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BIOCHEMICAL STUDY OF CARALLUMA SPECIES TO UNDERSTAND SPECIES HOMOLOGY

MADHURI VAJHA[1], AMRUTHA.V.AUDIPUDI*[2] & MURTHY K.S.R[1]

[1]Department of Bio-Technology, Acharya Nagarjuna university, GUNTUR-522512,

[2]Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur -522512, Andhra Pradesh., India

ABSTRACT: Demarcation of species is the prime endeavor of Biosystematics. conventional morphological parameters and the use of biochemical components in solving taxonomic disputes are gaining importance with the help bioactive phenolic compound as a genetic marker to assess species affinity. In present study methanolic extracts of six different Caralluma species were carried out through phytochemical screening and HPLTC analysis to ascertain relative relationship between active compounds., High Performance TLC on silica gel 60 F_{254} TLC aluminum sheet was used to separate the active compounds present in the extracts and scanned at 254 nm. The results revealed the presence of alkaloids, flavonoids, saponins and steroids. A partial biochemical profile for selected constituents of species was compared with that of other species in Caralluma by HPTLC to ascertain their relative phylogenetic position. From this phytochemical analysis, the paired affinity, group affinity and isolation value of each of these pair of species of Caralluma are expressed in percentage to study the species homology.

KEYWORDS: Caralluma, Paired affinity, group affinity, Isolation value, homology, HPTLC analysis

INTRODUCTION:

Caralluma, a cactus plant belongs to family Asclepiadaceae is a succulent, perennial herb, grow to a height of 1 to 10 ft and grow in different regions of India. The members of genus Caralluma are erect and fleshy. They have quadrangular stem, devoid of leaves and small flowers in several varieties of dark colour. The species of Caralluma found in India are edible and form a part of traditional medical system of country (Al-Yaha MA,2000). The present study aims to evaluate phyletic distance among six different species of Caralluma, *Boucerosea lasiantha* (BL), *Caralluma adscendens var.annuata* (CAA), *Caralluma stalagmifera* (CS), *Caralluma longipetale* (CL), *Caralluma adscendens var fimbriata* (CAF), *Boucerosea umbellate* (UMBL) distributed in peninsular India. This succulent Cactus contain glycosides, hydrocarbons, saponins as major phytoconstituents and reported for various biological activities such as rheumatism, diabetes, leprosy,antinociceptive, antipyretic, anti-helminthetic, antiobesity activities.(Rao R M et al.,1998; Jadge DR et al.,2009; Zakaria MN et al., 2001; Venkatesh S et al.,2003; Wadood A et al.,1999; Lawrence et al.,2004; Abdel-Sattar at al., 2002). The attraction of Pharmaceutical companies, Researchers for elucidation of bioefficacies and provide knowledge for the advancement of phytomedicine.

Species of caralluma have pregnane glycosides (Abdel- Sattar et al., 2007), stogmasterol and other further constituents (Bader A et al., 2003) which are well known for the array of biological activities and pocess antimicrobial, antidiabetic, antioxidant etc. Saponins and flavonoids (Kamil M et al., 1999) and predominantly found in Caralluma show in great interest because of the wide range of immunostimulating activities.

Present study made HPTLC fingerprinting for the comparative analysis in the form of qualitative relationship of active compounds on the basis of biochemical affinities. Attempts have been made to measure the similarity in pattern of distribution of the spots in all the species, but not to identify the chemical nature of individual species

MATERIALS AND METHODS

Plant material: The plant material Caralluma species were collected from Gooty, Tadiparthy, Penukonda areas of Ananthapur district of Andhra Pradesh, in April,
2010. They were identified by Prof. Pulliah. A voucher specimen was deposited in the depository of the Taxonomy Division at SK University.

Sample preparation:

Weighed about 1.0g of dried powder of each Caralluma species in to separate round bottom flask, added about 20ml of methanol and refluxed on a boiling water bath 80^0C for 30 min. Filtered and evaporated the filtrate on a water bath up to 10 ml. Made up the solution with methanol to 10 ml in volumetric flask. This is used as test solution.

HPTLC analysis:

The samples of Caralluma species were spotted with a Camag microlitre syringe on a pre-coated silica gel aluminum plates 60 F-254 (20 nm x 10nm) with 250 µm thickness, (E. Merck, Darm stadth, Germany) using a Camag Linimat IV (Multtenz, Switzerland) applicator. Linear ascending development was, carried out in 20 cm x 10cm twin through glass chamber (Camag Muttenz, Switzerland) using mobile phase consisting of Benzene: isopropyl alcohol (9 :1) and allowed it to equilibrate for 30 minutes at room temperature. (Katta Vijayakumar et al.,)

About 5ul test solution was applied separately to the chromatoplate and chromatogram was developed after running to a length of 8 cm from the point of application. After air drying, the TLC plates were scanned with a Camag TLC scanner III in absorbance mode at 254 nm, 366nm and 500nm, controlled by Cats software 4.03 version. Evaluation was peak areas with linear regression. To scan at 500nm, the plate was sprayed with anisaldehyde sulphuric acid and heated at 100c for 10 min. (Danish Iqbal at al,)

Analysis of Phytochemical data

The method adopted by Ellison et al. (1962) was followed to make the suitable comparisons in the form of qualitative relationships by HPTLC (Kaltsikes PJ et al., 1970; Wilbur J et al., 1972; Subash chandran G et al., 2010). Species were compared on the basis of their biochemical affinities. Values of paired affinity (PA), group affinity (GA) and isolation value (IV) were calculated as follows:

$$PA = \frac{\text{Spots common in species A and B X100}}{\text{Total spots in A and B}}$$

$$IV = \frac{\text{Number of unique spots in a species}}{\text{Total number of spots in all species}} \times 100$$

GA = Total PA value +100

RESULT & DISCUSSION

Previous studies revealed that the presence of effective ingradients and most popular compounds present in cucurbits possess a variety of biological activities. It have been reported effective in reducing blood lipids as an antioxidative, anti diabetic, antiobesity. (Ishrad M et al.,2010). HPTLC was used to separate active compounds present in cucurbits and their relationships were evaluated by Paired affinity (PA), Group affinity (GA) and isolation value (IV).Chemotaxonomic and Numerical taxonomic techniques are used to trace out variation pattern at interspecific level. (Sachdeva S.Ket al., 1979, Wilbur J et al., 1972; Saubhic Das et al., 1995)

High Performance TLC on silica gel 60 F_{254} TLC aluminium sheet was used to separate the active compounds present in the methanolic extracts of Caralluma. The TLC sheets were scanned at λ max 254 nm, and revealed 11 peaks of CAF, 9 peaks of CSF, 10 peaks of CAA and 8 peaks of CL, BL and UMBL (Fig I). The peaks were used for comparative analysis and revealed the active compounds. The active compounds of RF 0.13 is commonly found in all species except UMBL. However, active compounds of RF 0.14 and 0.48 exclusively found in UMBL. Similarly, active compound of RF 0.36 is common in CSF, CAA and CL where as RF 0.37 is common in CAF, BL and UMBL. Result indicated the distribution of common and species specific phytochemicals . (Fig II)

The concentration of active compound was assessed by peak area.(Fig II). Active compounds of RF 0.13 was found in highest concentration in BL and RF 0.36 was found in CSF. The peaks were also used for the evaluation of PA, GA and IV (Table I).

Table: I. Values of Pared affinity (PA), Group affinity (GA) and Isolation Value (IV) of Caralluma species.

254	CAF	CAA	CL	BL	UMBL	Total PA	GA	IV
CSF	20	36.84	35.29	11.76	0	100.89	200.89	10
CAF	---	19.04	10.52	15.78	5.26	70.26	170.26	0
CAA	19.04	---	22.22	11.11	5.55	94.76	194.76	3.33
CL	10.52	22.22	----	12.5	6.25	86.78	186.78	3.33
BL	15.78	11.11	12.5	----	6.25	57.24	157.24	13.33
UMBL	5.26	5.55	6.25	6.25	----	23.31	123.31	16.66

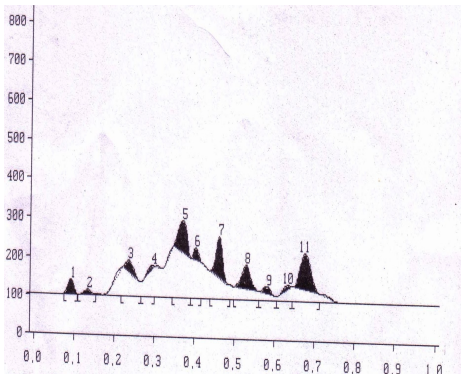
Attempts have been made to measure the similarity in pattern of distribution of the spots in all the species, but not to identify the chemical nature of individual species. Total no. of spots in all the species studied were 30, some spots were common among five species and some were exclusive for three species, the distinctive marker or index spots for that particular species.

Higher PA value was considered as an indication of close affinity between different species. PA value of 50% was considered as marker of close relationship. The PA value supported by Isolation value (IV) emphasizes distantly related species. (Suresh N Baitha et al., 2003). Highest PA value 36.84% was found between CSF and CAA and lowest value 0% was found between CSF and UMBL. The PA value between CSF and CL, CSF and CAF; CSF and BL were found to be in decreasing order (35.29%; 20%; 11.76% respectively). These values predict the close relationship of CSF with CAA and CL, distant relation with CAF and BL. However no relationship was found with UMBL. Indicating that UMBL is highly specific in its bioactive compound. The relationships were also confirmed through Group affinity (GA) and Isolation (IV) (Fig III).

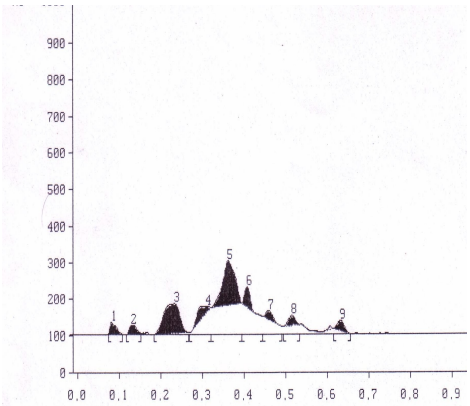
These findings supported that some of the active compounds are highly species specific and further needed to isolate the separated compounds to eleucidate the chemical structure and its bioefficacies. Synthesis of biochemical and Numerical systematics, at the same time making use of improved analytical procedures called HPTLC confirming the presence or absence of various compounds and their relative amounts gave good support to the species homology.

This relationship is expressed in a manner that presumably gives equal weight to both similarities and differences. These techniques were applied to a systematic evaluation of relationships in Caralluma (CAA, CAF, CSF and CL) and its related genera Boucerosea (BL and UMBL).

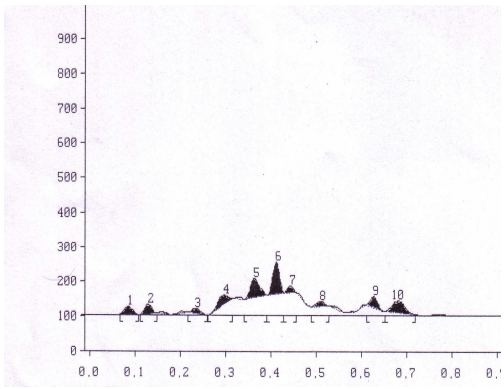
CAF



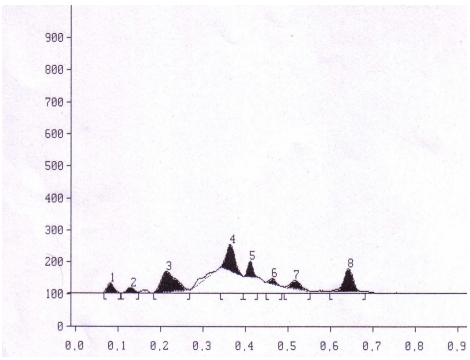
CSF



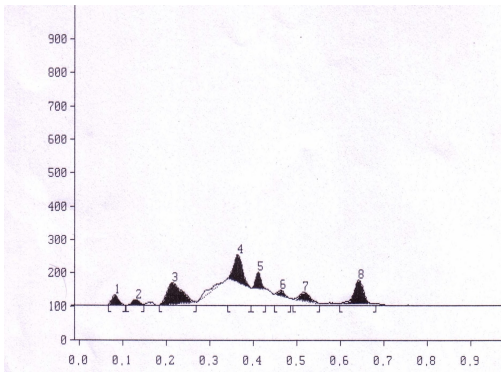
CAA



CL



BL



UMBL

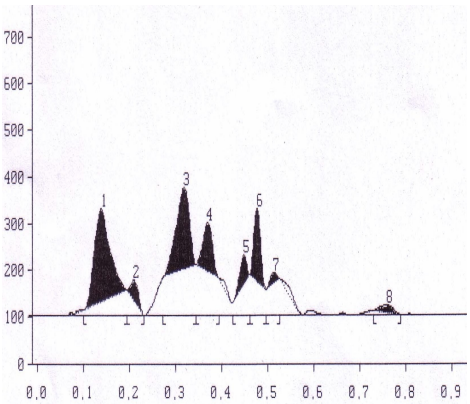


Fig I: HPTLC chromatogram obtained at 254nm. under UV illumination

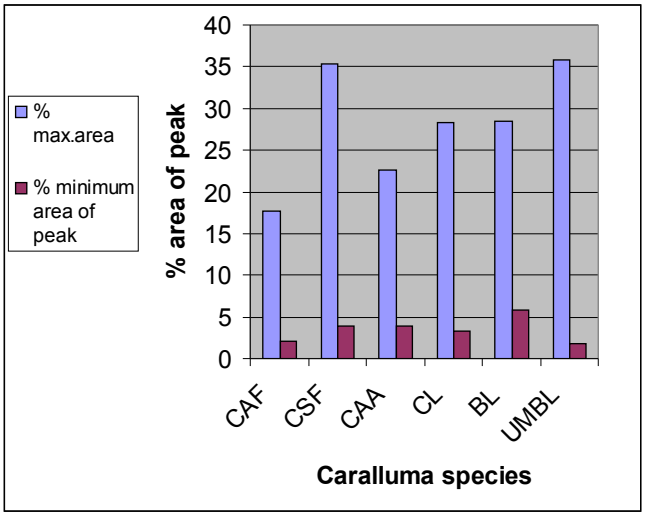


Fig II : Maximum and minimum % area of bioactive compounds of various species of Caralluma scanned at 254 nm. by HPTLC

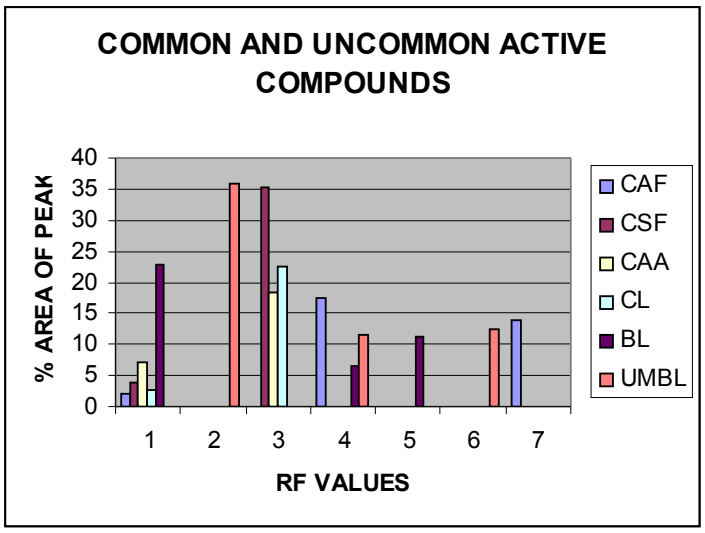


Fig III: Common and uncommon compounds of Caralluma species.
*Justification of RF values on X axis -1= 0.13; 2=0.14; 3=0.36; 4=0.47;5=0.48; 6=0.53

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