

Phytochemical Investigation of *Caralluma attenuata* (Wight) Roots.

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ABSTRACT

The aim of the present study was to investigate the phytochemicals of the rare endemic species *Caralluma attenuata*. No attempt was made to extract the compounds from the roots of *Caralluma attenuata* starting from Toluene, Ethyle acetate, Butanone and n-Butanol i.e., the extractions were carried out from the non-polar end and to the polar end. The results showed the presence of steroids / triterpenoids, saponins and flavonoids / their glycosides. Alkaloids and cardiac glycosides were found to be absent.

Key words: Polar solvents, non-polar solvents, *Caralluma*, secondary metabolites.

INTRODUCTION

Caralluma spp is a succulent plant native to Asia, the Mediterranean, and Africa¹. *Caralluma* is eaten as a food and has a number of traditional ethnobotanical uses that include: diabetes, leprosy, rheumatism, paralysis, joint pain, migraines, fever, malaria, and inflammation. The species *C. fimbriata* and *C. adscendens* var. *fimbriata* have been used in traditional Indian medicine in this manner. In addition, *C. tuberculata* has been used as a digestive aid and treat diabetes. Today most interest is centered on *Caralluma*'s use as an appetite suppressant (www.gencor-usa.com).

Caralluma attenuata (Wight) is a perennial, erect and fleshy herb. It grows to a height of 1-3 feet. Roots are fibrous. *Caralluma* is a succulent plant (cactus) from India and grows wild and is often used as a border in gardens and as a roadside shrub. It is also found in Africa, Saudi Arabia, Canary Islands, Afghanistan, and Southern Europe. Traditionally, Indian tribes chew chunks of *Caralluma* to keep from being hungry during a long hunt. These days, a solution that contains chemicals taken from the plant (extract) is used to decrease appetite for weight loss. It is also used to quench thirst and to increase endurance. In foods in India, *Caralluma* is cooked as a vegetable such as chutneys and pickles. It is also eaten raw. *Caralluma* sps have been extensively used for paralysis, joint pain and fever^{2,3}.

Literature survey reveals that *Caralluma* is a good source of steroids and their glycosides. Not many *Caralluma* have been investigated, earlier for their chemical constituents or for biological activity, the world over. In the present investigation an attempt has been made to know the phytochemical compounds of *C. attenuata* roots using nonpolar solvents like Toluene, Ethyl acetate, Butanone

and n-Butanol.

No attempt was made to extract the compounds from the roots of *C. attenuata* starting from Toluene, Ethyl acetate, Butanone and n-Butanol i.e., the extractions were carried out from the non-polar end and to the polar end.

MATERIALS AND METHODS

Experimental details

Solvents like Toluene, Ethyl acetate, Butanone and n-Butanol (SQ) (Qualigen) were used and were purified according to the procedures given in "A Text Book practical organic chemistry" by A.I. Vogel 3rd edition English language book society, London (1971). Silicagel 60-120 and 200 mesh, silicagel G (ACNE) were used for column and thin layer chromatography.

Extraction and Isolation from roots of *C. attenuata*

Plant material was collected from Tirumala forest of Seshachalam hills, Tirupati, Chittoor District, Andhra Pradesh and has been identified by Dr K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. The roots were carefully separated, washed and were air dried for 60 days under shade and powdered in a wearing blender using a mixer and passed through a 24-mesh sieve. The powder was stored at 4°C for further analysis.

In a blender, about 100 gm root powder was grinded with a certain quantity of alcohol (98%), shaken vigorously for 5-10min and left for 24 hours. The extract was filtered and the filtrate was fractionated with toluene, ethyl acetate, butanone and n-butyl alcohol. The solvents were removed under reduced pressure to get the respective extracts.

Chemical Tests: The following chemical tests were carried out on different prepared extracts to know the nature of the compounds present in them.

Liebermann-Burchard Test

TLC is performed according to Egon Stahl (1969) with a

Table: 1 Phytochemical analysis of *C.attenuata*.

| S.No. | Class of substances | Solvent systems | Spray reagents/ Tests | Toluene extract | Butanone extract | Ethylene acetate extract | n-Butanol extract |
|-------|----------------------------------|--|--|-----------------|------------------|--------------------------|-------------------|
| 1. | Alkaloids | Toluene: ethylacetate: diethylamine (70:20:10); Chloroform: diethylamine (90:10) | Dragendorff's Reagent | _____ | _____ | _____ | _____ |
| 2. | Cardiac glycosides | Ethylacetate: methanol:water (81:11:8); Chloroform: methanol:water (65:35:10) (Lower phase) | Kedde's reagent Legal reagent | _____ | _____ | _____ | _____ |
| 3. | Steroids / triterpenes | Petroleum ether: chloroform: acetic acid (7:2:1); Benzene: ethylacetate (50:50 and 25:75) | Sulfuric acid and vanillin-sulfuric acid | ++++ | ++++ | ++++ | ++++ |
| 4. | Saponins | Ethylacetate: methanol: water (81:11:8); Chloroform: methanol: water (70:30:4); Chloroform: Methanol (8:2) | Vanillin-sulfuric acid Froth test | _____ | _____ | ++++ | ++++ |
| 5. | Flavonoids and their glycosides. | Chloroform: methanol (7:3, 6:4, and 5:5); N-butanol: glacialacetic acid:water (4:1:5) (upper phase) | Visible and UV light (365nm) | _____ | _____ | ++++ | ++++ |

The extract / compound was dissolved in acetic anhydride, heated to boiling, cooled and then one ml of concentrated Sulphuric acid was added along the sides of the test tube. Red, pink or violet colour at the junction of the liquids indicates the presence of steroids / triterpenoids and their glycosides⁵.

b) Salkowski Test:

The extract / compound was dissolved in Chloroform Sulphuric acid and then one ml of concentrated Sulphuric acid was added. Red colour precipitate indicates the presence of steroids/Triterpenoids⁶.

Phytochemical analysis of the extracts

The chemical tests were performed on the toluene, ethyl acetate, butanone and n-butyl alcohol extracts of *Caralluma* root using standard procedure to identify the constituents as described by Sofowora⁷, Treasem⁸ and Evans and Herborne⁹.

Detection by TLC

slight modification¹⁰. Briefly, TLC plates were made by using a homogenous suspension of silica gel prepared by mixing 40 g of 200 mesh, silicagel G (ACNE) in about 85 ml distilled water. The suspension was then poured into TLC (UND PLAN model) spreader, which was adjusted to 0.25 mm thickness. Carrier plates (20 cm x 5 cm) of the same thickness were laid in a row on a template and coated in a single passage of the spreader over them. These plates were left on the template for air drying the transparency of the layer disappeared and dried at 110°C for 30 min and kept in desiccator. All the extracts prepared, i.e., the toluene, chloroform, ethyl acetate and n-butanol extracts were spotted on TLC plate at 2 cm from the edge of the TLC plate. The chromatogram was developed in a mixture of suitable solvent systems and dried at room temperature. The spots were visualized with UV light at 365 nm. The dried TLC plates were then sprayed with Methanol Sulphuric acid reagent.

Test for Alkaloids

TLC was carried out with all the extracts to know the presence of alkaloids. The solvent system used was Toluene: ethylacetate: diethylamine (70:20:10); Chloroform: diethylamine (90:10) and Dragendorff's reagent was used as the spray solution. A prominent yellow precipitation indicates the presence of alkaloids.

Test for cardiac glycosides

TLC was carried out with all the extracts to know the presence of cardiac glycosides. The solvent system used was Ethylacetate : methanol : water (81:11:8); Chloroform: methanol : water (65:35:10) (Lower phase) and Kedde's and Legal reagents were used for detection. Purple-violet (Kedde's reagent) and deep red colour (Legal reagent) indicates the presence of cardiac glycosides.

Test for Steroids/Triterpenoids

TLC was carried out with all the extracts to know the presence of Steroids/Triterpenoids and the solvent system used was Ethylacetate: methanol : water (81:11:8); Chloroform : methanol : water (70:30:4); Chloroform : Methanol (8:2). The plate was sprayed with Vanillin-sulfuric acid. Blue, pink and violet spots indicate the presence of steroids and tri terpenoids.

Test for Saponins

All the extracts were mixed with a little water in test tubes and shaken vigorously and kept undistributed for 15 minutes. The appearance of froth indicates the presence of saponins. TLC was also performed with all the extracts for further confirmation of saponins. The plates were developed with Ethylacetate: methanol: water (81:11:8); Chloroform: methanol: water (70:30:4); Chloroform: Methanol (8:2), sprayed with vanillin-sulfuric acid reagent and heated for a few seconds. Appearance of five characteristic colours indicates the presence of saponins.

Test for Flavonoids and their glycosides

TLC was performed with all the extracts to find out the presence of flavonoids and their glycosides. The spray solution used was Chloroform: methanol (7:3, 6:4, and 5:5); N-butanol: glacial acetic acid: water (4:1:5); (upper phase). Appearance of yellow spot at 365nm indicates the presence of flavonoids and their glycosides. The yellow colour was intensified when the plate was exposed to ammonia vapours.

For further confirmation Shinoda Test was also done. A few drops of all the extracts were treated with a few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The development of purple color within a minute or two indicates the presence of flavonoids.

Column Chromatography of the extracts

The ethyl acetate extract (3g) was column chromatographed over silica gel G 60-120 mesh (100G) and eluted with benzene: ethyl acetate (97:3) afforded a crystalline solid. The yield was 50 mg (0.0001%), melting point was 137-140°C. Liebermann-Burchard test was performed with this compound according to the protocol mentioned.

Further elution of column with ethylacetate: methanol (90:10) afforded a white compound with a yield of 300 mg (0.006%) and its melting point was between 262 and

264°C. Molisch and Liebermann-Burchard tests were performed as mentioned.

A yellow solid separated out during the concentration of the butanone extract of this plant. This was collected by filtration and washed with hot ethyl acetate and acetone, which yielded 2.1g (0.04%).

RESULTS AND DISCUSSION

Alkaloids, Cardiac glycosides were found to be absent in all the extracts i.e., toluene, butanone, ethylacetate, n-butanol extracts prepared from *C. attenuata*. All the extracts prepared, i.e., the toluene, butanone, ethylacetate and n-butanol extracts, gave spots on TLC characteristics of steroids/triterpenoids. Sulfuric acid and vanillin-sulfuric acid were used as spray reagents for TLC detection of these classes of compounds and characteristic coloured spots (blue, violet, pink etc.) were observed. The ethylacetate and the n-butanol extracts gave a froth which was stable for 15 minutes, when shaken with water in a test tube, thus indicating the presence of saponins. These two extracts also answered the Liebermann-Buchard Test as well as the Molisch Test, thus once again saponins in these two extracts is further indicated by TLC analysis. When plates were developed in polar solvent systems like ethylacetate : methanol : water (81:11:8) vanillin – sulfuric acid reagent and heated, five characteristic coloured spots were observed on the TLC plate. Thus polar compounds like the saponins are present in the ethylacetate and n-butanol extracts. The ethylacetate extract and the n-butanol extract tested positive for flavonoids and / or their glycosides, both in TLC as well as in test tube reaction. These extracts gave a characteristic purple colour when treated with magnesium and hydrochloric acid. When tested on TLC, these extracts showed three yellow coloured spots (visible light) in the solvent systems chloroform: methanol (7:3) and (5:5). The yellow colour of the spots got intensified when the plate was exposed to vapours of ammonia. There was quenching when the plate was observed in UV light (365nm). The results of phytochemical analysis of *Caralluma attenuata* have been summarized in Table – I. Column chromatography of Ethyl acetate crystalline solid gave a pink color with Liebermann-Burchard reagent. It corresponded to β - sitosterol on TLC (solvent system Benzene: ethyl acetate 97:3), gave a pink colour on TLC when sprayed with vanillin-sulfuric acid reagent. The result was superimposed over that of an authentic sample of β - sitosterol, isolated earlier from the fruits of *Terminalia chebula* in our lab (data not shown). This compound was characterized as β - sitosterol based on its melting point, mixed melting point, and TLC.

Further elution of column with ethylacetate: methanol (90:10) which afforded a white compound gave a red colour in the Liebermann-Burchard test, and violet color in the Molisch test indicating that it is a steroidal / triterpenoidal glycoside. On TLC it was found to be homogenous and correspond with β - sitosterol-D-glucoside (solvent system Chloroform: methanol 90:10, sprayed with vanillin-sulfuric acid reagent). From the melting point, mixed melting point and TLC this

compound was identified as β - sitosterol-D-glucoside. The butanone extract was found to be homogenous by TLC (solvent system Ethyl acetate: methanol: water 81:11:8; Chloroform: methanol 70:30, detection in UV at 365 nm). Thus phytochemical analysis of *C. attenuata* revealed the presence of steroids / triterpenoids, saponins and flavonoids / their glycosides. Alkaloids and cardiac glycosides were found to be absent. All *Caralluma* investigated so far, yielded a number of new steroidal glycosides. The latest example is the isolation of twenty new steroidal glycosides from *Caralluma negevensis*. Ten new steroidal glycosides and a new genin were isolated from *C. umbellata*.

Very few *Caralluma* species have been investigated for their pharmacological or biological actions. Butanolic extracts of fresh whole plants of *Stapelia nobilis* and *Caralluma stalagmifera* have shown significant anti-inflammatory activity on carragenan-induced rat paw edema and anti-arthritis activity on kaolin induced arthritis in rats¹¹ (Reddy 1996).

The flavonoid glycoside, luteolin-4'-O-neohesperidoside, isolated from *C. stalagmifera* was shown to possess significant anti-inflammatory activity¹² (Ramesh et al., 1998). A flavonoidal glycoside, luteolin-4'-O-neohesperidoside isolated from *C. attenuata* was shown to possess significant anti-inflammatory activity (Ramesh et al., 1998). Though this flavonoid glycoside was reported earlier from a few other sources, it was not screened for any biological actions. Flavonoid glycosides, in general, have varied biological actions. The ethanol (95%), chloroform and n-butyl alcohol extracts of fresh entire plant of *C. attenuata*, at 250 mg/kg dose showed significant anti-hyperglycemic activity in alloxan and glucose-induced hyperglycemic rabbits. The anti-hyperglycemic activity was studied by sub-acute treatment also¹³ (Venkatesh et al., 2003).

It is likely that some of them constitute a very good source of steroids and might yield them in higher amounts than the plants examined in the past. It is also possible that, some of these compounds have interesting biological actions. Oxypregnane glycosides are reported to possess cytotoxic activity.

CONCLUSIONS

The chemical investigation of *Caralluma adscendens* var *adscendens* one flavonoid glycoside and three steroidal / triterpenoidal glycosides were isolated. A number of nonpolar steroidal glycosides were isolated from the nonpolar (CHCl₃ or ether) fractions of other *Carallumas* like *C. resseliana*, *C. penicillata* and *C. negevensis*. In view of this it was felt that the nonpolar i.e., hexane, benzene, acetone fraction of *Carallumas* should be investigated for the presence of Steroid / triterpenoids.

The glycosides were isolated from the polar fractions i.e., ethylacetate and n-butyl alcohol fractions. In the non-polar fractions glycosides were not present. But steroid / triterpenoids are present. All along our attention was on the isolation and identification of steroid / triterpenoids from the non-polar fractions of *Caralluma*.

No attempt was made to extract the compounds from the roots of *Caralluma attenuata* starting from Toluene, Ethyle acetate, Butanone and n-Butanol i.e., the extractions were carried out from the non-polar end and to the polar end. So an attempt was made to extract the compounds from *Caralluma attenuata* root using nonpolar to polar solvents with a view to isolate compounds from different extracts. For further characterization, UV, IR, Mass spectrum, ¹H NMR and ¹³C NMR experiments are underway.

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