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PHYTOCHEMICAL PROFILING OF ETHANOLIC LEAVES EXTRACT OF *CASSIA AURICULATA*

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ABSTRACT

This study was conducted to assess the phytochemical constituents in Cassia auriculata Leaf extracts using standard methods. The qualitative analysis of bioactive compounds for the three extracts have been analyzed in this study and there is wide range of phytochemical compounds present in the three extracts. Cassia auriculata extract was found to have a wide range of bioactive compounds like alkaloids, carbohydrates, flavonoids, proteins, phenols, reducing sugars, steroids and tannins. In the present study ethanolic extract of Cassia auriculata was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) and the compound structures were identified with help of National Institute of Standards and Technology (NIST) library. GC-MS analysis of test plant revealed the presence of 48 bioactive compounds. The prevailing compounds were Orcinol (25.54%), Eptacosanol (10.22%), Utanol, -Dihydro-Benzofuran (5.76%), 3-Methyl-, Acetate (5.45%), Eptacosanol (4.50%), Eptacosanol (4.15%), Gmast-5-En-3-Ol, (3.Beta.)- (3.97%), 2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecy (3.63%), Benzamide, N-(2-Methoxyphenyl)-2-Pyrrolidi (3.52%) etc are important bioactive compounds which act as essential drugs for dangerous diseases and disorders and other compounds are used in antimicrobial, anti-inflammatory, antioxidant, cytotoxicity and cancer preventive activities.

KEY WORDS

Cassia auriculata, GC-MS analysis, Pharmacological, phytochemical, bioactive compounds.

INTRODUCTION

A great proportion of the natural products used as drugs are derived from plants. The poisonous or healing properties of plants were discovered by man in his search for food. Some plants were found to have very dramatic effect on the human body and some were found to cure certain diseases. The knowledge of these plants was passed on through the generations and thus man gathered considerable experience of drugs which could be obtained from the plants in his surroundings. It became the task of the medicine man to maintain this knowledge and pass it on to his successor. The medicine men were often also priests and thus the actual knowledge became enmeshed in a veil of myth and magic. This process can still be observed in the developing countries; consequently, the study of drugs used by traditional healers is an important object of pharmacognostical research. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry [1].

Medicinal plants are the source of many potent and powerful drugs. The plant derived drugs are healthier and safer alternate to the synthetic drugs [2]. Different parts of medicinal plants like root, stem, flower, fruit, seed etc. are used to obtain pharmacologically active constituents. Medicinal activities of plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, terpenoids and essential amino acids present in these plants. These active principles are isolated for direct use as drugs, lead compounds and or pharmacological agents [3]. Even

today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries [4]. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material can be useful for proper standardization of herbals and its formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards [5]. Nowadays there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation identification and structure determination of Phytochemicals. In GC-MS used to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc.,

The genus Cassia belongs to the order Leguminosae and sub-order Caesalpiniae which includes about 5000 species. About 45 species are found in India among which a several species have been introduced for medicinal purpose or to provide tanning material and some are ornamental [6]. They are also a part of traditional system of medicine and have been included in Indian, British and many other pharmacopoeias of the world. *Cassia auriculata* Linn. (Caesalpiniaceae, common name: Tanner's Cassia, Tanner's Senna) is a fast growing, profusely branched, tall, evergreen shrub generally 1.2-3.0 m in height and sometimes reaching a height of 6.0 m [7]. It is a common plant of wasteland in Asia that flower throughout year and also survives under adverse ecological conditions. In Indian ethnomedicine, this plant is commonly known as 'Avartaki', 'Avaram', 'Taravada', 'Aval', 'Avarike' and 'Hemapushpam [8]. The use of Plants with pharmaceutical properties has received increased interest nowadays from both homeopathic and allopathic branches. These medicinal plants play an important role in public health, especially in developing countries, where it is believed that the intense utilization of plants with therapeutic action does not lead to intoxication [9]. The cost of drugs in use today is too expensive for the majority of the population in the third world countries and therefore the search for some cheap sources of antimicrobial substances in nature become inevitable. Plants are good sources for new safe, biodegradable and renewable drugs. The use of plants as therapeutic agents in addition to being used as food is age long. *Cassia auriculata* is suitable for landscaping roadways and home gardens. It tolerates drought and dry conditions, but not much cold. The flowers in racemes are also attractive. This plant is said to contain a cardiac glucoside (sennapicrin) and sap, leaves and bark yield anthraquinones, while the latter contains tannins. The root is used in decoctions against fevers, diabetes, diseases of urinary system and constipation. The leaves have laxative properties. The dried flowers and flower buds are used as a substitute for tea in case of diabetes patients. It is also believed to improve the complexion in women. The powdered seed is also applied to the eye, in case of chronic purulent conjunctivitis. In Africa the bark and seeds are said to give relief in rheumatism, eye diseases, gonorrhea, diabetes and gout. Keeping this in view, the present study has been undertaken to investigate the phytoconstituents present in ethanolic extract of *Cassia auriculata*. Hence the present study focused on Phytochemical profiling of ethanolic extract of *Cassia auriculata* using Gas chromatography and mass spectrometry.

MATERIALS AND METHODS

Collection of plant samples

For the present study, the mature green leaves of *Cassia auriculata* belongs to family Fabaceae were collected from in and around area of Pattukkottai, Thanjavur District, Tamil Nadu, South India. The plant was identified with the help of manual of Tamil Nadu and Karnatic flora [10-11] with standard references [12].

Preparation of plant extract

The *Cassia auriculata* was collected, washed, cut into small pieces and dried at room temperature (28±1ºC) for two weeks and made into powder for further analysis. Extraction is a process, to separate or isolate the secondary metabolites from plant material. It is basically two types i.e. heat and cold extraction. Heat extraction has some advantage over cold extraction like time consistency and also no contamination by microbes. An apparatus called soxhlet did heat extraction. 100g of the plant leaf powder were packed into the thimble of a soxhlet apparatus. The ratio of the plant powder and solvents were maintained at 1:4. Preliminary phytochemical screening of methanol extract of *Cassia auriculata* was carried out to detect the

phyto-constituents using standard conventional protocols [13-15]. Alkaloids, carbohydrates, tannins and phenols, flavonoids, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Gas Chromatography-Mass spectrometry (GC-MS) analysis: The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µmdf capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (MHz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Identification of Compounds: The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. National Institute of Standards and Technology library sources were also used for matching the identified components from the plant material.

RESULT AND DISCUSSION

Qualitative phytochemical analyses for alkaloids, carbohydrates, tannins, phenols, gums and mucilage, fixed oils and fats, saponins, proteins, volatile oils, flavonoids and steroids were screened in ethanolic extracts of *Cassia auriculata*. The screening of the extract indicated the presence of alkaloids, tannins and saponin in the ethanolic extracts of leaves (Table 1). The plant extract yield percentage on the usage of methanol agreed with the earlier reported [16] obtained in *Hypochaeris radicata* L. The plant extract obtained using soxhlet is varied among the herbal plants to plant. In a plant, different parts having differently yielded [17]. The plant extract yield percentage on the usage of methanol agreed with the earlier reported obtained in *Brassica oleracea* [18]

Preliminary quantities of phytochemical screening of ethanolic extract of *Cassia auriculata* revealed the presence of alkaloids, flavonoids, terpenoids and phenolic compounds which are essential to prevent diseases and to maintain a state of well-being. Recent studies have been focused on finding the natural substance of medicinal plant that decrease the inflammation and reduce oxidative stress and there by counteracting the macromolecular damage. It is well known that reactive oxygen species interact with key bimolecular such as proteins and enzymes which regulate major metabolic path way and decrease their functional efficiency. Table 2 shows that *Cassia auriculata* contains rich amount of bioactive compounds which exhibit antioxidant property the quantitative analysis revealed that *Cassia auriculata* contain rich amount of phenolic compounds and flavonoids. It is well known that plant flavonoids and phenols in general are highly effective in scavenging free radical and providing antioxidant defense in living cells. Polyphenols and flavonoids isolated from medicinal plants have been used for the prevention and cure of various diseases which are mainly associated with free radicals [19].

Forty-eight compounds were identified in ethanolic fraction of *Cassia auriculata* leaf extract by GC-MS analysis. The chromatogram is obtained by ethanolic fraction of *C. auriculata* leaf. The active principle, area of the peak, Concentration (%), Retention Time (RT), Molecular formula and Molecular weight were presented in Table 3. The prevailing compounds were Orcinol (25.54%), Eptacosanol (10.22%), Utanol, -Dihydro-Benzofuran (5.76%), 3-Methyl-, Acetate (5.45%), Eptacosanol (4.50%), Eptacosanol (4.15%), Gmast-5-En-3-Ol, (3. Beta.)- (3.97%), 2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecy (3.63%), Benzamide, N-(2-Methoxyphenyl)-2-(Pyrrolidi (3.52%) etc. Fig. 1 shows the chromatogram with retention time, molecular weight, area, area% of the standards.

The differences between the compounds that we have found in the roots, stems and leaves of *Aristolochia clematitis* were studied by GC-FID. This study was performed on the alcoholic extracts of the three parts of the plant. From this study we have concluded that the compounds found in the root and steam are very

similar. The aristolochic acid derivatives are present in both extracts, but in the leaves these derivatives are in very low concentration [20]. Ramamurthy [21] reported that the roots and leaves of *H. indicum* were studied by GC-MS. This study was performed on the alcoholic extracts of the two parts of the plant. From this study we have concluded that the compounds found in the root and leaves are very dissimilar. The organic acid derivatives are present in both extracts, but in the leaves these derivatives are in very high concentration.

Plants are integral part of human civilization. Medicinal plants are also being relied upon by over 80% of the world population for their basic health care needs. Drugs based on the Plants are of prime importance for several remedies in traditional and conventional medicine throughout the world and serves as a substitute for drug supply in modern medicine [22]. Medicinal plants with therapeutic properties are used for the treatment of many infectious diseases of humans as they contain many bioactive phytochemical constituents which are of curative effects. By consuming medicinal plants, can boost the immune system and increase antioxidant activity in humans. The high level of use as a medicinal plant due to easily available, cheap and relatively no side effects [23]. Tetracosane, also called tetrakosane, is an alkane hydrocarbon. Tetracosane showed some cytotoxic activity against AGS, MDA-MB-231, HT-29 and NIH 3T3 cells [24]. It also used as a good antibacterial activity.

The analytical methods used GC/MS is suitable for medicinal herbs organic compounds determination. The sample preparation method is rapid and precise. There is a difference between the compounds extracted from herb by infusion and tincture but the important thing is that the organic acid and fatty acids derivatives are present in both of them. On the other side the study shows that their concentration is higher in the roots and steams. The present study focused on identification of several constituents present in the ethanolic leaves extract of *Cassia auriculata*. This type of GC-MS analysis is the first step towards understanding nature of active compounds in this medicinal plant and helpful for the further detailed study. In this plant contains various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners.

Table 1: Phytochemical screening of *Cassia auriculata*

Phyto-constituents	Observation
Alkaloids	+
Flavonoids	+
Terpenoids	+
Phenolic Compounds	+
Saponins	+
Tannins	+
Glycosides	+
Cardiac Glycosides	+
Coumarins	-

+: presence; –: absence

Table 2: Quantitative Phytochemical Analysis of *Cassia auriculata*

S. No.	Phytochemicals	Quantity of dry material
1.	Alkaloids (g/100g)	6.13 ± 1.14
2.	Terpenoids (g/100g)	4.79 ± 1.02
3.	Total phenols (g/100g)	56.5 ± 2.27
4.	Flavonoids (mg/g)	60.5 ± 0.42
5.	Tannin (mg/g)	41.5 ± 0.34
6.	Glycosidal (g/100g)	2.32 ± 1.19
7.	Crude Protein (g/100g)	13.4 ± 0.42
8.	Ascorbic acid (g/100g)	1.41 ± 0.17
9	β-Sistosterol (g/100g)	0.67 ± 0.03

Fig 1. GC-MS CHROMATOGRAM OF *CASSIA AURICULATA*

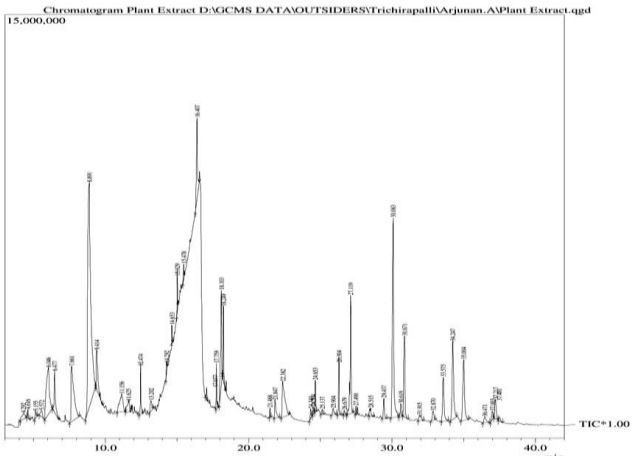

Table 3. GC-MS Profile of the Phytocompound of *Cassia auriculata*

Peak	R.T.	Name of the Compound	Molecular Formula	M.W	Area	Area%
1	4.292	2,6-dimethyl-6-nitro-2-	$C_9H_{15}NO_3$	185	3041893	0.65
2	4.616	1-hexanol, 2-ethyl-	$C_{10}H_{22}O_2$	174	1159873	0.25
3	5.155	2,5-anhydro-1,6-dideoxyhexo-3,4-diulose	$C_7H_9NO_2$	139	399508	0.30
4	5.572	Glycerin	$C_8H_8O_2$	136	134809	0.46
5	6.046	1-butanol, 3-methyl-, acetate	$C_{10}H_{18}O_2$	170	5411647	5.45
6	6.477	2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran	$C_9H_{16}O$	140	325907	1.79
7	7.661	2,3-dihydro-benzofuran	$C_9H_8O_2$	148	26845965	5.76
8	8.891	Resorcinol	$C_{10}H_{10}O_2$	162	119086981	25.54
9	9.414	3-[(trimethylsilyl)oxy]phenol #	$C_9H_8O_2$	148	10163578	2.18
10	11.156	xanthosine	$C_{15}H_{24}$	204	10125739	2.17
11	11.625	Decanoic acid	C_9H_9NO	147	4437489	0.95
12	12.474	Diethyl Phthalate	$C_{12}H_{24}O_2$	200	5251507	1.13
13	13.202	Ethyl. alpha.-d-glucopyranoside	$C_{16}H_{14}O_4$	270	2864013	0.61
14	14.292	Tetradecanoic acid	$C_{11}H_{12}O_2$	176	1327446	0.28
15	14.653	2(4H)-Benzofuranone, 5,6,7,7a-Tetrahydro-6-Hy	C_9H_9NO	147	1451033	0.31
16	15.029	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	$C_{14}H_{28}O_2$	228	3109409	0.67
17	15.478	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	$C_{11}H_{16}O_3$	196	1444605	0.31
18	16.407	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{18}H_{36}O$	268	14586595	3.13
19	17.077	Hexadecanoic acid, trimethylsilyl ester	$C_{15}H_{24}O$	220	1117639	0.24
20	17.759	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[R*	$C_{16}H_{26}O_3$	226	4161846	0.89
21	18.103	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{17}H_{34}O_2$	270	16414947	3.52
22	18.249	Octadecatrienoic acid	$C_{16}H_{32}O_2$	256	7178779	1.54
23	21.488	Celidoniol, Deoxy-	$C_{18}H_{36}O_2$	284	934427	0.20
24	21.847	Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxyme	$C_{21}H_{36}$	288	4096657	0.88
25	22.382	Benzamide, N-(2-Methoxyphenyl)-2-(Pyrrolidine	$C_{16}H_{22}O_4$	278	16390197	3.52
26	24.340	Dibenz[B,F]Oxepin-1,6-Diol, 8-Methoxy-7-Methy	$C_{11}H_6O_4$	202	1686972	0.36
27	24.504	9,10-Anthracenedione, 1,8-dihydroxy-3-methoxy-6-methyl-	$C_{10}H_9N$	143	1181114	0.25
28	24.653	Pentatriacontane	$C_{18}H_{18}O_7$	346	4145750	0.89

29	25.137	Tetracosanoic Acid, Methyl Ester	$C_{18}H_{18}O_7$	346	672756	0.14
30	25.904	Tetratriacontane	$C_{31}H_{50}O_2$	454	1514392	0.32
31	26.304	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethy	$C_{27}H_{56}O$	396	5711976	1.23
32	26.679	1,2,3,5-Tetraisopropylcyclohexane	$C_{29}H_{50}O$	414	901091	0.19
33	27.119	1-Heptacosanol	$C_{27}H_{56}O$	396	19350883	4.15
34	27.490	Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$	410	782042	0.17
35	28.515	Octadecane, 1-Chloro-	$C_{18}H_{37}Cl$	288	2055450	0.44
36	29.437	2,7,8-Trimethyl-2-(4,8,12-Trimethyltridecyl)-6-	$C_{28}H_{48}O_2$	416	3269907	0.70
37	30.083	1-Heptacosanol	$C_{27}H_{56}O$	396	47638716	10.22
38	30.619	Octacosanol trimethylsilyl ether	$C_{31}H_{67}OSI$	482	2129405	0.46
39	30.871	2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecy	$C_{35}H_{60}O_7$	592	16933078	3.63
40	31.915	1-Heptacosanol	$C_{27}H_{56}O$	396	2234463	0.48
41	32.870	Ergost-5-En-3-Ol, (3.Beta.,24R)-	$C_{28}H_{48}O$	400	2523487	0.54
42	33.575	Stigmasterol	$C_{29}H_{48}O$	412	11070152	2.37
43	34.247	1-Heptacosanol	$C_{27}H_{56}O$	396	20960773	4.50
44	35.004	Stigmast-5-En-3-Ol, (3.Beta.)-	$C_{29}H_{50}O$	414	18521587	3.97
45	36.471	Stigmast-7-En-3-Ol, (3.Beta.,5.Alpha.,24S)-	$C_{29}H_{50}O$	414	2392863	0.51
46	37.003	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	$C_{30}H_{50}O$	426	2098134	0.45
47	37.217	Lupeol	$C_{30}H_{50}O$	426	4936379	1.06
48	37.481	D:B-Friedo-B':A'-Neogammacer-5-En-3-One	$C_{30}H_{48}O$	424	1029395	0.22
					466203254	100

Source: - Dr. Duke's Phytochemical and Ethno botanical Databases

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