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Contributed Paper

# Antioxidant, Total Phenolics and Total Flavonoid Content of the Aqueous Extract of *Caesalpinia bonducella* Seeds

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## ABSTRACT

The objective of the present study was to determine the total phenolic and total flavonoid contents, and to evaluate the antioxidant potential of the aqueous extract of *Caesalpinia bonducella* seeds (AQCB) in various radical scavenging models. The DPPH radical scavenging activity of AQCB (20, 40, 50, 100 and 200 µg/mL) was increased in a dose-dependent manner, which was found in the range of 36.93-70.57% as compared to ascorbic acid (64.26-82.58%) with $IC_{50}$ values of 86.31 and 26.75 µg/ mL, respectively. The AQCB was also found to scavenge the superoxide generated by EDTA/NBT system. Measurement of total phenolic content of the aqueous extract of *C. bonducella* was achieved using Folin-Ciocalteau reagent and represented 59.87 mg/g of phenolic content. In addition, flavonoid contents in AQCB were determined to be 22.18 mg quercetin equivalents/g dry mass. The aqueous extract also inhibited the hydroxyl radical, nitric oxide, superoxide anions with $IC_{50}$ values of 139.95, 114.70 and 83.62 µg/mL, respectively. However, the $IC_{50}$ values for the standard ascorbic acid were noted to be 71.41, 66.01 and 36.69 µg/mL, respectively. The results obtained in this study indicate that *C. bonducella* could be considered as a potential source of natural antioxidant with enriched contents of phenolics and flavonoids.

**Keywords:** *Caesalpinia bonducella*, antioxidant, total flavonoids, phenolic content, aqueous seed extract

## 1. INTRODUCTION

*Caesalpinia bonducella* Fleming (Caesalpiniaceae), known as Bondoc nut, is a prickly shrub found throughout the hotter parts of India and Sri Lanka. Seeds are gray, hard, and globular in shape with a smooth shiny surface, and consist of a thick, brittle shell with a yellowish-white bitter fatty kernel [1]. *C. bonducella* is reported to have multiple of medicinal and therapeutic uses including antipyretic, antidiuretic, anthelmintic, antibacterial, antiviral, antiasthmatic, antiamebic and anti-estrogenic activities [2, 3, 4].

Natural antioxidants form medicinal plants either in the form of crude extracts or their chemical constituents are very effective to prevent the destructive processes caused

by oxidative stress [5]. Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts [5]. Recently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, because they possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance associated with reactive oxygen species (ROS) [6]. The role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human beings, such as diabetes, atherosclerosis, aging, immunosuppression and neurodegeneration [5]. An imbalance between ROS and the inherent antioxidant capacity of the body, directed the use of dietary and/or medicinal supplements particularly during the disease attack. Studies on herbal and medicinal plants have indicated the presence of antioxidants such as phenolics, and flavonoids [6]. Hence, the antioxidant contents of medicinal plants may contribute to the protection they offer from disease.

Although fewer reports are available in the literature on the antioxidant activity of *C. bonducella* seeds [7], no reports have been found on the antioxidant efficacy of aqueous extract of *C. bonducella* seeds. The present investigation evaluated the antioxidant activity and responsible phytochemicals such as phenolics and flavonoids present in the aqueous extract of *C. bonducella* seeds.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Seeds of *Caesalpinia bonducella* were collected from Jeevan Herbs Agro Farms, Sagar, MP, India, and were identified by herbarium in-charge of Dr. Hari Singh Gour University, Sagar, MP, India. The specimen (Bot/H/2692) was preserved at the Laboratory of Microbiology, Department of Botany, Dr. Hari Singh Gour University, Sagar, MP, India. The seeds were kept in airtight bottles for further studies.

### 2.2 Preparation of Aqueous Extract

The air-dried seeds (50 g) of *C. bonducella* were finely powdered (mesh size 20) and stirred with eight parts of distilled water at about 70°-80°C for 2 h. The liquid aqueous extract was filtered through a sieve (mesh size 200). The filtrate was concentrated up to two parts using a rotary vacuum evaporator (Model PBU-6D, Scientific India, Bangalore, Karnataka, India).

### 2.3 Determination of Total Phenolic Content

Total soluble phenolics in AQCB were determined with Folin-Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound [5]. About 1.0 mL of aqueous extract solution containing 1.0 g extract in a volumetric flask was diluted with 46 mL of distilled water. About 1.0 mL of Folin-Ciocalteau reagent was added and mixed thoroughly. Three minutes later, 3.0 mL of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue color that developed was measured at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract. The amount of total phenolics was calculated as gallic acid equivalents (GAE) from the calibration curve obtained from gallic acid standard solution and expressed as mg GAE/g dry mass.

### 2.4 Determination of Total Flavonoid Content

Total flavonoid contents in AQCB were

determined by the colorimetric method [5] with minor modifications. Briefly, 100 µL of aqueous seed extract or standard solution was mixed with 400 µL of ethanol, followed by the addition of the same volume of 2% solution $AlCl_3$ diluted in ethanol. After 1 h of incubation at room temperature, absorbance was measured at 517 nm. Quercetin was used to plot the standard curve, and results were expressed as the mean, in mg, of quercetin equivalents per gram of plant material from triplicate extracts.

### 2.5 Determination of DPPH Radical Scavenging Activity

The free radical scavenging activity of AQCB was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH [5]. About 0.1 mM solution of DPPH in ethanol was prepared and 1.0 mL of this solution was added to 3.0 mL of extract solution in water at different concentrations (20-200 µg/ mL). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as a reference compound. DPPH radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\text{Percentage inhibition} = ((A_0-A_t) / A_0 \times 100)$$

Where $A_0$ was the absorbance of the control (blank, without aqueous extract) and $A_t$ was the absorbance in the presence of the aqueous extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

### 2.6 Determination of Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging capacity of AQCB was measured using a modified method [6]. Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), $H_2O_2$ (10 mM) and deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 mL EDTA, 0.01 mL of $FeCl_3$, 0.1 mL of $H_2O_2$, 0.36 mL of deoxyribose, 1.0 mL of aqueous extract (20-200 µg/mL) each dissolved in distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.4) and 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 h. About 1.0 mL portion of the incubated mixture was mixed with 1.0 mL of (10%) TCA and 1.0 mL of (0.5%) TBA (in 0.025 M NaOH containing 0.025 M NaOH BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the aqueous extract was reported as the percentage of inhibition of deoxyribose degradation and was calculated according to the following equation:

$$\text{Percentage inhibition} = ((A_0-A_t) / A_0 \times 100)$$

Where $A_0$ was the absorbance of the control (blank, without aqueous extract) and $A_t$ was the absorbance in the presence of the sample of the aqueous extract. All the tests were performed in triplicate and the graph was plotted with the mean values. Ascorbic acid was used as a positive control.

### 2.7 Determination of Nitric Oxide Radical Scavenging Activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide [8], which interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric

oxide. Sodium nitroprusside (5 mM) in phosphate buffer saline (PBS) was mixed with 3.0 mL of different concentrations (20-200 µg/mL) of the AQCB and incubated at 25°C for 150 min. The samples were added to Griess reagent (1% sulphanilamide, 2% $H_3PO_4$ and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was measured at 546 nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Griess reagent as a positive control. The percentage of inhibition was measured by the following formula:

$$\text{Percentage inhibition} = ((A_0\text{-}A_t) / A_0 \times 100)$$

Where $A_0$ was the absorbance of the control (blank, without aqueous extract) and $A_t$ was the absorbance in the presence of the aqueous extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

### 2.8. Determination of Superoxide Radical Scavenging Activity

Superoxide activity of AQCB was measured using NBT (nitroblue tetrazolium reagent) method as described by Sabu and Ramadasan [9]. Test solution of aqueous extract (20-200 µg/mL) was taken in a test tube. To this, reaction mixture consisting of 1 mL of (50 mM) sodium carbonate, 0.4 mL of (24 mM) NBT and 0.2 mL of 0.1 mM EDTA solutions were added to the test tube. Further, about 0.4 mL of (1 mM) of hydroxylamine hydrochloride was added to initiate the reaction then reaction mixture was incubated at 25°C for 15 min and reduction of NBT was measured at 560 nm. Ascorbic acid was used as a reference compound. The percentage of inhibition of superoxide radicals in the reaction mixture was calculated according to the following equation:

$$\text{Percentage inhibition} = ((A_0\text{-}A_t) / A_0 \times 100)$$

Where $A_0$ was the absorbance of the control (blank, without aqueous extract) and $A_t$ was the absorbance in the presence of the samples of the aqueous extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

### 2.9 Statistical Analysis

All data were expressed as the mean ± SD. Analysis of variance using one-way ANOVA was performed to test the significance of differences between means at the 5% level of significance using the statistical analysis software, SAS (SAS 9.1 Version, NC, USA).

## 3. RESULTS AND DISCUSSIONS

### 3.1 DPPH Radical Scavenging Activity

DPPH method is widely reported for screening of antioxidants and its comparative effectiveness [10]. The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm, which is induced by the antioxidants. In this assay, the different concentrations of AQCB extract (20, 40, 50, 100 and 200 µg/mL) showed antioxidant activity in a dose-dependent manner (36.93, 41.14, 56.15, 65.16 and 70.57% inhibition), respectively (Figure 1). On the DPPH radical, AQCB had significant scavenging effect with increasing concentration when compared with that of ascorbic acid. The $IC_{50}$ values were calculated by using **linear** regression curve which were found to be 86.31 and 26.75 µg/mL for AQCB and ascorbic acid, respectively (Table 1). Positive DPPH test suggests that the samples were free radical scavengers. As reported previously, the amount

of DPPH scavenging activity appeared to depend on the phenolic concentration of the aqueous extract of *C. bonducella* seeds [6]. The AQCB at the used concentrations displayed potential effect of DPPH activity as percentage of free radicals inhibition. A higher DPPH radical-scavenging activity is associated with a lower $IC_{50}$ value.

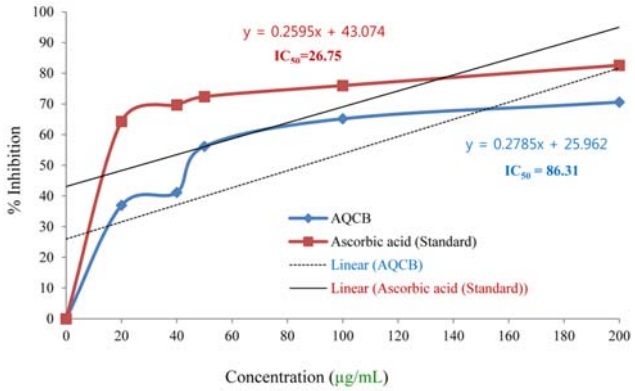


**Figure 1.** Antioxidant effect of *C. bonducella* aqueous seed extract in DPPH radical scavenging model. [◆] Solid line with tri-angle bullet represents AQCB; [■] solid line with rectangle bullet represents ascorbic acid (standard); [.....] dotted line represents linear curve for AQCB; [–] solid line represents linear curve for ascorbic acid (standard).

**Table 1.** Comparison of $IC_{50}$ value (µg/mL) of aqueous seed extract of *C. bonducella* and ascorbic acid.

| $IC_{50}$ value (µg/mL) | DPPH radical scavenging activity | Superoxide radical scavenging activity | Hydroxyl radical scavenging activity | Nitric oxide radical scavenging activity |
|---|---|---|---|---|
| AQCB | 86.31 | 83.62 | 139.95 | 114.70 |
| Ascorbic acid | 26.75 | 36.69 | 71.41 | 66.01 |

AQCB: Aqueous seed extract of *C. bonducella*.

**3.2 Hydroxyl Radical Scavenging Activity**

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity [11]. Among all the oxygen radicals, hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules [12]. The percent inhibitory effect of AQCB at 20, 40, 50, 100 and 200 µg/mL on scavenging hydroxyl radical was found to be 30.86, 35.47, 39.44, 44.95 and 56.88%, respectively (Figure 2). The aqueous extract of *C. bonducella* seeds may be capable of reducing DNA damage at different concentrations. In this assay, the Fenton reaction generates hydroxyl radicals (OH) 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 [13]. Cox et al. [14] reported that the water extract of *Smilax glyciphylla* also inhibited deoxyribose degradation. The ability of the extracts to quench hydroxyl radicals seems to be directly

related to the prevention of propagation of the process of lipid peroxidation and seems to be good scavenger of ROS, thus reducing the rate of the chain reaction. The results showed antioxidant activity of AQCB and ascorbic acid in dose-dependent manner with $IC_{50}$ values of 139.95 and 71.41 µg/mL, respectively (Table 1), and calculated by using linear regression curve (Figure 2). Yen and Hsieh [15] reported that xylose and lysine maillard reaction products had dose-dependent scavenging activity on hydroxyl radical which might have been attributed to the combined effects of reducing power, donation of hydrogen atoms and scavenging of active oxygen.

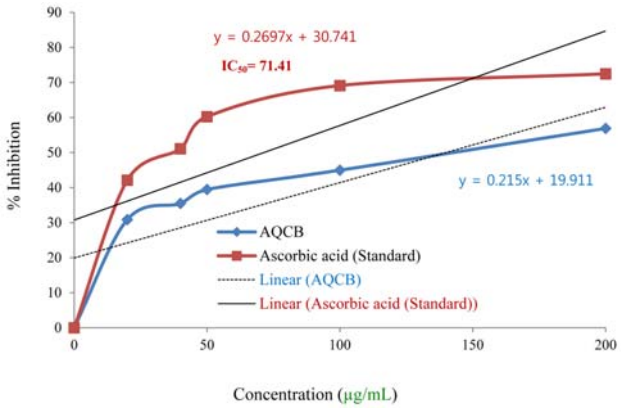


**Figure 2.** Antioxidant effect of *C. bonducella* aqueous seed extract in hydroxyl radical scavenging model. [◆] Solid line with tri-angle bullet represents AQCB; [■] solid line with rectangle bullet represents ascorbic acid (standard); [.....] dotted line represents linear curve for AQCB; [–] solid line represents linear curve for ascorbic acid (standard).

### 3.3 Nitric Oxide Radical Scavenging Activity

Nitric oxide, a free radical generated by endothelial cells, macrophages and neurons etc., involved in the regulation of various physiological processes [16]. The various concentrations of AQCB (20, 40, 50, 100 and 200 µg/mL) showed (34.75, 37.21, 41.70, 56.72 and 61.88% inhibition), respectively (Figure 3). Results showed that the percentage of inhibition was in a dose-dependent manner. The concentration of AQCB needed for 50% inhibition ($IC_{50}$) was calculated by using linear regression curve which was found to be 114.70 µg/mL, whereas 66.01 µg/mL was needed for ascorbic acid (Table 1, Figure 3). Nitric ions in the acidic environment can promote mutagenic and cell damaging reactions which may result in the formation of carcinogenic N-nitroso compounds. Several studies have confirmed that nitrite scavenging activity was high at acidic pH suggesting that nitrosamine production can be inhibited *in vivo* [17]. The relation between the antioxidant and the total phenolic content can be well established in the present study. In addition, the crude extracts of certain plants such as *Gingko biloba*, *Sanguisorbae radix*, *Caryophylli flos*, *Coptidis rhizoma*, *Granati cortex*, *Gallae rhois*, *Rhei rhizoma* and *Cinnamomi cortex* have been reported to inhibit NO [18, 19]. The high scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health [20].

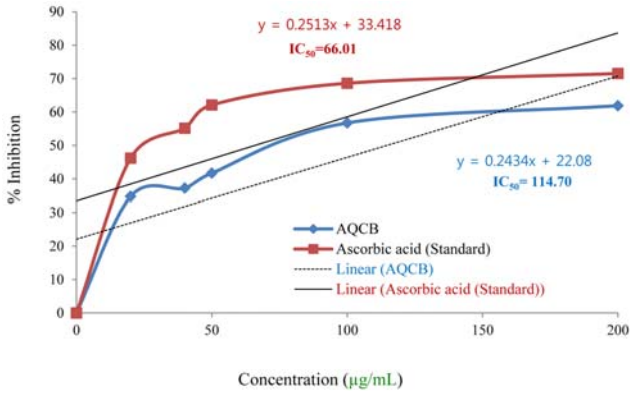


**Figure 3.** Antioxidant effect of *C. bonducella* aqueous seed extract in nitric oxide radical scavenging model. [◆] Solid line with tri-angle bullet represents AQCB; [■] solid line with rectangle bullet represents ascorbic acid (standard); [.....] dotted line represents linear curve for AQCB; [–] solid line represents linear curve for ascorbic acid (standard).

### 3.4 Superoxide Radical Scavenging Activity

Superoxide radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to tissue damage and various diseases [21]. In our study, super oxide radicals were generated by auto-oxidation of hydroxylamine in the presence of NBT (nitro blue tetrazolium) and the reduction of NBT in presence of AQCB was measured. The decrease of absorbance at 560 nm with antioxidant indicates the consumption of superoxide anion in the reaction mixture. Different concentrations of AQCB (20, 40, 50, 100 and 200 µg/mL) displayed strong superoxide radical scavenging activity (35.76, 45.33, 54.89, 64.46 and 73.12% inhibition), respectively (Figure 4). A 200 µg/mL of AQCB and ascorbic acid exhibited 73.12 and 85.42% inhibition, respectively (Figure 4). The $IC_{50}$ values of AQCB and ascorbic acid were calculated by using linear regression curve (Figure 4) which were found to be 83.62 and 36.69 µg/mL, respectively (Table 1). The AQCB exhibited superoxide radical scavenging activity in a dose-dependent manner. Nonetheless, when compared to ascorbic acid, the superoxide scavenging activity of the aqueous extract was found to be low. This could be due to the presence of reactive concentration of bioactive constituents and mixture of other nutrients in the aqueous extract. The antioxidant activity of phenolic compounds mainly due to their redox properties [22], that can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Our results also showed close agreement with antioxidant activity of ethanolic extract of *Paullinia cupana* [23]. Also Cox et al. [14] reported that the aqueous extract of *Smilax glyciphylla* (leaves) and methanolic extract of *Smilax excelsa* (leaves) quenched chemically-generated superoxide anion [24].

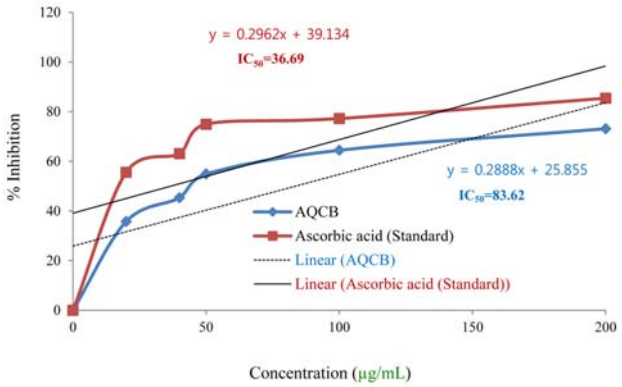


**Figure 4.** Antioxidant effect of *C. bonducella* aqueous seed extract in superoxide radical scavenging model. [◆] Solid line with tri-angle bullet represents AQCB; [■] solid line with rectangle bullet represents ascorbic acid (standard); [.....] dotted line represents linear curve for AQCB; [—] solid line represents linear curve for ascorbic acid (standard).

### 3.5 Total Phenolic Content

The total amount of phenolic content in AQCB was detected to be 59.87±5.12 mg/g gallic acid equivalent of phenols. These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. Similar relationship was also observed between phenolics and antioxidant activity in rosehip extracts [25]. Othman et al. [26] monitored phenolics and antioxidant capacity in beans from a range of geographical sources, including Ghana, and have demonstrated that there was a significant effect of "region of production" on both these parameters found a good correlation between total phenolic content and antioxidant capacity. Similarly, Gu et al. [27] also demonstrated a good correlation between total phenolics and antioxidant capacity in cocoa beans and their products. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [28].

### 3.6 Total Flavonoid Content

Flavonoids and other phenolic compounds are potent water-soluble antioxidants and free-radical scavengers that prevent oxidative cell damage [29]. Flavonoids have beneficial effects on human health. They are used to treat hypertension and diabetes [29]. Moreover, they possess antioxidant, antimicrobial, anti-carcinogenic effects [30]. Flavonoid intake has a protective role in human diet for the prevention of coronary heart disease [31]. Thus, these findings support the traditional use of *C. bonducella*. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. Quercetin is a well-known plant-derived flavonoid having anti-inflammatory and antioxidant properties [32]. The content of flavonoid compounds in AQCB was determined from the standard calibration curve and expressed in quercetin equivalents (QE). The flavonoid contents of the aqueous seed extract of *C. bonducella* were found to be 22.18±3.23 mg dry mass.

Phenolics and flavonoids are the classes of polyphenols that have mechanisms for protecting molecules (lipids, proteins and nucleic acids) from oxidative damage,

thereby show potent antioxidant activity [33]. Phenolics and flavonoids work together with the endogenous antioxidant defense system, and higher amount of polyphenol contents has been associated with higher antioxidant ability [34]. This might be the reason that in different scavenging model, AQCB showed the pattern of low to high antioxidant efficacy in a concentration-dependent manner in the tested *in vitro* scavenging models. Various plant extracts rich in phenolic and flavonoid contents have been proven to be the remarkable scavengers of free radicals in various *in vitro* systems [6].

## 4. CONCLUSION

Natural antioxidants can be advantageous for using in health care system as compared to synthetic ones due to their implications on human health. In this study, AQCB exhibited concentration-dependent antioxidant activity in different free radical scavenging models which might be attributed to the presence of various polyphenolic components (phenolics and flavonoids) of the aqueous extract of *C. bonducella* seeds. The findings of this study confirm that AQCB could be the potent source of natural antioxidants. However, further studies should be carried out to identify major bioactive molecules present in AQCB to confirm the *in vivo* antioxidant potential of *C. bonducella* seeds in animal model system.

## REFERENCES

[1] Nadkarni A.K., I*ndian Materia Medica*, Popular Prakashan, Bombay, India, 1954; **1**: 229-230.

[2] Neogi N.C. and Nayak K.P., *Indian J. Pharmacol.*, 1958; **20**: 95-100.

[3] Dhar L.M., Dhar M.M. and Dhawan B.N., Mehrotra B.N., Ray C., *Indian J. Exp. Biol.*, 1968; **6**: 232-247.

[4] Gayaraja S. and Shinde S., *Indian J. Pharmacol.*, 1978; **10**: 86-89.

[5] Saeed N., Khan M.R. and Shabbir M., *Alt. Med.*, 2012; **12**: e221. DOI 10.1186/1472-6882-12-221.

[6] Shukla S., Mehta A., John J., Singh S., Mehta P. and Vyas S.P., *Food Chem. Toxicol.*, 2009; **47**: 1848-1851. DOI 10.1016/j.fct.2009.04.040.

[7] Khan N., Kumar S., Singh R. and Dhankhar N., *Res. J. Pharm. Biol. Chem. Sci.*, 2012; **3(1)**: 480-496.

[8] Lee J.S., Shukla S., Kim J.A. and Kim M.H., *Plos One*, 2015; **10(2)**: e0118552. DOI 10.1371/journal.pone.0118552.

[9] Sabu M.C. and Amadasan R.K., *J. Ethnopharmacol.*, 2002; **81**: 155-160. DOI 10.1016/s0378-8741(02)00034-x.

[10] Vani T., Rajani M., Sarkar S. and Shishoo C.J., *Int. J. Pharmacog.*, 1997; **35**: 313-317.

[11] Babu B.H., Shylesh B.S. and Padikkala J., *Fitoterapia*, 2001; **72**: 272-277. DOI 10.1016/s0367-326x(00) 00300-2.

[12] Sanaka S., Tachibana Y. and Okada Y., *Food Chem.*, 2005; **89**: 569-575. DOI 10.1016/j.food chem.2004.03.013.

[13] Rajeshwar Y., Kumar G.P., Gupta M. and Mazumder U.K., *Eur. Bull. Drug Res.*, 2005; **3**: 31-39.

[14] Cox S.D., Jayasinghe K.C. and Markham J.L., *J. Ethnopharmacol.*, 2005; **101**: 162-168. DOI 10.1016/j.jep.2005.04.005.

[15] Yen G.C. and Hsieh P.P., *J. Sci. Food Agric.* 1995; **67**: 415-420. DOI 10.1002/jsfa.2740670320.

[16] Latha P.G., Evans D.A., Panikar K.R. and Jayavardhan K.K., *Fitoterapia*, 2000; **71**: 223-231. DOI 10.1016/s0367-326x(99) 00151-3.

[17] Choi D., Cho K.A., Na M.S., Choi H.S., Kim Y.O., Lim D.H., Cho S.J. and

Cho H., *J. Ind. Eng. Chem.*, 2008; **14**: 765-770. DOI 10.1016/j.jiec.2008.06.005.

[18] Marcocci L., Maguire J.J. and Droy M.T., *Biochem. Biophys. Res. Comm.*, 1994; **15**: 748-755. DOI 10.1006/bbrc.1994.1764.

[19] Yokozawa T., Chen C.P. and Rhyu D.Y., *Nephron*, 2002; **92**: 133-141. DOI 10.1159/000064483.

[20] Jagetia G.C., Baliga M.S., Malagi K.J. and Kamath M.S., *Phytomedicine*, 2002; **9**: 99-108. DOI 10.1078/0944-7113-00095.

[21] Halliwell B., Antioxidants in Disease Mechanisms and Therapy; in Seis H. ed., *Advances in Pharmacology* 38; Academic Press, New York, 1997: 3-17.

[22] Galato D., Ckless K., Susin M.F., Giacomelli C., Ribeiro R.M. and Spinelli A., *Redox Rep.*, 2001; **6**: 243-250. DOI 10. 1179/135100001101536391.

[23] Mattei R., Dias R.F., Espinola F.B., Carlini E.A. and Barros S.B.M., *J. Ethnopharmacol.*, 1998; **60**: 111-116. DOI 10.1016/s0378-8741(97)00141-4.

[24] Ozsoy N., Can A., Yanardag R. and Akev N., *Food Chem.*, 2008; **110**: 571-583. DOI 10.1016/j.foodchem.2008.02.037.

[25] Gao X., Bjork L., Trajkovski V. and Uggla M., *J. Sci. Food Agric.*, 2002; **80**: 2021-2027. DOI 10.1002/1097-0010(200011)80:14<2021::aid-jsfa745>3.0.co;2-2.

[26] Othman A., Ismail A., Ghani N.A. and Adenan I., *Food Chem.*, 2007; **100**: 1523-1530. DOI 10.1016/j.foodchem.2005.12.021.

[27] Gu L., House S.E., Wu X., Ou B. and Prior R.L., *J. Agric. Food Chem.*, 2006; **54**: 4057-4061. DOI 10.1021/jf060360r.

[28] Aneta W., Jan O. and Renata C., *Food Chem.*, 2007; **105**: 940-949. DOI 10.1016/j.food chem.2007.04.038.

[29] Mbaebie B.O., Edeoga H.O. and Afolayan A.J., *Asian Pac. J. Trop. Biomed.*, 2012; **2**: 118-124. DOI 10.1016/s2221-1691(11)60204-9.

[30] Andrade D., Gil C., Breitenfeld L., Domingues F. and Duarte A.P., *Ind. Crops Prod.*, 2009; **30**: 165-167. DOI 10.1016/j.indcrop. 2009.01.009.

[31] Peluso M.R., *Exp. Biol. Med.*, 2006; **231**: 1287-1299.

[32] Davis J.M., Murphy E.A., Carmichael M.D. and Davis B., *Am. J. Physiol. Reg. Integ. Comp. Physiol.*, 2009; **296**: 1071-1077. DOI 10.1152/ajpregu.90925.2008.

[33] Demrow H.S., *Circulation*, 1995; **91**: 1182-1188. DOI 10.1161/01.cir.91.4.1182.

[34] da Silva Oliveira C. and Maciel L.F., *Br. Food J.*, 2011; **113(9)**: 1094-1102. DOI 10.1108/00070701111174550.