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Research article

Efficient free radical scavenging activity of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous* leaves through DPPH (2, 2-diphenyl-1-picrylhydrazyl)

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Abstract

Free radical scavenging activity of three important plants *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous* was carried out to evaluate and explore new potential sources of natural antioxidants. For this purpose the leaves of the three plants were processed. In these experiments the order of the antioxidant activity was, maximum activity shown by methanolic extract of *Ginkgo biloba* followed by *Parthenium hysterophorous* and *Stevia rebaudiana*. Furthermore the ethanolic extract of *Ginkgo biloba* also showed maximum antioxidant activity seconded by *Stevia rebaudiana* and *Parthenium hysterophorous.*

Key words: *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous*, DPPH, radical scavenging activity

Introduction

The active ingredients of a medicinal plant are mainly its secondary metabolites, among which is the phenolic compound that is an important antioxidant [1, 2]. The phenolic compounds are general term for multiple aromatic groups including mainly flavonoids, phenols acid, bioflavonoid and anthocyanins. These ingredients are naturally produced during a plant's growth metabolic process, the active substances with antioxidant function such as scavenging reactive oxygen species (ROS), free radicals (hydroxyl radicals, $\cdot OH$ and superoxide anion radicals, $\cdot O_2$-) or non-free radical reactive oxygen species (peroxide, H_2O_2) production from body metabolism [3]. Nowadays, natural antioxidants have become a major area of interest in scientific research [4,5] therefore, the importance of searching for and exploiting natural antioxidants, especially those of plant origin, has increased greatly in recent years. There is a growing interest in natural additives as potential antioxidants [6-8]. Natural antioxidants are known to exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilatory activity. In fact, a fundamental property considered important for life is antioxidant activity and this property may give rise to anticarcinogenicity, anti-mutagenicity, and anti-aging activity, among others [9, 10].

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In recent developments it has been recorded that free radicals are involved in causing many diseases [11]. For instance unsaturated fatty acids in the biomembranes are attacked by free radicals causing in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane proteins leading to cell inactivation [12]. Free radicals also attack DNA and cause mutation leading to cancer [13]. For these reasons antioxidants are of interest for the treatment of many kinds of cellular degeneration [14]. Antioxidants are compounds that inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidant namely synthetic and natural ones, restriction on the use of synthetic anti-oxidantsis being imposed because of their carcinogenicity [15]. Thus the interest in natural antioxidants has been increased considerably. As resources of natural antioxidants much attention has been paid to plants [16, 17]. Especially, the antioxidants present in edible plants have recently been considered as food additives [18, 19].

Material and method

Plant material

Test plants were obtained from the local market (nurseries). Plant material consists of mature leaves of the three plants.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased (Sigma, Aldrich Germany). All other chemicals and reagents were purchased locally and were of analytical grade.

Preparation of the extract

The leaves of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous* were shade dried (28±2ºC), ground and sieved to get fine powder. Ethanolic extract of the plant was prepared by taking 10 g of dried leaves powder. With this 50 ml of ethanol was added and kept for 1 week with periodic shaking (The soaked material was stirred every 18 h using a sterilized glass rod). This procedure was repeated three times with fresh volume of ethanol. The final extracts were passed through Whatman filter paper No.1 (Whatman Ltd., England). Methanolic extracts of the plant material were also prepared in a similar manner. The pooled ethanol and methanol extracts were concentrated by rotary vacuum evaporator at 40ºC, dried and stored at 4ºC in air tight bottles (Jang, Y.S, *et al*, 2002).The stock solutions were prepared by dissolving pure extract 5mg in 20ml of methanol and ethanol independently.

Antioxidant activity (DPPH free radical scavenging activity)

Several methods have been developed to evaluate the total antioxidant activity of fruits or other plants and animal tissues. Among them, trolox equivalent antioxidant capacity, total radical absorption potentials, oxygen radical absorption capacity assays and the ferric reducing ability of plasma (FRAP) assay are commonly used and are the representative methods frequently used in various investigations. [20-24]. One of the methods is the DPPH assay, which can accommodate a large number of samples in a short period of time and is sensitive enough to detect natural compounds at low concentrations so it was used in the present study for the primary screening of antioxidants. The free radical scavenging activity of ethanolic and methanolic leaf extract of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous* was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Antioxidants react with DPPH which is a stable free radical, and convert it to 1; 1-diphenyl-2-picryl hydrazine. The DPPH solution was also prepared in methanol and ethanol independently. 3.96 mg of DPPH was dissolved in 20 ml of each solvent to get stock solution. With 0.5 ml of sample solution was added to 1 ml of DPPH solution separately. These solution mixtures were kept in dark for 30 min (incubation period) at room temperature. After which the absorbance was measured at 517 nm. All tests were carried out in triplicate. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Finally the radical scavenging

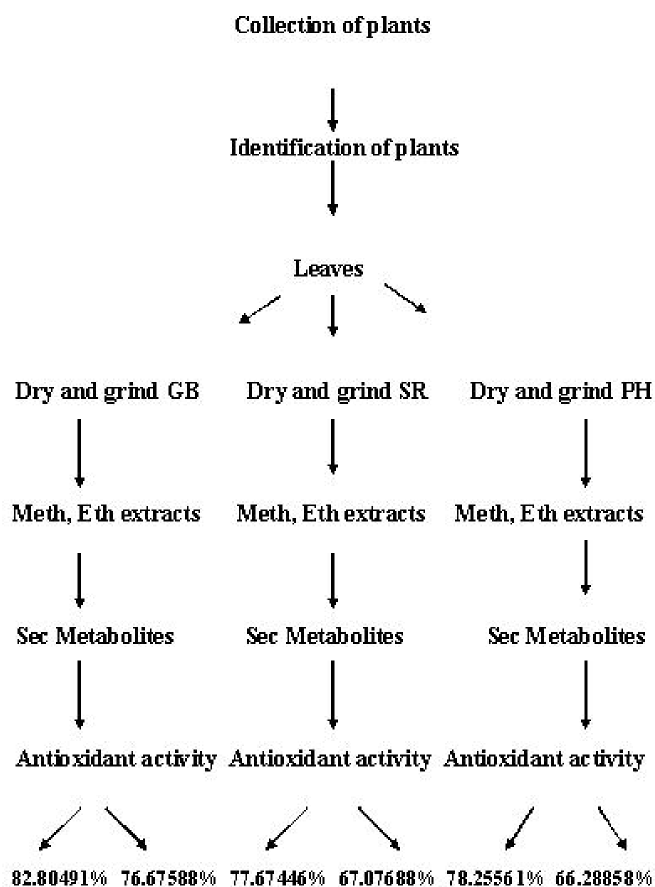
activity was calculated as percentage of DPPH discoloration using the equation;

% scavenging DPPH free radical = 100 × (1-AE/AD)

Where AE is absorbance of the solution, when extract has been added at a particular level and AD is the absorbance of the blank DPPH solution.

Results

In the present studies three important medicinal and economic plants were investigated for their antioxidant activities. The mean values are indicated in percentage inhibition as shown in the. Fig.1.The best inhibition was shown by methanolic extract of *Ginkgo biloba* followed by *Parthenium and Stevia*. The *Ginkgo biloba* ethanolic extract activity lead *Stevia* and *Parthenium* respectively.



DPPH radical scavenging activity of *Ginkgo biloba*:

Ginkgo biloba of the family Ginkgoaceae is native to China, cultivated in Europe, Australia, Japan, Korea, and USA, because of properties in health sector [25]. *G. biloba* has been used for 5000 years in China for traditional medicines. Ginkgo contains essential oils, flavors, carotenes, phenolic lipids and alkaloids which is important in medical and pharmacological properties [26]. Extracts from Ginkgo have a wide application for treating various cardiovascular and neurological diseases [27]. The Flavonoids glycosides in its extract are responsible for antioxidant activity and may prevent free radical damage [28]. The therapeutic role of *Ginkgo biloba* extract on impairment of visual function and pathological histology of the optic nerve was studied in alloxan induced diabetic rats, which showed an increase in the amplitude of Visual Evoked potentials indicating axonal protection [29]. There are many different antioxidants components present in *Ginkgo biloba* and it is very difficult to measure each antioxidant separately. This is based on evidence from diseased tissues for increased levels of free radicals, increased levels of free-radical–induced oxidative damage products of DNA, lipids or proteins or decreased levels of antioxidants [30]. The Flavonoids, terpenes and bioflavonoid constituents (the flavone glycosides) are thought to be the compounds that provide Ginkgo's antioxidant effects. Ginkgo has been shown to be active against a wide variety of free radicals and free radical generating substances including nitric oxide, the superoxide, hydroxyl and oxoferryl and peroxyl radicals [31-33]. Ginkgo's antioxidant properties help protect the brain, retina of the eyes and the cardiovascular system against free radical aging damage [34]. The flavonol contents of crude extracts from *Ginkgo biloba* leaves has been determined and their antioxidant properties have been checked [34]. The antioxidant activity of cultivated fruiting bodies of an endophytic Xylaria sp.strain from *Ginkgo biloba* of methanolic extract exhibited strong antioxidant capacity through DPPH assay. When DPPH radical is scavenged, the color of the reaction mixture changed from purple to yellow with decline of absorbance at 517 nm [35].

Among the methanolic and ethanolic extracts, using the DPPH method, the crude methanolic extracts of Ginkgo leaves shows 82.80491% activity and ethanolic shows 76.67588% activity of 5mg/20 ml respectively (Fig.1). The results indicate that the antioxidant activity of the crude extract in methanol is higher than that of ethanol. The antioxidant activity is presented in the Figure.2. Antioxidants react with DPPH which is a stable free radical, and converts it to 1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the radical scavenging potential of the antioxidant.0020

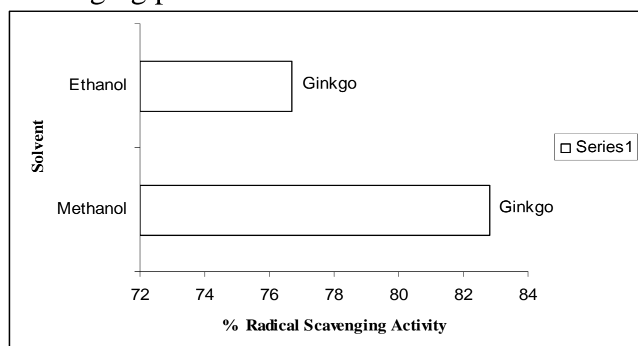


Fig 2 Free Radical Scavenging Activity of *Ginkgo bioloba* extracts.

DPPH radical scavenging activity of *Stevia rebaudiana.*

Stevia, commonly known as sweet leaf or sugar leaf of family Asteraceae, native to subtropical and tropical South America, Central America, Paraguay and Southern Brazil, also occur in Japan, Taiwan, Korea, Thailand and Indonesia [36]. For centuries, the Guarani tribes of Paraguay and Brazil used *Stevia* species, primarily *S. rebaudiana* as a sweetener in yerba mate and medicinal teas for treating heartburn and other ailments. Presently, this herb has become well-known for its high (about 4–20%) sweet diterpene content in the dried mass of the leaf, which includes stevioside, rebaudiosides A–F, and dulcoside A [37]. Many researchers have demonstrated that *Stevia* may be used for different therapeutic effects, such as hypoglyceamic activity [38] as hypotensive [35]. The World Health Organization (WHO) performed a thorough evaluation of recent experimental studies of stevioside and steviols conducted on animals and humans, and concluded that “stevioside and rebaudioside A are not genotoxic in vitro or in vivo and that the genotoxicity of steviol and some of its oxidative derivatives in vitro is not expressed in vivo” [39].

Stevia sweetener extractives have been suggested to exert beneficial effects on human health including antihypertensive [40, 41], antihyperglycemic non-cariogenic, antihuman rota virus activities, glucose metabolism [42] and renal function [43]. Aqueous extract of *S. rebaudiana* dried leaves induce systemic and renal vasodilation, causing hypotension, diuresis and natriuresis in rats [44].The highest antioxidant capacity of *Stevia rebaudiana* leaf extracts with methanol, followed by *Stevia rebaudiana* ethanol extracts has been detected [45]. The ethanolic extracts of leaves of *Stevia rebaudiana* have been found to be potential natural antioxidant [46].

One of the objectives of the present study was to assess the antioxidant potential of methanolic and ethanolic extracts of *Stevia rebaudiana* through DPPH assay. Methanolic extract of Stevia leaves showed significantly high antioxidant activity equivalent to methanolic extract activity of *Parthenium hysterophorous* and methanolic extract activity of Stevia is higher than ethanolic extract activity of Stevia, but the methanolic extract activity of Stevia is less than the methanolic extract activity of *Ginkgo biloba*. Fig.1. In the current observation it was investigated that methanolic extract activity is high than ethanolic that means more DPPH radical is scavenged by methanolic extract, the color of the reaction mixture changed from purple to yellow with decreasing of absorbance at 517 nm respectively. Fig.3. shows the percent inhibition of DPPH radical with different extracts of Stevia leaves. The percent inhibition of DPPH radical with methanolic extract of Stevia leaves were found to be 77.67446 % whereas it was found to be about 10 % greater than ethanolic extract which was record as 67.07688 % .

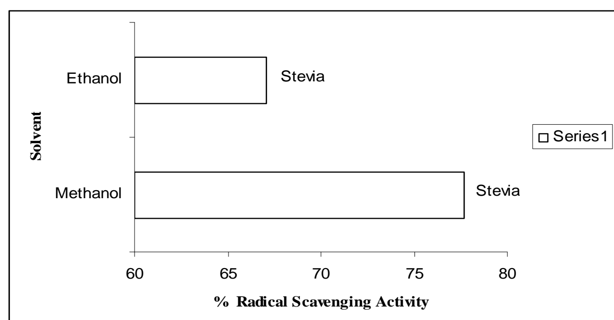


Fig 3 Free Radical Scavenging Activity of *Stevia rebaudiana* extracts.

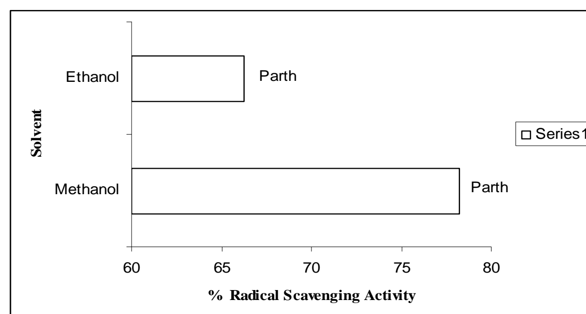


Fig 4 Free Radical Scavenging Activity of *Parthenium hysterophorous* extracts.

DPPH radical scavenging activity of *Parthenium hysterophorous.*

Parthenium hysterophorus L. belongs to family Asteraceae, native to the tropical Americas (Mexico, central and south America) [47, 48]. Since *P. hysterophorous,* popularly known as congress weed, star weed or ramphool is one of the 10 most invasive weeds in the world including Pakistan [49]. Parthenium has prolific seed bearing capacity producing a minimum of 330 crores of pollen per m^2 land area, of which 1500 to 20,000 seeds are produced per plant per year depending upon growth, habitat and longetivity and seeds germinate within two years if conditions are suitable [50].

To the best of our knowledge and from the cited literature, this is the first time to report the antioxidant activity of leaves of *Parthenium hysterophorous*. In order to measure antioxidant activity, DPPH (2, 2'-diphenyl-1 picrylhydrazyl) free radical scavenging assay was used. The method was carried out as described by [51]. The degree of discoloration indicates the radical scavenging potential of the antioxidant. Since the DPPH assay can accommodate a large number of samples in a short period of time and is sensitive enough to detect natural compounds at low concentrations, it was used in the present study for the primary screening of methanolic and ethanolic extracts free radical scavenging activity of *Parthenium*

Table 1 **Previous reports of Antioxidant activities of *Ginkgo biloba*, *Stevia rebaudiana.***

S.no	Plants specie	Part used	solvent	Activity	Reference
1	*Ginkgo biloba*	Secondary metabolites	_	Antioxidant activity	31
2	*Ginkgo biloba*	Secondary metabolites	_	Antioxidant activity	32
3	*Ginkgo biloba*	Samples	_	Antioxidant activity	34
4	*Ginkgo biloba*	leaves		Antioxidant activity	Kobus, *et al*., (2009).
5	*Ginkgo biloba*	Phenolics	methanol	Antioxidant activity	Wei Zheng and Shiow Y. Wang, 2001
6	*Ginkgo biloba*	Phenolics	acetone	Antioxidant activity	Wei Zheng and Shiow Y. Wang, 2001
7	*Ginkgo biloba*	Phenolics	methanol	Antioxidant activity	Liu, *et al*., 2007
8	*Ginkgo biloba*	_	extracts	Antioxidant activity	Oyama *et al*, 1996
9	*Ginkgo biloba*	Diterpenes and sesquiterpenes	_	Antioxidant activity	Oberpichler, *et al*, 1988
10	*Ginkgo biloba*	flavonoids and biflavonoids	_	Antioxidant activity	Chen, *et al*., 1999
11	*Ginkgo biloba*	_	_	Antioxidant activity	Joyuex, *et al*., 1995
12	*Ginkgo biloba*	Leaves	methanol	Antioxidant activity	Feng, et al., 2009
13	*Stevia rebaudiana*	Leaf	methanol	Antioxidant activity	Buran Phansawan1 and Supak Poungbangpho (2007)
14	*Stevia rebaudiana*	Leaf	ethanolic	Antioxidant activity	Buran Phansawan1 and Supak Poungbangpho (2007)
15	*Stevia rebaudiana*	Leaf	ethanolic	Antioxidant activity	Shukla, et al., (2009)

hysterophorous. The methanolic extracts of *Parthenium hysterophorous* have the second highest activity in whole experiment. The methanolic extracts of *Parthenium* have high antioxidant activity 78.25561%. After the *Ginkgo* the *Parthenium* leaves have second highest potential to scavenge the free radicals of DPPH in the experiment. But the ethanolic extracts of *Parthenium hysterophorous* have the least Free radical scavenging activity in the whole experiment, which was recorded 66.28858%.

Discusion

Plants can accumulate secondary metabolites. Antioxidant potential of these three plants was determined by using DPPH°-free radical. *Ginkgo biloba* had significantly higher antioxidant potential than other plants. The antioxidant testing can reveal various mechanisms of action, depending on features of the particular assay. Simple methods include free radical scavenging with use of colored, artificial stable free radicals such as 2,2 prime;-azinobis-(3-ethylbenzothiazoline- 6-sulfonate) (ABTS used in the TEAC assay — Trolox equivalent antioxidant capacity) and DPPH (1,1-diphenyl- 2-picrylhydrazyl free radical), as well as transition metal reduction that can be monitored by colorimetry. The metalbased methods include the reduction of ferric ions: FRAP — (ferric reducing ability of plasma) and ferric thiocyanate assays, or molybdenum ion — phosphomolybdenum (P-Mo) assay. These tests are easy and affordable and can be used in high throughput screening. Their main drawback is that their relevance to the real oxidizing life is somewhat limited. The first issue is the chemical context of the assays, which use artificial compounds or are conducted in unrealistic conditions. This problem is eliminated in methods based on naturally occurring reactive oxygen species, but the fate of a free radical is observed either indirectly with chromophore reagents or with more expensive ESR techniques (electron spin resonance). The scavenging of superoxide radical anion, hydroxyl radical, or nitric oxide can be observed. *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous* were used to determine antioxidant activity. Antioxidant activity was determined by using DPPH method. Two extract of *Ginkgo biloba* gave the best results which were 82.80491% and 76.67588%. *Stevia rebaudiana* shows 77.67446% and 67.07688% results. *Parthenium hysterophorous* showed 78.25561% and 66.28858% results.

Conclusion

The three important plants were subjected to two extractions with two solvent including methanol and ethanol. Highest antioxidant potential was found in *Ginkgo biloba* in methanolic extract followed by *Parthenium hysterophorous* and *Stevia rebaudiana*. From the present data methanol was the best solvent for extraction of all the medicinal plants. It means that methanol have potential to extract more active constituents from plants and showed maximum antioxidant activity. Ethanolic extract of three plants showed different results from methanolic. In methanolic extract *Ginkgo biloba* was the first plant which has high activity followed by *Parthenium hysterophorous* and *Stevia rebaudiana*, while in ethanolic extracts *Ginkgo biloba* was observed to have high potential for antioxidant activity followed by *Stevia rebaudiana* and *Parthenium hysterophorous*. From the experiment it was observed that these plants have certain important constituents which were responsible for Radical scavenging activity. Radical scavenging activity was observed when discoloration was occurred. *Ginkgo biloba* was observed to have high discoloration followed by *Parthenium* and *Stevia*. When the difference was high between the DPPH solution and sample, the percent free radical activity is high or the sample was high potential to scavenge the free radical of DPPH. This study reveals that tested plant materials have significant free radical scavenging activity. The result of the present study suggests that these plants can be used as a source of antioxidants for different diseases. These plants are an effective potential source of natural antioxidants. This free radical scavenging protocol is efficient for *Ginkgo biloba, Stevia rebaudiana* and *Parthenium hysterophorous* and need biotechnology techniques for feasible and increase continuous production of biologically active compounds at a comparable rate to commercially available antioxidants.

Cells are protected from free-radical–induced damage by a variety of endogenous radical scavenging antioxidant proteins, enzymes, and chemical (water or lipid soluble) compounds. These include metal ion sequester proteins such as transferrin or caeruloplasmin, enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, biothiols such as glutathione, and vitamins C and E. Cellular damage arising from an imbalance between these free radical generating and scavenging systems has been implicated in the pathogenesis of a wide range of disorders, including neurodegenerative disorders, cardiovascular disease, cancer and aging [52].

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