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Assessment of Variations in Phytochemical Components in Stem and Root Bark of *Dillenia pentagyna* from different Locations of Madhya Pradesh

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ABSTRACT: *Dillenia pentagyna* Roxb. (Dilleniaceae) is a pharmaceutically important plant species, in India, it is distributed in Himalayan terai from Punjab to Assam, South India, Andamans, Gujarat, Mizoram, and West Bengal. Secondary metabolites provide defence to plants against pests and pathogens and form the backbone of modern system of medicines. In the present investigation, preliminary phytochemical screening and quantification of secondary metabolites in stem and root bark of *D. pentagyna* collected from seven different forest divisions of Madhya Pradesh state were carried out to find out the variations in secondary metabolite concentrations. The preliminary phytochemical screening shows the presence of alkaloids, flavonoids, phenols, tannins, and terpenoids, and the quantification of secondary metabolites revealed the presence of alkaloids, flavonoids, phenols, tannins, and terpenoids to be in the range of 0.87±0.2 - 4.13±0.22 mg CE/g dry wt, 7.63±0.21 - 18.56±0.01 mg QE/g dry wt, 68.3±0.18 - 42.8±0.06 mg GAE/g dry wt, 3.29±0.04 - 12.04±0.4 (mg TAE/g dry wt) and 8.48±0.12 - 9.85±0.05 % respectively. The study showed the rich content of TAC in populations of Sagar, TFC in populations of Alirajpur, and TPC, TTC, TTrC in populations of North Balaghat which can further be investigated for individual chemical constituents.

Keywords: *Dillenia pentagyna*, Secondary Metabolites, Preliminary Phytochemical Screening, Alkaloids, Flavonoids, Phenols, Tannins, Terpenoids.

INTRODUCTION

Plants have a rich history of contributing to treating chronic diseases. The International Union of Conservation of Nature and the World Wildlife Fund predicts that approximately 50,000 – 80,000 flowering plant species are being used for therapeutical uses (Chen *et al*., 2016). *Dillenia pentagyna* is a member of Dilleniaceae family, commonly known as 'Karmal' or 'Karkat' or 'Nepali Elephant Apple' has been used in folklore medicine for its therapeutic properties (Patle *et al*., 2020). It is a moderately deciduous tree found in the dry deciduous forest of central India. *D. pentagyna* is indigenous to southern Asian countries like Bhutan, India, Indonesia, Malaysia, Myanmar, Nepal, Thailand, Vietnam, and Australia which are tropical or subtropical (Gandhi and Mehta 2013)

D. pentagyna exhibits a wide range of curative properties such as anti-tumor, anti-alpha glucosidase, anti-inflammatory, anti-diarrheal, anti-microbial, cytotoxic, and lymphocytic activities (Rosangkima *et al*., 2007). The stem and root bark extract has been evaluated for its cytotoxic properties against DL, MCF-7, and HeLa cell lines (Rosangkima *et al*., 2015; Rosangkima and Jagetia 2008). Decoction of the bark is used as a remedy against cuts, burns, dysentery, diarrhoea, cancer, and for post-partum bathing (Dubey *et al.,* 2009; Sharma *et al.*, 2001). The seeds and bark are widely used by the Koch-Rajbanshi tribes of north-eastern states of India for treating cancer (Yadav and Srivastava 2014). Antihyperglycemic activity has been indicated in the leaf extract of the plant species (Yadav and Srivastav 2014) and its leaf paste is also considered beneficial for bone fracture (Sikarwar *et al*., 2016). The fruit of the plant is edible and is quite popular among the tribal people of North-East and central parts of India, dried fruit of the plant is a major component of the widely used traditional medicine 'Malabar Nagakesara' which has been effective in treating gastrointestinal tract (GIT), skin, and bleeding disorders (Suresh *et al*., 2015) and fruit extract has the ability to inhibit the angiotensin-converting enzyme (ACE) (Nyman, 1998).

The bioactive extract of the bark of *D. pentagyna* appeared to help in reducing Dox-induced cardiotoxicity. Polyphenolic antioxidant substances such as gallic acid, syringic acid, and synaptic acid are considered to be responsible for the potent-cardioprotective impact, according to LC-QTOF-ESI-MS study of *D. pentagyna* and phenolic-rich fraction (Tene *et al*., 2021). *In silico* study with targeting isorhamnetin, lupeol, quercetin, kaempferol, and betulin phytocompounds of *D. pentagyna* showed these phytocompounds possess promising orally active drug-like activity (Alam *et al*., 2021). Sultana *et al.* (2022) performed *In vivo* analgesic, anti-inflammatory, and

antipyretic studies and concluded that methanolic extract of *D. pentagyna* possessing an elevated amount of bioactive phytoconstituents that can be a remarkable source of analgesic and antipyretic activities with moderate anti-inflammatory, antidiarrheal, and anticoagulant therapies.

Till date, study has not been conducted on variations in phytochemical constituents. Considering the therapeutical importance of *D. pentagyna*, the present study aimed at preliminary phytochemical screening and quantification of secondary metabolites in the stem and root bark collected from various locations of Madhya Pradesh for variation study. The qualitative phytochemical analysis will provide insight into different chemical compounds produced by plants and its quantification will help to extract, purify, and identify the bioactive compounds.

MATERIALS AND METHODS

Reagents and Chemicals. All the chemicals and solvents used were of analytical grade. Folin-Ciocatheu reagent, Ferric Sulphate, Glacial Acetic Acid, Sulphuric Acid, Hydrochloric Acid, Sodium Hydroxide, Phenanthroline, Ferric Chloride, Bismuth Nitrate, Potassium mercuric iodide, Aluminium Chloride, Potassium Acetate, Sodium bicarbonate, Phosphomolybdic acid, Sodium Tungstate, Phosphoric Acid, Ethanol, Methanol, n-Hexane, Petroleum Ether, Ethyl Acetate, and Chloroform. Reference standards Colchicine, Quercetin, Gallic Acid and Tannic Acid were purchased from Sigma-Aldrich (Mumbai, India).

Collection and processing of plant materials. The stem and root bark sample were collected from seven different locations of Madhya Pradesh. Collected samples were cleaned and sun-dried. The dried samples were grounded into a fine powder and stored in airtight bags for further phytochemical analysis.

Preliminary phytochemical analysis. 1g of dried and powdered stem and root bark samples were soaked for overnight in 25ml of ethanol, methanol, ethyl acetate, and chloroform solvents separately. The filtered extract was then subjected to preliminary phytochemical analysis (Table 1).

Table 1: Methods of preliminary phytochemical analysis.

Name of Phytochemicals	Test	Observations	References
Alkaloid	Dragandroff's Test	Cremish/Brown/Red Orange precipitate	Kundishora *et al*. (2020)
Cardiac Glycoside	Keller-kilianni test	Blue colour produced	Gul *et al*. (2017)
Flavonoid	Alkaline reagent test	Formation of intense yellow colour which becomes colourless on addition of dilute acid	Gul *et al*. (2017)
Phenol	$FeCl_3$ test	Formation of bluish black colour	Soloway and Wilen (1952)
Saponin	Frothing test	Formation of stable foam	Gul *et al*. (2017)
Steroid	Liebermann-Burchard test	Formation of blue-green colour	Nath *et al*. (1946)
Tannin	$FeCl_3$ test	Presence of blue-green precipitate.	Das *et al*. (2014)
Terpenoid	Salkowski test	Formation of reddish-brown coloration.	Das *et al*. (2014)

Quantitative Estimation of Secondary Metabolites

Estimation of Total Alkaloid Content (TAC). 0.1 g of the sample was extracted in 20 ml of 80% methanol. The extract was filtered and the filtrate was centrifuged at 4000 rpm for 10 minutes. 0.1 ml of the supernatant was taken and the volume was made up to 2 ml using 80 % methanol. The reaction mixture contained 2 ml of extract, 2 ml 0.025M $FeCl_3$ in 0.5M HCl, and 2 ml 0.05 M phenanthroline in ethanol. In a hot water bath at 70±2℃, the mixture was incubated for 30 minutes. At 510 nm, the absorbance of the red-colored complex was measured against the reagent blank. The standard curve of Colchicine (Fig. 1) was used to determine the TAC which was expressed as mg of colchicine equivalent per g of dry extract weight (mg CE/g dry extract wt) (Singh *et al*., 2004).

Estimation of Total Flavonoid Content (TFC). 0.5 g of the sample was weighed, added to 12.5 ml of 95% methanol, and allowed to stand overnight. The extract was filtered and volume was made up to 25 ml with 80% methanol. 10% aluminium chloride, 0.1M potassium acetate, and 2.5 ml of distilled water were added to 0.03 ml of the extract in a test tube and incubated for 30 minutes at room temperature. The absorbance of the reaction mixture was taken at 415 nm. The TFC was determined using the standard curve of quercetin (Fig. 2). Results were expressed as mg of quercetin equivalent per g of dry extract weight (mg QE/ g dry extract wt) (Kamtekar *et al*., 2014).

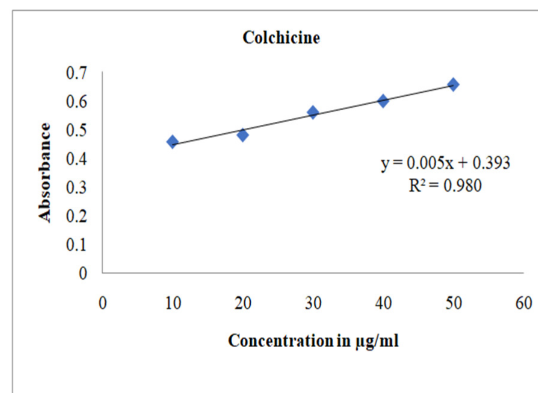

Fig. 1. Standard curve of Colchicine standard.

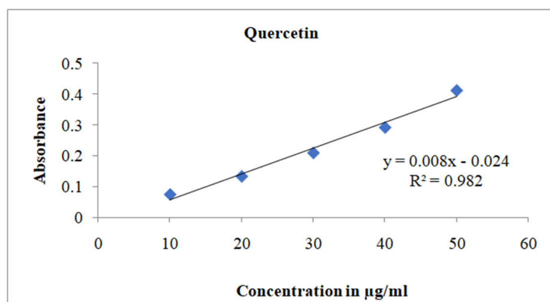

Fig. 2. Standard curve of Quercetin standard.

Estimation of Total Phenol Content (TPC). The TPC was determined using the Folin-Ciocalteau method. 25 ml of 80 % ethanol was added to 0.5 g of the powdered sample, which was kept on a rotary shaker for 1 hr. The mixture was filtered and 0.03 ml of the extract was considered for the analysis. The volume was made up to 3ml using distilled water to which 0.5 ml of Folin-Ciocalteau reagent was added. After incubation of 3 minutes, 2 ml of 20% Na_2CO_3 solution was added, mixed vigorously using a vortex mixer, and incubated in boiling water for 1min. The absorbance of the reaction mixture was measured at 650 nm against blank. The quantification of phenolic compounds was carried out using gallic acid standard curve (Fig. 3). Results were expressed as mg of gallic acid equivalent per g of dry extract weight (mg GAE/g dry wt) (Alhakmani *et al*., 2013).

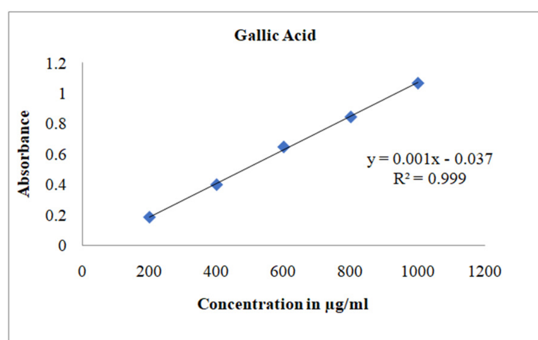

Fig. 3. Standard curve of Gallic Acid.

Estimation of Total Tannin Content (TTC). 0.5 g of the sample was taken in 75ml of distilled water and incubated in the hot water bath at 80±2°C for 30 minutes. The mixture was cooled down, filtered, and the volume was made up to 100 ml. 0.030 ml of the filtrate was taken and the volume was made up to 3ml using distilled water. 1ml of Folin-Denis reagent and 2 ml of sodium carbonate was added to the extract. The reaction mixture was used to take the absorbance at 700 nm against a blank. The TTC was determined using the standard curve of tannic acid (Fig. 4) and the results were expressed as mg of tannic acid equivalent per g of dry extract weight (mg TAE/g dry wt.) (Chandran *et al*., 2016).

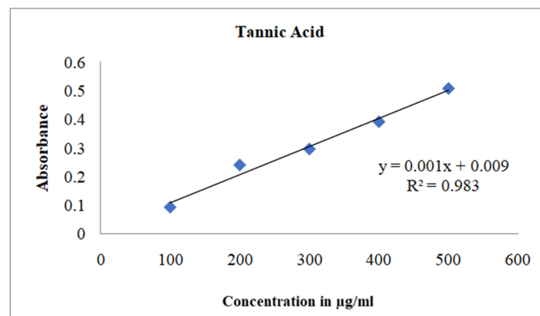

Fig. 4. Standard curve of Tannic Acid standard.

Estimation of Total Terpenoid Content (TTrC). 1g (w_i) of the sample was weighed, soaked in ethanol for 24 hr and filtered. The extract was separated using 10ml of petroleum ether thrice. The petroleum ether extract was taken in a pre-weighed glass beaker (w_a) and weighed again after complete evaporation in the hot water bath (w_b) (Malik *et al*., 2017).

Ether yield % was calculated with the formula:

$$\text{Ether Yield } \% = \frac{w_{i.} - w_f}{w_i} \times 100$$

where $w_f = w_b - w_a$

Statistical Analysis. The experiments were carried out in triplicates and the data was expressed as Mean ± Standard deviation. Statistical analysis of data was performed using one-way ANOVA in MS Excel. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Screening of phytochemicals in plant species is an important step to characterizing the bioactive compounds present in the plant species, which further gives an idea of the probable therapeutic properties of the plant. The secondary metabolites present in medicinal plants, such as alkaloids, flavonoids, saponins, phenol, and terpenoids offer a plethora of medical applications and are widely used in the pharmaceutical and medicine industries (Jeeva *et al*., 2011). Alkaloids, flavonoids, and glycosides are known to possess a variety of biological activities, including anti-inflammatory, antioxidant, anti-diabetic, antiviral, and anticancer activities (Kala *et al*., 2011).

The preliminary phytochemical investigation revealed ethanol and methanol to be the best solvent for the extraction of phytochemicals from *D. pentagyna*. Table 2 shows that both extracts indicated the presence of alkaloids, cardiac glycoside, flavonoid, saponin, steroid, tannin, and terpenoid. Solvents like chloroform, ethyl acetate, and n-hexane reported only the presence of flavonoids, whereas petroleum ether exhibited the presence of terpenoids, phenol, and flavonoids. The qualitative phytochemical analysis not only gives an overview of the secondary metabolites present in the sample but also gives an idea of the solvents which shows better extraction.

Table 2: Phytochemical screening of stem and root bark samples of *D. pentagyna.*

Phytochemicals	Plant Part	Chloroform	Ethanol	Ethyl Acetate	Methanol	n-Hexane	Petroleum Ether
Alkaloid	Stem Bark	-	+	-	+	-	-
	Root Bark	-	+	-	+	-	-
Cardiac Glycoside	Stem Bark	-	+	-	+	-	-
	Root Bark	-	+	-	+	-	-
Flavonoid	Stem Bark	+	+	+	+	+	+
	Root Bark	+	+	+	+	+	-
Phenol	Stem Bark	-	+	-	+	-	+
	Root Bark	-	+	-	+	-	-
Saponin	Stem Bark	-	-	-	-	-	-
	Root Bark	-	+	-	+	-	-
Steroid	Stem Bark	-	+	-	+	-	-
	Root Bark	-	+	-	+	-	-
Tannin	Stem Bark	-	+	-	+	-	-
	Root Bark	-	+	-	+	-	-
Terpenoid	Stem Bark	-	+	-	+	-	+
	Root Bark	-	+	-	+	-	+

+ indicates present, - indicates absent

Total alkaloid content was found to be highest in stem bark (4.13±0.22 mg CE/g dry wt) and lowest in root bark (0.87±0.2 mg CE/g dry wt) extract of Sagar Forest division (Table 3, Fig. 5). Alkaloids are particularly well known for their therapeutic uses as anesthetics, cardio protectants, and anti-inflammatory drugs. Numerous well-known alkaloids are employed in clinical contexts, including nicotine, ephedrine, strychnine, quinine, and morphine (Rajput *et al*., 2021).

Total flavonoid content was reported to be highest (18.56±0.01 mg QE/g dry wt) and lowest (8.01±0.09 mg QE/g dry wt) in stem bark extract of Alirajpur and root bark extract of Sagar Forest division respectively (Table 3, Fig. 5). Flavonoids have a variety of health advantages, such as antiviral, anticancer, antioxidant, and anti-inflammatory effects. Additionally, they have cardio-protective and neuroprotective properties. The kind of flavonoid, its (potential) method of action, and its bioavailability all affect these biological functions (Ullah *et al*., 2020).

The highest phenolic content (64.14±0.25 mg GAE/g dry wt) was reported in the root bark extract of North Balaghat, whereas the lowest (44.37±0.3 mg GAE/g dry wt) was reported in the stem bark extract of Panna Forest Division (Table 3, Fig. 5). Phenolic compounds have reportedly demonstrated cellular defence mechanisms against cancer and atherogenesis. The strong antibacterial potential is also exhibited by phenols and phenolic compounds (Edeoga *et al*., 2005).

North Balaghat reported the highest tannin content (12.04±0.4 mg TAE/g dry wt) in the root bark extract, while the stem bark extract of Panna Forest Division reported the lowest tannin content (2.7±0.06 mg TAE/g dry wt) (Table 3, Fig. 5). The evaluation of the tannin content in plant extracts plays a key role in understanding their potential use as pharmaceuticals and nutraceuticals. Tannins reduce bacterial growth by inhibiting the crucial enzymes involved in microbial metabolism. Tannins serve as a prime source of antioxidants (Geidam *et al*., 2007; Trease and Evans 1983).

The maximum terpenoid content (9.96±0.05 %) was reported from the stem bark extract of North Balaghat whereas the minimum content (8.48±0.12%) was reported in the root bark extract of Sagar Forest Division (Table 3, Fig. 5). A very small range of variation was noted in the terpenoid content of stem and root bark extracts within the locations. Terpenes enhance human health due to their antifungal, anti-inflammatory, analgesic, antibacterial, antihyperglycemic, anticancer, antiviral, and antiparasitic properties. It is likely the most diverse class of naturally occurring substances derived from plants and contains a wide range of physiologically relevant functions (Łukowski *et al*., 2022).

The estimated phytochemicals do not show any positive relationship, neither between the stem and root bark of the plant nor within the range of phytochemicals found

in them (Table 3 and Fig. 5). The variation observed in the secondary metabolite concentrations may be influenced by environmental variables such as temperature, altitude, soil, rainfall, humidity, drought, light intensity, high salinity, water availability, minerals, freezing temperatures, and CO_2. It is perceived that stressed conditions lead to the accumulation of secondary metabolites, which helps plants in cope with and overcome stress (Akula and Ravishankar 2011). The same kind of variation study in phytochemicals has also been found in plants such as *Urariapicta* (Saxena *et al*., 2016), *Hemidesmus indicus* (Saxena *et al.,* 2017), *Gloriosa superba* (Saxena *et al.,* 2017) and *Solanum indicum* (Saxena and Pawar 2019), which were collected in several agroclimatic regions of Central India.

Table 3: Quantification of secondary metabolites in stem and root bark of *D. pentagyna* from different locations of Madhya Pradesh.

Locations/ Forest Divisions	TAC (mg CE/g dry wt)		TFC (mg QE/g dry wt)		TPC (mg GAE/g dry wt)		TTC (mg TAE/g dry wt)		TTrC (%)	
	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem
South Balaghat	1.62±0.61[a]	2.91±0.22[a]	10.9±0.01[a]	11.76±0.01[a]	52.26±0.13[a]	60.9±0.03[a]	4.72±0.07[a]	7.61±0.17[a]	9.85±0.05[a]	9.80±0.03[a]
Panna	3.06±0.11[b]	3.04±0.08[b]	9.94±0.21[b]	7.63±0.21[b]	46.71±0.17[b]	42.8±0.06[b]	4.72±0.23[b]	2.70±0.06[b]	9.06±0.15[b]	9.61±0.06[b]
East Chhindwara	1.61±0.07[c]	1.33±0.07[c]	9.54±0.31[c]	10.43±0.31[c]	44.34±0.12[c]	57.35±0.27[c]	4.63±0.03[c]	5.02±0.02[c]	9.75±0.12[c]	9.74±0.09[c]
West Chhindwara	2.08±0.1[d]	2.91±0.01[d]	10.61±0.01[d]	11.81±0.01[d]	55.57±0.07[d]	54.13±0.03[d]	5.20±0.03[d]	6.31±0.03[d]	9.77±0.07[d]	9.76±0.2[d]
Alirajpur	2.90±0.14[e]	2.56±0.2[e]	15.83±0.01[e]	18.56±0.01[e]	56.9±0.03[e]	60.12±0.26[e]	3.29±0.04[e]	4.64±0.04[e]	9.79±0.19[e]	8.61±0.07[e]
North Balaghat	3.26±0.22[f]	1.39±0.2[f]	13.61±0.09[f]	15.58±0.1[f]	64.14±0.25[f]	68.3±0.18[f]	12.04±0.4[f]	6.06±0.26[f]	9.92±0.03[f]	9.96±0.05[f]
Sagar	0.87±0.2[g]	4.13±0.22[g]	8.01±0.09[g]	9.99±0.08[g]	44.37±0.3[g]	48.74±0.22[g]	4.54±0.15[g]	5.28±0.26[g]	8.48±0.12[g]	9.65±0.16[g]

Mean values within each column represented by different letters differ significantly ($p \leq 0.05$)

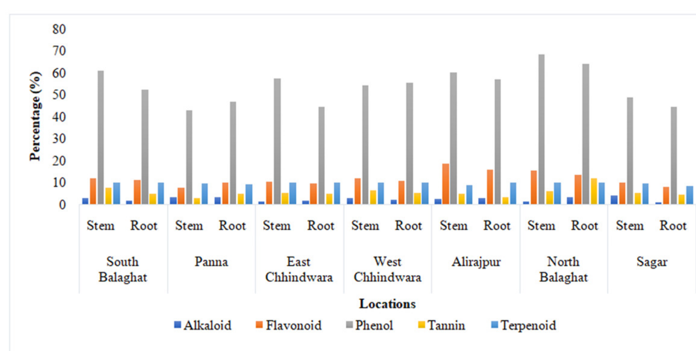

Fig. 5. Variation in secondary metabolites from various location in stem & root bark extract.

CONCLUSIONS

D. pentagyna has been extensively used in folklore medicine and the present work depicting the range of phytochemicals present in plant supports the medicinal importance of this plant. The present work is the first comprehensive evaluation on variations of various secondary metabolites in extracts of stem and root bark collected from various locations of Madhya Pradesh. The current finding will helpful in collection of samples for therapeutic purposes. Further research may also be carried out to isolate, characterize, and identify the individual compounds for drug development. Also, the study will help in the conservation as well as improvement programme of *D. pentagyna.*

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Conflict of Interest. None.

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