WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 8, Issue 7, 2440-2448. Research Article ISSN 2277– 7105

IN-VITRO ANTIOXIDANT ACTIVITY OF POLY HERBAL SIDDHA FORMULATION BRAHMI NEI

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Article Received on
25 May 2019,
Revised on 28 May 2019,
Accepted on 31 May 2019
DOI: 10.20959/wjpr20197-15254

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ABSTRACT

Siddha system of medicine is a renowned holistic system of traditional medicine emphasizing curative and preventive measures. The medicines used in siddha are of plant origin, metals, minerals and animal products. Kaya-karpam (Elixir science) is a treasure for the siddha system as they improvise the longevity of life through their anti-oxidant activities**.** Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms and leads to development of certain disease like cancer, atherosclerosis, cardiovascular diseases, ageing, and inflammatory diseases etc., In ancient time, the term Antioxidant has been clearly described in Siddha system of medicine as *Kaya Karpam* (Rejuvenation Therapy). The *Kaaya Karpam* helps to prevent the risk of disease development and ageing. *Brahmi nei (BN)* is a poly herbal Siddha preparation mentioned in ancient Siddha literature. The present study is aimed to evaluate the antioxidant effect of BN. In this study DPPH, Nitric Oxide and ABTS radical scavenging studies were performed. The results of this study shows that the percentages of inhibition in DPPH, Nitric Oxide and ABTS radical scavenging studies are 42.19 % (standard drug Ascorbic acid -83.99 %), 45.16

% (Gallic acid – 89.18 %) and 63.79 % (Gallic acid – 92.78 %) respectively and thus, findings provide evidence that BN could be a potential source of natural antioxidant and it may be used as rejuvenating medicine for vast therapeutic effects, gives a powerful body, mind and soul with long-lasting life.

KEYWORDS: Antioxidant, *Brahmi nei*, Siddha Medicine, Kaya karpam, Kaya kalpam.

INTRODUCTION

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules, viz., nucleic acids, proteins, lipids, and DNA and can initiate different degenerative diseases such as neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, and arthritis. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders. Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as superoxide dismutase, catalase, and antioxidant compounds, viz., ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione. Prior and Cao (1999) reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals. Plants have a longest history of use as a medicine, food source, and for a variety of daily needs. 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 centres 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 7000-7500 species are utilized in routine by traditional communities for their medicinal value. In India, drugs of herbal origin have been used by Ayurveda, Siddha, and Unani systems of medicines since ancient times. Siddha system of medicine is one of the oldest one from Dravidian culture. This system is mainly focused on food as medicine. Kayakarpam is also called as elixir science is unique and treasure of the siddha system. It is currently hypothesized that many diseases are due to oxidative stress that results from an imbalance between the formation and detoxification of prooxidants. Oxidative stress is initiated by reactive oxygen species (ROS), which are produced as a byproduct of electron transport in mitochondria. Prolonged oxidative stress can result in permanent damage to vital body organs, which may lead to chronic disorders such as heart diseases, diabetes, neurodegenerative diseases, cancer, and premature aging. It has been noted that about 95% of the pathologies observed in people above 35 years of age are associated with

production and accumulation of free radicals. In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases. Natural antioxidants such as α-tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer adverse reactions but their antioxidant activities are lower than the synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) which have been restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens. Therefore, there is a considerable interest in finding new and safe antioxidants from natural sources to replace these synthetic antioxidants. Recently, traditional plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids. Which prevent free radical damage, reducing risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular disease. In Siddha system of medicine many herbs are used in antioxidant medicinal formulations which are called as Kaya Kalpa medicines. These formulations have been used to treat the illness and help to regenerate the degenerative conditions and also help to prevent the ageing .In this BN is one of the Siddha antioxidant medicine which is widely used in practice for Poor memory, Convulsions, Nervous dibility, Delirium and Anxiety mentioned in Siddha Literature (Siddha Vaidhya Thitattu). Therefore, the objective of this study was to assess the free radical scavenging potential of the polyherbal Siddha formulation BN.

MATERIALS AND METHODS

Brahmi Nei (1): The test drug *Brahmi Nei* has been purchased from IMPCOPS Pharmacy, Chennai, Tamilnadu. It is a poly herbal Siddha preparation prepared from 13ingredients.

Table 1: Ingredients of *brahmi nei.*

S. no	Tamil Name	Botanical Name /English Name
1	*Brahmi*	*Bacopa Monneri*
2	*Vasambu*	*Acorus calamus*
3	*Sitraraththai*	*Alpinia galangal*
4	*Sivadhai veer*	*Operculina turpethum*
5	*Thippili*	*Piper longum*
6	*Vizhampala vidhai*	*Limonia acidissima*

7	*Chukku*	*Zingiber officinalae*
8	*Neer mulli*	*Emblica myrobalan*
9	*Kasthuri manjal*	*Curcuma aromatica*
10	*Indhuppu*	*Sodium chloride (Rock salt)*
11	*Seena karkandu*	*Sugar candy*
12	*Pasum paal*	*Cow's Milk*
13	*Nei*	*Cow's ghee*

Antioxidant activity

Dpph (2, 2-Diphenyl 1-2 Picrylhydrazyl) assay

The antioxidant activity of test drug sample BN was determined using the 2, 2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample BN extract was mixed with 95% methanol to prepare the stock solution in required concentration. From the stock solution 1ml, 2ml, 4ml, 6ml, 8ml and 10ml of this solution were taken in six test tubes and by serial dilution with concentration ranges from 10 µ g/ml, 20 µ g/ml, 40µ g/ml, 60 µ g/ml, 80 µ g/ml and 100 µ g/ml respectively. Ascorbic acid was used as standard was prepared in same concentration as that of the sample extract by using methanol as solvent. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample BN at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100µg/ml) was noted after 15 min incubation period at 370C. Absorbance wasread out using double-beam U. V, Spectrophotometer at 517 nm by using methanol as blank.

$$\% \text{ scavenging} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The effective concentration of test sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

Nitric oxide radical scavenging assay

The concentrations of test sample BN extract are made into serial dilution from 10−100µ g/mL and the standard gallic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% Naphthylethylene diaminedihydrochloride in 2.5% phosphoric acid immediately before use. A volume of

0.5 mL of10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the test drug (10−100 µ g/mL) and incubated at 25°C for 180 mins. The test drug BN was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the test drug but with an equal volume of buffer were prepared in a similar manner as done for the test samples. The absorbance was measured using a Spectra Max plus UV-Vis microplate reader at 546 nm (Molecular Devices, GA, and USA). Gallic acid was used as the positive control. The percentage inhibition of the test drug BN and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug BN and Gallic acid were calculated using the following formula:

percentage nitrite radical scavenging activity:

$$\text{nitric oxide scavenged } (\%) = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100,$$

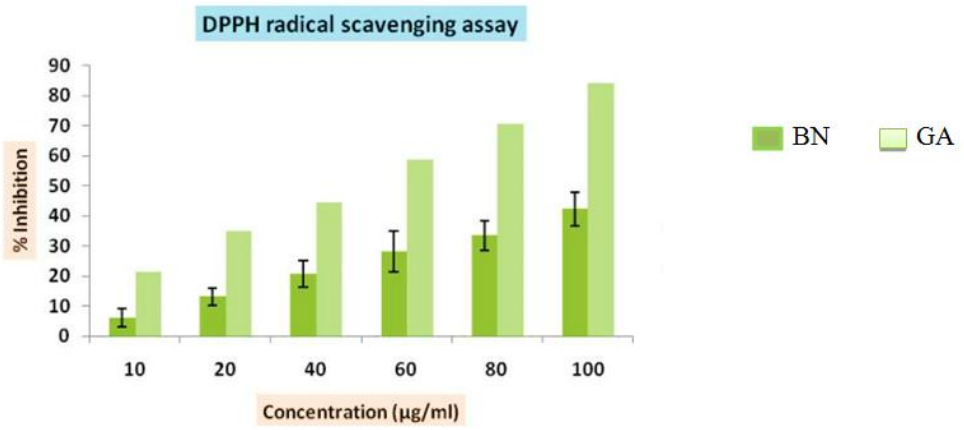
where A_{control} = absorbance of control sample and A_{test} = absorbance in the presence of the samples extracts or standards.

Abts assay

This assay carried out for the purpose of evaluating the anti-oxidant potential of BN extract against 2,2'- azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radicals. The ABTS radical cation method was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µ L of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1: 44, v/v). To determine the scavenging activity, 100 µ L ABTS reagent was mixed with 100 µ L of test sample (10- 100µ g/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured at 734 nm. 100% methanol was used as a control. Gallic acid with same concentrations of test drug BN was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample BN was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

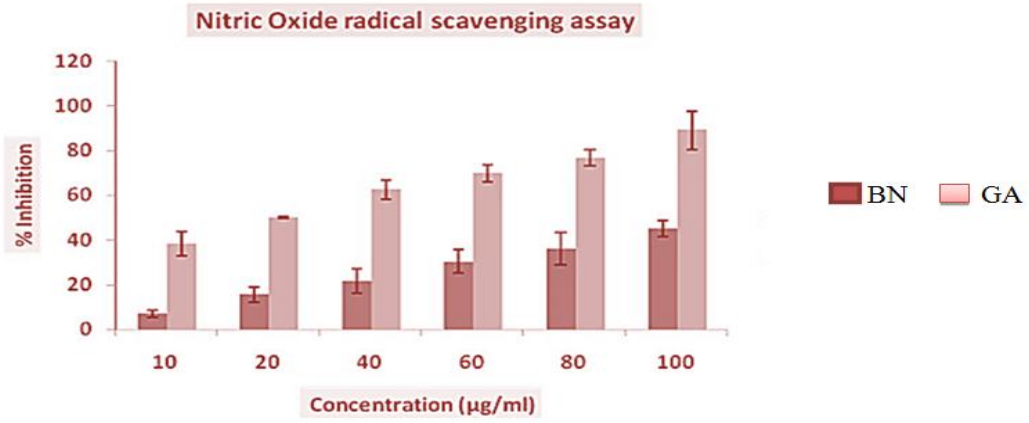
$$\text{Radical scavenging (\%)} = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100.$$

RESULTS AND DISCUSSION

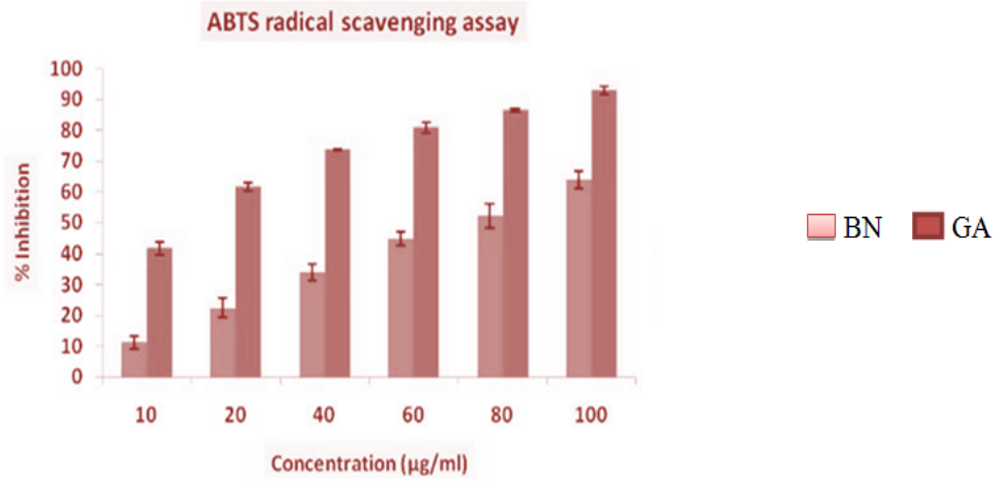


Nitric oxide (NO) is generated from amino acid Larginine by vascular endothelial cells, phagocytes, and certain cells of the brain. Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals. The toxicity of NO becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion (ONOO−)(2). The antioxidants from natural sources could be the alternative to synthetic antioxidants in counteracting oxidative stress associated diseases. A great number of naturally occurring substances have been recognized to have antioxidant abilities and various in vitro methods have been used to assess their free radical scavenging and antioxidant activity. In the present study free radical scavenging activities of BN was evaluated. Free radicals are involved in many disorders like neurodegenerative diseases, cancerand AIDS. Antioxidants duetotheirscavenging activity are useful for the management of those diseases. The reactivity of BN was analyzed with DPPH, a stable free radical. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is Spectrophotometrically related to the number of electrons gained.[21] The DPPH radical scavenging (%) activity is shown in the Fig: 1, BN exerted an inhibition of 42.19 % and that of Ascorbic Acid was 83.99 % at 100µ g/ml. Therefore, in the present study, BN at different concentrations were assessed for

their nitrite free radical scavenging activity in an in vitro model. The nitric oxide generated from sodium nitro prusside reacts with oxygen to form nitrite. The nitrite ions diazotize with sulphanilamide acid and couple with Naphthylethylenediamine, forming pink colour, which was measured at 546 nm.[22] As antioxidants donate protons to the nitrite radical, the absorbance is decreased. The decrease in absorbance was used to measure the extent of nitrite radical scavenging.[23] The Nitric Oxide radical scavenging (%) activity isshown in the Fig: 2, BN exerted an inhibition of45.16 % and that of Gallic Acid was 89.18 % at 100µ g/ml.



The ABTS scavenging assay, which employ a specific absorbance (734 nm) at a wavelength remote from the visible region and requires a short reaction time, can be used as an index that reflects the antioxidant activity of BN. In Fig. 3, BN extract was found to be effective in scavenging radicals and the increase was concentration dependent. At 100µ g/ml the inhibition of the extract was 63.79 % and that of Gallic acid 92.78%. This shows that BN presents a moderate ability to scavenge free radicals.



CONCLUSION

This study explores that the Siddha poly herbal preparation *Brahmi Nei* which possesses good antioxidant potential. Hence, further evaluation has to be carried out to isolate the specific bioactive compound.

REFERENCES

1. Formulary of Siddha medicines, published by the Indian Medical Practitioners' Cooperative Pharmacy and Stores Ltd., Adyar, Madras.
2. S. Karthik Nagarajan1*, M. Praveen2, N. J. Muthu Kumar3, V. Mahalakshmi4 and V. Banumathi5. In-Vitro Antioxidant Property of Siddha Polyherbal Formulation Thetran Ilagam, World Journal of Pharmacy and Pharmaceutical Sciences, Research Article, 7(9): 998-1006, ISSN 2278 – 4357.
3. D. M. Nagmoti, D. K. Khatri, P. R. Juvekar, andA. R. Juvekar, "Antioxidant activity and free radicalscavengingpotential of Pithecellobiumdulce Benth seed extracts," Free Radical and Antioxidants, 2011; 2(2): 37–43.
4. Y. Liu, G. Fiskum, D. Schubert, Generation of reactive oxygenspecies by the mitochondrial electron transport chain, J. Neu-rochem, 2002; 80: 780–787.
5. Agarwal, A. Aponte-Mellado, B. J. Premkumar, A. Shaman, and S. Gupta, "The effects of oxidative stressonfemale reproduction: a review," Reproductive Biology and Endocrinology, 2012; 10: 49.
6. P. Masoko and J. N. Eloff, "Screening of twentyfour South AfricanCombretum and six Terminalia species for antioxidant activities," African Journal of Traditional, Complementary and Alternative Medicines, 2007; 4(2): 231– 239,
7. J. E. Kinsella, E. Frankel, B. German, J. Kanner, Food Tech., 1993; 4: 85.
8. K. Imaida, S. Fukushima, T. Shirui, M. Ohtani, K. Nakanishi, N. Ito, Carcinogenesis, 1983; 4: 895.
9. D. L. Madhavi, D. K. Salunkhe, In D. L. Madhavi, S. S. Deshpande, D. K. Salunkhe, Editors, Food antioxidants, Dekker, New York, 1996; 239.
10. M. Hirose, Y. Takesada, H. Tanaka, S. Tamano,T. Kato, T. Shirai, Carcinogenesis, 1998; 19; 207.
11. G. Gazzani, A. Papetti, G. Masoolini, M. Daglia, Food Chem., 1998; 6: 4118.
12. M. Namiki, Crit. Rev. Food Sci. and Nutr, 1990; 20: 273.
13. T. Diplock, J. L. Charleux, G. Crozier-willi, F. J. Kok, C. Rice-Evan, M. Roberfroid, Brit. J. Nutr, 1998; 80S: S77.

14. F. B. Hu, Curr. Opin. Lipidol., 2000; 13: 3.
15. Juliet. L, Sivakkumar. S, Evaluation of Antioxidant Properties of Poly Herbal Siddha Preparation Karisalai Karpa Chooranam