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Research Article

Phytochemical screening and GC-MS analysis of bioactive compounds in *Caesalpinia bonduc* L. from Alagarkovil Reserve Forest (ARF), Dindigul District, South India

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Abstract

The popularly known *Kazharchi Kai* (Tamil), *Caesalpinia bonduc* L. (Fam: Caesalpiniaceae) is a traditional medicinal plant widely distributed in India which finds use as a therapeutic in indigenous systems of medicine such as Ayurveda, Siddha, Unani and Homoeopathy is a herbal remedy for treating of various ailments. The plant has been reported for its anticancer, hepatoprotective, antioxidant, antimalarial, antimicrobial, antipyretic, antifertility, anti-inflammatory and antimalarial properties. In particular *C. bonduc* seeds contain cassane diterpenoids viz., caesalpinins and caesalmins, cassane diterpenoids, and norcassane diterpenoids. This plant contains bioactive secondary metabolites (BASM) with unique structures and diverse mechanisms of action that can be well exploited to finetune novel therapeutic herbal formulations. In the present study an attempt has been made to screen for bioactive metabolites in the seed extracts of *C. bonduc* followed by GCMS analysis. GCMS analysis indicated the presence of 2,2,3,5-tetramethylheptane, (Z)-hept-2-enal, 2,2,3,5-tetramethylheptane, 3,3- dimethyloctane, 5-ethyl-2,2,3-trimethylheptane, 2,6,10-trimethyldodecane, 3- methylnonane, 3,8-dimethylundecane, 6-Ethyl-3,8-dimethylundecane, 2,3,4- trimethyldecane, 3-Methyldecane, (2E,4E)-deca-2,4-dienal, (1R,4E,9S)-4,11,11- trimethyl-8-methylidenebicyclo[7.2]undec-4-ene, Hexadecanoic acid, methyl (9Z,12Z)-octadeca-9,12-dienoate, (9Z)-octadeca-9,17-dienal, 2- (ethylhexoxycarbonyl) benzoic acid. Of all, compounds such as Hexadecanoic acid, methyl (9Z,12Z)-octadeca-9,12-dienoate, and (9Z)-octadeca-9,17-dienal with unusual bioactivity score could serve as promising lead candidate for the design of novel anticancer drug, *In-silico* predictions of molecular and biological activity of these bioactive compounds phytocompounds could pave way for its effective utilization in pharma industry scale as a potential candidate towards biomedical applications. However, before such an exercise, in-silico ADMET pharmacoinformatic studies followed by pre-clinical and clinical trials pursued in this exercise may well be required for its launch into the global pharma-market on commercial scale.

Keywords: Phytochemical Screening; Bioactive Secondary Metabolites (BASM); GCMS; Medicinal Plant; Ethnobotany; CBSPEE; PBNPs

INTRODUCTION

Ethnobotanical information on the traditional uses of *Caesalpinia bonduc* (syn. *C.bonducella*) states that different parts of the plant are used for varied but specific purposes by the local community as medicine[1]. In Ayurveda, this herb has been used to control diabetes and the long term complications associated with diabetics and Poly Cystic Ovary Syndrome (PCOS)[3]. The herb endowed with antidiarrheal property helps to cure diarrhea/ loose motion[4]. As a botanical it is used to treat joint pain and mitigate arthritis[5]. Traditionally the herb is used to cure many skin problems including leucoderma, leprosy, blisters, boils, and wounds[6]. Leaf extracts are reported in being used to treat elephantiasis[7] and stop growth of tumors[8,9]. Leaf paste reduces toothache[10]. Leaf extracts are used to elephantiasis[7], liver problem[11], and respiration odour[12]. Root bark is used to treat tumors, intestinal worms, fever, amenorrhea, cough, and helps to remove placenta after childbirth[6]. Leaf juice with honey is used to ward off the mucous secretions. It is used as astringent[13]. Paste of leaves finds use in treating toothache[10].

Fruits used to cure wounds, piles[14], urinary disorders[15], leucorrhoea, and treat menopause problems viz., no menses, intermittent menses, and relief abdominal pain during menopause offers a definite relief to women[1,3]. Gargling with boiled nut decoction *C. bonduc* cures sore throat[16]. Seeds are known for their anticonvulsive effect[17] and are used to treat smallpox, malarial fever, countering fever, and intermittent fever, besides it is also used to cure sweating deficiency and curb body odour[1,3,18]. Bonduc nuts are used to cure spleen disorders, liver problems, relieve hydrocele, skin diseases, inflammation, leprosy, and colic disorders[19]. Bonduc nuts are also used to treat intestinal worms, and relief inflammation of large intestine/ colic pain. Paste made of seed powder with castor oil is used as an ointment[20-23].

Seed oil is used to treat ulcers, paralysis, and convulsions[20]. Roasted seeds with castor oil are used to treat inflammatory

swelling[24], hydrocele, and inflamed piles[12]. Seed oil is used to treat rheumatism[25]. Paste of seeds helps to treat snakebite[26]. Seeds used to treat swelling and restraining haemorrhage[3,24]. Seed oil is used to treat paralysis and convulsions[27]. Decoction of roasted nut is used kernel used to treat asthma[1,22]. Nut kernel paste helps to get better relief from swelling and boils[15]. Nut kernel powder with salt, ginger, and honey is good for children's stomach problems[28]. Seed oil helps to remove freckles from face. It is also used as cosmetic and stops discharges from ears[29].

Ethno-veterinary uses encompass root, leaves and seeds used in treating bradycardia, tachycardia, tympanitis, tuberculosis, abdomen pain, fever, cough, and cold and liver fluke in ruminants[30] seed extract given to cattle for the management of Gut disorders and endoparasitic infestations[31]. Seed are used ornamentally adorn as beads to adorn bracelets, necklaces and rosaries[32] that this straggling climber can garner huge attention.

MATERIALS AND METHODS

Plant Material:

Fresh seeds of *C. bonduc* from the nearly mature pods were collected from foothills of Alagarkovil Reserve Forest (longitude/ latitude geographical coordinates 10.0748° N, 78.2131° E, Eastern Ghats) Dindigul District, Tamilnadu during Jun-Jul 2021, taken to laboratory, cleaned and preserved as Herbarium, part of the collected seed sample was shade dried, powdered and subjected to extraction. Botanical identity of the plant was established using flora and confirmed by PG Department of Botany, Sri Meenakshi Government Arts College for Women (A), Madurai, India.

Botanical affinities and description of the source plant

Kingdom: Plantae

Phylum: Magnoliophyta

Division: Magnoliopsida

Class: Angiospermae

Order: Fabales

Family: Fabaceae/Caesalpiniaceae

Genus: Caesalpinia

Species: bonduc

Habit: Climbers, prickly, yellowish pubescent throughout; prickles straight or somewhat re-curved; **Leaves** 30-45 cm; rachis with recurved prickles; pinnae 6-9 pairs, opposite; stipules deciduous, large, leaf like, usually lobed, lobes to 2 cm; leaflets 6-12 pairs, oblong, 1.5-4 × 1.2-2 cm, membranous, both surfaces pubescent, base oblique, apex rounded to acute, mucronate;

Inflorescence: racemes axillary, long pedunculate, densely flowered in upper part and sparsely so in lower part; Bracts: caducous at anthesis, reflexed, subulate, 6-8 mm, pubescent. Pedicel: 3-5 mm; Sepals: 5, ca. 8 mm, both sides ferruginous hairy;

Petals: yellowish; standard tinged with red spots, oblanceolate, clawed; Stamens: Filaments short, hairy in basal part;

Ovary: hairy; Fruit: Legume oblong, 5-7 × 4-5 cm, leathery, apex rounded and with beak, swollen, with dense, slender spines 5-10 mm;

Seeds: 2 or 3, greyish, shiny, ovoid to globose;

Flowering: Feb, Jul-Oct; Fruiting: Oct-May Summary of taxonomical attributes of *C. bonducella*

Foliage : Evergreen Root : Deep roots, taproots

Stem : Hard and woody, climber

Leaf : Bi-pinnately compound Leaf shape : Elliptical to ovate Leaf color : Green Leaf arrangement : Alternate Leaf surface :

Glossy Seed : Dicot Odour :

Characteristic Taste : Bitter

Sample preparation: Using direct method of extraction, approximately 10 g of powder was extracted with 100 ml of methanol. The extract was transferred in to glass vials. The process was repeated 3 times with fresh solvent. The solvent was removed by Rotavapor. The extracted residue was re-dissolved in the solvent to yield a final volume of 10mg/ml and the content was stored in cold (at 4°C) until further use.

Phytochemical Screening

The methanolic extracts were subjected to chemical tests for the detection of phytoconstituents using standard procedures[33-40].

TEST FOR ALKALOIDS

Mayer's test: Few drops of Mayer's reagent was added to 1 mL of plant extract, appearance of a deep yellow or white precipitate indicated the presence of alkaloids in the solution. (Mayer's reagent was freshly prepared by dissolving mercuric chloride (1.36 g) and potassium iodide (5.00 g) in 100 ml water).

Dragendorff's test: To 2 mL of the extract added 1 mL of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate indicated the presence of alkaloids. Dragendorff's reagent was prepared by Sol A: 0.85g bismuth subnitrate, 40mL water, and 10mL glacial acetic acid and Sol B: 8g potassium iodide and 20mL water. 5mL each of Sol A & B with 20mL of glacial acetic acid and 70-100 mL of water is mixed to prepare Dragendorff's reagent.

Hager's test: Hager's test was done by adding a few drops of Hager's reagent to plant extracts and appearance of a yellow-color precipitate indicated the presence of alkaloids in the solution. Hager's reagent is saturated solution of picric acid.

Wagner's test: Approximately, 1 ml of crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colour precipitate indicates the presence of alkaloids. Wagner's Reagent was prepared by mixing 2.5 gm iodine in 12.5 gm of potassium iodide (KI 2); add 250 ml of water to produce solution.

TEST FOR GLYCOSIDES

Test For Anthraquinones Glycosides

Borntragers test: 0.5 g of extract was boiled with 10% hydrochloric acid for few minutes in water bath. It was filtered and allowed to cool. Equal volume of CHCl3 was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Formation of rose – pink color indicates of n-hexane, chloroform, ethyl acetate and methanol of the presence of the anthroquinones.

Baljet test: Part of plant containing cardiac glycoside is dipped in sodium picrate solution; formation of a yellow to orange colour indicates the presence of aglycones or glycosides in the plant tissues.

Legal's Test: To the concentrated ethanolic extract few drops of 10% NaOH were added, to make it alkaline. Then freshly prepared sodium nitroprusside was added to the solution. Presence of blue coloration indicated the presence of glycosides in the extract.

TEST FOR CARDIAC GLYCOSIDES

Keller-Kiliani test: 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A browning of the interface indicates a deoxy-sugar characteristic of carotenoids. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

TEST FOR CARBOHYDRATES

Molisch's test: Small portion of the plant extract was put in a test tube; 10 ml of distilled water was added and shaken vigorously and gently. The mixture was then filters and divided into two portions. To the first portion, two drops of Molish's reagent was added followed by few drops of concentrated sulphuric acid by the wall of the test tube. Formation of brown or purple ring at the interphase indicated the presence of carbohydrates.

Fehling's test Equal volume of Fehling A and Fehling B reagents were mixed together and then add 2ml of crude extract in it and gently boiled. A brick red precipitate appeared at the bottom of the test-tube indicates the presence of reducing sugars.

Benedict's test 1 ml of crude extract was mixed with 2ml of Benedict's reagent and boiled. A reddish brown precipitate was formed which indicates the presence of the carbohydrates.

TEST FOR PHYTOSTEROLS

Libermann Burchard's Test: Dissolve one or two crystals of cholesterol in dry chloroform in a dry test tube. Add few drops of acetic anhydride and then 2 drops of concentrated H2SO4 and mix well. The formation of a green or green-blue colour after a few minutes indicates the presence of phytosterols. After the reaction, concentration of cholesterol can be measured spectrophotometry.

Salkowski's Test: On adding a few drops of conc. Sulphuric acid to the plant extract and allow the solution to stand for some time, formation of brown ring indicated the presence of phytosterols in the plant extract.

TEST FOR FLAVONOIDS

$FeCl_3$ Test: To 1 ml of the extract, 3 ml of distilled water followed by few drops of 10% aqueous Ferric chloride solution was added. Formation of blue or green colour indicates the presence of flavonoids. Shinoda Test: To 2 ml of the extract, 1 ml of 1% ammonia solution was added. Appearance of yellow colour indicates the presence of flavonoids.

TESTS FOR FIXED OILS AND FATS

Spot test Take the sample and place it between the folds of filter paper and rub it lightly. Presence of translucent spots on the filter paper confirms the presence of fats in in the plant material.

Saponification Take a sample a test tube, add strong alkali NaOH, boil the solution in a water bath for 5 min, add ethanol. Observe for the appearance of froth, formation of forth in the test tube indicates the presence of fat in the sample.

TEST FOR FREE AMINO ACID

Millon's test 1 ml of crude extract was mixed with 2ml of Millon's reagent; white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test 1 ml of crude extract was mixed with 2ml of 0.2% solution of Ninhydrin and boiled. A violet colour precipitate was appeared suggesting the presence of amino acids and proteins.

TEST FOR TANNINS 5% Ferric chloride test: 5 mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins. 10% Lead acetate test: 10 mg of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

TEST FOR SAPONINS

Foam Test: 2 ml of crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

GUMS & MUCILAGE Ruthenium red test: 50 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with a few drops of Ruthenium red solution. Observed for pink color indicates the presence of gums and mucilage.

GC-MS Analysis:

Seed samples of were collected from the fences of farmlands near Alagarkovil Reserve Forest (longitude/ latitude geographical coordinates 10.0748° N, 78.2131° E, Eastern Ghats) Dindigul District, Tamil Nadu, India. Phyto-components were identified using GC–MS detection system as described previously26, however with modification, whereby portion of the extract was analysed directly by headspace sampling. GC–MS analysis was accomplished using an Agilent 7890A GC system set up with 5975C VL MSD (Agilent Technologies, CA, and USA). Capillary column used was DB-5MS (30 m × 0.25 mm, film thickness of 0.25 µm; J&W Scientific, CA, USA). Temperature program was set as: initial temperature 50°C held for 1 min, 5°C per min to 100°C, 9°C per min to 200°C held for 7.89 min, and the total run time was 30 min. The flow rate of helium as a carrier gas was 0.811851 mL/ min. MS system was performed in electron ionization (EI) mode with Selected Ion Monitoring (SIM). The ion source temperature and quadruple temperature were set at 230°C and 150°C, respectively. Identification of phyto-components was performed by comparison of their retention times and mass with those of authentic standards spectra using computer searches in NIST 08.L and Wiley 7n.l libraries[40,41].

RESULTS AND DISCUSSION

The organoleptic description of the plant part (seeds) used in the study is provided in Table 1. Physiochemical parameters, nutrient and mineral composition of Bonduc nuts, *C. bonduc* seeds is provided in Table 2, 3, 4 respectively. The physiochemical properties, nutrient and mineral composition of Bonduc nuts are almost close to the data reported previously[10,11,42,43]. *C. bonduc* has been found to possess the following nutrients: crude fibre 12.79-14.07%, protein 18.65-20.32%, fat 6.54-7.23%, carbohydrate 16.91-18.56%, food energy (Kcal/100 g) 376.27-402.12, and food energy (Kcal/100 g) 376.27-402.12 similar to the one reported by Juvatkar and Jadhav[20]. Calcium ranges from 0.150% to 0.184%, Phosphorus from 0.17% to 0.22%, Sodium from 0.07 to 0.08%, Iron from 0.22% to 0.5%, Vitamin C from 0.016 to 0.043 (IU/g), and Vitamin A from 416.75 to 700.14 (IU/g).

Percentage yield[11] obtained during solvent extraction process of *C. bonducella* seeds in different solvent system viz., Petroleum Ether, Chloroform extract, and Methanol extract was 18.6, 8.6 and 4.0 respectively (Table 5).

Qualitative phytochemical investigation revealed the presence of alkaloids, flavonoids, gums & mucilage, carbohydrates, steroids, proteins & amino acids, fats & fxed oils, glycoside, phenols, and saponins (Table 6) similar to previous reports[10,11,44-46]. GCMS analysis of seeds indicated the presence of the following compounds with retention time, name of the compound, molecular formula, molecular weight, and the percent yield viz., 8.21 - 2,2,3,5-Tetramethylheptane (**$C_{11}H_{24}$**), 156.31, 2.8861; 8.35 - 2-Heptenal, (z)- ($C_7H_{12}O$), 112.17, 1.5880; 8.51 - Heptane, 2,2,3,5-tetramethyl- ($C_{11}H_{24}$), 156.31, 1.0960; 8.92 - Octane, 3,3-dimethyl- ($C_{10}H_{22}$), 142.28, 1.5210; 9.47 - Heptane, 5-ethyl-2,2,3-trimethyl- ($C_{12}H_{26}$), 170.33, 7.9563; 9.77 - Dodecane, 2,6,10-trimethyl- ($C_{15}H_{32}$), 212.41, 11.0032; 10.18 - Heptane, 5-ethyl-2,2,3-trimethyl- ($C_{12}H_{26}$), 170.33, 9.7909; 10.34 - Nonane, 3-methyl- ($C_{10}H_{22}$), 142.28, 8.9909; 10.61 - Undecane, 3-methyl- ($C_{12}H_{26}$), 170.33, 7.6780; 10.81 - 6-Ethyl-3,8-dimethylundecane ($C_{15}H_{32}$), 212.41, 2.2504; 11.06 - Decane, 2,3,4-trimethyl- ($C_{13}H_{28}$), 184.36, 2.5521; 11.46 - Decane, 3-methyl- ($C_{11}H_{24}$), 156.59, 1.2194; 15.27 - 2,4-decadienal ($C_{10}H_{16}O$), 152.12, 1.1017; 17.81 - Caryophyllene ($C_{15}H_{24}$), 204.35, 5.1918; 26.05 - n-Hexadecanoic acid ($C_{16}H_{32}O_2$), 256.42, 6.0711; 27.46 - 9,12-octadecadienoic acid (z,z)-, methyl ester ($C_{19}H_{34}O_2$), 294.47, 2.2379; 28.52 - 9,17-octadecadienal, (z)- ($C_{18}H_{32}O$), 264.45, 14.8972; 33.74 - 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester ($C_{16}H_{22}O_4$), 278.35, 9.7952 respectively (Table 7). Further, the compounds were web resource prospected for their biological activities - 2,2,3,5-Tetramethylheptane has bioresorbable, anticancer, antiviral; 2- Heptenal, (z)- antibacterial, nematicidal, antifungal; Heptane, 2,2,3,5-tetramethylbioresorbable, anticancer, antiviral; Octane, 3,3-dimethyl- biocontrol activity, bioelectrode, antibacterial, nematicidal; Heptane, 5-ethyl-2,2,3-trimethyl- bifunctional cytotoxic agent, Dodecane, 2,6,10-trimethyl- antimicrobial, anti-inflammatory, anaesthetic; Heptane, 5-ethyl-2,2,3-trimethyl- bifunctional cytotoxic agent, Nonane, 3-methyl- bioresorbable, bio-electrode, carbacyclin analogs; Undecane, 3-methylantimicrobial; 6-Ethyl-3,8 dimethylundecane - antimicrobial, anti-inflammatory; Decane, 2,3,4-trimethyl- NYR; Decane, 3-methyl- bioresorbable, bio-electrode, antifungal; 2,4-decadienal NFKBIA – NFKB, acetylcholinesterase (AChE), anticancer; caryophyllene acetylcholinesterase (AChE), antiviral; n-Hexadecanoic acid anti-inflammatory hepatoprotective, anticoronary, antiarthritic, anticancer; 9,12- octadecadienoic acid (z,z)-, methyl ester anticancer, antioxidant, antimalarial, antimicrobial, anti-inflammatory; 9,17-octadecadienal, (z)- antimicrobial; 1,2- benzenedicarboxylic acid, mono(2-ethylhexyl) ester NYR respectively. The summative information pertaining to the biological activity of the compounds is provided in Table 8.

CONCLUSION

Modern health care facilities with expensive drugs are still out of reach for the masses thriving in the rural segment of the developing country like India. As a result, it is important to evaluate the feasibility of herbal medicine to supplement the basic health-care needs. As a result, ethnomedical studies have gained prominence as it recognizes traditional medical virtues, mainly of plant origin. Though scores of plants are considered herbal, there is always a overwhelming need to be study and evaluate scientific technologies using phytochemical analysis, pharmacological screening, and clinical investigations in adherence of the state of the art approach.

Numerous studies involving *Caesalpinia* species have confirmed their efficacy as a natural source of new chemical entities with drug action. Investigations carried out in the past have examined the potential of many active ingredients that can be corroborated with the structural diversity of therapeutically competent substances. Seeds of *C. bonduc,* although, a known traded commodity in the local herbal markets in Tamil Nadu and elsewhere in India, has somehow eluded the attention of pharmacists. That in spite of the sting that the fruit hairs inflict, plant collectors dare to collect the seeds and venture trading of *C. bonduc* speaks of instant reception the plant has in the herbal market and signify the immensely important and unique medicinal properties vested with it.

Although antiulcer, anticancer, anti-diabetic, anti-inflammatory, anti-rheumatic, antimicrobial, antibacterial, and cytotoxic properties of *C. bonduc* have been experimentally demonstrated, data pertaining to analysis of the curative elements have not been forthcoming to match and vouch the claims made. It is this context that the present study seeks to provide the fillip. The basic details provided in this approach can lead to the development of novel drug prototypes provided ADMET, pre-clinical and clinical investigations are pursed in full swing. It is expected that the data presented at the moment will be required along with quality control systems to understand and realize the hitherto untapped potential of this wild plant.

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Table 1 Organoleptic description of the plant part used in the study

PART	SEED
Color	Greenish grey to bluish-grey
Odour	Characteristic odour
Shape	Globules or round
Taste	Bitter
Texture	Smooth and shiny

Table 2 Physiochemical parameters of *Caesalpinia bonducella* seeds

PARAMETERS ANALYSED	SEEDS
Total ash (%w/w)	3.52 ± 0.241
Water soluble ash (%w/w)	2.03 ± 0.692
Acid insoluble ash (%w/w)	0.58 ± 0.042
Water Soluble Extractive Value (%W/W)	6.54 ± 0.801
Ethanol Soluble Extractive Value (%W/W)	7.53 ± 0.034
Hexane Soluble Extractive Value (%W/W)	4.34 ± 0.032
Chloroform Soluble Extractive Value (%W/W)	2.59 ± 0.071
Ethyl Acetate Soluble Extractive Value (%W/W)	1.02 ± 0.012
Loss on dying (%w/w)	5.03 ± 0.124
Solubility in alcohol (%)	29.77 ± 1.692
Solubility in water (%)	31.52 ± 0.975
pH	4.13 ± 0.316

Values were in mean ± standard deviation, n=3

Table 3 Nutrient composition of *Caesalpinia bonducella* seeds

PARAMETERS	CONTENT
Energy value (kcal)	73.6
Carbohydrate (mg g^{-1})	18.4
Protein (mg g^{-1})	17.6
Total fat (mg g^{-1})	3.6
Crude fibre (mg g^{-1})	3.3
Free amino acids(mg g^{-1})	1.82
Free fatty acids(mg g^{-1})	0.03
Cholesterol (mg g^{-1})	0.02
Cellulose (mg g^{-1})	2.59
Thiamine (µg g^{-1})	10.6
Niacin (µg g^{-1})	22.6
Riboflavin (µg g^{-1})	89.6
Vitamin E (µg g^{-1})	6.09
Vitamin C (µg g^{-1})	4.2

Table 4 Mineral composition of *Caesalpinia bonducella* seeds

MINERALS	CONTENT
K (%)	42.59
O (%)	27.34
Ca (%)	13.33
Fe (%)	3.38
P (%)	3.31
S (%)	2.62
Mg (%)	1.96
Si (%)	1.37
Cl (%)	1.25
Pd (%)	0.61
Al (%)	0.57
Mo (%)	0.28
Cu (%)	0.16
Zn (%)	0.15
Na (ppm)	13.11
Pb (ppm)	7.08
Hg (ppm)	1.32
Cd (ppm)	<0.50

Table 5 % yield obtained during solvent extraction of *C. bonducella* seed samples

S.No	Species	Part Used	Solvent System (Yield in %)		
			Petroleum Ether	Chloroform Extract	Methanol Extract
1	***Caesalpinia bonduc***	Seed	18.6	8.6	4.0

Table 6 Qualitative Phytochemical Examination with solvent plant extracts

S. No	Plant constituents tested & Reagent used	*Caesalpinia bonduc* Seed		
		Petroleum Ether Extract (CBSPEE)	Chloroform Extract (CBSCE)	Methanol Extract (CBSME)
1	**TEST FOR ALKALOIDS**			
1.1	*Mayer's test*	-	-	-
1.2	*Dragendorff's test*	-	-	-
1.3	*Hager's test*	-	-	-
1.4	*Wagner's test*	-	-	-
2.1	**TEST FOR GLYCOSIDES - Anthroquinone**			
2.1.1	*Borntrager's test*	-	-	-
2.1.2	*Baljet test*	-	-	-
2.1.3	*Legal's test*	-	-	-
2.2	**TEST FOR GLYCOSIDES - Cardiac**			
2.2.1	*Keller-Killani test*	-	-	-
3	**TEST FOR CARBOHYDRATES**			
3.1	*Molish's test*	-	-	-
3.2	*Fehling's solution test*	-	-	-
3.3	*Bendict's reagent test*	-	-	-
4	**TEST FOR PHYTOSTEROLS**			
4.1	*Libermann Burchard's*	+	+	-
4.2	*Salkowski's test*	+	+	-
5	**TEST FOR FLAVONOIDS**			
5.1	*Ferric chloride test*	-	-	-
5.2	*Shinod's test*	-	-	-
6	**TEST FOR FIXED OILS AND FATS**			
6.1	*Spot test*	+	+	+
6.2	*Saponification*	+	+	+
7	**TEST FOR FREE AMINO ACIDS**			
7.1	*Million's reagent*	+	+	-
7.2	*Ninhydrin reagent*	+	+	-
8	**TEST FOR TANNINS**			
8.1	*5% Ferric chloride*	-	-	-
8.2	*10% Lead acetate*	-	-	-
9	**TEST FOR SAPONINS**			
9.1	*Foam test*	-	-	-
10	**GUMS & MUCILAGE**	-	-	-

Note: + indicates positive result; - indicates negative result

Table 7 GCMS profile of CBSPEE (*C. bonducella* Seed Petroleum Ether Extract)

RT	COMPOUNDS	MF	MW	PA (%)
8.21	2,2,3,5-Tetramethylheptane	**$C_{11}H_{24}$**	156.31	2.8861
8.35	2-Heptenal, (z)-	**$C_7H_{12}O$**	112.17	1.5880
8.51	Heptane, 2,2,3,5-tetramethyl-	**$C_{11}H_{24}$**	156.31	1.0960
8.92	Octane, 3,3-dimethyl-	**$C_{10}H_{22}$**	142.28	1.5210
9.47	Heptane, 5-ethyl-2,2,3-trimethyl-	**$C_{12}H_{26}$**	170.33	7.9563
9.77	Dodecane, 2,6,10-trimethyl-	**$C_{15}H_{32}$**	212.41	11.0032
10.18	Heptane, 5-ethyl-2,2,3-trimethyl-	**$C_{12}H_{26}$**	170.33	9.7909
10.34	Nonane, 3-methyl-	**$C_{10}H_{22}$**	142.28	8.9909
10.61	Undecane, 3-methyl-	**$C_{12}H_{26}$**	170.33	7.6780
10.81	6-Ethyl-3,8-dimethylundecane	**$C_{15}H_{32}$**	212.41	2.2504
11.06	Decane, 2,3,4-trimethyl-	**$C_{13}H_{28}$**	184.36	2.5521
11.46	Decane, 3-methyl-	**$C_{11}H_{24}$**	156.59	1.2194
15.27	2,4-decadienal	**$C_{10}H_{16}O$**	152.12	1.1017
17.81	Caryophyllene	**$C_{15}H_{24}$**	204.35	5.1918
26.05	n-Hexadecanoic acid	**$C_{16}H_{32}O_2$**	256.42	6.0711
27.46	9,12-octadecadienoic acid (z,z)-, methyl ester	**$C_{19}H_{34}O_2$**	294.47	2.2379
28.52	9,17-octadecadienal, (z)-	**$C_{18}H_{32}O$**	264.45	14.8972
33.74	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	**$C_{16}H_{22}O_4$**	278.35	9.7952

Table 8 Biological activity of bioactive compounds in CBSPEE

NAME OF COMPOUND	IUPAC NAME	BIOLOGICAL ACTIVITY
2,2,3,5-Tetramethylheptane	2,2,3,5-tetramethylheptane	Bioresorbable Anticancer Antiviral
2-Heptenal, (z)-	(Z)-hept-2-enal	Antibacterial Nematicidal Antifungal
Heptane, 2,2,3,5-tetramethyl-	2,2,3,5-tetramethylheptane	Bioresorbable Anticancer

		Antiviral
Octane, 3,3-dimethyl-	3,3-dimethyloctane	Biocontrol activity Bio-electrode Antibacterial Nematicidal
Heptane, 5-ethyl-2,2,3-trimethyl-	5-ethyl-2,2,3-trimethylheptane	Bifunctional cytotoxic agent
Dodecane, 2,6,10-trimethyl-	2,6,10-trimethyldodecane	Antimicrobial Anti-inflammatory Anaesthetic
Heptane, 5-ethyl-2,2,3-trimethyl-	5-ethyl-2,2,3-trimethylheptane	Bifunctional cytotoxic agent
Nonane, 3-methyl-	3-methylnonane	Bioresorbable Bio-electrode Carbacyclin analogs
Undecane, 3-methyl-	3,8-dimethylundecane	Antimicrobial
6-Ethyl-3,8 dimethylundecane	6-Ethyl-3,8-dimethylundecane	Antimicrobial Anti-inflammatory
Decane, 2,3,4-trimethyl-	2,3,4-trimethyldecane	
Decane, 3-methyl-	3-Methyldecane	Bioresorbable Bio-electrode Antifungal
2,4-decadienal	(2E,4E)-deca-2,4-dienal	NFKBIA - NFKB Acetylcholinesterase (AChE) Anticancer
Caryophyllene	(1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	Acetylcholinesterase (AChE) Antiviral
n-Hexadecanoic acid	Hexadecanoic acid	Anti-inflammatory Hepatoprotective Anticoronary Antiarthritic Anticancer
9,12-octadecadienoic acid (z,z)-, methyl ester	methyl (9Z,12Z)-octadeca-9,12-dienoate	Anticancer Antioxidant Antimalarial Antimicrobial Anti-inflammatory
9,17-octadecadienal, (z)-	(9Z)-octadeca-9,17-dienal	Antimicrobial
1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	2-(2-ethylhexoxycarbonyl)benzoic acid	NAR