International Journal of Nutrition and Metabolism

Volume 4 Number 3 March 2012
ISSN 2141-2340

Academic Journals

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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International Journal of Nutrition and Metabolism

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International Journal of Nutrition and Metabolism Vol. 4(3), pp. 45-50, March 2012
Available online at http://www.academicjournals.org/IJNAM
DOI: 10.5897/IJNAM11.043
ISSN 2141-2499 ©2012 Academic Journals

Review

Need of education and awareness towards zinc supplementation: A review

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Accepted 25 November, 2011

Zinc is an essential trace element and thus zinc deficiency may severely affect human health. Zinc supplementation is commonly used to prevent and treat human diseases due to zinc deficiency. Many studies published proved zinc supplementation as a boon for preventing and treating diseases related with zinc deficiency whereas in some cases adverse affects of excess zinc supplementation have also been reported, which clearly points out to the need of health education and programmes before zinc supplementation. This review highlights the need of health education and awareness programmes for effective zinc supplementation.

Key words: Zinc deficiency, zinc supplementation, health education, awareness.

INTRODUCTION

Zinc is an essential trace element for humans, animals and plants (Shah and Sachdev, 2001). It is involved in numerous aspects of cellular metabolism and is required for the catalytic activity of more than 100 enzymes (Sandstead, 1994; Institute of medicine, 2001) it plays a role in immune function (Shah, 2001; Prasad et al., 1997) protein synthesis (Prasad, 1997), wound healing (Lansdown et al., 2007), DNA synthesis (Institute of Medicine, 2001) and cell division (Prasad, 1995). Zinc also supports normal growth and development during pregnancy, childhood, and adolescence (Simmer and Thompson, 1985) and is required for proper sense of taste and smell (Heyneman, 1996).

Intake recommendations for zinc are provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies (formerly National Academy of Sciences).

The current RDAs for zinc are listed in Table 1 (Institute of Medicine, 2001). For infants aged 0 to 6 months, the FNB established an AI for zinc that is equivalent to the mean intake of zinc in healthy, breastfed infants.

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Zinc is primarily obtained from food. The major sources of zinc are (red) meat, poultry, fish and seafood, whole cereals and dairy products. Zinc is most available to the body from meat. The bioavailability of plant-based foods is generally lower due to dietary fiber and phytic acid which inhibit the absorption of zinc (Institute of Medicine, 2001).

Zinc is a component of more than 300 enzymes from all six classes. Zinc is important for the catalytic activity of carbonic anhydrase which in turn is a constituent of red blood cells and gastric juices and plays an important role in deposition of calcium salts in teeth and bones. Similarly it is also an important constituent of enzyme carboxy peptidase A, a pancreatic enzyme active in protein degradation. The enzyme alcohol dehydrogenase contains zinc as is essential for the conversion of alcohol to an aldehyde, thereby facilitating alcohol metabolism in the liver. Zinc is also a constituent of lactic acid dehydrogenase which is active in glycolysis, alkaline phosphatase active in maintaining phosphate levels near bone and glutamic dehydrogenase found in platelets. It is also essential for the proper activity of the RNA synthesizing enzyme RNA polymerase. Zinc is found in alpha-macroglobulin, an important protein in the body's immune system. This globulin firmly binds about 30% of plasma albumin, which functions primarily as a transport

protein. Thus zinc plays an important role in biological functioning of body and its deficiency affects human health a lot (Zinc-mineral, 2004)

Deficiency of zinc leads to a retardation of growth and development of growth and development in children, retarded genital development and hypogonadism, dermatitis and delayed wound healing, alopecia, poor pregnancy outcomes and teratology, and decreased immune function with a resulting increased susceptibility to infections (Maret and Sandstead, 2006). Zinc deficiency places children in many low income countries at an increased risk of illness and deaths from infectious diseases. Mild to moderate zinc deficiency may be common in developing world but the public health importance of this degree of zinc deficiency is not well defined as yet more than 400,000 children die each year due to zinc deficiency (Shah and Sachdev, 2001). Current estimates of the risk of zinc deficiency indicate that approximately one third of the world's population live in countries where the risk of zinc deficiency is high (WHO Report, 2007). Due to wide prevalence of zinc deficiency and the multitude of zinc's essential biological functions nutritional correction of zinc deficiency may have a significant impact on different aspects of human health. Following this rationale, over the years several hundred zinc supplementation studies have conducted, investigating the effects of nutritional zinc supple-mentation on different diseases, often with contradictory results, which points out the need for health education and awareness for community members before such zinc supplementation programmes.

This review aims to summarize various zinc supplementation studies mainly for immune function disorders in children, elderly and adults, and to illustrate the need for health education and awareness programmes for community members to gain effective results of zinc supplementation.

Zinc supplementation for disease prevention

Immune function

Severe zinc deficiency depresses immune function (Prasad, 1998), and even mild to moderate degrees of zinc deficiency can impair macrophage and neutrophil functions, natural killer cell activity, and complement activity (Rink and Gabriel, 2000). The body requires zinc to develop and activate T-lymphocytes (Sandstead, 1994; Beck et al., 1997). The immunological consequences of zinc deficiency may be responsible for decreased cell mediated immune functions and inflammatory reactions in zinc deficient subjects. Zinc influence immunity, tissue regeneration and promote protein synthesis. The effect of zinc deficiency on the immune response was studied in an experimental model of human recently (Prasad, 2000). Zinc deficiency causes imbalance between TH_1 and TH_2 functions and the production of INFg, IL-2 and TNFa (products of TH cells) are decreased (Prasad, 2000; 1998). Zinc supplementation increases IL-2 and INFg production. As a result of zinc deficiency, the ratio of $CD4^+$ $CD45RA^+$ to $CD4^+CD45RO^+$ was decreased suggesting that zinc may be required for the new $CD4^+$ T cells. Zinc deficiency caused decreased serum thymulin activity, which could be restored by zinc supplementation (Prasad, 1998). Zinc deficiency also decreased the percentage of $CD8^+$ $CD73^+$ T cells those are the precursor cells of cytotoxic T cells. IL-1b is involved in the zinc deficiency induced mucosal damage. Intestinal cell proliferation was also reduced by zinc deficiency.

The adverse effects of zinc deficiency on the immune system function are likely to increase the susceptibility of children to infectious diarrhea; persistent diarrhea contributes to zinc deficiency and malnutrition.

In children

Diarrhea

The adverse effects of zinc deficiency on immune system function are likely to increase the susceptibility of children to infectious diarrhea; persistent diarrhea contributes to zinc deficiency and malnutrition.

There is strong evidence to support role of zinc supplementation in diarrhea morbidity and mortality reduction. A study from India identified a 68% reduction in mortality in small-for-gestational-age term infants that were supplemented with zinc from 1 to 9 months of age (Bhutta et al., 1999). In addition, results from a pooled analysis of randomized controlled trials of zinc supplementation in developing countries suggest that zinc helps reduce the duration and severity of diarrhea in zinc-deficient or otherwise malnourished children (Black, 1998). Similar findings were reported in a meta-analysis published in 2008 and a 2007 review of zinc supplementation for preventing and treating diarrhea (Fisher Walker and Black, 2007; Lukacik et al., 2008). The effects of zinc supplementation on diarrhea in children with adequate zinc status, such as most children in the United States, are not clear. Studies show that poor, malnourished children in India, Africa, South America, and Southeast Asia experience shorter courses of infectious diarrhea after taking zinc supplements (Black, 2003). The children in these studies received 4–40 mg of zinc a day in the form of zinc acetate, zinc gluconate, or zinc sulfate (Black, 2003). The World Health Organization and UNICEF now recommend short-term zinc supplementation (20 mg of zinc per day, or 10 mg for infants under 6 months, for 10–14 days) to treat acute childhood diarrhea (WHO Report, 2004).

Wound healing

Zinc helps to maintain the integrity of skin and mucosa

Table 1. Recommended dietary allowances (RDAs) for Zinc (Institute of Medicine, 2001).

Age	Male	Female	Pregnancy	Lactation
0–6 months	2 mg*	2 mg*		
7–12 months	3 mg	3 mg		
1–3 years	3 mg	3 mg		
4–8 years	5 mg	5 mg		
9–13 years	8 mg	8 mg		
14–18 years	11 mg	9 mg	12 mg	13 mg
19+ years	11 mg	8 mg	11 mg	12 mg

*Adequate Intake (AI).

membranes (Anderson, 1995). Patients with chronic leg ulcers have abnormal zinc metabolism and low serum zinc levels (Wilkinson and Hawke, 1998), and clinicians frequently treat skin ulcers with zinc supplements (Lansdown et al., 2007). The authors of a systematic review concluded that zinc sulfate might be effective for treating leg ulcers in some patients who have low serum zinc levels (Wilkinson and Hawke, 1998, 2000).

The common cold

One disease for which the use of zinc has been extensively investigated is the common cold, and the results have already been summarized in detail elsewhere (Hulisz, 2004). These results are contradictory to some extent and design and sample size of several studies has been criticized.

Overall, it can be concluded that zinc is effective in shortening the duration of the common cold, but only if it is administered no later than 24 h within the onset of the symptoms (Hulisz, 2004). The mechanism by which zinc acts against the common cold is still not completely understood. It has been found that zinc inhibits the rhinovirus 3C protease, and hereby viral replication, but this effect was only observed in vitro and not in vivo (Turner, 2001). Also discussed is an interference of zinc with the binding of the rhinovirus to its cellular receptor, the adhesion molecule ICAM-1, or an interaction of zinc with host immune function (Hulisz, 2004).

Pneumonia

Zinc supplementation may also reduce the incidence of lower respiratory infections, such as inflammation of the lungs (‘pneumonia’). A growing body of research highlights the importance of zinc to child survival and to specifically reducing deaths from pneumonia. Zinc intake helps reduce the incidence of pneumonia and the severity of the disease. Specifically, research has shown that zinc intake during the acute phase of severe pneumonia decreased the duration and severity of pneumonia and reduced treatment failure rates when compared with a placebo intervention (Unicef/WHO, 2006).

A pooled analysis of a number of studies in developing countries demonstrated a substantial reduction in the total number of cases of pneumonia in children supplemented with zinc (Bhutta et al., 1999) A meta-analysis found that zinc supplementation reduced the incidence but not duration of pneumonia or respiratory tract illnesses in children less than five years of age (Aggarwal et al., 2007).

Malaria

Some studies have indicated that zinc supplementation may reduce the incidence of clinical attacks of malaria in children (Black, 2003). A randomized controlled trial in preschool-aged children in Papua New Guinea found that zinc supplementation reduced the frequency of health center attendance due to malaria by 38% (Shankar, 2000). Additionally, the number of malaria episodes accompanied by high blood levels of the malaria-causing parasite was reduced by 68%, suggesting that zinc supplementation may be of benefit in preventing more severe episodes of malaria.

However, a 6-month trial in more than 700 West African children did not find the frequency or severity of malaria episodes (Muller et al., 2001). Additionally, a randomized controlled trial in over 42,000 children aged one to 48 months found that zinc supplementation did not significantly reduce mortality associated with malaria and other infections (Sazawal et al., 2007).

Due to conflicting reports, it is not yet clear whether zinc supplementation can be used in treating childhood malaria.

In elderly and adults

Age-related declines in immune function have been associated with the vulnerability of the elderly to mild zinc deficiency. However, the results of zinc supplementation

trials on immune function in the elderly have been mixed.

In randomized controlled trials, certain aspects of immune function (e.g., increased levels of immune cells) in men and women over 65 years of age have been found to improve with zinc supplementation (Salgueiro et al., 1998; Fortes et al., 1998).

However, other studies have reported that zinc supplementation does not improve parameters of immune function, indicating that more research is required before any recommendations can be made regarding zinc and immune system response in the elderly.

Pregnancy complications

Poor maternal zinc nutritional status has been associated with a number of adverse outcomes of pregnancy, including low birth weight, premature delivery, labor and delivery complications, and anomalies in developing fetuses (Prasad, 1979). Association of maternal zinc deficiency with adverse pregnancy outcome is still an unresolved issue (Goldengerg et al., 1995).

Observational studies in human populations have produced strong associations between a poor maternal zinc status and various indicators of a poor pregnancy outcome but supplementation trials have not produced strong or even consistent results (Caulfield et al., 1998). Antenatal zinc supplementation did not improve birth outcome in Bangladeshi urban poor. Positive results were observed only in subgroups of the pregnant population in some studies (Goldenberg et al., 1995).

A review of 17 randomized controlled trials found that zinc supplementation during pregnancy was associated with a 14% reduction in premature deliveries; the lower incidence of preterm births was observed mainly in low-income women (Mahomed et al., 2007).

HIV/AIDS

Zinc is of particular importance for the development of T cells (Fraker and King, 2004; Wellinghausen et al., 1997). Hence, it seems reasonable to use it as a supporting therapeutic intervention for patients with HIV/AIDS. Studies show that short term supplementation of a relatively small group of five patients led to an improvement of immune function, with an increase in the number of activated (HLA-DR positive) T cells, augmented lymphocyte transformation by phytohaemagglutinin and concanavalin A, and increased phagocytosis by polymorphonuclear neutrophils (Zazzo et al., 1989). This was supported by another study which described an increase in the number of T helper cells and a protective effect against infections with Pneumocystis carinii and Candida (Mocchegianie et al., 1995). It has been shown that zinc deficiency is prevalent among HIV infected persons, especially in malnourished patients or users of illicit drugs (Baum et al., 2000, 2003). However, it can not be generalized that patients with AIDS are zinc deficient, since antiretroviral therapy can normalize the zinc status (Rousseau et al., 2000). A recent study has addressed the safety of zinc supplementation, using a moderate dose of 10 mg elemental zinc per day and the authors came to the conclusion that zinc supplementation has no adverse effects (Bobat et al., 2005). However, it was performed in HIV-infected South African children, a population with high prevalence of malnutrition and limited access to medication. Although the zinc status of the children has not been determined, it can be assumed that many of them were zinc deficient (Bobat et al., 2005; Green et al., 2006). Moderate supplementation to zinc-deficient patients can help stabilize their immune system; supplementation to zinc-sufficient ones may accelerate disease progression and increase mortality.

Health risks and zinc supplementations

Zinc supplementation at physiological doses is considered to be safe, although there are potential side-effects that need to be considered. The FNB has established Upper Intake Levels (UL) for zinc (Table 2). Long-term intakes above the UL increase the risk of adverse health effects (Institute of Medicine 2001).

Moderate doses of zinc supplements can give a metallic flavour and induce nausea and vomiting. These symptoms, however, have not been reported as significant side-effects in clinical trials that used short-term supplementation for the prevention or treatment of acute diarrhea or respiratory infections. Large oral doses of zinc can interfere with copper bio-availability as they compete for absorption, and clinical signs of immune dysfunction have been reported with daily doses in excess of 150 mg. In addition, a small, randomized clinical trial of 141 severely malnourished children in Bangladesh reported that children receiving 6 mg/kg of zinc for 15 days had a higher mortality than children receiving lower doses. In addition, in poorly ventilated mining industries and during galvanization of iron, welding and manufacture of brass, zinc in the air can reach toxic levels, posing a significant health risk to workers chronically exposed. Finally, a recent large study in the USA reported that men who consumed 100 mg/day had an increased risk of advanced prostate cancer. These findings were observed only in patients receiving high-dose supplements and chronic zinc deficiency has also been associated with an increased risk of prostate cancer. Elderly patients in the United States are currently recommended to consume moderate amounts of zinc as a preventive measure against age-related macular degeneration and prostate cancer. It is therefore prudent to recommend that further studies should use zinc supplementation at low to moderate doses and within

Table 2. Tolerable upper intake levels (ULs) for Zinc (Institute of Medicine, 2001).

Age	Male	Female	Pregnant	Lactating
0-6 months	4 mg	4 mg		
7-12 months	5 mg	5 mg		
1-3 years	7 mg	7 mg		
4-8 years	12 mg	12 mg		
9-13 years	23 mg	23 mg		
14-18 years	34 mg	34 mg	34 mg	34 mg
19+ years	40 mg	40 mg	40 mg	40 mg

physiological ranges (Luis et al., 2005). Two nutritional studies showed that increased intake of zinc in HIV-1 infected patients led to an augmented risk for the progression to AIDS (Tang et al., 1993) and lower survival (Tang et al., 1996). In the quartile of patients with the highest total daily zinc intake (>20 mg/day) combined from food and supplements, the risk for progression to AIDS and poorer survival was doubled compared to the quartile with the lowest intake of zinc (<11.6 mg/day) (Tang et al., 1993, 1996).

Health education and zinc supplementation

Health Education is to impart basic knowledge to people aware of all the aspects of keeping good health by avoiding diseases. Health Education is necessary for ensuring a good personal health as well as community health. Due to the lack of awareness several people have lost there lives in Nepal about, 15,000 children die from diarrhea, just because they do not have zinc to treat it, “According to health workers on the ground, factors hindering zinc coverage include inadequate supply of zinc tablets; weak logistical management; low awareness regarding zinc and its availability within the community; and inadequate understanding of the treatment among health service providers.” UNICEF is currently working to conduct a strategic review of Nepal’s zinc program and to increase public awareness of how this critical mineral can save lives (Nepal-Zinc Supplements, 2001).

The various elements of health education are knowledge of various nutrients present in various food materials, of making balanced diet from foods available, of the causes of various common diseases, of how various diseases spread, of the prevention measures for various diseases, of vaccines available for immunizing children, of the causes of environmental pollution, and of methods to protect environment from pollution.

Before imparting zinc supplementation programme to community members it is necessary to make them aware of various aspects of zinc, its recommended values as well as its dietary sources etc. through health education programmes. So that the community after gaining knowledge about this vital nutrient, may become attitudinal to its balanced consumption, and remain healthy.

Conclusions

1. Zinc supplementation has the potential to improve child survival.
2. Research to map out prevalence of zinc deficiency should be encouraged further.
3. Education programmes should be promoted before zinc supplementation programs at community levels for effective results.
4. People should be educated through media towards zinc deficiency and other micronutrient deficiencies and their preventive measures by appropriate dietary intake.

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International Journal of Nutrition and Metabolism Vol. 4(3), pp. 51-56, March 2012
Available online at http://www.academicjournals.org/IJNAM
DOI: 10.5897/IJNAM11.060
ISSN 2141-2499 ©2012 Academic Journals

Full Length Research Paper

Antioxidant activity of some wild edible plants of Meghalaya state of India: A comparison using two solvent extraction systems

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Accepted 30 December, 2011

The objective of the present study was to find out the antioxidant potential of some wild edible plants, traditionally used by the local people of Meghalaya state in India and also to investigate the effect of solvent extraction system (aq. methanol and acetone) on the total phenolic, flavonoids and flavonols content, reducing power and antioxidant activity of the plants. The total phenol content varied from 3.31±0.10 to 27.67±0.16 mg/g in the aqueous methanol extract and 2.61±0.13 to 6.85±0.13 mg/g in the acetone extract of the plants. Flavonoids content were between 8.11±0.071 and 52.14±0.004 mg/g in aqueous methanol extract and varied from 1.22± 0.01 to 52.17± 0.01 mg/g in the acetone extract. 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect of the extracts were determined spectrophotometrically. The highest radical scavenging was observed in the aq. methanol extract of *Gentiana pedicellata* with IC_{50} = 0.23±0.0007 mg dry material. The greater amount of phenolic compounds, flavonoids and flavonol content leads to more potent radical scavenging effect as shown by the aq. methanol extract of *G. pedicellata*. Flavonol content was observed highest in the aq. methanol extract of *G. pedicellata* (23.12 ±0.006 mg/g) and least in the acetone extract of *Gynocardia odorata* (0.09±0.008 mg/g). The reducing power of the extracts of the plants were also evaluated as mg AAE (ascorbic acid equivalent)/g dry material and highest reducing power (16.11 ± 0.03) observed in the aq. methanol extract of *Bauhinia purpurea*, which contain maximum amount of phenolic compounds (27.67±0.16 mg/g GAE). The results indicate that the type of extragent significantly influenced the antioxidant activity of these wild edible plants and could be utilized as potential source of natural antioxidant in the food or in pharmaceutical industry.

Key words: Wild edible plants, Meghalaya, phenolic, antioxidant activity, two different solvent extraction system.

INTRODUCTION

The main characteristic of an antioxidant is to inhibit the oxidation of lipids or other molecules and hence provides a protective effect against ROS (Reactive oxygen species) such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) and free radicals, such as the hydroxyl radical (·OH) and superoxide anion (O_2^-) (Ghimire et al., 2011). Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals and thus inhibit the oxidative mechanisms which are responsible for many disorders and diseases in humans such as infections, diabetes, arthritis, cardiovascular diseases, cancer, Alzheimer's diseases, AIDS etc. (Patel et al., 2010).

Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, barks, roots and crude plant drugs. Fruits and vegetables have long been viewed as a rich source of natural antioxidant compounds. Natural antioxidants are used to improve food quality and stability and also act as nutraceuticals to terminate free radical

chain reaction in biological systems and thus may provide additional health benefits to consumers (Nahak and Sahu, 2010).

The use of synthetic antioxdants like butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT) has been limited due to their toxicity and side effects and therefore search for the novel sources of natural oxidants is important (Pourmorad et al., 2006).

Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity (Uddin et al., 2008). It has been observed that the antioxidant activity of plant materials are strongly dependent on the nature of the different solvent extraction system due to the presence of different antioxidant components of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. Water, methanol, mixture of water-methanol, acetone have been widely used to extract antioxidant compounds from various plants and plant-based foods (fruits, vegetables etc.) (Sultana et al., 2009).

Though many other plant species have been investigated in the search for novel antioxidants but generally there is still a demand to collect more information regarding the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, much attention has been given towards the identification of plants with antioxidant ability.

The forests of Meghalaya (Northeastern region in India) provide a large number of plants whose leaves, fruits, seeds, tubers, shoots etc make an important contribution to the diet of the local people. These plants also provide some useful products like medicine, fibre, fodder, dyes etc (Kayang, 2007).

The present study was undertaken to evaluate the antioxidant potential of some wild edible plants, collected from different places of Meghalaya state, India. These plants are used by the tribals of Meghalaya for their day-to -day needs. The main target of our research was to examine the total phenolic content, flavonoid content, flavonol content and radical scavenging capacity related to antioxidant potential and reducing power of these nine wild edible plants. The objective of the present study was also to investigate the most effective solvent extraction system to extract potent antioxidant compounds from different wild edible plants which will guide us to obtain the best sources of dietary antioxidants.

MATERIALS AND METHODS

Plant materials

The nine plant materials e.g the leaves of *Bauhinia purpurea*, *Diplazium esculentum*, *Fagopyrum cymosum*, *Ficus clavata*, *Ficus geniculata*, *Ficus pomifera* , *Gentiana pedicellata*, flower of *Dillenia pentagyna* and seeds of *Gynocardia odorata* were collected from different tribal market of Meghalaya state, India on March 2010 and authenticated in our office. The voucher specimens were preserved in the Plant Chemistry department of our office under registry no BSITS 15, BSITS 16, BSITS 17, BSITS 20, BSITS 21, BSITS 22, BSITS 23, BSITS 24 and BSITS 25 respectively. The plant parts were shed-dried, pulverized and stored in an airtight container for further extraction.

Extraction of plant material (aqueous methanol and acetone extract)

One gram of each plant material were extracted with 20 ml each of aqueous methanol (20%, v/v) and acetone, with agitation for 18 - 24 h at ambient temperature. The extracts were filtered and diluted to 50 ml and aliquot were analyzed for their total phenolic, flavonoid and flavonol content, reducing power and their free radical scavenging capacity.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), ascorbic acid, quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA)., Folin-Ciocalteus's phenol reagent, gallic acid, potassium ferricyanide, Aluminium chloride, $FeCl_3$ and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

Estimation of total phenolics

The amount of total phenolic content of crude extracts was determined according to Folin-Ciocalteu procedure (Singleton and Rossi, 1965). 20 - 100 µl of the tested samples were introduced into test tubes; 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-visible spectrophotometer Hitachi U 2000 Japan). The total phenolic content was expressed as gallic acid equivalents (GAE) in miligram per gram (mg/g) of extract.

Estimation of total flavonoids

Total flavonoids were estimated using the method of Ordonez et al. (2006). To 0.5 ml of sample, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After one hour, at room temperature, the absorbance was measured at 420 nm (UV-visible spectrophotometer Hitachi U 2000 Japan). A yellow color indicated the presence of flavonoids. Total flavonoid contents were calculated as quercetin (mg/g) using the following equation based on the calibration curve:

$y = 0.0353x + 0.0566$, $R^2 = 0.9985$

Where y was the absorbance and x was the quercetin equivalent (mg/g).

Determination of total flavonols

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran, 2006. To 2.0 ml of sample (standard), 2.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm (UV-visible spectrophotometer Hitachi U 2000 Japan) was read after 2.5 h at 20°C. Total flavonol content was calculated as quercetin (mg/g) using the following equation based on the calibration curve:

$y = 0.0513x + 0.1658$, $R^2 = 0.9995$,

Table 1. Total phenolics content in the plants extracted by two different solvent.

Name of the plant	Local name at Meghalaya	Parts used	Total phenolics content (GAE mg / g of dry material) (Mean ± SEM)	
			Aq methanol extract	Acetone extract
Bauhinia purpurea	Megong	Leaves	27.67±0.16	3.47±0.48
Dillenia pentagyna	Agachi	Flower	9.33±0.15	2.61±0.13
Diplazium esculentum	Jhur- Tyrkhang	Leaves	15.01±0.32	3.77±0.05
Fagopyrum cymosum	Jarain	Leaves	9.22±0.08	6.85±0.13
Ficus clavata	Slachit	Leaves	14.47±0.32	5.23±0.53
Ficus geniculata	Mong lor	Leaves	12.07±0.20	6.04±0.10
Ficus pomifera	Jhu jri	Leaves	7.50±0.26	3.17±0.18
Gentiana pedicellata	Jamiaw	Leaves	23.46±0.32	3.07±0.22
Gynocardia odorata	So liang	Seeds	3.31±0.10	4.43±0.36

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

Where y was the absorbance and x was the quercetin equivalent (mg/g).

Measurement of reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Extracts (100 µl) of fruit extracts were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Reducing power is given in ascorbic acid equivalent (AAE) in milligram per gram (mg/g) of dry material.

Determination of free radical scavenging activity

The free radical scavenging activity of the plant samples and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1, 1-diphenyl-2-picrylhydrazyl) (Blois, 1958). Aliquots (20 - 100 µl) of the tested sample were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg L^{-1}) in methanol was added in each test tube and mixed. 30 min later, the absorbance was measured at 517 nm (UV-visible spectrophotometer Hitachi U 2000 Japan). The capability to scavenge the DPPH radical was calculated, using the following equation:

DPPH scavenged (%) = {(Ac – At)/Ac} x 100

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg / ml) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.
Values are presented as mean ± standard error mean of three replicates. The total phenolic content, flavonoid content, flavonol content, reducing power and IC_{50} value of each plant material was calculated by using Linear Regression analysis.

RESULTS AND DISCUSSION

Total phenol, flavonoid and flavonol content of the extracts

Phenolic components are very important plant constituents with scavenging ability because of its hydroxyl group. It has been established that phenolic compounds are the major plant compounds with antioxidant activity and this activity is due to their redox properties. Phenolic compounds are a class of antioxidant agents which can adsorb and neutralize the free radicals (Florence et al., 2011). Flavonoids and flavonols are regarded as one of the most widespread groups of natural constituents found in the plants. It has been recognized that both flavonoids and flavonols show antioxidant activity through scavenging or chelating process (Pourmorad et al., 2006).

Total phenolic contents of different plant materials, using two different solvent systems are presented in Table 1. The screening of the aq methanol and acetone extracts of nine wild plants revealed that there was a wide variation in the amount of total phenolics ranging from 2.61±0.13 to 27.67±0.16 mg GAE/g dry material (Table 1). The highest amount of phenolic content was found in the aq. methanol extract of *B. purpurea* (27.67±0.16 mg GAE/g dry material), while least amount was observed in the acetone extract of *D. pentagyna* (2.61±0.13 GAE). The aq methanol extract of *G. pedicellata* (23.46±0.32 GAE), *D. esculentum* (15.01±0.32 GAE), *F. clavata* (14.47±0.32 GAE) and *F. geniculata* (12.07±0.20 GAE) were also found to contain a very good amount of phenolic compounds and the phenolic content of the plants are very much comparable with some other wild edible plants e. g. *Morus indica* (24.94 ±0.58 GAE), *Parkia roxburghii* (49.39 ±0.25 GAE), *Prunus nepalensis* (10.49 ±0.14 GAE), *Terminalia bellirica* (95.40 ±0.74 GAE), collected from Meghalaya state, India (Seal, 2011). In this study the content of phenolic components extracted by aq methanol was

Table 2. Total flavonoids content in the plants extracted by two different solvent.

Name of the plant	Local name at Meghalaya	Parts used	Total flavonoids content (mg / g of dry material) (Mean ± SEM)	
			Aq. methanol extract	Acetone extract
Bauhinia purpurea	Megong	Leaves	23.19±0.009	5.39 ±0.04
Dillenia pentagyna	Agachi	Flower	52.14±0.004	2.74 ±0.07
Diplazium esculentum	Jhur- Tyrkhang	Leaves	34.81±0.003	2.49± 0.08
Fagopyrum cymosum	Jarain	Leaves	20.89±0.009	52.17± 0.01
Ficus clavata	Slachit	Leaves	34.81±0.003	10.30 ±0.08
Ficus geniculata	Mong lor	Leaves	41.73±0.011	7.35 ±0.03
Ficus pomifera	Jhu jri	Leaves	30.50±0.210	5.04 ±0.03
Gentiana pedicellata	Jamiaw	Leaves	34.79±0.013	19.76 ±0.17
Gynocardia odorata	So liang	Seeds	8.11±0.071	1.22± 0.01

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

much higher than that extracted by acetone. This may be due to the fact that phenolics are often extracted in higher amounts in more polar solvents such as aqueous methanol/ethanol as compared with absolute methanol/ ethanol or acetone (Sultana et al., 2009; Ghasemzadeh et al., 2011).

Total flavonoids content of different plant materials, using two different solvent systems are presented in Table 2. The flavonoid contents of the extracts in terms of quercetin equivalent were between 1.22 ± 0.01 to 52.17± 0.01 mg/g dry material. Highest amount of flavonoid content was observed in the acetone extract of *F. cymosum* (52.17± 0.01 mg/g). The aq. methanol extracts of all wild edible plants under investigation were found to contain greater amount of flavonoid than that of acetone extract except in case of *F. cymosum*. Results of the present study showed that the aq. methanolic extracts were better for flavonoid extraction.

In case of flavonol, the highest amount was observed in the aq. methanol extract of *D. esculentum* (23.20 ±0.03 mg/g) followed by *G. pedicellata* (23.12 ±0.006 mg/g) and *F. clavata* (23.10 ±0.005 mg/g) (Table 3). Appreciable quantities of flavonol were found in the aq. methanol extract of *B. purpurea* (15.50 ±0.004 mg/g) and *F. cymosum* (13.87± 0.005 mg/g) (Table 3).

The results strongly suggest that phenolics are important components of these plants. The other phenolic compounds such as flavonoids, flavonols, which contain hydroxyls are responsible for the radical scavenging effect in the plants. According to our study, the high content of these phenolic compounds in the aq. methanol extract of *G. pedicellata*, *F. clavata*, *B. purpurea*, *F. geniculata, D. pentagyna* and in the acetone extract of *F. cymosum* can explain their high radical scavenging activity.

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with the presence of reductones which breaks the free radical chain by donating a hydrogen atom (Subhasini et al., 2011). The reducing powers of the nine wild plants were evaluated as mg AAE/g dry material as shown in Table 4. The reducing abilty of the aq methanol extract of the nine wild edible plants in descending order was *B. purpurea* > *D. pentagyna* > *G. pedicellata* > *F. geniculata* > *F. pomifera* > *F. clavata.* The highest reducing power was exhibited by the aq methanol extract of *B. purpurea* (16.11 ± 0.03 mg/g AAE) which is also high in phenolic content (27.67±0.16 mg GAE/g dry material) and acetone extract of *G. odorata* showed lowest activity in terms of ascorbic acid equivalent. In general, the aqueous methanol extracts of the tested plant materials, exhibiting greater phenol, flavonoids and flavonol content, also depicted good reducing power in the present analysis. In this assay, the presence of antioxidants in the extracts reduced Fe^{+3}/ferricyanide complex to the ferrous form. This reducing capacity of the extracts may serve as an indicator of potential antioxidant activities through the action of breaking the free radical chain by donating hydrogen atom (Jamuna et al., 2011).

DPPH radical scavenging activity

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva et al., 2002). The antioxidant effect is proportional to the dis-appearance of the purple colour of DPPH in test samples. Thus antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 2, 2- diphenyl-1-hydrazine is formed and as a result of which the absorbance (at 517 nm) of the solution is decreased. Hence the more potent antioxidant, more decrease in absorbance is seen and consequently the IC_{50} value will

Table 3. Total flavonols content in the plants extracted by two different solvent.

Name of the plant	Local name at Meghalaya	Parts used	Total flavonols content (mg / g of dry material) (Mean ± SEM)	
			Aq. methanol extract	Acetone extract
Bauhinia purpurea	Megong	Leaves	15.50 ± 0.004	2.99 ± 0.14
Dillenia pentagyna	Agachi	Flower	6.73 ± 0.03	0.19± 0.02
Diplazium esculentum	Jhur- Tyrkhang	Leaves	23.20 ± 0.03	1.96± 0.006
Fagopyrum cymosum	Jarain	Leaves	13.87± 0.005	6.56± 0.02
Ficus clavata	Slachit	Leaves	23.10 ± 0.005	2.28± 0.11
Ficus geniculata	Mong lor	Leaves	5.31 ± 0.02	3.53± 0.02
Ficus pomifera	Jhu jri	Leaves	2.77 ± 0.02	7.50± 0.12
Gentiana pedicellata	Jamiaw	Leaves	23.12 ± 0.006	7.43± 0.02
Gynocardia odorata	So liang	Seeds	2.21± 0.07	0.09± 0.008

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

Table 4. Reducing power (Ascorbic acid equivalent) of the plants extracted by two different solvent.

Name of the plant	Local name at Meghalaya	Parts used	Ascorbic acid equivalent (AAE) (mg / g of dry material) (Mean ± SEM)	
			Aq. methanol extract	Acetone extract
Bauhinia purpurea	Megong	Leaves	16.11 ± 0.03	4.63±0.09
Dillenia pentagyna	Agachi	Flower	13.19±0.09	5.03±0.09
Diplazium esculentum	Jhur- Tyrkhang	Leaves	8.78±0.03	4.99±0.15
Fagopyrum cymosum	Jarain	Leaves	6.11±0.03	4.99±0.10
Ficus clavata	Slachit	Leaves	9.98±0.07	6.45±0.18
Ficus geniculata	Mong lor	Leaves	10.56±0.08	7.14±0.18
Ficus pomifera	Jhu jri	Leaves	10.33±0.05	4.75±0.10
Gentiana pedicellata	Jamiaw	Leaves	13.07±0.04	5.79±0.10
Gynocardia odorata	So liang	Seeds	8.46±0.25	3.19±0.09

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

be minimum.

The evaluation of anti-radical properties of nine wild edible plants was performed by DPPH radical scavenging assay. The 50% inhibition of DPPH radical (IC_{50}) by the different plant materials was determined (Table 5), a lower value would reflect greater antioxidant activity of the sample. In the present study the highest radical scavenging activity was shown by the aq. methanol extract of *G. pedicellata* (IC_{50} = 0.23±0.0007 mg dry material), whereas the acetone extract of *G. odorata* showed lowest activity (IC_{50} = 2.71±0.04 mg dry material).

Strong inhibition was also observed for the aq. methanol extract of *B. purpurea* (IC_{50} = 0.34±0.0004 mg dry material and *F. clavata* (IC_{50} = 0.31±0.0009 mg dry material). The high radical scavenging property of *G. pedicellata* may be due to the hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary component as a radical scavenger. The aq. methanolic and acetone extracts of all of the plants under investigation exhibited different extent of antioxidant activity and thus provide a valuable source of nutraceutical supplements. Depending on the values, some plants are more important than some others.

Conclusion

The result of present study showed that the aq. methanol extract of *B. purpurea* which contain highest amount of phenolic compounds and appreciable amount of flavonoids and flavonols exhibited the greatest reducing power and also showed strong radical scavenging activity. The highest radical scavenging activity and very strong reducing power of the aq. methanol extract of *G. pedicellata* may be due to the presence of a very good amount of total phenolics, flavonoids and flavonols contents in this plant. The radical scavenging activities of

Table 5. Free radical scavenging ability of the plant samples extracted by two different solvent by the use of a stable DPPH radical (Antioxidant activity expressed as IC_{50}).

Name of the plant	Local name at Meghalaya	Parts used	IC_{50} value (mg dry material) (Mean ± SEM)	
			Aq. methanol extract	Acetone extract
Bauhinia purpurea	Megong	Leaves	0.34± 0.0004	1.02±0.02
Dillenia pentagyna	Agachi	Flower	0.51± 0.006	2.11±0.06
Diplazium esculentum	Jhur- Tyrkhang	Leaves	0.92± 0.01	3.60±0.04
Fagopyrum cymosum	Jarain	Leaves	0.55± 0.002	0.68±0.008
Ficus clavata	Slachit	Leaves	0.31±0.0009	0.81±0.02
Ficus geniculata	Mong lor	Leaves	0.39± 0.001	0.93±0.01
Ficus pomifera	Jhu jri	Leaves	0.64± 0.005	1.55±0.02
Gentiana pedicellata	Jamiaw	Leaves	0.23± 0.0007	1.22±0.02
Gynocardia odorata	So liang	Seeds	1.97± 0.02	2.71±0.04

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

the selected plant extracts are still less effective than the commercial available synthetic like BHT. As the plant extracts are quite safe and the use of synthetic antioxidant has been limited because of their toxicity, therefore, these wild edible plants could be exploited as antioxidant additives or as nutritional supplements. However, further investigation is required to isolate and characterize the individual components from these plants which are actually responsible for their antioxidant activities and develop their applications for food and pharmaceutical industries.

ACKNOWLEDGEMENTS

Author of this paper is highly grateful to Dr. P. Singh, Director, Botanical Survey of India, Kolkata, Dr. M. Sanjappa, Ex-Director, Botanical Survey of India, Kolkata for their encouragement and facilities. I am also thankful to Mr. R. Shanpru, Scientist, Botanical Survey of India, Eastern Regional circle, Shillong, Meghalaya for identifying the plant specimens.

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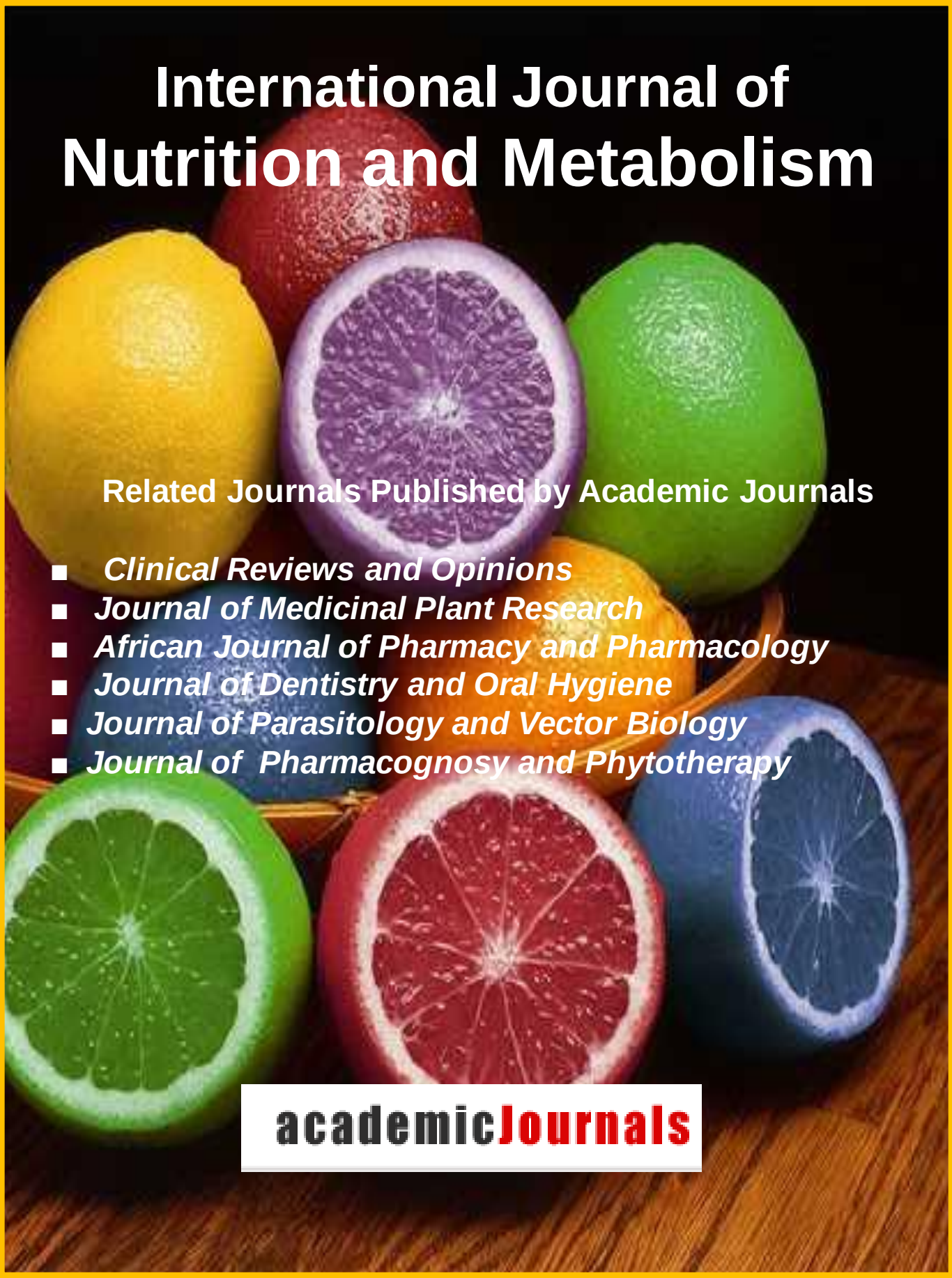

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