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PHYTOCHEMICAL SCREENING OF FLOWERS OF COUROUPITA GUIANENSIS AUBL

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ABSTRACT:

The phtocompounds that are found in the plants can scavenge free radicals are thus effective in ameliorating role in the progress of various types of diseases. Couroupita guianensis Aubl belonging to family Lecythidaceae is a common medicinal plant in India and South America, mainly it is used in Ayurvedic medicine as its antibiotic, antifungal, antiseptic, analgesic acitivities.

To separate and characterize the phytocompounds from methanolic extract of Couroupita guianensis Aubl flowers using High Performance Liquid Chromatography – Mass Spectroscopy. The phytocompounds with retention times 12.323, 13.642, 14.926, 19.413 and 20.418 may be identified as swietenine, sapropterin, usnic acid, lupeol and gamma tocopherol (vit. E) Respectively by using HPLC-MS technique. According to HPLC-MS technology of methanolic extract of flowers of Couroupita guianensis Aubl, our study identified only limited number of phytocompounds.

Keywords: Methanolic extraction, organic solvents, phytocompounds, HPLC-MS, Couroupita guianensis Aubl.

INTRODUCTION:

The coupling of High Performance Liquid Chromatography Ionosation Mass Spectroscopy combines efficiently the capability of HPLC and the excellent characteristics of Mass Spectroscopy (MS). It provides a powerful approach to identify a variety of polar and thermally labelled phytocompounds like flavonoids in crude extract of plant, Couroupita guianensis Aubl.

It deals with analysis of phytocompounds like alkaloids, fats and waxes, phenolics and terpenoids, flavonoids etc. are separated (Harbone, 1998). This served as a basis for the application of modern instrumental techniques such as HPTLC and HPLC-MS to develop phytochemical fingerprint and to identify marker for individual plants used in the study (Sethi, 1996).

It is reported that Couroupita guianensis used in fruit pulp as antimicrobial property (Shah G.N., et.al., 2012), evaluated anti-ulcer use of Couroupita guianensis Aubl leaves (A. Elumalai et.al., 2012) immunomodulatory action of flowers (Pradhan D. et.al., 2008), evaluation of antihelminthic activity of flowers (Rajamanickam V. et.al., 2009).

This promoted us to the present work and we have isolated and identified the five phytocompounds from Couroupita guianensis Aubl by HPLC-MS method. But they have improved if the study would have attempted for HPLC-MS analysis. It is a relatively simple technique and ideal for the rapid comparative study of plant samples. This method is an excellent technique for quality control of drug analysis (Zhang J., et.al., 2005).

MATERIALS AND METHODS:

The flowers of Couroupita guianensis Aubl were collected from Mumbai region and authenticated by Dr. Rajendra Shinde, Associate Professor, Depatment of Botany, St. Xavier's

college, Mumbai-400001, Maharashtra, India. Healthy flowers were spread to dry under sunlight exposure for 4-5 days and with the help of electric grinderground it in to fine powder form.

Preparation of extract:

Powdered mass of flowers was defatted with petroleum ether (60° to 80°c) to remove fat contents and the supernatant is discarded. The residue extracted in a soxhlet apparatus for at least 12 hours with methanol and extract used for experiment. The solvent from extract removed under reduced pressure and controlled temperature (40 to 50°c) (Vinod Gupta, et al. 2012).

METHODOLOGY:

Reagents:

All solvents were of HPLC grade (E. Merck, Mumbai, India) and reagents were of analytical grade and were purchased from Qualigens Fine Chemicals, Mumbai, India. Distilled water used for the analysis was prepared by double distillation using Milli Q water purifying system (Millipore, USA). All solvents were filtered through 0.5µ (Millipore) membrane and degassed in an ultrasonic bath. The solvent system for the analysis is given below.

Mobile Phase A consisted of 0.1 % Formic acid in water while mobile phase B consisted of 90 % acetonitrile in 10% water and 0.1% formic acid.

Standard volumetric flasks and pipettes of class A grade were used throughout the experiment.

Instrumentation:

1) Agilent Technologies, USA (1.290 Infinity UHPLC) coupled with 6550 iFunnel TOF/Q TOF mass spectrometer.

2) Electrospray ion trap instrument.

3) 2.1 ×100 mm, 1.8 micron Zorbox SB-C18 column.

Sample preparation:

One drop of sample was taken with the help of capillary and dissolved the sample in 2 ml methanol in the 20 ml beaker. The solution was then filtered through 0.22 µm filter named as DURAPORE GVWP 0.22 µm.

Table 1: Chromatogram of the methanolic extract of flowers of Couroupita guianensis.

Peak No.	Retention Time	Mass value (ESI –ve mode)
1	12.323,	568.26
2	13.642	241.12
3	14.926,	344.09
4	19.413	684.16
5	20.418	310.24

The table indicated that Retention time and mass value at negative mode of some phytochemicals present in the methanolic extract of Couroupita guianensis Aubl flowers were swietenine, sapropterin, usnic acid, lupeol and gamma tocopherol (vit. E).

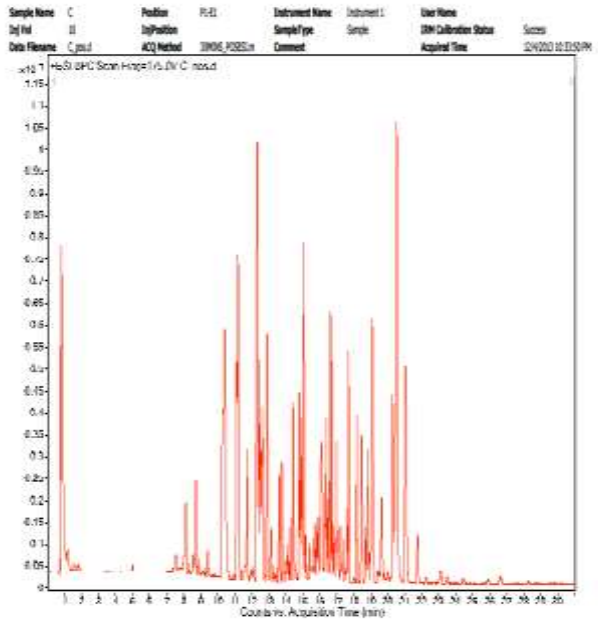


Figure 1: HPLC-MS chromatogram in water: methanol: acetonitrile (256:50:25).

RESULTS AND DISCUSSION:

HPLC-MS analysis of the methanolic extract of flowers of Couroupita guainensis:

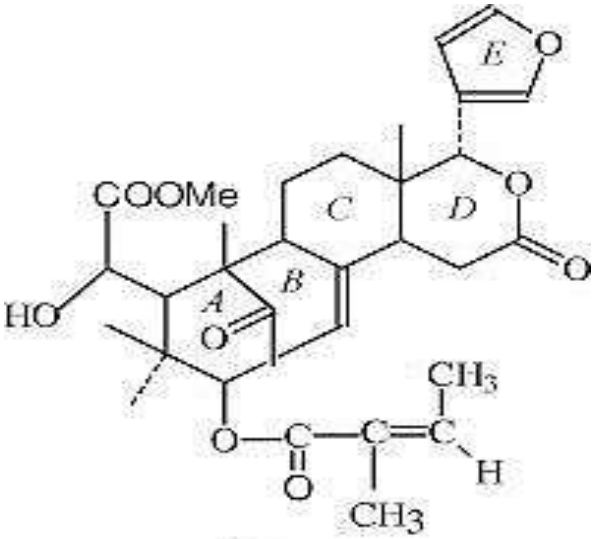
The present study we explored the usage of HPLC/ESI-MS analysis of the methanolic extract of Couroupita guainensis used three different solvent systems for the separation and identification of the phytocompounds rapidly (Harborne, 1998). The chromatograms obtained are shown in figure 1 and the results are also revealed in Table 1. The phytocompounds with their respective retention time and mass values given in the table 1 were swietenine, sapropterin, usnic acid, lupeol and gamma tocopherol (vit. E).

Swietenine is the type of tetranortriterpenoid that also isolated from Swietenia macrophylla seeds has anti-inflammatory property (KK Mak, et.al., 2021). Sapropterin is the kind of flavonoids that used as placebo controlled study of patients with liver cirrhosis and portal hypertension (Enric Reverter, et.al., 2015). Usnic acid extracted from lichen that has efficacy role to inhibit the cancer cell proliferation by suppressing the clonogenic potential, decreasing the expression of PCNA (proliferating cell nuclear antigen) and activating the tumor suppressor gene. (Kunal Kumar, et.al., 2019), Anti – cancer efficacy and mechanisms of usnic acid, Indian J. of Pharmaceutical and Biological Research 7 (03), 1-4).

Lupeol belongs to triterpenes that exhibited in various edible vegitables, fruits and many plants. It possesses antioxidants acidity, anti-inflammotory,anti-glycemic, anti-dislipidemic and anti-mutagenic effects (Fan-Shiu Tsai, 2016).

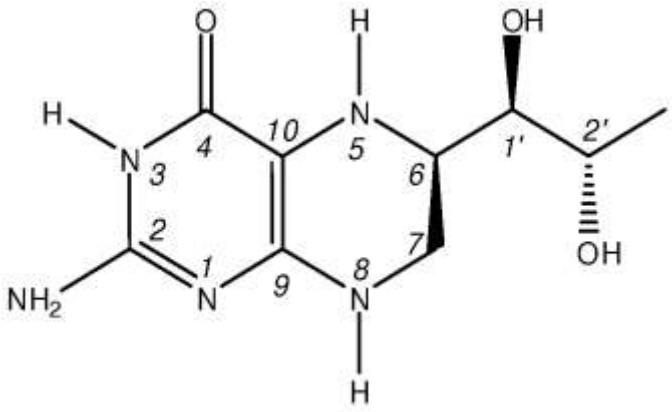
CONCLUSION:

The compound with mass value may be identified as swietenine. While others mass value may be identified as sapropterin, usnic acid, lupeol and gamma tocopherol (vitamin - E) respectively by using HPLC-MS technique. Our study identified only limited number of phytocompounds, but this result will help to identify the more other phytochemical compounds in the future.

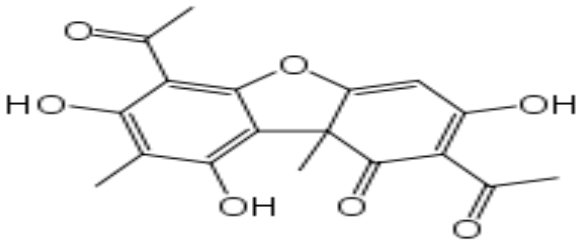


Swietenine

(I)



Sapropterin:



Usnic acid

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