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Phytochemical Screening of Plant Extracts and GC-MS Analysis of *n*-Hexane Extract of the Leaves of *Cassia alata* Linn

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ABSTRACT

The purpose of this investigation was to undergo the preliminary phytochemical screening of the plant extracts and identify the presence of pharmacologically bioactive constituents in the leaves of *Cassia alata* by using gas chromatography-mass spectrometry (GC-MS) technique. The preliminary phytochemical screening of different leaf extracts of *Cassia alata* revealed the presence of various phytochemical compounds such as terpenoids, steroids, flavonoids, phenolic compounds, quinones, carbohydrates, tannins and alkaloids. Qualitative and quantitative determination of different biologically active compounds from the crude *n*-hexane extract using gas chromatography-mass spectrometry disclosed 20 compounds with varying amounts where main components were identified as palmitic acid (26.65%), stearic acid (14.27%), (E)-9-octadecadienoic acid (11.40%), erucylamide (8.34%), 1,19-eicosadiene (5.15%), stigmasterol (4.68%), linoleic acid (4.06%), vitamin E (3.97%), methyl palmitate (3.93%) and methyl 11-octadecenoate (3.32%). The compounds identified through this investigation may be responsible for any of the pharmacological properties of *Cassia alata* and could be of considerable interests for the development of new drug leads.

Keywords: *C. alata*, Plant extracts, Phytochemical screening, GC-MS analysis, Bioactive constituents.

INTRODUCTION

Nature acts as an endless source of the medicinal entities, pharmacophores, novel chemophytes which contribute in the field of drug development for the betterment of the human illness since the ancient time. Medicinal plants have been used for thousands of years to cure various human diseases as the plants contain many constituents which have high therapeutic values [1]. In the field of plant based natural product, the genus *Cassia* covers a valuable space in the family of Fabaceae and the subfamily of Caesalpiniaceae because of being enriched with high therapeutic and biological activities [2]. The genus *Cassia* comprises about 580 species of herbs, shrubs and trees distributed throughout the world and among them 20 species are originated from India [3].

Cassia alata is a large shrubby ornamental plant with availably grown wild all over the world and is one of the most important members of the genus *Cassia* belonging to the family Fabaceae. In Bangladesh, it is wildly grown throughout the country mostly in Chittagong and Chittagong Hill tracts [4]. Almost every part of *Cassia alata* is claimed to have significant medicinal uses. Leaves are used as purgative and anti-parasitic. Traditionally, tea is made from the leaves and used to treat constipation and intestinal worms. Paste of the leaves is good for curing ringworm. The leaves are pounded and rubbed on the skin on affected area to fight against eczema, ringworm and white spot fungal skin infections [5, 6]. Seeds are used as vermifuge and stem bark is efficacious against eczema [7]. Decoction of leaves and flowers is efficacious as a cure for herpes in venereal diseases as an expectorant in bronchitis, astringent, wash for eczema, poisonous insect bites and a mouth wash in stomatitis [4].

Cassia alata has drawn the attention of the researchers throughout the world because of having high therapeutic values. Literature survey showed that many significant phytochemical inestigations have been done regarding this interesting plant. Fatty acids, steroids, flavonoids, anthraquinones and their glycosides have been isolated from the different parts of the plant *C. alata*[8-12]. The isolation of stigmasterol and betulinic acid from the stems and roots of this plant was reported by our research group in recent time [13]. Our present research is focused on the phytochemical screening of different extracts and identification of the compounds from the non-polar *n*-hexane extract of the leaves of *Cassia alata*.

MATERIALS AND METHODS

Collection of the plant

The fresh sample of the leaves of *Cassia alata* Linn was collected from different parts of Jahangirnagar University, Savar, Dhaka. The plant was then identified and authenticated by taxonomist of Bangladesh National Herbarium (BNH). Voucher specimen of this plant was deposited at BNH under the accession number DACB-38723 for *Cassia alata*.

Preparation of Plant Extracts

At first, the fresh plant sample (*C. alata* leaves) was cut severally into small pieces. It was ensured that the samples were cleaned properly. Freshly collected leaves at first were air dried at room temperature for several days under the shade and then powdered in the grinder to a coarse powder. Then the powdered sample was soaked with *n*-hexane, chloroform, ethyl acetate and methanol successively at room temperature and thus the corresponding plant extracts were prepared. All the extracts were subjected to phytochemical screening and the *n*-hexane extract was selected to undergo the GC-MS analysis.

Methodology of Phytochemical Screening

Standard phytochemical screening methods were used to indicate the presence of various plant metabolites in different extracts of the leaves of *Cassia alata* by using the following procedures [14, 15]. The result of this experiment is presented in Table-1.

Test for Steroids & Terpenoids

Liebermann-Burchard's test

Few drops of acetic anhydride were combined with 1 ml of concentrated sulphuric acid. About 10 mg of each of the extract was dissolved in chloroform and then treated with the combined solution which was added by the side of the test tube. A greenish color was produced which turned blue on standing indicating a positive reaction of steroids. On the other hand, the presence of pink appearance in the chloroform layer indicated the presence of terpenoids.

Test for Flavonoids

Shindo's test

10 mg of the extract was dissolved in methanol. Magnesium turnings were added into this followed by concentrated HCl. A pink color showed the presence of flavonoids.

Test for Carbohydrates

Molisch's test

2 ml of an aqueous filtrate of each crude extract (previously boiled with distilled water in a water bath) was taken in a test tube. 2 drops of freshly prepared 10% alcoholic solution of α-naphthol was added and mixed thoroughly. The mixture was shaken well and 1 ml of conc. H_2SO_4 was allowed to flow down the side of the test tube and allowed to stand. A red or reddish violet ring was formed in the two layers indicated a positive test. It was shaken and allowed to stand for two minutes and diluted with 5 ml of distilled water. A dull violet precipitate was formed immediately which indicated the presence of carbohydrates.

Test for Phenolic Compounds

10 mg of each extract was dissolved into chloroform. Then 1-2 drops of 2.5% ferric chloride was added into it. The reddish brown color confirmed the presence of phenolic compounds.

Test for Quinones

10 mg of each of the extract was dissolved into methanol and then treated with sulphuric acid. The formation of color indicated the presence of Quinone.

Test for Alkaloids

Mayer's Test

1.358 g of mercuric chloride and 5 g of potassium iodide were mixed in a 100 ml volumetric flask and made up to 100 ml with distilled water. Then 10 mg of the extract was dissolved in concentrated HCl separately. A few drops of this solution were taken in a test tube and a few drops of Mayer's reagent were added along the side of the test tube with the help of a glass rod. A white or creamy white precipitate or a white to yellowish precipitate at the junction of two liquids indicated the presence of alkaloids.

Test for Tannins

Lead acetate test

About 5 ml of an aqueous solution of each extract (previously boiled on water bath) was taken in a test tube and a few drops of 1% lead acetate solution were added. A yellow or red precipitate indicated the presence of tannins.

Instrumentation and methodology of GC-MS analysis

GC-MS is a unique analysis technique used for identification and quantification which is limited to analytes that are not only volatile and thermally labile but can also withstand the harsh partitioning conditions of the gas chromatograph [16]. A representative spectral output of all the ascertainable compounds from the empirical sample is displayed by this technique. The Gas-chromatography device has an injection port from where the process is initiated by injecting the sample to that port. After this, evaporation and separation of the components take place one by one and finally this equipment identifies the components present in the corresponding sample. A specific spectral pick is produced for each component which is recorded on a paper chart electronically.

In our present study, the *n*-Hexane extract of the leaves of *Cassia alata* was analyzed by Electron Impact Ionization (EI) method on a GC-17A gas chromatograph which was coupled to a MS 2010 plus mass spectrometer. The temperature of fused silica capillary column was kept 40°C with carrier gas helium at a constant pressure of 90 kPa. Sample was injected by splitting with the split ratio 10. The sample was dissolved in Chloroform. The operating conditions were as follows: name of column - RTS-5MS, diameter 30 cm, length 0.25 nm, temperature of the column-initial temperature 40°C (for 2 min), injector temperature- 220°C, holding time 5 min, column packing was done with 10% diethylene glycol succinate on 100-200 mesh diatomic CAW, splitting samples were injected by splitting with split ratio 10, carrier gas - helium gas at constant pressure 90 kPa, sample dissolved in chloroform and range of linear temperature increase 10°C per min[17].

The GC-MS analysis allowed identification and quantification of n-hexane soluble compounds from the leaves of the plant *Cassia alata*. Interpretation of mass spectrum in GC-MS instrument was conducted using data base of National Institute Standard and Technology (NIST) having more than 62000 patterns[16]. The spectrum of the unknown compound was compared with that of the known component stored in NIST library. The retention time, molecular weight and composition percentage of the sample materials of *Cassia alata* were recorded and presented in Figure-1 and Table-2.

RESULT AND DISCUSSIONS

Analysis of Phytochemical Constituents by Phytochemical Screening

The medicinal value of the medicinal plants lies in some chemical substances that have a definite physiological action on human body. The phytochemical screening of the *n*-hexane, chloroform, ethyl acetate and methanol extracts of the leaves of *Cassia alata* revealed the presence of terpenoids, steroids, flavonoids, carbohydrates, phenolic compounds, quinines, alkaloids & tannins. Results showed that the polar compounds like alkaloids, tannins and carbohydrates are present in comparatively polar extracts but terpenoids, steroids and phenolic compounds are present in all the extracts may be due to the availability of various substituents in their structures. This analysis was performed in the lab by using chemical methods.

From the literature studies, it can be said that the presence of carbohydrates can possibly increase the therapeutic potency of many important component [18]. Carbohydrates are claimed to be of special significance because they play pivotal role rising up the energy required for defenses and act as signals for monitoring the defense genes [19]. Alkaloids are deliberated as one of the chemo preventive phytochemicals that is relevant to the treatment of cancer delegating to the use of agents to suppress and hover tumorigenesis [20].

It is demonstrated that flavonoids can be named as nature's biological response modifiers and it can show anti-microbial, anti-allergic, anti-inflammatory & anti-cancer activities and tannins tend to possess cytotoxic & anti-tumor activities[21]. Our present experiment showed the presence of both of these phyto-constituents in the experimental plant sample.

It was also observed in our current study that *Cassia alata* is enriched with steroids and anthrquinones. Plant steroids are reported to possess significant pharmaceutical & agrochemical properties mainly antibacterial, immunosuppressive, hepatoprotective, anti-tumor, sex hormone, cytotoxic, plant growth hormone regulator, cardiotonic & antihelminthic activites[22]. Quinone govern the electron transfer reactions which outcomes as protection against reactive oxygen species (ROS) [23]. Quinones act as the excessive product of ROS causing oxidative damage which is a common scenario of anti-cancerous effects [24]. The plant sample may also show different pharmaceutical properties including cytotoxicity due to the presence of terpenoids[25].

Table 1: The results of phytochemical screening of the leaf extracts of *Cassia alata*

Tests for	*n*-Hexane Extract	Chloroform Extract	Ethyl Acetate Extract	Methanol Extract
Steroids	+ve	+ve	+ve	+ve
Terpenoids	+ve	+ve	+ve	+ve
Flavonoids	-ve	+ve	+ve	+ve
Carbohydrates	-ve	-ve	-ve	+ve
Phenolic comounds	+ve	+ve	+ve	+ve
Quinones	-ve	+ve	+ve	+ve
Alkaloids	-ve	+ve	+ve	+ve
Tannins	-ve	+ve	+ve	+ve

GC-MS analysis of the plant extract

The analytical GC-MS technique was used for the identification and quantification of the constituents present in the plant sample. Our present investigation revealed the presence of a total 20 *n*-hexane soluble compounds from the *n*-hexane extract of the leaves of *Cassia alata*. The identification of the compounds was assured by observing the molecular formula, retention time and peak area of the data. The whole result of the GC-MS analysis is shown in **Figure-1** and **Table-2** including TIC curve.

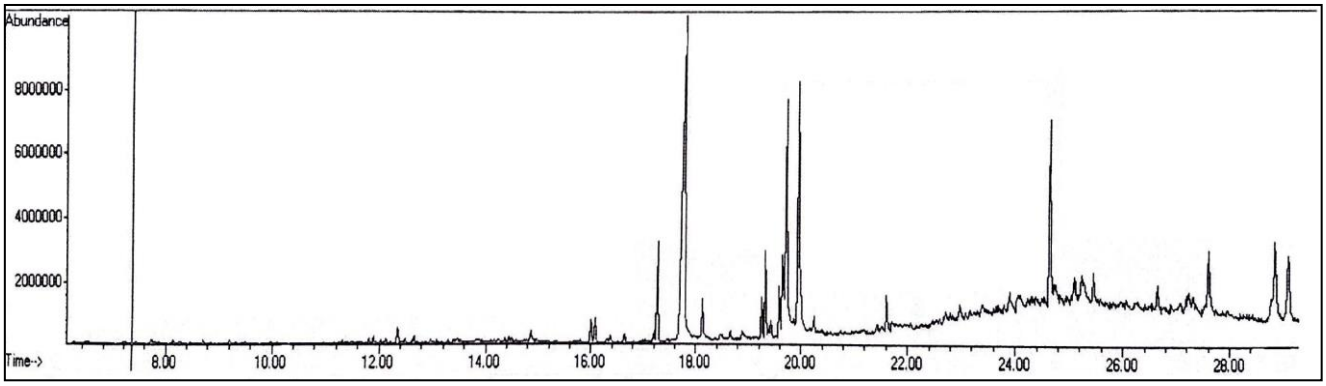


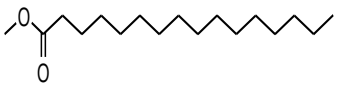
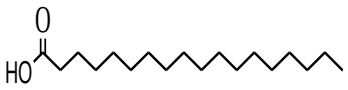
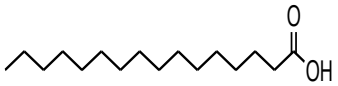
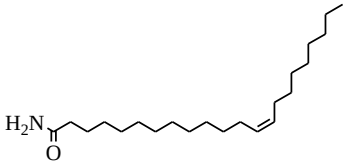
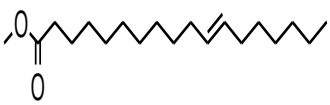
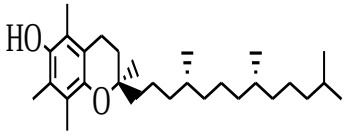
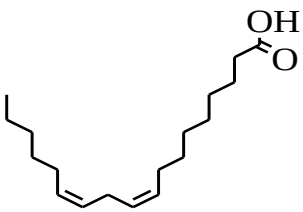
Figure 1: TIC of the n-hexane extract of leaves of the plant *Cassia alata* (GC-MS)

Table 2: GC-MS analysis of the *n*-hexane extract of leaves of the plant *Cassia alata*

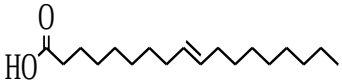
Sample No.	Retention time	Name of the Compound	Molecular weight	Molecular formula	Class of compound	Conc. (%)
PH-1	15.997	Neophytadiene	278.297	$C_{20}H_{38}$	Diene	1.01
PH-2	16.077	6,10,14-trimethyl-2-pentadecanone	268.277	$C_{18}H_{36}O$	Ketone	1.07
PH-3	17.267	Methyl palmitate	270.256	$C_{17}H_{34}O_2$	Fatty acid ester	3.93
PH-4	17.771	Palmitic acid	256.24	$C_{16}H_{32}O_2$	Fatty acid	26.65
PH-5	18.126	Ethyl hexadecanoate	284.272	$C_{18}H_{36}O_2$	Fatty acid ester	1.55
PH-6	19.259	Methyl linoleate	294.256	$C_{19}H_{34}O_2$	Fatty acid ester	1.36
PH-7	19.327	Methyl 11-octadecenoate	296.272	$C_{19}H_{36}O_2$	Fatty acid ester	3.32
PH-8	19.591	Methyl stearate	298.287	$C_{19}H_{38}O_2$	Fatty acid ester	1.70
PH-9	19.654	Linoleic acid	280.24	$C_{18}H_{32}O_2$	Unsaturated fatty acid	4.06
PH-10	19.722	(E)-9-Octadecenoic acid	282.256	$C_{18}H_{34}O_2$	Unsaturated fatty acid	11.40
PH-11	19.957	Stearic acid	284.272	$C_{18}H_{36}O_2$	Fatty acid	14.27
PH-12	21.61	4,8,12,16-Tetramethylheptadecan-4-olide	324.303	$C_{21}H_{40}O_2$	Terpenoid	1.37
PH-13	24.065	*tert*-Butyl 8-Methyl-10-azabicyclo[4.3.1] deca-3,7-diene-10-carboxylate	249.173	$C_{15}H_{23}NO_2$	Azabicyclo ester	1.31
PH-14	24.643	Erucylamide	337.334	$C_{22}H_{43}NO$	Unsaturated amide	8.34
PH-15	25.244	6-fluoro-4,6-cholestadien-3β-ol	402.33	$C_{27}H_{43}OF$	Steroid	1.08
PH-16	25.456	n-Eicosane	282.329	$C_{20}H_{42}$	Alkane	1.20
PH-17	26.64	Hexadecyleneoxide	240.245	$C_{16}H_{32}O$	Epoxide	1.46
PH-18	27.601	Vitamin E	430.381	$C_{29}H_{50}O_2$	Fat soluble vitamin	3.97
PH-19	28.854	1,19-Eicosadiene	278.297	$C_{20}H_{38}$	Diene	5.15
PH-20	29.106	Stigmasterol	412.371	$C_{29}H_{48}O$	Steroid	4.68

It can be observed from **Table-2** that the total of identified component from the crude *n*-hexane extract of the leaves of *Cassia alata was* 98.88% and the composition of nearly 1.12% remained unidentified. Here major components were identified as Palmitic acid (26.65%), Stearic acid (14.27%), (E)-9-octadecadienoic acid (11.40%), Erucylamide (8.34%), 1,19- Eicosadiene (5.15%), Stigmasterol (4.68%), Linoleic acid (4.06%), Vitamin E (3.97%), Methyl palmitate (3.93%), Methyl 11-octadecenoate (3.32%).

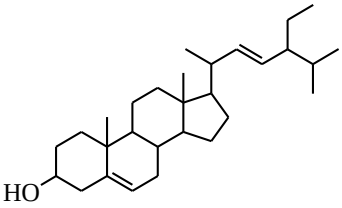
Table 3: Structure of the major components identified from the crude *n*-hexane extract of leaves of the plant *C. alata*

Name & Sample no. of the compounds	Chemical Structure	Name & Sample no. of the compounds	Chemical Structure
Methyl palmitate (PH-3)		Stearic acid (PH-11)	
Palmitic acid (PH-4)		Erucylamide (PH-14)	
Methyl 11-octadecenoate (PH-7)		Vitamin E (PH-18)	
Linoleic acid (PH-9)		1,19-Eicosadiene (PH-19)	

(E)-9-octadecenoic acid (PH-10)



Stigmasterol (PH-20)



CONCLUSION

Medicinal plants are indispensible in combating the human illness and are sources of the precursors for the synthesis of useful drugs that can be used as therapeutic purposes. These plants allocate a significant amount of secondary metabolites that do not function directly in respiratory processes, solute transport or protein synthesis, carbohydrates or lipids photosynthetic process and nutrient assimilation. Our present investigation was focused on the topic that showed the presence of such components in the leaves of *Cassia alata* which may have high therapeutic values and can play a indispensible role in the treatment of human ailment.

Conflict of interest

The authors declare no conflict of interests

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