



## Pharmaceutical Standardization

# Evaluation of comparative free-radical quenching potential of *Brahmi* (*Bacopa monnieri*) and *Mandookparni* (*Centella asiatica*)

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### Abstract

*Ayurvedic* texts describe rejuvenate measures called *Rasayana* to impart biological sustenance to bodily tissues. *Rasayana* acting specifically on brain are called *Medhya Rasayana*. *Brahmi* is one of the most commonly practiced herbs for the same. Yet there exist a controversy regarding the exact plant species among *Bacopa monnieri* L. Penn (*BM*) and *Centella asiatica* (L.) Urban (*CA*) to be used as *Brahmi* in the formulations. Though the current literature available has suggested a very good nootropic potential of both the drugs, none of the studies have been carried out on comparative potential of these herbs to resolve the controversy. Free-radical scavenging potential for these plants is studied to find out their comparative efficacy. The study revealed a very good *in vitro* free-radical scavenging properties of aqueous and ethanolic extracts of both the plants as evidenced by FRAP, DPPH, reducing power, and antilipid peroxidation assays. It can be concluded from the studies that both the plants, although taxonomically totally different at family level, showed similar type of *in vitro* activities. The total phenolic and flavonoid contents also revealed a significant similarity in the two plants. The *in vitro* study supports the *Ayurvedic* concept of *BM* and *CA* having a similar potential.

**Key words:** *Bacopa*, *Brahmi*, *Centella*, free-radical scavenging antioxidants

## Introduction

Plants have a longest history of use as a medicine, food source, and for a variety of daily needs.<sup>[1]</sup> Of the 250,000 known plant species on the Earth, more than 80,000 are utilized for medicinal purposes. India is one of the world's 12 biodiversity centers with the presence of over 45,000 different plant species. Of these, about 15,000-20,000 plants have a potent medicinal value. However, only 7,000-7,500 species are utilized in routine by traditional communities for their medicinal value.<sup>[2]</sup>

In India, drugs of herbal origin have been used by *Ayurveda*, *Siddha*, and *Unani* systems of medicines since ancient times. Among all traditional systems, *Ayurveda* is the most ancient yet still successfully practiced system, especially in India, Sri Lanka,

Germany, China, and many other countries.<sup>[3]</sup> It has a sound philosophical and experiential basis as well as a holistic approach to cure or combat the diseases to maintain a healthy state of mind and body. The sacred texts in *Ayurveda* have explained several plant species for their use in various ailments. Several classical literatures like *Charak Samhita* and *Sushrut Samhita* had described properties and uses of 1,100 and 1,270 species respectively.<sup>[2]</sup> More than 8,000 herbal remedies have been codified in *Ayurveda* till date.

In *Ayurveda*, there exists a unique concept of rejuvenation measures to impart biological sustenance to bodily tissues. This is called as *Rasayana* which is claimed to strengthen the whole biological system as well as impart subtle divine characteristics to the individual. Some of these *Rasayana* are tissue and organ specific. Those drugs having a specific action on brain and cognitive properties are called *Medhya Rasayana*. *Brahmi* is one of such *Medhya Rasayana* which is very commonly used among all *Ayurvedic* practitioners. There exists a dispute in the *Ayurvedic* literature about the identity of exact species of *Brahmi* since many years. The reason for this is the *Sanskrit* verse in the *Bhavaprakasha Nighantu* which states that *Brahmi* is the synonym for both *Jalabrahmi* (*Bacopa monnieri* L. Penn) and *Mandookaparni* (*Centella asiatica* (L.) Urban).<sup>[4-6]</sup>

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ब्राम्ही कपोतवंगा च सोमवल्ली सरस्वती ।  
 मंडूकपर्णी माण्डूकी त्वाष्ट्री दिव्या महौषधी ॥  
 ब्राह्मी हिमा रसा तित्ता लघुर्मध्या च शीतला ।  
 कषाया मधुरा स्वादुपाकाऽऽयुष्या रसायनी ॥  
 स्वर्या स्मानदतिप्रदा कुष्ठपाण्डुमहास्रकासजित् ।  
 विष शोथज्वरहरि तद्वन्मण्डूकपर्णिनी ॥  
 भावप्रकाश निघण्टू गुडूच्यादिवर्ग २८३

Taxonomically *Bacopa monnieri* and *Centella asiatica* are from totally different plant families (Scrophulariaceae and Apiaceae, respectively) and are very distinct in their morphology. *Bacopa monnieri* (BM) is a creeping, glabrous, somewhat succulent herb growing in wet places (photo plate 1). The plant is called *Aindri* and *Bramhi* in Sanskrit.<sup>[7,8]</sup> On the contrary, *Centella asiatica* (CA) is a slender, prostrate, glabrous herbaceous plant, rooting at the nodes. The leaves are simple, petiolate, palmately lobed<sup>[9]</sup> (photo plate 1). The Sanskrit name of the plant is *Aindri*, *Mandookaparni*, or *Bramhi* [Figure 1].<sup>[7,8]</sup>

Though some of the texts have tried to rule out the controversy regarding identity of exact species of *Brahmi*, whether to use BM or CA, still some *Ayurvedic* practitioners and pharmacies accept the use of both these plants as *Brahmi* while making different formulations and are believed that both are equipotent in regard to their medicinal values. The present literature on evaluation of potential of these two plants on neurological and behavioral systems elucidates a remarkable efficacy of both these plants. Yet no studies till date have reported the comparative efficacy of BM and CA to resolve the dispute of identity of *Brahmi*. The *Ayurvedic* literature is based on the clinical experiences by the sages for many years and is accepted to be true clinically. However the same has not yet been validated using experimental studies which can even throw a light on the probable mechanism of action of these plants. Thus it becomes imperative to evaluate and compare *in vitro* free-radical quenching potential of BM and CA which could help identify exact species of plants to be used for *Brahmi*. Also it will help to validate the *Ayurvedic* concept of similar action potential of these two different species for the same vernacular name.

## Materials and Methods

### Chemicals

All chemicals used for assays were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), TPTZ (2, 4,

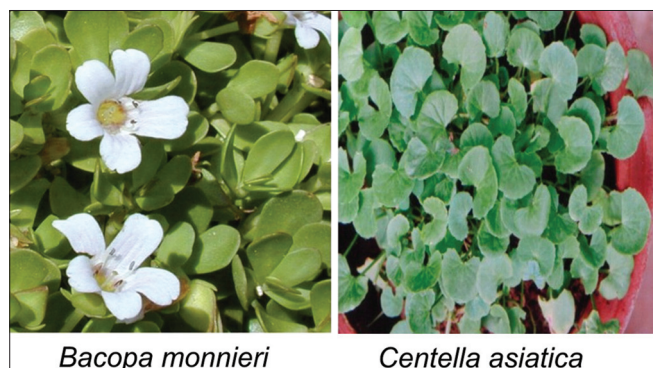


Figure 1: *Brahmi* and *Mandookaparni*

6-tripyridyl-s-triazine), Quercetin and gallic acid were procured from Sigma-Aldrich, USA. Acetic acid, sodium acetate, potassium persulfate ( $K_2S_2O_8$ ), sodium nitroprusside (SNP), ferric chloride ( $FeCl_3 \cdot 6H_2O$ ), hydrochloric acid (HCl), potassium hexacyanoferrate ( $K_3Fe(CN)_6$ ), ferrous sulfate ( $FeSO_4$ ), and tricarboxylic acid (TCA) were purchased from Qualigens Pvt. Ltd., Mumbai, India.

### Plant material

*Bacopa monnieri* (BM) and *Centella asiatica* (CA) were collected from their natural habitat, identified, authenticated, and deposited at Medicinal Plant Conservation Centre (MPCC), Pune, India. Fresh leaves of BM and CA were collected early in the morning from mature plants with uniform growth, washed thoroughly under running water followed by distilled water, shed dried, and crushed in a common grinder.

### Extraction

Ethanol and aqueous extracts of BM and CA were prepared by mixing 10% powder in respective solvent by constant agitation on a shaker (120 rpm, ambient temperature, 24 hours).<sup>[10]</sup>

### Biochemical evaluations

#### Total antioxidant activity (FRAP assay)

A slightly modified method of Benzie and Strain<sup>[11]</sup> was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer (3.1 g  $CH_3COONa$  and 16 ml  $CH_3COOH$ , pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM  $FeCl_3 \cdot 6H_2O$  solution. This assay involved (i) preparation of fresh FRAP solution by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml  $FeCl_3 \cdot 6H_2O$ , (ii) raising the temperature of the solution to 37°C, (iii) allowing plant extracts (150  $\mu$ l) allowed to react with 2,850  $\mu$ l of the FRAP solution for 30 minutes in the dark, and (iv) taking readings of the colored product (ferrous tripyridyl triazine complex) at 593 nm. The standard curve was linear between 200 and 1,000  $\mu$ M  $FeSO_4$ . Results are expressed in mM Fe (II)/g dry mass.

#### DPPH free-radical scavenging assay

Complementarities of the antioxidant capacity of the formulation was confirmed by the DPPH scavenging assay according to Brand-Williams *et al.*,<sup>[12]</sup> with slight modification. Different concentrations (100-1,000  $\mu$ g/ml) of the extracts and ascorbic acid (standard) were thoroughly mixed with 5 ml of methanolic DPPH solution (33 mg/l) in test-tubes and the resulting solution was kept standing for 10 minutes at 37°C before the optical density (OD) was measured at 517 nm. The measurement was repeated with three sets and an average of the reading was considered. The percentage radical scavenging activity was calculated from the following formula:

$$\% \text{ scavenging [DPPH]} = [(A_0 - A_1)/A_0] \times 100$$

where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance in the presence of the samples.  $IC_{50}$  values were calculated from the slope of the standard graph using the "y = mx + c" formula for every cases.

#### Reducing power assay

The  $Fe^{3+}$ -reducing power of the extract was determined by a method described by Hazra *et al.*,<sup>[13]</sup> with slight modification. The assay involved (i) mixing different concentrations (100-1,000  $\mu$ g/ml) of the extracts in a phosphate buffer (0.2 M, pH 6.6) with potassium hexacyanoferrate (0.1%), (ii) incubation at 50°C

for 20 minutes, (iii) arresting the reaction by addition of 10% tricarboxylic acid (TCA) and distilled water (2.5 ml), (iv) adding the FeCl<sub>3</sub> solution (0.01%) to the upper portion of the reaction mixture, (v) leaving the reaction mixture for 10 minutes at room temperature for color development, and (vi) measuring absorbance at 700 nm. All tests were performed in triplicate. Ascorbic acid was used as a positive control. A higher absorbance of the reaction mixture indicated greater reducing power.

#### Nitric oxide scavenging assay

Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, reacts with molecular oxygen to form nitrite ions. Sulfanilamide is quantitatively converted to a diazonium salt by reacting with nitrite under acidic conditions (5% phosphoric acid). This diazonium salt coupled with N-(1-naphthyl)-ethylenediamine (NED) forms an azo dye that can be measured quantitatively at 542 nm.<sup>[14]</sup>

Chemical reactions involved in the measurement of nitric oxide using the Griess reagent system: Briefly, the assay system involves (i) addition of buffer and sodium nitroprusside to various concentrations of extract, (ii) after incubation for 150 minutes at 25°C, addition of Griess reagent to initiate diazotization, (iii) addition of Griess B reagent to act as a chromophore, and (iv) finally, measuring the OD at 540 nm. The percentage radical scavenging activity was calculated from the following formula:

$$\% \text{ scavenging [NO]} = [(A_0 - A_1)/A_0] \times 100$$

where A<sub>0</sub> was the absorbance of the control and A<sub>1</sub> was the absorbance in the presence of the samples.

#### Antilipid peroxidation assay

Decomposition of lipid membrane in the body leads to formation of malondialdehyde (MDA) along with other aldehydes and enals as the end-product. These react with thiobarbituric acid to form colored complexes. Hence these are called the thiobarbituric acid reactive substances (TBARS). The complex of TBA-MDA is selectively detected at 532 nm using a double beam UV spectrophotometer.<sup>[15]</sup> Briefly, the assay involved (i) perfusion of goat brain with 0.15 M KCl, (ii) initiation of lipid peroxidation by addition of 1 mM FeCl<sub>3</sub>, (iii) stopping the reaction by adding ice-cold 0.25 N HCl containing TCA and TBA (this initiated the color development), (iv) addition of BHT, and (v) finally measuring the OD at 532 nm against solutions without FeCl<sub>3</sub> (normal) and without drug (induced).

#### Determination of the total phenolic content

The amount of total phenolics present in extracts of *BM* and *CA* was determined using the Folin-Ciocalteu (FC) reagent as described by Hazra *et al.*<sup>[13]</sup> A gallic acid standard curve (R<sup>2</sup> = 0.9) was used to measure the phenolic content [Figure 2].

#### Determination of the total flavonoid content

The amount of total flavonoids present in extracts of *BM* and *CA* was determined using aluminum chloride reagent as described by Asgarirad *et al.*<sup>[16]</sup> A Quercetin standard curve (R<sup>2</sup> = 0.9) was used to measure the total flavonoid content [Figure 3].

## Results

### FRAP

The FRAP values are represented in terms of μM Fe (II)/g of

dry mass of the sample. From the standard graph [Figure 4] of ferrous sulfate, the values for *BM* and *CA* were determined using the standard formula. Both aqueous and ethanolic extracts showed significant FRAP activity [Table 1].

### DPPH free-radical scavenging

Both *BM* and *CA* were found to be potent DPPH free-radical scavengers [Figure 5]. Comparatively higher activity (61-94% at conc. μg/ml) was observed in ethanolic extract of *CA* leaves while aqueous extracts showed lower activity. *CA* leaves showed higher free-radical scavenging activity compared to *BM* leaves in either solvent. IC<sub>50</sub> values for the aqueous extracts of *CA* and *BM* were 94.66 and 76.42 μg/ml, while those of ethanolic extracts were 475 and 469 μg/ml for *CA* and *BM*, respectively.

### Reducing potential assay

Although both aqueous and ethanolic extracts of the two plants have shown significant reducing potential, aqueous extract of leaves of *CA* showed maximum activity (OD 0.608 at conc. 1,000 μg/ml, at 700 nm). However, there was no significant variation in the activity for all the extracts [Figure 6].

### NO scavenging

For scavenging NO free radical *in vitro*, aqueous extract of *CA* exhibited maximum efficacy (46.27% at conc. 1,000 μg/ml) [Figure 7] and the activity was reduced in aqueous extract of *BM*.

### Antilipid peroxidation

Lipid peroxidation is one of the major events that occur during oxidative damage. Both aqueous as well as ethanolic extracts of *CA* and *BM* have antilipid peroxidation activity which is maximum at 1,000 μg/ml of the extracts. In the case of aqueous extracts, *CA* showed more protective potential for lipid peroxidation activity (97.37% at conc. 1,000 μg/ml) while *BM* had significantly less efficacy (79.02% at conc. 1,000 μg/ml). On the contrary, ethanolic extract of *BM* had more activity (95.78%) than that of *CA* (89.16%) at concentration 1,000 μg/ml [Figure 8].

### Total phenol content

Phenols play a vital role in the free-radical scavenging properties of plants.<sup>[17,18]</sup> The total phenolic content in ethanolic extracts of *BM* and *CA* was found to be 3.18 and 2.53 μg/ml of Gallic acid equivalent respectively, while that of aqueous extracts were 3.71 and 4.47 μg/ml of Gallic acid equivalent, respectively [Table 2].

### Total flavonoid content

The total flavonoid content was found to be higher in aqueous extracts of *CA* than *BM* (112.5 and 85.63 μg/ml of Quercetin equivalent, respectively) and comparable (116.8 and 115.0 μg/ml of Quercetin equivalent respectively) in ethanolic extracts. This shows that the two plants are significantly rich in flavonoid content [Table 3].

**Table 1: FRAP values (μM Fe (II)/g dry mass) of aqueous and ethanolic extracts of *BM* and *CA***

Name of the plant	Extracts	
	Aqueous	Ethanol
<i>BM</i>	502.64 ± 5.0	812.64 ± 5.0
<i>CA</i>	919.91 ± 7.5	628.09 ± 5.0

*BM*: *Bacopa monnieri* L. Penn, *CA*: and *Centella asiatica* (L.)

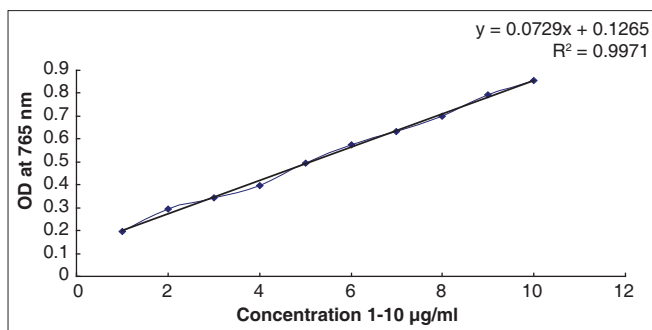


Figure 2: Standard graph of gallic acid

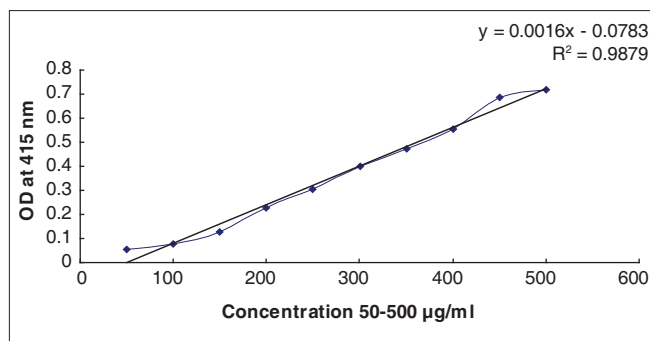


Figure 3: Standard graph of Quercetin

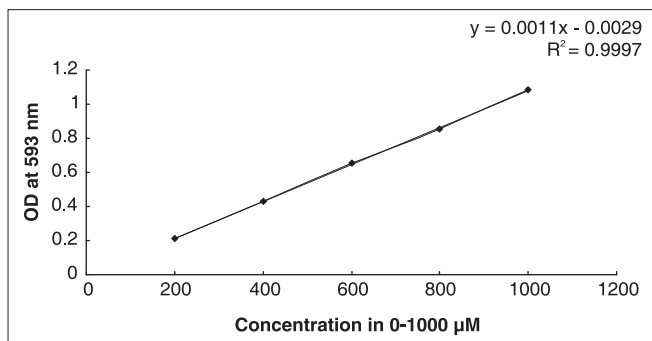


Figure 4: Standard graph of FeSO<sub>4</sub>

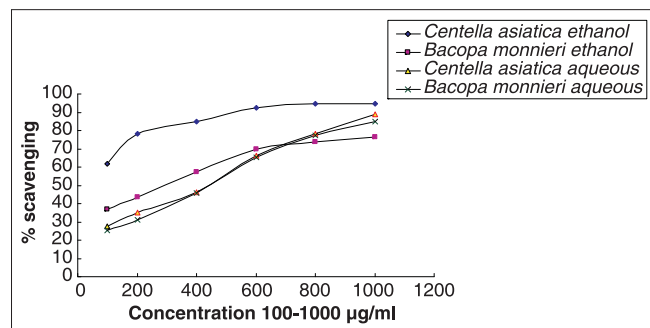


Figure 5: Comparative DPPH free-radical scavenging activity of *Bacopa monnieri* L. Penn and *Centella asiatica* (L.)

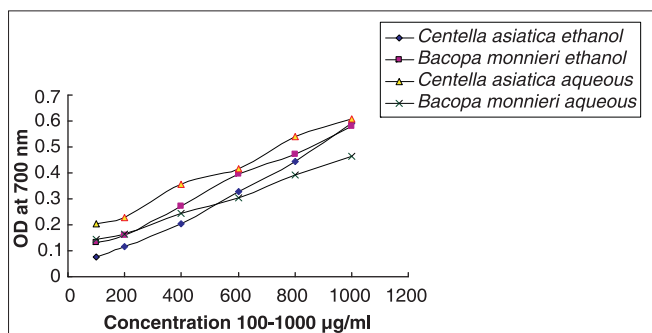


Figure 6: Comparative reducing potential by *Bacopa monnieri* L. Penn and *Centella asiatica* (L.)

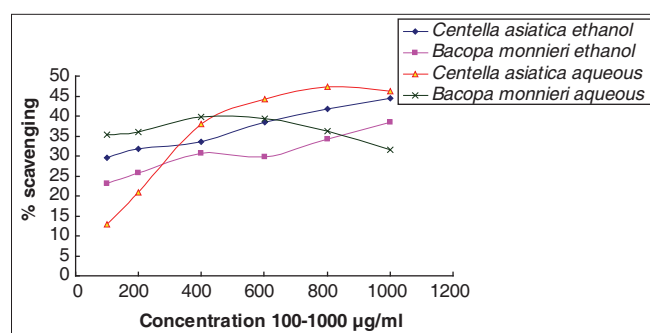


Figure 7: Comparative NO scavenging by *Bacopa monnieri* L. Penn and *Centella asiatica* (L.)

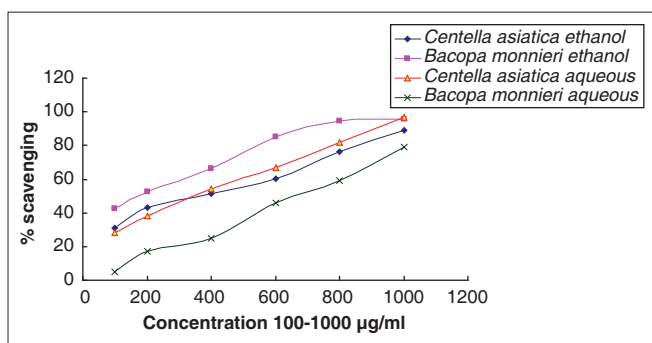


Figure 8: Comparative ALP potential of *Bacopa monnieri* L. Penn and *Centella asiatica* (L.)

## Discussion

BM is a well-known memory enhancer mentioned in the *Ayurvedic* system of medicine<sup>[19]</sup> where special mention is made about this plant in treatment of asthma, insanity, and epilepsy.<sup>[20]</sup>

The major chemical entity shown to be responsible for the said neuropharmacological effect, nootropic action, and anti-amnesic effects is saponin *Bacoside A*, along with *Bacoside A3*, *Bacoside II*, *Jujubogenin isomer of Bacopasaponin C*, and *Bacopasaponin C*.<sup>[19,21]</sup> The antioxidant properties and its ability to counter-balance the enzymes such as (i) SOD (superoxide dismutase) and (ii) catalase levels in the cells are also attributed to the same saponin (*Bacoside A*).<sup>[19]</sup> The aqueous extract of this plant is known to have a tranquilizing effect on experimental rats and dogs.<sup>[19]</sup>

CA leaves and entire plants are used therapeutically in the traditional *Ayurvedic* system to restore youth, memory, and longevity.<sup>[22]</sup> Cognitive enhancing effects of aqueous extract of CA have been shown to be associated with an antioxidant mechanism in the CNS in rats following oral administration.<sup>[23]</sup> *Ayurvedic* poly-herbal formulations, including CA, are used to retard age and prevent dementia, and the herb combined with milk is given to improve memory,<sup>[24]</sup> whereas CA has been used for combating physical and mental exhaustion<sup>[25,26]</sup> in traditional Chinese medicine (TCM).



**Table 2: Total phenol content (gallic acid equivalent/g dry mass) of aqueous and ethanolic extracts of *BM* and *CA***

Name of the plant	Total phenol content (gallic acid equivalent/g dry mass)	
	Aqueous	Ethanol
<i>BM</i>	2.53 ± 0.5	3.18 ± 0.5
<i>CA</i>	4.47 ± 0.5	3.71 ± 0.5

**DPPH free-radical scavenging and reducing power**

Plants with antioxidant activities have been reported to possess free-radical scavenging potentials.<sup>[27]</sup> In our studies, we have found that both *BM* and *CA* are potent DPPH free-radical scavengers. Aqueous as well as ethanolic extracts of both the plants have shown significant DPPH radical scavenging efficacy. However, there are no significant differences in the IC<sub>50</sub> value for both aqueous (94.66 and 76.42 µg/ml of *CA* and *BM*, respectively) as well as ethanolic extracts of *CA* and *BM* (475 and 469 µg/ml for, respectively). This suggests that these plants in their aqueous and ethanolic extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity.<sup>[27]</sup>

Free iron is a potential enhancer of reactive oxygen species (ROS) formation as it leads to reduction of H<sub>2</sub>O<sub>2</sub> and generation of the highly aggressive hydroxyl radical.<sup>[28]</sup> In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> by donating an electron. The amount of Fe<sup>2+</sup> complex can then be monitored by measuring the formation of Perl's blue at 700 nm. Increasing absorbance indicates an increase in reductive ability.<sup>[27]</sup> Also, reducing power of a compound serves as a significant indicator of its antioxidant activity.<sup>[29]</sup> When aqueous and ethanolic extracts of *BM* and *CA* were assayed for the reducing power activity *CA* showed maximum activity (OD 0.608 at conc. 1,000 µg/ml, at 700 nm), and no significant variation was observed in the reducing potential in either of the solvents used [Figure 6].

**Importance of NO-scavenging efficacy as a cognition enhancer**

Both aqueous and ethanolic extracts of *CA* and *BM* showed moderate *in vitro* nitric oxide-scavenging activity. *CA* aqueous extract showed highest activity of 46% at a concentration 1,000 µg/ml [Figure 7]. It is known that along with ROS, reactive nitrogen species (RNS) plays a pivotal role in several ailments like inflammation, cancer, and other pathological conditions.<sup>[30]</sup> NO is known to be a ubiquitous free-radical moiety, which is distributed in tissues or organ systems and is supposed to have a vital role in neuromodulation or as a neurotransmitter in the CNS.<sup>[31]</sup> Further, nitric oxide free radical (NO) can react with superoxide radical to form highly toxic peroxynitrite anion (ONOO<sup>-</sup>), which upon reacting with tissue or body fluid generates nitrotyrosines. These nitrotyrosines are detected in human brain and may be increased in neurodegenerative diseases, especially because glial cells and macrophages generate nitric oxide.<sup>[28]</sup> Thus *in vitro* NO-quenching potential of both aqueous and ethanolic extracts of *BM* and *CA* may provide a possible mechanistic clue to the traditional knowledge that these two plants act as a nootropic agent or enhance cognition.

**Table 3: Total flavonoid content (µg/ml of quercetin equivalent) of aqueous and ethanolic extracts of *BM* and *CA***

Name of the plant	Total flavonoid content (µg/ml of quercetin equivalent)	
	Aqueous	Ethanol
<i>BM</i>	85.63 ± 2.0	115.0 ± 2.0
<i>CA</i>	112.5 ± 2.0	116.8 ± 2.0

**Interrelation between DPPH radical and anti lipid peroxidation assay**

Lipid peroxidation is a major harmful consequence of ROS formation, as it reflects irreversible oxidative changes of membranes.<sup>[32]</sup> It is known that any free radical remains with sufficient energy to abstract a hydrogen atom from a methylene carbon of an unsaturated long chain fatty acid and can initiate a lipid peroxidation reaction, which increases in many oxidative damage-induced diseases. Reasons for this increased peroxidizability of damaged tissues include (i) inactivation of some antioxidants and/or (ii) leakage of antioxidants from the cell. Naturally occurring as well as cellular antioxidants protect these processes at different levels within cells by (a) preventing radical formation; (b) intercepting radicals when formed; (c) repairing oxidative damage caused by radicals; (d) increasing the elimination of damaged molecules.<sup>[33]</sup> In the anti-lipid-peroxidation assay, aqueous extracts of *CA* showed comparatively higher lipid peroxidation protection activity (97.37% at conc. 1,000 µg/ml) than that of *BM* (79.02% at conc. 1,000 µg/ml). On the contrary, the efficacy was comparable in ethanolic extract (89.16% for *CA* and 95.78% for *BM* at conc. 1,000 µg/ml). This activity of the extracts may be attributed to their hydrogen-donating ability.

It is also known that free radical causes harmful autooxidation of unsaturated lipids in food<sup>[34]</sup> and natural antioxidants and either (i) intercept the free-radical chain of oxidation or (ii) donate hydrogen from the phenolic hydroxyl groups; thereby forming a stable end product, which does not initiate or propagate further oxidation of lipid.<sup>[34]</sup> It is known that brain is rich in PUFAs, and is vulnerable to ROS-induced oxidative damage.<sup>[35,36]</sup> Both *BM* and *CA* are found to be potent free-radical scavenger, *in vitro*. Thus the antilipid peroxidation activity of these two plants may be due to their strong free-radical quenching potentials and also can be a possible clue for their neuro-protective potential as described by the *Ayurvedic* system.

**Correlation between flavonoid, phenol content and antioxidant**

Antioxidant activities are known to increase proportionally to the polyphenol content. This activity is believed to be mainly due to their redox properties,<sup>[17,18]</sup> which plays an important role in (a) adsorbing and neutralizing free radicals, (b) quenching singlet and triplet oxygen, and (c) decomposing peroxides.<sup>[37]</sup> Also according to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. Naturally occurring antioxidants such as phenols, flavonoids are well known to have very less or no side effects and hence are considered to be safe.<sup>[16]</sup> In our study, we found that the total phenolic content in aqueous extract of *CA* was higher than that of *BM*; while phenol content

in ethanolic extracts was comparable [Table 2]. This may be one of the causes for their strong free-radical scavenging potential. Similarly, total flavonoid content in the ethanolic extracts of the studied plants were comparable, while CA showed significantly higher flavonoid content in aqueous extract than that of BM [Table 3]. In plants, polyphenol compounds like flavonoids, which contain hydroxyl functional groups, are supposed to be responsible for the radical scavenging effect.<sup>[16,38,39]</sup> Flavonoids also play vital role as antitumoural, antiischemic, antiallergic, antiulcerative, and anti-inflammatory. They are known to inhibit several enzyme activities such as lipoxygenase, cyclooxygenase, monooxygenase, xanthine oxidase, glutathione-S-transferase, mitochondrial succino-oxidase, and NADH oxidase.

Many of the biological activities of flavonoids are attributed to their antioxidant properties and free-radical scavenging capabilities.<sup>[28]</sup> According to our study, the contents of these bioactive phytochemical compounds in BM and CA extracts can explain their antioxidant activity. Also, there exists a strong relation between total phenol and flavonoid content and polarity of the extraction solvent.<sup>[16]</sup> As our results suggest that aqueous extracts give a significant difference in the phenol as well as flavonoid content in these two plants, while there is a marginal difference in the ethanolic extracts.

All the above results highlight the potent free-radical quenching potentials of CA and BM and support their traditional uses in various ailments as well as *Medhya Rasayana*. The probable mechanism of action of these two plants on cognition and memory would be through prevention of neuronal damage which is due to increased oxidative stress.

## Conclusion

*Brahmi* is the one of the most studied *Ayurvedic* plant for its cognition enhancing activity. Our results suggest that both *Bacopa monnieri* and *Centella asiatica* are equipotent with regard to their free-radical quenching potentials and hence can be alternatively used. This also supports the *Ayurvedic* belief of the plants having similar activity as mentioned in ancient texts and opens a new arena of evaluation of these plants to elucidate their exact mechanism of action.

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## हिन्दी सारांश

# ब्राह्मी और मण्डूकपर्णी के फ्री रेडिकल पर प्रभाव का तुलनात्मक अध्ययन

सौरभ मुखर्जी, स्वप्निल दुगड, राहुल भण्डारे, नयना पवार, सुरेश जगताप, पंकज के. पवार, ओंकार कुलकर्णी

शरीर की कोशिकाओं को पुनर्जीवित करने तथा रक्षा करने के लिये आयुर्वेद के ग्रंथ में रसायन का उल्लेख किया है। जो रसायन मस्तिष्क तथा बुद्धि के लिये हैं उसको मेध्य रसायन कहते हैं। यद्यपि, ब्राह्मी ऐसा ही एक उल्लेखित, उपयोगी और नियमित रूप से प्रचलित मेध्य रसायन है, तथापि, बकोपा मोनेरी तथा सेंटैला असियाटिका इन दो वनस्पतियों का आयुर्वेदिक औषधियों में प्रयोग के संबंध में तीव्र द्विमत है। इस द्विमत का निराकरण करने के उद्देश्य में कोई भी संशोधन उपलब्ध नहीं हैं, जब कि दोनों वनस्पतियों की मेधा बढ़ाने की शक्ति की प्रशंसा हो चुकी है। इन दोनों वनस्पतियों की तुलनात्मक फ्री रेडिकल अपमार्जन क्षमता वर्तमान संशोधन में दिखायी गयी है। दोनों वनस्पतियों का जलिय एवं इथनलिक निष्कर्ष उत्कृष्ट फ्री रेडिकल अपमार्जक प्रमाणित हुआ जो DPPH, FRAP, Anti-lipid Peroxidation एवं Reducing power की परख करने से सिद्ध हुआ। इस अध्ययन से यह अनुमान किया जाता है, की यद्यपि दोनों वनस्पति दो अलग परिवार के अंतर्गत हैं, दोनों ने संपूर्ण क्रियाशीलता दिखायी है। दोनों वनस्पतियों का सम्पूर्ण फिनल एवं फलावोनयेड मात्रा में भी महत्वपूर्ण साम्य देखा गया। यह अध्ययन आयुर्वेद में बकोपा मोनेरी तथा सेंटैला असियाटिका का सामर्थ्य तथा उपयोग का पूर्ण समर्थन करता है।