



GCMS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF THE LEAVES OF CASSIA OBOVATA

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ABSTRACT

The chemical constituents and antibacterial activity of the leaves of *Cassia obovata* were investigated. It was found that the ethanolic extract of the leaves showed antimicrobial activity against both gram (+) and gram (–) bacteria. Maximal antibacterial activity was seen against *Staphylococcus aureus*. The GC-MS analysis of the ethanol extract revealed that the major chemical constituents of the leaves were n-Hexadecanoic acid, Oleic acid, 4H-1-Benzopyran-4-one, 7-hydroxy-2[4-hydroxyphenyl], Isopropyl stearate. *Cassia obovata* is thus a potential medicinal plant for therapeutic use.

KEYWORDS: *Cassia Obovata*, GCMS, antibacterial.

INTRODUCTION

Cassia obovata (Common names: Neutral Henna, *Senna italica* or *senna obovata*) grows in Egypt, Nubia (North eastern Africa), India and South China. The plant grows about two feet high with green leaves and yellow flowers. *Cassia* powder has been used for hair care as well as in Ayurvedic and Unani medicines since the ninth century. *Cassia obovata* contains a faint golden or slight yellow dye. It is commonly referred to as "neutral henna" because it has similar benefits of henna but does not give the hair the orange colour that results with the use of henna. It does, however, add a touch of blonde tint to your blonde and grey/white hair. The colour effects are not noticeable in darker hair. For centuries, cassia powder has been used to soothe and restore the most damaged hair. It works as a natural hair conditioner because of its ability to moisturize the hair and make it shiny, strong and healthy. *Cassia* is anti-fungal and anti-bacterial and highly effective against microbes and fungi.^[1] Therefore, it plays a significant role in fighting against dandruff, eczema and psoriasis. However, the chemical constituents of *Cassia obovata* leaves are not yet well known. Thus, the current work, analyses the chemical constituents and biological activity of *Cassia obovata* leaves.

MATERIALS AND METHODS

GC–MS: The ethanolic extract was subjected to GC-MS analysis on the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCMATE II GC-MS with Data system is a high resolution, double focusing instrument. Maximum

resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI) (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m x 0.25 mm I.D. Analysis conditions were 20 min. at 100°C, 3 min at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300°C. Run time was 22 min. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08 and Wiley08 library. Identification of components was based on comparison of their mass spectra.

Plant material: The leaves of *Cassia obovata* (Fig.1) were collected from Siluviniappatti village, Sivagangai District, Tamil Nadu. About 1.0 kg of shade-dried coarse powders of the plant material were extracted with 80%v/v aqueous ethanol by maceration at room temperature for 72 h. After the completion of each extraction, the extracts were filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50°C). The residues were stored in a vacuum desiccator for further use.



Fig.1: Leaves of *Cassia obovata*.

RESULTS AND DISCUSSION

Chemical composition by GC-MS analysis :

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. In the GC-MS analysis, 10 bio active phytochemical compounds were identified in the ethanol extract of leaves (Fig. 2 and Table 1)

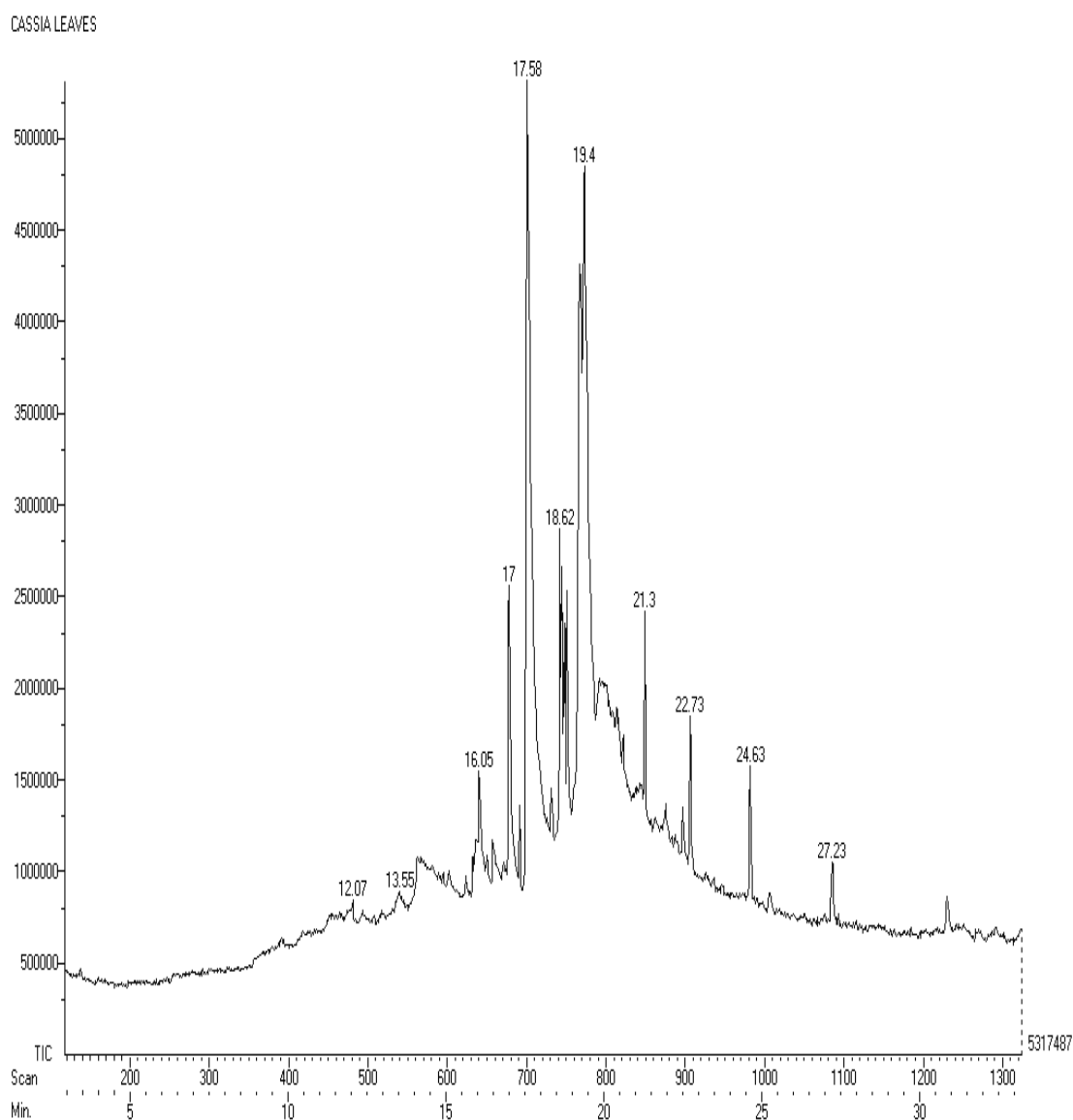


Fig.2: GC-MS chromatogram of leaves extract.

Table 1: Compound identified in the leaves of *Cassia obovata*.

RT	Name of the compound	Structure
27.23	[5(3-hydroxy-3-methyl-but-1-ynyl) furan-2-yl]-4[4-methoxy-phenyl] piperazin-1-yl]-methanone	
24.63	3,4-dihydroxy-1,6-bis-[3-methoxyphenyl]-hexa-2,4-diene-1,6-dione	
22.73	2a,3b,5a,6a-Tetramethoxycarbonylcarbonyl-bicyclo[2,2,2]oct-7-ene	
21.3	Isopropyl stearate	
18.62	Oleic acid	
17.58	n-Hexadecanoic acid	
17	4H-1-Benzopyran-4-one, 7-hydroxy-2[4-hydroxyphenyl]	
16.05	1,5-Naphthalenediol-decahydro	
13.55	3-Hydroxy-4-methoxybenzyl alcohol	
12.07	Phenol,2-propyl-	

There were ten phytocomponents present in the *Cassia obovata* leaves. Among the phytochemicals identified, 4H-1-Benzopyran-4-one, 7-hydroxy-2[4-hydroxyphenyl] is a flavanoid compound, commonly named as

Liquiritigenin, belongs to the chiral flavanone family and is an important ingredient present in liquorice and some Chinese medicinal herbs.^[2,3] Liquiritigenin has been reported as an important bioactive ingredient of the

popular Chinese herb Shao Yao Gan Cao Tang for its analgesic and anti-inflammatory properties.^[4] Previous studies have suggested some pharmacological activity for liquiritigenin including anticancer, anti-inflammatory, antidiabetic, and antioxidant activities.^[5-8] n-Hexadecanoic acid is found to be responsible for the peak at RT 17.58. This fatty acid has various activities like antioxidant, hypocholesterolemic activity.^[9] Oleic acid identified as the third major phyto compound with RT 18.62. The principal use of oleic acid is as a component in many foods, in the form of its triglycerides. It is a component of the normal human diet as a part of animal fats and vegetable oils. Oleic acid as its sodium salt is a major component of soap as an emulsifying agent, and it is used as an emulsifying or solubilising agent in aerosol products.^[10]

Assay of Antibacterial Activity

The leaves of *Cassia obovata* was shade dried at room temperature. The dried material was then homogenized to obtain coarse powder. 10gm powdered plant material was soaked in 20ml of ethanol overnight and then filtered through a Whatman No. 1 filter paper. The filtrate is used for the determination of antimicrobial activity.

Disc Preparation: The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. Then discs were mixed with chemical compounds separately and control discs were prepared.

Antibacterial activity test was carried out following the modification of the method originally described by Bauer

et al., (1966).^[11] Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The ethanol extract soaked discs were placed on the each petri plates and also placed control and standard (Nitrofurantoin) discs. The plates were incubated at 37 °C for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm. Plants are prospective source of antimicrobial agents in different countries.^[12] About 60 to 90% of populations in the developing countries use plant-derived medicine. Traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases.^[13] Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found *in vitro* to have antimicrobial properties.^[14] Global prevalence of infectious diseases caused by bacteria is a major public health problem. The bacterial agents including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* cause several human infections.^[15] The present study investigated the antimicrobial activity of leaves extract tested against human pathogenic bacteria like *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* compared with the standard, the diameters of inhibition zones increased for all the test pathogens (Table 2 & Fig.3). The leaves extract could inhibit different typical pathogenic bacteria. Thus, *Cassia obovata* could be considered as excellent broad-spectrum antibacterial agents.

Table 2: Assay of Antibacterial Activity.

S. No.	Bacteria	Zone of Inhibition (mm in diameter)		
		Control	Standard *	Leaf (20 mg)
1	<i>Escherichia coli</i>	-	20	24
2	<i>Klebsiella pneumoniae</i>	-	20	25
3	<i>Staphylococcus aureus</i>	-	18	26

*Nitrofurantoin (100µg)

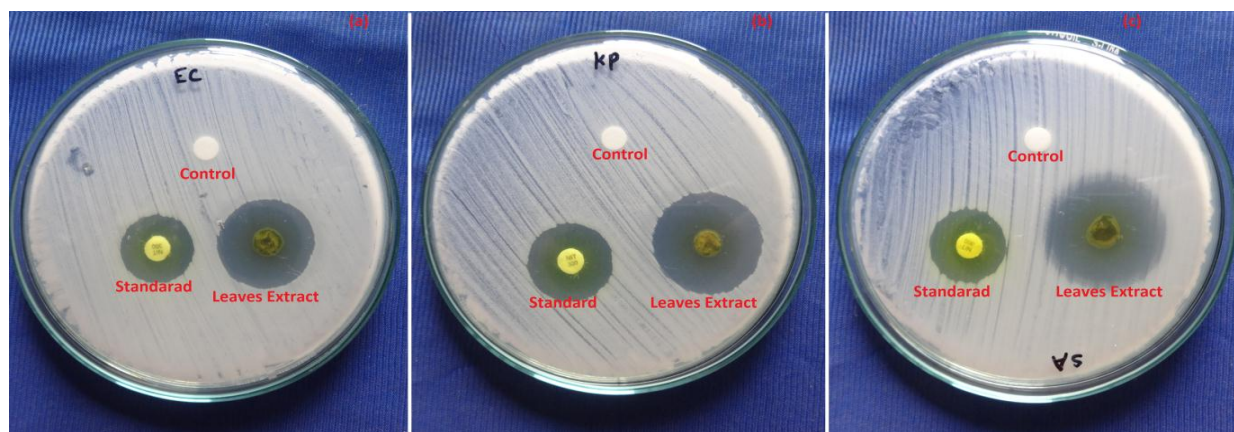


Fig.3: Antibacterial activity of *Cassia obovata* leaves on human pathogenic bacteria (a) *Escherichia coli* (b) *Klebsiella pneumonia* and (c) *Staphylococcus aureus*.

CONCLUSION

It was concluded that *C. obovata* showed potential antibacterial activity against human pathogens. Further phytochemical analysis of this plant will be helpful for elucidation of lead molecules.

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