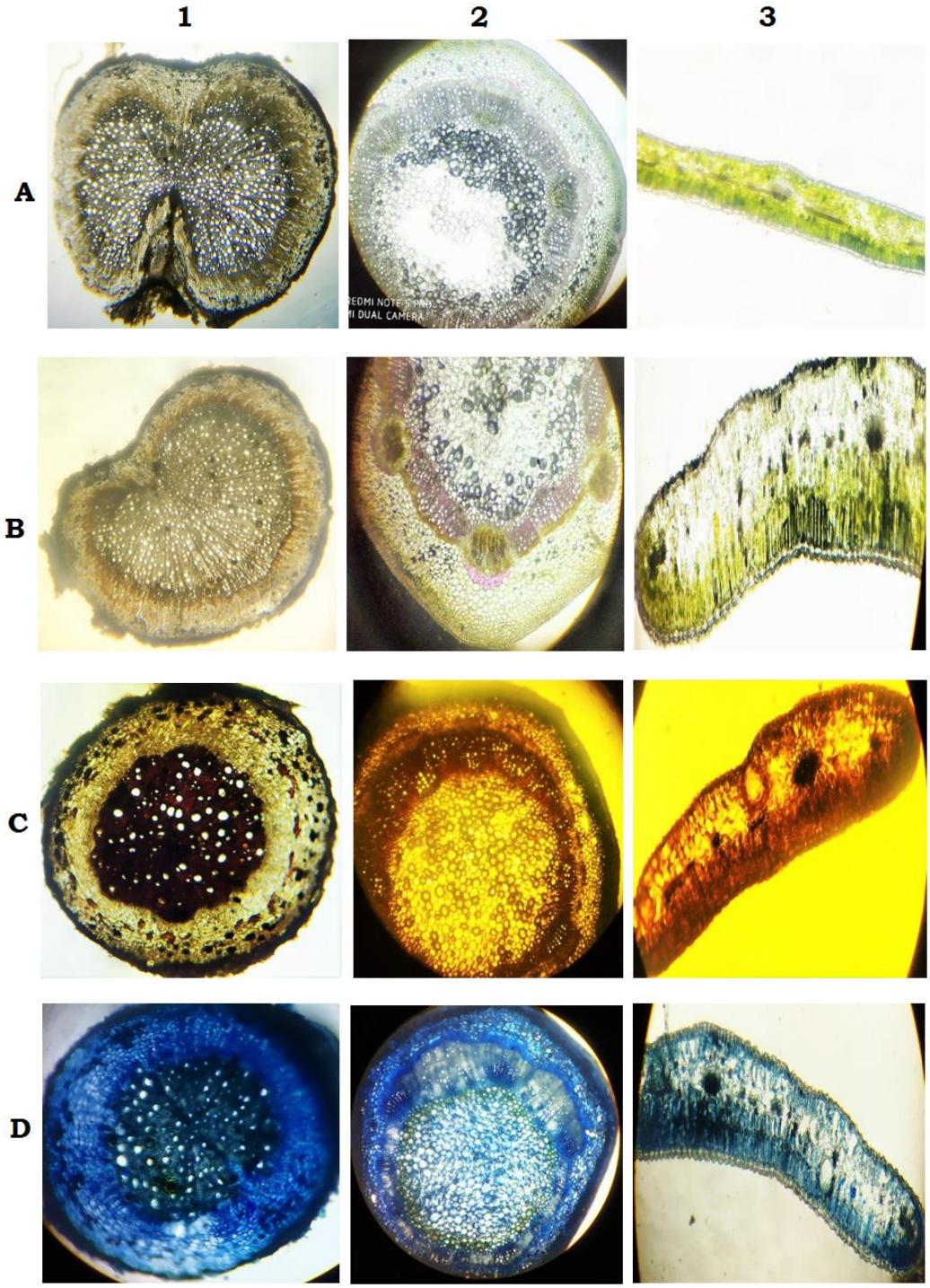

Fig. 4. Histochemical studies on 1) root, 2) stem and 3) leaf of *Ruta graveolens*
A - Unstained sections, B - Test for phenols, C - Test for alkaloids and D - Test for proteins

3.2 Qualitative Analysis

Preliminary phytochemical screening of four medicinal plants extracts was subjected to various tests specified to each compound (Table 1). The root extracts of *A. galanga* was found to contain the flavonoids, proteins, steroids, terpenoids, coumarins, quinones and phenols.

Whereas, stem and leaf extracts exhibit flavonoids, proteins, steroids, tannins, phenols, terpenoids, alkaloids and quinones. Studies by Singh and Singh, [26] reveals the presence of phytochemical compounds in methanolic extract of rhizome and flower of *A. galangal*. Subash et al. [27] recorded the presence of various constituents such as alkaloids, carbohydrates, Saponins, tannins, proteins, flavonoids etc., in ethanolic extracts of *A. galangal* rhizome.

C. pictus root extract showed the presence of tannins, flavonoids, proteins, steroids, terpenoids, coumarins, quinones, saponins and phenols. Whereas stem and leaf extracts exhibit flavonoids, proteins, steroids, tannins, phenols, terpenoids, alkaloids and quinones (Table 1). Studies by Malleshwari et al. [28], ethanol and methanol proved to be a better solvent compared to other solvents for the presence of phytochemicals. Leaves and rhizome were found to contain more phytochemicals compared to stem extracts. Meshram et al. [29] reveal the presence of phytochemical compounds such as alkaloids, flavonoids, glycosides, carbohydrates, saponins, tannins, proteins, reducing sugar, resins etc., in aqueous and methanolic extract of *C. pictus.*

C. roseus root, stem and leaf extracts revealed the presence of phenol, alkaloids, tannins, flavonoids, proteins, steroids, terpenoids, coumarins, quinones and Saponins (Table 1). According to Paikara et al. [30] investigate the preliminary phytochemical analysis in petroleum ether extract of *C. roseus*, which reveals the presence of some bioactive compounds like tannins, alkaloids, flavonoids, carbohydrates, terpenoids, proteins and cardiac glycosides. Studies by Kannabiran et al. [31] proved the presence of phytochemical constituents in aqueous and ethanolic leaf extract of *C. roseus*.

R. graveolens root extract showed the presence of flavonoids, proteins, steroids, terpenoids, coumarins, quinones, saponins and alkaloids. Whereas stem reveals the presence of proteins, steroids, terpenoids, coumarins, quinones, saponins and alkaloids (Table 1). Studies by Renugadevi and Meerabai [32] ethanol and methanolic extract of *R. graveolens* proved to be better solvents compared to chloroform, petroleum ether, acetone and hexane extracts for the presence of phytochemical compounds.

3.3 Quantitative Analysis

3.3.1 Total phenolic content

The phenolic content found in the extracts were determined using the linear regression equation using Gallic acid as standard ($y = 0.058x + 0.053$) $r^2 = 0.9978$. The leaf extract of *A. galanga* exhibit higher phenolic content (10.51 ± 0.05 mg/g). Whereas stem (09.74 ± 0.05) recorded a significant amount of total phenol compared to root extract (Fig. 5; Table 2). Studies by Malik et al. [33], the methanolic extracts of *A. galanga* showed higher phenolic content than ethanolic and aqueous extracts. According to Melanathuru et al. [34], the phenolic content of aqueous extracts of *A. galanga* rhizomes recorded the maximum amount of phenol compared to *A. calcarata.*

Table 1. Preliminary phytochemical analysis of *A. galanga, C. pictus, C. roseus and R. graveolens*

Sl. no.	Tests	*A. galanga*			*C. pictus*			*C. roseus*			*R. graveolens*		
		R	S	L	R	S	L	R	S	L	R	S	L
1)	Flavonoids	+	-	+	-	-	-	+	+	+	+	-	+
	$Fecl_3$	+	+	+	+	+	+	+	+	+	+	+	+
	Alkality												
2)	Proteins	+	+	+	+	+	+	+	-	+	+	+	+
3)	Steroids	+	+	+	+	+	+	+	+	+	+	+	+
4)	Saponins	-	-	-	+	+	+	+	+	+	-	-	-
5)	Tannins	+	-	+	-	-	-	+	+	+	+	-	+
	$Fecl_3$	-	-	-	-	-	-	-	-	-	-	-	-
	Gelatin												
6)	Phenols	+	-	+	+	+	+	+	+	+	+	-	+
7)	Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+
8)	Coumarins	+	+	+	+	+	+	+	+	+	+	+	+
9)	Quinone	+	+	+	+	+	+	+	+	+	+	+	+
10)	Alkaloids	-	+	+	-	-	-	+	+	+	-	+	+

The leaf extract of *C. pictus* exhibit higher total phenol content (16.76 ± 0.03 mg/g) followed by stem (03.18 ± 0.02 mg/g) and root extracts (02.38 ± 0.04 mg/g). Ramya and Dhamotharan [35] reported that the maximum percentage of phenols were recorded in ethanolic extract of leaf compared to other phytochemical constituents in *C. speciosus.*

In *Catharanthus roseus,* root extract (27.01 ± 0.05 mg/g) exhibited significant amount of total phenolic content followed by stem extract (10.64 ± 0.07 mg/g). Whereas leaf extract (01.54 ± 0.01 mg/g) showed lesser content of phenols. Studies by Kabesh et al. [36] methanolic extract of *C. roseus* recorded maximum content of phenols compared to aqueous extract of the leaf.

The leaf extract of *R. graveolens* (24.95 ± 0.03 mg/g) showed maximum content phenols. Whereas stem extract (17.36 ± 0.03 mg/g) reveals higher content of total phenols compared to root extract. Azlework et al. [37] the methanolic extract of *R. graveolens* recorded the maximum amount of phenolic contents. Studies by Ahmed et al. [38] reveals that the methanolic extract of *R. graveolens* leaf recorded a higher percentage of phenolic content.

3.3.2 Total flavonoid content

The total flavonoid content found in the different extracts were determined using the linear regression equation using quercetin as standard ($y = 0.047x + 0.0879$) $r^2 = 0.9939$ (Fig. 6; Table 2). The leaf extract of *A. galanga* exhibit higher flavonoid content (36.22 ± 0.09 mg/g) followed by stem (22.93 ± 0.04 mg/g) and root extracts (07.09 ± 0.05 mg/g). According to Devi et al. (2018), methanolic extract of *A. galanga* rhizome showed significant flavonoid content compared to other extracts.

The root extract of *C. pictus* exhibits higher flavonoid content (43.78 ± 0.06 mg/g). Whereas leaf (36.64 ± 0.07 mg/g) recorded significant amount of total flavonoid compared to stem extracts (13.13 ± 0.07 mg/g). According to Muthukumar et al. [39], quantitative estimation of methanolic extract of *C. igneus* leaf recorded higher content of saponins and total flavonoids.

The stem extract of *C. roseus* (22.72 ± 2.11 mg/g) noticed a higher amount of total flavonoid. Whereas root extract (19.04 ± 0.11 mg/g) recorded a moderate amount of flavonoid compared to methanolic leaf extract. According to Nayak and Babu [40], the presence of feather compost has significantly increased primary and secondary metabolites as compared to the control plant.

R. graveolens leaf extract (26.54 ± 0.02 mg/g) revealed the maximum flavonoid content. Whereas methanolic extract of stem exhibit (10.58 ± 0.05 mg/g) the higher content of total flavonoid compared to root extract (01.56 ± 0.01 mg/g). According to Benazir et al. [41] the methanolic extract of *R. graveolens* leaf and root exhibited a significant amount of total flavonoid content compared to methanolic extract of the stem.

3.3.3 Total tannin content

Total tannin content was calculated using standard curve of catechin ($y = 0.0073x + 0.0236$) $r^2 = 0.995$ (Fig. 7; Table 2). The leaf extract of *A. galanga* showed maximum tannin content (40.51 ± 0.16 mg/g). Whereas root (24.68 ± 0.07 mg/g) and stem extracts (23.19 ± 0.05 mg/g) exhibited moderate amount of tannin in *A. galanga*. Studies by Al-Enazi [42] *A. purpurata* extract reported the higher tannin content compared to *A. calcarata* and *A. zerumbet.*

C. pictus leaf extract (53.09 ± 0.09 mg/g) exhibited significant amount of tannin content followed by stem (18.99 ± 0.14mg/g) and root (17.15 ± 0.04 mg/g) extracts. Studies by Chibueze et al. [43] the aqueous extract of *C. afar* showed a significant amount of total tannin content compared to ethanolic extract.

The stem extract (36.61 ± 0.25 mg/g) of *C. roseus* recorded higher content of total tannin. Whereas root extract (18.72 ± 0.16 mg/g) showed a moderate amount of tannin content compared to leaf extract. Uadia et al. [44] reported the higher content of tannin in methanolic extract of stem compared to the root extract of *Vernonia amygdalina*.

In *R. graveolens* leaf extract (38.53 ± 0.07 mg/g) recorded a significant amount of tannin content. Whereas stem extract (27.64 ± 0.09 mg/g) exhibited moderate amount of tannin content compared to root extract (10.59 ± 0.03 mg/g). Studies by Azlework et al. [37] the methanolic extract of *R. graveolens* showed the presence of a higher amount of tannin content. Whereas aqueous extract of leaf showed the least amount compared to ethanol and chloroform extract.

Table 2. Total phenolics, flavonoids and tannin content of different plant extracts

Plant samples	Extracts (mg/g)	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)	Total tannins (mg CE/g)
A. galanga	Leaf	10.51 ± 0.05^{c}	36.22 ± 0.09^{c}	40.51 ± 0.16^{c}
	Stem	09.74 ± 0.05^{b}	22.93 ± 0.04^{b}	23.19 ± 0.05^{a}
	Root	03.65 ± 0.04^{a}	07.09 ± 0.05^{a}	24.68 ± 0.07^{b}
C. pictus	Leaf	16.76 ± 0.03^{c}	36.64 ± 0.07^{b}	53.09 ± 0.09^{c}
	Stem	03.18 ± 0.02^{b}	13.13 ± 0.07^{a}	18.99 ± 0.14^{b}
	Root	02.38 ± 0.04^{a}	43.78 ± 0.06^{c}	17.15 ± 0.04^{a}
C. roseus	Leaf	01.54 ± 0.01^{c}	09.71 ± 0.06^{a}	12.65 ± 0.10^{a}
	Stem	10.64 ± 0.07^{b}	22.72 ± 2.11^{c}	36.61 ± 0.25^{c}
	Root	27.01 ± 0.05^{a}	19.04 ± 0.11^{b}	18.72 ± 0.16^{b}
R. graveolens	Leaf	24.95 ± 0.03^{c}	26.54 ± 0.02^{c}	38.53 ± 0.07^{c}
	Stem	17.36 ± 0.03^{b}	10.58 ± 0.05^{b}	27.64 ± 0.09^{b}
	Root	02.33 ± 0.05^{a}	01.56 ± 0.01^{a}	10.59 ± 0.03^{a}

Values represent the Mean ± SD in triplicates. Means with the different letters in columns indicate significant differences at the 5% level

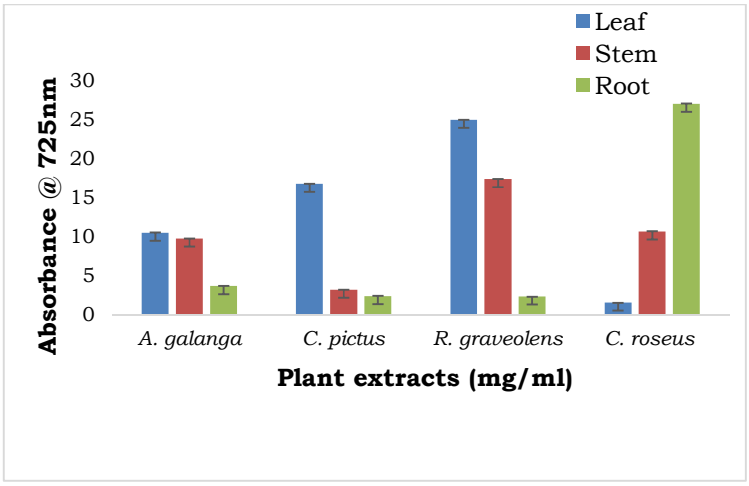

Fig. 5. Estimation of phenols in different plant extracts

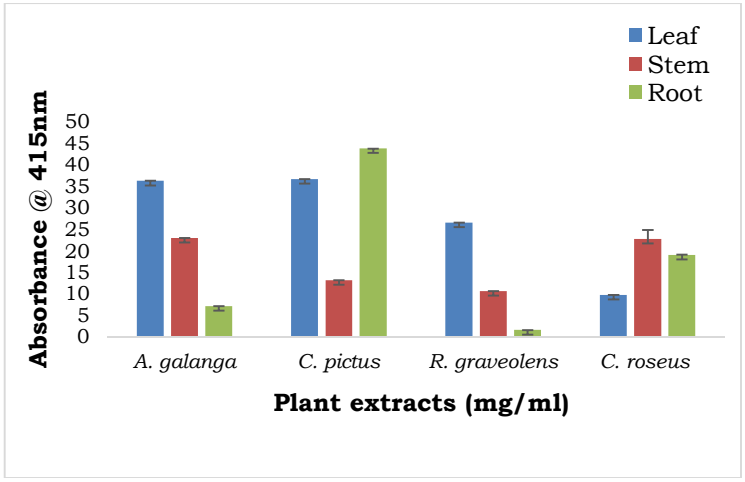

Fig. 6. Estimation of flavonoids in different plant extracts

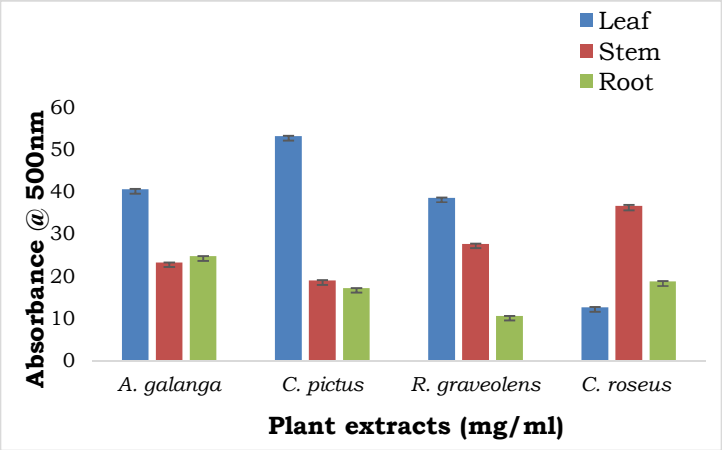

Fig. 7. Estimation of tannins in different plant extracts

4. CONCLUSION

In the present histochemical study reveals the presence of alkaloids, phenols and proteins in leaf, stem and root of four important medicinal plants. Qualitative analysis was done to investigate the active phytoconstituents present in *A. galanga, C. pictus, C. roseus* and *R. graveolens*. The extracts showed the presence of alkaloids, phenols, flavonoids, proteins, steroids, saponins, tannins, terpenoids, coumarins and quinones. *C. roseus* root exhibited higher content of phenols compared to other extracts. Whereas, total tannin and flavonoids were recorded in *A. galanga* leaf and *C. pictus* root. Further work will be concentrated on the isolation and characterization of four important medicinal plants.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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