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Preliminary phytochemical studies in the leaf, stem and root extracts of the traditional medicinal plant species, *Thalictrum javanicum* Blume

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Abstract. The leaf, stem and root parts of the traditional medicinal plant species, *Thalictrum javanicum* are effectively used against the disorder haematuria and the roots are prescribed as diuretic, purgative and tonic by the local healers of high hills of Nilgiris, the Western Ghats, India. Little works on the analysis of certain secondary metabolites of antioxidant properties have only been made in this species. Hence, the present study was aimed at determining the available major secondary metabolites in the study species, *T. javanicum* by preliminary analysis including TLC. The results of the study show the presence of many major secondary metabolites viz., tannins, saponins, resins, flavonoids, alkaloids, glycosides, terpenoids, cardiac glycosides and triterpenoids in all the three plant parts studied. A comparison of extracts prepared with petroleum ether, chloroform, methanol and water showed that the methanol extract contained a greater number of secondary metabolites than the other extracts. The mobile phases for the separation of various secondary metabolites were also standardized. The presence of different varieties of secondary metabolites of medicinal importance helps to rationalize the use of *T. javanicum* for therapeutic purposes by local healers of high hills of Nilgiris.

Keywords. Secondary metabolites, *Thalictrum javanicum*, TLC

1 Introduction

Thalictrum genus that belongs to the family, Ranunculaceae is an extremely important medicinal plant source, and more than 200 species are available worldwide. *Thalictrum* plants are rich in benzylisoquinoline derived alkaloids; atleast 250compounds of this category have been isolated from 60 species and most of them with strong biological activities. Extracts and alkaloid isomers from *Thalictrum* are known to exhibit various pharmacological activities, including antitumor, antimicrobial, antiamoebic and HIV antiviral activities (Chen *et al*. 2003). *Thalictrum* species are mainly growing in the temperate and cold zones of both hemispheres. Due to the temperature stress, almost all species of this genus are reported to have rich variety of secondary metabolites particularly the quartenary alkaloids like oxyberberine, thalrugosaminine, rugosinone, thalisopine, berberine, thalifendine, palmatine, berberine, columbanine, jatrorrhizine, magnoflorine and demethyleneberberine (Bahadur and Shukla 1983; Sahei *et al*. 1985; Khamidullinia *et al*. 2006). *T. javanicum*, a rare sighted traditional medicinal plant species inhabiting the high hills of Nilgiris (ca. 2400 m above msl, the Western Ghats, India) is being used by the rural communities against certain specific disorders like haematuria (Tiwari *et al*., 2006), and the roots are used as diuretic, purgative and tonic (Anonymous 1976; Sharma 2009). The parts below ground are used for extracting dye (Paulsamy 2009). As the phytochemicals are important factors for healing property of any species, study of their phytochemical profile is most essential. However, little information is available on the major phytochemical constituents of this species (Abinaya *et al*. 2013) Therefore, to address this lacuna, a preliminary phytochemical investigation of this plant was carried out.

2 Materials and Methods

The leaf, stem and root of *T. javanicum* were collected from the forest margins in high hills of Nilgiris were shade dried, powdered and extracted separately with the solvents, petroleum ether, chloroform, methanol and water for further studies.

2.1 Preliminary phytochemical analysis

Fifty (g) of leaf, stem and root powder were added separately into 300 mL of the solvents viz., petroleum ether, chloroform, methanol and water, and kept in soxhlet apparatus for hot extraction for 16 hours. The extracts were filtered separately and stored at 4˚C.

The presence of a variety of phytochemical compounds in the leaf, stem and root extracts of different solvents was determined by using the methods of Brindha *et al*. (1977) and Harbone (1988).

2.2 Thin layer chromatography

The TLC studies were performed by following the method of Wagner *et al*. (1984) by using silica gel-G thin layer chromatographic plate of 15x5 cm size with 3 mm thickness.

For the separation of phytochemical compounds, only the methanolic extracts of leaf, stem and root of *T. javanicum* were spotted manually using capillary tube. The spotted plates were put into a solvent system to identify the suitable mobile phase as per the method of Wagner *et al*. (1984). After the separation of phytochemicals, the spray reagents such as Dragondorff's reagent, Vanillin hydrochloric acid and Vanillin-phosphoric acid reagent were used to spot the compound. The colour of the spots was noted and the Retention factor (R_f) values were calculated by using the following formula:

$$\text{Retention factor } (R_f) = \frac{\text{Distance travelled by the respective solvent extract}}{\text{Distance travelled by the solvent}}$$

3 Results and Discussion

3.1 Phytochemical screening

The results of the study showed that depending on the polarity of the solvents, there was wide variation in the presence of the type of secondary metabolites observed (Table 1). It has been observed that in comparison to other solvent extracts, methanol extracts of all the three parts of *T. javanicum* contained a greater variety of secondary metabolites viz., tannins, saponins, alkaloids, steroids, phenols and triterpenoids with higher quantity in leaf than that of the other parts (Eloff 1998).

Table 1. Preliminary phytochemical analysis of various extracts of leaf, stem and root parts of *Thalictrum javanicum*.

*PE-Petroleum ether, CH- Chloroform, ME- Methanol, WA-Water.

Secondary metabolite	Leaf				Stem				Root			
	Solvent*											
	PE	CH	ME	WA	PE	CH	ME	WA	PE	CH	ME	WA
Tannins	-	-	+++	-	-	-	+++	++	-	-	+++	-
Saponins	-	-	+++	-	++	+	-	-	+++	-	+	-
Resins	-	+	+	-	-	-	+++	-	-	-	+++	++
Flavonoids	-	-	+++	+++	-	-	+++	+++	-	-	+	+
Alkaloids	-	-	+++	-	+++	+++	+++	+++	++	++	+++	+++
Glycosides	+	-	+	-	-	-	+	-	-	-	+++	-
Steroids	-	-	+	+++	-	+	+++	-	-	-	+++	-
Phenols	-	-	++	+	-	-	-	-	-	-	-	-
Terpenoids	+++	+++	-	-	-	-	+	+	-	-	+	-
Cardiac glycosides	+++	-	++	-	+++	++	+++	-	+	-	-	-
Triterpenoids	-	-	++	+++	-	-	+	-	-	+++	-	-

The methanolic extracts of other two parts analysed were also contained many types of secondary metabolites (resins, glycosides, triterpenoids and tannins in stem and glycosides, steroids and terpenoids in root). On the other hand, the petroleum ether extracts of all the three parts contained a lesser number of major secondary metabolites. This difference may be due to the relatively high polarity of methanol compared to the other solvents use (Eloff 1998). In addition, it has been noted that alkaloids were generally extracted by almost all solvents used. The species with high content of alkaloids are generally considered to have effective healing properties like diuretic, antispamodic, antiseptic and antidote actions (Ming Zhao *et al*. 2010; Gonzales *et al*. 2014; Melendez-Camargo *et al*. 2014).

3.2 Thin Layer Chromatography (TLC)

Many solvents and solvent mixtures were tried for the separation of alkaloids, flavonoids and terpenoids in the leaf, stem and root parts of the study species, *T. javanicum*. The results of the study revealed that the combination of relatively high polarity solvents viz., chloroform with methanol and water ($CHCl_3$:CH_3OH:H_2O) in different proportions was the most suitable solvent system for the separation of certain secondary metabolites like alkaloids

(Figure 1), flavonoids (Figure 2) and terpenoids (Figure 3). With respect to separation of alkaloids, in methanolic leaf, stem and root extracts, the solvent mixture $CHCl_3$:CH_3OH:H_2O in the ratio of 1:0.8:0.4, 1:0.7:0.2 and 1:0.6:0.2 respectively was the most suitable.

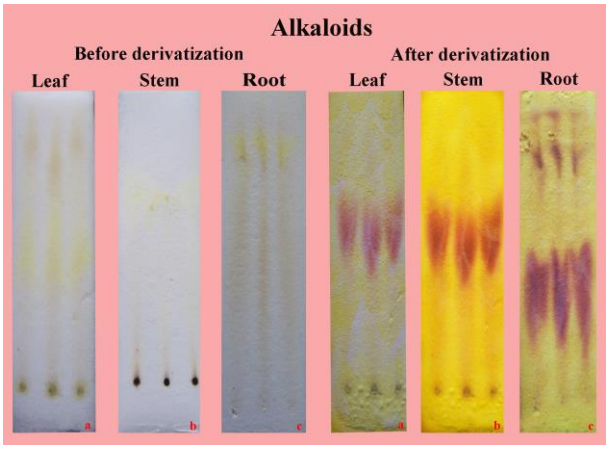

Fig 1. TLC for detection of alkaloids in methanolic leaf, stem and root extracts of *Thalictrum javanicum* (Ratio of Chloroform: Methanol: Water for leaf 1:0.8:0.4, for stem 1:0.7:0.2, and for root 1:0.6:0.2).

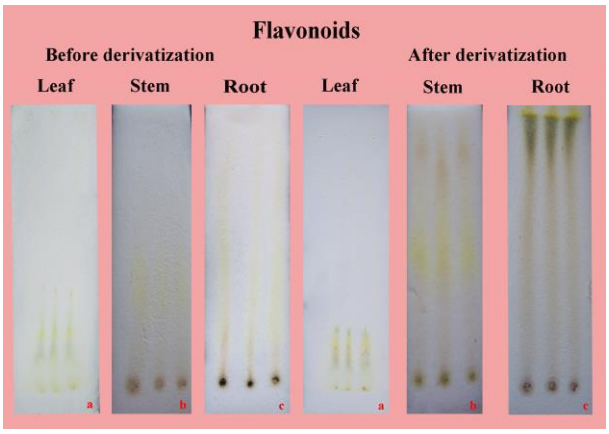

Fig 2. TLC for detection of flavonoids in methanolic leaf, stem and root extracts of *Thalictrum javanicum* (Ratio of Chloroform: Methanol: Water for leaf 1:0.6:0.3, for stem 1:0.5:0.3, and for root 1:0.5:0.4).

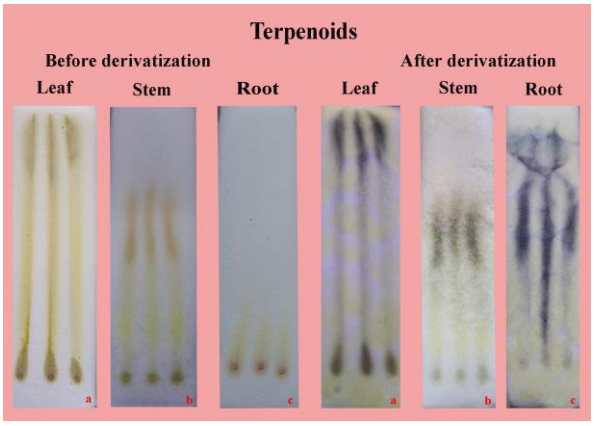

Fig 3. TLC for detection of terpinoids in methanolic leaf, stem and root extracts of *Thalictrum javanicum* (Ratio of Chloroform: Methanol: Water for leaf 1:0.7:0.2, for stem 1:0.7:0.1, and for root 1.6:0.9:0.5).

For the separation of flavonoids in the leaf, stem and root, the solvent system that was the most suitable mobile phase contained $CHCl_3$:CH_3OH:H_2O in the ratio of 1:0.6:0.3, 1:0.5:0.3 and 1:0.5:0.4 respectively. For the terpenoid separation, above solvents in the ratio of 1:0.7:0.2, 1:0.7:0.1 and 1.6:0.9:0.5 respectively was the most suitable. Results indicate that the alcoholic solvents are most effective as mobile phases for the separation of major secondary metabolites in the leaf, stem and root of the study species, *T. javanicum*. The TLC studies confirmed that the methanolic extracts of the leaf, stem and root parts of *T. javanicum* contained the most important secondary metabolites of medicinal importance viz., alkaloids, flavonoids and terpenoids (Table 2). According to Allen and Miller (1996), the species with more content of secondary metabolites such as alkaloids, flavonoids and triterpenoids naturally have antibacterial, anti-inflammatory, anti-allergic, antiviral, anti-neoplastic, antithrombotic, antioxidant, and vasotilatory properties etc. The presence of these phytochemical compounds help to support the traditional knowledge on medicinal usage of the species, *T. javanicum* by the local healers of high ranges of Nilgiris, the Western Ghats, for the treatment of various ailments. The results also support as well as recent findings pertaining to medicinal properties of this plant species by other investigators such as Abinaya *et al*. (2013, 2014).

Table 2. Preliminary separation of phytochemicals in methanolic extracts of the leaf, stem and root parts of *Thalictrum javanicum*.

Plant part	Mobile phase $CHCl_3$:CH_3OH:H_2O	Spray reagent	Colour of the spot/band	Rf value	Compound
Leaf	1: 0.8: 0.4	Dragondorff's reagent	Orange	0.57	Alkaloids
Stem	1: 0.7: 0.2	Dragondorff's reagent	Orange	0.85	Alkaloids
Root	1: 0.6: 0.2	Dragondorff's reagent	Orange	0.78	Alkaloids
Leaf	1: 0.6: 0.3	Vanillin hydrochloric acid	Yellowish spot	0.57	Flavonoids
Stem	1: 0.5: 0.3	Vanillin hydrochloric acid	Yellowish spot	0.28	Flavanoids
Root	1: 0.5: 0.4	Vanillin hydrochloric acid	Yellowish spot	0.43	Flavonoids
Leaf	1: 0.7: 0.2	Vanillin-phosphoric acid reagent (VPA)	Blue	0.90	Terpenoids
Stem	1: 0.7: 0.1	Vanillin-phosphoric acid reagent (VPA)	Blue	0.57	Terpenoids
Root	1.6: 0.9: 0.5	Vanillin-phosphoric acid reagent (VPA)	Blue	0.71	Terpenoids

4 Conclusion

The prelimnary phytochemical analysis showed that the traditional medicinal plant species, *T. javanicum* inhabiting the high ranges of Nilgiris, the Western Ghats, India contained rich variety of secondary metabolites of medicinal importance, thus which rationalizing the traditional uses of this plant species by local healers for a variety of medicinal purposes. However, further in depth pharmaceutical and pharmacological studies need to be carried out to confirm the therapeutic efficacy of this plant in the disease conditions for which local medical practitioners prescribe it.

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