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Phytochemical analysis of *Caralluma stalagmifera* C.E.C. Fisch, an endemic and important medicinal plant

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Abstract
The present study deals with preliminary phytochemical investigation of *Caralluma stalagmifera* C.E.C. Fisch, commonly known as dark purple *Caralluma*, belonging to the family Asclepiadaceae of sub family Apocynaceae. It is a perennial succulent plant, growing in rocky areas in dense clumps. The flowers are star shaped, dark purple with purplish yellow tips. The plant have many medicinal properties like anti-obesity, anti-diabetic, carminative, anthelmintic, antioxidant, antipyretic, anti-inflammatory etc. The plant mainly consists of pregnane glycosides and aglycone steroids. In the present study, plants were collected from Medikonda village from Jogulamba Gadwal District, Telangana. Plant powder was extracted in Soxhlet apparatus by using four different solvents like Petroleum ether, Ethyl acetate, Chloroform, Methanol. and analyzed for presence of different phytochemicals. The study revealed the presence of different phytochemicals like alkaloids, flavonoids, steroids, phenols, tannins, saponins, glycosides etc. plant extract. The preliminary screening is essential to understand the bioactive compounds and their pharmacological action of *Caralluma stalagmifera.*

Keywords: *Caralluma stalagmifera*, pharmacology, phytochemical analysis, anti-obesity

Introduction
Plants are capable of synthesizing diverse groups of chemical compounds, with various therapeutic properties. There has been a major resurgence in interest in traditional medicinal plants due to lesser side effects and eco-friendly nature. Presently, the research has been focused to exploring the indigenous plant resources to screen for development of novel pharmaceutical active compounds.

Caralluma species are important plants, with various medicinal properties. These are morphologically stem succulents, erect, creeping as well as scrambling herbs. These plants are widely distributed in South Asia, Africa, Middle East, and Spain etc. In India, these are represented by 13 species and 5 varieties (Jagtap A & N P Singh, 1999) [1]. Previously, genus *Caralluma* is grouped under the Asclepiadaceae family. However, modern molecular and genetic studies have suggested Asclepiadaceae to be treated as a subfamily Asclepiadoideae in the family Apocynaceae (Endress ME & Bruyns PV, 2000) [2]. Common members of this family are *Caralluma fimbriata, C. adscendens, C. stalagmifera, C. tuberculata, C. edulis, C. negevensis, C. sinaica, C. russeliana, C. dalzielii, C. Arabica*, C. *nilagiriana*, C. *indica* etc. Among all, *C. stalagmifera*, is of significant medicinal importance and not much explored species.

Caralluma stalagmifera C.E.C. Fisch, commonly known as dark purple *Caralluma*, belonging to the family Asclepiadaceae. *C. stalagmifera* is a perennial succulent plant growing mostly on rocky areas in dense clumps. The flowers are tiny, star shaped, dark purple with purplish yellow tips. The leaves are small, narrow and triangular or tooth-like, stalkless (Fig.1). The inflorescence is axillary, usually only one-flower, occasionally with two flowers. Flowers are bisexual, with fleshy corolla, wheel-shaped and dark purple coloured. The corolla tube is cream-coloured, with apex ciliate with spindle shaped or clavate hairs, characteristically hanging down like stalagmites (hence the name). Pollen is enclosed in pollinia and fruits are a pair of follicles. Flowering season is June.

In India, the species of *Caralluma* are edible, rich in protein, minerals, vitamins and a good source of nutrition (Rajikiran *et al*., 2011) [3]. Its stem and roots are not only eaten raw as famine food but also cooked due to its pharmacological potential.

The extracts of *Caramulla* found to be appetite suppressant and reduce obesity (Lawrence, R.M. & Choudary, 2004) [ ]. The medicinal properties of *Caralluma* include carminative, anthelmintic, anti-diabetic, antioxidant and anti-pyretic (Aruna *et al*., 2011) [5]. Chemically, *Caralluma* species are famous for presence of their key components i.e. glycosides specifically pregnane glycosides and aglycone steroids, saponins, triterpene etc. (*K*unert *et al*., 2009) [6]. The plant is used as utilized in various therapeutic purposes against different infectious agents and metabolic disorders (Adnan *et al*., 2015; Rauf *et al*., 2013) [9, 8].

At present, *Caralluma* species is gaining much importance as weight loss promoter by decreasing the appetite and also to increase the endurance. The present study was carried to investigate the phytochemicals from the aerial part of endemic species *Caralluma stalagmifera*.

Materials and Methods

Collection of plant material

The *C. stalagmifera* plants were collected from rural Medikonda village from Jogulamba Gadwal District, the southern part of Telangana state in India during the month of June-July in the year 2021. The plant was authenticated by the Department of botany of Osmania University & voucher specimen has been submitted to the Herbarium, Department of Botany Osmania University, Hyderabad).

Drying & Extraction

The collected *C. stalagmifera* plants were thoroughly cleaned and cut into pieces. Then, the pieces were dried in a hot air oven at 40 degrees centigrade to remove the moisture content. The dried herb was powdered by a mechanical grinder. The powder was sieved twice to get the fine powder. The extraction process was carried using different solvents successively in the order of increasing polarity. The powder was extracted successively in the Soxhlet apparatus by using four different solvents like Petroleum ether, Ethyl acetate, Chloroform, Methanol. The solvent was removed and the extract was isolated. The extracts from various solvents were used for analysis of presence of different phytochemicals Alkaloids, Flavonoids, Saponins, Steroids & Terpenoids, Phenolic compounds, Tannins, Glycosides etc.

Screening the plant extract for phytochemicals

Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds using standard procedures (Raman, 2006, & Sasikumar *et al*., 2014) [10, 11].

1. **Detection of Alkaloids:** Extracts were dissolved in dilute. Hydrochloric acid and filtered.

Mayer's Test: To 2.0 ml extract, two to three drops of Mayer's reagent were added by the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

2. **Test for Flavonoids:** To 2.0 ml of test solution, add few fragments of magnesium ribbon magnesium turnings. A few drops of concentrated HCl was added and Boiled for five minutes. A red colour (Tomato red color) indicates the presence of flavonoids.
3. **Test for Saponins:(frothing test):** About 0.5 g of the powdered drug was boiled gently for 2 min with 20 mL of water and filtered while hot and allowed to cool. 5 mL of the filtrate was then diluted with water and shaken vigorously. A frothing persistence indicates the presence of saponins.
4. **Detection of Steroids & Terpenoids Liebermann-Burchardt test**: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride, and 1 to 2 drops of concentrated sulphuric acid were added. Appearance of the dark green color showed the presence of steroids.
5. **Test for Phenolic compounds:** Small quantity of powdered sample was tested with the following reagents and the colour produced indicates the presence of phenolic compounds.

a. Ferric Chloride test

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

b. Lead acetate test

The extract (50 mg) is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

6. **Test for Tannins:** A small quantity of the powdered drug was extracted with solvents. To the 2 ml of filtered extract, few drops of ferric chloride solutions were added. A bluish black color indicates the presence of tannins.

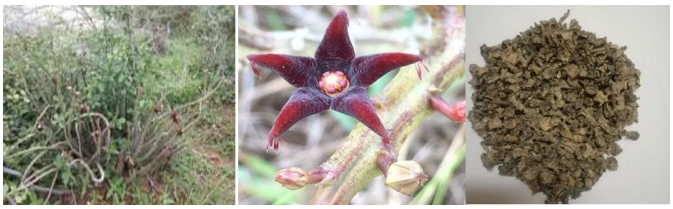
Test for Glycosides: 2 ml of extract was mixed with a little anthrone on a watch glass. One drop of concentrated sulphuric acid was added, made into a paste and warmed gently over a water bath. *A* dark green-blue coloration indicates the presence of glycosides.

Results and Discussion

Present study was under taken to qualitative essay of phytochemicals present in *Carulluma stalagifera* herb extract in different solvents. The results revealed the presence of a diverse group of phytochemicals, which were presented in tables (Table-1 & Fig. 2). Among different solvents, none of the compounds were observed in petroleum ether extract of plants.

The presence of Alkaloids and Flavonoids were noticed in Chloroform & Methanolic extract of plant and absent in the remaining solvents. Saponins were not observed in any of the solvent extracts. Presence of high amounts of steroids was observed in chloroform and low amounts in methanol extract, and absent in other solvents. Phenols were observed only in ethyl acetate extract and absent in other solvent extracts. High amounts of tannins were observed in chloroform extract and low amounts in methanol extract, but tannins were absent in other solvent extracts. Glycosides were observed in three different solvents i.e ethyl acetate, chloroform and methanol. Among these, high amount was observed in methanol, followed by chloroform extract.

In the present study, a qualitative assay for various phytochemicals in C. *stalagifera* was done using a range of solvents. The results showed the presence of Alkaloids, Flavonoids, Steroids, Phenols, Tannins, Glycosides in the plant extract. Previous studies on preliminary phytochemical analysis revealed the presence of these phytochemicals in some of the species of *Caramulla* i.e. *C. fimbriata* (Padwal *et al*., 2016) [12], C. *wissmannii* (Padwal *et al*., 2016) [12], *C. attentuata* (Kiranmayee *et al*., 2015) [14], *Caralluma indica* (Gnanashree & Mohamed Sirrajudeen, 2018) [15]. But no previous studies are reported in *C. stalagifera.* Hence this is the first report on phytochemical analysis of *Carulluma stalagifera.*



A B C

Fig 1: *Caralluma stalagmifera* plant, flower and dried ste M

A **B**

Fig 2: Extraction of *Caralluma stalagmifera* plant for phytochemical analysis.

Table 1: Preliminary phytochemical analysis of *Caralluma stalagmifera plant* in different solvents

Sl. No.	Phytochemicals	Petroleum ether	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	- ++	-	+++
2	Flavonoids	-	- +	-	+++
3	Saponins	-	- -	-	-
4.	Steroids	-	+	-	+
5	Phenols	-	- -	+++	-
6	Tannins	-	- ++	++	-
7	Glycosides	-	- ++	+	+++

(+) = Present, **(-)** = Absent
(+) =less amount, (++) = medium, (+++ and ++++) – more

Conclusions

The preliminary screening of phytochemicals is a valuable step in the detection of the bioactive principles of medicinally important plants. The present study confirms the presence of alkaloids, flavonoids, steroids, glycosides, phenols, tannins in the stem of *C. stalagifera.* The outcome of the present analysis will facilitate the quantitative estimation of phytochemicals and to study the pharmacological active principles of *Carulluma stalagifera*

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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