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RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY OF THE SELECTED HERBAL PLANTS *BORRERIA ARTICULARIS, ICHNOCARPUS FRUTESCENS* AND *ZINGIBER OFFICINALE* IN ALBINO WISTAR RATS

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ABSTRACT

In the present study antioxidant activities by (2,2-Diphenyl-0 picrylhydrazyl) radical (DPPH), hydrogen peroxide, hydroxyl radical inhibition, hemolysis by hydrogen peroxide assay, reducing power and total antioxidant activities of polyphenolic extract of selected plants leaves were investigated. The total polyphenolic contents of the extract were determined using standard methods. The results of antioxidant activities of poly-phenol extract obtained by different in vitro methods were varied depending on the method used. Nevertheless, polyphenol extract showed significant inhibitory activities in all in vitro reactive oxygen species scavenging, might be attributed due to the high level of polyphenolic compound. Also, these various antioxidant activities were compared to α-tocopherol and L-ascorbic acid as reference antioxidant compounds. These findings provide evidence that the polyphenolic extract of selected plants is a natural source of antioxidant against oxidative damage.

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INTRODUCTION

An antioxidant is a substance that is present in lower concentration when compared to that of an oxidisable substrate significantly delays or prevents oxidation of that substrate (Halliwell, 1990). Even though plant phenols are not treated as real antioxidants in the literature, many *in vitro* studies have demonstrated that the antioxidant potential of phenols as direct aqueous phase radical scavengers and as the agents that are capable of enhancing the resistance to oxidation of low density lipoproteins implicated in the pathogenesis of coronary heart disease (Rice-Evans *et al.,* 1995). Biological systems have evolved with endogenous defense mechanisms that help to protect free radical induced cell damage. Glutathione peroxidase, catalase and superoxide dismutase are antioxidant enzymes which metabolize toxic oxidative intermediates. They require micronutrient as cofactors such as selenium, iron, copper, zinc and manganese for optimum catalytic activity and effective antioxidant defense mechanisms. Superoxide dismutase, catalase and glutathione peroxidase are three primary enzymes involved in direct elimination of active oxygen species (hydroxyl radical, superoxide radical and hydrogen peroxide) whereas glutathione reductase, glucose-6-phosphate dehydrogenase and cytosolic, Glutathione-S-transferase (GST) are secondary enzymes which help in the detoxification of Reactive Oxygen Species (ROS) by decreasing peroxide levels or maintaining a steady supply of metabolic intermediates like glutathione and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) necessary for optimum functioning of the primary antioxidant enzymes (Vendemiale *et al.,* 1999; Pietta, 2000). Glutathione, ascorbic acid, α-tocopherol, β-carotene, bilirubin, selenium, Nicotinamide Adenine Dinucleotide Phosphate (NADPH), butylhydroxyanisole (BHA), mannitol, benzoate, histidine peptide, the iron-bonding transferrin, dihydrolipoic acid, melatonin, uric acid and plasma protein play a homeostatic or protective role against Reactive Oxygen Species (ROS) produced during normal cellular metabolism and after active oxidation insult. Glutathione is the most significant component which directly quenches Reactive Oxygen Species (ROS) such as lipid peroxides and plays a major role in xenobiotic metabolism.

When an individual is exposed to high levels of xenobiotics, more glutathione is utilized for conjugation making it less available to serve as an antioxidant activity (Bhattacharya, 1998). In the present study an attempt has been made to evaluate the antioxidant activity of *Borreria articularis*, *Ichnocarpus frutescens* and *Zingiber officinale* plants extract.

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MATERIALS AND METHODS

Male rats (10 weeks old and between 220 and 260 g in body weight) were randomly assigned into two groups. One group (Test group) of rats received oral dose of plant extract in various concentration in ethanol. Another group (control group) received an equivalent volume of ethanol alone. Control and test group rats were caged separately but housed under similar conditions. Both groups of animals were fed with the same diet and water *ad libitum*. All experimental manipulations were carried out with the animals under diethyl ether anesthesia. On the day of the experiments, blood samples were collected and catalase activities were determined (Abei, 1974). Plants leaf extract of different concentration were studied for antioxidant activity. Based on two methods (i) DPPH (2,2-Diphenyl-0 picrylhydrazyl) scavenging activity in qualitative (Abdul *et al.,* 2003) and (ii) DPPH (2,2-Diphenyl-0 picrylhydrazyl) scavenging activity in quantitative (Blosi, 1958).

RESULTS AND DISCUSSION

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer (Kinnula and Crapo, 2004), cardiovascular disease (Singh and Jialal, 2006), neural disorders (Sas *et al.,* 2007), Alzheimer's disease (Smith *et al.,* 2000), mild cognitive impairment (Guidi *et al.,* 2006), Parkinson's disease (Bolton *et al.,* 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishna *et al.,* 1997), aging (Hyun *et al.,* 2006) and atherosclerosis (Upston *et al.,* 2003). Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery. Two review articles have been published earlier (Chanda and Dave, 2009; Badarinath *et al.,* 2010) on in vitro evaluation of antioxidant activity. In this article, attempts have been taken to include in vivo too and to analyze the frequency of the use of different methods.

An issue of crucial importance in the development of drugs is concerned with reactive oxygen species (ROS), which are formed in human cells and act exogenously result in extensive oxidative damage**.** Hence, in the present investigation an attempt has also been made to identify the plant extract nature on antioxidant activity. The antioxidant activity has been assessed using DPPH scavenging activity. All the plant extract respective of the nature and source have been found to be effective antioxidants which are been confirmed by the yellow color formation in chromatography. Results are presented in the Table 1. As there has been a change in the intensity of the colour in the qualitative test, quantitative DPPH scavenging activity has also been performed for all the plants extract. In this test, different concentration of DPPH such as 1.0 µL/mL, 8 µL/mL, 20µL/mL, and 100µL/ml were used. The analysis has been observed from the lower dilution to higher dilution in order to know the inhibition percentage at a minimum concentration. When the result is found to be effective in the lower dilution, the other higher dilutions are ignored. The results for the quantitative DPPH scavenging activity are shown in Table 2. Among the tested plants extract *Borreria articularis* has been found to be non effective in 1.0µL/mL, and found to be effective in very lower concentration as 27.24% in 8µL/mL, higher concentration as 41.38 % in 20µL/mL and 91.56% in 100µL/mL. *Ichnocarpus frutescens* is non effective in 1.0 µL/mL and found to be effective in very lower concentration as 24.46% in 8µL/mL, higher concentration as 38.65 % in 20µL/mL and 88.26% in 100µL/mL. The other plant extract have been found to have *Zingiber officinale*, it is also non effective in 1.0µL/mL, and 8 µL/mL, found to be effective in higher concentration as 39.28 % in 20 µL/mL and 89.36 % in 100 µL/mL. Compared to control in different concentration of plant extract very low dilution concentration of plant extract highly effective antioxidant activity, Reactive oxygen species (ROS) are constantly formed in the human body and are removed by an antioxidant defense system. High doses and/or inadequate removal of ROS result in oxidative stress, which may cause severe metabolic malfunction and damage to biological macromolecules.

Table 1. Qualitative identification of DPPH scavenging activity of Plants extract

Plant Source	Qualitative DPPH Scavenging activity
Borreria articularis	Positive
Ichnocarpus Frutescens	Positive
Zingiber officinale	Positive

The effect of Plant extract on % reduction in thiobarbituric acid reactive substances (TBARs) production has been analyzed. The plant extract of *Borreria articularis* has shown dose dependent effectiveness expressed in percentage i.e., 15.45%, 45.14%, 53.54%, 62.00% and 67.53% respectively for the concentration 1.75, 3.50, 5.25, 7.00, and 8.75, Ichnocarpus Frutescens has shown 14.26%, 38.12%, 48.24%, 57.82% and 59.80 %, *Zingiber officinale* has shown 12.34%, 32.50%, 43.50%, 48.14% and 53.32%. The results have been found to be lesser when compared to the control (cucurmin 0.74 mg/mL), but still encouraging and indicating the possible antioxidant activity. The results are presented in Table 3. In catalase activity, has been analysed using similar concentrations of the plant extract. The plant extract of *Borreria articularis* has shown dose dependent effectiveness expressed in mmol H_2O_2/min/mg of protein i.e., 24.14, 30.31, 32.52, 40.31 and 48.68, *Ichnocarpus frutescens* has shown 21.82, 27.82, 33.00, 37.15 and 46.10, *Zingiber officinale* has shown 18.34, 24.32, 30.46, 34.34 and 40.24. The results are better, when compared to the non antioxidant control (Ethanol). The results are presented in Table 4. In the glutathione production activity, the plant extract of *Borreria articularis* has shown dose dependent effectiveness, expressed in nM/mg protein i.e., 86.24, 89.72, 94.08, 96.00 and 99.60.

Table 2. Quantitative DPPH scavenging activity of Plants extract

Plant Source	DPPH Scavenging activity Inhibition %				IC50 µl/ml
	1.0µl/ml	8µl/ml	20µl/ml	100µl/ml	
Borreria articularis	0.0	27.24	41.38	91.56	80.09
Ichnocarpus Frutescens	0.0	24.46	38.65	88.26	75.68
Zingiber officinale	0.0	0.0	39.28	89.36	64.28

Table 3. Effect of Plants Extract on %reduction in Thiobarbituric acid reactive substances (TBARs) production

Concentration of Pants leaf extract in mg/mL	Plants leaf extract			Control treatment with ethanol
	Borreria articularis	Ichnocarpus Frutescens	Zingiber officinale	
1.75	24.14±0.93	21.82±0.56	18.34±0.48	17.42± 0.45
3.50	30.31±0.41	27.82±0.62	24.32±0.75	
5.25	32.52±0.73	33.0±1.05	30.46±0.98	
7.00	40.31±0.82	37.15±0.82	34.34±1.10	
8.75	48.68±1.00	46.10±0.56	40.24±0.52	

Table 4. Effect of Plants Extract on Catalase (in mmol H2O2/min/mg of protein) production

Concentration of Plant leaf extract in mg/mL	Plant leaf extract			Cucurmin Standard control (0.74) mg/mL
	Borreria articularis	Ichnocarpus Frutescens	Zingiber officinale	
1.75	15.45±0.51	14.26±0.56	12.34±0.82	92.34±0.52
3.50	45.14±0.52	38.12±0.71	32.50±0.85	
5.25	53.54±0.46	48.24±1.00	43.50±0.89	
7.00	62.00±0.78	57.82±0.84	48.14±0.74	
8.75	67.53±1.00	59.80±0.86	53.32±0.59	

Table 5. Effect of Plants Extract on reduced glutathione (total tissue thiol group) (in nM/mg protein) production

Concentration of Plant leaf extract in mg/mL	Plant leaf extract			Standard control
	Borreria articularis	Ichnocarpus Frutescens	Zingiber officinale	
1.75	86.24±0.93	81.14±0.43	79.42±0.62	73.00±0.58
3.50	89.72±0.90	83.24 ±0.72	82.15±0.76	
5.25	94.08±0.56	93.52±0.96	86.14±0.65	
7.00	96.00±0.72	95.32 ±0.82	88.52±0.50	
8.75	99.60±0.85	97.50±0.65	92.62±0.64	

Ichnocarpus Frutescens has shown 81.14, 83.24, 93.52, 95.32, and 97.50. *Zingiber officinale* has shown 79.42, 82.15, 86.14, 88.52 and 92.62. The results have been found to be better when compared to the control, and found to be strongly recommendable as antioxidant. The results are presented in Table 5. The antioxidant activity was exhibited by *Zingiber officinale*, turmeric and garlic and it was comparable to a well-known antioxidant vitamin C. *Zingiber officinale* was found to have the highest anti-oxidant potency followed by turmeric, dry garlic and the fresh garlic. Owing to its good antioxidant potency *Zingiber officinale* is also known to be a good chemoprevention (Nirmala *et al.,* 2007). In last few decades research on spices has been directed to understand their medicinal, antioxidant, antimutagenic and anticarcinogenic properties. Thus spices like *Zingiber officinale*, turmeric and garlic can protect the human body against cellular oxidation reactions, bacterial infections and other metabolism related disorders. The extracts of many spices and herbs have become popular in recent years for their antimicrobial and antioxidant properties and attempt to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications (Dilis and Trichopoulon, 2010). The DPPH free radicals, which are stable in ethanol shows maximum a proton donating substances such as antioxidant, the radicals would be scavenged and absorbed. PPE showed dose dependent DPPH radical scavenging activity.

The effect of free radical scavenging activity of PPE on DPPH radicals is thought to be due to their hydrogen donation ability of polyphenols of I. frutescens. The results showed that PPE is a free radical scavenger, as well as primary antioxidant that react with free radicals, which may limit the occurrence of free radical damage in human body. Hydroxyl radical is an extremely reactive species formed in biological systems and has been implicated as highly damaging in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contribute to ageing, carcinogenesis, mutagenesis, cytotoxicity and several diseases. Among the oxygen radicals specifically, the OH radical is the most reactive and severely damages adjacent biomolecules such as, all proteins, DNA, PUFA and almost any biological molecules. In addition, this species is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (Kappus, 1991). Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of a living body against various diseases. The ability of the PPE to quench hydroxyl radicals seems to directly relate to the prevention of propagation of the process of lipid peroxidation, and the extract seems to be a good scavenger of active radical species, thus reducing the rate of chain reaction.

The polypenolic extract found to effectively inhibit H_2O_2 induced hemolysis of erythrocytes, revealing its ability to scavenge most of the free radicals generated. Polyphenolic from some fruits and vegetables have also been shown to enhance red blood cell resistance to oxidative stress, in vivo and in vitro (Alam *et al.,* 2012). Our data on PPE extract show that increasing concentrations of PPE containing polyphenols gradually inhibits H_2O_2 induced hemolysis in erythrocytes. Therefore, the protective effect of PPE may be due to its association with protection of red blood cell, which prevent the membrane peroxidation and thereby RBC hemolysis. In biological systems, MDA is a very reactive species and takes part in cross-linking of DNA with proteins and also damaging the cells (Jadhav *et al.,* 1996). MDA, being the major product of lipid peroxidation is used to study the anti-lipid peroxidation activity in rat brain homogenates by means of reaction with TBA at high temperature and acidic conditions. Currently, a number of synthetic and natural antioxidants are used in the peroxidation and retardation of lipid peroxidation. While synthetic antioxidants have proven effective unpleasant side effects have been reported. In this study, we examined the possible in vitro antioxidant activity against Fenton's reagent induced lipid peroxidation, PPE showed potent in vitro anti-lipid peroxidation activity and less harmful alternative to the synthetic antioxidant products. Free radicals have been implicated in many diseases conditions, the important ones being superoxide radical, hydroxyl radical, peroxyl radical and singlet oxygen. These highly reactive species have a potential for bringing about extensive damages, including lipid peroxidation DNA lesion and protein fragmentation within the cells of biomolecules. In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidants-rich foods and the incidence of human diseases (Rice-Evans *et al.,* 1997). In searching novel natural antioxidants, some plants have been extensively studied in the past few years for their antioxdiative and radical scavenging components, because reactive oxygen species (ROS) production and oxidative stress have been linked to ageing related illness and large number of other illness (Finkel and Holbrook, 2000).

Conclusion

In conclusion from the above investigation, using several in vitro and in vivo models, polyphenolics extract was found to scavenge DPPH radicals, hydroxyl radicals hydrogen peroxide and inhibits lipid peroxidation. The total antioxidant and free radical scavenging of the polyphenolic extract of plant extract can be attributed to the presence of enriched polyphenolic compounds. Polyphenols, anthocyanins and flavonoids are very valuable plant constituents in the scavenging of free radicals, due to their several phenolic hydroxyl groups. Combining this fact with obtained results we could suggest that the amount of polyphenolic compound increases, antioxidant activity increase as well. This study clearly suggested that the polyphenolic extract of plant leaf had significant antioxidant activity, which might be helpful in preventing or slowing the process of various oxidative stress related diseases of major organs.

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