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IDENTIFICATION OF PHYTOCHEMICALS OF *CASSIA TORA* BY GC-MS AND CORRELATION WITH REPORTED PHARMACOLOGICAL ACTIVITIES

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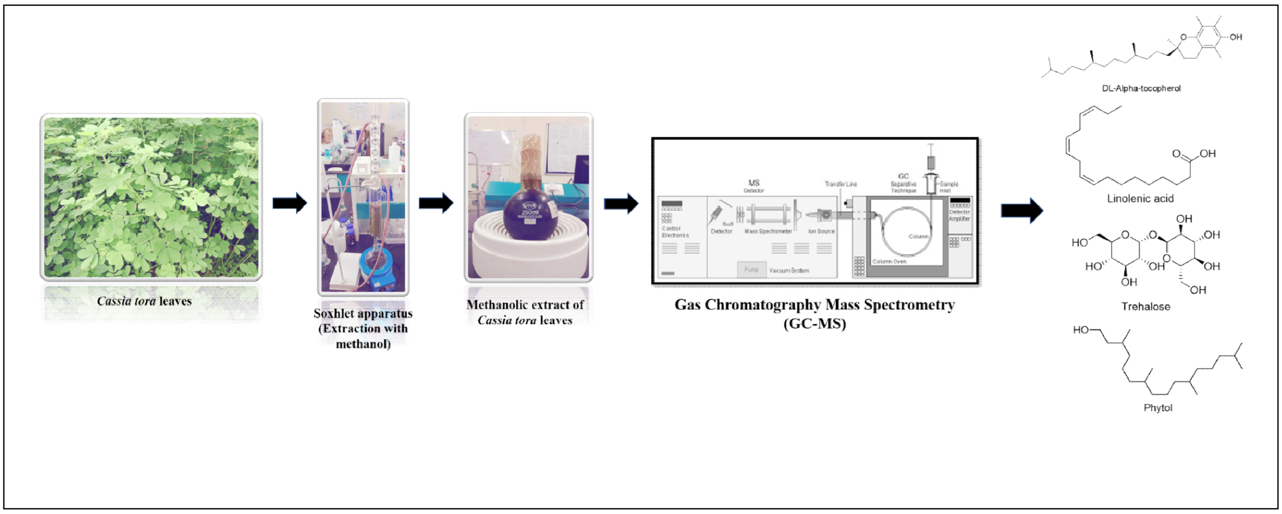
ABSTRACT

Cassia tora Linn. is a small shrub of significant medicinal importance. The fresh leaves and pods of this plant have been used as vegetable curry by tribal people in Amarkantak region, district Anuppur, Madhya Pradesh. The local tribal healers use the plant for mitigation of neurotoxicity and treatment of malaria. The present study was planned to identify the major phytochemicals of this plant and correlate them with reported pharmacological activity. The major components of methanol extract of fresh leaves of *C. tora* were identified by GCMS-NIST library. The details of major phytochemicals were searched and matched with available database on Pubchem. The pharmacological activities of the major components were searched from literature.

20 major compounds from the methanolic extract of *C. tora* leaves have been identified using GCMS-NIST library. The available data showed neuroprotective, anti-malarial, antioxidant, anti-inflammatory, antimicrobial, antibacterial, antiviral, antiasthmatic, anticancer, antituberculosis, antirheumatic and cholesterol-lowering activities of the identified compounds. Laminitol, the most abundant phytoconstituent has been reported to possess antimalarial activity.

The presence of major components with neuroprotective, anti-malarial, antioxidant activity as per literature, justifies the ethnopharmacological use of *C. tora* in this region.



Keywords*: Cassia tora*, Methanol Extract, GCMS, Phytochemicals, Pharmacological activity

INTRODUCTION

Medicinal plants have been associated with human civilization since time memorial and have been widely used for imparting health benefits **[1]**. Plants have been the basis of conventional medicines all over the world. As per World Health Organization, 80% of world's population relies on traditional treatment using plant extracts and their active components, for primary health care needs **[2]**. The Indian System of Medicine, especially Ayurveda is based on the healing potential of medicinal plants and herbo-mineral preparations **[3]**.

Cassia tora Linn., family Leguminosae/Fabaceae is well known medicinal plant of Indian subcontinent **[4]**. This plant has been used as laxative, antiseptic, antioxidant, antimicrobial, antidiarrhoeal, antidiuretic, antimutagenic and antiperiodic. It is also helpful in treatment of fever, bronchitis, cardiac diseases, hepatic disorder, leprosy, liver tonic, bowl problems (piles, hemorrhoids), ophthalmic and skin diseases (like ringworm, itching or body scratch, psoriasis, eczema and dermatomycosis) **[5]**. Amarkantak region of Madhya Pradesh, India is home of Baiga tribes and these tribes largely depends on forest produce for their health care needs. The region is rich in bauxite and a number of bauxite mines for extraction of Aluminium (Al) are located in this region. Since high concentration of Al have been identified as a contributing factor in neurological disorders including Alzheimer's disease **[6]**, we discussed with the local tribal healers about the possible management of Al toxicity. The local tribal healers informed that the fresh leaves and unripe fruits (pods) of *C. tora* plant has been used for mitigation of Al toxicity in this region. They also informed that the fresh leaves of the plant are also used in treatment of fever, especially, malaria. *C. tora* is available as wild in the low hills of Amarkantak region during rainy season. The leaves are dried by the local tribes and used throughout the year as '*Chakoda Bhaji*'. Although there are some reports on phytochemical analysis of *C. tora* from different geographical region in India **[7]**, the phytochemistry of *C. tora* available in Amarkantak region has not been explored. In this paper we are reporting the phytochemical analysis of *C. tora* along with correlation of these phytochemicals in management of different disorders, based on data available in literature.

Figure 1: *Cassia tora* plant

Figure 2: Fruits of *C. tora*

Figure 3: Seeds of *C. tora*

MATERIAL AND METHODS

Collection of plant material

The fresh leaves of *C. tora* were collected in the months of August to October (2018) from the surrounding hills of Indira Gandhi National Tribal University, Amarkantak, District Anuppur, Madhya Pradesh, India. The plant was authenticated by experts of Department of Botany, Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh, and a voucher specimen (IGNTU/2018/PS/01) was retained.

Preparation of plant extracts

The fresh leaves were washed under running tap water to clear the dust or any other surface impurities. The leaves were dried for one week in a well-ventilated place under shade, with intermittent mixing. The dried leaves were grinded to coarse powder using a mechanical blender. The leaf powder (150g) was extracted with methanol (AR grade) in a Soxhlet extractor for 24 hours. The methanol extract was filtered by using Whatman filter paper and concentrated to dryness using rotary evaporator. The concentrated extract was stored at 4^0C for further analysis.

Gas Chromatography Mass Spectrometry analysis and compound identification

Gas Chromatography Mass Spectrometry analysis was performed on Perkin Elmer, Claurs 680 GC coupled with PE SQ-8 C mass analyzer using Elite-5MS, 30 M X 0.25 mm, 0.25 µm capillary column. Carrier gas, helium; temperature programming, initial 40^0C, hold time 5.0 min, ramp rate 12^0C/min to 260^0C and finally held isothermally for 5 min. The injector temperature was 250^0C and carrier flow was at 1 mL/min, Programmable Split-Splitless Injector (PSSI) in split mode (1:50) with injector volume 1 µl using autosampler. The ion source temperature was set at 180^0C, transfer line temperature was 200^0C, and the ionization of the sample components was performed in electron ionization mode at an ionization voltage of 70eV.Mass range was used from m/z 50 to 550 amu. The compounds were identified by comparison of their mass spectra with those of the internal reference mass spectra library (NIST/Wiley) using standard methods **[8]**.

RESULTS AND DISCUSSION

GC-MS with NIST library is one of the widely used techniques for identification of the constituents of volatile matter, long chain and branched chain hydrocarbons, alcohols, acids, esters, etc.

We identified 20 major compounds from the methanolic extract of *C. tora* leaves using GCMS-NIST library **(Table 1)**. The Pubchem search was done for all these major compounds and data regarding their reported pharmacological activities was collected from existing literature. DL-Alpha-Tocopherol, Linolenic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Trehalose, Tocopheryl acetate, Phytol and Stearic acid as antioxidative and neuroprotective agents. Anti-inflammatory, antimicrobial, antibacterial, antiviral, antiasthmatic, anticancer, anti-malarial, antituberculosis, antirheumatic and cholesterol-lowering properties of the identified compounds have also been reported in the literature **(Table 1)**. The present report of identification of bioactive compounds of *C. tora* justifies its use in management of various ailments **[1]**. The presence of neuroprotective and antioxidant molecules justifies the use of this plant for mitigation of Al toxicity in this region. Laminitol has been identified as the major phytoconstituent in this study having 25.049% concentration. Ethanol extract of *Strychnos ligustrina* wood rich in Laminitol has been reported to possess antimalarial activity **[9]**.

CONCLUSION

The phytochemicals of *C. tora* chiefly possess antioxidant and neuroprotective activity. Anti-inflammatory, antimicrobial, antibacterial, antiviral, antiasthmatic, anticancer, anti-malarial, antituberculosis, antirheumatic and cholesterol-lowering properties have also been reported from these compounds. The high concentration of Laminitol may be responsible for its antimalarial activity.

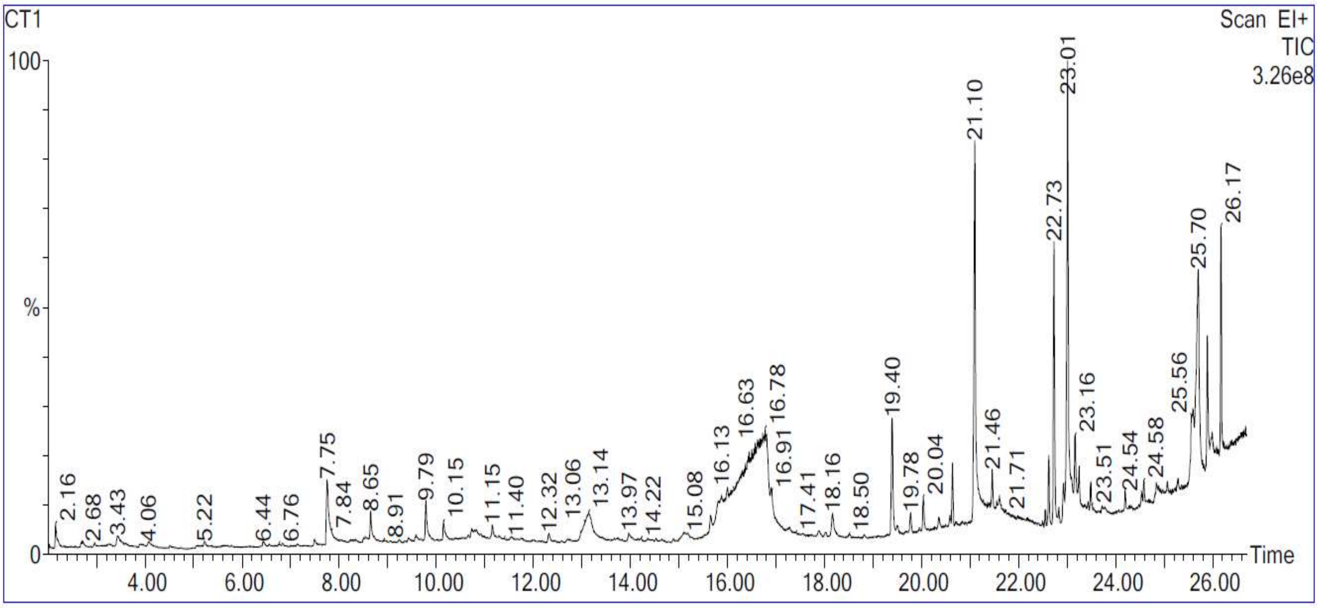
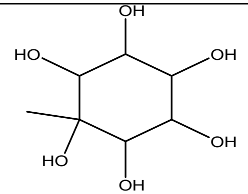
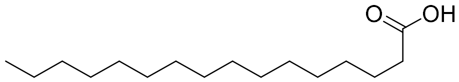
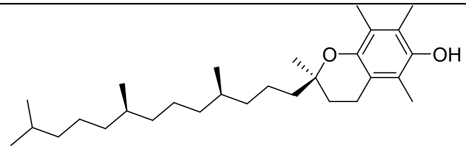
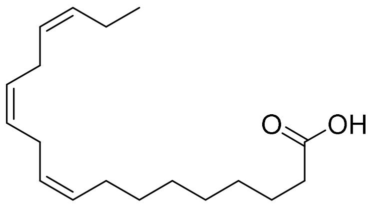
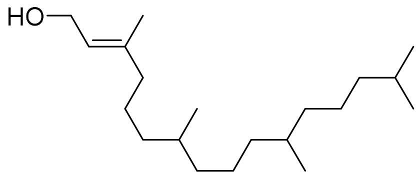
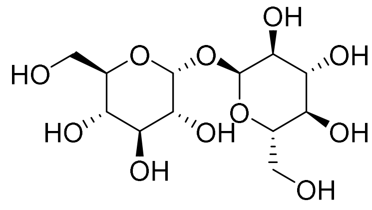
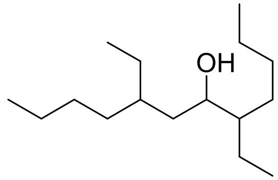
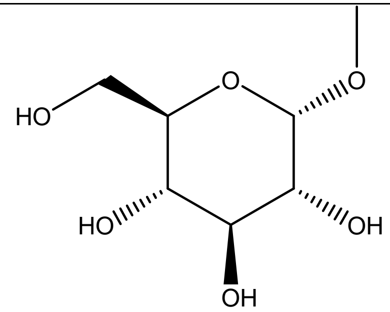
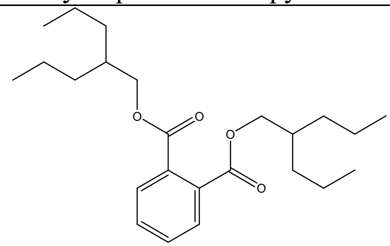
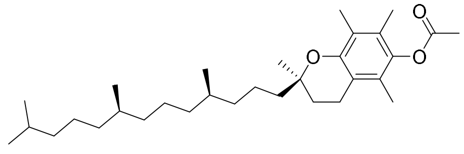
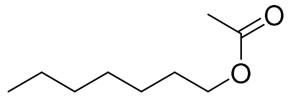
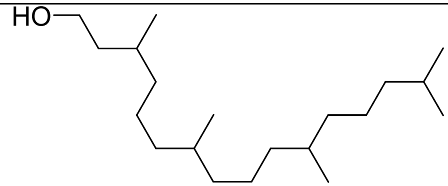
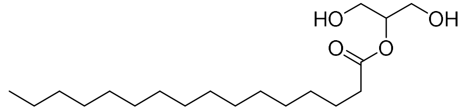
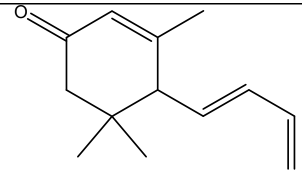
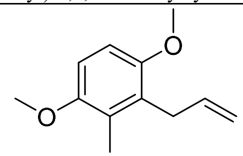


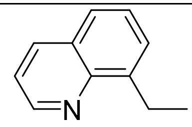
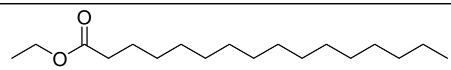
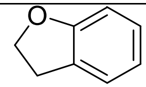
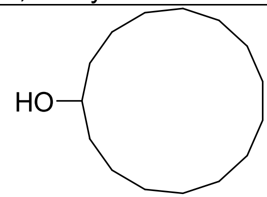
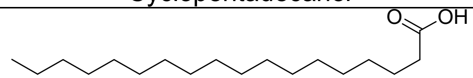
Figure 4: GC-MS spectral chromatogram of methanolic extract of *C. tora* leaves

Table 1: GC-MS analysis of methanolic extract of *C. tora* leaves and biological properties of the phytocompounds

S. No.	Retention Time	Peak area%	Name of the compound	Molecular Formula	Molecular weight	Chemical structure	Reported biological activity
1.	16.779	25.049	Laminitol or 1-Methyl-1,2,3,4,5,6-cyclohexanehexol	$C_7H_{14}O_6$	194.18g/mol	 1-Methyl-1,2,3,4,5,6-cyclohexanehexol	Anti-malarial [9]
2.	21.096	8.247	Palmitic acid or n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43 g/mol	 n-Hexadecanoic acid	Not reported
3.	25.703	6.187	DL-ALPHA-TOCOPHEROL or Alpha-tochopherol	$C_{29}H_{50}O_2$	430.71 g/mol	 DL-Alpha-tocopherol	Antioxidant, Neuroprotective [10]
4.	23.012	6.075	Linolenic acid	$C_{18}H_{30}O_2$	278.43 g/mol	 Linolenic acid	Antioxidant, Neuroprotective [11]
5.	22.727	3.361	Phytol or 3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.50 g/mol	 3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Neuroprotective, Antimicrobial, Antiasthmatic [12]

6.	**15.884**	**3.012**	**Trehalose**	$C_{12}H_{22}O_{11}$	**342.29 g/mol**		**Neuroprotective [13]**
7.	**16.899**	**2.652**	**5,8-Diethyl-6-dodecanol**	$C_{16}H_{34}O$	**242.44 g/mol**		**Not reported**
8.	**13.143**	**2.642**	**Methyl-alpha-D-Glucopyranoside**	$C_7H_{14}O_6$	**194.18 g/mol**		**Pro-apoptotic activity [14]**
9.	**26.168**	**2.231**	**Phthalic acid, di (2-propylpentyl) ester**	$C_{24}H_{38}O_4$	**390.56 g/mol**		**Not reported**

10.	**25.588**	**2.095**	**Tocopheryl acetate or Vitamin E acetate**	**$C_{31}H_{52}O_3$**	**472.75 g/mol**	 Vitamin E acetate	**Neuroprotective [15]**
11.	**7.751**	**1.932**	**Heptyl acetate**	**$C_9H_{18}O_2$**	**158.24 g/mol**	 Heptyl acetate	**Not reported**
12.	**19.395**	**1.913**	**Phytol**	**$C_{20}H_{40}O$**	**296.53 g/mol**	 Phytol	**Neuroprotective, Antimicrobial, Antiasthmatic [12]**
13.	**25.893**	**1.731**	**2-Palmitoylglycerol**	**$C_{19}H_{38}O_4$**	**330.50 g/mol**	 2-Palmitoylglycerol	**Not reported**
14.	**15.999**	**1.685**	**Megastigmatrienone or 4-(1,3-Butadienyl)-3,5,5-trimethylcyclohex-2-en-1-one**	**$C_{13}H_{18}O$**	**190.28 g/mol**	 4-(1,3-Butadienyl)-3,5,5-trimethylcyclohex-2-en-1-one	**Not reported**
15.	**21.601**	**1.163**	**2-Allyl-1,4-dimethoxy-3-methyl-benzene**	**$C_{12}H_{16}O_2$**	**192.25 g/mol**	 2-Allyl-1,4-dimethoxy-3-methyl-benzene	**Not reported**

16.	**15.649**	**1.137**	**8-Ethylquinoline**	**$C_{11}H_{11}N$**	**157.21 g/mol**	 8-Ethylquinoline	**Anti-malarial, Anti-tuberculosis, Anti-rheumatic, Anesthetic, Anti-bacterial [16]**
17.	**21.456**	**1.089**	**Ethyl palmitate**	**$C_{18}H_{36}O_2$**	**284.48 g/mol**	 Ethyl palmitate	**Anti-inflammatory [17]**
18.	**9.786**	**0.852**	**2,3-Dihydrobenzofuran**	**C_8H_8O**	**120.15 g/mol**	 2,3-Dihydrobenzofuran	**Antimicrobial, Antiviral, Antioxidative, Anti-inflammatory [18]**
19.	**25.988**	**0.818**	**Cyclopentadecanol**	**$C_{15}H_{30}O$**	**226.40 g/mol**	 Cyclopentadecanol	**Not reported**
20.	**23.162**	**0.809**	**Stearic acid**	**$C_{18}H_{36}O_2$**	**284.48 g/mol**	 Stearic acid	**Neuroprotective, cholesterol-lowering [19]**

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