J Radioanal Nucl Chem (2009) 281:53–58
DOI 10.1007/s10967-009-0081-z

Elemental analysis of brahmi (*Bacopa monnieri*) extracts by neutron activation and its bioassay for antioxidant, radio protective and anti-lipid peroxidation activity

A. N. Garg · A. Kumar · A. G. C. Nair · A. V. R. Reddy

Received: 19 April 2009 / Published online: 18 June 2009

Abstract Brahmi (*Bacopa monnieri*) leaves, known as nervine tonic in Ayurveda, and its aqueous (BA), methanolic (BM) and aqueous–methanolic (BAM) extracts were analyzed for 7 minor (Al, Fe, Na, K, Ca, P, Cl) and 18 trace (As, Au, Ba, Br, Co, Cr, Cu, Hf, Hg, La, Mn, Rb, Se, Sm, Sr, Th, V, Zn) elements by INAA. BAM extract showed maximum contents of Na, K, Cl and significant amounts of Mn, Co, Zn. It was also found as effective scavenger of DPPH radicals with 33.5% total phenolic content, highest γ-ray radioprotective effect and higher anti lipid peroxidation activity.

Keywords *Bacopa monnieri* leaves · Antioxidant activity · Neutron activation analysis · Radioprotective effect · Anti-lipid peroxidation activity · Phenolic content

Introduction

Majority of the world population especially in the developing countries depends on the traditional medicines derived from plants. Several hundred genera used herbal preparations in the indigenous systems of medicine in different countries as sources of very potent and powerful drugs [1]. The World Health Organization (WHO) reported that ~80% of the world's population relies chiefly on traditional medicines based on plants or its extracts containing chemical compounds some of which act as antioxidants [2]. Antioxidant compounds in foods [3] play an important role as health-protecting factor and provide protection against oxidative damage by repair or removing damaged molecules [4].

All aerobic organisms, including human beings, have antioxidant defense against oxidative damage. Hence it is of great interest to investigate antioxidant and therapeutic activity of medicinal plants where natural antioxidants may act as potential therapeutic agents. Several workers from China [5], Japan [6], Korea [7] and India [8] have analyzed medicinal plants for their biological activity and active constituents.

Brahmi (*Bacopa monnieri*), family *scrophulariaceae* is a reputed nerve tonic used for enhancing memory, curing epilepsy and as a mild sedative in Ayurveda. It benefits the mind and spirit, and improves the intellect and consciousness, the cognitive disorders of aging [9], assists in heightening mental acuity and supports the physiological processes involved in relaxation. Brahmi is hepatoprotective with anti-ulcer properties [10] and has curative potential towards neurodegenerative Alzheimer and Parkinson diseases. Organic extracts of brahmi have been investigated for its antioxidant [11], lipid peroxidation activity [12] and protective role on morphine induced brain mitochondrial enzyme activity in rats [13]. Bhattacharya et al. [14] reported antioxidant effect of brahmi in the rat frontal cortex.

Since brahmi is neuroprotective and has been demonstrated to enhance memory we examined the possibility of correlating its antioxidant property and nutrient element contents. Different extracts of brahmi were evaluated towards DPPH radical scavenging, anti-lipid peroxidation capacity, and DNA strand break. Antioxidant activity is correlated positively with the phenolic content which is mainly because of their redox properties. The extracts were

A. N. Garg (✉) · A. Kumar
Department of Chemistry, Indian Institute of Technology, Roorkee 247667, India
e-mail: agargfcy@yahoo.com

A. G. C. Nair · A. V. R. Reddy
Radiochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India

analyzed for their elemental contents by instrumental neutron activation analysis (INAA) to understand the role of essential elements.

Experimental

Preparation of extracts

Brahmi powder was procured from Yogi Pharmacy, Haridwar, India. Brahmi powder (50 g) was equilibrated twice with 250 mL methanol. The residue was extracted with 1:1 aqueous–methanol and then with water. The extracts are designated as methanol (BM), aqueous–methanol (BAM) and aqueous (BA), respectively, and were evaporated to dryness.

Irradiation and counting

About 50 mg powder each of the dried extracts and 2–3 SRMs were weighed accurately and packed in polythene/aluminum foil (Superpure) for short (1 and 5 min) and long (14 h and 3 days) irradiations, respectively. Irradiations were carried out in E8 position of the APSARA reactor at BARC, Mumbai at $\sim 10^{11}$ n cm^{-2} s^{-1}. A 1 min short irradiation was carried out at $\sim 10^{13}$ n cm^{-2} s^{-1} using pneumatic carrier facility in Dhruva reactor (BARC). High resolution gamma spectrometry with 80 cm^3 coaxial HPGe detector (EG & G ORTEC) and 4 k MCA was used for the measurement of γ activity first at the reactor site and later at our laboratories in Roorkee. Counting was followed at different intervals up to 3 months [15]. Elemental contents were calculated using different SRMs (1547 and 1573) as comparators. Phosphorus was determined by counting β–activity of ^{32}P on an end window G.M. counter using a 27 mg cm^{-2} Al filter [16].

1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging assay

An ethanolic solution of DPPH (100 μM) was incubated with an aqueous solution of the test compound (0.1–1.0 μM) and the absorbance was measured at 517 nm. The concentration or inhibition capacity ($IC_{0.2000}$) of the test compound that reduced absorbance by 0.20 in a 30 min duration observation was taken as the free radical scavenging potency.

Anti lipid peroxidation assay

Small unilamellar vesicles (SUV) were prepared from phosphotidyl choline following the procedure of Patro et al. [17] and peroxidation was initiated either by 50 μM ferrous ammonium sulfate and 500 μM ascorbic acid or by 50 mM AAPH. In both cases volume was 0.5 mL in 10 mM potassium phosphate buffer (pH 7.4). The test extracts were added before the initiation of lipid peroxidation. In both cases the peroxidation was terminated by the addition of 1 mL thiobarbituric acid (TBA)-thiochloroacetic acid (TCA)-HCl (0.375 w/v-15%, w/v-0.25 N) solution followed by incubation for 15 min at 100 °C. After the development of color, it was extracted in equal volume of water saturated *n*-butanol and absorbance was measured at 532 nm.

Gamma-ray induced DNA strand break assay

DNA samples in presence or absence of the test samples were prepared in 20 μL and irradiated in ^{60}Co Gamma Chamber for 3 min at a dose rate of 6.64 Gy/min. In all the experiments, the concentration of super-coiled pBR322 DNA was 10 mg/L in a 10 mM potassium phosphate buffer (pH 7.4). After irradiation two plasmid forms supercoiled (Form I) and open circular (Form II) were separated by electrophoresis, stained with ethidium bromide and seen under UV light. The relative intensities of the bands were determined using a Bio-Rad gel documentation system.

Estimation of total phenolic content

It was measured spectrophotometrically by redox reaction using Folin-Ciocalteu (FC). 2.5 mL of 10 times diluted FC reagent was added to 500 μL extract followed by 2 mL of Na_2CO_3 (75 g/L) within 0.5–8 min. Sample was incubated for 5 min at 50 °C, cooled and absorbance measured at $\lambda_{max.} = 760$ nm.

Results and discussion

Elemental contents in raw brahmi and its extracts including residue are listed in Table 1, where ± represent uncertainties calculated on the basis of replicate analyses, multiple irradiations or different photo peaks. It has been emphasized that many trace elements such as Mn, Fe, Cu and Zn play a vital role in biochemical processes including transport of oxygen, normalizing the nervous system and stimulating the growth, maintain and repair of tissues and bones [18]. It is particularly crucial to have them at optimum concentration and in bioavailable form. It is important to study the relationship of minerals to the human health. Brahmi is enriched in essential nutrients such as Ca, Fe, K, P, Cr, Mn, Cu and Zn which play a vital role in biochemical/enzymatic processes and are likely to be responsible for its medicinal properties. Some of the elements are integral parts of various antioxidant compounds.

Table 1 Elemental contents in raw and different extracts of brahmi

Element	Raw sample (50 g)	BM (2.35 g)	BAM (4.99 g)	BA (2.77 g)	Residue (39.1 g)
Al (mg/g)	4.39 ± 0.44	0.15 ± 0.02	0.14 ± 0.01	2.00 ± 0.21	4.21 ± 0.42
(mg)	(219 ± 22)	(0.35 ± 0.04)	(0.71 ± 0.07)	(5.55 ± 0.59)	(165 ± 16)
As (μg/g)	0.71 ± 0.01	–	–	–	0.79 ± 0.01
(μg)	(35.4 ± 2.0)				(31.0 ± 0.24)
Au (ng/g)	12.4	24.5	18.1	24.2	8.4
(ng)	(620)	(57.6)	(90.3)	(67.0)	(328)
Ba (μg/g)	66.7 ± 0.7	–	–	–	99.8 ± 1.2
(μg)	(3.34 ± 0.04)				(3.90 ± 0.05)
Br (μg/g)	7.56 ± 0.88	34.1 ± 4.0	21.8 ± 2.55	25.6 ± 2.8	2.94 ± 0.34
(μg)	(378 ± 44)	(80.1 ± 9.4)	(109 ± 13)	(70.9 ± 7.8)	(115 ± 13)
Ca (mg/g)	27.5 ± 1.5	–	–	3.50 ± 0.36	12.3 ± 1.3
(mg)	(560 ± 75)			(9.70 ± 1.0)	(481 ± 51)
Cl (mg/g)	2.83 ± 0.22	15.59 ± 1.75	6.00 ± 0.67	4.90 ± 0.46	0.15 ± 0.01
(mg)	(98.3 ± 11.1)	(36.6 ± 4.1)	(29.9 ± 3.3)	(13.6 ± 1.3)	(5.71 ± 0.55)
Co (μg/g)	0.64 ± 0.12	0.82 ± 0.16	0.54 ± 0.10	0.59 ± 0.12	0.50 ± 0.08
(μg)	(32.1 ± 2.0)	(1.94 ± 0.37)	(2.67 ± 0.51)	(1.62 ± 0.34)	(19.4 ± 3.7)
Cr (μg/g)	5.91 ± 0.04	2.74 ± 0.01	2.53 ± 0.01	5.27 ± 0.03	5.69 ± 0.03
(μg)	(296 ± 2)	(6.43 ± 0.02)	(12.6 ± 0.05)	(14.6 ± 0.1)	(222 ± 1.17)
Cu (μg/g)[a]	26.1 ± 0.2				
Fe (mg/g)	3.43 ± 204	–	–	1.53 ± 82	3.27 ± 0.13
(mg)	(171 ± 10)			(4.23 ± 0.23)	(128 ± 5)
Hf (μg/g)	0.304	–	–	–	0.370
(μg)	(15.2)				(14.5)
Hg (ng/g)	34.0 ± 1.6	–	3.79 ± 0.28	31.1 ± 1.5	40.7 ± 2.0
(μg)	(1.70 ± 0.08)		(0.02 ± 0.01)	(0.086 ± 0.004)	(1.59 ± 0.08)
K (mg/g)	16.0 ± 1.3	25.1 ± 2.1	35.7 ± 3.3	76.2 ± 2.9	11.4 ± 1.1
(mg)	(800 ± 65)	(59.0 ± 4.9)	(178 ± 16)	(211 ± 8)	(446 ± 43)
La (μg/g)	5.68 ± 0.16	–	–	1.36 ± 0.04	5.73 ± 0.16
(μg)	(284 ± 8)			(3.77 ± 0.11)	(224 ± 6)
Mn (μg/g)	173 ± 10	4.56 ± 0.42	15.2 ± 1.6	82.2 ± 10.3	117 ± 11
(mg)	(6.15 ± 0.50)	(0.011 ± 0.001)	(0.076 ± 0.008)	(0.23 ± 0.03)	(4.57 ± 0.43)
Na (mg/g)	1.05 ± 180	1.99 ± 0.29	1.33 ± 0.20	3.87 ± 0.37	0.70 ± 0.07
(mg)	(52.5 ± 9.0)	(4.67 ± 0.69)	(6.65 ± 0.97)	(10.7 ± 1.0)	(27.2 ± 2.8)
P (mg/g)	2.92 ± 0.24	1.31 ± 0.11	2.17 ± 0.18	9.86 ± 0.81	2.26 ± 0.19
(mg)	(146 ± 12)	(3.08 ± 0.26)	(10.8 ± 0.9)	(27.3 ± 2.2)	(88.4 ± 7.4)
Rb (μg/g)[a]	7.26 ± 0.5				
Se (ng/g)	295 ± 4	–	–	152 ± 3	255 ± 4
(μg)	(9.75 ± 0.20)			(0.42 ± 0.01)	(9.97 ± 0.20)
Sm (μg/g)	1.18 ± 0.23	–	–	0.31 ± 0.06	1.26 ± 0.24
(μg)	(59.0 ± 11.5)			(0.84 ± 0.16)	(49.2 ± 9.4)
Th (μg/g)*	0.42 ± 0.05				
Sr (μg/g)*	64.7 ± 0.5				
V (μg/g)*	2.78 ± 0.11				
Zn (μg/g)	69.2 ± 2.4	69.1 ± 2.4	34.5 ± 1.2	52.5 ± 1.8	56.6 ± 2.0
(mg)	(3.46 ± 0.12)	(0.162 ± 0.006)	(0.172 ± 0.006)	(0.145 ± 0.005)	(2.21 ± 0.08)

The values in parenthesis are obtained by material balance; ± represent uncertainties

[a] These values were obtained for a different fraction

Therefore, we shall discuss antioxidant and radioprotective properties of brahmi and correlate these with elemental contents.

Scavenging potential of the nitrogen-centered DPPH radical is a measure of the antioxidant properties of test compounds. It is observed that all the three extracts exhibited DPPH scavenging activity. BAM extract showed ~100% at 100 μg/mL whereas methanolic extract (BM) showed minimum activity (<20%) as plotted in Fig. 1. Phenolic content in a plant extract, a measure of its antioxidant capacity and expressed in terms of equivalent of standard gallic acid for BM, BAM and BA were found to be 3.56%, 33.5% and 4.12%, respectively. It indicates that antioxidant capability of plant products is due to their constituent chemicals, especially the polyphenolic compounds which may remain complexed with Mn, Co and Zn exhibiting beneficial properties [19].

It is a free radical-related process in biological systems that may occur (i) under enzymatic control, e.g., for the generation of lipid-derived inflammatory mediators or (ii) non-enzymatically. The latter form is mostly associated with cellular damage as a result of oxidative stress. Oxygen radicals and lipid peroxidation play a vital role in the damage during central nervous system and more generally in neurotoxicity [20]. Anti-lipid peroxidation activity of brahmi extracts were measured by Fe^{2+}/ascorbate and AAPH protocols. AAPH is a radical generator itself and iron does not play any role in the peroxidation induced by it. On the contrary Fe^{2+} is known to react with lipid hydroperoxides to yield highly reactive alkoxyl radical. Brahmi prevents lipid peroxidation induced by Fe^{2+}/ascorbate as well as AAPH in a concentration dependent manner as shown in Fig. 2. In both cases, BAM showed superior effectiveness to prevent lipid peroxidation than

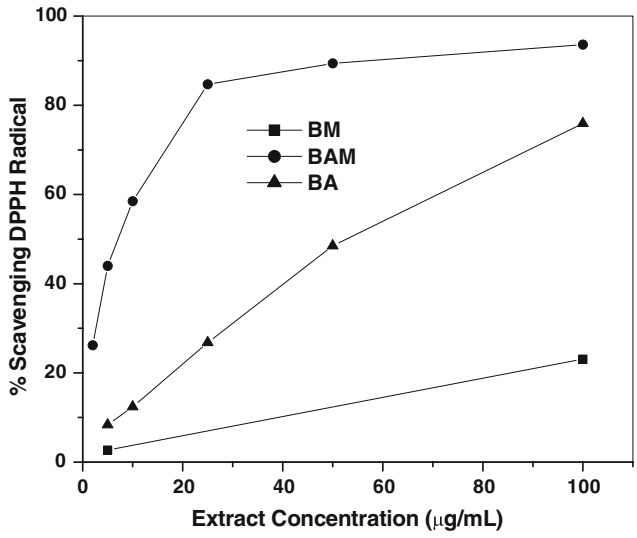

Fig. 1 Scavenging activity of brahmi extracts for DPPH radical

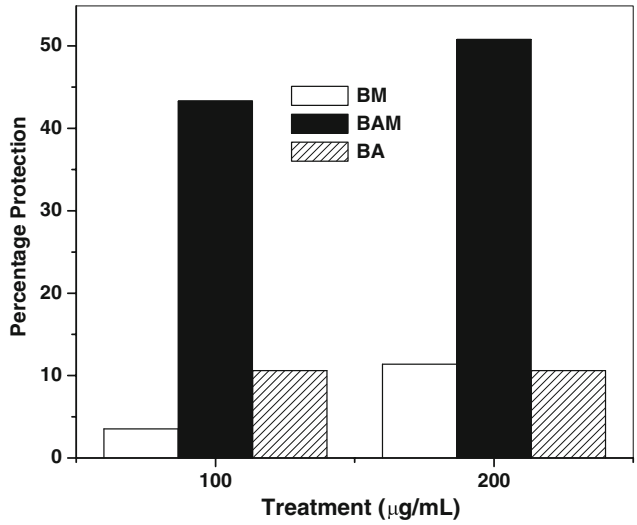

Fig. 2 Anti-lipid peroxidation activity of brahmi extracts by APPH

BM and BA extracts. IC_{50} values for BAM were found to be 8.57 ± 0.93 μg/mL and 144 ± 29.7 μg/mL for Fe^{2+}/ascorbate and AAPH systems, respectively.

Due to the polyanionic nature DNA binds with transition metal ions that mediate hydroxyl radical generation. Free radical damage to DNA has been proposed to lead to several types of cancers [21]. The biological consequences of ionizing radiations especially with respect to causing mutation and carcinogenesis are believed to be due to scission of DNA molecules. A pre-requisite of radical-mediated DNA strand break is the degradation of its sugar moiety via a direct or indirect process.

Radiation exposure of biological systems in presence of water results in the formation of primary radiolysis products, e^-_{aq}, $^{\bullet}OH$ and $H^{\bullet}$ which can abstract hydrogen atom at the C (4′) position of deoxyribose or generate DNA base radicals. It damages the sugar moiety leading to DNA strand breakage. In aerobic cells, the electron is readily accepted by the easily reducible oxygen molecule generating reactive oxygen species (ROS), all of which can damage DNA. Brahmi extracts could effectively prevent γ-ray induced damage to plasmid DNA and the extent of prevention depends on the concentration as shown in Fig. 3 where maximum undamaged % DNA occurred at 200 μg/mL. At higher concentrations, however, a little decrease is observed. BM and BA extracts at 50, 100 and 150 μg/mL showed much less % undamaged DNA compared to BAM and not shown. Thus, BAM showed highest radioprotection (~70%) compared to other extracts.

Several studies have reported intake of trace elements through tea and plant extracts taken as medicine [22]. A perusal of data in Table 1 shows that only a fraction of a few elements except Na, K and Cl are extracted whereas major amounts of Ca, As, Ba and Se are left in the residue.

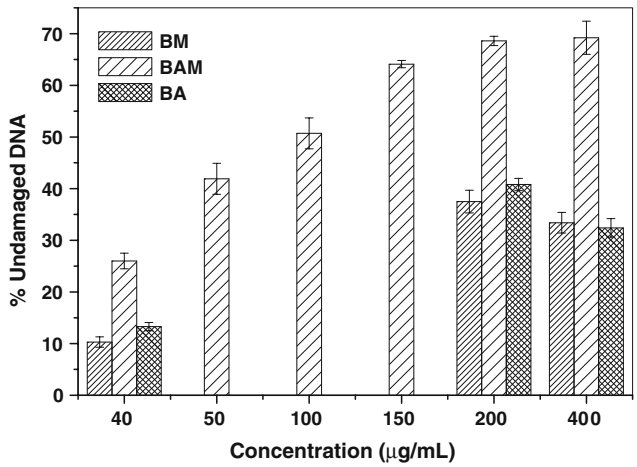

Fig. 3 Radioprotective effect of brahmi extracts against γ ray induced damage of DNA

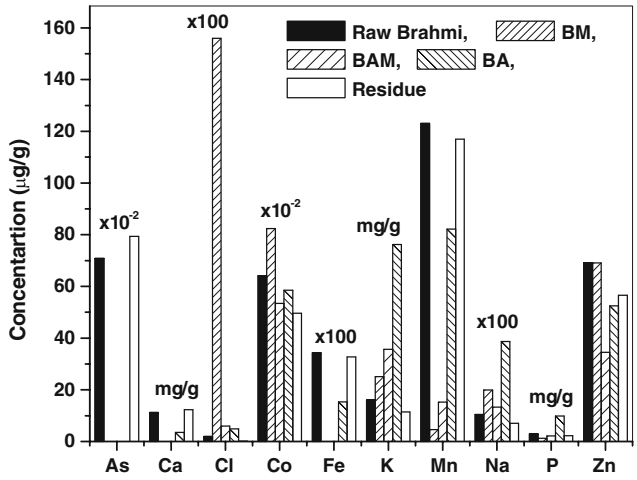

Fig. 4 Variation in elemental contents in raw and different extracts of brahmi including residue

In order to further compare the elemental contents, bar plots of elemental concentrations are shown in Fig. 4. It is observed that ~42% Na, 56% K and 81% Cl are extracted in BAM. Interestingly, BM extract showed minimum amounts of Na (8.9%) and K (7.4%) whereas a maximum of 20.4% of Na and 26.4% of K are extracted in BA. However, in the case of Cl an opposite trend is observed with a maximum of 37.2% in BM and a minimum of 13.8% in BA which may be due to easy solubility of some chloro compounds in methanol. It means that BAM extracts moderate amounts of essential nutrients such as Mn, Co and Zn which also exhibit radical scavenging activity. Incidentally BAM extract also exhibits maximum activity due to DPPH radical scavenging and anti-lipid peroxidation including DNA strand break. Since residue itself is about 78%, it is obvious that many elements go along with it and are not able to play any role even if these are beneficial for biological functions. Au, an important element in Ayurveda is present in all the fractions but again its maximum amount is left in residue.

Conclusions

Brahmi is enriched in several essential nutrient elements. Its aqueous–methanolic extract showed higher antioxidant, radioprotective and anti-lipid perosxidation activity and polyphenolic content compared to methanolic and aqueous extracts. It also showed higher Mn, Co and Zn contents. Study of ethanol and hot water extracts may help to further explore the role of essential nutrient elements in brahmi.

Acknowledgement Financial assistance from the Board of Research in Nuclear Sciences, DAE, Government of India, via Grant no 2000/37/5 is gratefully acknowledged.

References

1. Paranjpe, P.: Indian Medicinal Plants: Forgotten Healers. Chaukhamba Sanskrit Pratistha, Delhi (2001)
2. Traditional Medicine: Report by the Secretariat, 56th World Health Assembly. World Health Organization, Geneva (2003)
3. Halliwell, B., Gutteridge, J.M.C.: Free Radicals in Biology and Medicine, 3rd edn. Oxford University Press, Oxford, UK (1999)
4. Migdal, C.A.: Antioxidants in lubricants additive chemistry and applications. Chem. Ind. **90**, 1–27 (2003)
5. Li, L.N.: Biologically active components from traditional Chinese medicines. Pure Appl. Chem. **70**, 547–554 (1998)
6. Nomura, T., Fukai, T., Hano, Y.: Chemistry and biological activities of isoprenylated flavonids from medicinal plants (moraceous plants and Glycyrrhiza species). In: Atta-ur-Rahman (ed.) Studies in Natural Products Chemistry, vol. 28, 199 pp. Elsevier, Amsterdam (2003)
7. Hwang, K.H.: Monoamine oxidase inhibitory activities of Korean medicinal plants classified as cold drugs by the theory of KIMI. Food Sci. Biotech. **12**, 238–243 (2003)
8. Choudhury, R.P., Acharya, R., Nair, A.G.C., Reddy, A.V.R., Garg, A.N.: Availability of essential trace elements in medicinal herbs used for diabetes mellitus and their possible correlations. J. Radioanal. Nucl. Chem. **276**, 85–93 (2008)
9. Russo, A., Izzo, A.A., Borrelli, F., Renis, M., Vanella, A.: Free radical scavenging capacity and protective effect of Bacopa monniera on DNA damage. Phytother. Res. **17**, 870–875 (2003)
10. Sairam, K., Rao, C.V., Babu, M.D., Goel, R.K.: Prophylactic and curative effects of Bacopa monniera in gastric ulcer models. Int. J. Phytother. Phytopharmacol. **8**, 423–430 (2001)
11. Krishnaraju, A.V., Sundararaju, D., Rao, T.V.N., Kumar, K.A., Subbaraju, G.V.: Antioxidant activity and brine shrimp lethality of Bacopa monniera. Asian J. Chem. **15**, 1554–1560 (2003)
12. Tripathi, Y.B., Chaurasia, S., Tripathi, E., Upadhyay, A., Dubey, G.P.: Bacopa monniera Linn. As an antioxidant: mechanism of action. Indian J. Exp. Biol. **34**, 523–526 (1996)
13. Sumathy, T., Subramanian, S., Govindasamy, S., Balkrishna, K., Veluchamy, G.: Protective role of Bacopa monniera on morphine induced hepatotxicity in rats. Phytother. Res. **15**, 643–645 (2001)
14. Bhattacharya, S.K., Bhattacharya, A., Kumar, A., Ghoshal, S.: Antioxidant activity of Bacopa monniera in rat frontal cortex, striatum and hippocampus. Phytother. Res. **14**, 174–179 (2000)

15. Garg, A.N., Kumar, A., Nair, A.G.C., Reddy, A.V.R.: Analysis of some Indian medicinal herbs by INAA. J. Radioanal. Nucl. Chem. **271**, 611–619 (2007)
16. Garg, A.N., Kumar, A., Choudhury, R.P.: Phosphorus in biological standards and samples by thermal neutron irradiation and β-counting. J. Radioanal. Nucl. Chem. **271**, 481–488 (2007)
17. Patro, B.S., Rele, S., Chintalwar, G.J., Chattopadhyay, S., Adhikari, S., Mukherjee, T.: Protective activities of some phenolic 1, 3 diketones against lipid peroxidation: possible involvement of the 1,3 diketone moieties. Chem. Biochem. **3**, 364–370 (2002)
18. O'Dell, B.L., Sunde, R.A. (eds.): Handbook of Nutritionally Essential Mineral Elements. Marcell Dekker Inc., New York (1997)
19. Choudhury, R.P., Kumar, A., Garg, A.N.: Analysis of Indian mint (Mentha spicata) for essential, trace and toxic elements and its antioxidant behaviour. J. Pharm. Biomed. Anal. **41**, 825–832 (2006)
20. LeBel, C.P., Bondy, S.C., Meneghini, R.: Oxygen radicals: common mediators of neurotoxicity. Neurotoxicol. Teratol. **23**, 783–786 (1997)
21. Meneghini, R.: Iron homeostasis, oxidative stress and DNA damage (subcellular distribution of chelatable iron: a laser scanning microscopic study in isolated hepatocytes and liver endothelial cells). Free Radic. Biol. Med. **23**, 783–792 (1997)
22. Burns, J., Gardner, P.T., O'Neil, J., Crawford, S., Morecroft, I., McPhail, D.B., Lister, C., Matthews, D., MacLean, M.R., Lean, M.E.J., Duthie, G.G., Crozier, A.: Relationship among antioxidant activity, vasodilation capacity and phenolic content red wines. J. Agric. Food Chem. **48**, 220–230 (2000)