Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(7):2083-2088

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phytochemical and FT-IR spectral analysis of *Caralluma geniculata* Grev. et Myur. an endemic medicinal plant

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ABSTRACT

The present investigation was focused on the preliminary phytochemical and Fourier Transform Infrared Spectral analysis of Caralluma geniculata Grav. et Myur. The aqueous and organic solvent extracts (Petroleum ether, chloroform, ethanol and acetone) from the aerial part of Caralluma geniculata (Asclepiadaceae) were tested for the availability of flavonoids, phenolic compounds, saponins, terpenoids, steroids, proteins, coumarins, quinones and phytosterols. The FT-IR spectrum showed the presence of alkyne (C-H), methylene (C-H), isocyanate (-N=C=O), organic nitrates, aliphatic nitro compounds, ammonium ion and aromatic nitro compounds. The results confirm the fact that this plant posses' important bioactive constituents useful for our health so further scientific investigation is needed.

Key words: *Caralluma*; FT-IR; bioactive constituents

INTRODUCTION

Plants produce bioactive molecules in a diverse range making them a rich source of different types of medicines [1-5]. Traditionally herbal extracts were known to be effective against microorganisms as a result; plants form the basis of modern medicine. Plants produce phytochemicals to protect themselves; but recent studies indicate that many phytochemicals can also protect humans against infectious diseases [5-10]. *Caralluma geniculata* is an attractive, succulent medicinal plant of the family Asclepiadaceae. It is an endemic plant distributed in Maruthuvamalai, Aramboli and Valliyur hills of Kanyakumari District, Tamilnadu, India [11]. The family Asclepiadaceae comprises about 200 genera and 2500 species [12] with a global distribution and represented in all types of habitats. A total of 16 species and 8 varieties of *Caralluma* occur in India out of which 5 species and 5 varieties are solely endemic to Peninsular India [13]. They grow in arid, rocky regions in the foot hills of Western Ghats and Eastern Ghats [11].

Caralluma species present in India are edible and also take part in traditional medicine of our country [14]. People in semi arid areas of Pakistan used the species of *Caralluma* for centuries as emergency foods [15]. Pallliyars of Western Ghats, Tamilnadu used the stems of *C. adscendens* R.Br. var. *attenuata* (Wight) Grav. & Mayuranathan) (Periyasirumankeerai) and *C. lasiantha* (Wight) N.E.Br (Sirumankeerai) as edible plant [16] whereas, Karuppusamy documented that Paliyan tribes of Sirumalai Hills, Southern India utilized burned stems of *C. umbellata* (Roxb.) Haw. (Kallimulayan-local name) in direct fire and eaten for five days regularly in empty stomach to cure ulcer and sliced stem of *C. adscendens* (Roxb.) Haw. with salt was taken orally for diuretic condition [17]. Similarly 10 grams of fresh rootless plant of *C. lasiantha* Wight & N.E.Br (Sirumankeerai) was taken as such twice a day for a period of three days to reduce body heat [18]. People of Puttaparthi Mandal belongs to Sri Sathya Sai taluk of Anathapur District, Andhra Pradesh used succulent stems of *C. adscendens* Grav. & Mayur (Telugu Name-Kundelu Kommulu) and *C. umbellata* Haw. (Telugu Name – Kundeti Kommulu) to treat inflammation and stomach disorders [19]. Farmers in Dindigul District, Tamilnadu, India believed that feeding leaves of *C. adscendens* R.Br. (Muyalkathu, Muyal Kurabu) in odd numbers i.e., 3,5,7 or 9 can relieve bloat and also the mixture of paste with

ghee and leaves of *Carulluma* cure mastitis in animals [20]. Roasted plants of *C. umbellata* Haw. (Chirukalli) is made in to paste and applied for indigestion by the malayali tribals in Kollihills of Tamilnadu, India [21]. Many recent studies revealed that *Caralluma* is an important medicinal plant. Keeping the values of *Caralluma* in mind the present investigation was carried out to screen the biomolecules present in aqueous, petroleum ether, chloroform, ethanol and acetone extracts of the aerial part of *Caralluma geniculata* collected from Maruthvamalai, Kanyakumari District, Tamilnadu, India and to determine their functional group using (FT-IR) spectral analysis.

EXPERIMENTAL SECTION

The aerial part of *C. geniculata* was collected from Maruthuvamalai, Kanyakumari District, Tamilnadu, India. The plants were examined carefully and old, infected and damaged parts were removed. Extracts were prepared from fresh aerial portion. 50 grams of aerial parts were collected, smashed and soaked in 200ml of distilled water, petroleum ether, chloroform, ethanol and acetone respectively. These flasks were kept in a shaker at room temperature for 24h. After incubation, the extracts filtered through Whatman No. 1 filter paper, collected and stored in a refrigerator at 4°C. The extracts were concentrated using a vacuum evaporator and dried at 60°C. Preliminary phytochemical screening was performed using standard procedures [22].

Fourier Transform Infrared Spectroscopic Analysis (FT-IR)

The whole plant of *C. geniculata* was oven dried at 60° C and ground into fine powder using a mortar and pestle. Two milligrams of the sample was mixed with 100mg KBr (FT-IR grade) and then compressed to prepare a salt disc (3mm diameter). The disc was immediately kept in the sample holder and FT-IR spectra were recorded in the absorption range between 400 and 400cm^{-1}. All investigations were carried out with a Shimadzu FT-IR spectrometer.

RESULTS

Preliminary phytochemical screening was done in aqueous, petroleum ether, chloroform, ethanol and acetone leaf extracts of *C. geniculata*. Of the five solvent extracts tested, steroids showed their presence in four extracts, terpenoids and proteins in three extracts, carbohydrates, phenolic compounds, saponins, coumarins and phytosterols in two extracts, flavonoids and quinones were noticed in only one extract. Glycosides and alkaloids were completely absent in all the extracts (Table 1).

The FT-IR spectrum was used to identify and detect the characteristic peaks and functional groups of the active components based on the peak value in the region of infrared radiation (Table 2; Figure 1). The results revealed the presence of different phytochemicals which are formed during the plants normal metabolic processes. The extract of *C. geniculata* was subjected to FT-IR analysis and the functional groups of the components were separated based on their peak ratios. The result confirmed the presence of normal polymeric (OH stretch), alkyne (C-H stretch), methylene (C-H stretch), isocyanate (-N=C=O stretch), terminal alkyne, cyanide or thiocyanate ion and related ions, open-chain imino (−C=N–), organic nitrates, aliphatic nitro compounds, methyl (C-H) asym./sym.bend, ammonium ions, aromatic nitrocompounds, tertiary amine (C N stretch), sulfonates, thio ethers (C-S stretch), disulfides (S-S stretch) and aryl disulfides (S-S stretch) which showed major peaks at 3371.34/3203.54, 3319.26, 2925.81/2856.38, 2268.13, 2115.77, 2007.76, 1668.31, 1633.59, 1556.45, 1452.30, 1400.22, 1338.51, 1195.78, 1112.85, 653.82, 605.61 and 464.81 cm¯1 respectively (Figure 1; Table 2).

DISCUSSION

Preliminary phytochemical screening of *Carulluma adsendens* showed the availability of wide range phytoconstituents present. There are several reports to show that *Caralluma* is an important source of bioactive molecules. Phytochemical screening done in the methanolic extract of the whole plant of *C. fimbriata* was rich in alkaloids, flavonoids, glycosides, phenolic compounds, saponins and quinones [23]. Vajha and his coworkers separated the active compounds like alkaloids, flavonoids, saponins and steroids present in the methanolic extract of *Carulluma* species [24].

Pregnane glycosides named (5α,17s)-12-O-benzoyl-3β,8β,12β,14β-tetrahydroxypregnan-20-one, (5α, 17s)-12-O-benzoyl-3β,8β,12β,14β-tetrahydroxypregnan-20-one -3- O- β- cymaropyranoside, (5α,17s) -12-O-benzoyl - 3β, 8β, 12β, 14β-etrahydroxypregnan- 20-one- 3-O-β- cymaropyranosyl- (1→4)-β-cymaropyranoside, (5α,17s)-12-O-benzoyl-3β,8β,12β,14β-tetrahydroxypregnan-20-one-3-O-β-cymaropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-cymaropyranoside, (5α, 17s)-12-O-benzoyl-3β,8β,12β ,14β-tetrahydroxypregnan-20-one-3-O-β-cymaropyranosyl-(1→4)-β -cymaropyranosyl-(1→ 4)-β-cymaropyranosyl-(1→ 4)-β -cymaropyranoside, (5α, 17s)-12-O-benzoyl-3β,8β,12β,14β-tetrahydroxypregnan-20-one-3-O-β -cymaropyranosyl-(1→4)-β -cymaropyranosyl-(1→ 4)-β -

cymaropyranosyl-(1→ 4)-β - cymaropyranosyl-(1→ 4)-β -cymaropyranoside, (5α, 17s)-12-O-benzoyl- 3β, 8β, 12β, 14β- tetrahydroxypregnan-20-one-3-O- (acetyl -β -thevetopyranosyl)-(1→4)-β -cymaropyranoside, (5α, 17s)-12-O-benzoyl-3β,8β,12β,14β-tetrahydroxypregnan-20-one-3-O-β -glucopyranosyl-(1→ 6)- β-glucopyranosyl-(1→4)-(2-acetyl-ß-theretopyranosyl)-(1→4)-β cymaropyranoside, (5α, 17s)-12-O-(4-methylpent-3 enoyl)- 3β,8β,12β,14β-tetrahydroxypregnan-20-one-3-O-β -glucopyranosyl-(1→ 6)- β-glucopyranosyl-(1 →4)-(2-acetyl-β-theretopyranosyl)-(1→4)-β cymaropyranoside, (5α, 17s)-12-O-acetyl-19-O-benzoyl-3β,8β,12β,14β-pentahydroxypregnan-20-one-glucopyranosyl -(1→6)-β-glucopyranosyl-(1→4)-(2-acetyl-β -theretopyranosyl)-(1→4)-β cymaropyranoside, (5α, 17s)- 12-O-acetyl-3β,8β,12β,14β, 19-pentahydroxy-19-O-(4-methylpent-3-enoyl)pregnan -20-one-3-O-β-glucopyranosyl-(1→6)- β-glucopyranosyl-(1→4)-(2-acetyl-β -theretopyranosyl)-(1→4)-β cymaropyranoside, (5α, 17s)- 12-O-acetyl-19-O-benzoyl-3β,8β,12β,14β, 19-pentahydroxypregnan-20-one-3-O-β -glucopyranosyl-(1→ 6)- β-glucopyranosyl-(1→ 4)-β-thevetopyranosyl-(1→ 4)-β -cymaropyranoside, (5α, 17s)- 12-O-acetyl-3β,8β,12β,14β, 19-pentahydroxy-19-O-(4-methylpent-3 enoyl) pregnan-20-one-3-O-β -glucopyranosyl-(1→ 6)-β -glucopyranosyl-(1→ 4)-β - thevetopyranosyl-(1→ 4)-β-cymaropyranoside were isolated from *C. adscendens* var. *fimbriata* [25].

Minor pregnanes 12β,20-O-dibenzoyl-5α,6-dihydrosarcostin β-Oleandropyranosyl-(1 →4)-β -cymaropyranosyl-(1→ 4)-β-digitoxypyranosyl-(1→4)-β -cymaropyranosyl-(1→ 4)-β cymaropyranoside, 12β-O-benzoyl-3β,11α,14β,20R-pentahydroxy-pregn-5-ene, 11α-O-benzoyl-3β,12β,14β,20R-pentahydroxy-pregn-5-ene were identified in *C. adscendens* var. *gracilis* and *pauciflora* [26]. Significant anti-inflammatory activity was noticed by Naik and Jadge in the ethanolic and aqueous extracts of whole plant of *C. adscendens* [27]. Ethyl acetate fraction of the vegetative part of *C. edulis* resulted in the separation of eighteen fatty acids, four hydrocarbons and sterols [28]. Two new pregnane compounds named as 3β-hydroxy-pregn-5-ene (CRUR – 1) and 3β, 14β-dihydroxy pregn-5-ene (CRUR II) were isolated from the roots of *C. umbellata* [29].

Flowers of *C. europaea* (Guss.) N.E.Br collected from Lampedusa Island was analyzed for volatile constituents by a headspace GC method which resulted in the identification of 41 components. The important components are terpinolene (23.3%), α-terpinene (19.1%) and linalool (18.4%) from the monoterpenoids, whereas, heptanal (2.0%), octanoic acid (2.4%) and hexanoic acid (1.7%) from the carboxylic compounds, nitrogen containing compound, indole (0.8%) and a sulphur containing compound, dimethyl sulphide (t) note worthy [30]. Similar study reveals that stems and fruits of *C. europaea* possess 16 aromatic and 58 non-aromatic compounds. Stems and fruits contained 1.4% and 2.7% of aromatic compounds respectively. At the same time non-aromatic were 88.3% and 88.8%. fruits exhibited a higher diversity in aromatic compound [31].

Braca *et al*. isolated twenty new pregnane glycosides from the whole plant of *C. negevensis* [32]. Ability of *C. fimbriata* extract on appetite, food intake and anthropometry in adult Indian men and women was examined and observed significant variation over 2 months period [33]. Habibuddin *et al*. studied anti-diabetic effect of *C. sinaica* L. on *Streptozotocin* – induced diabetic rabbits and identified significant effect of plant extract to manage diabetes mellitus and also noticed that the plant extract possess chemical constituents like phenolic compounds, alkaloids, glycosides, flavonoids, coumarins, steroids and tannins [34]. Pregnane glycosides Caratuberside (C,D,E,F,G) were isolated from *C. tuberculata* [35]. Mali *et al*. examined the hypoglycemic activity of *C. adscendens* in alloxan induced diabetic rats and noticed significant ($p<0.01$) hypoglycemic activity [36].

Antioxidant and Hypolipidemic effect of *C. adscendens* Roxb. in alloxanized diabetic rats was studied by Tatiya *et al*. found significant lowering of blood glucose by butanolic extract and also decrease in total cholesterol, LDL, triglyceride and TC/HDL and increase in HDL in the treated diabetic animal group [37]. Immunostimulating activities of *Boucerosea lasiantha*, *C. adscendens* var. *attenuata*, *C. stalagmifera* and *C. longipetala* were investigated. Methanolic extract of CAA exhibited highest free radical scavenger activity and anti-inflammatory activity. Methanolic extracts of four species showed anti-diabetic activities and anti-microbial activity, whereas *C. stalagmifera* and *C. longipetala* showed highest anti-microbial activity [38]. Tambe *et al*. identified the wound healing properties of *C. adscendens* Roxb. in rats using methanol extract [39]. Ethanolic and aqueous extract of *C. adscendens* showed prominent antibacterial activity against *S. typhi* and *E. coli* [40].

C. umbellata also exhibited significant antinociceptive activity, this is due to the presence of novel pregnane glycoside named Carumbelloside II and V, and anti-inflammatory activity due to the presence of pregnane glycoside Carumbelloside II and III [41-43]. Similarly leaf extract of *Caralluma fimbriata* exhibited analgesic activity [44]. Saivasanthi and her coworkers evaluated and identified the analgesic, anti-inflammatory and anxiolytic activity of *C. fimbriata* [45]. Leaf extract of *C. fimbriata* shows significant anti-nociceptive activity [44]. Experimented the appetite suppressant and antiobesogenic effects of *C. fimbriata* extract on a sample of rats fed with cafeteria diet and observed the potential of CFE to curb obesity and the pathologies linked to obesity [46]. The gastroprotective effect of *C. adscendens* var. *fimbriata* due to its antioxidant property was proved [47].

Table 1: Preliminary phytochemical screening of various extracts of *Caralluma geniculata*

Phytoconstituents	Solvents				
	Aqueous	Petroleum Ether	Chloroform	Ethanol	Acetone
Carbohydrate	+	-	+++	-	-
Glycoside	-	-	-	-	-
Flavonoid	-	-	-	-	+
Phenolic	+	-	-	-	++
Saponins	+	-	-	-	+
Terpenoids	-	-	++	+++	+++
Steroids	+	-	++	++	++
Proteins	-	-	+	+	+++
Coumarins	+	-	-	+	++
Quinones	-	-	-	-	+
Alkaloids	-	-	-	-	-
Phytosterols	-	++	+++	-	-

Key : + Present, - Absent

Figure 1FT-IR spectrum of *Caralluma geniculata*

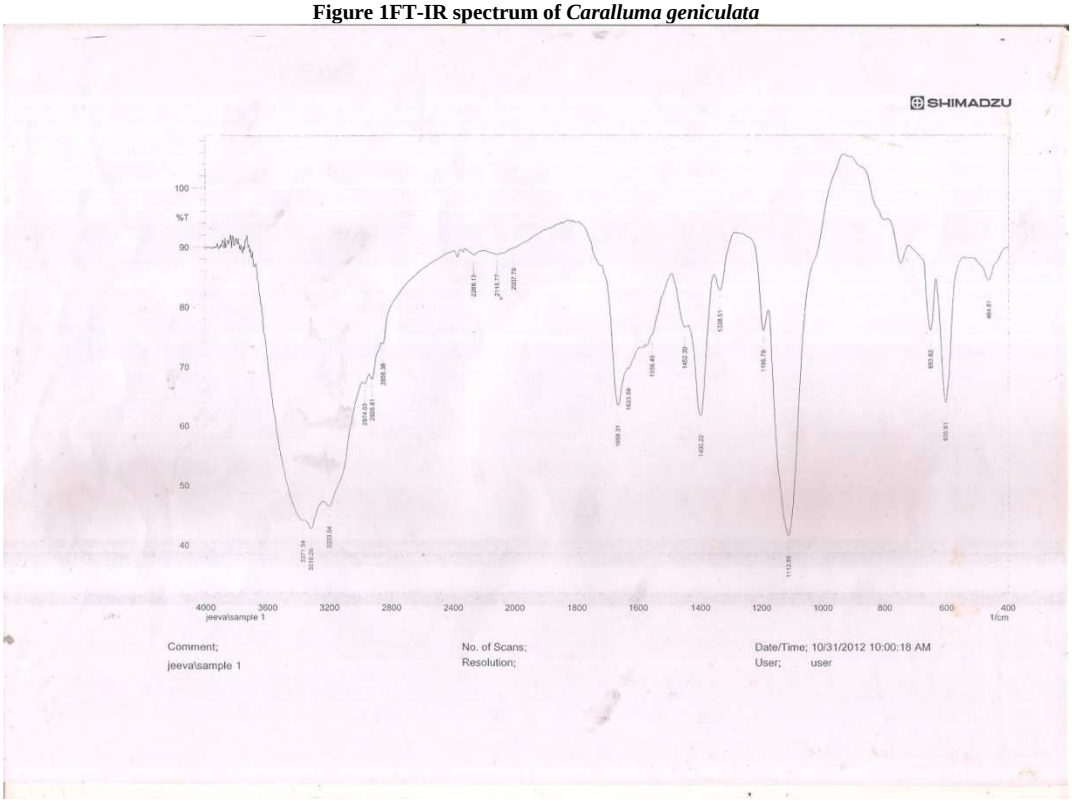

Table 2 Compounds identified in the extract of *Caralluma geniculata* by FTIR

Origin	Group frequency	Peak value	Assignment
O-H	3400-3200	3371.34	Normal "polymeric" OH stretch
C-H	3320-3310	3319.26	Alkyne C-H stretch
O-H	3400-3200	3203.54	Normal "polymeric" OH stretch
(=CH^{-2})	2935-2915	2925.81	Methylene C-H asym,/ sym stretch
(=CH^{-2})	2865-2845	2856.38	Methylene C-H asym,/ sym stretch
	2276-2240	2268.13	Isocyanate (–N=C=O asym. Stretch)
CΞC	2140-2100	2115.77	Terminal alkyne (Mono substituted)
	2200-2000	2007.76	Cyanide ion- thiocyanate ion. And related ions
	1690-1590	1668.31	Open-chain imino (−C=N–)
	1640-1620	1633.59	Organic nitrates
	1560-1540	1556.45	Aliphatic nitro compounds
-CH_3	1470-1430	1452.3	Methyl C-H asym./sym. Bend
	1430-1390	1400.22	Ammonium ion.
	1355-1320	1338.51	Aromatic nitro compounds
C−N	1210-1150	1195.78	Tertiary amine, C N stretch
	1200-1100	1112.85	Sulfonates
	660-630	653.82	Thioethers, CH_3-S- (C-S stretch)
	620-600	605.61	Disulfides (S-S stretch)
	500-430	464.81	Aryl disulfides (S-S stretch)

CONCLUSION

The result of the present study along with previous studies showed the presence of valuable compounds present in the plant. The study also justified the uses of the plant in the treatment of various diseases, ornamental and edible values. Continuation of study on the same plant is required to identify, isolate, characterize and elucidate the structure of bioactive compounds present in it as there is no detailed study on this plant.

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