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Phytochemical Analysis and Partial Characterization Caralluma Attenuata Extract by TLC

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Abstract

Medicinal and natural herbal plant products are traditionally used from long time in many countries. The current work was to evaluate the flavonoid rich fraction in Caralluma attenuate *and other phytochemical analysis of stem of the plant. Preliminary phytochemical analysis revealed the presence of phytochemicals such as alkaloids, polyphenols, flavonoids and tannin content in methanol extracts of stem then they were determined spectrometrically. The present study provided, a detailed report on the isolation and characterization of Thin Layer Chromatography from stem of* Caralluma attenuate. T*he methanol extract were used for various biological properties and in vivo assays which is used discovering new drugs.*

Keywords: *Caralluma attenuata, phytochemical screening, medicinal uses and TLC.*

Introduction

Traditional medicinal plants has focused on the discovery of valuable drugs during the past few decades (Buenz *et al*., 2004). Polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. Fruits like grapes, apple, pear, cherries and berries. Typically a glass of red wine or a cup of tea or coffee contains about 100 mg polyphenols. Cereals, dry legumes and chocolate also contribute to the polyphenolic intake (Scalbert *et al*., 2005 and Spencer *et al*., 2008). Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens (Beckman, 2000).

Stem and bark paste is used for treating skin diseases, headaches and to arrest bleeding wounds (Balasubramanian *et al*., 1997, Ajit *et al*., 2007, Lenin and Venkat, 2009). Stem and branches are used as fuel and its ashes are used as detergents in interior eastern India (Mahadeswara Swamy, 2006). On the other hand, seeds are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses (Divya *et al*., 2011).

Bio-flavonoids comprise a group of phenolic secondary plant metabolites that are widespread in nature. Major flavonoids that have well categorized structures and well defined structure function-relationships are: flavans, flavanones, flavones, flavonols, flavanols, flavanonols, cetechins, anthocyanidins and isoflavones. Bio-flavonoids are well-known for their multi-directional biological activities including anti-diabetic efficacy (Brahmachari, 2009).

Tannins are naturally occurring, high molecular weight polyphenols which can be divided into hydrolysable tannins and condensed tannins. Tannins are the most abundant antioxidants in the human diet and they exhibit many biologically important functions which include protection against oxidative stress and degenerative diseases.

Materials and methods

Collection and preparation of plant extracts

Stem of *Caralluma attenuata* were obtained from in and around Thiruvellore District, Tamilnadu, India and the plants are authenticated and identified at Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106 Tamil Nadu, India.

Phytochemical analysis of caralluma attenuate

The following qualitative analysis are performed to find out the presence of bioactive compounds qualitatively.

Test for alkoloids

To 0.05ml of *Caralluma attenuata* extract 2ml of HCl was added. To this acidic medium 1ml of Dragendroffs reagent was added on, orange or red precipitate produced immediately indicate the presence of alkaloids.

Test for flavonoids

A portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

Test for terpenoids (Salkowski test)

Five ml of *Caralluma attenuata* extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for phenols

5ml of filtered extracts were taken and 1ml of $FeCl_3$ (1%) and 1ml K_3 (Fe (CN) $_6$) (1%) were added. The appearance of fresh radish blue color indicated the presence of polyphenols.

Flavonoid extraction from caralluma attenuata

The herb (10 g) was extracted exhaustively with 70% aqueous methanol, combining maceration (24 h) with subsequent extraction at 60°C. The aqueous methanol extracts were evaporated in vacuum to a thick residue and left for 10 -12 h at 5–10°C. The dark green resinous solid was separated by filtration, treated with hot water, cooled, and filtered. The purified aqueous solution was extracted successively with EtOAc, and *n*-BuOH (Kovalev, 2009).

The Partial characterization of flavonoid rich fraction from caralluma attenuata by TLC

The flavonoid fraction of *Caralluma attenuata* extract were loaded on to pre coated TLC (60 F_2 54) and it was developed using solvent system in the ratio of Petroleum ether, Chloroform and methanol (1:0.5:0.1, V/V/V) was used for the development of the exudates on silica gel plates silica gel 60 F_{254} (10x20cm, 0.2mm layer). Visible and the non-visible spot given and it is fluorescent with UV light at 360nm and 240nm.

Results

Phytochemical screening of aqueous methanol extracts from the caralluma attenuata

Recent interest in plant secondary metabolites has focused on their potential benefits to human health. The polyphenols, tannin, alkaloids are capable not only to reduce oxidative stress but also to inhibit carbohydrate hydrolyzing enzymes and thus preventing hyperglycemia Many research and investigations of oral anti-hyperglycaemic agents of natural plant origin were used in traditional medicine have been studied and many of them have been found to posess the positive activity (Kirti *et al.*, 2008). The phytochemical screening of the *Caralluma attenuata* were studied showed the presence of alkaloids, flavonoids, tannins, terpenoids, glycosides and phenols (Table -1 and Figure -1).

Table 1. Phytochemical screening of aqueous methanol extracts from the Caralluma attenuata

S.No.	**Constituents**	**Test**	**Qualitative results of *Caralluma attenuata* Aqueous methanol extract**
1.	Alkaloids	Mayer's test	+ +
2.	Flavonoids	Lead acetate test	++
3.	Polyphenols	Ferrozine test	+
4.	Terpenoids	Salkowski test	-
5.	Tannins	$FeCl_3$ Test	+
6.	Glycosides	Keller-Killani test	+
7.	Saponins	Froth test	+

-- = Negative (absent); + = Positive (present)

Figure 1. Phytochemical screening of aqueous extracts from the *Caralluma attenuata*

Total flavonoid content of stem extract of caralluma attenuata

In this context, the preliminary experiments revealed that 80% methanol was the best solvent for the extraction of flavonoids from *Caralluma attenuata* at 60 °C for 60 min since it afforded a maximum yield of flavonoids. The yields stem of *Caralluma attenuata* extracts ranged from 43% (w/w). Therefore, the total phenolic contents were reported as rutin equivalents (Figure-1 and Table-2).

Table 2. Total flavonoid content *Caralluma attenuate* stem extract

Sample	**Yield of extract (g/100 g of defatted Content)**	**Total flavonoid content (mg rutin equivalents per gram flavonoid rich fraction)**
Flavonoid exctract of *Caralluma attenuata*	42.1±1.7[a]	127.2±1.3[b]

[a]Data are expressed as mean ± standard deviation (n = 3) on a fresh weight basis.

[b]Means in each column sharing the same letter are not significantly (P = 0.05) different from other.

The Partial characterization of Caralluma attenuata by TLC

The flavonoid extract of *Caralluma attenuata* loaded on Pre-coated TLC plates (60 F_2 54 Merck) and developed with a solvent system of Toluene, Tetrahydrofuran, and Glacial aceticacid in the ratio of 9.5:2.5:0.4 were efficient to extract the compounds it is used for further studies. The developed plate was viewed under UV 240nm and 360nm (Table-3 and Figure-2).

Table 3. Partial characterization of Caralluma attenuata flavonoid extract by TLC.

S.No	**Rf Values of *Caralluma attenuata* flavonoid extract**		
	UV 240 nm Rf value	**UV 360 nm Rf value**	**Visible Light Rf value**
1.	0.84	0.84	-
2.	0.56	0.56	-
2.	0.50	0.50	-
4.	0.44	0.44	-
5.	0.26	0.26	-

TLC under normal light

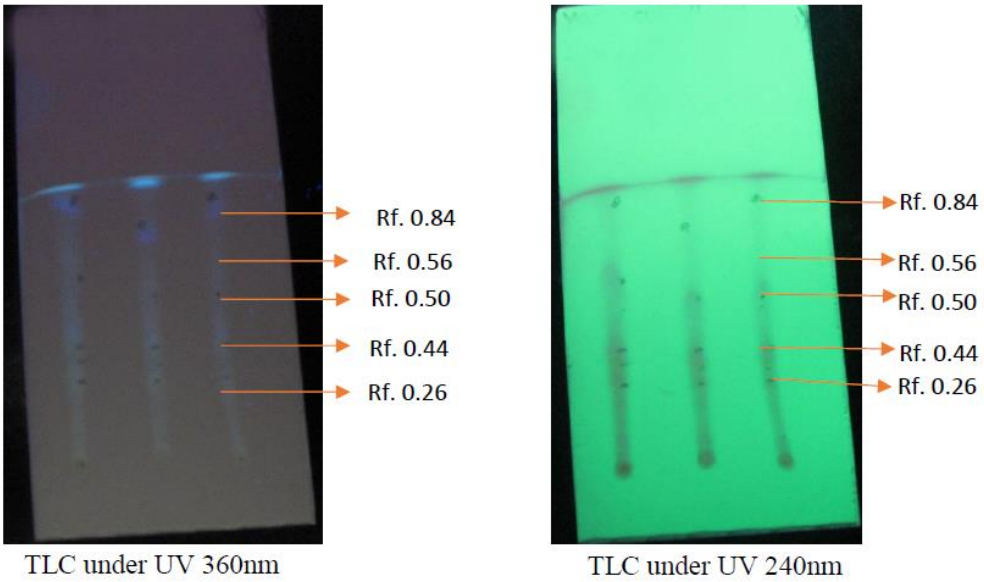

Figure. 2. Partial characterization of Caralluma attenuata flavonoid extract by TLC.

Conclusion

The present study of the phytochemical screening in flavonoid rich fraction of *Caralluma attenuata* stem showed that, this plant could be a potential source for natural antioxidants. It has been reported that most active principles in *Caralluma attenuata* are frequently alkaloids, flavonoids and phenols and these may be responsible for many of the pharmacological actions of the particular plant. If these plants are examined for further biological studies, it could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions. Furthermore, detailed studies on the isolation and characterization of HP-TLC in the plant extract and its validation will be interesting in discovering new drugs.

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[13]. Composition of Reagents:

Dragendroffs reagent

8g of bismuth nitrates Bi $(No_3)_3.5\ H_2O$ was dissolve in 20ml of HNo_3 and 2.72g of Potassium iodide in 50ml of H_2O. These were mixed and allowed to stand for deposition of KNo_3 Crystals. The Supernatant was decanted off and made up to 100ml with distilled water.