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● *RESEARCH ARTICLE* ●

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An investigation to antioxidant activity of *Caesalpinia bonducella* seeds

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ABSTRACT

The present study envisages the antioxidant potential of *caesalpinia bonducella,* a widely grown plant of medicinal importance throughout India. Chloroform extract of *caesalpinia bonducella* seeds were screened for antioxidant activity using, DPPH free radical scavenging activity, total phenolic content (tpc) estimation and β- carotene bleeching assay. The results showed Ic_{50} of chloroform extract 170±4.08µg/ml and that of ascorbic acid is 2.03±0.16µg/ml. Total phenolic content was found to be 21.96±2.12 (for 1000µg/ml) and total antioxidant activity (taa) 24.96±0.31 while 'taa' of standard BHA was found to be 46.70±0.43. The study revealed the presence of antioxidant activity in chloroform extract of *caesalpinia bonducella* seeds.

Key words : Herbal antioxidants, Nata Karanja, Total phenolic content, Seed extract, Natural cure

INTRODUCTION

Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases (Huy *et al.*, 2008). Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function (Neogi and Nayak, 1958). Reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function (Ali *et al.,* 2008).

Most of the potentially harmful effects of oxygen are believed to be due to the formation and activity of reactive oxygen species acting as oxidants *i.e.* compounds with a tendency to donate oxygen to other substances. Many reactive oxygen species are free-radicals. A free radical is any chemical species that has one or more unpaired electrons. Antioxidants help organisms deal with oxidative stress, caused by free radical damage, which are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability (Ali *et al.,* 2008). Therefore, the oxidants held responsible for precipitation of number of diseases, and antioxidants can be remedy for the diseases caused by such substances being capable to prevent their effect.

Natural products have received huge attention in the field of pharmaceutical science and technology over past few decades because of their good biocompatibility and cost effectiveness (Sachan and Bhattacharya, 2009a). The biological diversity responds to a number of new, emerging concerns including thrust for research and developments (Mukharjee *et al.,* 2007; Sachan and Bhattacharya, 2009b). In this 'herbal boom worldwide' many researchers have focused on the natural products as source of new medicines as well as an alternative of existing synthetic drugs. Many herbs have proven to have natural antioxidants and are being used in the formulation of ayurvedic and modern drug dosage forms. In this chain, the present study was performed to examine a wild herb *Caesalpinia bonducella* for the presence of antioxidant activity.

Caesalpinia bonducella commonly known as Nata Karanja, a prickly shrub found throughout the hotter parts of the India, Myanmar and Sri lanka, has grey, hard, globular shaped seeds with a smooth shining surface. Seed consist of thick brittle shell with a yellowish white bitter fatty kernel (Nadkarni, 1954). Seeds contain bitter substance phytosternine, saponin, fatty oil 20 to 24%, starch, 2-phytosterols; bitter amorphous glycoside bonducin

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isolated from the oil. The constituent fatty acids are: palmitic, stearic, lignoceric, oleic, and mixture of unsaturated acids of low molecular weights. α- Caesalpin , M.P. 187 °C, β caesalpin, M.P 243°C, γ – caesalpin and δ – caesalpin have been isolated from seeds. Seeds also contain diterpene ζ – caesalpin, isolated from seeds kernels, diterpinoid bonondinolide and novel nor- cassane type diterpine are reported from seeds. Leaves contain diterpinoids, amino acids, and sugars (Neogi and Nayak, 1958).

Plant is reported to have multiple therapeutic properties like antibacterial (Neogi and Nayak, 1958), antianaphylactic and antidiarrhoeal (Iyengar and Pendse, 1965), antiasthamatic (Gayaraja *et al.,*1978) antiviral (Khalaf *et al.,* 2008), antiamoebic and antiestrogenic, antipyretic, antihelmitic activity (Neogi and Nayak, 1958; Iyengar and Pendse, 1965; Dhar *et al.,* 1968*;* Raghunathan and Mitra, 1982; El-Agbar *et al.,* 2008).

MATERIALS AND METHODS

Materials:

1,1Diphenyl -2-picrylhydrazyle (DPPH), β- carotene, Linoleic acid (>=99%), Butylated hydroxyanisole were obtained from Sigma Aldrich Co. All other chemicals used were of analytical grade laboratory reagents.

Preparation of plant extract:

Plants were collected from Kukrail Forest Research Centre, Lucknow and authenticated from NBRI Lucknow (CIF/Re./08/2008/32). Plant material consisted of dried seeds of *Caesalpinia bonducella*, extraction was performed by Soxhlet extraction process in two steps. Firstly the powdered seeds are deffated under soxhlet assembly using 250mL of 98% ether for 6 hours. This is followed by 9 hours soxhlation of defatted seeds powder by using 250mL of 98% chloroform as solvent. The final extract was passed through No. 1 Whatman filter paper. The filtrate obtained was concentrated under vacuum in a rotary evaporator at 40°C and stored at 4°C for further use. The crude extract was obtained by dissolving a known amount of dry extract in 98% methanol to obtain a stock solution of 1000 ìg/ml concentration. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions (25, 50, 100, 125,150, 200, 250, 300 and 500 ìg/ml).

Antioxidant activity (DPPH Free radical scavenging activity) of chloroform extract:

Antioxidant activity of the plant extract and the standards was assessed on the basis of the radical scavenging effect of the stable DPPH free radical (El-Agbar *et al.,* 2008). The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 25 to 500µg/ml. We prepared 0.002% DPPH in methanol. Then 2 ml of this solution was mixed with 2 ml of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 20 min and optical density was measured at 517 nm using spectrophotometer (Shimadzu Pharmatech 1700) against methanol. The blank was used is 2 ml of methanol with 2 ml of DPPH solution (0.002%). The optical density was recorded and per cent of inhibition was calculated using the formula given below (Panovaska *et al.*, 2005):

% of inhibition of DPPH activity = (A–B/A) × 100

where 'A' is optical density of the blank and 'B' is optical density of the sample.

Evaluation of antioxidant activity*:*

The antioxidant activity of the extract was evaluated using a β-carotene/linoleate model system. A solution of β-carotene was prepared by dissolving 2.0 mg of β-carotene in 10 ml of chloroform. One milliliter of this solution was then pipetted into a round-bottom flask. After chloroform was rotary evaporated at 40°C under vacuum, 20 mg of purified linoleic acid, 200 mg of Tween 40 emulsifier and 50 ml of distilled water were added to the flask under vigorous shaking. Aliquots (5 ml) of this emulsion were transferred into a series of tubes containing 2 mg of each extract or 2 mg of BHA (butylated hydroxyanisole) for comparison. An aliquot (5 ml) of emulsion without any further addition was used as control. As soon as the emulsion was added to each tube, the zero time absorbance was read at 470 nm. Subsequent absorbance readings were recorded at 10-min intervals by keeping the sample in a water bath at 50 °C until the visual color of -carotene in the control sample disappeared it takes about 120 min (Panovaska *et al.,* 2005; Khalaf *et al.,* 2008; Percival, 1998).

Antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using a formula given below (Huy *et al.*, 2008).

$$AA = \left[1 - \frac{(A_0 - A_1)}{A_0^0 - A_t^0}\right] x\,100$$

where *A0* and *A°0* are the absorbance values measured at initial time of the incubation for samples and control, respectively, while *At* and *A°t* are the absorbance values measured in the samples or standards and control

Table 1 : Ic_{50} value, TPC, antioxidant activity of seed extract of *C. bonducella*

Test compound	DPPH assay Ic_{50} (µg/mL)*	β-carotene/linoleic acid (%AA)	TPC (in GAE)*
C.bonducella (Chloroform)	170±4.08	24.96±0.31	21.96±2.12
Ascorbic acid	2.03±0.16	-	-
BHA	-	46.70±0.43	-

*Values are means ± SD of three determinations

at t = 120 min.

Total phenol content (TPC):

TPC was determined using the Folin-Ciocalteu's reagent using the method as per Yan and Asmah (2001). Samples of 0.3 ml were introduced into test tubes followed by 1.5 ml of Folin- Ciocalteu's reagent (diluted 10 times with water) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min. Absorption at 765 nm was measured. If the sample absorbance exceeded 1, the sample was appropriately diluted to give a reading of less than 1. Total phenol contents were expressed in gallic acid equivalents (mg per 100 g fresh fruit). The gallic acid standard line has the equation $y = 0.0111x - 0.0148$ ($R2 = 0.9998$), where y is absorbance at 765 nm and x is concentration of gallic acid in mg/L. All absorptions were measured in three replicates.

Statistics:

Experiments were carried out in triplicate and the results are expressed as mean ± SD. Statistical analyses were performed using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Chloroform extract of *Caesalpinia bonducella* seeds and standard ascorbic acid was screened for *in vitro* antioxidant activity using the DPPH method, the chloroform extract showed antioxidant activity with Ic_{50} value of 170±4.08µg/ml. The Ic_{50} value for Ascorbic acid was 2.03±0.16µg/ml. The result indicates that the antioxidant activity of chloroform extract of *Caesalpinia bonducella* seeds was lesser than that of ascorbic acid as its IC_{50} value was higher than ascorbic acid.

The antioxidant activity of extract of the *Caesalpinia bonducella* and standard BHA was examined using β-carotene/linoleic acid model system; readings are shown below in Table 1. Chloroform extract showed the antioxidant activity (24.96 ± 0.31) where as BHA showed the value of 46.70 ± 0.43. Chloroform extract showed lower antioxidant activity than BHA in the β-carotene/ linoleic acid model system.

The content of Phenolic compound (mg/g) in chloroform extracts expressed as GAE was found to be 21.96 ± 2.12. The extract has shown free radical scavenging activity (170 ± 4.08µg/ml) and total antioxidant activity (24.96 ± 0.31). Presence of phenolic compounds suggests that the antioxidant activity may be due to the polyphenol content.

Conclusion:

Identification of all chemical constituent in seed extract that are responsible for antioxidant activity requires further investigation, the crude chloroform extract merits further experiments *in vivo*. However, present study showed new natural antioxidant that can replace the synthetic ones to be used in foods and cosmetics.

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