

Investigation of secondary metabolites from *Couroupita guianensis* through GC-MS

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Abstract

Medicinal plants have a crucial role in human culture and civilization. In this study, the bioactive compounds of *Couroupita guianensis* have been evaluated using GC-MS. Ethanolic extract of plant leaves samples was tested by GC-MS. This investigation used for identified bioactive compounds in *C. guianensis*. Totally 8 compounds peak were found in GCMS spectrum. That one major peak with retention time 18.88 min and their respective area percentage is 81.84%. As per our current reports showed metabolites/compounds as 10-Octadecenoic acid, methyl ester which is present in the *C. guianensis*. It have more pharmaceutical properties, hence this plant as more vital role in drug discovery.

Keywords: Bioactive compounds, GC-MS analysis, *Couroupita guianensis*, Octadecenoic acid.

1.Introduction

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [1]. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [2]. According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries. The premier steps to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation.

Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, anti-diarrheal, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of certain natural or herbal products. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim. Clinical trials directed towards understanding the pharmacokinetics, bioavailability, efficacy, safety and drug interactions of newly developed bioactive compounds and their formulations (extracts) require a careful evaluation. Clinical trials are carefully planned to safeguard the health of the participants as well as answer specific research questions by evaluating for both immediate and long-term side effects and their outcomes are measured before the drug is widely applied to patients. So, the plant derived

drug/ bioactive compounds is an urgent need for modern medicine in order to avoid the multi-drug resistant problem, side effects and chemical treatment methods.

The *Couroupita guianensis* tree was selected for further investigations. *Couroupita guianensis* tree is a botanical curiosity. It bears its flowers on thick, tangled extrusions that grow on the trunk, below the foliage branches. The extrusions are from 2 - 6 feet long. At night, the 6 petaled, orange-red flowers are strongly perfumed. There are many stamens in the center of the flowers. The flowers are attached to an upwardly bent, white, fleshy disk. The tree gets its common English name from its heavy, brown, spherical fruits. While flowers are short-lived, fruits take up to a year to mature. The fruits do not open but fall from the tree and rot with an unpleasant odor, unlike that of the flowers. Where native, *C. guianensis* is a tree of river banks and lowlands, subjected to periodic flooding. Although a plant of moist soils, it grows well under dry conditions. A stout, straight, tree, it possesses a dense, often narrow crown, with leaves clustered at the tip of branches. In the present investigation, the main objective is to find the bioactive compounds/ secondary metabolites from the ethanolic extraction of *Couroupita guianensis*.

2. Materials and methods

2.1 Study area and sampling

The plant materials were collected from Pudukkottai district of Tamil Nadu in India during the period of January to February 2014. The shade dried *C. guianensis* fine powder was sterilized at 121°C for 15 min.

2.2 Ethanolic extraction of plant samples

Sterilized fine powder, 20 g each was taken each plant, mixed with 200 ml of Milli Q water and kept in boiling water bath at 60°C for 10 min. The extracts were filtered with Whatman No. 1 filter paper and the filtered extracts were stored in a refrigerator at 4°C and it's used as test samples for basic preliminary study. The 250 g of sterile fine powder was processed in soxhelt apparatus for attaining of ethanolic extraction of the plant sample. The ethanolic extraction of plant sample was used for analysis of bioactive compounds through several techniques.

2.3 Secondary metabolites screening

This ethanol extracts of plant sample were sonicated for 20 min in sonicator 20 µl from sonicated extracts was passed through 0.45 µm filter. Filtrate was used for GCMS analysis. Gas Chromatography Mass Spectrometry (GC-MS) is a technique for the analysis and quantitative of organic volatile and semi-volatile compounds. Gas chromatography (GC) is used to separates mixtures into individual components using a temperature-controlled capillary column. Smaller molecules with lower boiling points travel down the column more quickly than larger molecules with higher boiling points. The maximum allowable temperature for this method is 300°C. The GCMS system (Agilent 7890A GC-MS QToF 7200 series) was used. Chromatographic analysis was carried out using a INNOWAX 30 m x 0.250 mm x 0.25 µm column at temperature: ambient. Running conditions included: injection volume HS 2.5 mL syringe, HS SPME injection technique; mobile phase: Helium. Samples were filtered through an ultra-membrane filter (pore size 0.45 µm) prior to injection in the sample loop. Retention time and concentration of Metabolites were analyzed by using in-built GCMS software.

3. Result and discussion

Gas chromatography with Mass Spectrum (GCMS) was used for the estimation of secondary metabolites from leaves of *C. guianensis*. The GC-MS chromatogram of the eight peaks of the compounds detected was shown in Figure-1 and 2 Retention time (Rt) and percentage peak of the individual compounds. GCMS analysis of samples revealed that one major peak as item 5 with retention time 18.88 min, and their respective area percentage is 81.84%. As per our current reports showed metabolites/ compounds peak no 5 as 10-Octadecenoic acid, methyl ester which is present in the *C. guianensis*. Other seven minor compounds such as Heptadecanoic acid,16-methyl-,methyl ester (7.90% at Rt-19.13), Hexadecanoic acid, methyl ester (7.11% at Rt-17.15), methyl tetradecanoate (1.12% at Rt-15.12), Undeconic acid, 10-methyl-,methyl ester (0.72% at Rt- 12.95), Hexadecanoic acid,14-methyl-,methyl ester (0.40% at Rt-18.18), 1,2,4-trioxolane-2-octanoic acid,5-octyl-,metyl ester (0.02% at Rt-20.98), 9-Hexadecanoic acid (0.01% at Rt-16.97). The identified major compounds possess some important biological potential for future drug development. The 10-Octadecenoic acid, methyl ester with highest % peak area (81.84%) has previously been reported for antibacterial activity [3]. Glycerin decreases intracranial pressure in numerous disease states, including Reye's syndrome, stroke, encephalitis, meningitis, pseudotumor cerebri, central nervous

system tumor, and space occupying lesions. It is also effective in lowering intraocular pressure in glaucoma and shrinking the brain during neurosurgical procedures [4].

The interpretation of this spectrum confirmed the presence of polyphenolic type of compounds and nitrogen containing alkaloid type of compounds. Extraction of the dried fruits from the cannon ball tree, *Couroupita guianensis* Aubl., yielded 6,12-dihydro-6, 12-dioxindolo[2,1-b]quinazoline (tryptanthrin), indigo, indirubin and isatin [5]. Ahmed John and Koperuncholan [6] 2012 have reported the presence of isatins in *Couroupita guianensis*. The structure of indirubin contains two NH and two -CO groups and the molecular weight of indirubin is 262. Since the GC-MS spectrum of *Couroupita guianensis* plant extract reveals the presence of 'N' and 'CO' group, in the fraction at 10.88, with higher molecular ion peak at m/e 300, it may be concluded that this extract contains a higher derivative of indirubin. Roy et al [7] (2009) have done the phytochemical analysis of *Andrographis paniculata* extract and subjected it to GC-MS. GC-MS results revealed phenols, aromatic carboxylic acids and esters in the chloroform extract to be the molecules responsible for the antimicrobial activity of *A. paniculata*. Phytochemical screening of three different oil fractions, obtained from n-hexane extract of *Prunus domestica* shoots analyzed by GC and GC-MS resulted in the identification of 9, 16 and 24 compounds, which represented 92.56%, 90.6% and 90.69% of these oil fractions, respectively [8]. In another study, the essential oil from the fresh leaves of *Schinus terebinthifolius* was extracted and subjected to GC-MS analysis. This showed that the major constituents of the essential oil were sabinene, pinene, phellandrene, pinene, terpinene-4-ol, trans ocimene and myrcene [9]. *Henophyton deserti* was characterized with respect to its chemical composition, antioxidant potential and antimicrobial activity of its methanolic extract. Fourteen compounds were identified by LC/MS, GC/MS, and GC in leaf and seed extracts [3]. The essential oils from the flowers, leaves, barks, roots and fruits of *A.brachypus* were individually extracted by hydrodistillation, and their chemical constituents were isolated and characterized by means of GC and GC-MS [10].

Table. Secondary metabolites screening by GCMS

Name	Retention Time	Area	Area %
Peak-1	12.95	5409428	0.72
Peak-2	15.12	8436276	1.12
Peak-3	17.15	53702660	7.11
Peak-4	18.18	3016892	0.40
Peak-5	18.88	617990792	81.84
Peak-6	19.13	59642360	7.90
Peak-7	20.77	3092104	0.41
Peak-8	20.98	125308	0.02
Peak-9	23.00	3685208	0.49
Totals		755101028	100.00

Figure 1: GCMS Spectrum of *C. guianensis*

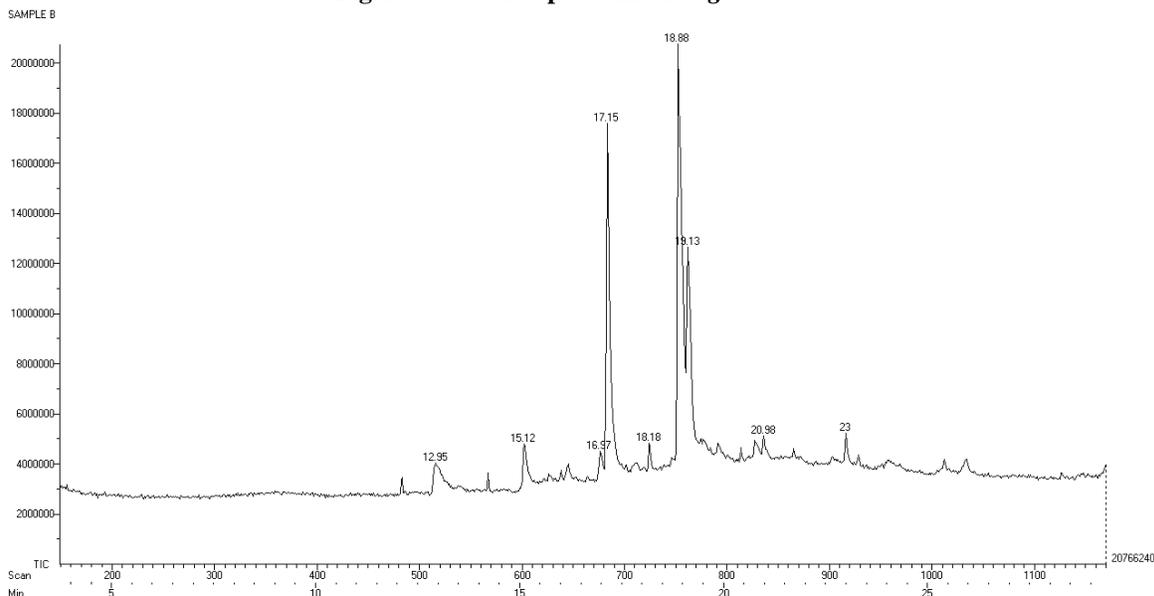
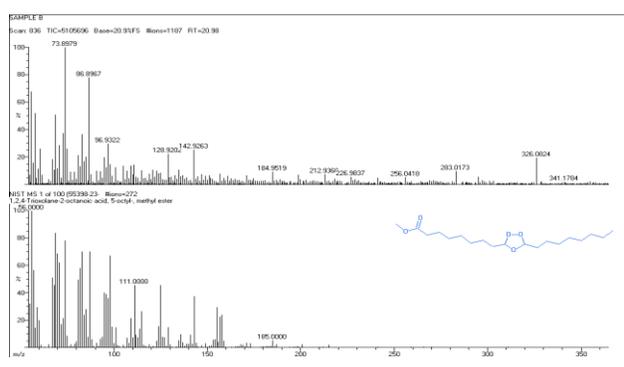
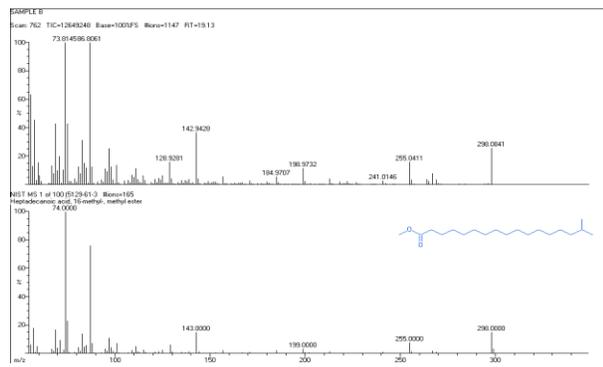
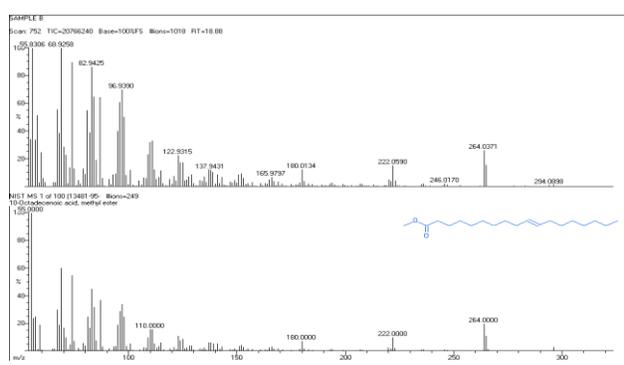
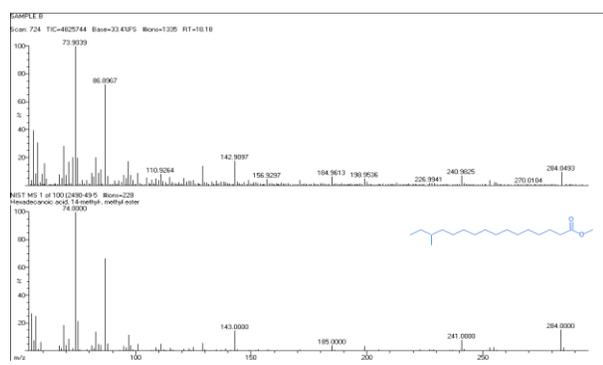
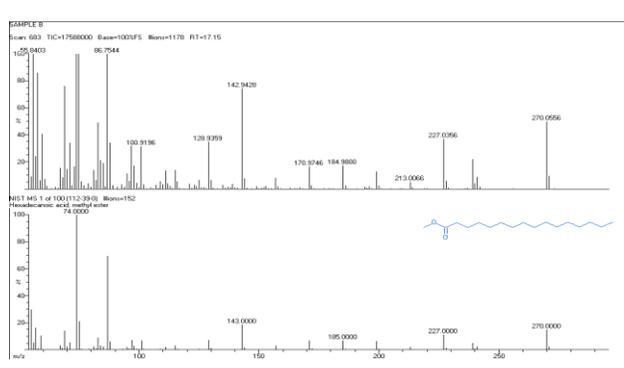
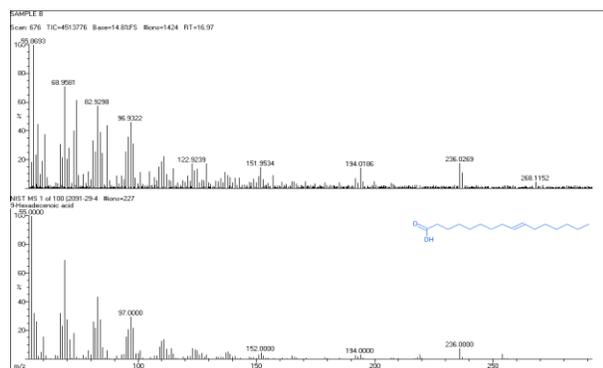
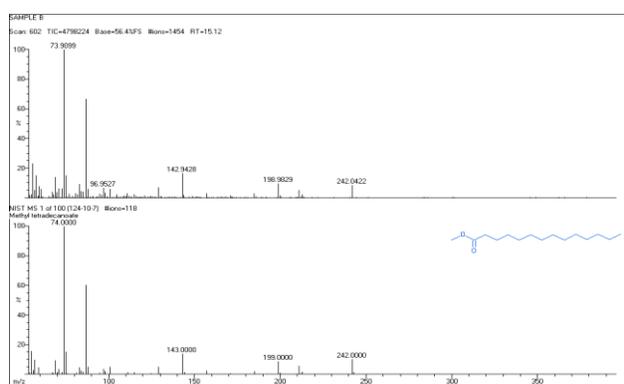
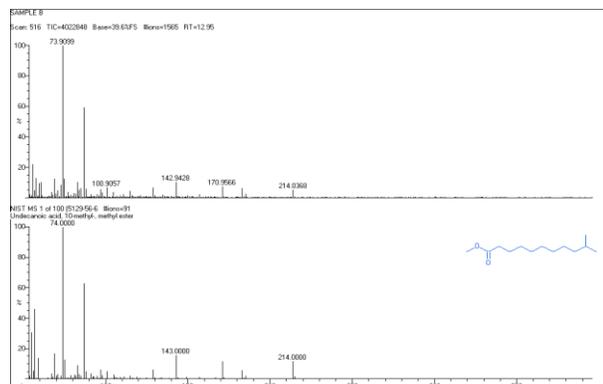


Figure 2: GC-MS Spectrum fractions of *C. guianensis*



4. Conclusion

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. In the present study eight chemical constituents have been identified from ethanol extract of the leaves of *C. guianensis* by Gas Chromatogram Mass spectrometry (GC-MS) analysis. The presence of various phytochemicals contributes to the medicinal activity of the plant

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