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Isolation and Structural Elucidation of Bioactive Compound from Ethanolic Extract of *Cassia tora* Leaves

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Present study describes the isolation of extract from the leaves of plant *Cassia tora* (Linn.). During the present study, fresh leaves after shade drying was used for the isolation of extract using Soxhlet apparatus and percentage yield was obtained (3.22 % in ethanol). Then, preliminary phyto-chemical screening of the extract was done and certain secondary metabolites *viz*. alkaloids, glycosides, saponin, triterpenes, tannin and flavonoid were confirmed in the *Cassia tora* extract. Then thin layer and column chromatography of the extract was done and R_f value *viz*. 0.91, 0.96 and 1.00 % were calculated and obtained fractions (CT1 to CT5) were tested on Wistar albino rats for wound healing activities. Active fraction of extract were sent to SAIF, CDRI Lucknow for spectral analysis *viz*. IR, UV, ^{1}H NMR, ^{13}C NMR and Mass for the detection/confirmation of bio-active compound present in the fraction and finally emodin was elucidated.

Keywords: Bioactive compounds, *Cassia tora,* Secondary metabolites, Traditional medicines.

INTRODUCTION

Plants produce several secondary metabolites for their defense which are basically bioactive compounds *viz*. saponins, alkaloids, glycosides, steroids, flavonoids, tannins, resins, phenols, *etc*. These compounds are available in traditional medicines. The world health organization supports the use of traditional medicine and proved that traditional medicines are efficacious and safe. One survey conducted by the WHO report that more than 80 % of the world's population still depends upon the traditional medicines for various diseases. In the developed countries 25 % of the medicines are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries[1-5]. In developing countries like India a large number of people lives in extreme poverty and some are suffering and dyeing due to deficiency of safe drinking water and medicines. Moreover, they do not have any alternative for primary health care. Therefore, the necessity to use of medicinal plants as alternatives to orthodox (conventional) medicine in the provision of primary health care cannot be over emphasized. So that herbal medicines have received more attention as sources of leading compounds since they are considered as time tested and relatively safe not only for human beings but environment friendly also. Moreover, they are also low-cost, easily available and affordable. Therefore, there is a need to look inwards to search for herbal medicinal plants with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of bio active compounds.

EXPERIMENTAL

In the present study, plant *Cassia tora* was identified and authenticated by Taxonomist Dr. P.G. Diwakar, Joint Director, Botanical Survey of India, Pune and was procured in the Herbarium Record at Se. No. Rajesh1. About 1 kg fresh leaves were collected and brought into the laboratory for shade drying at room temperature and pulverized to powdered at 40-60 mesh size and used for the isolation of crude extract by Soxhletion[6] by applying different solvents in increasing order of polarity. The obtained crude extracts were filtered using Whatman's filter paper No. 1 and extract evaporated under reduced pressure by using rotary vacuum evaporator (RE 100 Model) to get semisolid crude. The percentage yield of crude extracts was also noticed as shown (Table-1). Preliminary phytochemical screening of the plant extract (Table-2) was carried out as per the standard methods and presence of different phyto-constituent was confirmed by applying the various tests. The presence of different constituent and their separation in extract of the plant was confirmed by thin layer chromatography[7] and column chromatography[8] and R_f value with active fractions were obtained (Tables 3 and 4).

Bioactive fractions were sent to SAIF, CDRI, Lucknow (U.P.) for spectral analysis (IR, UV, ^{1}H NMR, ^{13}C NMR and Mass) and obtained graphs were used for the interpretation of the bio-active compound (Figs. 1-5).

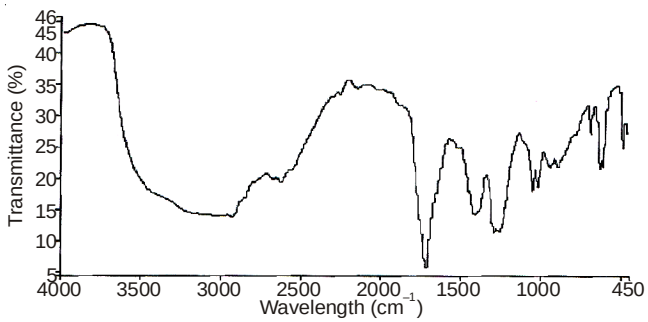


Fig. 1. IR spectral analysis of *Cassia tora* leaves purified fraction (CT-1)

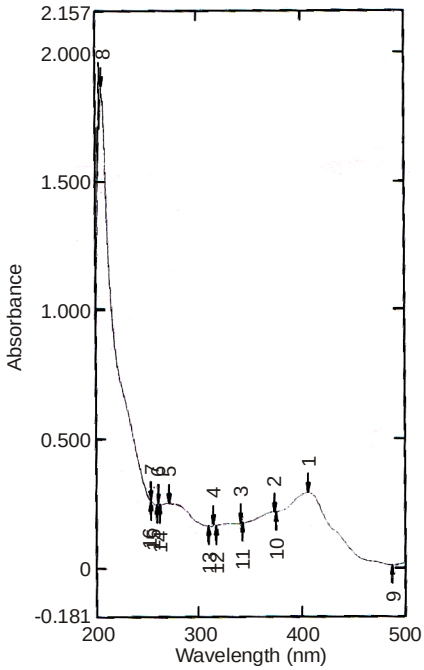


Fig. 2. UV spectral analysis of *Cassia tora* leaves purified fraction (CT-2)

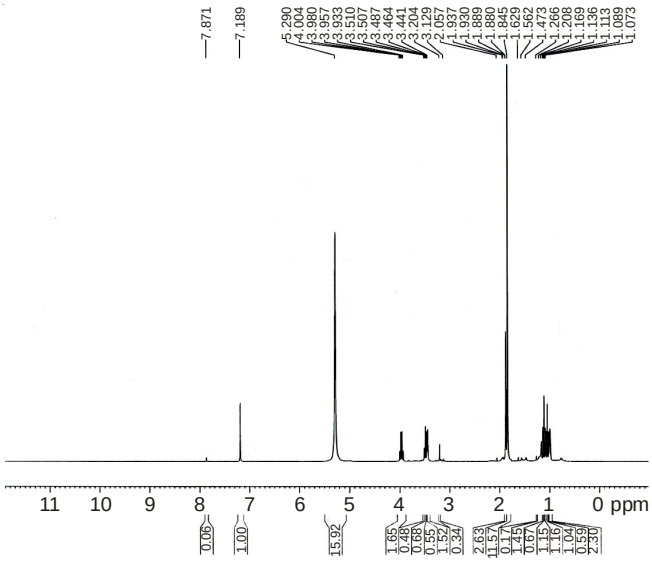


Fig. 3. ^{1}H NMR spectral analysis of *Cassia tora* leaves purified fraction (CT-3)

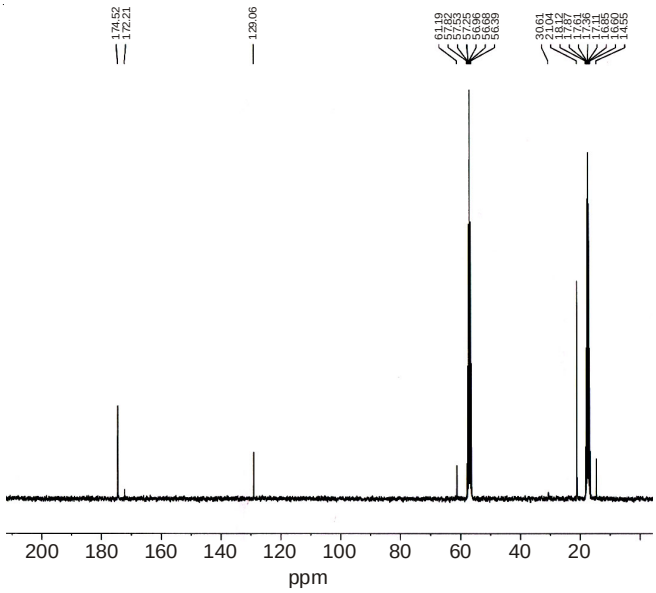


Fig. 4. ^{13}C NMR spectral analysis of *Cassia tora* leaves purified fraction (CT-4)

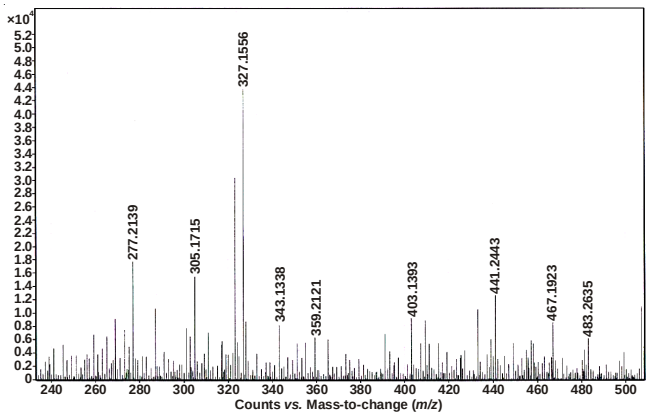


Fig. 5. Mass spectral analysis of *Cassia tora* leaves purified fraction (CT-5)

RESULTS AND DISCUSSION

In the present study, folklore information was gathered from the tribal peoples for wound healing properties of the *Cassia tora* plant and leaves of this plant were shade dried and extracted in ethanol by using Soxhlet apparatus and percentage yield of extract was obtained 3.22 % in ethanol (Table-1). Preliminary phytochemical screening of *Cassia tora* extract showed the presence of alkaloids, glycosides, saponin, triterpenes and phytosterol, tannin, flavonoid (Table-2). Sharma *et al.*[9] have reported preliminary phytochemical screening of alcoholic extract of *Cassia tora* which revealed the presence of anthraquinone glycosides, phenolic compounds, saponin

TABLE-1
ISOLATION OF EXTRACTS FROM POWDERED MATERIAL BY SOXHLETION

Weight of powdered materials (g)*	Solvent used in extraction	Extract obtained (g)	Yield of crude extract (%)
Cassia tora leaves 150 g	Ethanol 900 mL	4.84	3.22

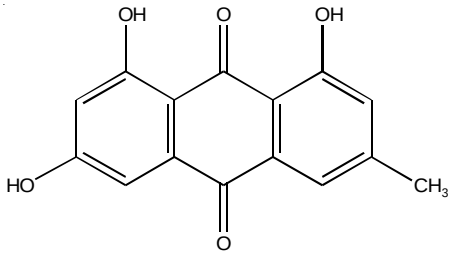
*Powdered material defatted with in *n*-hexane

TABLE-2
PRELIMINARY PHYTO-CHEMICAL SCREENING OF *Cassia tora* EXTRACTS

Constituents of plants	Ethanolic extract
Alkaloids	+
Glycosides	+
Saponins	+
Triterpenes and phytosterol	+
Tannin	+
Flavonoids	+

glycoside and while aqueous extract of *Cassia tora* showed presence of glycosides, phenolic compounds and saponin glycoside. In high performance thin layer chromatography of each plant extract, solvent system benzene (80): methanol (16): acetic acid (04) was used and three spots were obtained with the R_f values *viz*. 0.91, 0.96 and 1.00 % in TLC of *Cassia tora* extract (Table-3). John *et al*.[10] have also performed HPTLC on 10 cm × 10 cm HPTLC plates coated with 0.25 mm layer of silica gel 60 F254 (Merck, Germany). The column chromatography of the *Cassia tora* extract was also done by using benzene (80): methanol (16): acetic acid (04) solvent systems and 5 fractions of plant extract were obtained (Table-4). In the same manner, John *et al*.[10] have described column chromatographic analysis of ethyl acetate fraction of *Cassia tora* leaves extract which showed about 12 components and most of the components were coloured which shown fluorescence at 366 nm and quenching of fluorescence was observed at 254 nm. HPTLC studies confirmed the presence of flavonoid, quercetin and anthraquinone glycosides in the ethyl acetate fraction of the *Cassia tora* leaves extract. Finally, purified fraction of *Cassia tora* extract done by thin layer and column chromatography were used for wound healing activities.

Cassia tora leaves extract showed characteristic bands in the IR spectrum of the emodin (CT-1) fraction and their structural assignments elucidated with the help of available literatures[11-15]. Fraction (CT-2) has displayed absorption band in the UV spectrum at 270.60 nm in heavy ethanol (ETOD) which confirmed the presence of compound emodin. Some important peaks observed in the ^{1}HNMR spectrum of emodin (CT-3) fraction of *Cassia tora* and the structural assignments inferred with the help of available literature[16-18]. This ^{1}H NMR spectrum was found to be in complete conformity of the structure assigned to emodin (CT-3) which is supported by the available literature[19]. ^{13}C NMR spectra of *Cassia tora* fraction (CT-4) was found to be proton decoupled revealed a single line (peaks or signals) for each chemically non-equivalent carbon atom in the emodin. The data of the compound was found in full agreement as reported by Cong[20] and Silverstein[21]. The significant fragmentation pattern observed in the FAB-MS of *Cassia tora* (CT-5) elucidated a compound Emodin with molecular formula of $C_{15}H_{10}O_5$ and molecular weight of 265.30 g/mol on the basis of the interpretation of spectral data and literature described by Yen *et al*.[22], Jain and Patil[23] that is mentioned below:



Emodin

Conclusion

It can be concluded that the biologically active purified fraction of *Cassia tora* leaves ethanolic extract, on the basis of spectral analysis (IR, UV, HNMR, ^{13}C NMR and mass) confirmed the presence of active principle Emodin for their wound healing activity.

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TABLE-3
THIN LAYER CHROMATOGRAPHY OF EXTRACT OF *Cassia tora* LEAVES

Plant extract	Solvent system used	Spots	R_f value	Colour		
				Visual light	UV-light	Iodine chamber
Cassia tora leaves	Benzene:Methanol:Acetic acid (80:16:04)	1	0.91	Light brown	Brown	Dark brown
		2	0.96	Yellow	Yellow	Dark yellow
		3	1.00	Green	Green	Dark green

TABLE-4
COLUMN CHROMATOGRAPHY OF *Cassia tora* PLANT EXTRACTS

Plant extract	Solvent system used	Fractions obtained	Fractions obtained (g)	Colour
Cassia tora leaves	Benzene:Methanol:Acetic acid (80:16:04)	CT-1	0.55	Brown
		CT-2	0.80	Light brown
		CT-3	0.45	Yellow
		CT-4	0.30	Light green
		CT-5	0.10	Green

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