Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(9); 1444-1457

ISSN: 0975-4873

Research Article

Pharmacognostical Assessment and Anticonvulsant Activity of Whole Plant of *Cassia occidentalis* Linn

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Available Online: 10th September, 2016

ABSTRACT
Objective: To survey the pharmacognostical characteristics and *in vivo* anticonvulsant activity of chloroform, alcoholic and aqueous extracts of whole plant of *Cassia occidentalis* Linn. Methods: *Cassia occidentalis* Linn Negro coffee whole plant were successively extracted using chloroform, alcoholic and aqueous solvents. The extracts were screened for phytochemicals using HPTLC and GCMS techniques. The extracts were also screened for acute toxicity and anticonvulsant activity, against MES and PTZ induced convulsions, using Wistar albino rats. Results: The phytochemical screening study reveals the presence of more chemical constituents in chloroform extract followed by alcoholic and aqueous extract. We found no significant changes in average body weight of animals, up to tested oral dose of 3000 mg/kg, during acute toxicity study. The *in vivo* study reveals the anticonvulsant activity of chloroform and alcoholic extract against MES and PTZ induced convulsions. The chloroform extract is found to be more potent, similar to Phenytoin, in controlling both MES and PTZ induced convulsions than alcoholic and aqueous extracts. Conclusions: The results obtained suggest that the chloroform extract of whole plant of *Cassia occidentalis* Linn has remarkable anticonvulsant activity. Also, our study indicates the potential application of *Cassia occidentalis* Linn whole plant in the treatment of convulsive disorders as a need of modern health science. However, the further studies are needed to screen the active constituent having anticonvulsant effect.

Keywords: Negro coffee, Kasamardah, anticonvulsant activity, TLC, HPTLC, and GCMS

INTRODUCTION

Herbal medicine is also called botanical medicine or phytomedicine. It is referring to using a plant's bark leaves seeds, berries, roots or flowers for medicinal purposes. In conventional medicine, Herbalism has a long tradition of use was reported[1] and herbal medicine is an integral part of traditional medicine (TM) and has a broad range of characteristics elements and which earned it the working definition from the World Health Organization (WHO). Globally, people developed unique indigenous healing traditions adapted and defined by their culture, beliefs and environment, which satisfied the health needs of their communities over centuries was studied[2]. The use of herbal medicinal products and supplements has increased enormously over the past 3 decades with not less than 80% of people worldwide relying on them for various part of primary health care system and this history has obviously witnessed a tremendous flow in recognition in natural drug therapies by public interest both in developing and developed countries[3-5]. The global pharmaceutical market was worth US $550 billion in 2004 and is expected to exceed US $900 billion by the year 2009. According to WHO estimates, the present demand for medicinal plants is US $14 billion a year and by the year 2050. In India, around 25,000 effective plant-based formulations are used in traditional and folk medicine. More than 1.5 million practitioners are using the traditional medicinal system for health care in India and it is estimated that more than 7800 manufacturing units are involved in the production of natural health products and traditional plant-based formulations, in India which requires more than 2000 Tons of a medicinal plant raw material annually was studied[6]. Name Epilepsy is of a brain disorder, characterized by recurrent and unpredictable interruptions of normal brain function, called epileptic seizures[7,8]. The prognosis of anticonvulsants seizures in most patients by currently available drugs of anticonvulsant, suffer the neurotoxicity, teratogenic and other dose related side effects[9].

Botanical name: *Cassia occidentalis* (Linn)
Synonym: Senna occidentalis
Family: Leguminose
Sub Family: Caesalpiniaceae
English: Negro coffee, Stinking weed
Hindi: Kasaumdi, Barikasaumdi
Kannada: Doddagace
Malayalam: Ponnaviram, Ponnariviram
Sanskrit: Kasamardah
Tamil: Ponnavirai, Peraviral, Nattam takarai

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Figure 1: Cassia Occidentalis Linn whole plant

Table 1: The physicochemical characteristics of *Cassia occidentalis* Linn whole plant are shown in table1.

S. No	Parameters	As Per Literature	Observation
	Physical Tests		
	Nature	Coarse powder	Coarse powder
1.	Color	Yellowish color	Yellowish color
	Odour	Odourless	Odourless
	Taste	Tasteless	Tasteless
	Extractive Value		
	Pet. Ether (40-60)	--	2.978
2.	Chloroform	--	2.627
	Alcoholic	--	3.150
	Aqueous	--	4.274
3.	Loss on Drying		2.383
	Ash values		
	Total ash	--	3.76 % w/w
4.	Acid insoluble ash	--	2.6 3%
	Water soluble ash	--	1.26% w/w
	Fluorescence analysis	--	Dark fluorescence

Table 2: The Percentage Yield of *Cassia occidentalis* whole plant are shown in table 2.

S. No	Extract	Nature of Extract	Weight (g)	% Yield w/w
1	Chloroform (300gm)	Semisolid viscous	3.721	1.090
2	Alcohol (300gm)	Semisolid viscous	4.31	2.100
3	Aqueous (300gm)	Semisolid viscous	6.643	3.567

Telugu: Kasinda (Fig 1)

Throughout India, it grows abundantly on wastelands immediately after the rains. A diffuse offensively odorous under shrub with furrowed sub glabrous branches, leaflets 3-5 pairs, flowers yellow, in short peduncled few flowered racemes, fruits cylindrical or compressed, transversely septate glabrous pods containing 20-30 seeds ovoid, smooth and shiny dark olive green or pale brown. The plant is bitter, sweet, and thermogenic, purgative, expectorant. It is useful in cough, bronchitis, constipation, fever, epilepsy and convulsions was shown[10] and scattered from the Himalayas to the Western Bengal, South India, Burma and Ceylon was reported[11]. Growing throughout India up to an altitude of 1,500 m. it is used in expectorant, diuretic, leaves used internally and externally in scabies, ringworm and other skin disease was reported[12]. The pharmacological studies carried out by several workers that indicate *Cassia occidentalis* possesses Toxicological reproductive were testimony[13] and Antidote of poison, blood purifier, expectorant, anti-inflammatory agent, liver disease was reported[14]. In Unani system of medicine melanoblast cell line[15], traditional medicine anti-allergic, anti-inflammatory and anti-oxidant was also studied[16]. The relaxant[17], anti-nociceptive activity[18] and skin diseases like psoriasis; leprosy was premeditated[19]. In CCl_4 induced oxidative stress[20] and antifungal, anti-diabetic[21], Hepato-protective was also studied[22]. The *in vitro* cytotoxicity and antibacterial against eight human cancer cell lines from six different tissues and four bacterial strains[23], the mitotic activity of root tip cells of *Allium cepa* and in vitro Anti-malarial was reported[24]. Ant anxiety and antidepressant[25], Nephroprotective is repoted[26] and also *leaf* extracts of *Cassia occidentalis* were investigated against eleven

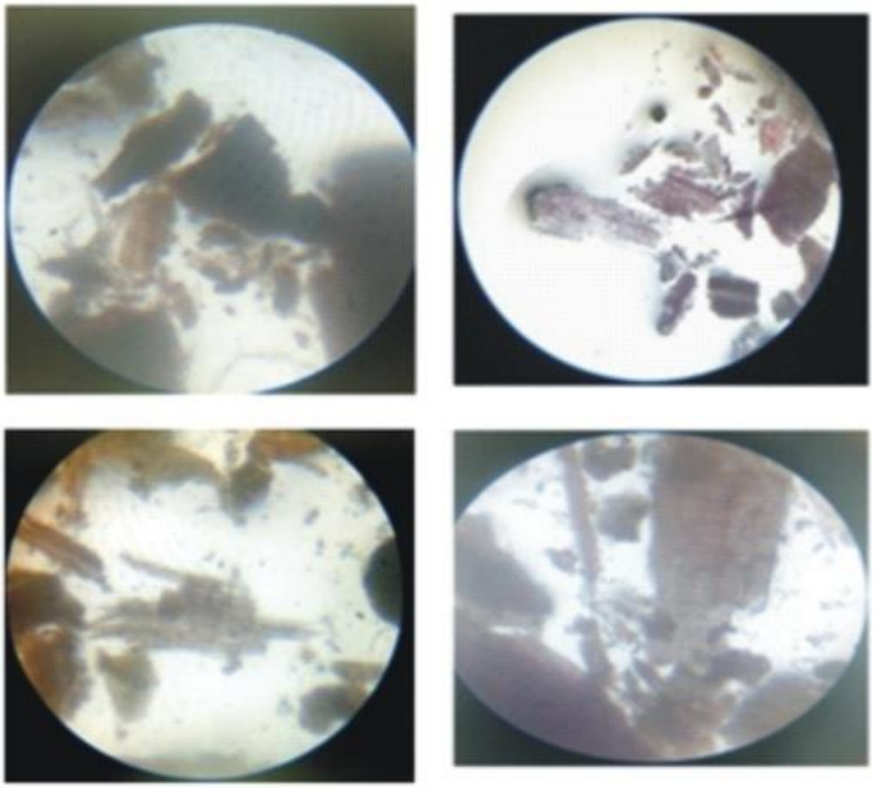


Figure 2: Powder characteristics *Cassia occidentalis* Linn

Table 3: Results of Qualitative Chemical Investigation of whole plant of *Cassia occidentalis* Linn

Phytochemical Name	Solvents Name			
	Pet. Ether	Chloroform	Alcohol	Aqueous
Alkaloids	-	-	+	+
Carbohydrates	+	+	+	+
Glycosides	-	-	+	+
Flavonoids,	-	+	+	+
Triterpinoids	-	+	+	-
Tannins & Phenolic comp	-	+	+	+
Steroids	+	+	+	+
Proteins	-	+	+	+
Amino Acids	-	-	-	+
Lipids	+	-	-	-
Saponine	+	+	+	+

+: present, -: absent

Gram-positive and four Gram-negative bacteria isolates was reported[27].

MATERIALS AND METHODS

Collection and authentification of plant material

In present study, the whole plant of *Cassia occidentalis* Linn was collected from waste place surrounding area of modala vittalapura, Shimoga (District), Karnataka. The whole plant was authenticated from botanist Dr. S. R. Yadav, Head of the Department, Department of botany Shivaji University, Kolhapur, Maharashtra, India. (University TKCP/SU/BOT/ 164/2011). After authentification, the whole plant was dried at room temperature, until they were free from the moisture and subjected to physical evaluation with different parameters.

Physicochemical constant study

Physicochemical parameters of powder drug such as total ash, water-soluble ash, acid insoluble ash value and the moisture content, by loss on drying method, were determined[28] Pet ether, chloroform, alcohol and water soluble extractive values were determined to find out amount of Pet ether, chloroform, alcohol and water soluble components[29]. Fluorescence analysis of the Drug was also performed[30].

Macroscopic Examination

Macroscopic study of *Cassia occidentalis* like colour, shape size taste and odour of the powder were determined. The powder was cleared with chloral hydrate and stained with concentrated hydrochloric acid, phloroglucinol to identification of lignified elements, the presence of ruptured xylem vessel, pericyclic fibers, secretory glands, calcium oxalate crystals and medullary ray cells[31].

Preparation of Extracts

The shade-dried whole plant of *Cassia occidentalis* Linn were reduced to fine powder (# 40 size mesh) and around 300 gms of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether, chloroform and ethanol. Finally, the drug was macerated with chloroform-water. Each time before extracting with the next solvent the powdered material was air dried in hot air oven below 50^0 C. After the effective extraction, the solvents were redistilled, the extract was then concentrated on water bath and the extract obtained with each solvent was weighed. Its percentage was calculated in terms of air-

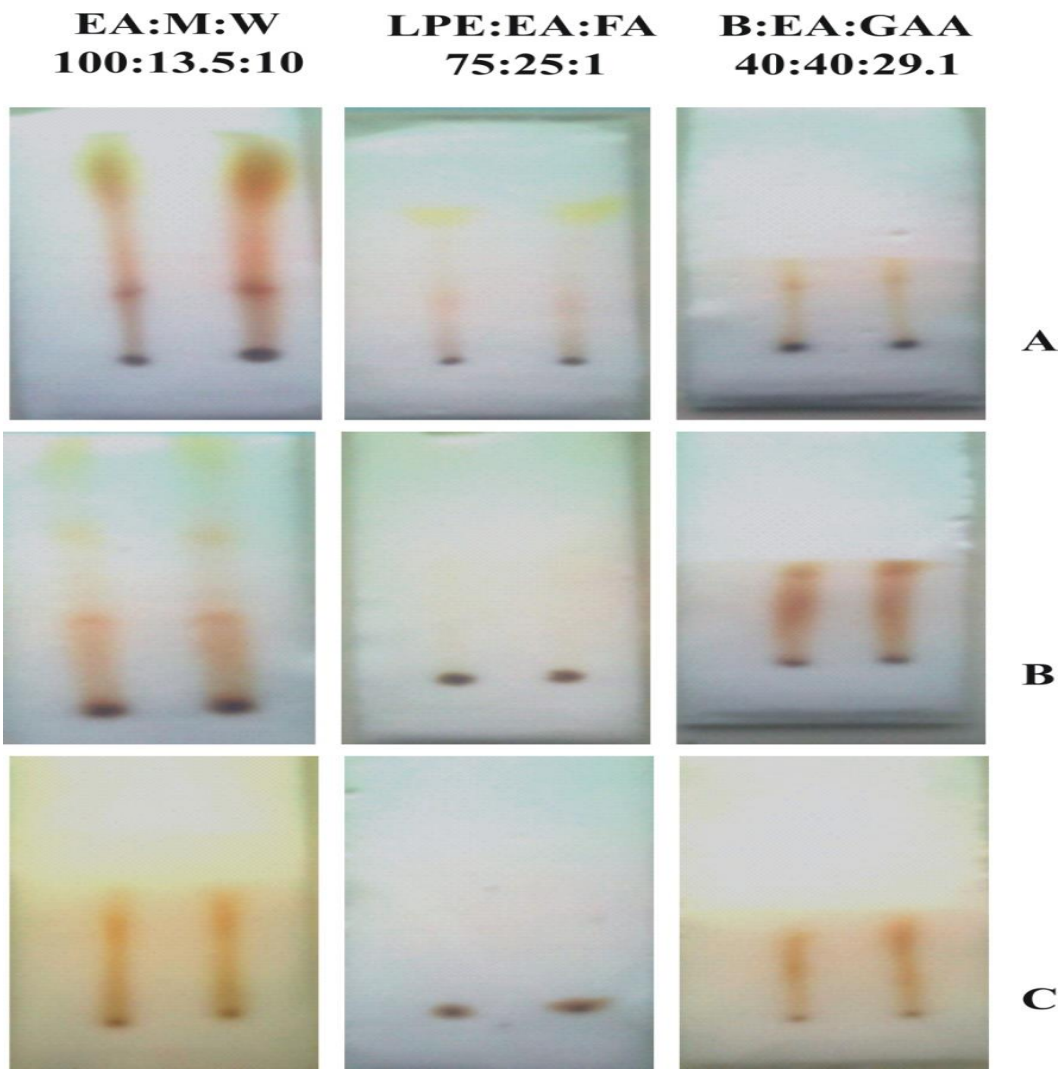


Figure 3: TLC of (A) Chloroform, (B) alcoholic and (C) aqueous extracts of *Cassia occidentalis* Linn whole plant

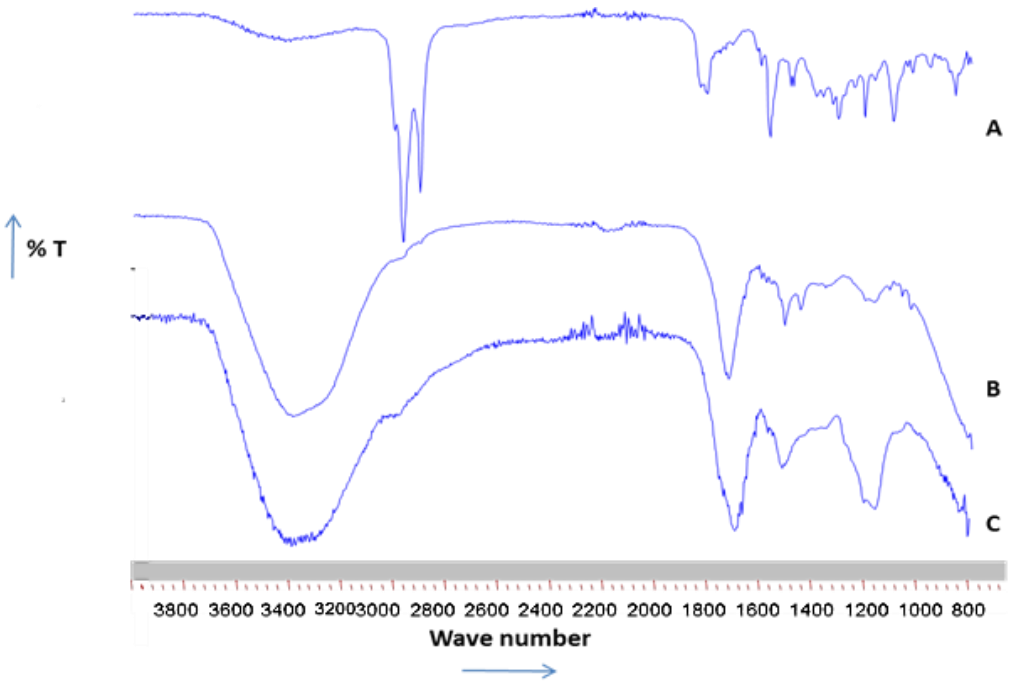


Figure 4: Interpretation of IR of (A) chloroform, (B) ethanol and aqueous extracts of *Cassia occidentalis* whole plant

dried weight of plant material. The colour and consistency of the extracts was noted.

Phytochemical Screening

The presence of various phytoconstituents viz alkaloids, carbohydrates, glycosides, flavonoids, triterpinoids, phenolic, steroids and proteins, aminoacids, fats, fixed oil and saponins were determined using suitable chemical test[32].

TLC Analysis

Analytical TLC plates were prepared by pouring the silica gel G (TLC-grade; Merck India) slurry on the glass plates. Thin layer plates were dried for 30 minutes in air and then in an oven at 110 ℃ for another 30 minutes. For qualitative exertion, the CE, AE and AqE extracts were spotted, in duplicate, on TLC plate, about 2cm above from the bottom, by using capillary tubes. The range of sample volume was controlled, spreading not more than 0.5cm. For each extract, three different solvent systems were used as developing systems. For chloroform extract the solvent systems used were CE-EA: M: W (100:13.5:10), CE-LPE: EA: FA (75:25:1) and CE- nB: EA: GAA (40:40:29:1). The aforesaid solvent systems were also used for alcoholic extract (AE) and aqueous extract (AqE). The standard abbreviations used are: CE= chloroform extract, EA= ethyl acetate, M= methanol and W= water, LPE= light pet ether, FA= formic acid, nB= n-butenol and GAA= glacial acetic acid. The plates were placed in previously saturated TLC chamber with mobile phase. The plates were developed in respective mobile phase up to 80%, dried and spots were visualized by exposing the plates to iodine vapor[33].

FT-IR analysis

The IR spectrum of chloroform, alcoholic and aqueous extract of whole plant of *Cassia occidentalis* Linn was obtained using FT-IR Agilent-630 over the frequency range from 4000-650 cm^{-1}. The spectra were plotted against Wave number cm^{-1} Vs Transmittance (%)[34].

HPTLC Fingerprint

CAMAG HPTLC system equipped with Linomat 5 applicator, TLC scanner-3, repro star 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software was used. All solvents used for HPTLC analysis were purchased from MERCK. 100 mg of extract was dissolved in 5ml of methanol and used, as tests solution, for HPTLC analysis. 10µl samples were spotted in the form of bands of width 8mm with a Camag microlitre syringe on precoated silica gel glass plate 60F-254. The distance between two tracks was 10 mm. An application volume of 10µl was applied at a position of 10mm from the bottom on the plate and the length of chromatogram run was 80mm from the application position. The sample loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapor for 30 minutes) containing mobile phase. The ethyl acetate: methanol: water (3:5:8), Light pet. ether: ethyl acetate: formic acid (3:5:8) and n-butanol: ethyl acetate: GAA (3:5:8) were employed as mobile phases. Aforesaid mobile phases were used for analysis of CE, AE, and AqE of *Cassia occidentalis* whole plant. The linear ascending development was carried out in 20cm x 10cm twin trough glass chamber saturated with the mobile phase. The developed plate was dried by hot air to evaporate solvents from the plate and the plate was fixed in scanner stage and scanning was done at 254nm. Densitometric scanning was performed on CAMAG TLC Scanner-3 and operated by CATS software (V 4.63, Camag). The scanner converts bands into peaks, peak height and area which were related to the concentration of the substance on the spot[35].

GC-MS Analysis

GC-MS analysis of the extracts was performed with Shimadzu system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS, 2010) equipped with an Elite-1 fused silica capillary column (RTZ i 5ms; 30 mm x 0.25 mm 1D). For GC-MS detection, and electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow

Table 4: Observed functional groups of (A) chloroform (B) alcoholic and (C) aqueous extracts of *Cassia occidentalis* whole plant

(A) chloroform extract	
Wave length (cm^{-1})	Interpretation
2921.95	Alkenes asymmetric C-H stretching vibration band
2852.67	Alkenes asymmetric C-H stretching vibration band
1709.84	C=O stretching vibration band
1460.07	Alkane-CH_2-bending vibration band
1492.29	Alkane-CH_2-bending vibration band
1379.71	-NO_2 Nitro compounds
1269.96	C-C stretching vibration band
1186.02	C-N Amines
1035.65	C-O Alcohols, ethers, carboxylic acid, esters stretching vibration
1117.90	C-N Amines
1081.11	C-O Alcohols, ethers, carboxylic acid, esters stretching vibration
969.40	Vinyl C=C bending vibration band
665.01	C-H Aromatic rings
	(B)Alcoholic extract
3339.41	C≡C-H stretching vibration band
2933.04	Alkenes asymmetric C-H stretching vibration band
1626.46	Aromatic carbon skeleton C=C stretching vibration band
1399.99	-NO_2 Nitro compounds
1350.84	-NO_2 Nitro compounds
1224.71	C-C stretching vibration band
1033.70	C-O Alcohols, ethers, carboxylic acid, esters stretching vibration
	(C)Aqueous extract
3324.93	C≡C-H stretching vibration band
3240.64	Hydrogen –bonded alcohols, phenols
2936.96	Alkenes asymmetric C-H stretching vibration band
2298.15	C≡C Alkynes
1397.24	-NO_2 Nitro compounds
1234.29	C-C stretching vibration band
1043.28	C-O Alcohols, ethers, carboxylic acid, esters stretching vibration

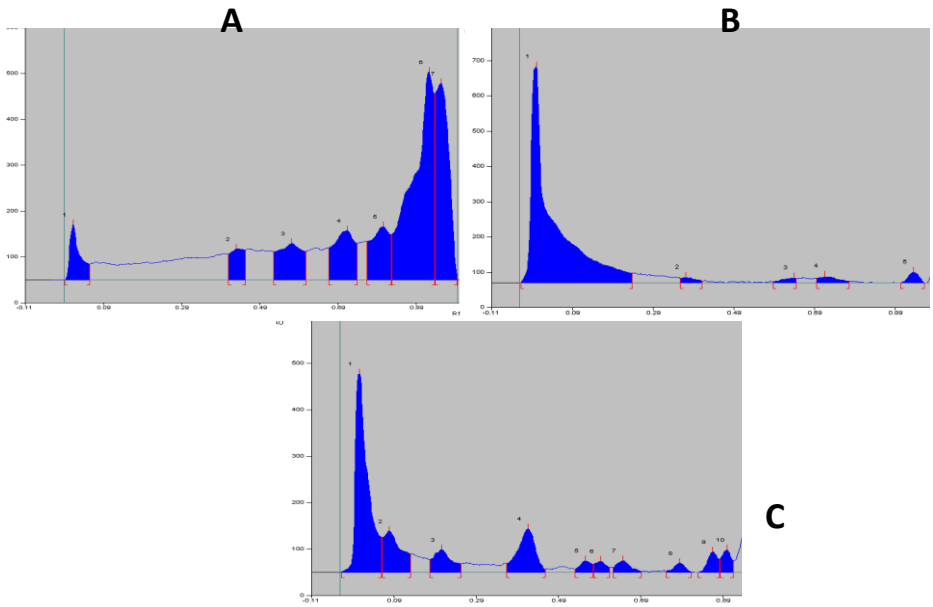


Figure 5: HPTLC - spectral analysis of (A) chloroform, (B) ethanol and (C) aqueous extracts of *Cassia occidentalis*

spectra and chromatograms was a turbo mass.

Table 5: HPTLC - spectral analysis of chloroform ethanol and aqueous extracts of *Cassia occidentalis* whole plant

Samples	Peak	R_f	Max Height	Max %	Area	Area %	Assigned substances
	1	0.01	121.1	8.80	2591.3	5.15	Unknown
	2	0.43	67.9	4.94	2122.0	4.22	Unknown
Chloroform	3	0.57	80.0	5.81	4142.8	8.23	Unknown
	4	0.71	108.1	7.85	4865.0	9.67	Unknown
	5	0.81	116.1	8.44	4617.4	9.18	Unknown
	6	0.92	453.8	32.98	19826.7	39.41	Unknown
	7	0.95	429.0	31.18	12143.4	24.14	Unknown
	1	0.00	612.2	89.06	25501.1	92.0	Unknown
Ethanol	2	0.37	15.0	2.18	455.3	1.84	Unknown
	3	0.64	13.0	1.89	393.0	1.42	Unknown
	4	0.72	17.2	2050	707.9	2.55	Unknown
	5	0.93	30.0	4.36	858.8	2.38	Unknown
	1	0.01	427.9	50.47	10761.2	46.24	Unknown
	2	0.08	88.9	10.49	3176.7	13.65	Unknown
	3	0.21	49.4	5.83	1820.4	7.82	Unknown
Aqueous	4	0.42	93.9	11.08	3338.9	14.35	Unknown
	5	0.55	25.1	2.96	595.6	2.58	Unknown
	6	0.59	24.0	2.83	544.8	2.34	Unknown
	7	0.85	25.2	2.97	723.8	3.11	Unknown
	8	0.78	20.3	2.39	459.0	1.97	Unknown
	9	0.86	44.3	5.22	900.1	3.87	Unknown
	10	0.90	48.9	5.76	948.7	4.08	Unknown

rate of 1 ml/min and an injection volume of 2µl was employed (Split ratio of 50:50). The injector temperature of 250 ℃ and ion-source temperature of 250 ℃ was used. The oven temperature was programmed from 80 ℃ (isothermal for 3 min), with an increase of 10 ℃/min, to 280 ℃, ending with a 10 min isothermal at 280 ℃. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 40 to 550 Da. The total GC running time was 33 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas. The software adopted to handle mass

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of 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 NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Acute Toxicity Study

The experimental protocol was approved by the Institutional Animal Ethics Committee of Tatyasaheb Kore College of Pharmacy, Warananagar, (Maharashtra),

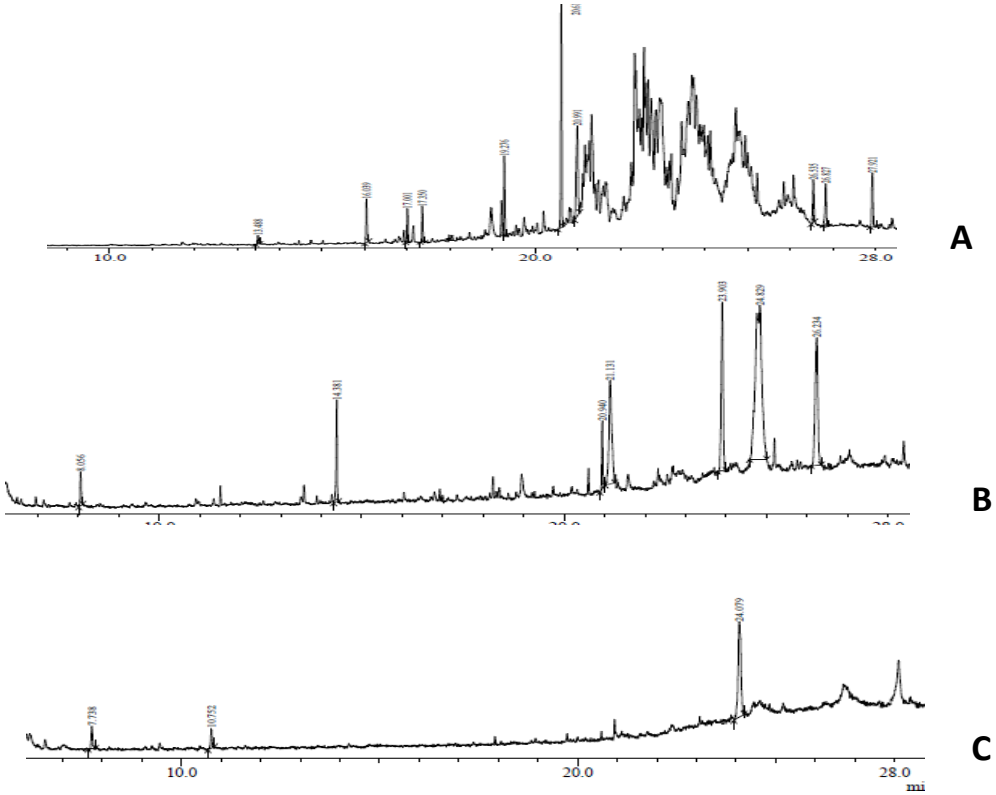


Figure 6: GC-MS Analysis of (A) Chloroform (B) Alcoholic and (C) Aqueous extracts of *Cassia occidentalis* whole plant

India (Ref.No.IAE/TKCP/2012/11, date: 21/12/2012) and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The acute toxicity of the extract was determined by the method of Reed and Meunch[36] on Wistar albino rats. 35 Wistar albino rats of either sex, weighing 185-200g were divided into 7 groups, each containing five animals. The rats were fasted for 18 hours, with water and libitum. The animals were administered with solution of chloroform extract of *Cassia occidentalis* Linn whole plant in distilled water and Tween 20 mixture. The aqueous solutions of extracts containing 2% Tween 20 were administered to rats orally. The dose was administered by gavage using a stomach tube. Group 1 was kept as untreated control. Group 2, 3, 4, 5 and 6 were administered orally with a dose of 250, 500, 1000, 2000 and 3000 mg/kg body weight, respectively. Group 7 was given 2% Tween 20 in distilled water and kept as vehicle control. The number of animals dead in each group, after 72 hours of administration of the drug was recorded and results were tabulated. Table 6[37].

Anticonvulsant Activity

Anticonvulsant activity of CE, AE, and AqE of *Cassia occidentalis* Linn whole plant was screened against MES and PTZ induced convulsions on group of six albino rats of either sex. The activity was compared with standard Phenytoin [38].

MES Induced Method: 1. Animals were weighed, numbered and divided into five groups each consisting 6 rats. One group was used as control (Saline treated) and the other as reference standard (Phenytoin treated). 2. Animal were placed with ear clip electrode and electric current of 150mA was applied for 0.2sec and noted the different stages of convulsions, i.e. a) tonic flexion, b) tonic extensor, c) clonic convulsion d) stupor and e) recovery or death. Also recorded the time (in sec) spent by the animal in each phase of convulsions. Repeated the same for other animals of the control group. 3. The animals were injected with Phenytoin intraperitonially (25mg/kg i.p) and after 30 minutes the animals were subjected to electro convulsions as described in step two. 4. Noted the reduction in time or abolition of tonic extensor phase of MES convulsions. 5. The CE, AE, and AqE of *Cassia occidentalis* Linn whole plant were dissolved in water containing 2% Tween-20. And the steps from 2 to 4 were repeated to determine the anticonvulsant activity of CE, AE, and AqE of *Cassia occidentalis* Linn whole plant with group 3, 4 and 5, respectively. 6. The extracts were tested at a dose of 200 mg/kg orally and the results are tabulated.

PTZ Induced Method: 1. Animals were weighed, numbered and divided into five groups each consisting 6 rats. Group one was used as control (pentylenetetrazol treated; 80 mg/kg) and the group 2 was used for studying the protective effect of diazepam (at dose of 4mg/kg). 2. The control group animals were injected with pentylenetetrazol (80 mg/kg) intraperitonially and noted the onset of action (indicated by straub's tail, jerky movements of whole body and convulsions) and severity of convulsion. 3. The second group animals were first injected with diazepam intraperitonially and after 30 minutes, the pentylenetetrazol was injected to the same

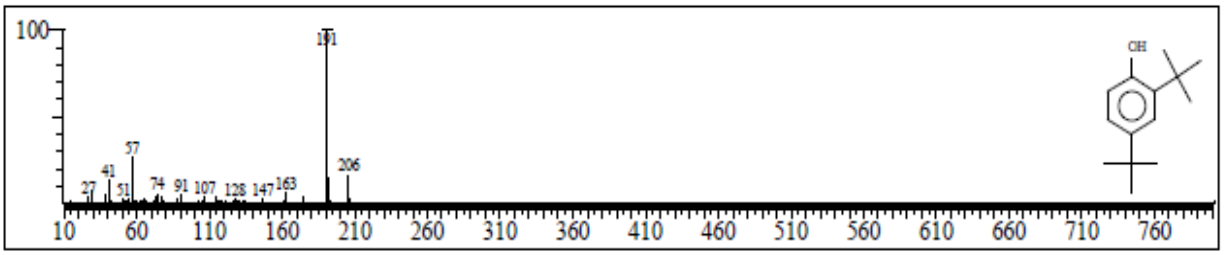


Figure 7: MS of Phenol, 2, 4 bis (1,1dimethylethyl) Phenol: $C_{14}H_{22}O$; MW: 206

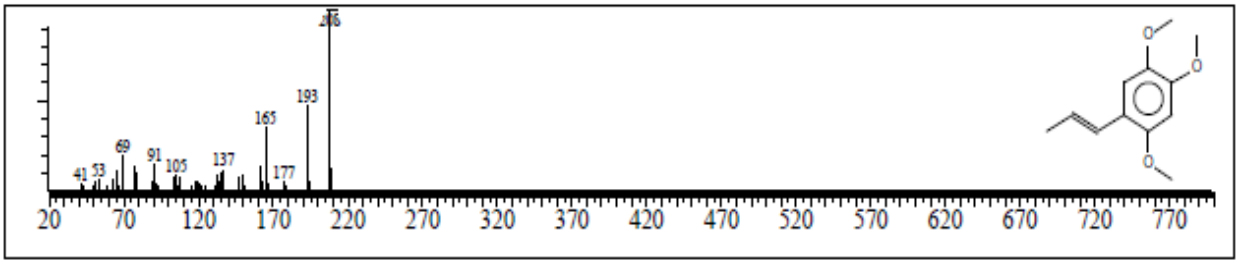


Figure 8: MS of Asarone: $C_{12}H_{16}O_3$; MW: 208

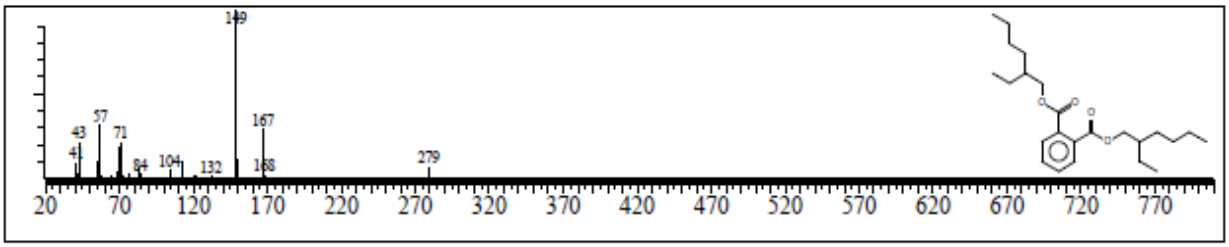


Figure 9: MS of 1, 2-Benzenedicarboxylic acid: $C_{24}H_{38}O_4$; MW: 390

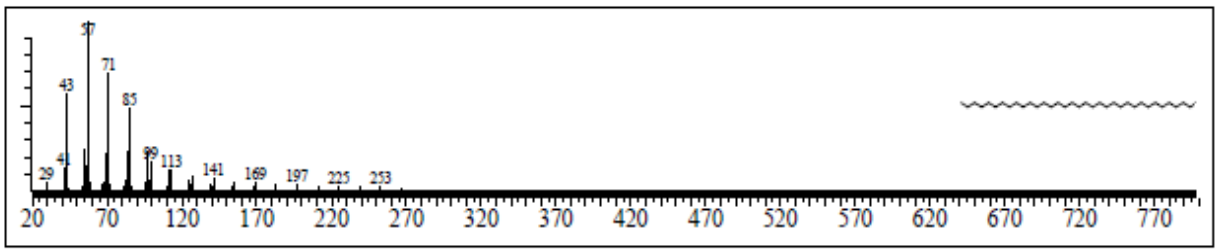


Figure 10: MS of Dotriacontane: $C_{32}H_{66}$; MW: 450

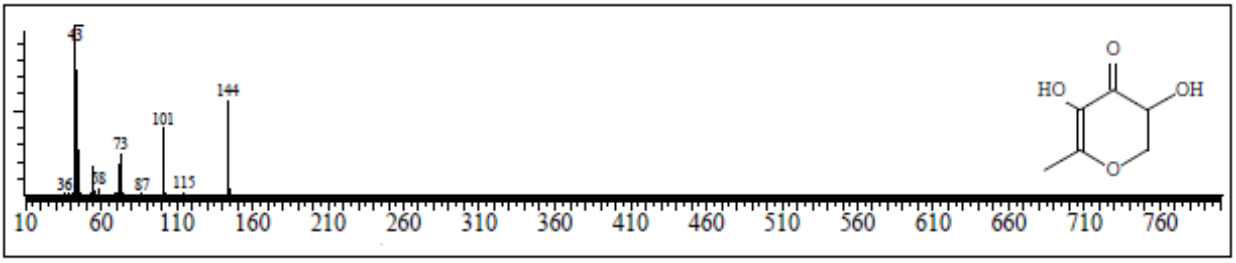


Figure 11: MS of 4H-Pyran-4-one: $C_6H_8O_4$; MW: 144

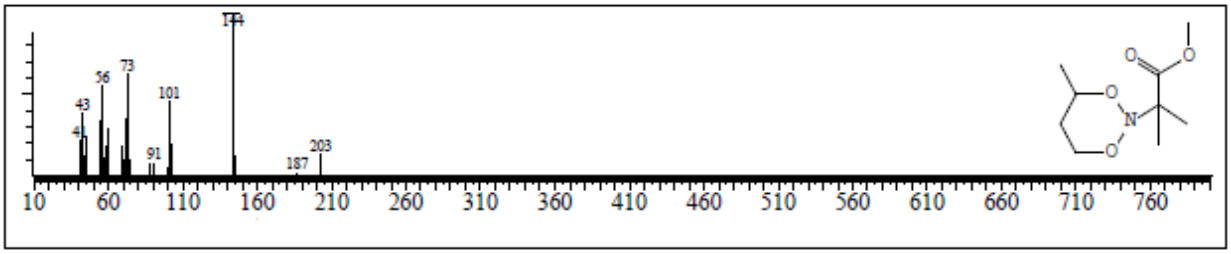
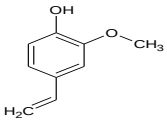
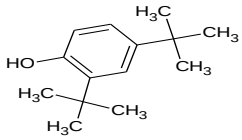
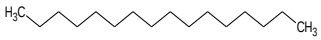
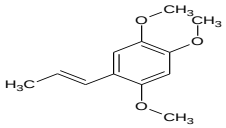
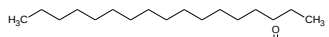
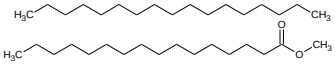
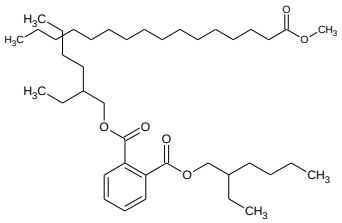
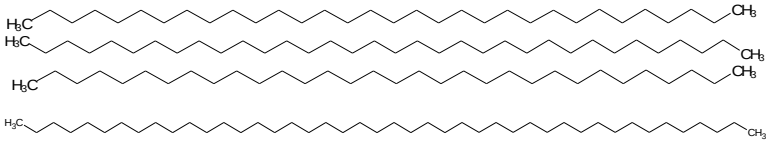
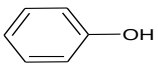
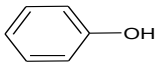
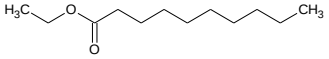
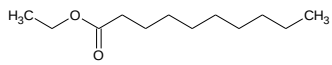
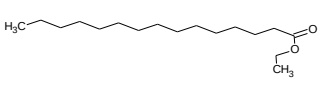
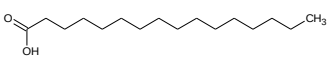
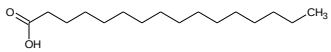
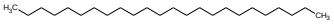
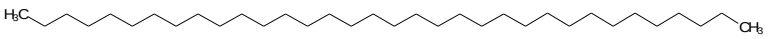
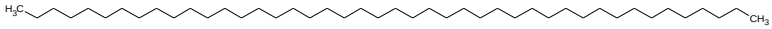
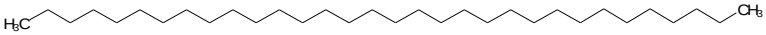
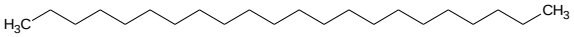
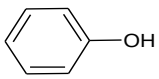
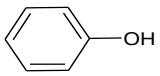
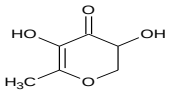
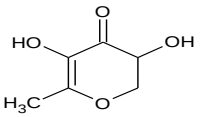
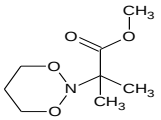


Figure 12: MS of 1, 3-Diazacyclo octane-2-thione: $C_6H_{12}N_2$ S; MW: 144

animals and noted either delay or complete abolition of convulsions. 4. The CE, AE, and AqE of *Cassia occidentalis* Linn whole plant were dissolved in water containing 2% Tween-20 and the step 2 and 3 were repeated to determine the anticonvulsant activity, of CE, AE, and AqE of *Cassia occidentalis* Linn whole plant, with group 3, 4 and 5, respectively. 5. The extracts were tested at a dose of 200 mg/kg orally and the results are tabulated.

Table 6: GC-MS investigation of chloroform extracts of *Cassia occidentalis* (Linn)

S. No	Compound Name	R. Time	Peak Area	% Peak Area	Structure
	(A) Chloroform extract of *Cassia occidentalis* whole plant				
1	4-Hydroxy-3-methyl acetophenone: $C_9H_{10}O_2$ MW: 150	13.488	403698	1.37	
2	Phenol, 2,4-bis(1,1dimethyl) phenol: $C_{14}H_{22}O$; MW: 206	16.039	184031	6.24	
3	Hexadecane: $C_{17}H_{36}$; MW: 240	17.001	120361	4.08	
4	Octadecane:$C_{18}H_{38}$; MW: 254	17.350	136361	4.62	
5	Docosane: $C_{22}H_{46}$; MW: 310	19.276	279816	9.48	
6	Asarone: $C_{12}H_{16}O_3$; MW: 208	20.614	889158	0.13	
7	Heneicosane: $C_{21}H_{44}$; MW: 296	20.991	622539	21.09	
8	Hexadecanoic acid: $C_{17}H_{34}O_2$; MW: 270	26.535	186965	6.34	
9	Octadecanoic acid: $C_{21}H_{38}O_4$; MW: 354	26.827	214416	7.27	
10	1, 2-Benzenedicarboxylic acid: $C_{24}H_{38}O_4$; MW: 390	27.921	277148	9.39	
11	Nonacosane: $C_{29}H_{60}$; MW: 408				
12	Tetratetracontane: $C_{44}H_{90}$; MW: 618				
13	Dotriacontane: $C_{32}H_{66}$; MW: 450				
14	Tetrapentacontane: $C_{54}H_{110}$; MW: 758				
	(B) Alcoholic extract of *Cassia occidentalis* whole plant				
1	Phenol: C_6H_6O; MW: 94	8.056	625294	1.60	
2	Phosphonic acid(P-hydroxyphenyl): $C_6H_7O_4$; MW: 174	14.381	168690	4.32	
3	Decanoic acid: $C_{12}H_{24}O_2$; MW: 200	20.940	106653	2.73	
4	Pentadecanoic acid: $C_{17}H_{34}O_2$; MW: 270	21.131	472557	12.10	
5	Undecanoic acid: $C_{13}H_{26}O_2$; MW: 214	23.903	503622	12.90	

6	Hexadecanoicacid: $C_{16}H_{32}O_2$; MW: 256	24.829	159909	40.95	
7	Pentadecanoic acid: $C_{15}H_{30}O_2$; MW: 242	26.234	557199	14.27	
8	Dotriacontane: $C_{32}H_{66}$; MW: 450	28.923	435016	11.14	
9	Nonacosane: $C_{29}H_{60}$; MW: 408				
10	Tetrapentacontane: $C_{54}H_{110}$; MW: 758				
11	Tetratetracotane: $C_{44}H_{90}$; MW: 618				
12	Tetracosane: $C_{24}H_{50}$; MW: 338				
13	Pentatriacontane: $C_{35}H_{72}$; MW: 492				
	(C)Aqueous extract of *Cassia occidentalis* whole plant				
1	Phenol: C_6H_6O; MW: 94	7.738	455353	9.48	
2	Phosphonic acid (P hydroxyphenyl): $C_6H_7O_4$; MW174	10.752	388410	8.09	
3	4H-Pyran-4-one: $C_6H_8O_4$; MW: 144	24.079	395815	82.43	
4	3-dioxane(1-Methoxycarbonyl-1-methyl):$C_9H_{17}NO_4$; MW: 203				
5	1,3-Diazacyclooctane-2-thione: $C_6H_{12}N_2S$; MW: 144				
6	Pentatriacontane: $C_{35}H_{72}$; MW: 492				

RESULTS AND DISCUSSION

Physicochemical analysis

Macroscopic Examination

The Powder was characterized on its morphological features as color; yellowish, taste; tasteless and odour; odourles of the powder were determined. The powder was cleared with chloral hydrate and stained with concentrated hydrochloric acid, phloroglucinol to identification of lignified elements, the presence of ruptured xylem vessel, pericyclic fibers, secretory glands, calcium oxalate crystals, medullary ray cells were observed (Fig 2)

Pytochemical Screening

The preliminary phytochemical analysis revealed the presence of glycosides, flavonoids, triterpinoids, carbohydrates, steroids, phenolic compounds, saponins, amino acids and proteins (Table 2).

TLC Analysis

The CE, AE and AqE of whole plant of *Cassia occidentalis* Linn were subjected to TLC analysis in order to identify the phytochemicals. The TLC of CE, AE and AqE of whole plant of *Cassia occidentalis* Linn are shown in Fig 3. The figure clearly depicts very predominant spots for CE as compared to AE. All spots were observed, no tailing. However, no spot was observed with AE in LPE: EA: FA solvent system. The less intense spots, as compared to CE, were observed with AE in all three solvent systems. In case of AqE we found very less intense spot and tailing with

LPE: EA: FA solvent system and found no spots with LPE: EA: FA solvent systems. The TLC profiling of plant extracts in different solvent system reveals the presence of diverse group of phytochemicals in CE and AE as compared to AqE.

FT-IR analysis

Infrared spectroscopy is one of the powerful analytical techniques which offer the possibility of chemical identification. The technique is based on the simple fact that chemical substance shows selective absorption in

Table 7: Acute Toxicity Studies of *Cassia occidentalis* whole plant

Group	Ave, Weight of Animals	Dose mg/kg	Death After			No. of Survived Animals	Ave, Weight of Animals
			24 Hrs	48 Hrs	72 Hrs		
I	187.4±8.79	250	-	-	-	5	183.4±4.27
II	173.8±11.38	500	-	-	-	5	175.1±10.10
III	191.2±4.49	1000	-	-	-	5	192.1±2.20
IV	185.0±11.95	2000	-	-	-	5	186.0±9.70
V	185.8±11.71	3000	-	-	-	5	182.9±8.51
VI	188.4±6.61	-	-	-	-	5	189.0±8.31

Note: The animals were reused, after 10 days of washing period, to perform toxicity study of alcoholic and aqueous extracts of *Cassia occidentalis*

infrared region. After absorption of IR radiations, the molecules vibrate, giving rise to absorption spectrum. It is an excellent method for the qualitative analysis because except optical isomers, the spectrum of compound is unique. It is most useful for the identification of purity and gross structural details. This method is useful in the field of natural products, forensic chemistry and in industrial analysis of competitive products. The IR spectrum of chloroform, alcoholic and aqueous extract of *Cassia occidentalis* whole plant are shown in the fig 4. The absorption peaks corresponding to different functional groups of CE, AE and AqE extracts are shown in table 3.

HPTLC Analysis

HPTLC technique is most simple and fastest separation technique available today which gives better precision and accuracy with extreme flexibility for various steps. The HPTLC fingerprinting of CE, AE and AqE are shown in Fig. 5A, 5B and 5C, respectively. The results showing number of peaks, maximum R_f value, maximum height and total % area are given in Table 4. The Fig 5A, 5B and 5C clearly indicate that all samples constituents are clearly separated without any tailed and diffuseness. It is evident from Fig 5 that the AqE shows more number of peaks (10 peaks) as compared to CE (7 peaks) and AE (5 peaks). The CE shows 7 peaks and the maximum percentage area covered is by peak 2 (Rf value 0.92 and 0.95) (Fig. 5A). The AE shows 5 peaks and the maximum percentage area covered is by peak 1(Rf value 0.00) (Fig. 5B). The AqE shows 10 peaks and the maximum percentage area covered is by peak 1 (Rf value 0.01) (Fig 5C). The HPTLC results clearly reveals that the more number of phytochemicals are present predominantly in aqueous extract as compared to chloroform and alcoholic extracts which shows only one predominant component. The other components present in the extracts are found to be less predominant and found in less concentration. Form the HPTLC study it is very clear that the CE, AE and AqE extract containing not only a single compound but a mixture of compounds.

GC-MS Analysis

GC-MS is a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra[39] (Ronald Hites., 1997). Thus, GCMS analysis is the first step towards understanding the nature of active principles of the medicinal plant and this type of study will be helpful for further detailed study. In the present study we analyzed the CE, AE and AqE of *Cassia occidentalis* whole plant using GC-MS in order to identify the number and type of phytochemicals present in them. The components present in CE, AE and AqE of *Cassia occidentalis* whole plant were identified by GC-MS analysis (Fig 6). The name of phytochemicals with their retention time (RT), peak area, % peak area, molecular formula, molecular weight and chemical structure are shown in Table 6. The result clearly reveals the presence of total of 14, 13 and 6 compounds in CE, AE and AqE, respectively. The major component present in CE is heneicosane (21.09%) and the other components of CE includes docosane (9.48%), 1,2 benzenedicarboxylic acid (9.39 %), octadeconoic acid (7.27%), hexadeconoic acid (6.34%). From the results it is found that the hexadecanoic acid (40.95%) and Pentadecanoic acid (14.27%) are major components of AE followed by undecanoic acid (12.90%) and dotriacontane (11.14%), We, in AqE, found 4H, pyran 4-one (82.43%), phenol (9.48%), 3-dioxane, 1,3 diazacyclooctane 2-thione and phosphonic acid (8.09%) as chemical constituents. The GC-MS analysis study concludes the presence of more components in CE as compared to AE and AqE.

Toxicity study of Cassia occidentalis

The results of acute toxicity study of CE, AE, and AqE of *Cassia occidentalis* whole plant are shown in Table 7. No mortality was observed, at the tested dose up to 3000 mg/kg, during the study and this indicates that the LD50 of the extracts was found to be more than 3000 mg/kg by oral route. Also, we found, no significant changes in the body weight of animals after 72 hr of dose administration indicating the extracts were nontoxic at the tested dose up to 300mg/kg. The similar results were observed with AE and AqE of *Cassia occidentalis* whole plant

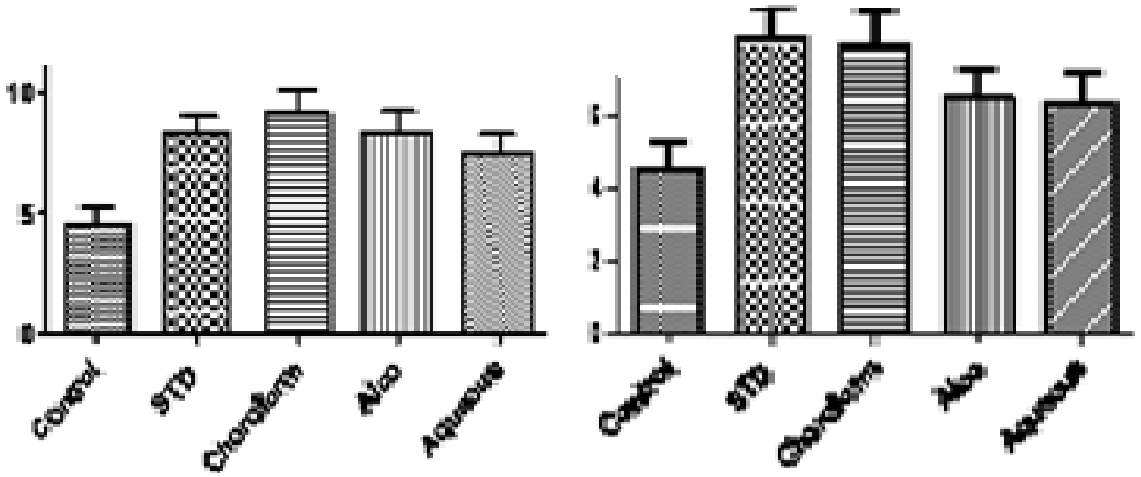


Figure 13: Anticonvulsant activity of stem extract against (A) MES and (B) PTZ induced convulsion

Table 8: The Effect of MES Indused convulsions of *Cassia occidentslis* whole Plant

Drug	Dose mg / kg b.w	Route of Administration	Time (Sec) in various phases of convulsions (Mean + SEM				Recovery/ Death
			Flexion	Extensor	Clonus	Stupor	
Control (Salaine)		Oral	3.50±0.76	13.33±1.30	5.60±1.49	122.16±5.79	Recovery
Standard Phenytoin	25	Intra peritoneal (i.p)	2.83±0.60	00±00	00±00	00±00	Recovery
Chloroform	200	Oral	1.83±0.47	2.83±0.70	00±00	19.00±3.07	Recovery
Alcoholic	200	Oral	2.50±0.76	5.00±1.57	00±00	153±6.17	Recovery
Aqueous	200	Oral	3.83±0.94	7.66±2.14	4.00±0.96	55.83±5.61	Recovery

Data was analyzed using one-way ANOVA followed by dunnet's test. Values are mean ± SEM, N=6. ***p<0.05 when compared with standard group.

Anticonvulsant activity

For inducing convulsion by electro shock, a rectangular pulse current of high voltage 150mA is employed. The electro shock was given to each rat for 0.2 seconds with the help of convulsio meter through pinna electrodes. Drug likely to be effective in Grandmal epilepsy usually confer protection against electrically induced convulsion in animals. Group 1 received saline, group 2 received Phenytoin (25mg/kg) and group 3, 4, and 5 received 200mg/kg body weight of CE, AE and AqE of *Cassia occidentalis* whole plant, respectively. The results are tabulated in Table 8. The CE AE and AqE Phenytoin treated groups exhibited significant ($p<0.0001$) reduction in various phases of epileptic seizure on comparison with the control group and the CE and AE treated groups showed slight decrease in flexion phase(1.83±0.47 and 2.50±0.76), similar extensor(2.83±0.70 and 5.00±1.57) and clonus phases, and significantly increased stupor phase (19.00±3.07and 153±6.17) on comparison to standard, Phenytoin treated, group (Figure 13A) and this can be better correlate to the presence of few phytochemicals in CE and AE following GC-MS analysis. The results reveal that the AqE *Cassia occidentalis* whole plant shows less anticonvulsant activity on comparison to control group (Table 8 and Figure 8A). The anticonvulsant activity of CE, AE and AqE of *Cassia occidentalis* whole plant is also tested using PTZ method (Table 9 and Figure 13B). The clonic seizures were induced in rats by intraperitonial injection of 80mg/kg body weight PTZ. The latency to the onset of clonic convulsions in non-protected rat and lethality during the following 24hrs was recorded and compared with standard and Extract treated groups to evaluate the anticonvulsant activity. Group 1 received diazepam (4mg/kg) intraperitonially, as a reference standard, 30 minutes before PTZ. The animals were observed for onset of convulsion up to 30 minute after PTZ administration and later up to 24hrs. The latency (onset of clonic), onset of tonic convulsions, and the status of animals were recorded (Table 9). The Phenytoin and extracts treated groups showed significant anticonvulsant activity as compared to control group ($P<0.0001$). The CE of *Cassia occidentalis* whole plant showed anticonvulsant activity similar to Phenytoin. We observed no convulsions and mortality with CE treated group as like with Phenytoin treated group. In AE and AqE treated groups we observed convulsions in 50% of rats and this indicates poor anticonvulsant activity of AE and AqE as compared to Phenytoin and CE. However, during the study, no mortality was observed with AE and AqE treated group. GABA is the major inhibitory neurotransmitter in the mammalian CNS, and is widely implicated in epilepsy, mediating inhibition of neuronal responsiveness (excitability) and activity by increasing the chloride ion

Table 9: The Effect of PTZ Indused convulsions of Cassia *occidentslis* whole Plant

Drug	Dose mg / kg b.w	Route of Adminstration	Onset of (Sec. + SEM)			Martility %
			Onset	No. of Animal Survived	No. of Animal Convulsed	
Control PTZ	80	Oral	00±00	0/6	6/6	100%
Standard Diazepam+PTZ	4+80	Intra peritoneal (i.p)	00±00	6/6	0/6	0 %
Chloroform	200	Oral	00±00	6/6	0/6	0%
Alcoholic	200	Oral	00±00	6/6	0/6	0%
Aqueous	200	Oral	198.16±5.61	6/6	2/6	33.33%

Data was analyzed using one-way ANOVA followed by dunnet's test. Values are mean ± SEM, N=6. ***p<0.05 when compared with standard group.

conductance through opening of the chloride ion channel[40]. Epilepsy is not a singular disease unit but a variety of disorders reflecting underlying brain dysfunction that may result from several dissimilar causes and for that reason, there is continuing demand for new anticonvulsant agent requirement in advancement of some novel anticonvulsant drug with more selective activity and lower toxicity for the effective treatment of epilepsy[41]. Electrical stimulation causes seizures which passes through phases of tonic limb extension, tonic limb flexion and clonus period[42]. The generalised tonic clonic and cortical focal seizures predicts activity against MES, while PTZ tests activity against petitmal epilepsy or absence seizures and reduction in duration of hind limb extensor phase, delay in the latency of seizures are considered as anticonvulsant agents important parameters[43]. On observation and reference to reported data from Phytochemical tests it was clear that, CE, AE and AqE extracts of *Cassia occidentalis* whole plant showed the presence of glycosides, flavonoids, alkaloids, triterpinoids, carbohydrates, steroids, phenolic compounds, saponins, amino acids and proteins have been implicated various pharmacological actions on central nervous system(CNS) including anticonvulsant activity. The anticonvulsant activity may be due to the presence of glycosides, flavonoids and sterols in the extracts. From the results we can conclude that the CE, and AE of *Cassia occidentalis* whole plant possesses anticonvulsant activity against MES and PTZ induced convulsions. The CE of *Cassia occidentalis* whole plant is found more potent in showing anticonvulsant activity than AE and AqE and additional studies are required to find and isolate active principles, to determine the mechanism of their anticonvulsant action. Also our study suggests the application of *Cassia occidentalis* whole plant in the treatment of convulsive disorders as a need of modern health science.

CONCLUSION

The present studies conclude and indicated that the activity is mediated by a combination of two or more molecules and the anticonvulsant effect of the chloroform extract of *Cassia occidentalis* whole plant may be via non-specific mechanisms and further experiments will be necessary to identify the active molecules(s) and their mechanism(s) of action.

ACKNOWLEDGEMENTS

Authors are thankful to Mr. G. D. Patil, Secretary, Shree Warana Vibhag Shikshan Mandal, and Principal, Tatyasaheb Kore College of Pharmacy, Warananagar for their continuous support in carrying out this project.

Conflict of interest

The authors declare that there are no conflicts of interest.

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