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RESEARCH ARTICLE

Chemical Fingerprinting of Flavonoids in Tuber Extracts of *Tacca leontopetaloides* (L.) O. Ktze

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Abstract

Western Ghats of Maharashtra are known to be the 12th major biological hotspot that supports plant diversity and endemism. Members of the Taccaceae are famous for their use as medicinal herbs. *Tacca leontopetaloides* is erect perennial herb occurs as undergrowth in moist shady places in forests of Maharashtra. In the present investigation, flavonoid analysis of the tuber extracts of *Tacca leontopetaloides* by HPTLC was evaluated. Our findings showed recorded flavonoids like rutin, diosmin, saponin, chlorogenic acid and quercetin. This study highlights the biochemical and ethnopharmacological significance of *Tacca leontopetaloides*.

Keywords: Western Ghats, *Tacca leontopetaloides*, tuber extracts, flavonoid, ethnopharmacology.

Introduction

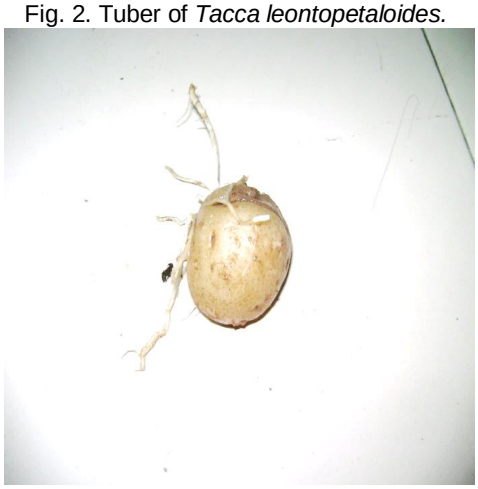
Tacca leontopetaloides is a tuberous marshy monsoon perennial, flowering in Aug-Sep, found in shades of moist deciduous forest in peninsular parts of India. In India it is reported from Travancore (Kerala), Nallamalais (Andhra Pradesh), Anantpura (Karnataka), Gujarat, Singhbhum and Manbhum (Orissa), Hajaribag (Bhihar), in Maharashtra it was found in Khinsi near Nagpur, Worli, Parel, Trombay, Kalyan, Bombay Ajara, Ramtirth, Tillari of Kolhapur district (Gamble, 1957; Shah, 1978; Ugemuge, 1986; Yadav and Sardesai, 2010). *Tacca leontopetaloides* is the only genus in the family Taccaceae, a newly-developed plant family carved out of the Dioscoreaceae, but both families still share a close taxonomic relationship (Caddick *et al.,* 2002). The plant is native to Malaysia and the Pacific Islands (Purseglove, 1972) and it is naturally distributed from Western Africa, through Southern Asia to northern Australia. *Tacca leontopetaloides* leaves are large and deeply divided, 30 to 70 cm long and up to 120 cm in width. The leaf upper surface has depressed veins and the under surface is shiny with bold yellow veins. Flowers are borne on tall stalks in greenish-purple clusters, with long trailing bracts. The plant is usually dormant for part of the year and dies down to the ground. Later, new leaves will arise from the round underground tuber. The tubers are hard and potato-like, with a brown skin and white interior (Wagner *et al*., 1990). The plants are cultivated in Travancore for tubers and foliage. The tubers used medicinally and source of starch (Mulla and Kulkarni, 1991; Okwu, 2004). The fresh tubers are acrid, bitter, poisonous and used for treatment of piles. The macerated fresh tubers repeatedly washed and salt water yield a nutritive starch of excellent culinary properties used to prepare porridges, cakes and other meats, can also used as laundry starch. The flour from tuber has following composition: water; 18.0%, fiber; 0.05%, total nitrogen; 0.01%, ether extractives; 3.0% and starch; 76.0%. The presence of sitosterol, ceryl alcohol and taccallin (0.003%), also gives positive tests for alkaloids (Watt, 1893; Kirtikar and Basu, 1937; Peters *et al.,* 1960; Scheur *et al.,* 1963). Phytochemicals are chemical compounds formed during the plants' normal metabolically processes (Okigbo *et al.,* 2009). These chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids. Most of these phytochemicals are produced through biosynthesis in metabolic pathways (Heldt, 2005). Phytochemical studies on the genus Taccahave led to the isolation of ca. 122 compounds including steroidals, diarylheptanoids, and terpenoids (Jiang *et al.,* 2014). The tuber contains starch, ceryl alcohol, steroidal saponins and a bitter principle, Taccalin (Caddick *et al.,* 2002). According to Borokini and Ayodele (2012), there were presence of alkaloids, saponins and tannins in *T. leontopetaloides* leaf while only alkaloids were present in tubers. Phytochemical analysis of *T. involucrata* showed the presence of reducing sugars, tannins, flavonoids, steroids, glycosides and hydrogen cyanide (Bosha *et al.,* 2015). Most of the phytochemicals present in *Tacca involucrata* like reducing sugars, flavonoids, tannin, steroids, alkaloid and saponins have various nutritional, physiological and pharmacological uses in the body of individuals (Ubwa *et al.,* 2011). Keeping the above facts in view, in the present study, chemical fingerprinting of flavonoids by High performance thin layer chromatography (HPTLC) in tuber extracts of *Tacca leontopetaloides* was evaluated.

Materials and methods

Sampling: Fresh samples of *Tacca leontopetaloides* tubers were collected from Uttan, district, Thane and Tamini Ghat, district, Pune region of Western Ghats of Maharashtra (Fig. 1 and 2). Fresh tubers were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The material was ground into coarse powder using mechanical grinder. This coarse powder was sieved by 1 mm pore size sieve. The powder was stored in air tight containers at room temperature till further phytochemical screening of secondary metabolites.

Fig. 1. Habit of *Tacca leontopetaloides.*

Fig. 2. Tuber of *Tacca leontopetaloides.*



Soxhlet extraction: Exhaustive Soxhlet extraction was performed using a classical Soxhlet apparatus with accurately weighed 10 g of the crude powder of plant material for 18-40 h. Extraction was performed with water, methanol, chloroform and acetone as the extracting solvent. The extraction was conducted for 6-8 h/d and finally all the extracts were evaporated under vacuum. The water, methanol, chloroform and acetone extracts of tubers of the plant were prepared according to standard methods (Harbone, 1998). These extracts were sealed in airtight containers and stored at -4°C.

Flavoinoids analysis by HPTLC: The standards Quercetin, Kaemferol, Catechin gallate, Rutin hydrate and Hesperdin were procured from Sigma Aldrich USA. All the standard solutions were prepared in ethanol where as hesperdin in water. Chromatography was performed on silica gel $60F_{254}$ (10 cm X 10 cm; 25 mm layer thickness; Merk) with aqueous, methanolic, chloroform and acetone extracts of *Tacca leontopetaloides* tuber. The fraction residues were collected and (10 µL) subjected for HPTLC (CAMAG, Switzerland) analysis. The fractions were impregnated on silica gel $60F_{254}$ TLC plate. The plate was air-dried and then inserted in CAMAG-twin through lass chamber containing solvent system of composition with ethyl acetate, acetic acid, formic acid and water (100:11:11:27) as a gradient mobile phase for 20 min. The well eluted TLC plate was then dried at 105°C for 15 min and scanned using Scanner 3 (CAMAG, Switzerland) at 254 and 366 nm using Win Cat 4 software.

Results and discussion

Flavonoids are important group of polyphenols widely distributed among the plant flora. Over four thousand flavonoids are known to exist and some of them are pigments in higher plants. Quercetin, kaempferol and quercitrin are common flavonoids present in nearly 70% of plants. Other group of flavonoids include flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, calchones and catechin and leucoanthocyanidins (Godstime *et al.,* 2014). The hydrophilic flavonoids were detected in aqueous extracts of all the plants. Additionally, the hydrophobic flavonoids were found in rest of the organic extracts. The significant test was observed in methanolic extracts of all the plants. These results prompted us to investigate and identify the various types of flavonoids by HPTLC method. Flavonoids were found profusely in all the extracts, which impelled us to evaluate the anti-oxidant activity of all the plant extracts. Flavonoids and saponins are known to be antioxidant. They prevent the damage caused by free radicals to cells. They can mediate in most cases of chronic diseases such as cancer and diabetes. They are also slow or even can stop the proliferation of cancer cells (Bruneton, 1994; Ubwa *et al.,* 2011; Trease and Evans, 2009).

Determination of flavonoids by HPTLC: HPTLC is the most recent evolution of planar chromatography, whose mission is to change the weakness of TLC into strength. HPTLC rose from a need for major separation capacity, obtained by the use of precoated plates with smaller particles (2 µm vs. 15 µm), i.e. a more active surface, in order to obtain the efficacy needed for plant mixtures. In the modern HPTLC, the plate is the central tool of a complex automatic instrumentation system developed to control analysis conditions, to optimize reproducible results and to allow a complete comparison between different laboratories.

Being a multistep process, HPTLC performance requires a separated device for each step of the sequence: sample application, chromatogram development, derivatization, visualization and documentation. HPTLC chromatograms of standard compounds (quercetin, rutin, hesperidin, Kaempferol and catechin) are presented in Figs. 3 to 6. Water, methanol, chloroform and acetone extracts, as well as of spots were characterized by R_f values and colour under UV light before (UV) and after spraying with 2-aminoethyl diphenylborinate (UV-NA). The results of two-dimensional HPTLC analyses showed that different flavonoids, phenolic compounds and phenolic acids are present in the investigated plant extracts. A large number of flavonoids (Rutin, Quercetin, Epigenin, Hesperidin, Diosmin, Kaempferol, Catechin, Astrangnlin, Luteolin, Isoquericitrin) and some unidentified flavonoid-glycosides, phenolic acids (Chlorogenic, Caffeic acid, Coumaric and Vanillic acid) and Saponins were identified by R_f values (Table 1).

Fig. 3. HPTLC chromatogram for Rutin.

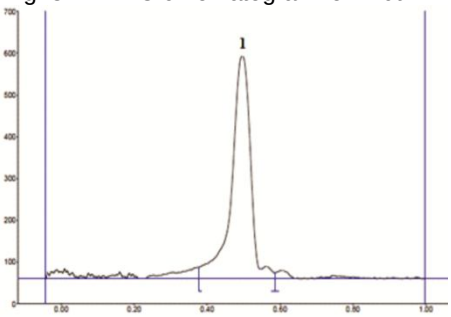


Fig. 4. HPTLC chromatogram for Quercetin.

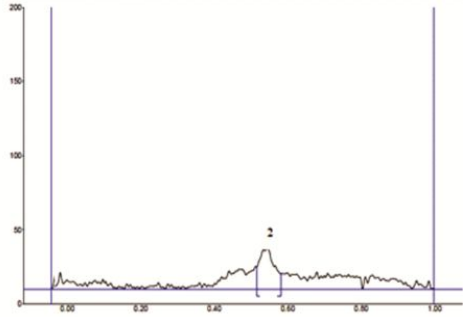


Fig. 5. HPTLC chromatogram for Kaempferol.

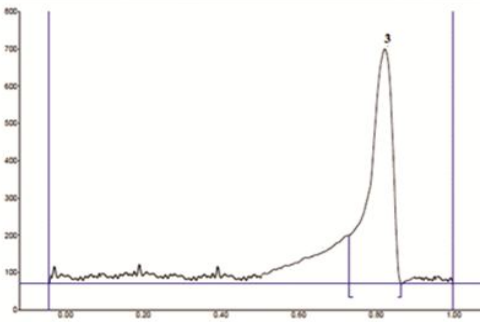


Fig. 6. HPTLC chromatogram for Catechin.

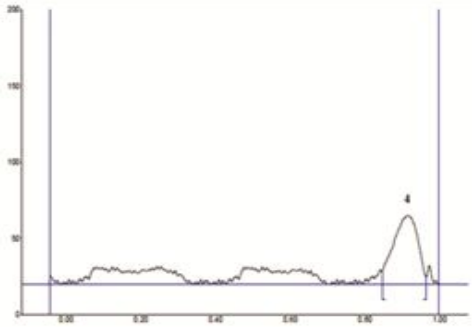


Fig. 7. HPTLC chromatogram for Hesperidin.

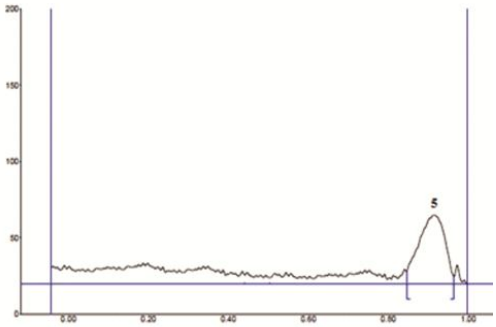


Table 1. R_f values of standard flavonoids.

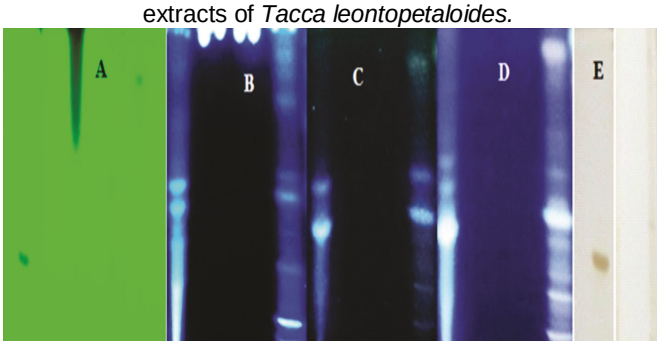
S. No.	Name of flavonoid	R_f value	Reference
1	Kaempferol	0.87	STD R_f values
2	Rutin	0.46	
3	Quercetin	0.98	
4	Hesperian	0.58	
5	Catechin	0.97	
6	Luteolin	0.34	Pavel *et al.* (2011)
7	Epigenin	0.50	
8	Saponanin	0.20	Joseph and Bernard (2003)
9	Apiin	0.39	
10	Diosmin	0.31	
11	Astrangnlin	0.65	
12	Isoquericitrin	0.53	
13	Caffeic acid	0.79	Gordana *et al.* (2003)
14	Coumaric acid	0.92	
15	Chlorogenic acid	0.64	
16	Vanilic acid	0.99	

In the chromatogram of *Tacca leontopetaloides* using water extract, total 6 and 7 peaks were obtained under UV light at 254 nm and 366 nm respectively as shown in the Fig. 9. Two unidentified flavonoids were observed, whereas Diosmin (R_f=0.29), Rutin (R_f=0.45), Epigenin (R_f=0.49), Hesperidin (R_f=0.58), Phenolic acid (R_f=0.73), Caffeic acid (R_f=0.76) were identified at 254 nm. At 366 nm, one unknown flavonoid was observed and peaks of Rutin, Epigenin, Phenolic acid, Caffeic acid were disappeared from the chromatogram.

Table 2. Chemical profiling of tuber extracts of *T. leontopetaloides* at 254 nm and 366 nm after derivatization (AD).

Plant extract	254 nm AD				366 nm AD			
	R_f value	Height (mm)	Area (AU)	Assigned substances	R_f value	Height (mm)	Area (AU)	Assigned substances
Water	0.13	5.4	39645.7	Unknown	0.13	7.0	44465.2	Unknown
	0.31	13.0	710.3	Diosmin	0.32	13.3	772.5	Diosmin
	0.44	12.2	2108.1	Rutin	0.57	4.6	3038.1	Hesperidin
	0.50	25.1	786.9	Epigenin				
	0.57	0.3	909.5	Hesperidin				
	0.63	3.6	168.6	Unknown				
	0.74	22.3	671.9	Phenolic acid				
	0.78	5.4	397.4	Caffeic acid				
Acetone	-0.01	0.4	281.5	Unknown	-0.01	2.2	198.1	Unknown
	0.06	1.2	227.6	Unknown				
	0.16	2.3	284.0	Unknown				
	0.63	13.8	373.7	Chlorogenic acid				
	0.67	10.0	262.3	Unknown				
Chloroform	0.01	29.1	905.8	Unknown	-0.02	19.1	275.0	Unknown
	0.05	4.6	577.3	Unknown	0.05	1.9	795.5	Unknown
	0.75	0.7	167.0	Unknown	0.12	0.2	20277.4	Unknown
Methanol	0.11	1.6	16016.7	Unknown	0.21	5.3	1476.3	Saponin
	0.21	2.0	2358.8	Saponin	0.33	3.5	627.2	Luteolin
	0.32	4.9	427.2	Luteolin	0.38	12.0	424.1	Unknown
	0.48	0.5	461.5	Rutin	0.48	6.8	685.0	Rutin
	0.55	8.8	1096.0	Isoquercetin	0.55	22.2	1939.7	Isoquercetin
	0.98	7.9	292.3	Quercetin	0.59	5.4	444.2	Hesperidin

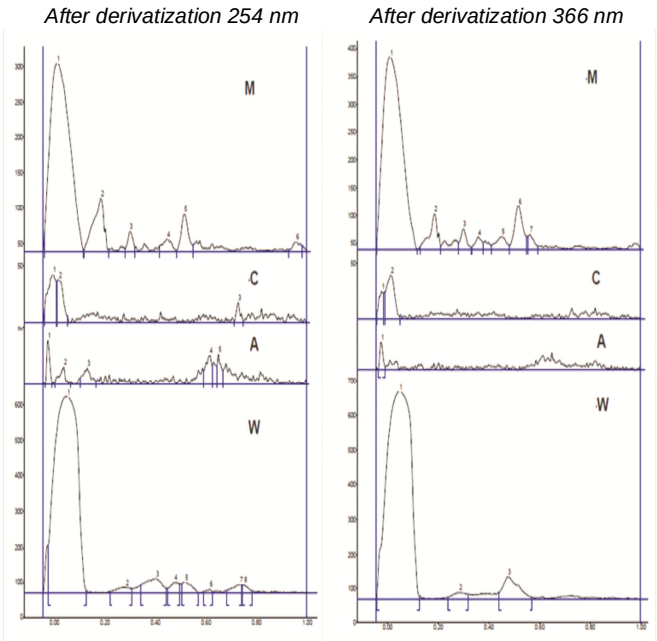
Fig. 8. HPTLC-Chemical profiling of flavonoids in tuber extracts of *Tacca leontopetaloides.*



A. Water, methanolic, chloroform and acetone extracts of *T. leontopetaloides* under UV 254 BD.
B. Water, methanolic, chloroform and acetone extracts of *T. leontopetaloides* under UV 366 BD.
C. Water, methanolic, chloroform and acetone extracts of *T. leontopetaloides* under UV 254 BD.
D. Water, methanolic, chloroform and acetone extracts of *T. leontopetaloides* under UV 366 AD.
E. Water, methanolic, chloroform and acetone extracts of *T. leontopetaloides* under visible light AD.

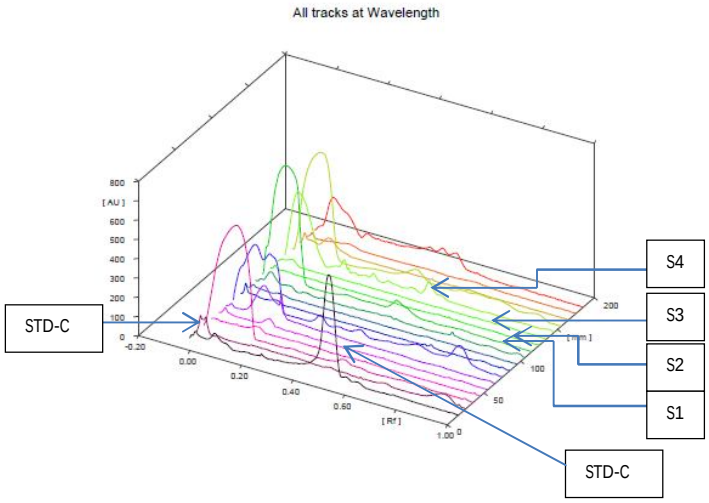
The chromatogram of acetone plant extract was investigated under UV light. Total 4 and 1 peaks were observed at 254 nm and 366 nm respectively. Four unknown flavonoids were observed, whereas Chlorogenic acid (R_f=0.63) was identified at UV light 254 nm and at 366 nm, one unknown spot was observed. The peak of Chlorogenic acid disappeared in the chromatogram. Three peaks were observed in the chromatogram of chloroform plant extract at 254 nm. All 3 peaks were unidentified. Additional 1 peak of unknown flavonoids was located at 366 nm.

Fig. 9. HPTLC peaks at 254 nm and 366 nm after derivatization of M: Methanol, C: Chloroform, A: Acetone and W: Aqueous tuber extracts of *Tacca leontopetaloides.*



In methanolic plant extract, the chromatogram displayed 6 peaks; one remained unidentified, while 5 were identified as flavonoids and phenolic acids. Saponin (R_f=0.21), Luteolin (R_f=0.32), Rutin (R_f=0.48), Isoquercetin (R_f=0.55), Quercetin (R_f=0.98) were identified at 254 nm. At 366 nm, 7 peaks were obtained.

Fig. 10. HPTLC peaks of standard and tuber extracts of *Tacca leontopetaloides.*



All peaks at 366 AD (R-STD Rutin, C-STD Catechin, S1-Water, S2-Acetone, S3-Chloroform and S4-Methanol).

Among 7 peaks, 2 unknown flavonoids have been located and a new peak of Hesperidin (R_f=0.59) was observed. The peak of Quercetin disappeared in the chromatogram. The results are depicted in Table 2 and Figs. 8, 9 and 10.

Conclusion

Tacca leontopetaloides have an ancient history of the multiple indigenous uses and is one of the most highly commercialized indigenous traditional medicines from India. Investigations of the phytochemicals and their biological activity have provided scientific support for many of its traditional uses. The HPTLC technique expressed for the determination of flavonoid from *Tacca leontopetaloides* tuber is simple, precise and can be used for standardization of biological compounds in the plant extracts. This HPLC technique is highly adaptable, because of the precision and repeatability of compound analysis in plant extracts. The detection of flavonoids like Diosmin, Rutin, Epigenin, Saponin, Hesperidin, Phenolic acid, Chlorogenic acid, Quercetin, Isoquercetin by HPTLC reveal strong medicinal value in all the tuber extracts. The structural characterisations (FTIR, NMR studies) of isolated flavonoids from various extracts of *Tacca leontopetaloides* are in progress. The use of such highly nutritious and medicinally important tubers in dietary conditions is required. The cultivation practices of wild *Tacca leontopetaloides* on commercial scale is needed for source of food and energy.

Acknowledgements

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