

GC-MS ANALYSIS OF *Caesalpinia bonducella*

P. EZHILARASAN¹, S. SIVAKRISHNAN^{1*}, S.VIGIL ANBIAH²

1: M.Pharm (Pharmacology), Department of Pharmacy, FEAT, Annamalai University

1*: Assistant Professor, Department of Pharmacy, FEAT, Annamalai University

2: Associate Professor and Head i/c, Central Animal House, Rajah Muthiah Medical College,
Annamalai University, AnnamalaiNagar-608002

*Corresponding Author: E Mail: sivacdm82@gmail.com

Received 22nd July 2019; Revised 7th Oct. 2019; Accepted 7th Jan. 2020; Available online 1st June 2020

<https://doi.org/10.31032/IJBPAS/2020/9.6.5075>

ABSTRACT

Use of plants as a source of medicine has been inherited and is an important component of the health care system. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. *Caesalpinia bonducella* is an important medicinal plant for its traditional uses against different types of diseases. In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species. This purpose of this study was to evaluate the GC-MS analysis of ethanolic extract of leaves of *Caesalpinia bonducella*. The leaves of *Caesalpinia bonducella* were collected from puthupalayam village, Villupuram District, Tamil Nadu, India. The extract was prepared from ethanol by hot continuous percolation method in soxhlet apparatus for 24 hours and evaluate the phytochemical activity of ethanolic extract of seeds of *Caesalpinia bonducella* and identification of active biochemical constituents using Perkin-Elmer Gas Chromatography-Mass Spectroscopy. The GC-MS analysis of ethanolic extract of seeds of *Caesalpinia bonducella* showed 17 bioactive compounds, the highest compound was named as 2,3-Di-O-Methyl-D-Xylopyranose (31.842%) and lowest was named as 2,4,4-Trimethyl-3-

Hydroxymethyl-5A-(3-Methyl-But-2-Enyl)-Cyclohexene (0.817%). In the present study, we concluded that the GC–MS analysis of the ethanolic extract of leaves of *Caesalpinia bonducella* shows various bioactive constituents and it will be helpful to treat various disorders.

Keywords: Medicinal Plants, GC-MS, *Caesalpinia bonducella* , Phytol, Ethanol, Leaves

INTRODUCTION

These medicinal plants are used to treatment for variety of diseases without any serious side effects. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines. *Caesalpinia bonducella* L. (Family: Fabaceae) is an important medicinal plant, which is widely distributed in the tropical and subtropical regions of Asia and the Caribbean.[1] In Bangladesh, this plant is abundant in forests and village thickets of Dhaka, Chittagong, Khulna, Tangail, and North Bengal. A large straggling, very thorny shrub, branches armed with hooks and straight hard yellow prickles; leaves bipinnate, large, stipules, foliaceous, pinnae 7 pairs, leaflets 3-8 pairs with 1-2 small recurved prickles between them on the underside; flowers yellow, in dense long peduncled supra-axillary racemes at the top; fruits inflated pods, covered with wiry prickles, seeds 1-2 per pod, oblong or

globulat, hard, grey with a smooth shiny surface. The plant is known as Nata in Bengali and fever nut or nicker nut in English. Different parts of the plant have extensive uses in folk medicines for the treatment of a variety of diseases. [2, 3] Seeds contain stearic, palmitic, octadeca-4, octadeca-2, ligoceric, linolenic and oleic acids. They also contain bonducellin, arginine, citrulline and aspartic acid. The whole plant of *Caesalpinia bonducella* are used as a medicine. The leaves and the juice or paste is used to relieving smallpox, elephantiasis, liver diseases and for eliminating bad odour in perspiration. It is also used for reducing toothache. *Bonducella* has febrifuge, antiperiodic, anthelmitic and tonic properties. Pods are roasted and the powder is used as a substitute for quinine. The root bark has been used for relieving intestinal worms, fevers, tumors, cough, amenorrhea and to remove placenta after childbirth. The flower tastes bitter and induce warming effect on the body. It improves the balance of vata and kapha [4] and the ash is useful in ascites. Fruits of *Caesalpinia*

bonducella used for treating piles, haemorrhoids, [5] wounds [6], leucorrhoea and urinary disorders. It also produces heat in the body, shows astringent activity on bowels and is known as an aphrodisiac. Boiled leaves can be used for gargling to relieve sore throat. The seeds have astringent properties and are being used to relieve inflammation, contagious disease, skin diseases [7], hydrocele, colic and leprosy. Seed sprouts are useful in relieving tumours. Leaves and seeds after roasting in castor oil can be applied to reduce piles, inflammatory swellings orchitis and hydrocele. It has been reported that seeds of the plant possess antidiarrhoeal, antiviral, antibacterial, antimicrobial, antifungal, antidiabetic, antitumor, antipyretic and analgesic,

antifilarial, anxiolytic, anti-inflammatory, antioxidant, immunomodulatory, adaptogenic, anticonvulsant, antispasmodic, nootropic, antifeedant, antiamoebic, antiestrogenic, diuretic, insecticidal as well as trypsin and chymotrypsin inhibitor properties [8-12]. Most of herbal medicines and their derivative products were often prepared from crude plant extracts, which comprise a complex mixture of different phytochemical constituents. The chemical features of these constituents differ considerably among different species. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetics, drugs, pharmaceutical or food industries.

Table 1: Taxonomy of *Caesalpinia bonducella*

Scientific Classification	
Kingdom	Plantae
Phylum	Magnoliophyta
Division	Magnoliopsida
Class	Angiospermae
Order	Fabales
Family	Fabaceae
Genus	Caesalpinia
Species	bonduc
Synonyms	
English Name	Fever nut, bonduc nut, nicker nut and nicker seed
Tamil Name	Kalarci ver, Kalarci Koluntu, Kalarci paruppu, Kazharchikkaai, Kalichikai and Kazarci.
Hindi Name	Kantkarej, Kantikaranja and Sagar Gota.
Sanskrit Name	Kakachika, Kantakikaranja, Kuberaksi, Latakaranja, Prakirnah and Putikaranja
Bengali Name	Nata
Malayalam Name	Ban-karetti, Kaka-moullou, Kazhanji, Kalanci and Kajanchikkur
Telugu Name	Mulluthige and Gaccakayai
Kannada Name	Gajjiga, Kiri gejjuga and Gajikekayi

MATERIALS AND METHODS

Collection and Identification of *Caesalpinia bonducella*

The leaves of *Caesalpinia bonducella* were collected from puthupalayam village, Villupuram District, Tamil Nadu, India. They were authenticated by Dr. N. Srinivasan., M.Pharm (Pharmacognosy), Assistant Professor, Department of Pharmacy, FEAT, Annamalai University, Annamalai Nagar, Chidambaram.

Extraction and Isolation of *Drynaria Quercifolia*

The leaves of *Caesalpinia bonducella* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve [13-15]. The powdered plant materials were stored in an air-tight container. The above powdered materials were successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus for 24 hours [16, 17]. The extract was concentrated by using a rotary evaporator [18, 19] and subjected to freeze drying in a lyophilizer [20] till dry powder was obtained.

Gas Chromatography - Mass Spectroscopy Analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95%

dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 230 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components [23] were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library (Table 2).

Identification of phytocomponents

Analysis on mass-spectrum GC-MS [24, 25] was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was correlated with the spectrum of known components stored in the NIST library. The structure, name and molecular weight of the components of the test materials were confirmed. [26]

Table 2: GC-MS Information

Instrument information		Acquisition parameters		Mass condition (EI)	
Make	Perkin Elmer	Oven	Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min,	Solvent Delay	2.00 min
GC-model	clarus 680	Total Run Time	32.00 mins	Transfer Temperature	230°C
Mass Spectrometer	clarus 600 (EI)	Inj.auto Volume Split	260°C 1 µL 10:1	Source Temperature	230°C
Software	TurboMassver 5.4.2	Flow Rate Carrier Gas	1 mL/mins Helium	Scan	50 to 600Da
Library year	NIST-2008	Column	Elite-5MS (30.0m, 0.25mmID, 250µm df)		

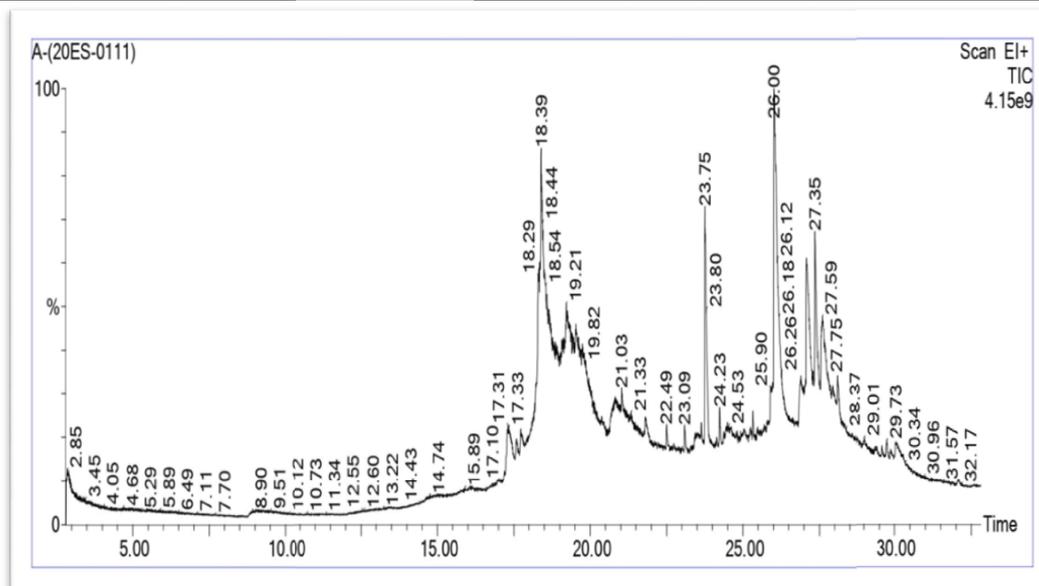
RESULTS AND DISCUSSION

GC-MS analysis of ethanolic extract of leaves of *Caesalpinia bonducella* revealed various compounds with help of NIST library [27]. Totally 17 most abundant compounds with their retention time, molecular formula, molecular weight, peak area and applications were listed in **Table 3** and GC-MS chromatogram of the seventeen peak of the compounds detected was shown in **Figure 1**. The ethanolic extract of seeds of *Caesalpinia bonducella* showed 17 bioactive compounds namely, Phytol, 2,3-Di-O-Methyl-D-Xylopyranose, Alpha.-D-Mannofuranoside, 1-Nonyl --, Hexadecanoic acid, 1-(Hydroxymethyl) - 1,2-Ethanediy ester, Undecanoic acid, Octadecanoic acid, Sulfurous acid, Octadecyl 2-Propyl ester, 11-Eicosenoic acid, Trimethylsilyl ester, 2, 6,

10, 14, 18, 22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E)-, Pregna-1,4-Diene-3, 20-Dione, 11, 22-Diacetoxy-16, 17-Propylidenedioxy-, 3 - (1-Acetyl-2-OxoPropylsulfanyl) - 3, 3 - Difluoro - 2 -Trifluoromethyl - Propionic acid methyl ester, 4, 4, 6A, 6B, 8A, 11, 11, 14B - Octamethyl 1, 4, 4A, 5,6, 6A, 6B,7,8, 8A,9,10, 11,12, 12A, 14, 14A, 14B - Octadecahydro - 2, URS-12-EN-3-OL, Acetate,(3.BETA.)- and 2, 4, 4-Trimethyl - 3-Hydroxymethyl - 5A- (3-Methyl-But-2-Enyl) - Cyclohexene. The GC-MS of ethanolic extract of *Caesalpinia bonducella* shows a more number of active constituents. These active principles were used as various pharmacologic properties, cosmetic preparations and also used as a energy sources.

Table 3: Phytochemicals identified in ethanolic extract of leaves of *Caesalpinia bonducella*

S. No	RT	Compound Name	Mol. Formula	Mol Wt	%Peak Area	Applications
1.	17.309	Phytol	C ₂₀ H ₄₀ O	296	2.178	Commonly used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. Anti-inflammatory, Antimicrobial, Anti-inflammatory
4.	18.395	2,3-Di-O-Methyl-D-Xylopyranose	C ₇ H ₁₄ O ₅	178	31.842	Antioxidant
5.	19.215	Alpha.-D-Mannofuranoside, 1-Nonyl--	C ₁₅ H ₃₀ O ₆	306	13.995	Not reported
6.	19.525	Hexadecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediy ester [28]	C ₃₅ H ₆₈ O ₅	568	15.403	Exhibits antioxidant, hypocholesterolemic, antiandrogenic, hemolytic and alpha reductase inhibitor activity
7.	20.375	Undecanoic acid	C ₁₁ H ₂₂ O ₂	186	1.113	Antifungal agent, to treat ringworm and athlete's foot
8.	20.816	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	3.252	commonly used in the production of detergents, soaps, and cosmetics, such as shampoos and shaving cream product and also used as an antifungal, antitumor, and antibacterial
9.	21.026	Sulfurous acid, Octadecyl 2-Propyl ester	C ₂₁ H ₄₄ O ₃ S	376	2.162	Antibacterial
10.	21.331	11-Eicosenoic acid, Trimethylsilyl ester	C ₂₃ H ₄₆ O ₂ Si	382	1.357	surfactant, emulsifier, nutrient, membrane stabilizer, energy source and storage.
11.	23.747	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E)-	C ₃₀ H ₅₀	410	3.894	Anticancer, antioxidant, drug carrier, detoxifier, skin hydrating, and emollient activities.
13.	26.893	Pregna-1,4-Diene-3,20-Dione, 11,22-Diacetoxy-16,17-Propylidenedioxy-	C ₂₈ H ₃₆ O ₈	500	1.223	Not reported
14.	27.068	3-(1-Acetyl-2-Oxo-Propylsulfanyl)-3,3-Difluoro-2-Trifluoromethyl-Propionic acid Methyl ester	C ₁₀ H ₁₁ O ₄ F ₅ S	322	4.035	Not reported
15.	27.348	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadecahydro-2	C ₃₀ H ₄₈ O	424	2.220	Not reported
16.	27.588	URS-12-EN-3-OL, Acetate, (3.BETA.)-	C ₃₂ H ₅₂ O ₂	468	1.918	Not reported
17.	30.054	2,4,4-Trimethyl-3-Hydroxymethyl-5A-(3-Methyl-But-2-Enyl)-Cyclohexene	C ₁₅ H ₂₆ O ₂	222	0.817	Antibacterial, Antioxidant [18]

Figure 1: GC-MS Chromatogram of ethanolic extract of leaves of *Caesalpinia bonducella*

CONCLUSION

The GC-MS study on the leaves of *Caesalpinia bonducella* revealed the presence of various bio-active compounds. From this study it can be concluded that the *Caesalpinia bonducella* may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds. These results further support the traditional use of this plant in medicine. This preliminary study gives an idea to isolate the major active constituents present in the plant and also helps to develop potential pharmacologically active compounds.

FINANCIAL SUPPORT

None.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

All the authors contributed equally to the paper.

REFERENCES

- [1] Asolkar LV, Kakkar KK, Chakre OJ, *Second Suppl.* Part 1, To glossary of Indian medicinal plants with active principles, 1992, New Delhi: PID-CSIR, 150
- [2] Nazeerullah K, Sunil K, Pal SR, Neelam D, A Pharmacognostic and

pharmacological overview on *Caesalpinia bonducella*, Res J Pharma, Biol and Chem Sci, 3, 2012, 480-496.

- [3] Moon K, Khadabadi SS, Deokate UA, Deore SL, *Caesalpinia bonducella* F- An overview, Report and Opinion, 2, 2010, 83-90.
- [4] S. Sivakrishnan and J.Kavitha, Herbal Drugs, Diet and Lifestyle Changes for Liver Diseases- A Review, Journal of Global Pharma Technology, 11(03), 2019, 12-21.
- [5] M. Swamivelmanickam and S. Sivakrishnan, Ethnopharmacology of Boraginaceae Family- An Update, Journal of Global pharma Technology, 11 (9), 2019, 10-24.
- [6] S. Sivakrishnan and M Swamivelmanickam, A comprehensive review of hepatotoxins, International Research Journal of Pharmacy, 6(10), 2019, 1-4.
- [7] Sivakrishnan S and Swamivelmanickam M, A comprehensive review of *Albizia procera* (Roxb.) Benth.-An update, Int J Pharm Sci & Res, 10(9), 2019, 4129-4144.
- [8] Kshirsagar Sunil N, Nootropic activity of dried seed kernels of *Caesalpinia crista* Linn against scopolamine induced amnesia in

- mice, Int J Pharma Tech Res, 3, 2011, 104-109.
- [9] Emmanuel N, Swaran D, Biological effects of *Caesalpinia crista* seed extracts on *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its predator, *Coccinella septempunctata* (Coleoptera: Coccinellidae), J Asia-Pacific Entomol. 9, 2006, 159-164.
- [10] Raghunathan K, Mitra R, Pharmacognosy of indigenous drugs, part-I. Edited by: Raghunathan K, Mitra R, New Delhi: Central Council for Research in Ayurveda Siddha, 1982, 484-510.
- [11] Khedkar A, Mandavkar YD, Shinde G, Khalure P, Pravin D, Diuretic effect of *Caesalpinia bonduca* in rats, Bangladesh J Pharmacol, 6, 2011, 61-63.
- [12] Arindam B, Shruti R, Babu CR, A trypsin and chymotrypsin inhibitor from *Caesalpinia bonduca* seeds: Isolation, partial characterization and insecticidal properties, Plant Physiol and Biochem, 45, 2007, 169-177.
- [13] Sivakrishnan S and Kottaimuthu A, Phytochemical Evaluation of Ethanolic Extract of Aerial Parts of *Albizia procera*, British Biomedical Bulletin, 2(1), 2014, 235-241.
- [14] S. Sivakrishnan and A. Kottai Muthu, *In Vitro* free radical scavenging activity of aerial parts of ethanolic extract of *Albizia procera* (Family: Mimosoideae), Int. J of Pharm. and Pharmaceu. Sci., 5(3), 2013, 352-354.
- [15] S.Sivakrishnan, J.Kavitha and A. Kottai Muthu, Antioxidant potential, total phenolic and flavonoids content of aerial parts of ethanolic extract of *Albizia procera* (Family: Mimosoideae), Asian Journal of pharmaceutical and Clinical Research, 6(1), 2013,108-110.
- [16] S.Sivakrishnan and A.KottaiMuthu, *In vivo* Antioxidant Activity of ethanolic extract of aerial parts of *Albizia procera* roxb (benth.) against paracetamol induced liver toxicity on wistar rats, Journal of Pharmaceutical Science and Research, 5(9), 2013, 174 – 177.
- [17] S.Sivakrishnan and G.Veeramani, Phytochemical activity of aerial parts of *Cordia obliqua* Willd, Int. Res. J. Pharm, 10(7), 2019, 94-98.
- [18] M. Swamivelmanickam and S. Sivakrishnan, Antioxidant Potential,

- Total Phenolic and Flavonoids Content of Aerial Parts of Ethanolic Extract of *Cordia Obliqua*, Journal of Global Pharma Technology, 11(8), 2019, 5-7.
- [19] G.Veeramani, S.Sivakrishnan and A.Kottaimuthu, Isolation of Active Compounds from Aerial Parts of *Albizia procera*, Journal of Global pharma Technology, 11(3), 2019,1-6.
- [20] A. Kottaimuthu and S.Sivakrishnan, Evaluation of hepatoprotective activity of squalene isolated from *Albizia procera* against paracetamol induced hepatotoxicity on wistar rats, World journal of pharmacy and pharmaceutical sciences, 3(3), 2014, 1351-1362.
- [21] Sivakrishnan S, Kottaimuthu A and Kavitha J, GC-MS analysis of ethanolic extract of aerial parts of *Albizia procera* (Roxb.) Benth, Int. J. Pharmacy and Pharm. Sciences, 5, 2013, 702-704.
- [22] Sivakrishnan S and Pradeepraj D, "Gas chromatography – mass spectroscopy analysis of ethanolic extract of leaves of *cordia obliqua willd*", Asian Journal of Pharmaceutical and Clinical Research, 12(6), 2019, 110-112.
- [23] Ronald Hites A, Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry, 1997, 609-611.
- [24] Jayapriya G, Gricilda Shoba F, GC-MS analysis of bioactive compounds in methanolic leaf extracts of *justicia adhatoda Linn*, Journal of Pharmacognosy and Phytochemistry, 4(1), 2015,113-1176.
- [25] Kanthal LK, Dey A., Satyavathi, K., Bhojaraju, GC-MS analysis of bioactive compounds in methanolic extract of *lactuca runcinata* DC, Pharmacognosy Res, 6(1), 2015, 58–61.
- [26] Ezhilan BP, Neelamegam R, GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense L*, Phcog Res, 4(1), 2012, 4-11.
- [27] Mass Spectrometry Data Centre, Eight peak index of mass spectra: The eight most abundant ions in 31,101 mass spectra, indexed by molecular weight, elemental

composition and most abundant ion (4 volume set). 2nd edition, Alder maston: Mass Spectrometry Data Centre, 1974.

[28] B Perumal Ezhilan and Ramasamy Neelamegam, GC-MS analysis of phytochemicals in the ethanol extract of *Polygonum chinense* L, Pharmacognosy Res, 4(1), 2012, 11–14.

[29] Sunita Arora and Ganesh Kumar, Gas Chromatography - Mass Spectrometry (GC - MS) determination of bioactive constituents from the methanolic and ethyl acetate extract of *Cenchrus setigerus Vahl* (Poaceae), The Pharma Innovation Journal, 6(11), 2017, 635-640.