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Original Article

FREE RADICAL SCAVENGING ACTIVITY OF THE BACOSIDE FRACTION FROM *BACOPA MONNIERI*

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ABSTRACT

Objective: *Bacopa monnieri* is a herb which has been used in Indian traditional medicine for a long period. Bacosides, the major constituents of *Bacopa monnieri*, have antioxidant, anticancer and memory enhancing properties.

Methods: The bacoside fraction was isolated from the dried powder of the aerial parts of *Bacopa monnieri*. The free radical scavenging activity of the bacoside fraction was determined by different assays (DPPH, ABTS, $SO^{\bullet}$, $NO^{\bullet}$, $OH^{\bullet}$ and H_2O_2) at concentrations ranging from 5 µg to 200 µg.

Results: The scavenging activity was increased in a dose-dependent manner from 5µg to 50 µg and a plateau in activity was observed from concentrations higher than 50 µg of the bacoside fraction.

Conclusion: Higher concentrations did not show further increase in scavenging activity. Various *in vivo* and *in vitro* studies are needed to assess the medicinal potential of the bacoside fraction

Keywords: *Bacopa monnieri*, bacoside fraction, and free radical scavenging activity.

INTRODUCTION

Bacopa monnieri, which is commonly known as "Brahmi", belongs to the family Scrophulariaceae. It is a creeper growing in wetlands and in marshy areas throughout India, Taiwan, China, Sri Lanka and Vietnam. It has known the traditional medicinal value for almost 3000 y [1]. It is widely used as a revitalizing herb, digestive aid, memory enhancer [2], anti-inflammatory, analgesic [3], antipyretic, sedative [4] and for respiratory functions [5]. The biologically active compounds present in the *Bacopa monnieri* are alkaloids (brahmine and herpestine), flavonoids (luteolin and epigenin), saponins and sterols. The saponins include her saponin [5], bacoside A and B [6], bacoside A2 [7], bacoside A3 [8], bacopa sides A, B, and C [9], bacopaside I and II [10], bacopa sides III, IV, V [11], bacopa sides VI–VIII [12], bacopa saponins A, B and C [13], bacopa saponin D [8], and bacopa saponin G [12].

Secondary metabolites are produced in plants for defence mechanisms to protect themselves. They are used as dyes, fibres, glues, oils, waxes, flavouring agents, perfumes, natural drugs, antibiotics, insecticides and herbicides [14]. Oxidative stress represents an imbalance between the production of reactive oxygen species and the biological system's ability to readily scavenge the reactive intermediates or to repair the resulting damage. Antioxidants of plant origin are effective in scavenging the reactive species generated in the biological system and from the environment. There are studies reporting that *Bacopa monnieri* plays a role in memory enhancing [15, 16] and antioxidant activity [17]. A methanolic extract of *Bacopa monnieri* has been shown in our studies to possess good antioxidant and anti-cancer activity [18]. Ethanol and aqueous extracts of *Bacopa monnieri* were also shown to have free radical quenching activity [17]. In the present study, the bacoside (triterpenoid saponin) fraction was isolated, and the antioxidant activity was assessed by various free radical scavenging assays.

MATERIALS AND METHODS

The plant was collected from Kovilpalayam, Coimbatore district, Tamil Nadu, India. The plant was identified and certified by Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore. The aerial parts of the plant were washed, shade dried and coarsely powdered. The plant material was first defatted separately with n-hexane and acetone and then extracted with methanol in a Soxhlet apparatus at 60 °C. The methanol fraction was further sub-fractionated to get the n-butanol fraction, which is reported to be rich in bacosides, the major active components of *Bacopa monnieri* [19]. The free radical scavenging activity of the bacoside fraction was assessed by the following assays at various concentrations from 5µg to 200 µg.

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay [20]

To 0.01 ml of the fraction, 0.5 ml of 0.4 mM methanolic solution of DPPH and 0.49 ml of methanol was added. The mixture was allowed to react at room temperature for 30 min in the dark. Methanol served as a blank and DPPH in methanol, without a fraction, served as a positive control. After 30 min of incubation, the decolourization of the purple to yellow colour was measured at 518 nm*. The radical scavenging activity was calculated as,

$$\% \text{ Scavenging} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) scavenging assay [21]

ABTS radical cations (ABTS+) were produced when 7 mM ABTS solution reacted with 2.45 mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 h before use. To 0.01 ml of the fraction, 0.3 ml of ABTS solution was added, and the final volume was made up to 1 ml with ethanol. Ethanol served as a blank and ABTS in ethanol without fraction served as a positive control. The absorbance was read at 745 nm* and the percent inhibition was calculated.

Superoxide scavenging assay [22]

To 0.01 ml of the fraction, 0.2 ml of 0.1M EDTA, 0.1 ml of 1.5 mM NBT, 0.05 ml of 0.12 mM riboflavin and 2.64 ml of 0.067M phosphate buffer was added. The assay mixture without sample was considered as control. All the tubes were vortexed, and the initial absorbance was read at 560 nm*. The tubes were illuminated uniformly in white light for 30 min. The absorbance was read again

at 560 nm*. The difference in the optical density before and after illumination was taken as the measure of superoxide generation and the percentage inhibition was calculated.

Nitric oxide scavenging assay [23]

To 0.01 ml of the fraction, 2 ml of 100 mM sodium nitroprusside and 0.49 ml phosphate buffered saline were added and incubated at 25ºC for 30 min. Griess reagent (0.5 ml) was added and allowed to stand for 30 min. The control tube was prepared without fraction. The absorbance of the pink coloured chromogen was read at 546 nm* against a reagent blank, and the percent inhibition was calculated.

Hydroxyl radical scavenging assay [24]

The reaction mixture contained 0.1 ml of 2.8 mM deoxyribose, 0.1 ml of 0.1 mM ferric chloride, 0.1 ml of 0.1 mM EDTA and 0.1 ml of 1 mM H_2O_2, 0.1 ml of 0.1 mM ascorbate, 0.01 ml of the fraction and the final volume was made up to 1 ml with 20 mM KH_2PO_4-KOH buffer (pH 7.4). The reaction mixture was incubated at 37 °C for 1 hour. At the end of the incubation period, 0.5 ml of 70% ethanol and 1 ml of 1% thiobarbituric acid (TBA) were added and heated at 95 °C for 20 min to develop the colour. After cooling, 0.5 ml of acetone was added, and the formation of thiobarbituric acid reactive substances (TBARS) was measured spectrophotometrically at 532 nm* against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing the absorbance of the control with those of the samples. The percent extent of TBARS production for the positive control (H_2O_2) was fixed at 100% and the relative percent TBARS production was calculated for the fraction treated groups.

Hydrogen peroxide scavenging assay [25]

To 0.01 ml of the fraction, 0.6 ml of 40 mM H_2O_2 containing phosphate buffer solution was added, and the final volume was made up to 3 ml with the same buffer. After 10 min, the absorbance values of the reaction mixtures were recorded at 230 nm* against a blank containing phosphate buffer without H_2O_2 for each concentration. The percent scavenging was calculated.

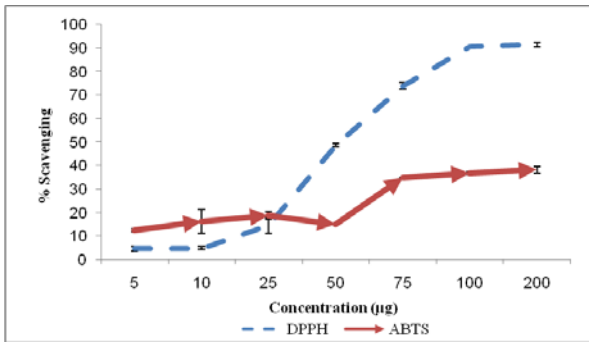

Fig. 1: The percent scavenging activity of the bacoside fraction from *Bacopa monnieri* against DPPH and ABTS

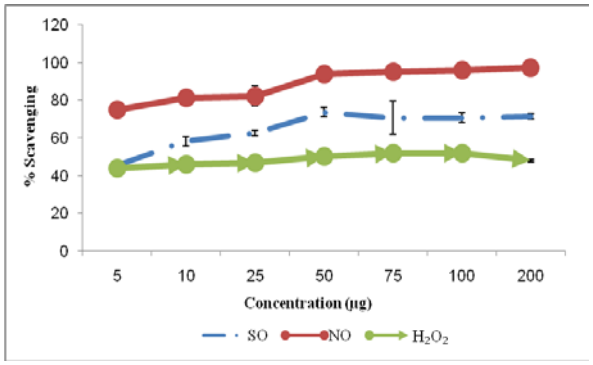

Fig. 2: the percent scavenging activity of the bacoside fraction from Bacopa monnieri against SO •, NO • AND H_2O_2

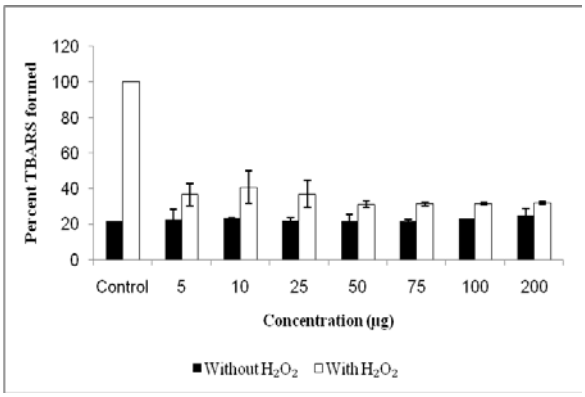

Fig. 3: The percent tbars formed in the hydroxyl radical scavenging activity of the bacoside fraction from *Bacopa monnieri*

RESULTS

In the present study, free radical quenching activity of the bacoside fraction from *Bacopa monnieri* was determined. The inhibition of free radicals generated *in vitro* increased in a dose-dependent manner up to 100µg of the bacoside fraction in DPPH and up to 75µg in ABTS assays (fig. 1), whereas in SO•, NO•, H_2O_2 (fig. 2), hydroxyl scavenging assays (fig. 3), the scavenging activity was increased till 50µg concentration. After that, a plateau was observed in all the assays.

DISCUSSION

Saponins have been shown to have an increased traditional and industrial application in medicine as anti-inflammatory, molluscicidal, antimicrobial, antispasmodic, antidiabetic, antitumor, antioxidant, as well as adjuvants. ROS, composed of superoxide, hydrogen peroxide, hydroxyl radical and peroxynitrite, mainly generated by the normal mitochondrial respiration, are critical intracellular signaling messengers [26]. If it is not effectively scavenged by the antioxidant system, it leads to various oxidative stresses related diseases. Therefore, it is very essential to scavenge the excess free radicals generated *in vivo* in order to protect the biomolecules. In the present study, the bacoside fraction, a triterpenoid saponin was tested against a battery of free radicals such as DPPH, ABTS, superoxide, nitric oxide, hydroxyl and hydrogen peroxide. The bacoside fraction showed a concentration dependent scavenging activity of the free radicals.

DPPH radicals are scavenged by antioxidants through the donation of hydrogen, thus forming reduced DPPH-H, which changes the colour from purple to yellow following reduction and is quantified by analyzing absorbance at wavelength 517 nm. The ABTS assay is based on the antioxidant capacity of the samples to prevent the oxidation of ABTS to ABTS++radical cation [27]. The bacoside fraction exhibited a DPPH and ABTS scavenging activity in a dose-dependent manner. Our results are similar with the methanolic extract of both leaves and rhizome of *Curcuma amada*, which exhibited the maximum scavenging activity of DPPH and ABTS [28]. Our results were also supported by the aqueous leaf extract of *Ceaselpinia crista* and *Caesalpinia asiatica*, which scavenged DPPH radicals at a concentration of 24.35µg/ml and 139.5µg/ml respectively [29]. The ABTS scavenging activity of the agathi leaf protein extract increased with increase in concentration up to a certain extent (3.44µM) and then leveled off with the further increase, which was in accordance with our results [30].

Hydrogen peroxide is an important reactive oxygen species due to its ability to penetrate into the biological membrane [26]. The bacoside fraction showed a concentration-dependent hydrogen peroxide scavenging activity. The ethanol extract of the leaves of *Solanum surattense* was capable of scavenging H_2O_2 in a dose-dependent manner [31], which correlates with our results.

OH• has a short half-life and is the most reactive, known to be capable of abstracting hydrogen atoms from cell membranes and bring about peroxidic reactions of lipids [32]. The bacoside fraction

scavenged hydroxyl radical, in a concentration-dependent manner. The aqueous extract of root bark of *Cassia sieberiana* had maximum hydroxyl radical scavenging activity [33]. A similar result was observed in the phenolic extract of the citrus peels (orange, grapefruit, and shaddock), which exhibited hydroxyl radical scavenging activity in a dose-dependent manner [34].

Superoxide radical, a precursor of the more reactive oxygen species and causative agent in various diseases, was also evaluated. The scavenging of superoxide radical by the bacoside fraction was concentration-dependent. An aqueous extract of seed of *Abelmoschus moschatus Medik.* was found to be an efficient scavenger of superoxide radical, which were in accordance with our results [35]. Our results are supported by the report of inhibition of superoxide radical anion generation by the water extract of *Eclipta prostrate L* at a concentration of 1 mg/ml [36].

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions that can be estimated with the use of Griess reagent [37]. The bacoside fraction exhibited a higher nitric oxide scavenging activity which increased with higher concentration. Our results were in agreement with the inhibition of nitric oxide production *in vitro* by the petroleum ether, chloroform, and methanol extracts of the fruits of *Dregea volubilis* Benth [38]. It shows that the bacoside fraction showed a concentration dependent free radical quenching activity against natural and synthetic-free radicals.

CONCLUSION

From the present study, it is clear that bacoside fraction is an efficient free radical quencher *in vitro*. The scavenging ability was increased with increasing in concentrations of the fraction up to 50µg. Higher concentrations did not show a further increase in scavenging activity. Various *in vivo* and *in vitro* studies are needed to assess the medicinal potential of the bacoside fraction.

CONFLICT OF INTERESTS

Declare none

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