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Chemical profiling of *Rhinacanthus nasutus* (L.) Kurz leaves using GC-Ms analysis

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Abstract

Rhinacanthus nasutus (L.) Kurz.is a herbaceous shrub of the acanthaceae family. It is widely distributed in some regions of the subcontinent of India, China, and Southeast Asia. *Rhinacanthus nasutus* plant is widely used in folk medicine preparations, and recent research has begun to analyze its chemical composition and pharmacological properties. The purpose of this study was to identify the phytocomponents present in the chloroform extract of the *Rhinacanthus nasutus* leaves using GC-MS analysis. As a result of the GC-MS analysis, 18 compounds were identified. GC-MS analysis of the bioactive fraction revealed the highest peaks in the chromatogram. Chloroform extract of the *Rhinacanthus nasutus* (L.) Kurz. leaves contain a wide range of biologically important compounds. Therefore, our research's primary goal is to isolate these bioactive compounds and utilize them as therapeutic targets.

Keywords: GC-MS analysis, chloroform extract**,** *Rhinacanthus nasutus* (L.) Kurz, Ethno medicine

Introduction

Some parts of the sub-continent of India, China, and some regions of Southeast Asia are home to *Rhinacanthus nasutus* (L.) Kurz (family - Acanthaceae). There are several synonyms, as well as local or common names for this herbaceous shrub. This plant is also called snake jasmine, and it is one of the medicinal plants that are used extensively in Indonesia. Phytochemical studies have revealed that *R. nasutus* (L.) Kurz contains secondary metabolites such as flavonoids, steroids, terpenoids, anthraquinones and especially naphthoquinone as the significant compound (Bukke and Ps 2011) [1].

R. nasutus (L.) Kurz ointment is used in alternative medicine for treating skin disorders. Ointments are semi-solid preparations applied externally (Ministry of Healthy Republic of Indonesia 2013) [2]. Research was conducted to develop a skin disease ointment that was made using *R. nasutus* (L.) Kurz (Fadillah *et al* 2014) [3]. It has been reported that preparations from the roots and leaves of this herb are used to treat prickly heat and scurf, and the whole plant is used for treating ringworm and skin problems such as eczema (Achararit 1983[4]., Atsusi and Yoshioki, 1993) [5] as well as pulmonary tuberculosis (Sattar *et al* 2004) [6]. *R. nasutus* (L.) Kurz reduced the activity of viruses that cause herpes, namely herpesvirus (HVS)-1 and HVS-2 (Chaliewchalad *et al* 2013) [7].

A number of pharmaceutical companies and research organizations have recently expressed renewed interest in investigating higher plants as sources for novel lead structures and for the development of standardized phytomedicines that offer reasonable efficacy, safety, and quality (Odunayo *et al* 2007) [8]. It is therefore necessary to look for secondary metabolites in plants. Thus, this led to the discovery of potentially useful preliminary active ingredients, which can be used to make new drugs from *R. nasutus* (L.) Kurz, by proving through GC-MS analysis

Materials and Methods

Collection and identification of plant material

Leaf samples were collected from Marthandam, Kanyakumari District, Tamil Nadu, India in December 2020. Herbarium was deposited in Holy Cross College (Autonomous) Nagercoil after identification of the plant.

Preparation of plant sample extract

R. nasutus (L.) Kurz were shade dried for two weeks and ground into a fine powder. Chloroform was used as the solvent in the Soxhlet apparatus to extract 100 grams of powdered leaves. In a rotatory evaporator, the extract was cooled to room temperature and evaporated to dry under reduced pressure (Raj *et al* 2015) [9]. The extraction was done for six hours. The final extract was then subjected to GC-MS analysis.

GC-MS analysis

GC-MS is an integrative technique for separating, identifying, and quantifying chemicals in a sample (Leary *et al* 2021) [10]. Data quantification is an important step in data analysis, and different software is used to determine the retention time corresponding to specific peaks (Koelmel *et al* 2019) [11]. Its application in the pharmaceutical industry is extensive, as it aids in the identification of bioactive compounds and any impurities present in plant extracts (Chauhan *et al* 2014) [12]. In present study, several compounds have been identified from leaf extract.

Chloroform extract of leaves of *R. nasutus* (L.) Kurz were subjected to GC-MS analysis (Agilent Technologies 7820A Network GC system for gas chromatography). Helium was used as the carrier gas at a flow rate of 1mL/min. The temperature was programmed at 100˚C for 5 min then increased to 270˚C at the rate of 10˚C /min. 2µL of plant extract was injected with a Hamilton syringe into the GC-MS manually.

Identification of Components

Willey and NIST libraries were used to identify components, as well as comparisons of their retention indices. After comparing the constituents with those in the computer library (NIST and Willey) attached to the GC-MS, the results were tabulated.

Identification of Components

Results and Discussion

Gas chromatography-mass spectrometry is a technique used to separate phytochemicals and detect them at the same time (Chauhan *et al.* 2014) [12]. GC-MS analysis of chloroform extract of leaves of *R. nasutus* showed the presence of phenolic and fatty acids, as well as other bioactive compounds present in the leaves.. GC-MS studies have extracted 18 compounds from chloroform extracts of leaves. They were1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester, 1,1,3,3-Tetraallyl-1,3-disilacyclobutane, 1,1,1,3,5,5,5-Heptamethyltrisiloxane, Di-n-decylsulfone, Silicic acid, diethyl bis(trimethylsilyl) ester, Tris (tert-butyldimethylsilyloxy) arsane, 2-((But-3-enyloxy)carbonyl) benzoic acid, Phthalic acid, di (cycloheptyl) ester, Phthalic acid, oct-3-yl pentyl ester, Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl, 1,2-Bis(trimethylsilyl)benzene, Tris (tert-butyldimethylsilyloxy) arsane, 2-Methyl-7-phenylindole, 2-Ethylacridine, N-Methyl-1 adamantaneacetamide, 1,2,4-Benzenetricarboxylic acid, 4-dodecyl dimethyl ester, 5,5'-Di (ethoxycarbonyl)-3,3'-dimethyl-4,4'-dipropyl-2,2'-dipyrrylmethan, Benzo[h]quinoline, 2,4-dimethyl, and N-Methyl-1-adamantaneacetamide, 2-Ethylacridine.

Table 1: Biologically important compounds in *Rhinacanthus nasutus* (L.) Kurz using GC-MS analysis

Sl. No.	Compound name	Molecular Formula	Molecular weight	Biological activity
1.	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	$C_{15}H_{23}NO_3$	265.35	Antiviral, Antiinflammatory, Antifungal, Antioxidant
2.	1,1,3,3-Tetraallyl-1,3-disilacyclobutane	$C_{14}H_{24}Si_2$	248.51	Antineoplastic, Antibacterial, Anticatract, Antihelmentic
3.	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{21}O_2Si_3$	221.50	
4.	Di-n-decylsulfone	$C_{20}H_{42}S$	346.6	Antiulcerative, Antituberculosic, Antitoxic, Anticarcinogenic
5.	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	168.30	Antibacterial
6.	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	468.7	Antihelmentic, Antiinfective, Antiulcerative, Antifungal
7.	2-((Butanol-3-enyloxy)carbonyl)benzoic acid	$C_{12}H_{12}O_4$	220.22	Antiparkinsonian, Antipsoritic, Antinephritic, Antihypertensive,
8.	Phthalic acid, di(cycloheptyl) ester	$C_{22}H_{30}O_4$	358.47	Antiinfective, Antiseptic, Antitoxic, Antimycobacterial
9.	Phthalic acid, oct-3-yl pentyl ester	$C_{24}H_{38}O_4$	390.6	Vasoprotector, Oxygen scavenger, Cytoprotectant, oxidizing agent
10.	Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl	$C_{11}H_{13}NOS$	207.29	Antiinflammatory, Antithrombolite, Antibacterial, Antifungal
11.	1,2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222.47	Antiinflammatory, Anticonvulsant, Antialcoholic, Antiinfective
12.	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	468.7	Antiviral, Antiprotozoal, Anticatract, Antimicrobial
13.	2-Methyl-7-phenylindole	$C_{15}H_{13}N$	207.27	Antileprosy, Antipyrotic, Cardioprotectant, Coagulant
14.	2-Ethylacridine	$C_{15}H_{13}N$	207.27	Antiinfective, Antialcoholic, Antiinflammatory, Insulin promoter
15.	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	207.31	Anticonvulsant, Antiobesity, Aniasthmatic, Antidiabetic
16.	1,2,4-Benzenetricarboxylic acid, 4-dodecyl dimethyl ester	$C_{20}H_{28}O_6$	364.4	Antihypertensive, Antiulcerative, Antparasitic, Antiinfective

17.	5,5'-Di(ethoxycarbonyl)-3,3'-dimethyl-4,4'-dipropyl-2,2'-dipyrrylmethane	$C_{23}H_{34}N_2O_4$	402.5	No activity reported
18.	Benzo[h]quinoline, 2,4-dimethyl	$C_{15}H_{13}N$	207.27	Antiinflammatory, Antiulergic, Insecticide, Antifungal

Table 1 lists the biologically active compounds in chloroform extracts of leaves of *R. nasutus* (L.) Kurz. Figure 1 displays the phytoconstituents identified from the extract with retention times between 15.50 and 21.38. 2-Methyl-7-phenylindole recorded the highest peak area (22.56%) in the chromatogram and the lowest peak area is recorded in hexadecanoic acid and n-octyl ester (3.81%) respectively.

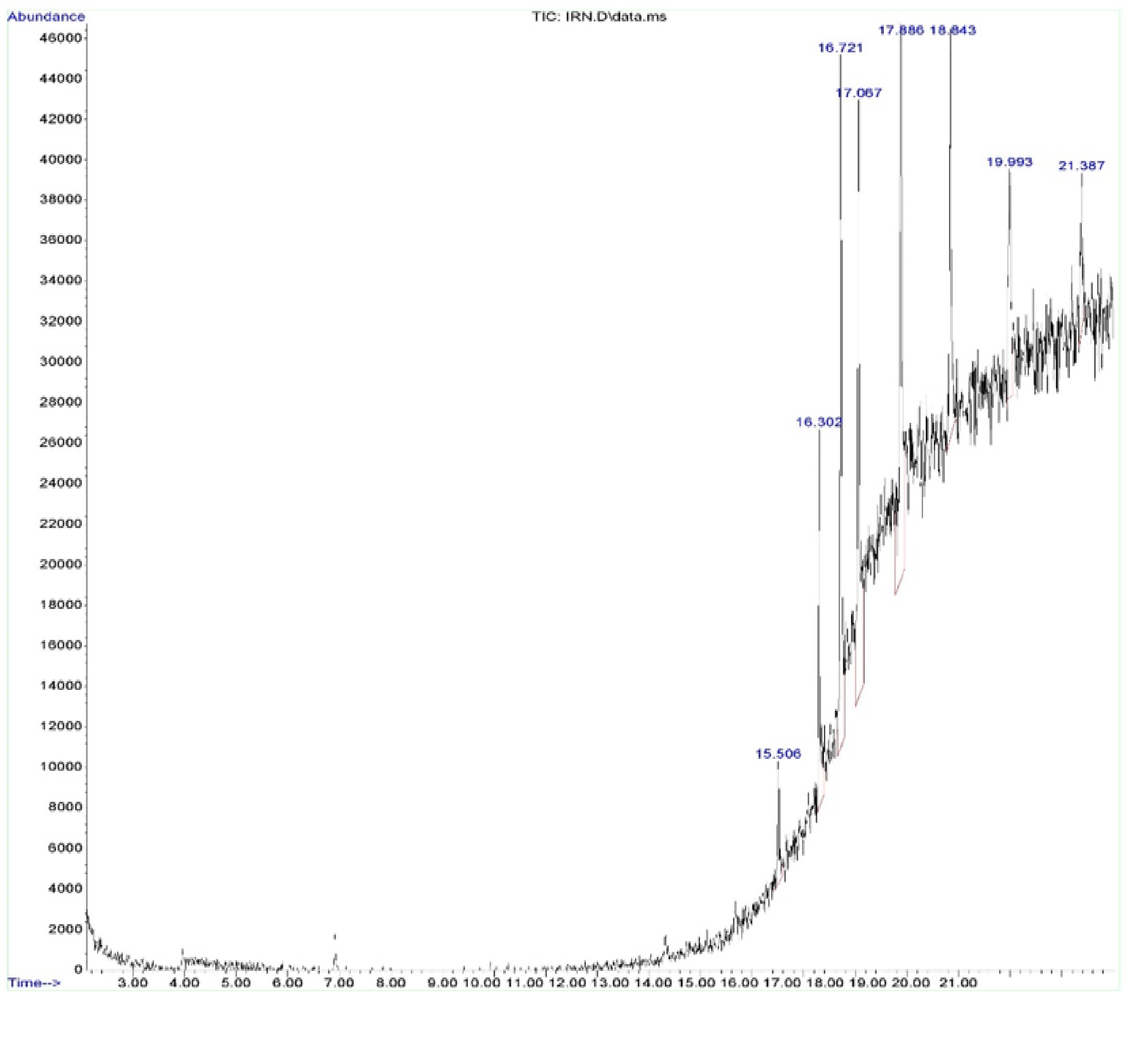

Fig 1: Chromatogram of the plant extract

Research has indicated that many of the compounds in this list have medicinal efficacy as antibacterial, anti-inflammatory, and antiviral compounds. Plants in different climatic and geographical locations produce different compounds in their extracts (Policegoudra *et al* 2012) [13]. Several of these compounds have been discovered in other plants. All of the major molecules are biologically active and are considered to be part of the plants' defense system, as part of a larger group of protective molecules called phytoanticipins or phytoprotectants. (Hossain *et al* 2011[14]; Johnson *et al* 2010[15]; Liu *et al* 2008[16]). 1-Benzazirene-1-carboxylic acid is a fatty acid compound (Kale *et al* 2011) [17], reduces oxidative stress and able to cure neurodegenerative disorders (Jung Choi *et al* 2009) [18], hypo-cholesterolemic activities (Sathish *et al* 2012) [19]. As a volatile methyl siloxane, hexamethyltrisiloxane is used to make silicone liquids and is used in lotions, perfumes, and skin care products. (Wang *et al* 2009) [20]. Antioxidant, antimicrobial, anticancer and antitumor properties are found in 1,2-bis(trimethylsilyl)benzene (Alokprakash and Suneetha 2014) [21]. Silicic acid, diethyl bis(trimethylsilyl) ester, a non-toxic molecule with antimicrobial activity, is an important component of plants against human pathogens (Kathirvel *et al*., 2014) [22]. The siloxane compound 1, 1, 1, 3, 5, 5, 5-heptamethyltrisiloxane exhibited anti-inflammatory and antimicrobial properties, as well. (Ismaila and Clement, 2017) [23].

Conclusion

As the backbone of traditional medicine, medicinal plants have undergone extensive pharmacological studies in the last few decades, mainly due to their value as potential sources of new compounds with therapeutic value and as potential lead compounds in drug development. Therefore, the identification of bioactive compounds in *R. nasutus* (L.) Kurz was done by GC-MS analysis which shows the presence of 18 compounds. Compounds identified in this study have antioxidant, antimicrobial, anticancer, antidiabetic, and anti-inflammatory effects. Due to the presence of these phytochemicals and bioactive compounds, this study suggests that *R. nasutus* may serve as a potential new source of medicines

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