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Screening of antioxidant and antimicrobial activities of *Caesalpinia bonducella* Flem., leaves (Caesalpiniaceae)

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SUMMARY

The study was aimed at evaluating the antioxidant and antimicrobial activities of methanol extract of *Caesalpinia bonducella* leaves (MECB) (Family: Caesalpiniaceae). The effect of MECB on antioxidant activity, reducing power, free radical scavenging (DPPH radical, nitric oxide radical, superoxide anion radical, hydroxyl radical and hydrogen peroxide radical scavenging), total phenolic content and antimicrobial activities were studied. The antioxidant activity of MECB increased in a dose dependent manner. About 50, 100, 250 and 500 g of MECB showed 53.4, 61.2, 69.1 and 76.2 % inhibition respectively on peroxidation of linoleic acid emulsion. Like antioxidant activity, the effect of MECB on reducing power increased in a dose dependent manner. The free radical scavenging activity of MECB was determined by DPPH radical scavenging method. The potency of this activity was increased with increased amount of extract. MECB was found to inhibit the nitric oxide radicals generated from sodium nitroprusside (IC_{50} = 102.8 g/ml) whereas the IC_{50} value of curcumin was 20.4 g/ml. Moreover, the MECB was found to scavenge the superoxide generated by photoreduction of Riboflavin. MECB was also found to inhibit the hydroxyl radical generated by Fenton reaction, where the IC_{50} value is 104.17 g/ml compared with catechin 5 g/ml, which indicates the antioxidant activity of MECB. The MECB capable of scavenging hydrogen peroxide in a concentration-dependent manner. The amounts of total phenolic compounds were also determined. Antimicrobial activities of MECB were carried out using disc diffusion methods with five Gram positive, four Gram negative and four fungal species. The results obtained in the present study indicate that MECB leaves are potential source of natural antioxidant and antimicrobial agents.

Keywords: *Caesalpinia bonducella*; Antioxidant activity; Free radical scavenging; DPPH test; Antimicrobial

INTRODUCTION

Reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals ($OH^·$) and non free-radical species such as H_2O_2 and singled oxygen ($^1O2$), are various forms of activated oxygen (Halliwel and Gutteridge, 1999; Yildirim *et al.*, 2000; Gulcin *et al.*, 2002). The importance of free radicals and ROS has attracted increasing attention over the past decade (Gulcin *et al.*, 2002). These molecules are exacerbating factors in cellular injury and aging process (Lai *et al.*, 2001).

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In living organisms, various ROS can form in different ways. Normal aerobic respiration stimulates polymorphonuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of ROS include tobacco smoke, certain pollutants, organic solvents, and pesticides (Halliwell and Gutteridge, 1989; Davies, 1994; Robinson *et al.*, 1997).

ROS have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer (Hertog *et al.*, 1993; Tanizawa *et al.*, 1996; Duh, 1998; Alho and Leinonen, 1999; Yildirim *et al.*, 2001).

O_2^- is an oxygen-centred radical with selective reactivity. This species is produced by a number of enzyme systems in autooxidation reactions and by nonenzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complex such as cytochrome *c*. H_2O_2 is not a radical and can be formed *in vivo* by many oxidize enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds. OH^- is a highly reactive oxygen-centred radical, which attacks all proteins, deoxyribonucleic acid (DNA), and polyunsaturated fatty acid (Aruoma, 1998).

ROS are continuously produced during normal physiologic events and are removed by antioxidant defence mechanisms (Halliwell *et al.*, 1992). There is a balance between the generation of ROS and inactivation of ROS by the antioxidant system in organisms. Under pathological conditions, ROS are overproduced and result in lipid peroxidation and oxidative stress. ROS are formed when endogenous antioxidant defenses are inadequate. The imbalance between ROS and antioxidant defense mechanisms leads to oxidative modification in cellular membrane or intracellular molecules (El-Habit *et al.*, 2000).

Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers (Yen and Duh, 1994; Duh, 1998). Among them one of the plant *Caesalpinia bonducella* (L.) Flem., Fever nut; Bunduc nut, (Family: Caesalpiniaceae) commonly known as Nata Karanja (Hindi), a prickly shrub; found throughout the hotter parts of India, Myanmar and Sri Lanka. The leaves of this plant are traditionally used for the treatment of liver disorders, inflammation and tumors (Kirtikar and Basu, 1975). It has also been to possess multiple therapeutic properties like antipyretic, antidiuretic, anthelmintic, antibacterial, anticonvulsant, anti-anaphylactic and antidiarrheal, antiviral, antiasthmatic, antiamebic and antiestrogenic activities. Currently, the hepatoprotective and antioxidant role of *Caesalpinia bonducella* on paracetamol-induced liver damage in rats (Gupta *et al.*, 2003) anti-inflammatory, analgesic and antipyretic activity (Gupta *et al.*, 2004). Antitumor activity and antioxidant status of *C. bonducella* against Ehrlich ascites carcinoma in Swiss albino mice were also reported from our laboratory (Gupta *et al.*, 2004). The chemical constituents of the plant include flavonoids, (Purushothaman *et al.*, 1982) triterpenoids (Lai *et al.*, 1977), diterpenoids (Peter *et al.*, 1997), and steroids (Lyder *et al.*, 1998). A number of reports on flavonoids, triterpenoids, and steroids indicate that they exert multiple biological effects due to their antioxidant and free radical scavenging abilities. These phytoconstituents produced protective effects against tumors, heart disease, and different pathologies (DeFeudis *et al.*, 2003; Takeoka and Dao, 2003). Realizing these facts, this work was carried out to evaluate the antioxidant and antimicrobial activity of the methanol extract of *Caesalpinia bonducella* leaves (MECB).

MATERIALS AND METHODS

Plant material and extraction

The plant *Caesalpinia bonducella* (Family: Caesalpiniaceae) was collected from Kolli Hills of Tamilnadu, India. The plant material was taxonomically identified by the Botanical Survey of India, Kolkata. A voucher specimen (No. GMS-2) has been preserved in our laboratory for the future reference. The leaves were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container. The dried powder material of the leaf (500 g) was defatted with petroleum ether (60-80 ℃) in a soxhlet apparatus. The defatted powder material thus obtained was further extracted with methanol for 72 h in the soxhlet. The solvent was removed by distillation under suction and the resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue (8.78 %). Phytochemical screening of the extract revealed the presence of alkaloids, saponins, flavonoids, triterpenoids, tannins, and steroids.

Chemicals

Ammonium thiocyanate was purchased from E. Merck. Ferrous chloride, ferric chloride, polyoxyethylene sorbitan monolaurate (Tween-20), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), nicotinamide adenine dinucloetide (NADH), EDTA, butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), α-tocopherol, curcumin, quercetin, catechin, nitroblue tetrazolium, thiobarbituric acid, calf thymus DNA, trichloroacetic acid, phenazine methosulphate and potassium ferricyanide were purchased from Sigma Chemical Co. Ltd, USA. All other unlabeled chemicals and reagents were analytical grade.

Total antioxidant activity determination

The antioxidant activity of MECB was determined according to the thiocyanate method (Mistuda *et al.*, 1996). About 10 mg of MECB was dissolved in 10 ml water. Then 1.0 mg of MECB in 1 ml of water was added to linoleic acid in potassium phosphate buffer (2.5 ml, 0.04 M pH 7.0). The mixed solution was incubated at 37 ℃ in a glass flask. The amount of peroxide was determined by measuring the absorbance at 500 nm, after reaction with 0.1 ml of $FeCl_2$ (0.02 M) and 0.1 ml of thiocyanate (30 %) at several intervals during incubation (Yen and Chen, 1995). To eliminate the solvent effect, control sample, which contains the same amount of solvent, added into the linoleic acid emulsion in the test sample and reference compounds used (α-tocopherol-20 μg/ml). All data are the average of five parallel measurements. The percentage inhibition of lipid peroxide generation was measured by using the following equation:

$$\% \text{ Inhibition} = 100 - [(A_1/A_O) \times 100]$$

Where A_O is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of MECB (Duh *et al.*, 1999).

Reducing power

The reducing power of MECB was determined according to the method of Oyaizu (1986). 10 mg of MECB extract in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1 %). The mixture was incubated at 50 ℃ for 20 min. A portion (2.5 ml) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %) and the absorbance was measured at 700 nm. The reductive capability of MECB was compared with butylated hydroxy toluene (BHT). Increased absorbance of the reaction mixture indicated increasing reducing power.

Free radical scavenging activity

The free radical scavenging activity of MECB was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Blois (1958). 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of MECB solution in water at different concentrations (50-250 g). After 30 min, absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH concentration in the reaction medium was calculated from the following calibration curve, determined by linear regression:

Absorbance = 2.4928 × [DPPH˙] + 0.0392

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by Griess reaction (Green *et al.*, 1982; Marcocci *et al.*, 1994). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and the MECB in different concentrations was incubated at 25 ℃ for 150 min. At intervals, samples (0.5 ml) of incubation solution were removed and 0.5 ml of Griess reagent (1 % sulphanilamide, 2 % H_3PO_4 and 0.1 % naphthylethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test compounds.

Superoxide anion radical scavenging activity

Measurement of superoxide anion scavenging activity of MECB was done based on the method described by Nishimiki (1972) and slightly modified. About 1 ml of nitroblue tetrazolium (NBT) solution (156 μM NBT in 100 mM phosphate buffer, pH 7.4) 1 ml of NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution of MECB in water were mixed and the reaction started by adding 100 μl of phenazine methosulphate (PMS) solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 25 ℃ for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition was measured by using the following equation:

$$\% \text{ Inhibition} = [(A_O - A_1) / A_O] \times 100$$

Where A_O is the absorbance of the control, and A_1 is the absorbance of MECB or standard (Ye *et al.*, 2002)

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by Fe^{3+}-ascorbateEDTAH_2O_2 system. (Fenton reaction). The hydroxyl radicals attack deoxyribose that eventually results in TBARS formation. The reaction mixture contained in a final volume of 1.0 ml, 100 μl of 2-deoxy-2-ribose (28 mM in KH_2PO_4-K_2HPO_4 buffer, pH 7.4), 500 μl solutions of various concentrations of MECB in buffer, 200 μl of 1.04 mM EDTA and 200 M $FeCl_3$ (1:1 v/v), 100 μl of 1.0 mM H_2O_2 and 100 μl of 1.0 mM ascorbic acid was incubated at 37 ℃ for 1 hour. The free radical damage imposed on the substrate, deoxyribose was measured as thiobarbituric reactive substance (TBARS) by the method of Ohkawa (1979). 1.0 ml of thiobarbituric acid (1 %) and 1.0ml of trichloroacetic acid (TCA) (2.8 %) were added to the test tubes and were incubated at 100 ℃ for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in

triplicate. The percentage inhibition was determined by comparing the results of the test compounds and control.

Hydrogen peroxide radical scavenging activity

The ability of MECB to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*, (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically measuring absorption with extinction coefficient for H_2O_2 of 81 M^1cm^{-1}. Extracts (12.5 - 62.5 g/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of MECB and standard compounds was calculated:

$$\%\text{Scavanged } [H_2O_2] = [\,(Ao - A_1)\,/Ao] \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the sample of MECB extract or standards.

Determination of total phenolic compounds

Total soluble phenolics in the MECB were determined with FolinCiocalteu reagent according to the method of Slinkard and Singleton (1977). Briefly, 1.0 ml of extract solution containing 1.0 g extract was transferred into 100 ml Erlenmeyer flask then final volume was adjusted to 46 ml by addition of distilled water. Afterwards, 1ml of FolinCiocalteu reagent (FCR) was added to this mixture and after 3 min. 3 ml of Na_2CO_3 (2 %) were added. Subsequently, mixture was shaken on a shaker for 2 h at room temperature and then absorbance was measured at 760 nm. The concentration of total phenolic compounds in MECB was determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graph. The equation is given below;

Absorbance = 0.001 × Pyrocatechol (g) + 0.0033

Preparation of test microorganisms

Pseudomonas aeruginosa (ATCC 9027, gram negative), *Escherichia coli* (ATCC 9837, gram negative), *Salmonella typi* (ATCC 43579, gram negative), *Shigella dysenteriae* (ATCC 13313, gram negative), *Vibrio cholerae* (ATCC 14033, gram negative), *Staphylococcus aureus* (ATCC 6538, gram positive), *Streptococcus pneumoniae* (ATCC 49619, gram positive), *Micrococcus luteus* (ATCC 10240, gram positive), *Staphylococcus epidermidis* (ATCC 12228, gram positive) were used for antibacterial activity. Fungal organism such as *Candida albicans* (ATCC, 10231), *Aspergillus niger* (ATCC 16404), *Aspergillus flavus* (ATCC 9643) and *Alternaria solani* (ATCC 20476) strains were employed for determination of antifungal activity.

Bacteria and fungi were obtained from the stock cultures of Central Drugs Laboratory, Kolkata (CDL), Indian Institute of Chemical Biology (IICB), Kolkata, and Mycology and Plant Pathology Laboratory, Kolkata, India. The bacterial and fungal stock cultures were maintained on Muller Hinton Agar slants, respectively, which were stored at 4℃. For the purpose of antimicrobial evaluation, thirteen microorganisms were used. These bacteria were maintained on Nutrient agar base. The fungus was maintained on sabouraud-dextrose agar, which is often used with antibiotics for the isolation of the pathogenic fungi.

Antimicrobial activity determination

Agar cultures of the test microorganisms were prepared as described by Mackeen *et al.* (1997). Three to five similar colonies were selected and transferred with loop into 5 ml of Tryptone soya broth. Tryptone soya broth is a highly nutritious

versatile medium, which is recommended for general laboratory use and used for the cultivation of aerobes and facultative anaerobes, including some fungi. The broth cultures were incubated for 24 h at 37 ℃. For screening, sterile, 6-mm diameter filter paper disc were impregnated with 25-200 μg of the MECB. They were dissolved in sterile water for the assay by magnetic stirrer. Then the paper discs placed onto Mueller Hinton agar. The inoculum for each organism was prepared from broth cultures. The concentration of cultures was to 10^8 colony forming units (1×10^8 cfu/ml). The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses. Ofloxacin (5 μg/disc), and antifungal miconazole nitrate (40 μg/disc) were used as reference standards, which recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

Statistical analysis

Experimental results were mean ± S.D. of five parallel measurements. The values $P < 0.05$ were regarded as significant and the values < 0.01 highly significant.

RESULTS AND DISCUSSION

Total antioxidant activity determination in linoleic acid emulsion

Total antioxidant activity of MECB was determined by the thiocyanate method. This method was used to measure the peroxide level during the initial stages of lipid oxidation. The antioxidant activity of MECB might be due to hydroperoxides, inactivating of free radicals or complexing with metal ions, or combinations thereof. This good antioxidant activity of MECB might be attributed to the presence of phytochemicals, such as flavonoids and triterpenoids. MECB exhibited effective antioxidant activity at all concentrations. The effects of various amounts of MECB (from 50 to 500 μg/ml) on peroxidation of linoleic acid emulsion are shown in Fig. 1. The antioxidant activity of MECB increased with increasing concentration. MECB at the concentration 500 μg/ml (76.2 %) showed nearly equal to that of 500 μg/ml (77.9 %) concentration of α -tocopherol.

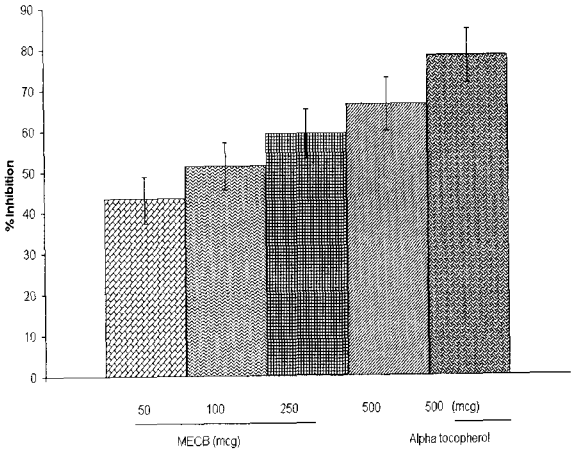


Fig 1. Percentage inhibition of lipid peroxidation by Alpha tocopherol and different doses of methanol extract of Caesalpinia bonducella (MECB) in the linoleic acid emulsion

Reducing power

Fig. 2 shows the reductive capabilities of MECB compared with BHT. For the measurements of the reductive ability, we investigated the $Fe^{3+}Fe^{2+}$ transformation in the presence of MECB using the method of Oyaizu (1986). The antioxidant activity has been reported to be concomitant with the development of reducing power (Tanaka *et al.*, 1998). Okuda *et al.*, 1983, reported that the reducing power of tannins prevents liver injury by inhibiting the formation of lipid peroxides. The reducing capacity of MECB may be due to the presence of tannins. The reducing capacity of a compound may serve as a significant

indicator of its potential anti- oxidant activity (Meir *et al.*, 1995). The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim *et al.*, 2001). Like Antioxidant activity, the reducing power of MECB increased with increasing amount of sample. All of the amounts of MECB showed higher activities than control and these differences were statistically significant ($P<0.01$).

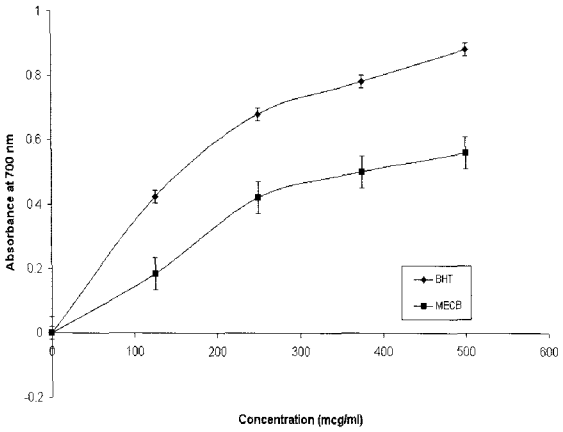


Fig 2. Reducing power of methanol extract of Caesalpinia bonducella (MECB) and BHT

Free radical scavenging activity

DPPH· is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The reduction capability of DPPH· radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, DPPH· is often used as a substrate to evaluate antioxidative activity of antioxidants (Duh *et al.*, 1999). Fig. 3 illustrates a significant ($P < 0.05$) decrease in the concentration of DPPH radical due to the scavenging ability of the MECB and standards. We used BHA as a standard. These results indicate that MECB has a noticeable effect on scavenging free radical. Free radical scavenging activity also increased with increasing concentration.

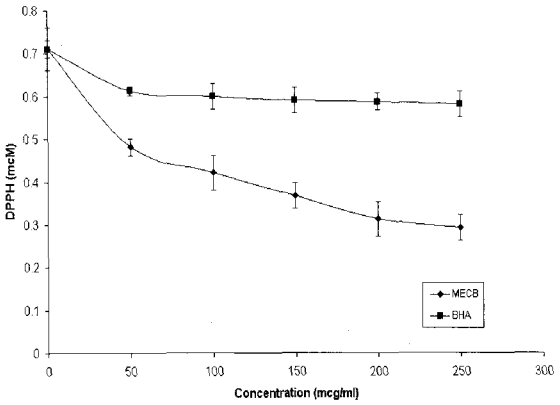


Fig 3. Frre radical scavenging activity of different concentration of methanol extract of Caesalpinia bonducella (MECB) and BHA on 1,1-diphenyl-2-picryl hydrazyl radicals

Nitric oxide radical scavenging activity

It is well known that nitric oxide has an important role in various types of inflammatory processes in the animal body. In the present study, the crude extract of the leaves was checked for their inhibitory effect on nitric oxide production. Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by MECB. Fig. 4 illustrates the percentage inhibition of nitric oxide generation by MECB. Curcumin was used as a reference compound. The concentration of MECB needed for 50 % inhibition was found to be 102.8 g/ml whereas 20.4 g/ml for curcumin.

Superoxide anion radical scavenging activity

Superoxide anions indirectly initiated lipid oxidation as a result of superoxide and hydrogen peroxide serving as precursors of singlet oxygen and hydroxyl radicals (Okuda *et al.*, 1983). Robak and Glyglewski reported that the

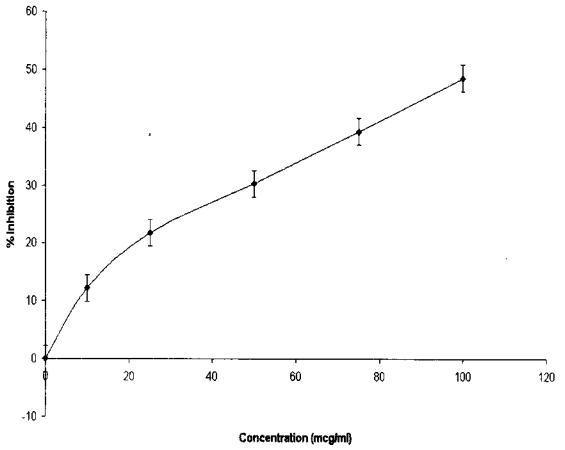
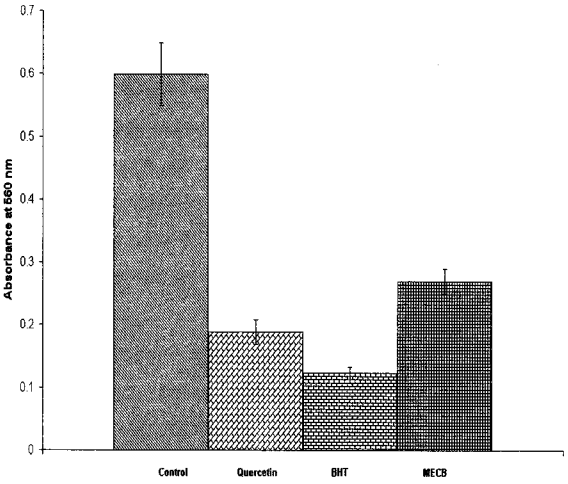


Fig 4. Percentage inhibition of nitric oxide radical by methanol extract of Caesalpinia bonducella (MECB)



Results are mean + S.D of five parallel measurements. P<0.05 when compared with control.

Fig 5. Superoxide anion scavenging activity of methanol extract of Caesalpinia bonducella (MECB) and same doses of quercetin and BHT by PMS/NADH-NBT method

antioxidant properties of flavonoids are effective mainly via the scavenging of superoxide anion. MECB was found to possess good scavenging activity on superoxide anions at all the tested concentration. It may be due to the presence of flavonoids. In the PMSNADHNBT system, superoxide anion derived from dissolved oxygen by PMSNADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Fig. 5 shows the percentage inhibition of superoxide radical generation by MECB and comparison with same concentrations of BHT and Quercetin was found to be 54.9%, 79.5% and 68.5% respectively. MECB had strong superoxide radical scavenging activity when compared than that of standards. The results were found statistically significant ($P<0.05$).

Hydroxyl radical scavenging activity

The effect of MECB on the inhibition of free radical-mediated deoxyribose damage was assessed by means of the Iron (II)-dependent DNA damage assay. The Fenton reaction generates hydroxyl radicals ($OH^{\cdot}$) which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. Attack at a sugar ultimately leads to sugar fragmentation, base loss and strand break with a terminal fragmented sugar residue. Addition of low concentrations of transition metal ions such as Iron to DNA causes degradation of the sugar into malondialdehyde and other related compounds, which form a chromogen with TBARS. Table 1 shows the effect of the extracts on the Iron (II)-dependent deoxyribose damage. The MECB was the capable of reducing DNA damage at all concentrations. The IC_{50} value of MECB was found to be 104.17g/ml Catechin, used as a standard was highly effective in inhibiting the oxidative DNA damage, showing an IC_{50} = 5 μg /ml.

Hydrogen peroxide radial scavenging activity

The ability of the MECB to scavenge hydrogen peroxide was determined according to the

Table 1. Effect of methanol extract of *Caesalpinia bonducella* MECB and Catechin on inhibition of Hydroxyl radical

Sample	% Inhibition			IC_{50}
	1 μg/ml	10 μg/ml	100 μg/ml	(μg/ml)
Catechin	40	59	74	5
MECB	31	48	63	104.17

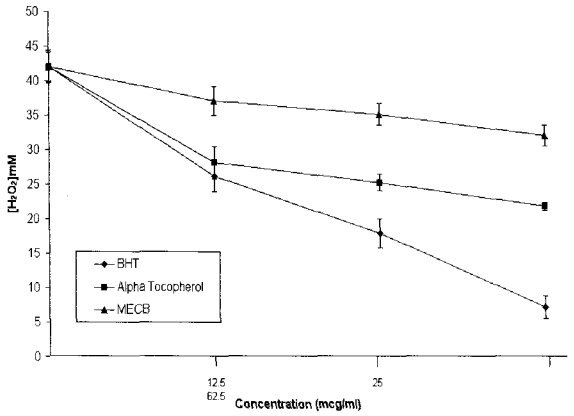


Fig 6. Effect of methanol extract of Caesalpinia bonducella (MECB) , Alpha tocopherol and BHT on hydrogen peroxide radical scavenging activity

method of Ruch *et al.*, (1989). The scavenging ability of MECB on hydrogen peroxide is shown in Fig. 6 and compared with that of BHT and α-tocopherol as standards. The MECB was capable of scavenging hydrogen peroxide in a concentration-dependent manner. The MECB at the concentration of 62.5 μg/ml exhibited 44.3% scavenging activity on hydrogen peroxide respectively. In the other hand, BHT, and α -tocopherol exhibited 86, and 57% hydrogen peroxide scavenging activity at the same dose. These results showed that MECB had stronger hydrogen peroxide scavenging activity. Those values are lower than that of BHT and α -tocopherol. There was statically significant correlation between those values and control (P<0.05). Hydrogen peroxide it self is not very reactive, but it can some times be toxic to cell because it may give rise to hydroxyl radical in the cell (Halliwell, 1991). Thus, removing h_2o_2 as well as $o^{\cdot}{}_2$ is very important for protection of food system.

Determination of total phenolic compounds

Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups (Hatano *et al.*, 1980). In MECB (1 mg) 68.7μg pyrocatechol equivalent of phenols was detected. The phenolic compounds may contribute directly to the antioxidative action (Duh *et al.*, 1999). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables (Tanaka *et al.*, 1998). Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants (Rice-Evans *et al.*, 1995). Thus, therapeutic properties of *Caesalpinia bonducella* may possibly attributed to the phenolic compounds present.

Antimicrobial activity

Disc diffusion methods are extensively used to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of substances to be examined. In the case of solutions with a low activity, however, a large concentration or volume is needed. The limited capacity of discs means that holes or cylinders are preferably used (Bartner *et al.*, 1994). Most of the bacterial species and the fungi species were inhibited antimicrobial activity as it is shown in Table 2. In this study, nine different microbial and four fungi species were used to screen the possible antimicrobial activities of MECB. The MECB showed broad spectrum of activity against all the bacterial strains at the tested concentration (25 - 200 μg/ disc) and also active against fungal organism.

Table 2. Antimicrobial activities of methanol extract of *Caesalpinia bonducella* (MECB), ofloxacin and miconazole nitrate on selected bacterial and fungal strains

Microorganism μg/ml/disc	Diameter of inhibition zone (mm)					
	MECB μg/ml/disc				Standards μg/ml/disc	
	25	50	100	200	(Ofl) 5	(Mic) 40
Pseudomonas aeruginosa	7	9	12	15	22	-
Escherichia coli	-	8	9	11	26	-
Salmonella typi	9	10	13	16	24	-
Shigella dysenteriae	7	9	10	13	21	-
Vibrio cholerae	-	8	9	11	20	-
Staphylococcus aureus	-	7	8	10	14	-
Streptococcus pneumoniae	-	8	9	11	22	-
Micrococcus luteus	7	8	10	12	19	-
Staphylococcus epidermidis	-	7	9	11	23	-
Candida albicans	-	7	9	13	-	21
Aspergillus niger	-	-	7	10	-	19
Aspergillus flavus	-	-	7	10	-	19
Alternaria solani	-	7	8	12	-	22

Ofl: Ofloxacin (5 μg/ml/disc); Mic: Miconazole (40 g/ml/disc), (-): in active.
The results are the mean values of triplicate tests repeated three times after 24 - 72 h of inhibition at 37 ℃.

The height growth inhibition of *Pseudomonas aeruginosa* and *Salmonella typi* were observed with the MECB. Ofloxacin (5 μg/disc) and Miconazole nitrate (40 μg/ disc) were used as positive controls for bacteria and fungi.

CONCLUSION

MECB showed strong antioxidant activity, reducing power, DPPH radical and superoxide anion scavenging, hydrogen peroxide scavenging, and hydroxyl radical scavenging activities when compared with different standards such as BHT, BHA and -tocopherol. In addition, 200 g of MECB possessed noticeable antimicrobial activity against selected bacterial and fungal strains when compared with standard. Previous reports from our laboratory stated that *Caesalpinia bonducella* leaves showed hepatoprotective and antioxidant activities (Gupta *et al.*, 2003), anti-tumor and antioxidant status (Gupta *et al.*, 2004), anti-inflammatory, analgesic and antipyretic activity (Gupta *et al.*, 2004). The results of this study show that the *in vitro* antioxidant and antimicrobial activities of *Caesalpinia bonducella*. From the above studies we suggested that MECB could be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry and also it may be extensively used for the treatment of some degenerative diseases such as cancer, anti-inflammatory, liver disorder etc. Therefore, it is suggested that further works should be performed on the isolation and identification of

the antioxidant components in MECB. This could ultimately lead to the inclusion of this compound(s) in different antioxidant pharmaceutical formulation.

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