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Determination of bioactive compounds using GCMS and evaluation of antioxidant activity of *Caesalpinia bonducella* seeds kernel

M Hasen Mothi, G Senthil Kumar
Department of Microbiology, AVVM Sri Pushpam College, (Affiliated to Bharathidasan University), Poondi, Thanjavur, Tamil Nadu, India

Abstract
The aim of the study was to investigate the phytochemical constituents present in *Caesalpinia bonducella* seeds kernel (CBSK) extract using Gas chromatography and mass spectrum analysis and evaluate the antioxidant activity. Twenty-nine phytochemical compounds identified Gas chromatography and mass spectrum analysis. The important phytoconstituents were 3-O-Methyl-d-glucose -12.39 (16.89%), 17-(1-acetoxy-ethyl)-10,13-dimethyl-3-oxo 2, 3, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-dodecahedron-1H-cyclopenta [a] phenanthrene-11-yl(ester) (17.03%), Acetic acid, α-D-Glucopyranoside, O-α-D-glucopyranosyl -(1. fwdarw.3) -β-D-fructofuranosyl-9.41 (13.92%), 5, 16, 20-Pregnatriene-3beta, 20-diol diacetate-29.08 (7.22%), 8, 11, 14-Eicosatrienoic acid, (Z,Z,Z) -22.92 (6.05%). Antioxidant activity was expressed as ascorbic acid corresponding antioxidant capacity (AEAC). Gas chromatography and mass spectrum helps to predict the structure and formula of phytocompounds in *Caesalpinia bonducella* seeds kernel which can be used as herbal medicines for various ailments and *Caesalpinia bonducella* seeds kernel has good antioxidant activity.

Keywords: *Caesalpinia bonducella*, GC-Ms, antioxidant activity

Introduction
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 (Sarma and Das, 2009) [1]. *In vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigation. It has been reported to have multiple remedial Parcels similar as an analgesic, anti-inflammatory, antioxidant, antipyretic, antidiarrheal (Iyengar and Pendse, 1965) [2], antidiabetic, antiestrogenic (Raghunanathan and Mitra, 1982) [3], antiviral, antibacterial, immunomodulatory, antifilarial and hepatoprotective (Dayanand and Kumar, 2011) [4]. Plant produces these substances to guard itself but current research proves that emphasizes the plant source of most of these defensive, disease-preventing compounds (Chakrabarti *et al*., 2003) [5]. Oxidative stress is an imbalance condition between antioxidant defense and ROS production. Any natural or artificial compound with antioxidant possessions might donate toward the partial or total mitigation of this type of damage (Halliwell and Gutteridge, 1999) [6]. Antioxidants work by transporting under control the rogue and unbalanced oxygen particles that have an odd number of electrons. The antioxidants effort in agreement and the usefulness of one antioxidant be depending upon the accessibility and attentiveness of alternative. Fundamentally, antioxidants effort by contributing an electron to the uneven free radical. This alleviates the free radical and changes it into a inoffensive composite that may safely be detached from the body (Ozsoy *et al.*, 2008) [7]. Free radicals are designed from particles via the breakage of a biochemical bond such that each piece keeps one electron, by cleavage of a essential to give another radical and, also via redox responses. The aim of this study is to regulate the phyto compounds and antioxidant activity present in the *C. bonducella* seed kernel extract.

Pods and Seeds Kernel

Fig 1: *Caesalpinia bonducella* L.

Materials and Method

Plant material Collection

The fresh dried CBSK was collected from a local Country shop at Thanjavur, Tamil Nadu, India.

Plant Material Preparation

The CBSK were washed to void the stuck distant measureable and were washed under valve water, air dehydrated, homogenized to fine grease paint using the electrical blender. The powdered material was kept in hermetically sealed bottles until use.

Preparation of the extract

The air-dried CBSK (50 g) were pulverized and also uprooted with 500 ml of ethanol by using a soxhlet outfit. The crude excerpt was filtered using No. 1 Whatman sludge and the excerpt was dried in a vacuum rota-vapour. This crude excerpt was dissolved in detergent and used for the assessment of antioxidant exertion and GC-MS analysis to find the bioactive factors.

GCMS analysis

Gas chromatography and mass spectrum analysis was done by the Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India. The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 (5 Diphenyl/ 95 Dimethyl polysiloxane), 30 mx0.25 nm ID x 10.25 µm df) and the factors were separated using Helium as carrier gas at a constant inflow of 1 mL/ min. The 1µL sample excerpt fitted into the instrument was detected by the Turbo gold mass sensor (Perkin Elmer) with the aid of the Turbo mass5.1 software. During the 36th nanosecond GC birth process, the roaster was maintained at a temperature of 1100C with3.50 twinkles holding. The injector temperature was set at 2800 C (mass analyser). Interpretation of Mass-Spectrum was carried out by using the database of National Institute Standard and Technology (NIST) having further than patterns (Table1).

Determination of antioxidant activity by (1-1-diphenyl 2-picryl hydroxyl-DPPH) radical-scavenging activity

The free revolutionary-scavenging exertion of the CBSK extract was measured in terms of hydrogen giving or radical-scavenging capability using the stable radical DPPH (Blios, 1958) [8]. Radical-scavenging exertion was expressed as the inhibition chance of free revolutionary by the sample and was calculated using the following formula DPPH Scavenging effect (%) or % inhibition = ((Absorbance of control - Absorbance of test) / Absorbance of control x100).

Results and Discussion

GC-MS analysis

GC-MS is the stylish fashion to identify the ingredients of unpredictable matter, fanned chain hydrocarbons, long-chain, alcohols acids, ester etc. The active principles with their Retention time (RT), molecular weight and peak area as a chance are presented in (Table 1). The GC-MS analysis of CBSK revealed the actuality of factors at the retention time of 3-O-Methyl-d-glucose-12.39 (16.89), 17- (1-acetoxy-ethyl) cyclopenta (a) phenanthrene-11-yl (ester)-30.47 (17.03), Acetic acid, α-D-Glucopyranoside, O-α-D-glucopyranosyl-41 (13.92), -Pregnatriene--diol diacetate-29.08 (7.22), -Eicosatrienoic acid, (Z, Z, Z)-22.92 (6.05). The factors present in the CBSK linked by the GC-MS chromatogram are shown in (figure 2). The natural conditioning attained through the GC-Ms analysis of CBSK have been tabulated (Table 2).

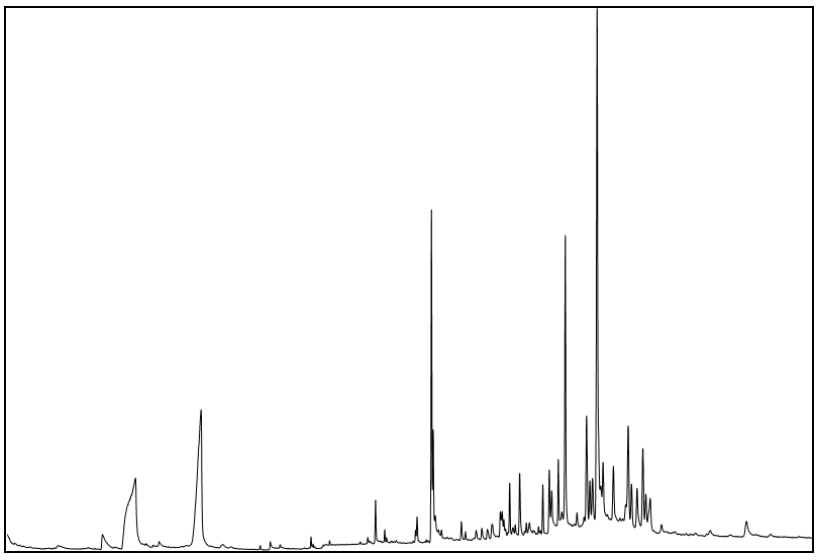

Fig 2: Gas Chromatographic and mass spectrum of CBSK

Table 1: Phyto-compounds were screened in the CBSK using GCMS techniques

S. No	RT (min)	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
1	7.94	6-Acetyl-β-d-mannose	$C_8H_{14}O_7$	222	0.07
2	9.41	α-D-Glucopyranoside, O-α-D- glucopyranosyl-(1.fwdarw.3)-β-D- fructofuranosyl	$C_{18}H_{32}O_{16}$	504	13.92
3	12.39	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	16.89
4	18.29	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	0.14
5	20.37	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	0.77
6	20.79	4-Heptadecyne, 1-chloro-	$C_{17}H_{31}Cl$	270	0.23
7	22.20	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	0.21
8	22.27	Retinal, 9-cis-	$C_{20}H_{28}O$	284	0.57
9	22.92	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	6.05
10	23.00	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	3.58
11	24.29	Androst-4-en-11-ol-3,17-dione, 9- thiocyanato-	$C_{20}H_{25}NO_3S$	359	0.45
12	18.29	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	0.14
13	25.22	Androst-4-en-9-thiocyanomethyl-11-ol-3,17-Dione	$C_{21}H_{27}NO_3S$	373	0.35
14	26.06	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	352	4.05
15	26.49	5,16,20-Pregnatriene-3beta,20-diol diacetate	$C_{25}H_{34}O_4$	398	1.07
16	26.95	6β-Hydroxymethandienone	$C_{20}H_{28}O_3$	316	1.56
17	27.25	5,7,9(11)-Androstatriene, 3-hydroxy-17-oxo-	$C_{19}H_{24}O_2$	284	0.25
18	27.99	1-Phenanthrenecarboxylic acid, tetradecahydsro -7- (2-methoxy-2- oxoethylidene)-1,4a,8-trimethyl-9-oxo, methyl ester, [1S-(1α,4aα,4bβ,8β,8aα,10aβ)]-	$C_{22}H_{32}O_5$	376	1.01
19	28.29	psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	$C_{42}H_{64}O_2$	600	1.60
20	28.41	Fluprednisolone	$C_{21}H_{27}FO_5$	378	1.72
21	28.71	5-Androsten-17α-ethynyl-3β,17β-diol	$C_{21}H_{30}O_2$	314	1.66
22	29.08	5,16,20-Pregnatriene-3beta,20-diol diacetate	$C_{25}H_{34}O_4$	398	7.22
23	29.99	17Alpha-ethynyl-6beta-methoxy-3alpha,5-cyclo-5alpha-androstane-17beta,19-diol	$C_{22}H_{32}O_3$	344	3.78
24	30.14	17Alpha-ethynyl-17beta-hydroxy-6beta methoxy-3alpha, 5-cyclo-5alpha-androstan- 19-oic acid 2, 3, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-dodecahydro-1H-cyclopenta[a] phenanthre n - 11-yl (ester)	$C_{22}H_{30}O_4$	358	1.88
25	30.26	Prednisolone hemisuccinate	$C_{25}H_{32}O_8$	460	1.91
26	30.47	Acetic acid, 17-(1-acetoxy-ethyl)-10,13-dimethyl-3-oxo-	$C_{25}H_{34}O_5$	414	17.03
27	31.21	Phorbol 12,13,20-triacetate	$C_{26}H_{34}O_9$	490	3.70
28	31.88	1H-Cyclopropa[3,4] benz [1,2-e] azulene-4a,5,7b,9,9a (1aH) -pentol, 3-[(acetyloxy) methyl]-1b,4,5,7a,8,9- hexahydro-1, 1,6, 8-tetramethyl-, 5,9,9a- triacetate, [1aR-(1aα, 1bβ, 4aβ, 5β,7aα, 7bα, 8α, 9β, 9aα)]-	$C_{28}H_{38}O_{10}$	534	3.73
29	32.29	Pregn-5-ene-3,11,12,14,20-pentol, 11-acetate 12-(3-methylbutanoate), (3β,11α,12β,14β)-	$C_{28}H_{44}O_7$	492	1.82

The GC-MS has been the stylish fashion used for webbing, identification and quantification of numerous susceptible emulsions in factory extracts and helping understand the nature of medicinal parcels in this medicinal factory of seed kernel extract. The phytochemical analysis conducted on the CBSK revealed the presence of ingredients that are known to parade medicinal parcels similar as anti-inflammatory, antibacterial, antioxidant, antiviral, antitumor, immunomodulatory, anticancer. The composites linked by the GC-MS analysis have

numerous uses in the medicinal field. Each composite is linked to have its unique character to treat colourful conditions. Hence the report of the outline can be used pharmaceutical chemicals for the identification of CBSK.

Table 2: Biological properties of CBSK recognized in major compounds

S. No	Nature of Compounds	Biological Activity
1	6-Acetyl-β-d-mannose	Anticancer
2	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D- fructofuranosyl	Antimicrobial
3	3-O-Methyl-d-glucose	Anticancer
4	9-Hexadecenoic acid	Antibacterial, Antifungal, Anti-inflammatory
5	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	Anti-inflammatory
6	4-Heptadecyne, 1-chloro-	Antimicrobial
7	Linoleic acid ethyl ester	Antioxidant, Antibacterial
8	Retinal, 9-cis-	Antimicrobial
9	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	Antimicrobial
10	9,12-Octadecadienoyl chloride, (Z,Z)-	Antimicrobial
11	Androst-4-en-11-ol-3,17-dione, 9-thiocyanato-	Antimicrobial, Fungicide
12	9-Hexadecenoic acid	Anti-inflammatory
13	Androst-4-en-9-thiocyanomethyl-11-ol-3,17-Dione	Anti-inflammatory
14	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester,	Antidiabetic, Antipyretic
15	5,16,20-Pregnatriene-3beta,20-diol diacetate	Antiviral, Anti-inflammatory, Antitumor
16	6β-Hydroxymethandienone	Antibacterial
17	5,7,9(11)-Androstatriene, 3-hydroxy-17-oxo-	Anticancer
18	1-Phenanthrenecarboxylic acid, tetradecahydro-7-(2-methoxy-2- oxoethylidene)-1,4a,8-trimethyl-9-oxo-,methyl ester, [1S-(1α,4aα,4bβ,8β,8aα,10aβ)]-	Anticancer
19	psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy	Antibacterial
20	Fluprednisolone	Antiallergic, Anti-inflammatory
21	5-Androsten-17α-ethynyl-3β,17β-diol	Antimicrobial
22	5,16,20-Pregnatriene-3beta,20-diol diacetate	Antioxidant, Antibacterial
23	17Alpha-ethynyl-6beta-methoxy-3alpha,5-cyclo-5alpha-androstane-17beta,19-diol	Antitumor, Anti-inflammatory
24	17Alpha-ethynyl-17beta-hydroxy-6beta- methoxy 3alpha,5-cyclo-5alpha-androstan- 19-oic acid	Antibacterial, Anti-inflammatory
25	Acetic acid, 17-(1-acetoxy-ethyl)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren- 11-yl (ester	Antiviral, Anticancer
26	Prednisolone hemisuccinate	Anti-inflammatory, Immunomodulatory
27	Phorbol 12,13,20-triacetate	Antitumor, Antimicrobial
28	1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3- [(acetyloxy)methyl]-1b,4,5,7a,8,9- hexahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1Ar (1aα,1bβ,4aβ,5β,7aα,7bα,8α,9β,9aα)]-	Antitumor, Antiviral, Antibacterial
29	Pregn-5-ene-3,11,12,14,20-pentol, 11-acetate 12-(3-methylbutanoate), (3β,11α,12β,14β)-	Antioxidant, Anticataractogenic

Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl revolutionary is considered to be lipophilic revolutionary. The 2,2-diphenyl-1-picrylhydrazyl radical was extensively used to assess the free-radical hunting ability of antioxidants (Soares *et al*., 1997) [9]. The scavenging outcome of CBSK and standard ascorbic acid on 2,2-diphenyl-1-picrylhydrazyl revolutionary was matched. On the 2, 2-diphenyl-1-picrylhydrazyl revolutionary, the CBSK had a significant scavenging effect with adding attention in the variety of 10-50 µg/ ml. when matched with that of ascorbic acid, the scavenging effect of CBSK was lower. The attention of CBSK showed the implicit effect of 2,2-diphenyl-1-picrylhydrazyl exertion as the chance of free revolutionaries inhibition (Table 3), (Shukla *et al*.,) [10].

Table 3: Antioxidant (DPPH Method) Activity of *Caesalpinia bonducella* seeds kernel

Inhibition %		
DPPH radical-scavenging activity		
Concentration of sample (µg/ml)	***Caesalpinia bonducella* seeds kernel**	**Ascorbic acid (standard)**
10	20.03	26.21
30	22.87	54.09
50	24.37	75.63
IC_{50} values (µg/ml)	127.2	26.68

The CBSK exhibited strong antioxidant exertion by obstructing 2, 2-diphenyl-1-picrylhydrazyl conditioning when related with standard ascorbic acid. The antioxidant conditioning plant *in vitro* trial was only reflective of the implicit health benefit. Therefore the *C. bonducella* seeds kernel ethanol extract can be used as a source of accepted antioxidants with subsequent healthiness benefits.

Conclusion

The present study concluded that a rich source of phytochemicals found in *Caesalpinia bonducella* seed kernel (CBSK) ethanol extract identified by GC MS technique and possesses potential antioxidant activity was confirmed through the *in vitro* antioxidant model. The present study suggests a contribution from these compounds to pharmacological activity in the future.

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