

FTIR and GCMS Analysis of Bioactive Phytocompounds in Methanolic Leaf Extract of Cassia Alata

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ABSTRACT

The methanolic extract of plant Cassia alata was prepared by using soxhlet apparatus. FTIR and GCMS analysis were done to this plant extract to find out the bioactive phytocompounds. The FTIR results of this plant extract showed 21 peaks indicate the presence of the bioactive compounds such as sulfates, sulfonamides, sulfones, sulfonyl chlorides, sulfates, sulfonamides, alkanes, aromatic, aromatic, alkenes, ester, alkenes, ketenes, isocyanates, isothiocyanates, acetylene, nitrile, phosphine, phosphine, aldehyde, alkane, amide, alcohol and alcohol. The GCMS results showed 13 peaks. The retention time (RT) of all these thirteen peaks indicate the presence of functional group such as 1-Butanol, 3-methyl-1,6-Anhydro-β-D-glucopyranose (levoglucosan), 3-O-Methyl-D-glucose, Oxirane, 10-Methyl-E-11-tridecen-1-ol propionate, L-(+)-Ascorbic acid 2,6-dihexadecanoate, (R)-(-)-14-Methyl-8-hexadecyn-1-ol, Oleic Acid, Vitamin E acetate and 1,2-Bis(trimethylsilyl)benzene.

Keywords: Methanolic extract, Cassia alata, bioactive phytocompounds, functional groups and soxhletion.

Are Mangiferin and Mangiferin-Containing Plant Extracts Helpful for Iron-Loaded Transfusion-Dependent and Non-Transfusion-Dependent Thalassaemia Patients?

INTRODUCTION

Plants play a vital role in our lives. Addition to this, they are mainly used to cure the various human diseases, treatment of ailment and improve the health of affected organs from the period of time immemorial (Sofowora,1993). About 80% of the world's population relies solely or largely on traditional remedies for their healthcare needs. Today, about 70,000 to 80,000 plant species are used for medicinal or aromatic purposes globally. This is because of some biological active and naturally occurring phyto-chemical present in the various parts of plants. Plant produce these chemical compounds as part of their normal metabolic activities to protects their own cells from

environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Gibson, et al, 1998, Mathai et al,2000 and Izhaki, 2002), which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg,1999).Even today, large number of peoples in the developing countries are using the plants and plant based preparations to cure various diseases by their inherent traditional knowledge because they believe the herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway (Zaidan et al., 2005).Addition to this, the side effects associated with synthetic drugs continue to make researchers to look for natural remedies which are safe and effective



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(Gijtenbeek et al, 1999 and Johnson and William, 2002). A single plant may contain a great number of bioactive phytochemicals and a combination of plants even more. This complexity is one of the most important challenges to phytoscientists to identify and sort out which bioactive compounds has the potential to cure which disease. Without screening the active compounds from particular plant, the researcher cannot invent a new medicinal drug to cure particular disease. Hence, in the present investigation, the plant *Cassia alata* have been selected to screen the possible bioactive compounds by GCMS method.

Plant description

Cassia alata belongs to family Fabaceae and sub family Caesalpinioideae is commonly known as King of the forest, emperor's candlesticks, candle bush, candelabra bush, Christmas, Ringworm Bush, Dadrughna, Dadmardan, Dadmari (Daad=Ringworm) Desay, Fleur, Impetigo bush, Ringworm tree, Candelabra bush, Guajava Empress candle plant, Seven Golden Candlestick, and Christmas candle. It is a medium growing, soft wooded medicinal shrub plant reaching up to height of 6-8 feet. This plant is growing in open wastelands near watery places. It is one of the oldest known medicinal plant of Central America, world wide, particularly in Asia Pacific countries where people used it for curing skin diseases, worms, fever, bites of insects, ring worm, goiter, hook worm infestation, sexually transmitted diseases, constipation and other skin diseases, blemishes, scabies, ringworm and other fungal skin infections.

Common names in various countries

Dadmurdan (Hindi), Seemaigathi (Tamil), Simayakatti (Malayalam), Aaku pero (Bosnia and Herzegovina), Akapulko (Philippines), Akapulko (West Africa), Akoria (West Africa), Awunwon (West Africa), Ayengogo (Guinea), Bai nicagi (Guinea), Bakua (Guinea), Balilang (Malaysia), Barajo (Guatemala), Candelabra bush (Thailand), Candle tree (Malaysia), Christmas blossom (Nicaragua), Chumhet yai (Thailand), Dadmardan (India), Cortalinde (Guinea-Bissau), Dadmurdan (Fiji), Dadrughna (India), Galinggang hutan (Indonesia), Gelenggang (Malaysia), Gelenggang (Indonesia), Grili (Papua New Guinea), Kabaiura (Papua New Guinea), Ketapeng (Indonesia), King of the Forest (Jamaica),

Kinkeliba (Gabon), Kislin (Nicaragua), La au fai lafa (Nicaragua), Maliof (Papua-New Guinea), Mata pasto (Brazil), Mhingu (Tanzania), Mongrang-jangtong (India), Mulu mula (India), Mulu mulu (Papua), Njepaa (Sierra Leone), Okpo Ndichi (Sierra Leone), Palotsina (Philippines), Pui-chi (Bangladesh), Qanabisi (Nicaragua), Ringworm bush (Fiji, Guyana, West India), Ringworm cassia (Malaysia), Roman candle tree (Fiji), Sengseng (India), Serocontil (Nicaragua), Sindjo-el (Guinea-Bissau), Sussaika (Nicaragua), Sustara saika (Nicaragua), Suswaha tara (Nicaragua), Tarantan (West Indies), Telelango (West Indies), Totoncaxihuitl (Mexico), Wasemu (Papua), Wild senna (West Indies).

Cassification

Kingdom: Plantae
Order: Fabales
Family: Fabaceae
Subfamily: Caesalpinioideae
Tribe: Cassieae
Subtribe: Cassiinae
Genus: *Senna*
Species: *Senna alata*

Distribution

This plant is mostly found in tropical and subtropical regions. i.e., India, Pakistan, Burma, Sri Lanka, Philippines and most of the African countries.

Habitats

They are grown in rather open vegetation such as roadsides, river banks, rain forest edges, lake shores, pond and ditch margins, open forest, orchards and around villages, at elevations up to 1,400 metres, occur 2,100 metres and fast-growing, but short-lived plant. This plant is mostly found in moister areas in the tropics. It is reported to tolerate a mean annual rainfall of 600 - 4,300 mm and average yearly temperatures of 15 - 30°C, grow well in well drained both heavy and sandy, acid to slightly alkaline, soil of a sunny position, wastelands, flood plains, highly adaptable, tolerating both drought and waterlogged soils.

Morphology

This plant is erect shrub, able to grow up to 3 - 4 m tall. Foliage contains pinnately compound leaves with 6 - 12 pairs of leaflets (30 - 60 cm long).

Leaflets are oblong, smooth and thinly leathery (6 - 15 cm long, 3.5 - 7.5 cm wide) in appearance. They have a rounded tip with a slight indentation in the middle. The flowers are arranged in a vertical column and bloom from the base of the column. The inflorescence is raceme type, resembles a lit, yellow candle, because the flowers at the base are yellow, while the unopened flower buds at the top are covered by orange bracts. Fruits are in the form of winged pods with dark purple to black colour, smooth and 4-sided. Each pod contain 50 - 60 flattened, triangular to squarish seeds.

Medicinal uses

In rural Tamilnadu, this plant is used to treat various skin diseases caused by bacteria and fungal infection and by insect bite. *Cassia alata* is widely used as traditional medicine in India and Southeast Asia (Reezal et al., 2002). This plant is reported to possess insecticidal, anti-inflammatory, hydragogue, sudorific, diuretic, pesticidal properties. Fresh leaves juice is used for ring worm, snakebite, scorpion bite, skin diseases, impetigo, syphilis sores, itching, mycosis (washerman's itch), herpes and eczema. Roots, leaves and flowers of this plant possess many biological properties such as antibacterial, antifungal, anti-inflammatory, antitumor, expectorant and also useful in urinary tract problems (Quattrocchi, 2012). The leaves have been reported to be useful in the treatment of convulsions, gonorrhoea, heart failure, abdominal pains, oedema and also The leaves

have been reported to be useful in the treatment of convulsions, gonorrhoea, heart failure, abdominal pains, oedema and also as a purgative (Ogunti and Elujoba, 1993). *Cassia alata* has been reported to contain anthraquinones and the methanol fractions were found to be active against *Aspergillus flavus* (Ogunti and Elujoba, 1993 and Owoyale et al, 2005).

MATERIALS AND METHOD

Shade dried leaves were ground well by using mixer grinder to get fine powder. This powder was stored in a air tight contained for later usage. 25 grams of this powder was packed in the thimble of the soxhlet extractor and the methanol was loaded into the distillation flask. The leaf extract obtained at the flask finally collected after the completion of soxhlation. This methanol extract was taken to the FTIR and GCMS analysis.

FTIR and GCMS Analysis

FTIR analysis was done by the instrument FT/IR-6300 type, S.No-A021261024 BMDLABS Company in standard light sources by using TGS detector at resolution 4 cm^{-1} . The GCMS analysis was done by GCMS- Perkin Elmer.

The bioactive compound of methanol extract of leaves of plant *Cassia alata* was traced out by FTIR spectrophotometer (Thermo electron Scientific). Totally 21 peaks were obtained for the

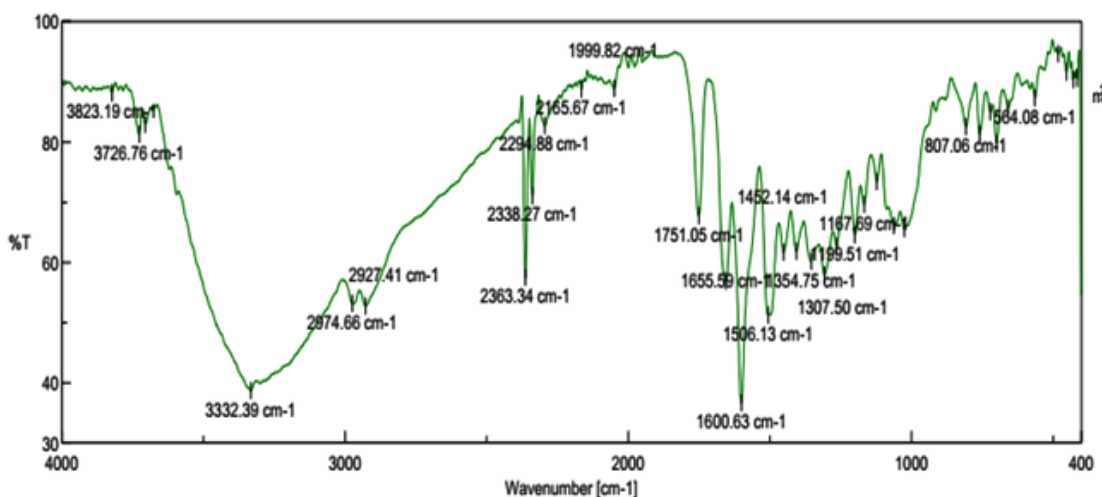


Fig. 1: FTIR Spectra of methanol extract of leaf of *Cassia alata*

FTIR analysis of *Cassia alata* methanol extract. The IR spectrum of *Cassia alata* sample showed an absorption band at 564.08 cm^{-1} which corresponds to C-Cl stretching showed the presence of alkyl halides, band at 807.06 cm^{-1} is C-H stretching exhibit the presence of , aromatic, bands at 1167.69 cm^{-1} , 1199.51 cm^{-1} , 1307.50 cm^{-1} , 1354.75 cm^{-1} , 1452.14 cm^{-1} , 1506.13 cm^{-1} , 1600.63 cm^{-1} , 1655.59 cm^{-1} , 1751.05 cm^{-1} , 1999.82 cm^{-1} , 2165.67 cm^{-1} , 2294.88 cm^{-1} , 2338.27 cm^{-1} , 2363.34 cm^{-1} , 2927.41 cm^{-1} , 2974.66 cm^{-1} , 3332.39 cm^{-1} , 3726.76 cm^{-1} and 3823.19 cm^{-1} indicate the presence of bioactive compounds such as sulfones, sulfonyl, chlorides, sulfates, sulfonamides, sulfones, sulfonyl chlorides, sulfates, sulfonamides, alkanes, aromatic, aromatic, alkenes, ester, alkenes, ketenes, isocyanates, isothiocyanates, acetylene, nitrile, phosphine,

phosphine, aldehyde, alkane, amide, alcohol and alcohol.

GC-MS chromatogram of the ethanol leaf extract of *Cassia alata* (Figure 1) showed 13 peaks indicating the presence of thirteen compounds with the retention time range between 2.72 and 34.54 (Figure 1). The active principles in the methanol leaf extract of *Cassia alata* were confirmed based on retention time (RT), molecular formula, molecular weight (MW) and structures. The phytochemical compounds with their retention time (RT), molecular formula, molecular weight (MW) and structures are presented in Table 1. The first compound identified with less retention time (4.181min) was 1-Butanol, 3-methyl-, whereas 1,2-is(trimethylsilyl)benzene, was the last compound which took longest retention

Table 1: Peak values, band type and functional group for FTIR Spectra of methanol leaf extract of *Cassia alata*

S. No	Frequency (cm^{-1})	Types of compound	Bond responsible	Intensity
1	564.08	Alkyl halides	C-Cl or C-Br	Strong
2	807.06	Aromatic	C-H	Strong
3	1167.69	Sulfones, Sulfonyl Chlorides, Sulfates, Sulfonamides	S=O	Strong
4	1199.51	Sulfones, Sulfonyl Chlorides, Sulfates, Sulfonamides	S=O	Strong
5	1307.5	Alkanes	C-H	Strong
6	1354.75	Aromatic	C=C	Strong
7	1452.14	Aromatic	C=C	Medium to weak
8	1506.13	Alkenes	C-C	Medium to weak
9	1600.63	Ester	C-O	Variable two bonds
10	1655.59	Allenes, Ketenes, Isocyanates, Isothiocyanates	X=C=Y	Strong
11	1751.05	Acetylene, nitrile	C=N, C=C	Medium to Strong
12	1999.82	Phosphine	P-H	Strong
13	2165.67	Phosphine	P-H	Medium
14	2294.88	Aldehyde,	C-H	Medium
15	2338.27	Alkane	C-H	Weak
16	2363.34	Amide	N-H	Medium to strong
17	2927.41	Alkanes	C-H	strong
18	2974.66	Alkanes	C-H	Strong
19	3332.39	Carboxylic acids	O-H	Medium
20	3726.76	Alcohol	O-H	Strong and broad
21	3823.19	Alcohol	O-H	Strong and broad

time (16.055min) to identify. The phyto chemicals identified through GC-MS analysis of methanol leaf extract of *Cassia alata* showed many biological activities relevant to this study are listed in Table 2.2.

The present a GC-MS analysis result of *C. alata* leaves all thirteen compounds possess many biological properties. For instance, Oleic acid shows some beneficial effect on cancer, autoimmune and inflammatory diseases, besides its ability to facilitate wound healing and may improve the immune response associated to a more successful elimination of pathogens such as bacteria and fungi, by interfering in many components of immune system such as macrophages, lymphocytes and neutrophils (Sales-Campos et al, 2013). Vitamine E acetate at retention time 14.709 (RT) is a potent antioxidant compound. It exhibits antioxidant activity by virtue of the phenolic hydrogen on the 2H-1-benzopyran-6-ol nucleus. It has four methyl groups on the 6-chromanol nucleus. The natural d form of alpha-tocopherol is more active than its synthetic dl-alpha-tocopherol racemic mixture. 10-Methyl-E-11-tridecen-1-ol propionate obtained

at retention time 10.607(RT) was also identified in GSMS study of *Premna serratifolia* by Vasantha and Maruthasalam (2015) has no any remarkable bioactivity. l-(+)-Ascorbic acid 2,6-dihexadecanoate observed at retention time 10.176 (RT) shows Antioxidant, antiscorbutic, antiinflammatory, antinociceptive, anti- mutagenic, wound healing property (Vasthi Gnana Rani and Murugaiah, 2015). The bioactive compound oxirane observed at retention time 9.669,9.783 and 9.841 exhibited baccidal fungicidal and sporicidal activities. Its is also used as effective antimicrobial agent to control a variety of micro organisms including virus and also used as sterilizing agent (Pub-chem,2004). ,6-Anhydrohexopyranoses observed at 7.672min have proven to be valuable synthons for the preparation of biologically important and structurally diverse products such as rifamycin S, indanomycin, thromboxane B2, (+)-biotin, tetrodotoxin, quinone, macrolide antibiotics and modified sugars. phytocompound 1,2-Bis(trimethylsilyl)benzene at retention time 15.651 and 16.055 min has antioxidant, antimicrobial, anticanserous and antitumerous activity (Alok prakash and Suneetha,2014)

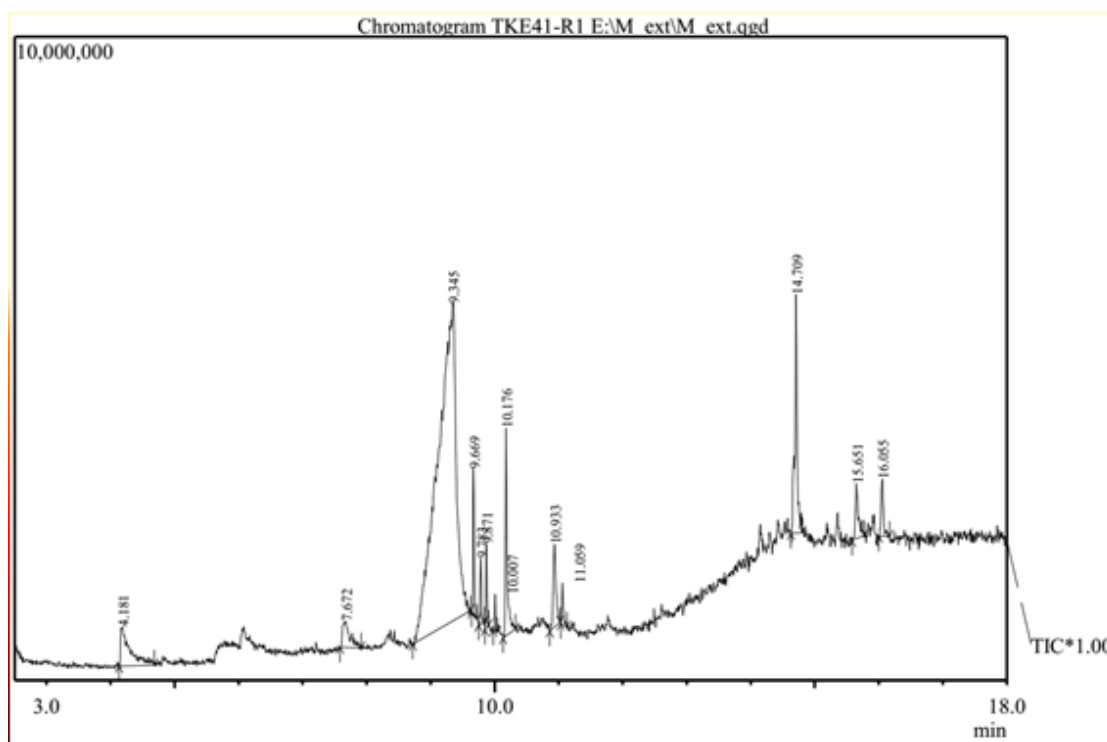


Fig. 2: GC-MS Chromatogram of methanolic leaf extract of *Cassia alata*

Table 2: Bioactive compounds identified in the methanol leaf extract of *Cassia alata*

S. No	Name of the Compound	Molecular Formula	MW	RT	% Peak area	Structure
1	1-Butanol, 3-methyl-,	C ₆ H ₁₂ O ₂	116	4.181	4.34	
2	1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)	C ₆ H ₁₀ O ₅	162	7.672	2.14	
3	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	194	9.345	70.69	
4	Oxirane,	C ₁₈ H ₃₆ O	268	9.669	2.00	
5	Oxirane	C ₁₈ H ₃₆ O	268	9.783	0.95	
6	Oxirane,	C ₁₈ H ₃₆ O	268	9.841	1.25	
7	10-Methyl-E-11-tridecen-1-ol propionate	C ₁₇ H ₃₂ O ₂	268	10.607	0.46	
8	l-(+)-Ascorbic acid 2,6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	652	10.176	4.39	
9	(R)-(-)-14-Methyl-8-hexadecyn-1-ol,	C ₁₇ H ₃₂ O	252	10.933	2.75	
10	Oleic Acid ,	C ₁₈ H ₃₄ O ₂	282	11.059	0.84	
11	Vitamin E acetate,	C ₃₁ H ₅₂ O ₃	472	14.709	6.65	
12	1,2-Bis(trimethylsilyl)benzene,	C ₁₂ H ₂₂ Si ₂	222	15.651	2.05	
13	1,2-Bis(trimethylsilyl)benzene,	C ₁₂ H ₂₂ Si ₂	222	16.055	1.58	

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