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Phytochemical Screening and Thin Layer Chromatography Profiling of Various Extracts of *Achyranthes aspera* and *Cissus quadrangularis*

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ABSTRACT

Achyranthes aspera and *Cissus quadrangularis* are important medicinal plants possessing wide biological activity. The natural products obtained from plants provides an unique opportunity for the development of new drugs but due to their complex nature there is a need to isolate and purify the bioactive compounds from plant extracts by separation techniques. Our earlier findings revealed that methanol extracts of *A. aspera* and *C. quadrangularis* and aqueous extract of *C. quadrangularis* exhibited antibacterial activity. This prompted to take up further research to reveal these plants' potential valuable phytochemicals and therefore an attempt has been made to separate the phytochemical constituents of methanol and aqueous extracts of *A. aspera* and *C. quadrangularis* by thin layer chromatography. Phytochemical screening of both plant extracts revealed more concentrated phytochemicals in methanol extracts than in aqueous extracts. The TLC profiling showed that methanol extract of *A. aspera* and *C. quadrangularis* are rich in flavonoids and phenolic compounds. Flavonoids were separated with n-butanol:ethyl acetate:water (1:2:3) solvent system at Rf value of 0.66 and was confirmed by post-development derivatization with 3% boric acid and 10% oxalic acid spray. After derivatization, the flavonoid spot could be further visualized under UV light at 254nm. Phenolic compounds were separated with methanol:water (2:1) solvent system at Rf value of 0.88 and confirmed with 10% ferric chloride spray. From the present study, suitable mobile phase for separation of flavonoids and phenolic compound fraction from methanol and aqueous extracts of both plants and TLC profiling have been developed.

Keywords: Phytochemicals, TLC, *Achyranthes aspera*, *Cissus quadrangularis*.

INTRODUCTION

Achyranthes aspera and *Cissus quadrangularis* are important plants possessing a wide biological activity. *Achyranthes aspera* is a medicinal plant, used in traditional medicinal systems in India as anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases and in the treatment of irregular menstruation, fever, dysentery and asthma [1, 2, 3]. *Cissus quadrangularis* is an indigenous medicinal plant of India traditionally used as an anthelmintic, antidyspeptic, digestive tonic, analgesic and treatment for scurvy and asthma [4, 5, 6]. Western Ghats in southern region of Tamil Nadu is rich in medicinal plants and traditional healers possess rich knowledge about the medicinal plants available in this region. *Achyranthes aspera* and *C. quadrangularis* plants are used by the traditional healers for common ailments in animals.

The natural products obtained from plants provides a unique opportunity for the development of new drugs but due to their complex nature there is a need to isolate and purify the bioactive compounds from crude plant extract by advanced separation techniques and instrumentation. The compounds which are isolated from different natural plant sources by using various solvent systems and chromatographic techniques are very important. Practically most of them have to be purified by the combination of several chromatographic techniques. Our earlier findings revealed that methanol extracts of *A. aspera* and *C. quadrangularis* and aqueous extract of *C. quadrangularis* exhibited antibacterial activity [7]. Hence, the present study has been taken up to reveal these plants' potential valuable phytochemicals and an attempt has been made to separate the phytochemical constituents of methanol and aqueous extracts of *A. aspera* and *C. quadrangularis* by thin layer chromatography (TLC).

MATERIALS AND METHODS

Preparation of plant material

Achyranthes aspera and *Cissus quadrangularis* plants (stem and leaves) were collected from its natural habitat in and around Tirunelveli, Tamil Nadu and were authenticated by Department of Botany, Rani Anna Government College for Women, Tirunelveli. The plants were shadow dried at room temperature and coarsely powdered using a mechanical grinder as described by us earlier [7].

Preparation of plant extracts

Aqueous extracts of *A. aspera* and *C. quadrangularis* were prepared as per the procedures of Uma *et al*., 2012 [8]. The powdered plant materials were extracted with distilled water at 100°C for 4h, centrifuged at 5,000g for 15min, and filtered using Whatman No.1 filter paper. The filtrate was vacuum evaporated and the yield was calculated according to Isah 2012 [9].

Methanol extracts of both the plants were prepared by following the methods described by Gawande and Goel 2015 [10]. The powdered plant materials were subjected to simple maceration process by using methanol for 72h with orbital shaking at room temperature. The extracts were filtered through Whatman No.1 filter paper and the solvents were evaporated with the help of the rotary evaporator under reduced pressure at 45°C. The concentrated extracts were kept at 4°C in a refrigerator until further use.

Phytochemical Screening

Preliminary phytochemical tests of extracts of *A. aspera* and *C. quadrangularis* were performed for phytochemical screening viz., alkaloids, flavonoids, amino acids, carbohydrates, saponins and tannins and phenolic compounds by following the standard procedures [11, 12].

Thin layer chromatography separation

Chromatography was performed on TLC silica gel $60F_{254}$, Aluminium sheets of size 7.5cm x 10cm (Merck, Germany). The aqueous and methanol extracts of *A. aspera* and *C. quadrangularis* were resuspended in respective solvents at a concentration of 100mg/mL and used for TLC analysis. The extracts of 10µL was manually applied to the plate as spot using the Hamilton 50µL syringe, positioned 1cm from the bottom and 1.5cm from side of the plate, on each plate with four applications. The space between two spot was 1.5cm. The application parameters were identical for all the analyses performed. The spotted TLC plates were subjected to development in the TLC developing glass chamber pre-saturated with different solvent systems as mobile phase (Table.1). The developing distance was 80mm and the developed plate was removed from the chamber and dried over the hot plate for the evaporation of solvents used as mobile phase. The different spots developed in each solvent system were identified by means of post-development derivatization with different spraying agents (Table.1) and the Rf value were correspondingly calculated and interpreted as described by Mehta *et al*., 2017 [13].

Table 1: Different solvent system and confirmatory test spray agents used for TLC profiling of photochemical

Photochemical	Solvent system	Confirmatory test spray agents
Alkaloids	Ethyl Acetate: Chloroform: Water 5: 3: 1	Mayer's reagent spray
Flavonoids	n-Butanol: Ethyl Acetate: Water 1: 2: 3	3% boric acid + 10% oxalic acid spray
Tannins	Chloroform: Water 3: 2	10% Ferric chloride spray
Phenols	Methanol: Water 2: 1	10% Ferric chloride spray

RESULTS AND DISCUSSION

The percentage yield of *A. aspera* and *C. quadrangularis* methanol extract was 6% and 4% respectively. Whereas, 12.1% and 23.5% is the yield from aqueous extracts of *A. aspera* and *C. quadrangularis* respectively (Table 2). The percentage of yield was highest in aqueous extracts than methanol extracts of both plants.

Table 2: Percentage yield of various solvent extracts of *A. aspera* and *C. quadrangularis*

Sl.No.	Plant	Methanol Extract	Aqueous extract
1	*A. aspera*	6%	12.1%
2	*C. quadrangularis*	4%	23.5%

Phytochemical screening

The results of phytochemical screening of both methanol and aqueous extract of *A. aspera* and *C. quadrangularis* are shown in Table 3 and Fig. 1 to 4. The results revealed the presence of various phytochemical constituents including alkaloids, flavonoids, amino acids, carbohydrates, saponins, tannins and phenolic compounds. However, methanol extracts exhibited more concentrated phytochemicals when compared to that of aqueous extracts of both plants, except alkaloids and amino acids, which were enriched in aqueous extracts. These findings are in accordance with Shabi Ruskin *et al.,* 2014 who showed that phenol, alkaloids, tannins and flavonoids were present in the ethanolic extract of *C. quadrangularis*, while saponin and carbohydrates were absent [14]. Whereas, Chidambara Murthy *et al*., 2003 reported that ethyl acetate fraction of both fresh and dry stem extracts of *C. quadrangularis* showed the presence of sterols, vitamin C, and tannins as phytoconstituents [15].

Table 3: Qualitative analysis of phytochemicals of methanol and aqueous extracts of *A. aspera* and *C. quadrangularis*

Sl. No.	Phytochemical constituents and Test	*Achyranthes aspera*		*Cissus quadrangularis*	
		Methanol extract	Aqueous extract	Methanol extract	Aqueous extract
1	**Alkaloids**				
	Dragendorff's test:	++	+++	++	+++
	Mayer's test:	+	++	+	++
2	**Flavonoids**				
	Ferric chloride test:	+++	++	++++	+
3	**Amino Acids**				
	Ninhydrin Test:	+	+++	+	+++
4	**Carbohydrates**				
	Fehling's test:	++	+	++	+
5	**Saponins**				
	Foam test:	++	++	+	++
	Lead acetate test:	+	+++	+	+++
6	**Tannins and Phenolic Compounds**				
	Ferric chloride test:				
	Lead acetate test:	+++	++	++++	++
		++	++	+++	+

+++ Appreciable; ++ Moderate; + Trace amount

Figure 1: Alkaloids – Dragendorff's test: Reddish-brown precipitate

Figure 2: Saponins – Lead acetate test: White recipitate

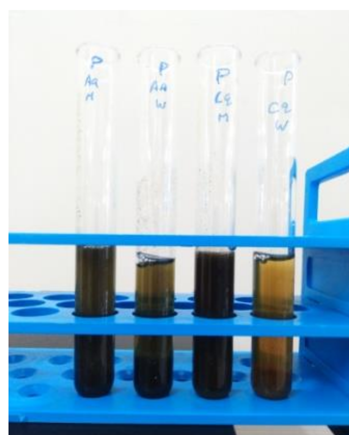

Figure 3: Tannins and Phenolic compounds – Ferric chloride test: Dark green colour

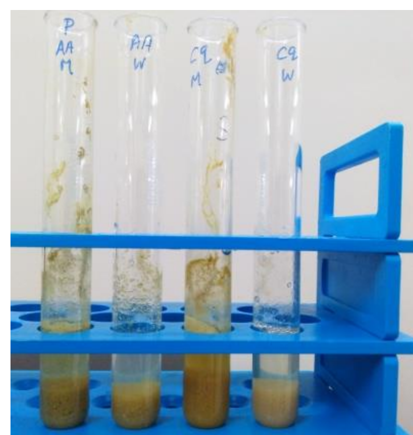

Figure 4: Tannins and Phenolic compounds - Lead acetate test: White precipitate

Thin layer chromatography separation

In the present study an attempt has been made to separate the phytochemical constituents of methanol and aqueous extracts of *A. aspera* and *C. quadrangularis* by thin layer chromatography (TLC). TLC profiling of both plant extracts were carried out with different solvent systems for different phytochemicals (Table 1). Flavonoids were better separated with n-butanol:ethyl acetate:water (1:2:3) solvent system. TLC separation followed by post-development derivatization of the TLC plate with 3% boric acid + 10% oxalic acid spray revealed the presence of flavonoids in the plant extracts at Rf value of 0.66 (Fig. 5 to 6). Thereby, the presence of flavonoids was confirmed by post-development derivatization of the TLC plate, which is in accordance with Mehta *et al.*, 2017 [13]. In the present

study, after derivatization, the flavonoid spot could be further visualized when the plates were viewed under UV light at 254nm, which was more dense in methanol extract of *C. quadrangularis* when compared to methanol extract of *A. aspera* (Fig. 7). Based on the intensity of the spot under UV light at 254nm, it was found that flavonoid was enriched in methanol extract of *C. quadrangularis* followed by methanol extract of *A. aspera*, aqueous extract of *A. aspera*, while aqueous extract of *C. quadrangularis* showed traces only.

Phenolic compounds were better separated with methanol:water (2:1) solvent system and confirmed by post-development derivatization with 10% ferric chloride spray, which exhibited dark green colour spot at Rf value of 0.88 (Fig. 8 and 9). Based on the intensity of the spot, it was observed that methanol extract of *A. aspera* showed highest concentration of phenolic compounds followed by methanol extract of *C. quadrangularis*, aqueous extract of *A. aspera*.

Figure 5: TLC separation of flavonoids: before derivatization.

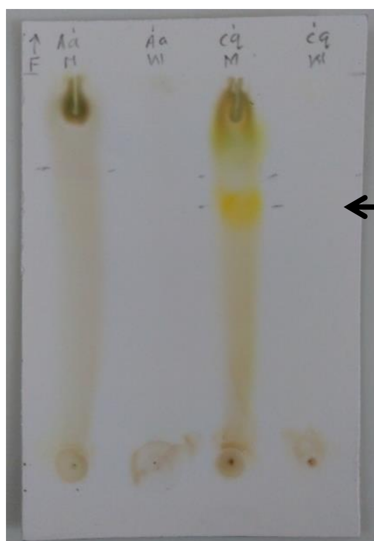

Figure 6: TLC separation of flavonoids: after derivatization with confirmatory spray agent 3% boric acid + 10% oxalic acid.

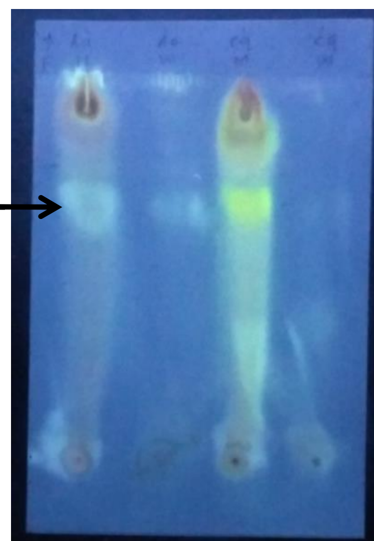

Figure 7: TLC separation of flavonoids: after derivatization, viewed under UV light at 254 nm.

Figure 8: TLC separation of phenolic compounds: before derivatization.

Figure 9: TLC separation of phenolic compounds: after derivatization with confirmatory spray agent 10% ferric chloride.

Aa-M: *Achyranthes aspera* Methanol extract; Aa-W: *Achyranthes aspera* Aqueous extract
Cq-M: *Cissus quadrangularis* Methanol extract; Cq-W: *Cissus quadrangularis* Aqueous extract

The TLC profiling results showed that methanol extract of *A. aspera* and *C. quadrangularis* are rich in the presence of flavonoids and phenolic compounds. Further, the results of the present study indicated that suitable mobile phase for separation of flavonoids and phenolic compound fraction from methanol and aqueous extracts of *A. aspera* and *C. quadrangularis* have been developed.

CONCLUSION

Phytochemical screening of methanol and aqueous extracts of *A. aspera* and *C. quadrangularis* revealed more concentrated phytochemicals in methanol extracts when compared to that of aqueous extracts of both plants except alkaloids and amino acids,

which were enriched in aqueous extracts. Flavonoids were better separated with n-butanol:ethyl acetate:water (1:2:3) solvent system at Rf value of 0.66 by TLC and was confirmed by post-development derivatization with 3% boric acid and 10% oxalic acid spray. Phenolic compounds were better separated with methanol:water (2:1) solvent system at Rf value of 0.88 and confirmed with 10% ferric chloride spray. The outcome of the present study encourages taking up further research to isolate and characterize the active principles from the *A. aspera* and *C. quadrangularis* plant extracts to exploit the potential pharmacological activity and to find novel pharmacological uses of these plants beyond their ethnic use.

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