PHYTOCHEMICAL SCREENING AND GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS OF CALOTROPIS PROCERA LEAF EXTRACT

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Abstract

Objective: The leaves of Calotropis procera were subjected to a preliminary phytochemical examination as well as gas chromatography and mass spectrometry (GC-MS) for the purposes of this study.
Methods: In order to conduct the GC-MS analysis, the secondary electron multiplier that is a part of the GC and MS JEOL GC mate equipment was utilized.
Results: The GC-MS analysis identified 12 different phytoconstituents, some of which were *cyclohexene, cyclopropane* tetra decanoic acid, etc.
Conclusions: According to the findings of this study, C. procera leaves are an excellent source of phytocompounds with antioxidant and anticorrosive characteristics.
Keywords: *Calotropis procera,* Phytochemical screening, GCMS.

Introduction

Plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. According to study conducted by the World Health Organization in 2008, more than 80 percent of the world's population relied mostly on traditional medicine as their primary method of receiving medical treatment [1]. As a primary supply of bioactive compounds, higher plants are an important component in the upkeep of human health. According to the research that has been conducted, green plants have a variety of powerful chemotherapeutants [2-4] that are not toxic to plants, have a wider spectrum of effects, and breakdown faster than conventional medications. The secondary metabolites that may be discovered in plants each exhibit distinctive qualities on a biological level. These secondary metabolites are distinguished by their one-of-a-kind activities and the many structural forms that they may take [5]. Learning about plant chemicals is beneficial for several reasons, including the potential to uncover useful folk cures and new sources of economically viable

hytocompounds for the synthesis of complex chemicals [6]. This is why it is beneficial to study about plant chemicals: not only is it desired to find new therapeutic agents, but also because doing so could lead to the discovery of important folk remedies. As a result of the fact that various phytochemicals work together and have a similar mode of action, there is a new area of research in the scientific community that is dedicated to the comprehensive validation of herbal medicines. In order to conduct out direct tests of herbal remedies and other types of traditional medicines, it is common practice to combine chromatographic separation techniques, such as gas chromatography (GC/MS), with mass spectrometry. The purpose of doing this is to improve the reliability of the analysis. Due to its efficacy in detecting non-polar components including volatile essential oil, fatty acids, lipids, and alkaloids, GC-MS investigations have gained popularity in the evaluation of medicinal plants in recent years [7]. This pattern is probably because GC-MS studies are better than others in detecting non-polar components. It's possible that GC-MS research, which has improved in recent years, is to responsible for this trend.

Plant materials

Leaves of *C.procera* were collected from local area of Newai Rajasthan. Maximum weight was given to factors such as local availability, ease of identification, lack of toxicity, and affordability while making the plant material selections. Calotropis procera, which is both harmless and cheap, appears to be widely available there, according to a literature search. Botanist Dr. Afroz alam from the Banasthali Vidyapith, Rajasthan, Department of Bioscience and Biotechnology verified the legitimacy of the selected plant.. The authentification no of selected plant is BURI-1704/2022.

Preparation of extract

Fresh leaves of calotropis procera were washed with double distilled water, dried in the shade and then ground into a powder. The leaves were pulverized, then extracted with methanol at 60°C, and the residue was concentrated to get a thick extract.

Soxhlet extraction or hot continuous extraction

The Soxhlet apparatus was employed, and the sample that had been crushed was placed in the chamber that looked like a thimble. Following heating of the methanol extraction solvent in the bottom flask, the solvent vaporizes in the sample thimble before condensing in the condenser and dripping back into the sample thimble. The process was repeated until the level of the liquid reached the siphon arm, at which time the liquid was poured back into the bottom flask.

Phytochemical screening

Phytochemical screening chemical tests were carried out on the extract using standard procedures to identify the phytoconstituents. [8]

Phytochemical evaluation of methanol extract of *Calotropis procera*		
Phytochemical constituents	Test/Reagents	Result
Alkaloids	Mayer's test	+ve
Tannins	Acetic acid test	+ve
Flavonoids	NaOH test	+ve
Carbohydrates	Benedict and Molisch's test	+ve
Terpenoids	Libermann- Burchardt test	+ve
Saponins	Foam test	+ve
Protein	Ninhydrin test	+ve
Anthraquinones	Borntrager's test	+ve

Gas Chromatography Mass Spectrometry

The methanolic extract was analyzed by means of gas chromatography and mass spectrometry with the assistance of a GC and MS JEOL GC mate that was outfitted with a secondary electron multiplier. High resolution, double-focusing, and a data system are all features of the GC-MS device known as the JEOL GCmate II. This HP5 column has a length of 50 meters and an inner diameter of 50 millimeters and is constructed out of fused silica. The following is a list of the parameters that were used for the analysis: "20 minutes at 100°C, 3 minutes at 235°C for the column temperature, and 240°C for the injector temperature; the split ratio was 5:4; the carrier gas that was employed was helium; the injector temperature was 240°C; the split ratio was 5:4." In a splitless injector, one microliter of the chemical was heated to 300°C, and it was then evaporated. To complete everything required 22 minutes of work. The GC-MS technique was applied in order to determine the ingredients.

In chromatography, a mobile phase carries a mixture while coming into contact with a stationary phase that selectively absorbs certain substances. Its analytical value in guaranteeing the reliability and security of phytotherapeutics cannot be overstated. [9] The mobile and stationary phases used in various chromatographic techniques are what set them apart from one another. In gas chromatography, also known as gas-liquid chromatography, a sample is vaporized before being deposited onto the head of the chromatographic column. An inert gas (the mobile phase) transports the sample down the column. The column's inert solid is being adsorption by a liquid

stationary phase. The principles of gas chromatography are based on adsorption and partition. Among the several chromatography-based methods, gas chromatography (GC) is widely used. James and Martin presented one of the best techniques for separating volatile compounds for the first time in 1952. Gas chromatography has several applications outside of the oil industry, such as in the management of food quality, the study of environmental pollutants, and the diagnosis of medical conditions.[10] These are but a few of gas chromatography's many applications. Due to its speed, sensitivity, and strong resolving power, gas chromatography is an excellent instrument for separating challenging compounds. The direct identification of previously unknown substances is also made possible by the simple coupling of mass spectrometry (MS) and other spectroscopic methods.

Identification of components

The GC-MS mass spectrum was interpreted using NIST's massive database of spectral patterns (including over 62,000 peaks). Mass spectra from the NIST08 and Wiley08 libraries were used to cross-reference with the unknown component's spectra. The mass spectra of the various components were compared in order to determine their identities. Compounds were identified in electronic signals when they eluted from the column and were separated. Compounds were broken down into their constituent ions as they passed down the gas chromatographic column and into the electron ionization detector. The pieces turned out to be charged ions of a certain mass. The resultant graph, known as the mass spectrum graph, serves as a calibrator for the m/z ratio as it represents the molecular fingerprint.

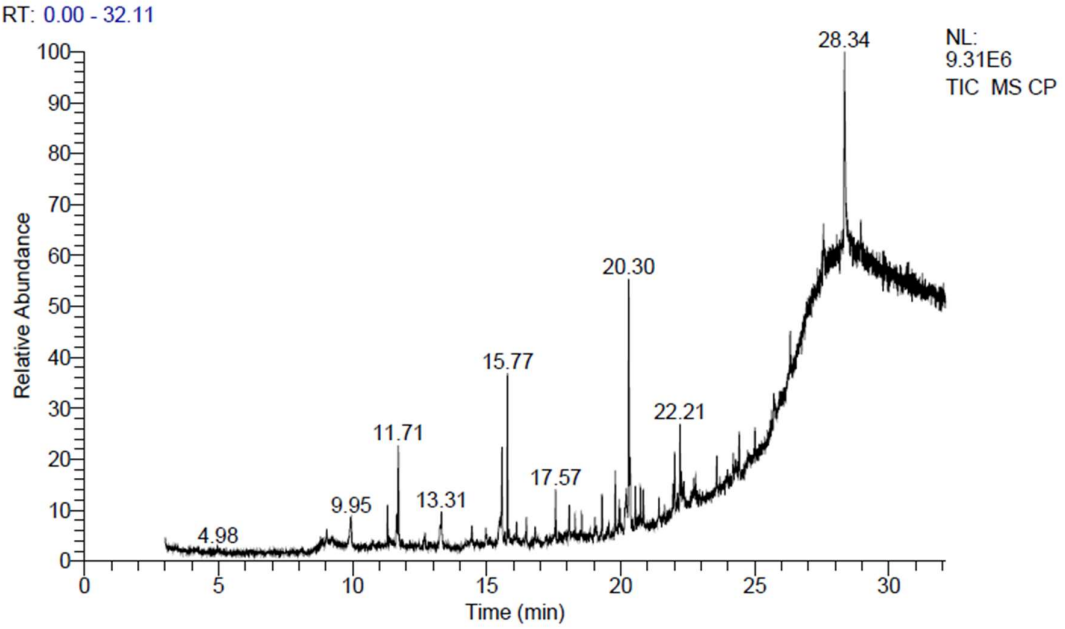
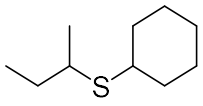
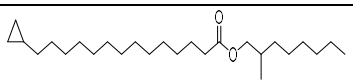
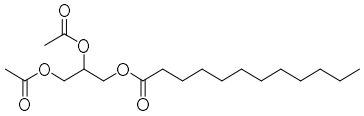
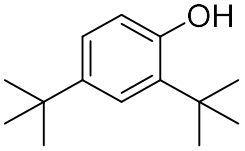
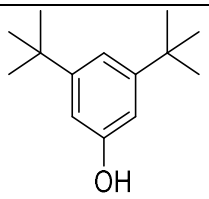
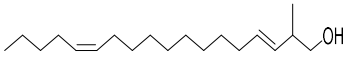
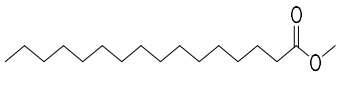
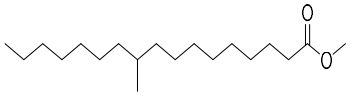


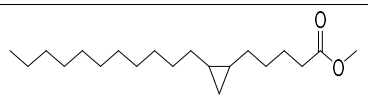
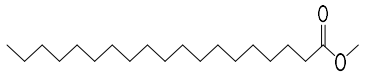
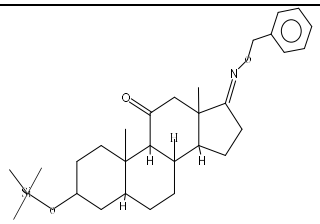
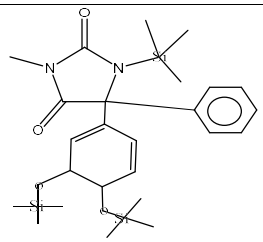
Fig. Phytochemical characterization of Calotropis procera extract by GC-MS

Result and discussion

The GC-MS spectrum of Calotropis procera leaf extract is shown on the graph. In this work, 17 compounds were isolated from a C. procera leaf extract in methanol; however, only 12 compounds showed significant antioxidant and anticorrosive activity. Peak area (representing the proportion of the compound), molecular formula, and molecular weight were used for

compound identification in this investigation. Retention times 4.98, 9.95, 11.71, 13.31, 15.77, 17.57, 20.30, 22.21, and 28.34 were found to be the most prominent on the GCMS chromatogram of the methanolic extract of leaf in the current investigation. Cyclohexene ($C_{10}H_{20}S$) was detected at retention time 11.70 with peak area 6.16%, while cyclopropane tetra decanoic acid ($C_{26}H_{50}O_2$) was observed at retention time 13.31 with peak area 5.14%, both being the most abundant compounds in *C. procera* leaves.

S. No.	Phytochemical compound	RT (min)	Formula	Molecular weight	Area %	Chemical structure	References
1.	"Cyclohexane, [(1-methylpropyl)thio]"	11.70	$C_{10}H_{20}S$	172	6.16		[11]
2.	"Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester"	13.31	$C_{26}H_{50}O_2$	394	5.14		[12]
3.	"Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester"	15.57	$C_{19}H_{34}O_6$	358	10.34		[13]
4.	"2,4-Di-tert-butylphenol"	15.77	$C_{14}H_{22}O$	206	9.54		[14]
5.	"Phenol, 3,5-bis(1,1-dimethylethyl)-"	15.77	$C_{14}H_{22}O$	206	9.54		[15]
6.	"2-Methyl-E, E-3,13-octadecadien-1-ol"	20.21	$C_{19}H_{36}O$	280	6.06		[16]
7.	Hexadecanoic acid, methyl ester	20.30	$C_{17}H_{34}O_2$	270	19.90		[17]
8.	Heptadecanoic acid, 10-methyl-, methyl ester	22.21	$C_{19}H_{38}O_2$	298	6.37		[18]

9.	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	22.21	$C_{20}H_{38}O_2$	310	6.37		[19]
10.	Nonadecanoic acid, methyl ester	22.21	$C_{20}H_{40}O_2$	312	6.37		[20]
11.	"Androstane-11,17-dione, 3-[(trimethylsilyl) oxy]-, 17-[O-(phenylmethyl)oxime], (3à,5à)"	26.32	$C_{29}H_{43}NO_3Si$	481	4.92		[21]
12.	"2,4-Imidazolidinedione, 5-[3,4-bis[(trimethylsilyl)oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-"	27.56	$C_{25}H_{40}N_2O_4Si_3$	516	6.86		[22]

The antioxidant and anticorrosive effects of 12 out of these 17 compounds are demonstrated. The chemicals are both cosmetically pleasing and therapeutically useful. Since just a little amount of plant material is needed for analysis (a few grams), the GC-MS technique is ideal for the rapid and precise identification of chemicals. Antioxidant and anticorrosive capabilities addthe significance to the present research.. In the current investigation, 12 chemical components of Calotropis procera methanolic plant extract were isolated and identified using Gas Chromatogram / Mass spectrometry (GC/MS). These bioactive chemicals found in *C.procera* plants provide support for their potential application in medicine.

Conclusion

The presence of so many phytocomponents in *C,procera* lends credence to its use by the local community as a plant with 'medicinal properties' and also holds promise for the production of novel pharmaceuticals as well as a nutraceutical. To further understand the synergistic effect of compounds for therapeutic applications, it would be beneficial to further isolate the compounds and establish their specific activity. Because of the chemicals' antioxidant and anticorrosive qualities, this plant is more useful in any economic or ecological conditions.

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