International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
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SCREENING EFFECT OF FLAVONOIDS IN CARALLUMA NILAGIRIANA USING CHROMATOGRAPHIC TECHNIQUE

B.Renuka[1]*

[1]Department, SITRA (South India Textile Research Association) Coimbatore, Tamilnadu, India.

ABSTRACT

The present study investigated to determine the bioflavonoid compounds in the methanol, ethanol, chloroform, acetone, ethyl acetate and petroleum benzene extracts of Caralluma nilagiriana shoot. It can be done by qualitative chemical analysis using specific reagents for specific constituents followed by confirmation with different chromatographic techniques, like TLC, HPTLC, HPLC, and GC-MS and etc.HPTLC is a major advancement and used for fast screening and quantification of plant extracts that are cost effective. This study was extended by analyzing the potent bioflavonoid compounds in the caralluma nilagiriana plant using HPTLC. Bioflavonoids have a good medicinal potential. It needs further research on toxicological aspects to develop safe drug.

Keywords:- *Phytochemical, Bioflavonoid, Caralluma nilagiriana, HPTLC etc.*

Correspondence Author

B.Renuka
Department, SITRA (South India Textile Research Association)
Coimbatore, Tamilnadu, India.

Email: chemistryrenuka@gmail.com

INTRODUCTION

Phytochemical characterization of plant material is important as it relates to the therapeutic action. Among the thousands of naturally occurring constituents so far identified in plants and exhibiting a long history of safe use, there are none that pose- or reasonably might be expected to pose- a significant risk to human health at current low levels of intake when used as flavoring substance. Due to their natural origin, environmental and genetic factors will influence the chemical composition of plant essential oils. Factors such as species and subspecies, geographical location, harvest time, plant part used and method of isolation all affect chemical composition of the crude material separated from the plant. The screening of plant extracts and natural products for antioxidative and antimicrobial activity has revealed the potential of higher plants as a source of new agents, to serve the processing of natural products (Hard cover, 2011). In India *Caralluma* species is edible and used in traditional medicine. It is commonly used in treating rheumatism, diabetes, leprosy, tumor, fungal diseases, snake and scorpion bites; also have an antipyretic, antiobesity and, antihelmintic and analgesic properties[(2).] *Caralluma nilagiriana* is the new endemic species found in 1976[(3).]*C. nilagiriana* is a succulent plant depleted because of, over exploitation, lack of organized cultivation and completely eaten by sheep and goats, the wild population has become restricted to Nilgiris,

Tamilnadu. Regrettably, this species has a now been added to the list of Indian endemic plants[4]. It shows there is no report on the event of phytochemicals in C*. nilagiriana.*Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include Alkaloids, terpenopids, flavonoids, steroids, tannins, phenolic compounds and etc[5].These compounds are synthesized by primary or rather secondary metabolism of living phytochemical evaluation of plant is essential to study the pharmacological activities[6].The aim of the present study was to investigate the presence of phytochemical and to determine the total flavonoids content of the selected medicinal constituent in*Caralluma nilagiriana.*It can be done by qualitative chemical analysis using specific reagents for specific constituents followed by confirmation with different chromatographic techniques, like TLC, HPTLC, HPLC, and GC-MS and etc. HPTLC is a major advancement of TLC principle requiring shorter time and a chromatographic technique used for fast screening and quantification of plant extracts that are cost effective and has been commonly used in screening studies[7].Further we have also doinganother phytochemicals.

MATERIALS AND METHODS

Plant material and Collection

The individuals of *Caralluma nilagiriana* were collected from foot hills of Nilgiris, Tamilnadu and it is binomial authenticity was confirmed with the voucher specimen deposited in the Department of Botany, Government Arts College (Autonomous) Coimbatore[1].

Preparation of extractions

A fresh plant was dried in room temperature for two weeks. Then the dried pieces were powdered with a hand mill and stored in room temperature. About 1 g of powdered material was then subjected to extractions using Soxhlet apparatus in AR grade Methanol, ethanol, chloroform, ethyl acetate, acetone, and petroleum ether was successfully treated with6 hours. All the extracts were filtered and concentrated in rotary evaporator under reduced pressure (Vacuum 175 mbar for bp at 40^{o}C) to get thick green crude extracts[8].

Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituent of bioflavonoids in the medicinal plant under study were carried out in extracts using the standard procedures as described by Sofowara(1993), Trease and Evans (1989) and Harborne (1973).

Detection of Flavonoids constituent

(a) Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids [9].

(b) Lead acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids [10].

(c) Shinoda's Test

In a test tube containing 4 ml of extracts solution were treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution 5-6 drops of con HCL acid was added and red color was observed for flavonoids and orange for flavones [11].

HPTLC analysis

Instrumentation

The TLC technique was adopted to separate the flavonoids and identified[12].It was done using CAMAG HPTLC systems equipped with a sample applicator Linomat5, twin trough development

chamber (10x10) size, TLC Scanner, Visualizer, winCATS integration software was used.

Reagents and Chemicals

Analytical grade ethanol, ethyl acetate, water, glacial acetic acid and formic acid, were obtained from Merck and SD fine Chem. Ltd, Mumbai. Precoated TLC aluminium sheets silica gel 60F254 (10 x 10 cm, 0.2 mm thick) were obtained from E. Merck Ltd, Mumbai.

Preparation of Test Sample

Each crude extracts of the sample was diluted with subjected solvents up to 50 ml, sonicated for 15 min, centrifuged and 25 µL of the test sample was applied as band on plate for determination.

Chromatographic Conditions

Extracts of *C.nilagiriana* were spotted on a precoated TLC aluminium sheet silica gel 60 F254(10 x10 cm, 0.2 mm thick) as 8mm wide band using automatic TLC applicator Linomat 5 at 8 mm from the bottom. The mobile phase used was ethyl acetate: water: acetic acid: formic acid (8:1:0.5:0.5 v/v) and the plates were kept for saturation in twin trough chamber for 30min. After development the plates were dried in air and scanned at 366 nm by using CAMAG TLC-Scanner, Linomat 5 samples applicator equipped with a 100 µL syringe. A constant application rate of 25µL s-1 was used. Automatic scanning was done with CAMAG TLC Scanner in remission absorbance mode controlled by winCATS software resident in the system. The slit dimensions were 6.00 x0.45 mm, micro and the scanning speed was 20mm s-1.The radiation source was deuterium lamp and W emitting continuous UV radiation between 190-500 nm. The plates were photographed at 254 nm and 366 nm by using CAMAG Visualizer.

RESULTS AND DISCUSSION

Phytochemical studies

Qualitative analyses of flavonoids were carried out by Harborn and Kokate method. Maximum extractions of flavonoids were found to be present in ethanolic extract when compared with methanol and acetone (Table-1). The phytochemical screening of bioflavonoids in ethanolic extract contains may be the Rutin, Quercetin, Genisteinand ect[13]. These compounds can be responsible of several medicinal activities of *caralluma nilagiriana.*

Table-1

Phytochemical Analysis

S.No	EXTRACTS	FLAVONOIDS
1	METHANOL	+++
2	ETHANOL	+++++
3	CHLOROFORM	---
4	ETHYL ACETATE	---
5	ACETONE	++
6	PETROLUM ETHER	---

HPTLC: Bioflavonoids in all extracts of *caralluma nilagiriane* by HPTLC report

The presence of bioflavonoids in all extracts of caralluma nilagiriana is represented by graphical method. Bioflavonoid compounds were identified in caralluma nilagiriyana by HPTLC analysis. The active flavonoid with their retention time (Rt-0.21) is found. The major flavonoid compound present in ethanolic extract was shown in 3D fig-1. All extracts densitograme were shown in Fig: 2-7. Photo documentation of all extracts 254, 366 respectively shown in Fig-8, 9.

Fig-1: 3D densitograme of all extracts

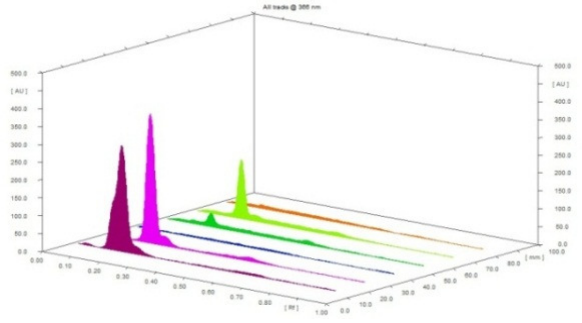


Fig.2. Chromatogram of Methanol Extract at 366 nm

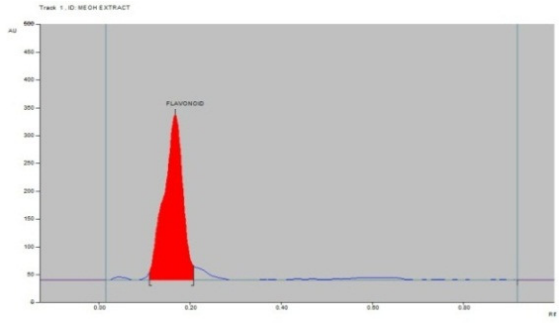


Fig.3. Chromatogram of Ethanol Extract at 366 nm

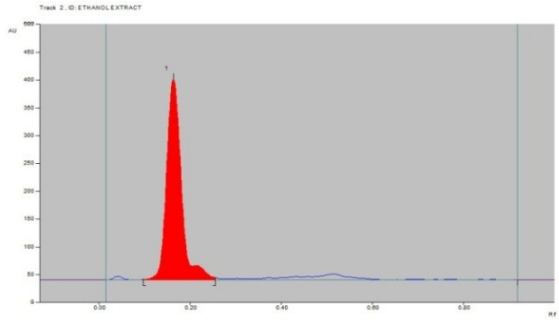


Fig.4. Chromatogram of Chloroform Extract at 366 nm

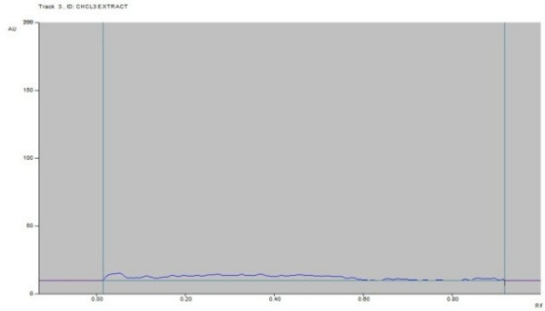


Fig.5. Chromatogram of Ethyl acetate Extract at 366 nm

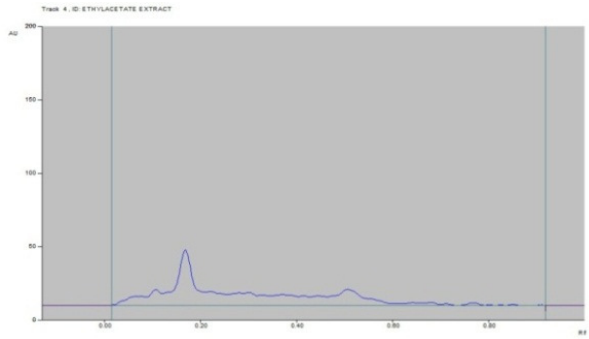


Fig.6. Chromatogram of Acetone Extract at 366 nm

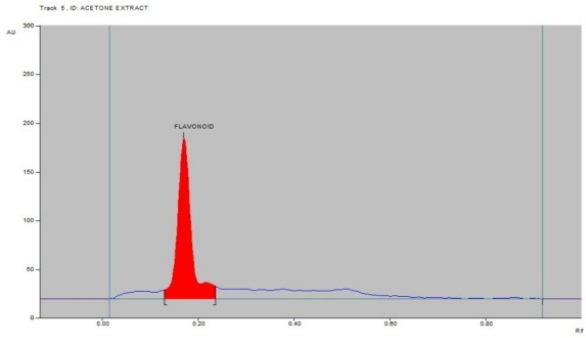


Fig.7. Chromatogram of Petroleum benzene Extract at 366 nm

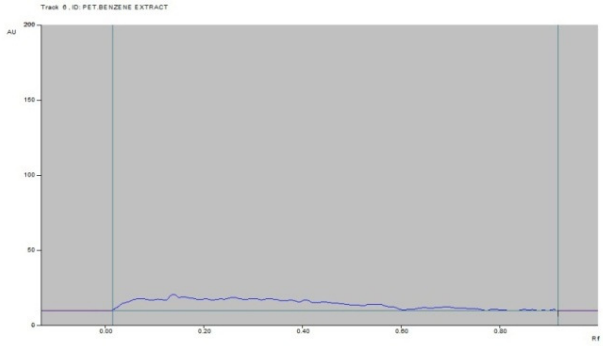
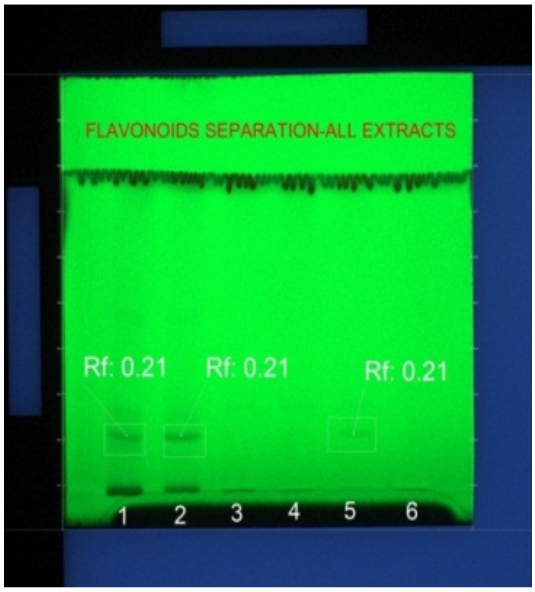
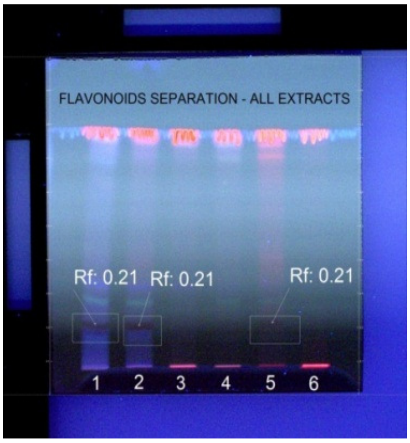


Fig.8. Photo documentation of all extracts Sample at 254 nm



Developed photo at 254 nm 1.Methanol, 2.Ethanol, 3.Chloroform, 4.Ethylacetate, 5.Acetone, 6.Petroleum benzene

Fig.8. Photo documentation of all extracts Sample at 366 nm



Developed photo at 366 nm 1.Methanol, 2.Ethanol, 3.Chloroform, 4.Ethylacetate, 5.Acetone, 6.Petroleium benzene

DISCUSSION

Flavonoids are polyphenolic compounds with high capacityof inhibition of free radicals in human beings which derives fromstress, antibiotic consumption, pollution, etc. This is calledantioxidant activity; a diet without flavonoids or polyphenols would imply a degradation or aging of the cells in human organism[(14).] The flavonoids have aroused considerable interest recentlybecause of their potential beneficial effects on human health. Theyhave been reported to have antiviral, anti-allergic, anti-platelet,anti-inflammatory and antitumor activities[(15).] A researching of 2002 in the Netherlands said that theconsumption of flavonoids per person was 23mg per day, which70% wasquercetin (Ochoa, C. 2004).The major unknown flavonoid compound identified by HPTLC in ethanolic extract is medicinally valuable and posses various pharmaceutical application. The identified phyto constitutions are needs further research on toxicological aspects to develop safe drug.

CONCLUSION

The plant screened for phytochemical constituent seemed to have the potential to act as a source of usefuldrugs and also to improve the health status Available online on www.ijprd.com of theconsumers as a result of the presence of flavonoids compounds that are vital for good health.

ACKNOWLEDGEMENT

The authors are grateful and thankful to the management and the Director Dr.Prakash vasudevan, SITRA Coimbatore, for providing the necessary facilities to carry out the research work.

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