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PHYTOCHEMICAL EVALUATION OF *CASSIA AURICULATA* USING ANALYTICAL STUDIES

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Keywords:

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ABSTRACT: Medicinal plants, herbs, spices, and herbal remedies are integral components of the alternative system of medicine since while's ancient *Cassia auriculata* Linn is a potential folklore medicinal plant (Caesalpiniaceae) used for Ayurveda and Siddha systems of medicine. Plants contain a wide variety of phytochemical constituents, which are secondary metabolites and are used either directly or indirectly in the pharmaceutical industry. For centuries, man has effectively used various components of plants or their extracts for the treatment of many diseases. The aim of the study was to investigate the phytochemical compounds of *Cassia auriculata* and GC-MS analysis. The presence of phytochemical compounds was screened by a qualitative method using gas chromatography and mass spectroscopy (GC-MS). An aqueous and ethyl acetate extract obtained were subjected to GC-MS for the determination of bioactive volatile compounds. GC-MS analysis was carried out using claries 680 GC with clarus 600 (EI) MS. The GC-MS analysis of the aqueous and ethyl acetate extract revealed the presence of 26 bioactive compounds with valuable biological activities. The major chemical constituents were ethyl – 1 – thio - beta.- d-glucopyranoside (39.52%), (14.97%), tritetracontane (13.66%), hexatriacontane (11.19%), tetratetracontane (10.78%), neopentane-1,1-dioldiacetate(9.37%),1,6;3,4-dianhydro-2-deoxy-.beta.-d-lyxo-hexopyranose (6.62%). The presence of various bioactive compounds in *C. auricular* taproved the pharmaceutical importance. It can be concluded that the plant investigation has opened up a new perspective in pharmaceutical research and plants can be used for the development of potential, novel antioxidant agents for the treatment of many diseases.

INTRODUCTION: Medicinal plants have been used as conventional treatments for abundant human diseases for thousands of years and in various parts of the world [1]. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety, and efficiency [2]. The cost of drugs in use today is too expensive for the majority of the population in third-world countries. Therefore the search for some cheap sources of antimicrobial substances in nature becomes inevitable [3].

Plants are a good source for new safe, biodegradable, and renewable drugs. The plant is used as a therapeutic agent regions of India. Avaramsenna is a much-branched shrub with smooth cinnamon-brown bark and closely pubescent branchlets. In Indian ethnomedicine, this plant is commonly known as 'Avartaki in addition to being used as food is age-long.

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So, there is a great awareness of the use and significance of these medicinal floras by the World Health Organization in several resource-poor nations [4]. This has led to intensified efforts on the documentation of medicinal plants [4]. *Cassia auriculata* Linn (Family: Caesalpiniaceae), commonly known as *Tanners Senna*, is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine [5]. It occurs in the dry, 'Avaram', 'Taravada', 'Aval', 'Avarike' and 'Hemapushpam. The plant has been reported to possess antipyretic [6], hepatoprotective [7], anti-diabetic, anti-peroxidative, and antihyperglyceamic and microbial activity. The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation [8]. Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample [9]. We have reported the phytochemical analysis of bioactive compounds using Gas Chromatography-Mass Spectroscopy from aqueous and ethyl acetate extract in the present investigation. This study will help to design the new traditional drugs for the treatment of various diseases.

Literature Review:

Guruprasad C. Nille *et al.*, (2015) [10]: Was reported that *Cassia auriculata* used for a long period in various chronic diseases therapeutically. The current review is to search literature for the pharmacological properties, safety/ toxicity studies, pharmacognostic studies, and phytochemical investigation of *Cassia auriculata* plant. Particulars of pharmacological activities, phytochemical isolation, toxicity studies *etc*., were extracted from the published reports focusing on the safety profile of the plant. The safety of the whole plant was concluded in the review. The compiled data may be helpful for the researchers to focus on the priority areas of research yet to be discovered.

Gaurav M. Doshi, *et al.* (2011) [11]: Was reported an Antibacterial potential of *Cassia auriculata* flowers Medicinal herbs as a potential source of therapeutic aids have attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. *Cassia auriculata* has wide range of pharmacological actions; hence present study was undertaken to evaluate its efficacy against gram-positive and gram-negative microorganisms. Antibacterial potential of methanolic extract of dry flowers of Cassia *auriculata* was conducted using agar disc diffusion method. The microorganisms used include *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Escherichia coli*. The maximum activity was observed against all micro-organisms; the minimum inhibitory concentration was determined depending on microorganisms. *Cassia auriculata* was observed to have anti-bacterial activity and can be used to combat against vast flora of microorganisms.

1. **J. Syam Praveen Kumar, *et al.*, (2014) [12]:** The present study was designed to explore the protective effect of *Cassia auriculata* L. flower extract (CAE) in a high fat diet and streptozotocin-induced type 2 diabetic (T2DM) rats.

2. **J. Meenupriya, *et al.*, (2012) [13]:** Was proposed that medicinal values of avaram: *Cassia auriculatalinn* a review}: Nature has been a powerful source of powerful medicines for thousands of years, and number of modern drugs has been extracted and exploited from natural sources, for its use in traditional medicine. Traditional herbal medicines have a long history of use and are generally considered to be safer than synthetic drugs. Over 50% of all modern clinical drugs are natural products that play an important role in drug development in the pharmaceutical industries. The present communication constitutes a review on the distribution, phytography, medicinal properties and pharmacological actions of *Cassia alata* and *Cassia auriculata.* These plants are known to contain various active principles of therapeutic value and to possess biological activity against a number of diseases.

3. **Sachin Chaudhary, *et al.*, (2014) [14]:** Was reported the Phytochemical Analysis and Assessment of *In-vitro* Anthelmintic Activity of *Cassia auriculata* Linn leavesThree different extracts namely Ethanol, Chloroform, Petroleumether of *Cassia auriculata* Linn (Family: Caesalpiniaceae) leaves were screened for their phytochemical composition. The phytochemical studies of *Cassia auriculata* leaves show the presence of alkaloids, tannins flavonoids, glycosides, saponins along with proteins. Three extracts *viz.* Petroleum ether,

Methanol and Chloroform extracts of *Cassia auriculata* leaves were investigated for the anthelmintic activity against earthworms [*Megascoplex konkanensis*]. Three concentrations [20, 40, 60 mg/ml] of each extract were studied which included the determination of time of paralysis and time of death of earthworms. Albendazole [10 mg/ml] was used as a standard drug and distilled water containing 2% Tween 80 was used as control. All the extracts exhibited dose dependentanthelmintic activity. The decreasing order of activity of extracts was assessed to be Methanol, Petroleum ether and Chloroform.

Rukshana MS, *et al.*, (2017) [15]: Was reported this study was implement to actuate the chemical components of Pergulariadaemia leaves using Perkin- Elmer Gas Chromatography-Mass Spectrometry, our results of GCMS compounds in the extract was relevant to the National Institute of Standards and Technology (NIST) library.

GC/MS analysis of ethanolic extract of *Pergularia daemia* leaves confess the presence Hexadecanoic acid, methyl ester (33.42), Pentadecanoic acid, 14-methyl-methyl ester (36.23, Ethyl 9,12,15-octadecatrienoate (33.12 and 4-(4-Chlorobenzoyl)-1-cyclohexyl - 5 - tosylamino-1 H-1, 2, 3 - triazole (31.24). Qualitative phytochemical screening of the ethanolic extracts of the leaves revealed the presence of many compounds such as flavonoids, tannins, alkaloids, terpenoids, steroids and phenols. This study result will make a way for the production ofherbal medicines for various ailments by using *Pergularia daemia* leaves.

Objective of Research: The objective of the present study will subject to the traditionally well-known plant of *Cassia auriculata*in aqueous and ethyl acetate extraction in soxhlet apparatus and the chemical constituents were analyzed by using GC-MS analytical techniques. The work is designed to be carried out in the following phases:

Phase I: Phytochemical Studies:
Phytochemical Studies:

- Collection and authentication of leaves of *Cassia auriculata*.
- Extraction of *Cassia auriculata*.
- Preliminary phytochemical tests of extracts of *C. auriculata*.

PHASE II: Analytical Studies:

- Isolation of plant constituents by thin layer and column chromatography.
- Characterization of isolated compounds by FTIR, 1H NMR, 13C NMR and GC-Mass-spectral studies.

MATERIALS AND METHOD:

Preparation of Powder Material: After authentication from the Botanical Survey of India (A.NO: BSI/SRC/23/2020/Tech/708), the fresh, healthy aerial parts of the plant of *Cassia auriculata* dried properly in the shade for 3 weeks, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an airtight container and used for further studies.

Preparation of Extracts [16, 17]: About 1 kg of air-dried aerial parts of plant *Cassia auriculata* was extracted in Soxhlet assembly successively with ethyl acetate and water. Each time before extracting with the next solvent, the powdered material was dried at room temperature. Each extract was concentrated by using a rotary vacuum evaporator. The extract obtained with each solvent was weighed, and the percentage yield was calculated in terms of the dried weight of the plant material. The colour and consistency of the extract were also noted. All the solvents used for this entire work were of analytical reagent grade (Merck, Mumbai).

GC-MS Analysis: The gas chromatography study includes an important optimization process such as**.** Introduction of sample extract onto the GC column, ii) separation of its components on an analytical column, and iii) detection of target analysis by using mass spectrometry (MS) detector. 5 ml of sample was evaporated to dryness and reconstituted into 2 ml methanol. The extracts were then subjected to GC-MS analysis.

Chromato-graphic separation was carried out with instrument GC-MS-QP 2010 [SHIMADZU] instru-ment with Db 30.0 column (0.25 µm diameter × 0.25 µm thickness). The oven temperature was programmed from 70 °C (isothermal for 5 minutes), with an increase of 10 °C / min, to 200 °C, then 5 °C / min to 280 °C, ending with a 35 min isothermal at 280 °C. Mass spectra were taken at 70 eV; scan interval of 0.5 seconds and scan range

from 40–1000 m/z. Helium was used as the carrier gas at 99.99% pressure with flow 1.0 ml/min and electronic pressure control on. Samples were dissolved in ethanol and injected automatically.

Analytical Condition: The injection temperature at 240 °C, the interface temperature at 240 °C and ion source temperature at 70 °C were determined. The injection was performed in split-less mode.

Identification of Compounds (Data Analysis) [18]: The compounds were identified by spectral studies like GC-MS, 1H NMR, 13C NMR, and mass spectrum.1H NMR spectra were recorded at 400 MHz on a Bruker AMX 400 MHz Spectrometer (Bruker Bio Spin GmbH, Ettlingen, Germany**)** using deuterated chloroform (CDCl3) as solvent and TMS (tetramethylsilane) as internal standard.13C NMR spectra were recorded at 400 MHz on a Bruker AMX 400 MHz (Bruker Bio Spin GmbH, Germany**)** using standard parameters using CDCl3 as solvent.

All the NMR measurements were made on 5 mm NMR tubes. For recording the 1HNMR spectrum, solutions were prepared by dissolving10 mg of the active principle in 0.5 ml of CDCl3, while for 13C NMR spectra, about 20 mg of the compound was dissolved in the same volume of the solvent.

Here, TMS was used as an internal standard. The mass spectrum was recorded using Varian 1200 L Mass Spectrometer (Varian India Pvt. Ltd., Powai, Mumbai).

The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV and the detector operator in scan mode from 40 to 1000 m/z atomic mass units.

The identification of the compounds in the GC-MS of the sample was based on the database of the National Institute Standard and Technology (NIST), which had standards for more than 62,000 compounds. The known components in the mass spectrum were compared with the spectra of known components stored in the NIST library, through which the name, molecular weight, and structure of the compounds were disclosed. Identification based on the molecular weight, molecular formula, retention time and peak area %. It is done in order to determine whether this plant species contain any individual compound or group of compounds that may substantiate its current commercial and traditional use as herbal medicine.

RESULTS AND DISCUSSION: Twenty- six compounds were identified in ethylacetate, and aqueous fraction of *Cassia auriculata* extract by GC-MS analysis. The chromatogram was obtained by ethylacetate and aqueous fraction of *C. auriculata*. The active principle, area of the peak, Concentration (%), Retention Time (RT), Molecular formula, and Molecular weight were presented in **Table 1, 2 & Fig. 1, 2** shows the chromatogram with retention time, molecular weight, area, area% of the standards of ethyl acetate and aqueous extracts of *C. auriculata*.

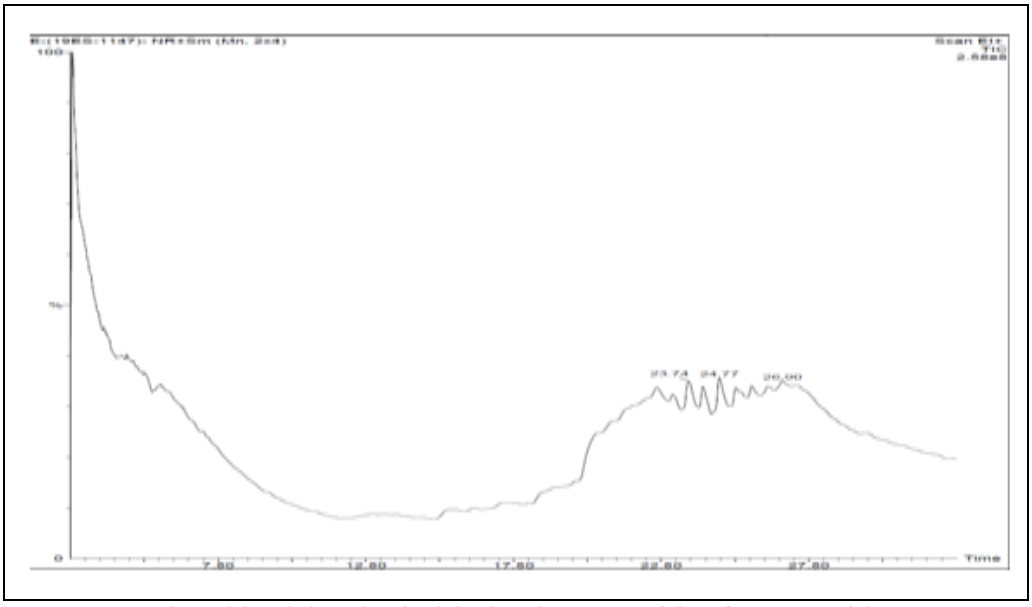

FIG. 1: GS-MS CHROMATOGRAM OF ETHYLACETATE EXTRACT

TABLE 1: PHYTOCHEMICAL COMPOUNDS IDENTIFIED IN ETHYLACETATE EXTRACTC

S. no	Compound Name	% of Peak Area	Retention time (RT)	Molecular Formula (MF)	Molecular Weight (MW)
1.	1,6;2,3-dianhydro-4-deoxy-.beta.-d-ribo-hexopyranose	6.690	20.67	$C_6H_8O_3$	128
2.	2,6-pyrazinediamine	7.757	21.11	$C_6H_8O_3$	128
3.	ethyl-1-thio-.beta.-d-glucopyranoside	39.523	22.58	$C_8H_{16}O_5S$	224
4.	alpha.-d-mannofuranoside, methyl	6.527	23.17	$C_7H_{14}O_6$	194
5.	neopentane-1,1-diol diacetate	9.374	23.71	$C_9H_{16}O_4$	188
6.	pentanoic acid, 2-(aminooxy)-	5.450	24.17	$C_5H_{11}O_3N$	133
7.	1,6;3,4-dianhydro-2-deoxy-.beta.-d-lyxo-hexopyranose	6.623	24.78	$C_6H_8O_3$	128
8.	sulfurous acid, 2-propyl tridecyl ester	4.750	25.30	$C_{16}H_{34}O_3S$	306
9.	1,6;3,4-dianhydro-2-deoxy-.beta.-d-ribo-hexopyranose	3.450	25.83	$C_6H_8O_3$	128
10.	1,3-bis-t-butylperoxy-phthalan	2.340	26.36	$C_{16}H_{24}O_5$	296
11.	di-n-decylsulfone	2.758	26.87	$C_{20}H_{42}O_2S$	346
12.	1-monolinoleoylglycerol trimethylsilyl ether	4.757	27.33	$C_{27}H_{54}O_4Si_2$	498

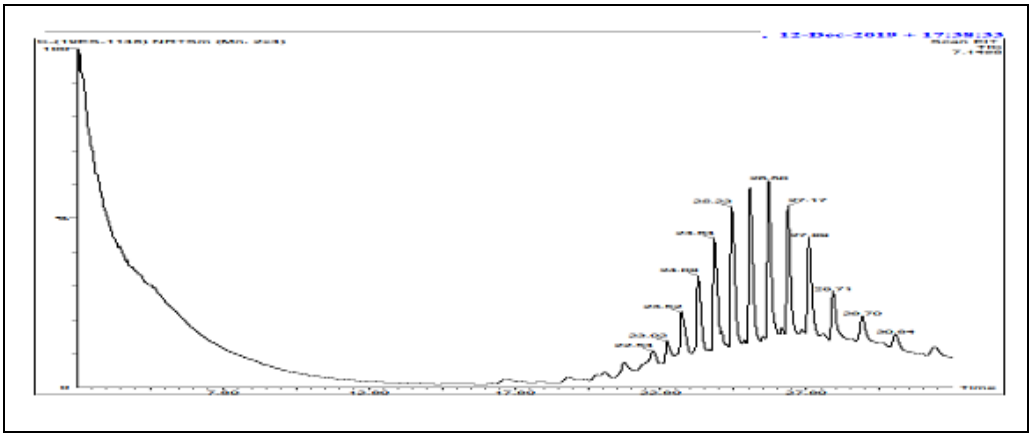

FIG. 2: GS-MS CHROMATOGRAM OF AQUEOUS EXTRACT

TABLE 2: PHYTOCHEMICAL COMPOUNDS IDENTIFIED IN AQUEOUS EXTRACT

S. no.	Compound Name	% of Peak Area	Retention time (RT)	Molecular formula (MF)	Molecular weight (MW)
1.	methylene chloride	0.703	3.21	CH_2C_{l2}	84
2.	methylene chloride	0.744	3.26	CH_2C_{l2}	84
3.	cis-2-methyl-7-octadecene	1.307	21.54	$C_{19}H_{38}$	266
4.	sulfurous acid, 2-propyl tetradecyl ester	1.647	23.01	$C_{17}H_{36}O_3S$	320
5.	nonadecane	4.498	23.50	$C_{19}H_{40}$	268
6.	hexatriacontane	7.020	24.06	$C_{36}H_{74}$	506
7.	hexatriacontane	11.193	24.63	$C_{36}H_{74}$	506
8.	cis-9,10-epoxyoctadecan-1-ol	0.818	24.89	$C_{18}H_{36}O_2$	284
9.	tetratetracontane	13.593	25.21	$C_{44}H_{90}$	618
10.	tetratetracontane	14.972	25.84	$C_{44}H_{90}$	618
11.	tritetracontane	13.664	26.49	$C_{43}H_{88}$	604
12.	tetratetracontane	10.787	27.16	$C_{44}H_{90}$	618
13.	heptacosane, 1-chloro	5.109	28.71	$C_{27}H_{55}Cl$	414
14.	nonadecane, 1-chloro	3.340	29.71	$C_{19}H_{39}Cl$	302
15.	sulfurous acid, pentadecyl 2-propyl ester	1.885	30.83	$C_{18}H_{38}O_3S$	334

GC-MS analysis revealed that the presence ofbioactive compounds in both the fraction of the plant were 1, 6; 2, 3-dianhydro-4-deoxy-.beta.-d-ribo - hexopyranose (6.690), 2, 6-pyrazinediamine (7.757), ethyl-1-thio-.beta. -d-glucopyranoside (39.52%), alpha.-d-mannofuranoside (6.527), methyl, neopentane-1, 1-diol diacetate (9.374), pentanoic acid (5.450), 2-(aminooxy)-, 1,6;3,4-dianhydro-2-deoxy - beta. - d - lyxo – hexopyranose (6.623), sulfurous acid, 2-propyl tridecyl ester (4.750), 1, 6; 3, 4-dianhydro-2-deoxy-.beta.-d-ribo- hexopyranose (3.450), 1, 3 – bis - t-butylperoxy - phthalan

(2.340), Di – n -decylsulfone 1, 3- (2.758), 1-monolinole-oylglyceroltrimethylsilyl ether (4.757). Methylene chloride (0.74%), cis-2-methyl-7-octa-decene (1.307), sulfurous acid (1.647), 2-propyl tetradecyl ester (1.647), nonadecane (4.498), hexatri-acontane (11.193), cis-9,10-epoxyoctadecan-1-ol (0.818), tetratetracontane (14.972), tritetracontane (13.664), tetratetracontane (10.787), heptacosane, 1-chloro (5.109), nonadecane, 1-chloro (3.340), sulfurous acid, pentadecyl 2-propyl ester (1.885).

The analytical methods used GC/MS is suitable for medicinal herbs organic compounds determination. The sample preparation method is rapid and precise. There is a difference between the compounds extracted from the herb by infusion and tincture, but the important thing is that the organic acid and fatty acids derivatives are present in both of them. The present study focused on the identification of several constituents present in the ethylacetate and aqueous extract of *Cassia auriculata*. This type of GC-MS analysis is the first step towards understanding the nature of active compounds in this medicinal plant and helpful for further detailed study.

FTIR Analysis: FTIR analytical spectrum of *cassia auriculata* presented in **Fig. 3 and 4** which shows the presence of a functional group at various positions in both ethyl acetate and aqueous extract of the plant.

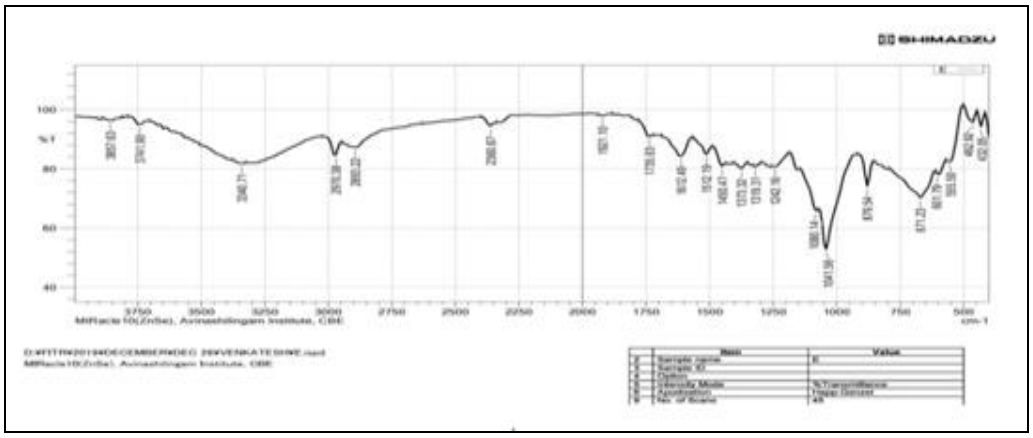

FIG. 3: FTIR SPECTRUM OF ETHYL ACETATE EXTRACT

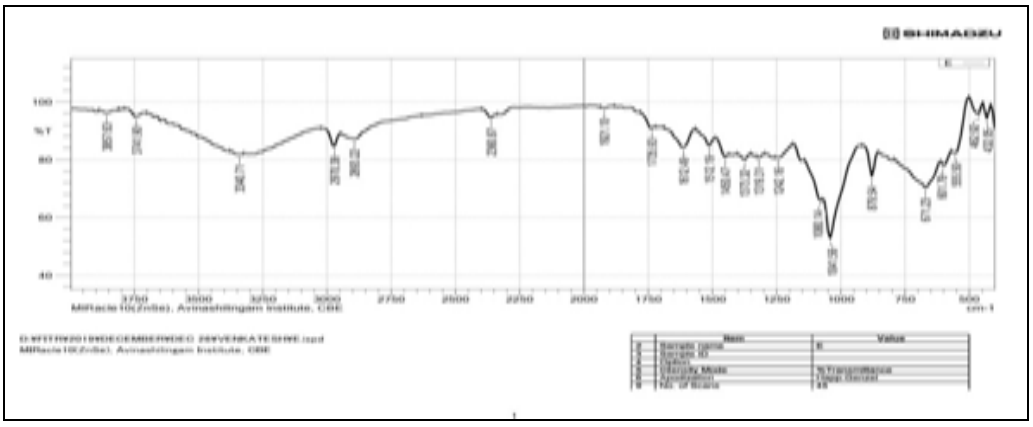

FIG. 4: FTIR SPECTRUM OF AQUEOUS EXTRACT

FT-IR analysis shows important characteristic peaks found in the region of 3857.63 to 3340.71 cm^{-1} is assigned to stretching of O-H and N-H groups.

Like that, the peaks in the region 2970.38 to 2360.87cm^{-1} indicates the stretching of =CH-H, –CH, and -CH_3 groups. The functional groups –C=C and-C=O are presented in the region of 1921.10 to 1512.19 cm^{-1}. The peaks in the region of 1450.47 to 1041.56 cm^{-1} show the presence of –C-N, C-O-C, and C=C.

Nuclear Magnetic Resonance (NMR) Studies:

1H NMR: The 1H NMR spectrum of ethyl acetate and aqueous extract of *Cassia auriculata* is presented in **Fig. 5** and **6**.

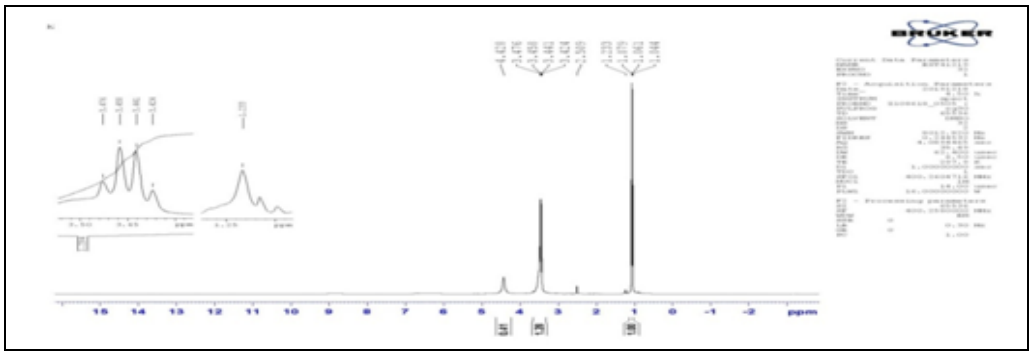

FIG. 5: 1H-NMR OF ETHYL ACETATE EXTRACT

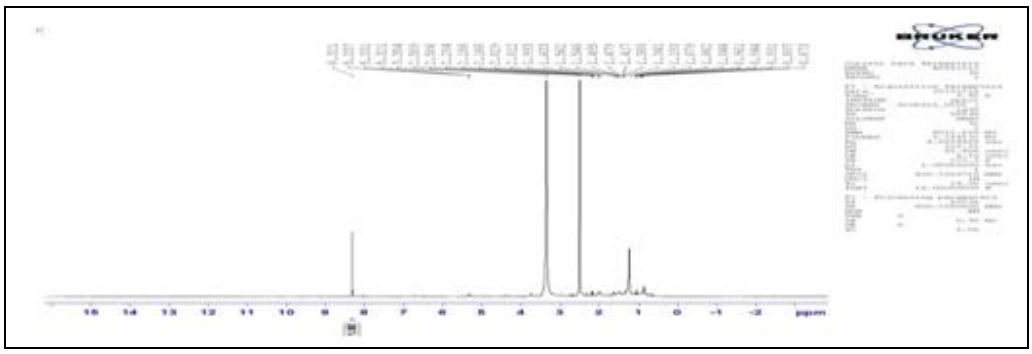

FIG. 6: 1H-NMR OF AQUEOUS EXTRACT

It showed three singlet methyl groups at δ 1.04, 1.06, 1.07, (each 3H, s, CH3 × 3) and 2.50 (3H, s) on an aromatic proton.

A singlet of one methyl proton at δ 3.45 (1H) and another singlet of one proton attached to halogen at δ 4.42 (1H).

It shows δ value at 8.31 (1H) due to aromatic or aldehyde proton, and δ value at 1.54 indicate the presence of tertiary methyl proton.

13C NMR: The 13C NMR spectrum of both the extract of the plant was shown in **Fig. 7** and **8**.

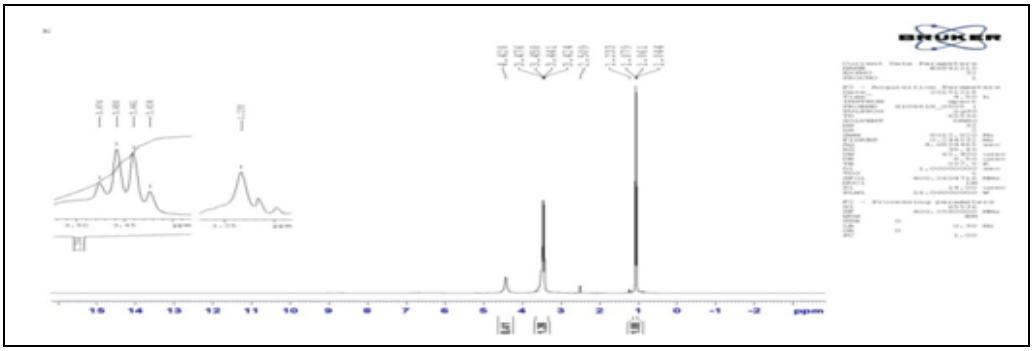

FIG. 7: 13C-NMR OF ETHYL ACETATE EXTRAC

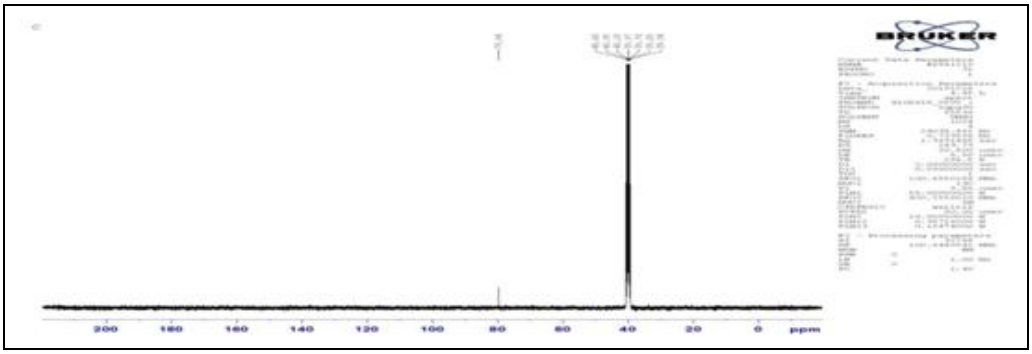

FIG. 8: 13C-NMR OF AQUEOUS EXTRACT

The presence of oxygenated carbon resonates at δ 79.64.The gem-dimethyl carbons groups appear at δ 18.87. The Halogenated carbon appears at δ 40.01.The hydroxyl group attached carbon appears at δ 56.52 and 60.06. Obviously, the remaining signals at δ 70.30, 71.37, 72.85, 73.06, and 74.70 are due to methyne carbons, respectively.

CONCLUSION: This study has revealed the presence of many bioactive phyto-components in the plant*cassia auriculata*, which might be of a very important medicinal value, and further plan of study include isolation and purification of bioactive phyto components. It has been reported that the presence of bioactive substances in plants plays a role in preventing colorectal carcinoma and renal calculi. In this plant contains various bioactive compounds that justifies the use of the whole plant for various ailments by traditional practitioners.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: The authors declare that the study was conducted in the absence of any commercial or financial dealings that could be construed as a potential conflict of interest.

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