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EVALUATION OF *IN-VITRO* FREE RADICAL SCAVENGING ACTIVITY OF *BETA VULGARIS* (BEET ROOT) EXTRACT

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ABSTRACT

Medicinal plants and natural products have attracted global attention due to their safety as well as their considerable antioxidant content that helps to prevent or ameliorate various disorders including memory impairments. The phenolic compound in fruits, vegetables, herbs and spices possess potent antioxidant, anti-inflammatory, antigenotoxic and anticancer activities. This study was conducted to investigate the effect of *Beta vulgaris* root extract using Fenton reaction. The dried root of *Beta vulgaris* was extracted with methanol using a Soxhlet extractor. The total phenolics content of leaf as determined by Fenton reaction and was found to be good antioxidant activity as different dose concentrations. The antioxidant activity of plant extract was carried out with ascorbic acid as a standard reducing agent. The present results were made with the use of UV-Visible Spectrophotometer. In this plant *Beta vulgaris* Extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. In conclusion the present study indicates that *Beta vulgaris* root extract may be a potential source of natural antioxidant.

KEYWORDS

Antioxidant activity, Fenton Reaction, Hydroxyl radical, ascorbic acid, *Beta vulgaris* and TBARS.

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INTRODUCTON

Nature is and will still serve as the man's primary source for the cure of his ailments. However, the potential of higher plants as sources for new drugs is still largely unexplored. It is worldwide accepted that antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias (Groff and Gropper, 2000)[1]. High rate of reactive oxygen

species and free radicals production due to oxidative stress can lead to peroxidation of biomolecules such as lipids, the result as proteins and damage to DNA that cause memory impairments (Sulakhiya *et al*, 2016)[2] Brain tissue is more sensitive to the effect of free radicals due to its high oxygen demands, abundant lipid content, and relative paucity of antioxidant enzymes compared to other organs (Rasoolijazi *et al*, 2015[3], Vincent *et al*, 2004)[4]. The use of medicinal plants with high levels of antioxidant components is one of the most efficient ways to minimize the adverse effects of free radicals and treat diseases. The synthetic drugs may be unsafe for human and the environment, medicinal plants and their derivatives are growingly accepted worldwide due to their safety (Sulakhiya *et al*, 2016[2], Zaidi *et al*, 2014)[5]. In this regard, it has been frequently shown that antioxidant agents reverse scopolamine-induced memory impairments (Hosseini *et al*, 2015[6], Yang *et al*, 2009)[7].

Beetroot (Beta vulgaris) is a plant from the family Chenopodiaceae. A beta vulgaris plant is native to Mediterranean regions and is extensively cultivated in different countries including Iran (Sulakhiya *et al*, 2016)[2]. Roots and leaves of B. vulgaris are used in traditional medicine, to treat different diseases. B. vulgaris leaves have diuretic, purgative, laxative, and aphrodisiac activity (Sulakhiya *et al*, 2016[2], Jain *et al*, 2011[8], El Gamal *et al*, 2014)[9]. Plants leaves contain various phytoconstituents such as betalains, flavonoids, polyphenols, vitamins, and minerals (Sulakhiya *et al*, 2016[2], Jain *et al*, 2012[10], Bolkent *et al*, 2007[11], Mroczek *et al*, 2012)[12]. B. vulgaris leaves have antioxidant, anticancer, hepatoprotective, nephroprotective, wound healing, and anti‑inflammatory activities (Sulakhiya *et al*, 2016[2], El Gamal *et al*, 2014[9], Babu *et al*, 2010[13], Jain *et al*, 2011[8], Sacan *et al*, 2010)[14], but its potential neuroprotective effects have not yet been tested. B. vulgaris leaves possess diuretic, purgative and anti-inflammatory activity, seeds known to possess expectorant and carminative properties, roots possess sedative and emenogogue effects (Kirtikar and Basu, 1975)[15]. It is also used as a natural food colour in dairy and meat products (Bias *et al*, 2000)[16]. Betalaine comprises of two main groups, the red violet betacyanin group and the yellow betaxanthine group. Active component of B. vulgaris as betalaine have contains about 50 red pigments and 20 yellow pigments. Betanine accounts for ca. 75-90 % of total betacyanin content and betaxanthnin comprises vulgaxanthine I and vulgaxanthine II (Bias *et al*, 2000)[16]. Therefore, we have planned out the antioxidant activity of the *Beta vulgaris* extract using Fenton reaction.

MATERIAL AND METHODS

Plant material

Beta vulgaris root was collected from Local Market, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples

The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532nm, UV Spectrometer made by Varian.

Preparation of extract

Dried powdered of *Beta vulgaris* root (15 g) were extracted by continuous mixing in 500ml 50% methanol and water, 24h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 0c temperature. The final extract was kept in air tied box.

Deoxyribose assay to assess OH -radical scavenging activity

The OH- radical scavenging activity of *Beta vulgaris* root extract (10–100ug/ml) was determined according to the deoxyribose method reported of (Halliwell *et al*, 1987)[17]. In the protocol the presence of 100lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100lM EDTA, 1 mM H2O2, 100lM L- ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38ºC, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbances were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The result of lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \text{Abs: } \frac{532 \text{ nm Control Abs.} - 532 \text{ nm sample Abs.} \times 100}{532 \text{ nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

RESULTS AND DISCUSSION

The results of the effects of the examined *Beta vulgaris* root extract as well as control solutions on OH- radical production. They show that all extract of *Beta vulgaris* root *extract* and control solutions as a ascorbic acid inhibited the production of OH-radicals. The % of free racial scavenging activity of hydro-methanolic extract of *Beta vulgaris* presented in Table No.1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

The Indian medicinal system remains the most ancient yet living traditional with sound philosophical and experimental basis. It is known to be complete medical system that comprised physical, physiological, philosophical, ethical, and spiritual health (Semwal *et al*, 2015)[18]. In countries beyond India, Ayurveda therapies and practices have been integrated in general wellness applications and in some cases in medical use (Populorum and Michael Alexander, 2008)[19].

The natural and medicinal plants are able to produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables (Suffredini *et al*, 2004)[20]. Many of plants metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. In the worldwide it is proved that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Narasinga Rao, 2003)[21].

The roots and leaves of the Beta vulgaris have been used in traditional medicine to treat a wide variety of ailments (Grubben and Denton, 2004)[22]. As per the daily dietetic point of view, the red beet is the most interesting beet variety because of its medicinal properties. It is known as a potent anticancerous food, a virtue that derives from its wealth in flavonoids, mainly because of the red pigment betaine.

Several parts of Beta vulgaris plant are used in medicinal system such as anti-oxidant, anti-depressant, anti-microbial, anti-fungal, anti-inflammatory, anticancerous, diuretic, expectorant and carminative. It is one of the natural food which boosts the energy in athletes as it has one of the highest nitrates and sugar contents plant. Beetroot has attracted much attention as a health promoting functional food (Wettasinghe *et al*, 2005)[23].

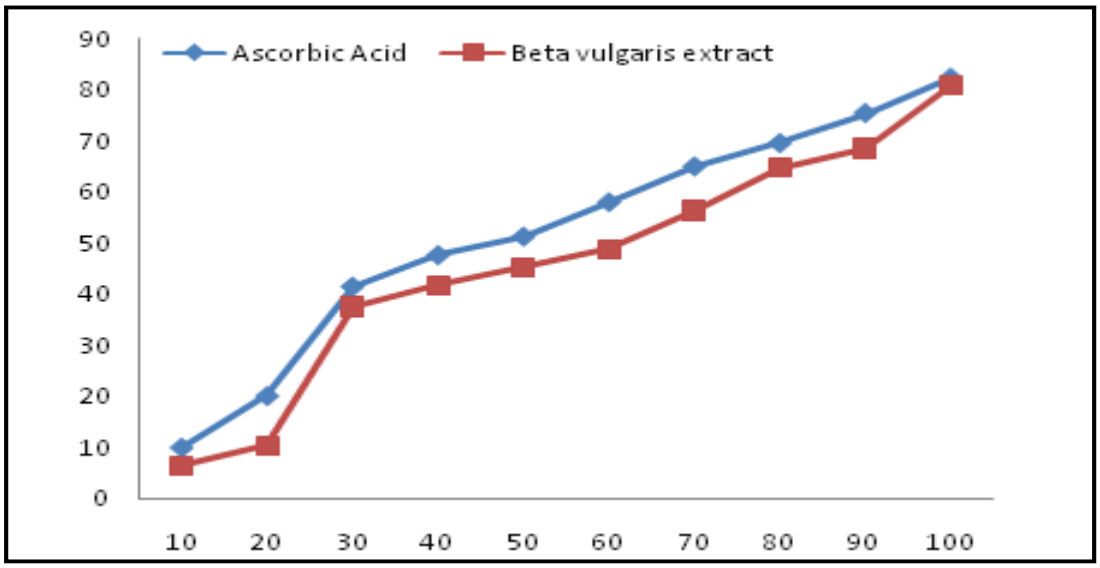
The results of the effects of the examined *Beta vulgaris* extract as well as control solutions on OH - radical production. They show that all extract of Beta vulgaris extract and control solutions as Ascorbic acid inhibited the production of OH-radicals. The Free radical scavenging activity of hydro-Ethanolic and Methanolic extract of Beta vulgaris presented reducing power, the free radical OH- scavenging activity of the extract increase with increasing the concentration. Extent of hydroxyl radical scavenged was determined the increasing in intensity of light pink coloured, which was determined at 532nm. The oxidant activity was compared with Ascorbic acid as a positive control. Beta vulgaris active compound like betacyanin and betaxanthin which act as free radical hunter and avoid free radical mediated oxidation of biological molecules. Plant extract showed strong antioxidant capacity *in vitro* and the extract can be considered a good source of natural antioxidant.

Table No.1: Antioxidant activities of *Beta vulgaris* root extract using Fenton reaction

S.No	Concentration (in µl)	Ascorbic acid (Mean + SE)	Beta vulgaris extract (Mean + SE)
1	10	10.12 ± 1.90	6.49 ± 2.17
2	20	20.20 ± 3.01	10.27 ± 0.96
3	30	41.57 ± 1.51	37.53 ± 1.33
4	40	47.72 ± 1.15	41.69 ± 0.99
5	50	51.33 ± 1.25	45.17 ± 1.37
6	60	58.03 ± 1.00	48.68 ± 1.02
7	70	65.03 ± 0.91	56.24 ± 0.73
8	80	69.59 ± 1.01	64.51 ± 0.65
9	90	75.44. ± 1.41	68.38 ± 0.34
10	100	82.34 ± 0.87	80.55 ± 0.33

Table No.2: Inhibitory concentration 50% values (IC_{50}) of Ascorbic acid and Beta *vulgaris* root extract using Fenton reaction

S.No	Group	IC_{50} Value
1	Ascorbic acid	46.0 (µg/ml)
2	Beta vulgaris	62.0 (µg/ml)



Graph No.1: Antioxidant activities of Ascorbic acid and Beta *vulgaris* root extract using Fenton reaction

CONCLUSION

Plants are responsible for preventing disease and promoting health have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Gibson *et al*, 1998[24], Mathai, 2000)[25]. Many of advance research clearly proved that they have roles in the protection of human health, when their dietary intake is significant. Antioxidant molecule inhibits the oxidation of other molecules from the body. Natural and artificial oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiol or ascorbic acid (vitamin C) terminate these chain reactions. The natural and synthetic antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as Cancer or coronary heart disease. The hypothesis that antioxidant supplements might

promote health has not been confirmed experimentally (Abner *et al*, 2011[26], Bjelakovic *et al*, 2013)[27]. Beta vulgaris the traditional uses, the plant is reported for a number of pharmacological activities such as anti-oxidant, anti-depressant, anti-microbial, anti-fungal, anti-inflammatory, anticancerous, diuretic, expectorant and carminative. It is one of the natural food which boosts the energy in athletes as it has one of the highest nitrates and sugar contents plant. Beetroot has attracted much attention as a health promoting functional food (Wettasinghe *et al*, 2005)[23]. In the present study, the phenolic content of Beta vulgaris root was found to be high which might have responsible for its antioxidant and free radical scavenging activity in the in vitro study models. *B. vulgaris* root extract can be used as a beneficial medicinal herb for reducing the free radicals present in body due to high levels of polyphenolic antioxidant compounds.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Groff J and Gropper S. Advanced Nutrition and Human Metabolism, *Belmont: Wadsworth,* 3rd Edition, 2000, 225-227.
2. Sulakhiya K, Patel V K, Saxena R, Dashore J, Srivastava A K, Rathore M. Effect of Beta vulgaris Linn leaves extract on anxiety-and depressive-like behavior and oxidative stress in mice after acute restraint stress, *Pharm Res,* 8(1), 2016, 1-7.
3. Rasoolijazi H, Mehdizadeh M, Soleimani M, Nikbakhte F, Farsani M E, Ababzadeh S. The effect of rosemary extract on spatial memory, learning and antioxidant enzymes activities in the hippocampus of middle-aged rats, *Med J Islam Repub Iran,* 29, 2015, 187-193.
4. Vincent A M, Russell J W, Low P, Feldman E L. Oxidative stress in the pathogenesis of diabetic neuropathy, *Endoc Rev*, 25(4), 2004, 612-28.
5. Zaidi S K, Hoda M N, Tabrez S, Ansari S A, Jafri M A, Shahnawaz Khan M. Protective effect of Solanum nigrum leaves extract on immobilization stress induced changes in rat's brain, *J Evid Based Complementary Altern Med*, Article ID 912450, 2014, 7.
6. Hosseini M, Mohammadpour T, Karami R, Rajaei Z, Sadeghnia H R, Soukhtanloo M. Effects of the hydro-alcoholic extract of Nigella Sativa on scopolamine-induced spatial memory impairment in rats and its possible mechanism, *Chinese J Integ Med*, 21(6), 2015, 438-444.
7. Yang M H, Yoon K D, Chin Y W, Park J H, Kim S H, Kim Y C, *et al*. Neuroprotective effects of Dioscorea opposita on scopolamine-induced memory impairment in *in vivo* behavioral tests and *in vitro* assays, *J Ethnopharm*, 121(1), 2009, 130-134.
8. Jain S, Garg V K, Sharma P K. Anti-inflammatory activity of aqueous extract of Beta vulgaris L, *J Basic Clin Pharm*, 2(2), 2011, 83-89.
9. El Gamal A A, AlSaid M S, Raish M, Al-Sohaibani M, Al-Massarani S M, Ahmad A. Beetroot (Beta vulgaris L) extract ameliorates gentamicin-induced nephrotoxicity associated oxidative stress, inflammation, and apoptosis in rodent model, *Mediators Inflamm*, Article ID 983952, 2014, 12.
10. Jain N K, Singhai A K. Protective role of Beta vulgaris L leaves extract and fractions on ethanol-mediated hepatic toxicity, *Acta Pol Pharm*, 69(5), 2012, 945-950.
11. Bolkent Ş, Yanardağ R, Tabakoğlu-Oğuz A, Özsoy-Saçan Ö. Effects of chard (Beta vulgaris L var cicla) extract on pancreatic B cells in streptozotocin-diabetic rats: a morphological and biochemical study, *J Ethnopharmacol*, 73(1-2), 2007, 251-259.
12. Mroczek A, Kapusta I, Janda B, Janiszowska W. Triterpene saponin content

in the roots of red beet (Beta vulgaris L) cultivars, *J Agric Food Chem*, 60(50), 2012, 12397-12402.

13. Babu V A, Gowri R. Evaluation of antioxidant activity of Beta vulgaris root extract in rats, *Asian J Chem*, 22(5), 2010, 3385-3390.
14. Sacan O, Yanardag R. Antioxidant and antiacetylcholinesterase activities of chard (Beta vulgaris L var cicla), *Food Chem Toxicol,* 48(5), 2010, 1275-1280.
15. Kirtikar K R and Basu B D. Indian Medicinal Plants, *Periodical Experts,* Delhi, India, 3, 1975, 2310-2311.
16. Bias H P, Madhusudhan R, Bhagyalakshmi N, Rajashekaran T, Ramesh B S and Ravishankar G A. Influence of polyamines on growth and formation of secondary metabolites in hairy root cultures of *Beta vulgaris* and *Tagetes patula*, *Acta Physiol. Plantarum,* 22(2), 2000, 151-158.
17. Halliwell B, Gutteridge J M, Aruoma O I. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals, *Anal Biochem*, 165(1), 1987, 215-219.
18. Semwal D K, Mishra S P, Chauhan A, Semwal R B. Adverse health effects of tobacco and role of Ayurveda in their reduction, *J Med Sci*, 15(3), 2015, 139-146.
19. Populorum, Michael Alexander. Trendsund Beschäftigungs felderim Gesundheits- und Wellness-Tourismus: Berufsentwick lung, Kompetenz profile und Qualifizierungsbedarf in wellness-bezogenen Freizeitund Gesundheitsberufen (in German), *LIT Verlag Münster,* 2008.
20. Suffredini I B, Sader H S, Gonçalves A G, Reis A O, Gales A C, Varella A D, Younes R N. Screening of antibacterial extracts from plants native to the brazilian amazon rain forest and atlantic forest, Braz. J. Med. Biol. Res, 37(3), 2004, 379-384.
21. Narasinga Rao. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention, *Asia Pacific Journal of Clinical Nutrition*, 12(1), 2003, 9-22.
22. Grubben G J H and Denton O A. Plant Resources of Tropical Africa 2. Vegetables, *PROTA Foundation, Wageningen; Backhuys, Leiden; CTA, Wageningen*, 2004, 668.
23. Wettasinghe M, Lee C H, Bolling W B, Ji L L and parkin Kirk L. Betaine, Phase 2 Enzyme – Inducing components from Red Beetroot (Beta vulgaris L.) Extracts, *Nutrition and Cancer,* 53(1), 2005, 91-103.
24. Gibson E L, Wardel J, Watts C J. Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children, *Appetite*, 31(2), 1998, 205-228.
25. Mathai K. Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, ed. L.K. Mahan and S. Escott-Stump, 10th Edition, 271, 2000, 274-275.
26. Abner E L, Schmitt F A, Mendiondo M S, Marcum J L, Kryscio R J. Vitamin E and all-cause mortality: a meta-analysis, *Current Aging Science,* 4(2), 2011, 158-170.
27. Bjelakovic G, Nikolova D, Gluud C. Meta-regression analyses, meta-analyses, and trial sequential analyses of the effects of supplementation with beta-carotene, vitamin A, and vitamin E singly or in different combinations on all-cause mortality: do we have evidence for lack of harm, *PLoSONE,* 8(9), 2013, e74558.

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