HPTLC Fingerprinting Method to Distinguish Total Extract of Caralluma fimbriata from the Modified Extracts of Caralluma fimbriata

T. Lakshmi*, R. Rajendran2, Vijay Raghavan2 and Antony Silvester2

1Department of Pharmacology, Saveetha Dental College & Hospitals, Chennai, India. Green Chem Herbal Extracts and Formulations, Bangalore, India.

doi: http://dx.doi.org/10.13005/bbra/1338

To establish the fingerprint profile of total extract of Caralluma fimbriata using high performance thin layer chromatography (HPTLC) technique. HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. HPTLC finger printing of Caralluma fimbriata leaf extract revealed that the chromatogram obtained with the reference solution shows in the middle part a Dark blue zone [Slimalumaside A] at RF 0.33 and in the upper part a Blue zone [Slimalumaside B] at RF 0.94 was observed. It can be concluded that HPTLC fingerprint analysis of total extract of Caralluma fimbriata can be used as a diagnostic tool for the appropriate identification various extracts.

Key words: Caralluma adscendens var. fimbriata, HPTLC, Chromatogram, Isolated compounds.

Standardization of herbal extracts is mandatory. Several pharmacopoeia containing monographs of the herbs describe only the physicochemical parameters. Hence the modern methods describing the identification, isolation and quantification of active constituents in the herbal extracts is quite useful for proper standardization of herbs and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards.

HPTLC offers better resolution and estimation of active constituents and can be done with reasonable accuracy in a shorter time.

Caralluma adscendens var. fimbriata, belongs to the family Asclepiadaceae. Caralluma fimbriata is the most prevalent of the genus, as it grows wild in urban centers, is planted as a roadside shrub, and is commonly used as a boundary marker in gardens. This so-called vegetable is eaten daily in several different forms – cooked as a regular vegetable, placed in preserve like chutneys and pickles, and sometimes eaten raw.

Caralluma fimbriata is an traditional Indian “famine food” with no history of adverse effects, which also contains pregnane glycosides. Many species of Caralluma are commonly used as traditional medicine for the treatment of rheumatism, diabetes, leprosy, paralysis, and inflammation and have antimalarial, antitrypanosomal, anti-ulcer, antioxidant, antinoceptive, and antiproliferative activities.

The genus is known for compounds like pregnane glycosides, flavonoid glycoside, flavones, magastigmane glycosides, pregnane steroids, steroidal glycosides, saturated and unsaturated...
hydrocarbons, aromatic and nonaromatic volatile compounds, and β-sitosterol. A total extract of C. fimbriata (Slimaluma® of Green Chem, India and Gencor Nutrients, Anaheim, CA, USA) is used as an anti-obesity agent and appetite suppressor. The total pregnane glycosides extracted and identified as anti-obesity and appetite-suppressant compounds. Extract of Caralluma fimbriata is capable of decreasing appetite, prevent deposition of fats, reduce obesity, enhances thermo genesis to burn adipose fat, moderately reduces excess sugar, helps in relieving joint pain and improves memory power. This is because Caralluma fimbriata blocks the formation of 2 enzymes i.e., Acetyl Co-Enzyme A and Malonyl Co-Enzyme A, which are the building blocks of fat synthesis. Caralluma inhibits fat synthesis also increase burning of fats. As per reported information, the whole herb is eaten as such. Hence it is essential to use the extract containing the major compounds present in the herb to get the above said benefits. If the extract is modified to remove the key compounds, the health benefits may not be achieved. Therefore effort was made to develop a sensitive method of HPTLC to distinguish the modified extracts so that potentially unsafe extracts are not consumed without ensuring safety.

In this present study the HPTLC fingerprinting of Caralluma fimbriata leaf extracts has been performed which may be used as markers for quality evaluation and standardization of the drug.

**HPTLC fingerprinting of caralluma fimbriata extract**

HPTLC studies were carried out following the method of Harborne and Wagner et al.14-17

**MATERIAL AND METHODS**

**Plant material**

Caralluma fimbriata aerial parts where collected from the wild, forest of Krishnagiri, Tamil nadu. Aerial parts were dried in open air under shade. 100g of dried C. fimbriata was used in each extraction Method. Plant material was authenticated by NISCAIR, New Delhi. Reference standards Slimalumaside A and Slimalumaside B, Sample of C. fimbriata extract Working Standard were procured from M/s. Green Chem, Bangalore.

**Extraction Procedure**

**Method 1**

The powdered dried parts of C. fimbriata extracted with 30% Ethanol, at 75 °C temperature, for 2 hours, in a round bottom flask with condenser attached. Collect the extract. Repeat extraction with 30% Ethanol twice. Collect and combine all the three extracts. Filter the extracts. Wash the combine and filtered extract (Part A) with Hexane twice to remove resinous mater. Separate the aqueous alcoholic layer in a separation funnel. The aqueous alcoholic layer was evaporated to dryness under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 50 - 55°C. Powdered caralluma extract was obtained. (US patent 7,060,308 B2 method).

**Method 2**

The powdered dried parts of C. fimbriata extracted with 80% Ethanol, at 55 to 60 °C temperature, for 2 hours, in a round bottom flask with condenser attached. Collect the extract. Repeat extraction with 80% Ethanol. Collect the extract. Marc was again extracted with 50% ethanol twice at a temperature of about 55 to 60°C, for 2 hours. The extracts are distilled. Solvent was removed under pressure in a Buchi Rotary evaporator at 30-35°C to obtain a concentrate. The concentrate was chilled at temperature about 6 – 10 °C for a period of about 7-8 hours to remove the resinous matter. Chilled concentrate was filtered. Filtrate was collected. Filtrate was evaporated to dryness under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 50 - 55°C. Powdered caralluma extract was obtained.

**Method 3**

The powdered dried parts of C. fimbriata extracted and powdered by Method 2. The concentrate was chilled at temperature about 15-20 °C.

**Method 4**

The powdered dried parts of C. fimbriata extracted and powdered by Method 2. The concentrate was cooled to temperature about 25-30 °C.

**Chemicals & reagents**

Ethyl acetate, methanol, water [all Reagents of analytical grade, E-Merck] and silica plate with Linomat applicator precoated TLC aluminium plates [E-Merck].
Instrumentation
CAMAG High Performance Thin Layer Chromatography System (HPTLC) comprising of: Applicator – Linomat 5, Digistore – 2, Multiwavelength Scanner, Transparent Chromatographic Tank, and HPTLC pre-coated silica plate, Silica Gel 60 F254, 10 X 10 cm (Merck)

Preparation of sample solution

Working Standard Preparation A
Extract 200mg of *Caralluma fimbriata* Working Standard [CFE/WS/10/70.9%] with 50 ml Methanol for 30 minutes. Filter and concentrate to 10 ml. Proceed for spotting.

Sample Preparation B

Sample Preparation C
Extract 200mg of *Caralluma fimbriata* Extract [Alternate process where resins are removed by chilling at Temp 15-20º C - Method 3] with 50 ml Methanol for 30 minutes. Filter and concentrate to 10 ml. Proceed for spotting.

Sample Preparation D
Extract 200mg of *Caralluma fimbriata* Extract [Alternate process where resins are removed by chilling at Temp 20-30º C - Method 4] with 50 ml Methanol for 30 minutes. Filter and concentrate to 10 ml. Proceed for spotting.

Standard Preparation E

Standard Preparation F
Extract 5mg of Slimalumaside B/Reference Standard [SLM B/REF/10] with 10 ml Methanol for 30 minutes. Filter and concentrate to 10 ml. Proceed for spotting.

Sample Preparation G
Extract 200mg of *Caralluma fimbriata* Extract [Alternate process where resins are removed by chilling at Temp 6 -10 ºC- Method 2] with 50 ml Methanol for 30 minutes. Filter and concentrate to 10 ml. Proceed for spotting.

Mobile phase
Ethyl Acetate: Methanol: Water (7.7: 1.7: 1)

1) Chromatography
1. Transfer 10 ml of mixture of Ethyl Acetate: Methanol: Water (7.7: 1.7: 1) to the chromatographic tank. Place a Whatman Filter paper disc and close with the lid (for faster saturation of the tank with the solvent system). Allow the tank to saturate for 30 minutes.
2. Apply 10 micro liter of sample(s) and 10 micro liter Standard (as 10 mm bands separated by a distance of 15 mm; at 10 mm from the base) on a HPTLC silica plate using a Linomat HPTLC applicator.
3. Leave the plate in Fume hood to let the solvent to evaporate. Place the plate in the tank as near vertical as possible ensuring that the line of application is well above the solvent level. Replace the lid tightly and allow the solvent to ascend to 1.5cm below the top of the plate.
4. Remove the plate and let it to air dry in fume hood

Detection
1) Anisaldehyde Sulphuric Acid Reagent
2) After Spraying UV 366nm

RESULTS AND DISCUSSION

Result I : Anisalehyde Sulphuric acid in white light
The chromatogram obtained with the reference solution shows in themiddlepart a Dark blue zone [Slimalumaside A] at RF 0.33 and in the upper part a Blue zone [Slimalumaside B] at RF 0.94 was observed.

The chromatogram obtained with the test solution shows a Dark blue zone corresponding to the zone of Slimalumaside A & a blue zone corresponding to the zone of Slimalumaside B. Further more other zones were present in the chromatogram obtained with the test solution.

Result II : After Spraying UV 366nm
The chromatogram obtained with the reference solution shows in themiddlepart a Greenish blue flourescent zone [Slimalumaside A] at RF 0.33 and in the upper part a Greenish blue flourescent zone [Slimalumaside B] at RF 0.94 was observed.

The chromatogram obtained with the test
solution shows a two greenish blue fluorescent zone corresponding to the zone of Slimalumaside A & Slimalumaside B. Further more other zones were present in the chromatogram obtained with the test solution.

**CONCLUSION**

The above finger prints conclude that in chilling process, many important bioactive Pregnane Glycosides are getting removed from
the Caralluma herb, whereas the Green Chem’s patented process where Hexane is used to selectively remove resinous matters, gives Caralluma extract containing the key bioactive Pregnaneglycosides. Therefore the product obtained by Hexane treatment is more suited for antiobesity and other health benefits than the product obtained by chilling process.

The finger print profile of compounds present in Caralluma extract as per the process of US patent 7,060,308 B2 complies with the herb, thus making the product as safer as the tribal consuming the whole herb. Hence this extract is not a “modified extract”.

The extract obtained by the chilling process does not give a similar finger print profile of the herb, thus confirming it is a “modified extract”.

HPTLC profile proves that a major pregnane glycoside Slimalumaside-B and many other compounds are removed from the herb, proving the status of “modified extract”. Hence this modified extract can not be treated as the total extract of Caralluma and is different from the extract obtained by the process described in US patent 7,060,308 B2.

This modified extract may not give the anti obesity activity and other health benefits; the toxicity should be examined very carefully.

The above HPTLC method is sensitive to identify the potentially unsafe extracts of Caralluma.

REFERENCES