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# Research Article
# Studies on Nutraceutical Properties of *Caesalpinia bonducella* L.: An Indian Traditional Medicinal Plant

[1]S. Manikandaselvi, [2]V. Vadivel and [2]P. Brindha

[1]PG and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Mannargudi, Tamilnadu, India
[2]Centre for Advanced Research in Indian System of Medicine, SASTRA University, Thanjavur, Tamilnadu, India

## Abstract

*Caesalpinia bonducella* L. belongs to the family Caesalpiniaceae is distributed throughout India and commonly known as Kazhichikkai in Tamil. In the present study, attempts were made to assess the physicochemical, phytochemical, nutritional and pharmacological properties of *C. bonducella* seeds. Powder microscopy revealed characteristics features of *C. bonducella* seeds. Physicochemical properties such as foreign matter (0.97%), loss on drying (8.83%), total ash (3.37%), solubility in water (28.8%) and extractive value in water (6.7%) were revealed by the sample. Phytochemical analysis revealed the presence of alkaloid (0.12 mg $g^{-1}$), phenol (0.60 mg $g^{-1}$), flavonoid (0.33 mg $g^{-1}$), tannin (4.90 mg $g^{-1}$) and lignin (74.7 mg $g^{-1}$). The nutritional profile of *C. bonducella* revealed the presence of carbohydrate (18.4 mg $g^{-1}$), proteins (17.6 mg $g^{-1}$), fat (3.6 mg $g^{-1}$), fibre (3.3 mg $g^{-1}$) and energy value (73.6 kcal). Presence of different phytochemicals was confirmed in the hexane and chloroform extracts of *C. bonducella* using HPTLC and GC-MS techniques. The aqueous extract (1000 µg $mL^{-1}$) recorded anti-inflammatory activity in terms of inhibition of protein denaturation (94.15%), inhibition of protease activity (32.40%) and HRBC membrane stabilization (81.66%). Strong antioxidant activity was noticed in aqueous extract based on the results of DPPH radical scavenging (90%) and lipid peroxidation (99.69%) assays, which are higher than that of BHT standard. Thus the present investigation provides scientific evidence for the folk-lore claims for medicinal value of *C. bonducella* seeds.

**Key words:** Indian folk-lore drug, physicochemical, nutrients, phytochemicals, antioxidant, anti-inflammatory

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**Corresponding Author:** V. Vadivel, Center for Advanced Research in Indian System of Medicine, SASTRA University, Thanjavur, Tamilnadu, India
Tel: 8973830858



**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Peoples of all ages in both developing and undeveloped countries use plants in an attempt to cure various diseases and to get relief from physical sufferings. For centuries, plant and plant products have been used for treating various illnesses. Current researchers primarily focused on plants, since they can be sourced easily and selection is mostly based on their ethno-medicinal claims (Chaudhari *et al*., 2013). Usage of plant materials in preventing and treating inflammatory, oxidative stress and microbial mediated diseases over the past few years has attracted the attention of scientists worldwide (Seth and Sharma, 2004). Today, several medicinal plants and their products are still being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market. However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries is the lack of documentation and stringent quality control. There is a need for documentation of research work that has to be carried out on traditional medicines. Therefore, it has become extremely important to make an effort towards standardization of the plant material to be used as medicine as well as nutraceutical (Kumar *et al*., 2011).

Nutrition is a basic requirement and any imbalance in the requirement will leads to various risk factors leading to diseases/disorders such as cancer and diabetes. In India, nearly 20% of the total population and 44% of young children are under-nourished conditions and are also underweight (Pandey *et al*., 2013). Nutritional deficiency has thrown up a major challenge in the form of "Lifestyle diseases". Nutraceuticals can play an important role in preventing these diseases and increasing healthy society. About 2000 years ago, hippocrates correctly emphasized "Let food be your medicine and medicine be your food" (Rajasekaran *et al*., 2008).

In this connection, the present work was attempted to evaluate the nutraceutical properties of aqueous extracts of seeds of *Caesalpinia bonducella* L. (Family, Caesalpiniaceae), a common Indian folk-lore drug. The seeds of *C. bonducella* are claimed to be styptic, purgative, anthelmintic and cures inflammations; useful in colic, malaria, hydrocele, skin diseases and leprosy (Yunani) (Singh and Raghav, 2012). In and around Chennai, an ointment is made from the *C. bonducella* seed powder and castor oil and applied externally for curing hydrocele and orchitis (Handa and Kaul, 1996). The seeds are considered as tonic, febrifuge, anthelmintic and anti-blennorrhagic (Nazeerullah *et al*., 2012). The oil from the seeds is used in convulsions and paralysis (Moon *et al*., 2010). The antimicrobial activity of ethanolic extract of *C. bonducella* was reported by Subramani *et al*. (2014) while, Deepika *et al*. (2014) revealed its anti-cancer property. The seeds of *C. bonducella* are found to contain various chemical constituents such as furanoditerpenes, phytosterinin, β-sitosterol, flavonoids, bonducellin, aspartic acid, arginine, citrulline and β-carotene (Williamson, 2002). Even though some traditional uses and medicinal properties of *C. bonducella* seeds were reported, no scientific investigation is performed. Hence, the purpose of the present study was to evaluate the phytochemical properties, chemical composition, nutritional value and medicinal properties such as anti-inflammatory and antioxidant activities of *C. bonducella* seeds.

## MATERIALS AND METHODS

**Sample collection:** The seeds of *Caesalpinia bonducella* L. were collected on 02 February, 2015 from local market, Thanjavur, Tamilnadu, India. Identification and authentication of seeds were carried out by Dr. N. Ravichandran, Botanist, Department of CARISM, SASTRA University, Thanjavur. The collected materials were cleaned, shade dried and coarsely powdered and used for further studies.

**Physicochemical properties:** Powder microscopic characteristics were observed under light microscope. The sensory nature and physicochemical properties of dry powder of selected plants will be analyzed according to IMHF (1989). Different chemical tests including fluorescence analysis were performed to qualitatively and quantitatively to identify the major chemical constituents (Trease and Evans, 1989; Harborne, 1998; Edeoga *et al*., 2005). Biochemical profile such as carbohydrate (Southgate, 1969), free amino acid (Chen *et al*., 2009), protein and energy value (Rajashree *et al*., 2012), fat (Eller and King, 1996), fibre (Van Soest, 1973), cholesterol (Valsta *et al*., 2004), thiamine (Chaikelis, 1947), riboflavin (Weisberg and Levin, 1937), niacin (Okwu and Josiah, 2006), vitamin E (Jayasree *et al*., 1985), vitamin C (Sarkiyayi and Ikioda, 2010), catalase (Sinha, 1972), lipase (Arzoglou *et al*., 1992), amylase (Huggins and Russel, 1948), acid phosphatase (Kolari and Sarjala, 1995) and alkaline phosphatase (Olusegun *et al*., 2013) were estimated. X-ray fluorescence spectrophotometer, flame photometry, atomic absorption spectroscopy were used to analyze the mineral composition while, the phytochemical profile was evaluated by GC-MS and HPTLC techniques.

**Pharmacological properties:** Aqueous extract of seeds of *C. bonducella* was prepared by soaking the coarse powder of seeds (100 g) in distilled water (1 L) and kept in shaker for 48 h at room temperature. Then, the content was filtered through Whatman filter paper (No. 42) and the filtrate was collected, frozen and lyophilized. The extract thus obtained was re-suspended in water at 1 mg $mL^{-1}$ ratio and used for experiments. The anti-inflammatory activity was investigated based on *in vitro* methods such as inhibition of protein (Albumin) denaturation activity (Alhakmani *et al*., 2014), protease inhibition activity (Chandra *et al*., 2012) and membrane stabilization activity in human red blood cells (hypo-tonicity induced haemolysis) (Sadique *et al*., 1989). Results were compared with aspirin (250 μg $mL^{-1}$) treated samples. Similarly, antioxidant activity was determined using DPPH free radical scavenging assay (Alhakmani *et al*., 2013) and lipid peroxidation assay (Kabir *et al*., 2014). In this study, BHT is used as positive control.

## RESULTS AND DISCUSSION

**Physicochemical properties:** The powder microscopy of *C. bonducella* revealed the presence of palisade like elongated linea lucida cells, parenchyma cells with starch grains, osteosclereids filled with brown contents, prismatic type of calcium oxalate crystals, xylem vessels with pitted thickening (Fig. 1). Starch grains are simple and compound with round, oval, polygonal and irregular shaped margins with closely striated and central linear hilum. Fatty oil globules and piriform fibres are also seen.

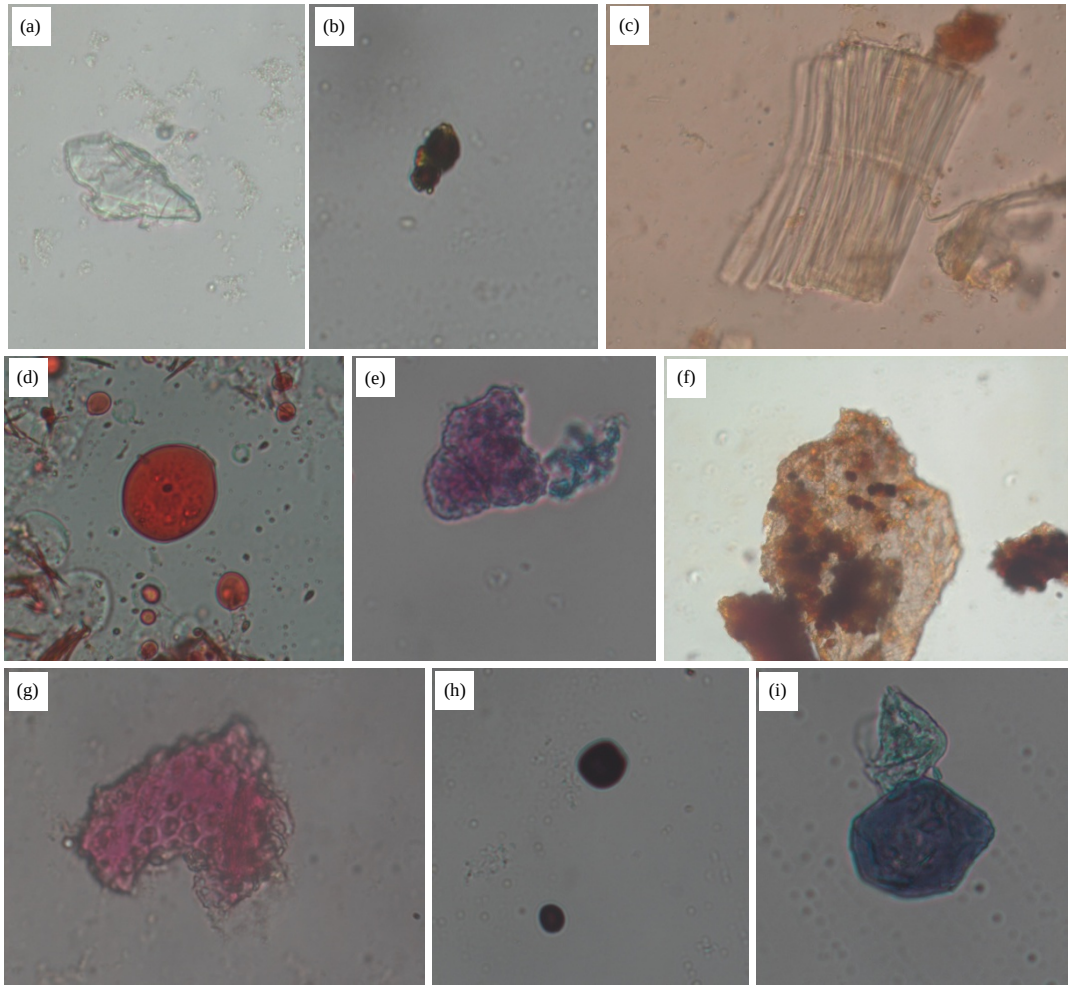


Fig. 1(a-i): Powder microscopic details of *Caesalpinia bonducella* L. seeds, (a) Calcium oxalate crystals, (b) Compound starch grains, (c) LL, D: Oil globules, (e) Parenchyma cells, (f) Parenchyma with starch grains, (g) Sclereids, (h) Starch grains and (i) Stone cells

The data obtained on sensory evaluation revealed that the selected part of *C. bonducella* is astringent in taste, light brown colour with characteristic odour (Table 1). Loss on drying is 8.83%, which implies that the shelf life for this plant material appears to be longer. Ash content 3.37% reveals that the plant is rich in mineral contents. Solubility in water (28.8%) is greater than that of in alcohol (26.8%). The extractive value suggests that the sample satisfies purity standards and is also rich in high polar compounds. Among the different solvents, water extract of *C. bonducella* was found to have maximum yield (6.7%) followed by hexane, ethanol, chloroform and ethyl acetate.

Preliminary phytochemical analysis on water, hexane, ethanol, chloroform and ethyl acetate extract of *C. bonducella* exhibits the presence of carbohydrates, saponins, alkaloids, phenolic compounds, tannins and lignins (Table 2). Lignin recorded higher percentage of yield (74.7 mg $g^{-1}$), when compared to tannin (4.90 mg $g^{-1}$), phenol (0.60 mg $g^{-1}$), flavonoid (0.33 mg $g^{-1}$) and alkaloid (0.12 mg $g^{-1}$) in the seeds of *C. bonducella* (Table 3). Secondary metabolites play both a defensive role against herbivore, pathogen attack and inter-plant competition and an attractant role towards beneficial organisms such as pollinators or symbionts (Wink and Schimmer, 1999). Plant secondary products also have protective actions in relation to abiotic stresses such as those associated with changes in temperature, water status, light levels, UV exposure and mineral nutrients. Furthermore, recent study has indicated potential role of secondary products at the cellular level as plant growth regulators, modulators of gene expression and in signal transduction (Kaufman *et al*., 1999). Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al*., 2007). Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, etc. (Ali *et al*., 2008). A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers (Singh, 2006). Flavonoids present in the plant might be responsible for anti-inflammatory properties (Kunle and Egharevba, 2009). Tannins help in wound healing and as an anti-parasite and could reduce the risk of coronary heart diseases. Alkaloids are a diverse group of secondary metabolites found to exhibit antimicrobial activity. Alkaloids are also known for decreasing blood pressure, balancing the nervous system in case of mental illness and also possess anti-malarial properties (Batista *et al*., 2009).

Table 1: Physicochemical properties of *Caesalpinia bonducella* L. seeds

| Parameters | Physicochemical properties |
|---|---|
| Taste | Astringent |
| Color | Light brown |
| Odour | Characteristic odour |
| Foreign matter (%) | 0.979 |
| Loss on drying (%) | 8.83 |
| Total ash (%) | 3.37 |
| Acid insoluble ash (%) | 0.49 |
| Water soluble (%) | 1.69 |
| Sulphated ash (%) | 4.37 |
| Solubility in alcohol (%) | 26.8 |
| Solubility in water (%) | 28.8 |
| Extractive value in hexane (%) | 4.3 |
| Extractive value in chloroform (%) | 2.58 |
| Extractive value in ethyl acetate (%) | 0.92 |
| Extractive value in ethanol (%) | 2.92 |
| Extractive value in water (%) | 6.7 |

Table 2: Phytochemical screening of various extracts of *Caesalpinia bonducella* L. seeds

| Tests | Reagents used | Hexane | Chloroform | Ethyl acetate | Ethanol | Water |
|---|---|---|---|---|---|---|
| Alkaloids | Dragendroff's | - | - | - | - | + |
| | Mayer's | - | - | - | - | + |
| | Wagner's | - | - | - | - | + |
| | Hager's | - | - | - | - | + |
| Reducing sugar | Fehling's | + | - | + | - | - |
| Carbohydrates | Molisch's | + | + | - | + | - |
| Saponins | Foam's | + | + | - | - | + |
| Glycosides | Anthrone | - | - | - | - | - |
| Steroids | Liebermann burchard | - | - | - | - | - |
| Flavonoids | Shinado's | - | - | - | - | - |
| Phenolic compound | Ferric chloride | + | - | - | - | + |
| Tannin | Lead acetate | - | - | - | - | + |
| Quinone | Sulphuric acid | - | - | - | - | - |
| Anthraquinone | Aqueous ammonia | - | - | - | - | - |
| Lignin | Phloroglucinol | + | - | + | + | - |
| Proteins | Millon's | - | - | - | - | - |
| Amino acids | Ninhydrin | - | - | - | - | - |

Table 3: Estimation of major phytoconstituents of *Caesalpinia bonducella* L. seeds

| Phytoconstituents | Content (mg $g^{-1}$ sample) |
|---|---|
| Flavonoid | 0.33 |
| Alkaloid | 0.12 |
| Lignin | 74.7 |
| Tannin | 4.90 |
| Phenol | 0.60 |

Table 4: Fluorescence analysis of *Caesalpinia bonducella* L. seeds

| | Chromogen at UV light | | |
|---|---|---|---|
| Powder+reagent | Chromogen at visible light | 254 nm | 366 nm |
| Powder | Light brown | Light brown | Grayish white |
| Powder+$H_2So_4$ | Yellowish brown | Brown | Brown |
| Powder+$HNO_3$ | Yellow | Yellowish orange | Yellow |
| Powder+$CH_3COOH$ | Reddish brown | Light brown | Grey |
| Powder+$NH_4OH$ | Dark brown | Maroon | Grey |
| Powder+$I_2$ | Brown | Reddish brown | White |
| Powder+$Fecl_3$ | Green | Greenish brown | Black |
| Powder+picric acid | Yellow | Green | Greenish orange |
| Powder+NaOH | Reddish brown | Brown | Grayish yellow |

Table 5: Nutritional value and biochemical composition of *Caesalpinia bonducella* L. seeds

| Parameters | Content |
|---|---|
| Energy value (kcal) | 73.60 |
| Carbohydrate (mg $g^{-1}$) | 18.40 |
| Protein (mg $g^{-1}$) | 17.60 |
| Total fat (mg $g^{-1}$) | 3.60 |
| Crude fibre (mg $g^{-1}$) | 3.30 |
| 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 $g^{-1}$) | 10.60 |
| Niacin (µg $g^{-1}$) | 22.60 |
| Riboflavin (µg $g^{-1}$) | 89.60 |
| Vitamin E (µg $g^{-1}$) | 6.09 |
| Vitamin C (µg $g^{-1}$) | 4.20 |
| Catalase (µg $g^{-1}$) | 9.60 |
| Lipase (µg $g^{-1}$) | 12.90 |
| Amylase (µg $g^{-1}$) | 12.30 |
| Alkaline phosphatase (mg $g^{-1}$) | 0.56 |
| Acid phosphatase (mg $g^{-1}$) | 0.25 |

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. The seed powder as such and after treatment with various solvents was subjected to fluorescence analysis (Table 4). Observations were made under visible light and under UV light of short wavelength and long wavelength separately. Many phytochemicals fluorescence fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non-fluorescent compound may fluorescence, if mixed with impurities that are fluorescent. Hence, it is useful in detecting the adulterants and substituent. Some constituents show fluorescence in the visible range in many natural products (e.g., alkaloids like berberine), which do not visibly fluorescence in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacology evaluation (Ali, 2008).

Table 6: Mineral composition and heavy metal content of *Caesalpinia bonducella* L. 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 |

**Nutritional value:** Nutritional value of *C. bonducella* is clearly depicted in the Table 5. Energy value (73.6%), crude fibre (3.3 mg $g^{-1}$) and cellulose (2.59 mg $g^{-1}$) contents of the selected plant material were observed. Intake of dietary fibres can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ishida *et al*., 2000). The RDA of fibres essential for children, adults, pregnant and lactating mothers are 19-25, 21-38 and 28-29%, respectively. Thus *C. bonducella* can act as a valuable source of dietary fibre in human nutrition. Other nutritional constituents found are total fat (3.6%), free amino acids (1.82%), protein (17.6%) and carbohydrates (18.4%). Free fatty acid content is present as little as 0.03 mg $g^{-1}$. In addition to this, the plant is rich in vitamins such as vitamin E (6.09 µg $g^{-1}$), vitamin C (4.2 µg $g^{-1}$), thiamine (10.6 µg $g^{-1}$), niacin (22.6 µg $g^{-1}$) and riboflavin (89.6 µg $g^{-1}$).

The XRF and flame photometry data suggested that the plant is rich in minerals especially K, Ca, Fe, P, S, Mg, Si, Cl, Pb, Pd, Al, Mo, Cu and Zn (Table 6). From this data, it can be deduced that seeds of *C. bonducella* has high content of K, Ca and Fe. Deficiency of calcium and phosphorous leads to the classical bone symptoms associated with rickets, such as bowlegs, knock knees, curvature of the spine and pelvic and thoracic deformities. Fe could be used in the treatment of anemia. Magnesium plays important role in the structure and

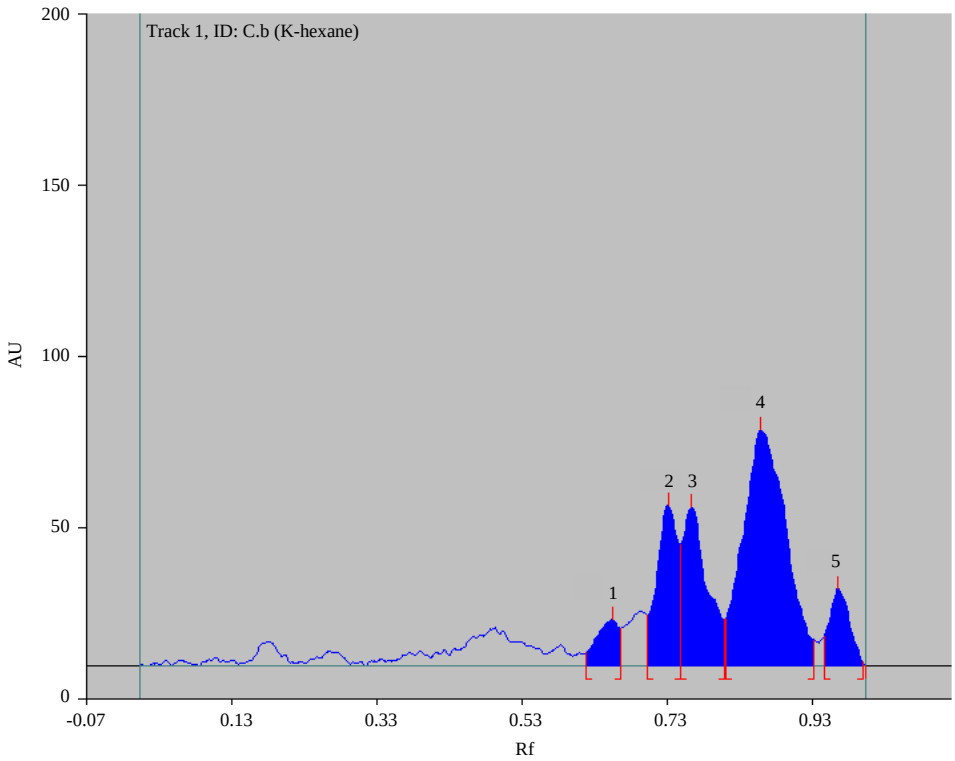


Fig. 2: HPTLC profile of hexane extract of *Caesalpinia bonducella* L. seeds

the function of the human body. Iron, zinc, copper and manganese are playing an important role in the improvement of antioxidant system. The positive impact of zinc supplementation on the growth of some stunted children and on the prevalence of selected childhood diseases such as diarrhoea, suggests that zinc deficiency is likely to be a significant public health problem, especially in developing countries (Osendarp *et al*., 2003; Hussain *et al*., 2009). According to FAO's food balance data, it has been calculated that about 20% of the world's population could be at risk of zinc deficiency with the average daily intake less than 70 µg per day (Brown *et al*., 2004).

Enzymes such as lipase (12.9 µg $g^{-1}$), amylase (12.3 µg $g^{-1}$), catalase (9.6 µg $g^{-1}$), alkaline phosphatase (0.56 µg $g^{-1}$) and acid phosphatase (0.25 µg $g^{-1}$) were present in *C. bonducella* seeds (Table 5). Catalase converts the reactive oxygen species hydrogen peroxide to water and oxygen and thereby mitigates the toxic effects of hydrogen peroxide thereby, it prevent oxidative stress. Oxidative stress is hypothesized to play a role in the development of many chronic or late-onset diseases such as diabetes, asthma, Alzheimer's disease, systemic lupus erythematosus, rheumatoid arthritis and cancers, which have been associated with decreases in catalase activity. A study from the Institute of Cytology and Genetics found that oxidative stress, accumulation of protein and DNA damage could be reduced in the presence of antioxidant enzymes catalase in the cytosol and mitochondrial extracts from liver cells of rats (Sinitsyna *et al*., 2006). This study also found that dietary supplements for increasing the activity of catalase in the liver mitochondria in rats led to reduced mitochondrial dysfunction and slowed the process of aging in these animals. One of the most familiar functions of lipase in the body is digestion of dietary fat. Another important function of lipases is to help the body package cholesterol for transport and also helps the biosynthesize the vitamins A, D, E and K. The ALP hydrolyzes the phosphate moiety and makes tyrosine available for conversion to catecholamines that are then used to cross-link proteins during sclerotization (Lunan and Mitchell, 1969).

**Phytochemical profile:** In the last two decades High Performance Thin Layer Chromatography (HPTLC) method has emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulation. This includes TLC fingerprint profile and estimation of chemical markers. The HPTLC fingerprinting of the plant was presented with Rf values under 254 and 366 nm that confirm the presence of different type of phytochemical compounds in the aqueous extract of seeds of *C. bonducella*. Five bands with blue (Rf 0.66, 0.74 and 0.81), pink (Rf 0.93 and 1.00) were noted in hexane extract (Fig. 2) while, four bands with green colour were observed with Rf values of 0.41, 0.52,

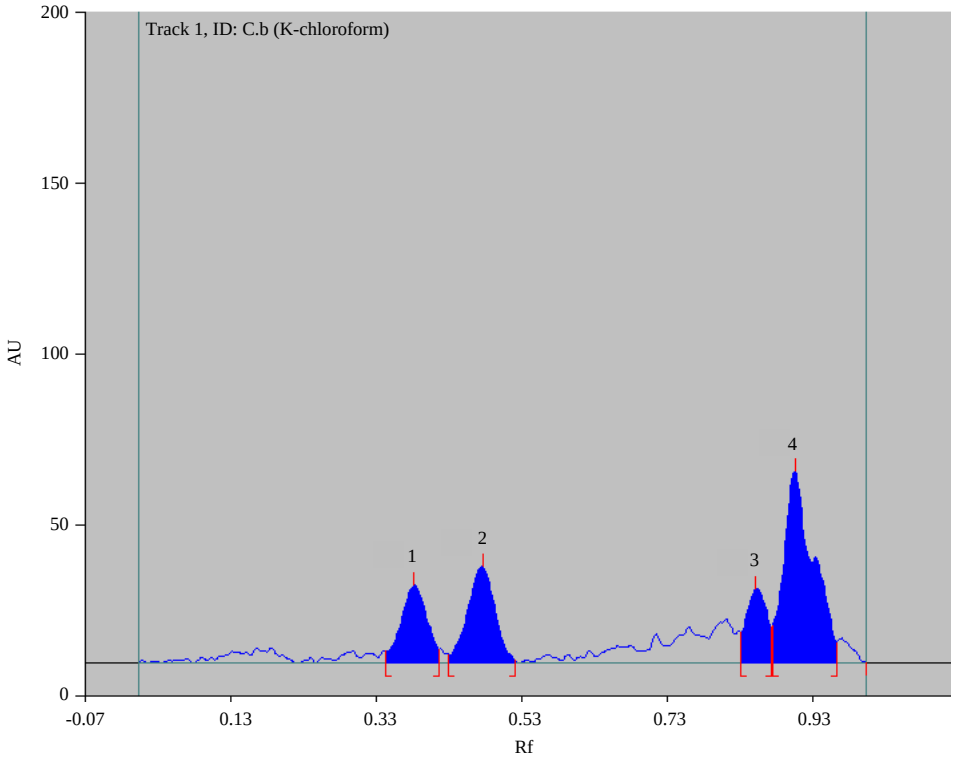


Fig. 3: HPTLC profile of chloroform extract of *Caesalpinia bonducella* L. seeds

0.87 and 0.96 at 254 nm in chloroform extract (Fig. 3). Presence of such bands with different colour and Rf value indicated the occurrence of different types of phytochemicals in the extract of *C. bonducella*.

The GC-MS analysis of the hexane and chloroform extract of *C. bonducella* were performed using a Perkin-Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30×0.25 µm ID×0.25 µm df). In the present study, GC-MS analysis of *C. bonducella* hexane extract revealed the presence of 18 major compounds such as heptane, 2,2,3,5-tetramethyl-, 2-heptenal, (z)-, heptane, 2,2,3,5-tetramethyl-, octane, 3,3-dimethyl-, heptane, 5-ethyl-2,2,3-trimethyl-, dodecane, 2,6,10-trimethyl-, heptane, 5-ethyl-2,2,3-trimethyl-, nonane, 3-methyl-, undecane, 3-methyl-, undecane, 3,8-dimethyl-, decane, 2,3,4-trimethyl-, decane, 3-methyl-, 2,4-decadienal, caryophyllene, n-hexadecanoic acid, 9,12-octadecadienoic acid (z, z) methyl ester, 9,17-octadecadienal, (z)- and 1,2-benzenedicarboxylic acid mono(2-ethylhexyl) ester (Fig. 4 and Table 7). The chloroform extract exhibited 11 major compounds like octane, hexanal, 2-heptenal (z)-, hexanoic acid, 2-decenal, (e)-, 2,4-decadienal, 2,4-decadienal, (E, E)-, benzene, (1-pentylheptyl)-, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, n-hexadecanoic acid and 9,12-octadecadienoic acid (z,z) (Fig. 5 and Table 8).

**Anti-inflammatory activity:** Inflammation is a complex process, very often associated with pain. Anti-inflammatory compounds can act on various levels of patho-physiological process by blocking the biosynthesis of pro-inflammatory mediators, by decreasing the enzyme expression or by reducing substrate levels or by inhibiting the release of performed stored mediators, by blocking mediator-receptor interaction on target cells and immune stimulation, which results in less aggressive response to allergen challenge (Mujumdar *et al*., 2000). Inflammation is described as the succession of changes in a living tissue, when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality, as well injure living microcirculation and related tissues (Sanderson, 1971). Inflammatory response to tissue injury involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane and Botting, 1995). Inflammation can be classified as either acute or chronic (Coussens and Werb, 2002). Acute inflammation is the initial response of the body to harmful

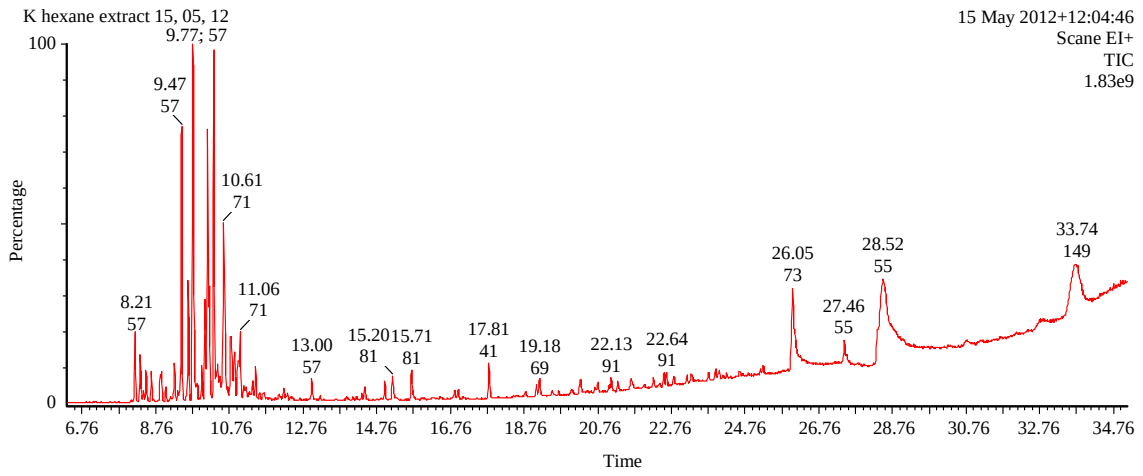


Fig. 4: GC-MS profile of hexane extract of *Caesalpinia bonducella* L. seeds

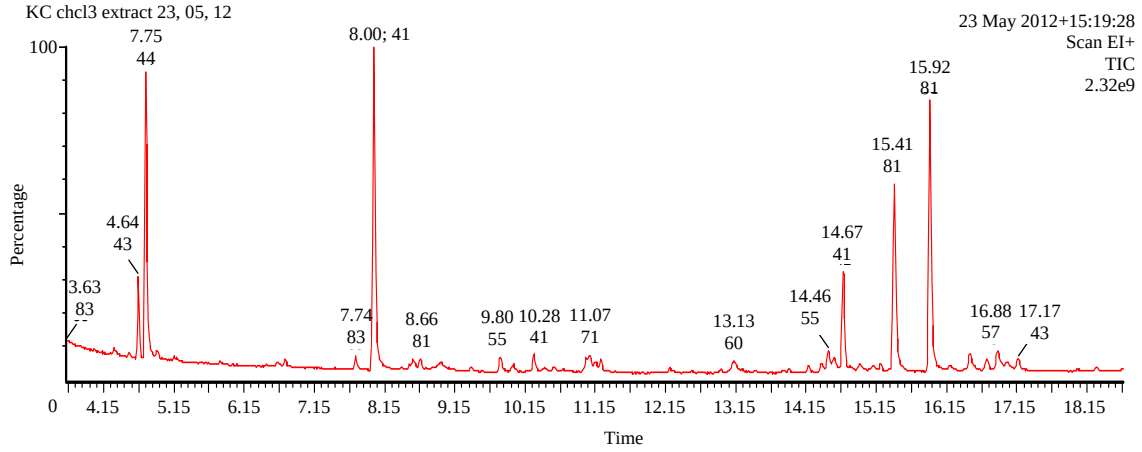


Fig. 5: GC-MS profile of chloroform extract of *Caesalpinia bonducella* L. seeds

Table 7: GC-MS profile of hexane extract of *Caesalpinia bonducella* L. seeds

| Peak names | Formula | Molecular weight | Retention time | Peak area | Peak area (%) |
|---|---|---|---|---|---|
| Heptane, 2,2,3,5-tetramethyl- | $C_{11}H_{24}$ | 156 | 8.21 | 12097773 | 1.8861 |
| 2-heptenal, (z)- | $C_7H1_2O$ | 112 | 8.35 | 10185596 | 1.5880 |
| Heptane, 2,2,3,5-tetramethyl- | $C_{11}H_{24}$ | 156 | 8.50 | 7029960 | 1.0960 |
| Octane, 3,3-dimethyl- | $C_{10}H_{22}$ | 142 | 8.92 | 9756205 | 1.5210 |
| Heptane, 5-ethyl-2,2,3-trimethyl- | $C_{12}H_{26}$ | 170 | 9.47 | 51033712 | 7.9563 |
| Dodecane, 2,6,10-trimethyl- | $C_{15}H_{32}$ | 212 | 9.77 | 70577472 | 11.0032 |
| Heptane, 5-ethyl-2,2,3-trimethyl- | $C_{12}H_{26}$ | 170 | 10.18 | 49973040 | 7.7909 |
| Nonane, 3-methyl- | $C_{10}H_{22}$ | 142 | 10.34 | 57669852 | 8.9909 |
| Undecane, 3-methyl- | $C^{12}H_{26}$ | 170 | 10.61 | 49248872 | 7.6780 |
| Undecane, 3,8-dimethyl- | $C_{13}H_{28}$ | 184 | 10.81 | 17569720 | 2.7392 |
| Decane, 2,3,4-trimethyl- | $C_{13}H_{28}$ | 184 | 11.06 | 14434844 | 2.2504 |
| Decane, 3-methyl- | $C_{11}H_{24}$ | 156 | 11.46 | 7821664 | 1.2194 |
| 2,4-decadienal | $C_{10}H_{16}O$ | 152 | 15.20 | 7066797 | 1.1017 |
| Caryophyllene | $C_{15}H_{24}$ | 204 | 17.81 | 7644363 | 1.1918 |
| n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 26.05 | 32527574 | 5.0711 |
| 9,12-octadecadienoic acid (z,z)-, methyl ester | $C_{19}H_{34}O_2$ | 294 | 27.46 | 14354317 | 2.2379 |
| 9,17-octadecadienal, (z)- | $C_{18}H_{32}O$ | 264 | 28.52 | 82469656 | 12.8572 |
| 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester | $C_{16}H_{22}O_4$ | 278 | 33.74 | 69070088 | 10.7682 |

Table 8: GC-MS profile of chloroform extract of *Caesalpinia bonducella* L. seeds

| Peak names | Formula | Molecular weight | Retention time | Peak area | Peak area (%) |
|---|---|---|---|---|---|
| Octane | $C_8H_{18}$ | 114 | 4.64 | 15451957 | 2.2506 |
| Hexanal | $C_6H_{12}O$ | 100 | 4.75 | 74878912 | 10.9060 |
| 2-heptenal, (z)- | $C_7H_{12}O$ | 112 | 8.00 | 98459864 | 14.3405 |
| Hexanoic acid | $C_6H_{12}O_2$ | 116 | 8.94 | 7048877 | 1.0267 |
| 2-decenal, (e)- | $C_{10}H_{18}O$ | 154 | 14.67 | 27702498 | 4.0348 |
| 2,4-decadienal | $C_{10}H_{16}O$ | 152 | 15.41 | 69594144 | 10.1363 |
| 2,4-decadienal, (e,e)- | $C_{10}H_{16}O$ | 152 | 15.92 | 89079616 | 12.9743 |
| Benzene, (1-pentylheptyl)- | $C_{18}H_30$ | 246 | 23.01 | 9686806 | 1.4109 |
| 3,7,11,15-tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 296 | 24.62 | 35051964 | 5.1053 |
| N-hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 27.40 | 106674760 | 15.5370 |
| 9,12-octadecadienoic acid (z,z)- | $C_{18}H_{32}O_2$ | 280 | 31.79 | 77697776 | 11.3166 |

Table 9: *In vitro* anti-inflammatory activity of aqueous extract of *Caesalpinia bonducella* L. seeds

| | Anti-inflammatory activity | | |
|---|---|---|---|
| Concentration of the extract (µg) | Protein denaturation activity (%) | Protease inhibition activity (%) | Membrane stabilization (%) |
| 1000 | 94.15±19.26 | 32.40±3.37 | 81.66±0.63 |
| 500 | 75.83±12.71 | 31.90±3.81 | 54.16±2.61 |
| 250 | 61.53±11.80 | 31.76±5.01 | 51.56±0.35 |
| 125 | 55.02±11.01 | 29.96±6.08 | 45.21±3.53 |
| 62.5 | 47.71±10.53 | 27.74±6.52 | 39.04±1.37 |
| 31.25 | 40.73±7.68 | 26.06±5.78 | 33.41±5.65 |
| 15.6 | 34.22±2.08 | 23.27±6.45 | 32.54±0.25 |
| 7.81 | 24.42±3.13 | 21.45±6.61 | 21.52±0.12 |
| 3.90 | 19.92±6.64 | 18.40±5.68 | 21.36±2.33 |
| 1.95 | 10.91±22.01 | 15.02±1.33 | 11.16±0.42 |
| Aspirin | 38.58±12.05 | 27.12±15.71 | 91.91±0.84 |

stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells within the injured tissues. Prolonged inflammation, known as chronic inflammation leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction.

Successful predication of natural compounds from plant material largely depends on the type of solvent used in the extraction process. Traditional healers use primarily water as the solvent, hence, in the present study also aqueous extract was used to evaluate the medicinal properties of *C. bonducella* seeds. Denaturation of proteins is a well-documented cause of inflammation. Since during inflammation condition, protein of the cell gets denatured, albumin protein is used as a model, whose protection by the plant extract during heat-induced denaturation was evaluated (Gupta *et al*., 2013). Inhibition of protein denaturation (albumin) activity of aqueous extract *C. bonducella* seeds revealed high anti-inflammatory activity (94.15% inhibition at 1000 µg $mL^{-1}$ concentration) (Table 9), which is higher than that of standard aspirin. The neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further protein denaturation and subsequent tissue inflammation and damage (Chou, 1997). Thus, the aqueous extract of *C. bonducella* seeds may possibly inhibit the protein denaturation caused by the release of lysosomal content of neutrophils at the site of inflammation.

Proteases have been implicated in arthritic reactions. Neutrophiles contain neutral serine protease in their liposomal granules. Leukocyte protease plays an important role in the development of tissue damage during inflammatory reactions and significant level of protection is provided by protease inhibitors (Sakat *et al*., 2010). Protease inhibition study revealed that the aqueous extract of seeds of *C. bonducella* showed moderate activity of 32% inhibition at 1000 µg $mL^{-1}$ concentration (Table 9). However, this value is higher than that of standard aspirin (27.12%).

Stabilization of liposomal membrane is important in limiting the inflammatory response by inhibiting the release of liposomal constituents of activated neutrophil such as bactericidal enzymes and protease, which cause further tissue inflammation and damage upon extracellular release (Da Silveira e Sa *et al*., 2013). Human Red Blood Cell Membrane (HRBC) is analogous to the liposomal membrane and its stabilization implies that the extract may stabilize liposomal membranes. Hypo-tonicity induced HRBC membrane damage can be taken as an *in vitro* measure of anti-inflammatory activity of the selected plant extracts (Ballabeni *et al*., 2010).

Membrane stabilization method revealed significant membrane stabilizing activity of aqueous extract of *C. bonducella* seeds (81.66%), which was comparable to that of standard drug (Table 9). The aqueous extract of *C. bonducella* seeds was effective in inhibiting the hypo-tonicity induced hemolysis of erythrocyte membrane. This property provides evidence for membrane stabilization as an additional mechanism of anti-inflammatory effect of *C. bonducella* seed extract.

**Antioxidant property:** The mechanism of inflammation is attributed to the release of Reactive Oxygen Species (ROS) from activated neutrophils and macrophages. Over-production of ROS results in tissue injury by damaging macromolecules and membranes. In addition, it propagates inflammation by stimulating the release of cytokines such as interleukin (IL-1), Tumour Necrosis Factor (TNF-α) and interferon-γ, which are responsible for the recruitment of additional neutrophils and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory responses (Matcha *et al*., 2013). Free radicals are important in the regulation of signal transduction, gene expression and activation of receptors (Ajith and Janardhanan, 2007). However, an excess of free radicals is toxic to almost every biological molecule in living cells (Liu *et al*., 2008) and can cause oxidative damage to functional macromolecule, if not eliminated quickly (Cui *et al*., 2012). Free radicals and other ROS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals (Bagchi and Puri, 1998). Excess generation of free radicals can lead to many age-related disorders like cancer, atherosclerosis, neurogenerative diseases and inflammation (Sagar *et al*., 2009).

The mechanism of inflammatory injury is attributed, in part to the release of ROS from activated neutrophils and macrophages. The over-production of ROS leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes (Roy *et al*., 2010). In addition, ROS propagates inflammation by stimulating the release of cytokines such as interleukin-1, tumor necrosis factor and interferon-γ, which stimulate recruitment of additional neutrophils and macrophages (Hamid *et al*., 2013). Thus free radicals are important mediators that provoke or sustain inflammatory process. In addition to ROS, hydrogen peroxide can also easily cross the cell membrane by convert into water and attack different sites (Chen *et al*., 2008). It can cause DNA damage in the form of single and double strand breaks believed to be the initial step in the induction of cancer (Mothana *et al*., 2009). Neutralization of ROS by antioxidants can attenuate inflammation (Singh *et al*., 2010). Currently available synthetic antioxidants like Butylated Hydroxyl Anisole (BHA), Butylated Hydroxyl Toluene (BHT), tertiary butylated hydroquinone and gallic acid esters have been suspected to cause negative health effects. Moreover, these synthetic antioxidants are lipophilic in nature and hence show low water solubility. Hence, due to these limitations in employing synthetic antioxidants, there is an urgent need to identify a natural source of antioxidants. Antioxidant compounds from plant can minimize the generation of free radicals (Dragland *et al*., 2003) and alleviate diseases caused by oxidative stress (Ozen *et al*., 2010).

Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants especially in reducing free radical-induced tissue injury (Pourmorad *et al*., 2006). Some well-known and traditionally used natural antioxidants such as tea, wine, fruits, vegetables, spices are already explored commercially as nutritional supplements (Schuler, 1990). Nutraceuticals with antioxidant property are non-toxic or may have minimum side effects than synthetic compounds. It has been well established that antioxidant activity of plants might be due to their phenolic constituents (Duh *et al*., 1999). Flavonoids are group of polyphenolic compounds known for a wide spectrum of biological properties which include free radical scavenging, enzymes inhibitory and anti-inflammatory properties (Sakat *et al*., 2010). The DPPH is a synthetic free radical used to evaluate the antioxidant property of plant extracts. The seeds of *C. bonducella* exhibited 90% of maximum free radical scavenging activity at a concentration of 1000 μg $mL^{-1}$ and low effect (22.39%) was observed at a concentration of 1.95 μg $mL^{-1}$ (Table 10). This result confirms the dose-dependent antioxidant activity of aqueous extract of *C. bonducella* seeds.

Table 10: *In vitro* antioxidant potential of aqueous extract of *Caesalpinia bonducella* L. seeds

| | Antioxidant activity | |
|---|---|---|
| Concentration of the extract (μg) | DPPH free radical scavenging activity (%) | Lipid peroxidation (%) |
| 1000 | 90.00±9.19 | 99.69±0.41 |
| 500 | 78.94±6.15 | 98.99±0.56 |
| 250 | 76.84±2.61 | 97.74±0.91 |
| 125 | 74.19±5.37 | 96.34±0.07 |
| 62.5 | 68.49±0.98 | 88.89±0.98 |
| 31.25 | 59.59±0.84 | 84.74±1.48 |
| 15.6 | 46.24±4.17 | 75.44±0.35 |
| 7.81 | 35.49±0.14 | 68.09±2.82 |
| 3.90 | 27.29±4.10 | 42.79±1.69 |
| 1.95 | 22.39±1.55 | 22.84±1.20 |
| BHT | 85.24±21.21 | 94.75±1.67 |

Malondialdehyde, a lipid peroxidation product is a mediator of ROS generation in the tissue (Sultana *et al*., 2009). During lipid peroxidation, secondary oxidizised products of lipids form pink chromogen with thiobarbituric reagent and the amount of TBARS can be used as an index of lipid peroxidation (Akinmoladun *et al*., 2007). Lipid peroxidation method revealed that the aqueous extract of seeds of *C. bonducella* possess high antioxidant effect at 1000 μg $mL^{-1}$ (99.69%), which is higher than that of standard BHT (Table 10). As phenol antioxidants are suggested to act as inhibitors of lipid peroxidation by means of free radical scavenging, it is expected that the presence of phenolic compounds in the aqueous extract of *C. bonducella* seeds might be responsible for inhibiting the lipid peroxidation by donating the hydrogen atom (Sakat *et al*., 2010).

## CONCLUSION

Results of the present study depicted that the seeds of *Caesalpinia bonducella* L. possess good physicochemical properties, high nutritional value, ambient phytochemical compounds and medicinal properties such as anti-inflammatory and antioxidant activities. This justifies and provides scientific evidences for its usage in folk-lore claims on the medicinal use of *C. bonducella*. Thus, the seed materials of *C. bonducella* could be used to formulate a nutraceutical product with significant nutritional and medicinal properties. The physicochemical properties and phytochemical profiles investigated in the present study could be helpful in authentication of *C. bonducella* material and also useful as quality control tools.

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