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Research Article

Evaluation of antioxidant activity of *Alpinia purpurata rhizome* (*Vieill*)

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ABSTRACT

Research field with endless potential is especially important in countries possessing great interest to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen. The present study investigates the quantitative analysis of secondary metabolites and antioxidant activity of three various extracts of *Alpinia purpurata rhizome*. Using DPPH, ABTS and Ferric reducing antioxidant power assay FRAP to investigate the antioxidant potential of crude extracts. The extracts showed the inhibition in a dose dependant manner. The results were expressed as IC_{50} values, considering the growing interest in assessing the antioxidant capacity of herbal medicine. The ethanolic extract of *Alpinia purpurata* rhizome exhibited a DPPH radical scavenging higher activity, FRAP and ABTS.

Keywords: *Alpinia purpurata*; Secondary metabolites; Antioxidants; FRAP; ABTS; DPPH.

1. INTRODUCTION

Antioxidants have received increased attention by nutritionists and medical researchers for their potential. Epidemiological studies have provided evidence of an inverse association between diets rich in fruits, vegetables and disease. These health promotion effects may be related to components in the foods with antioxidant activity (Kaur and Kapoor. 2001). The ability of antioxidants to scavenge free radicals in the human body and thereby decrease the amount of free radical damage to biological molecules like lipids and DNA may be one of their protective mechanisms. However, clinical trials using 'nutritional' antioxidants in food such as vitamins C and E have given equivocal results (Emmert and Kirchner, 1999; Hercberg *et al.,* 1999). Free radical is any atom or a molecule, which has a single electron on its external orbit. Unstable free radicals are produced in normal metabolism when oxygen is used to burn food for energy. A free radical is defined as "any species capable of independent existence that contains one or more unpaired electrons. Generally, free radicals are more reactive than non radicals and will react with them to produce new free radicals in a chain reaction (Halliwell *et al.,* 1995). The study of free radicals and antioxidants is producing huge advances in medicine. Reactive oxygen species (ROS) play key roles in chronic degenerative diseases including cancer, inflammatory, cardiovascular and neurodegenerative diseases and aging (Andlauer and Furst, 2003; Finley, 2003; Golden *et al.,* 2002). In the last years, interest in the antioxidant activity of plant extracts, or isolated substances from plants, has grown, due to the fact that free radicals have been related to some diseases, as well as to the aging process (Walker *et al.,* 2009). Reactive oxygen species (ROS) are generated by normal metabolic processes in all oxygen utilizing organisms. It is estimated that about 1% of the total oxygen consumed by mitochondria is transformed into superoxide anion (Boveris and Chance, 1973). Damage induced by ROS includes DNA mutation, protein oxidation and lipid peroxidation, contributing to the development of cancer, diabetes, atherosclerosis, inflammation, and premature aging (Finkel and Holbrook, 2000). About 95% of the pathologies observed in people above 35 years of age are associated with production and accumulation of free radicals (Gordon, 1996). In search for sources of novel antioxidants, in the last few years some medicinal plants have been extensively studied for their radical scavenging activity (Joyeux et al., 1995; Malick and Sing, 1980). The rhizomes of Zingiberaceae plants are widely used as spices or traditional medicine in Asian countries, eaten raw, or cooked as vegetables and as flavouring. Leaves of several Zingiberaceae have also been used for food flavouring and in traditional medicine (Harborne, 1973). In the phytochemical study was focused to reveal the quantitative and qualitative analysis, Tannins, Resins, Proteins, Alkaloids, Flavonoids, Glycosides, Phenols and Saponins presented in ethanolic extract of parts of *Alpinia purpurata* rhizome. The purpose of the present work is to determine the antioxidant activities of *Alpinia purpurata* rhizome by DPPH radical assay, ABTS+ radical assay, Ferric reduc-

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ing ability of plasma (FRAP) assay and measurement of total Phenols, Flavonoids, Alkaloids, and Tannins contents.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh plant material was collected from Kovaipudhur, Coimbatore District, Tamil nadu, India. Efforts were made to collect the plant in rhizomes and flowering conditions for the correct botanical identification. The plant material was brought to the laboratory and identified with the help of Agriculture university of Coimbatore, Tamil Nadu State.

2.2. Sample preparation

Crude extracts was obtained from rhizome of *Alpinia purpurata* Coarse powder of the plant material was extracted by soxhlet method using successive solvents such as petroleum ether, chloroform, ethanol, methanol and aqueous in increasing polarity for 48 hours respectively. The extracts were concentrated and dried under reduced pressure.

2.3. Antioxidant Determinations

Ascorbic acid content was determined using the 2, 6-dichlorophenol-indophenol titration method described (Trease and Evans, 1982). Lascorbic acid was used to prepare a standard solution (1 mg/mL). The ascorbic acid concentration was calculated by comparison with the standard and expressed as mg/ 100 g fresh mass. Total phenolics content was determined by the Folin–Ciocalteu method, which was adapted from (Harborne, 1973). The 150 mL of extract, 2400 mL of nanopure water, and 150 mL of 0.25 N Folin–Ciocalteu reagents were combined in a plastic vial and then mixed well using a Vortex. The mixture was allowed to react for 3 min then 300 mL of 1NNa2CO3 solution was added and mixed well. The solution was incubated at room temperature (37°C) in the dark for 2 h. The absorbance was measured at 725nm using a spectrophotometer and the results were expressed in gallic acid equivalents (GAE; mg/100 g fresh mass) using a gallic acid (0–0.1 mg/mL) standard curve. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

2.4. Determination of total flavonoid content

Flavonoids were estimated (Cameron et al., 1943). The plant samples (0.5g) were extracted first with methanol: water mixture (2:1) and secondly with the same mixture in the ratio 1:1. The extracts were shaken well and allowed to stand overnight, pooled the supernatants and measured the volume. This was concentrated and then used for the assay. An aliquot of the extract was pipetted out and evaporated to dryness. Vanillin reagent (4.0) ml was added and the tubes were heated for 15minutes in a boiling water bath. Varying concentrations of the standard were also treated in the same manner. The optical density was read at 340nm. The standard curve was constructed and the concentration of flavonoids was calculated. The values are expressed as mg flavonoids/g sample.

2.5. Determination of total phenol content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Malick and Sing, 1980; Tepe, and Sokmen, 2007). The extract samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 650nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg/ ml solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a common reference compound.

2.6. Determination of total Alkaloid content

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Halliwell *et al.,* 1995).

2.7. Determination of Tannins Content

Estimation of total tannins (Sadipo *et al.,* 1991). Weighed 0.5g of the powdered material and transferred to a 250ml conical flask. Added 75ml water. Heated the flask gently and boiled for 30 minutes, centrifuged at 2000 rpm for 20 minutes and collected the supernatant in 100 ml volumetric flask and made up to 100ml. Transferred 1ml of the sample extract to a 100ml volumetric flask containing 75 ml water. Added 5ml for folin's Denis reagent, 10ml of sodium carbonate solution and diluted to 100ml with water shook well and read the absorbance at 700nm after 30 minutes. Standard graph was prepared by using 10-100µg tannic acid, blank was prepared with water instead of the sample; the amount of tannin was expressed as mg/g of sample.

2.8. Antioxidant Activity Determinations

2.9. DPPH Photometric Assay

This method was given (Finley, 2003) and later modified (Re, Roberta et al., 1999). It is one of the most extensively used antioxidant assay for plant samples. Recently the assay has been used to determine antioxidant activity in Tanacetum (Silva *et al.,* 2005; Swain and Hillis, 1959). This method is based on scavenging of the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) from the antioxidants, which produces a decrease in absorb-

Table 1: Different concentration of *Alpinia purpurata rhizome* in DPPH photometric assay

Concentration	Aqueous Extract (%)		Ethanolic Extract (%)		Chloroform Extract (%)		Standard (Ascorbic acid)	
	Inhibition %	IC_{50} µg /ml	Inhibition %	IC_{50} µg /ml	Inhibition %	IC_{50} µg /ml	Inhibition %	IC_{50} µg /ml
25µg	1.52 ± 0.05	375	2.78 ± 0.04	90	2.04 ± 0.03	110	27± 0.17	50
50 µg	6.18 ± 0.09		14.85 ± 0.10		12.28 ± 0.12		50± 0.20	
125 µg	28.24 ± 0.11		69.29 ± 0.51		60.32 ± 0.58		97± 0.36	
250 µg	40.64 ± 0.23		79.48 ± 0.38		75.06 ± 0.64		181± 0.35	
500 µg	60.12 ± 0.42		81.24 ± 0.41		80.82 ± 0.71		322± 0.36	
CD (p<0.05)	1.07							

Values are mean ± SD of three samples in each column

Table 2: Different concentration of *Alpinia purpurata rhizome* in ABTS assay

Concentration	Aqueous Extract (%)		Ethanolic Extract (%)		Chloroform Extract (%)		Standard (Ascorbic acid)	
	% Inhibition	IC_{50} mg /ml	% Inhibition	IC_{50} µg /ml	% Inhibition	IC_{50} µg /ml	% Inhibition	IC_{50} µg /ml
100 µg	18.14 ± 0.10	120	39.39 ± 0.31	233	22.46 ± 0.16	480	90±0.26	50
250 µg	34.52 ± 0.19		61.49 ± 0.46		40.08 ± 0.35		181±0.35	
500 µg	39.37 ± 0.21		69.05 ± 0.59		52.82 ± 0.27		322±0.36	
1000 µg	44.60 ± 0.27		71.28 ± 0.63		63.02 ± 0.33		532±0.22	
CD (p<0.05)	0.94							

Values are mean ± SD of three samples in each column

ance at 517 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of colour. This delocalization is also responsible for the deep violet colour, characterized by an absorption band in ethanol solution at about 520 nm.

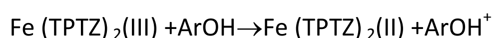
Representing the DPPH radical by Z* and the donor molecule by AH, the primary reaction is: $Z^* + AH \rightarrow ZH + A^*$

2.10. ABTS•+ *Radical Cation Scavenging Activity*

Antioxidant activities of the samples before and after the gastric and duodenal phases of digestion were also analysed by investigating their ability to scavenge the ABTS•+ free radical using a modified methodology previously reported by (Ozgen *et al.,* 2006). When combined with an oxidant (2.45 mM potassium persulfate), ABTS (7 mM in 20 mM sodium acetate buffer, pH 4.5) reacts to create a stable, dark blue-green radical solution following 12–16 h of incubation in the dark (4°C). The solution was then diluted to an absorbance of at 734 nm to form the test reagent. Reaction mixtures containing 20 µL of sample and 3 mL of reagent were incubated in a water bath at 30°C for 30 min. As unpaired electrons are sequestered by antioxidants in the sample the test solution turns colourless and the absorbance at 734 nm is reduced. An aliquot from each test tube was removed and placed into a cuvette to prevent occlusion of the light pathway by sediment in the juice before the absorbance of each sample was measured. The percentage inhibition was calculated against a control and compared to a Trolox standard curve 10–100 mM and expressed as fold change compared to the sample.

2.11. FRAP *Ferric-Ion Reducing Antioxidant Power*

Ferric reducing ability of plasma (FRAP) assay is a technique to determine the total antioxidant power interpreted as the reducing capability. The FRAP assay was first given by (Benzie and Strain, 1999). This assay was very recently used (Pulido *et al.,* 2000). The FRAP reagent is prepared by adding 200ml of acetate buffer; 20 ml TPTZ; 20ml $FeCl_3$ and 24 ml distilled water. Keep the reagent in desiccators a discard if the solution tube blue and prepare fresh solution after rinsing the bottle thoroughly with demineralised water. Mix and keep in water bath at 37°C. Run a set of blank and washed down cuvettes with distilled water. Add 1 ml FRAP reagent vigorously to each cuvette and mix the contents thoroughly and set the spectrophotometer at 593 nm. The temperature is kept at 37°C for 4 min. After 4 min, zero the blank and press run record.



$$Fe\,(TPTZ)_2(III) + ArOH \rightarrow Fe\,(TPTZ)_2(II) + ArOH^+$$

2.12. Statistical Analysis

Tests were carried out in triplicates. Statistical analyses were carried out using the statistical package SPSS version (10). Differences among the tested antioxidants were analysed by using two-way ANOVA. Values are expressed as the Mean ± SD and differences between groups were considered to be significant if $p<0.05$.

3. RESULTS AND DISCUSSION

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, various extracts extract. This indicates a highly potential as free radical scavengers (Table 1). *Alpinia purpurata* extracts of rhizome showed a higher capacity of scavenging the DPPH free radical.

ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods (Huang and Prior, 2005). In this assay, ABTS is converted to its radical cation by addition of sodium persulfate. This radical cation is blue in color and absorbs light at 734 nm (Pulido *et al.,* 2000). The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols and Vitamin C (Walker *et al.,* 2009). During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form.

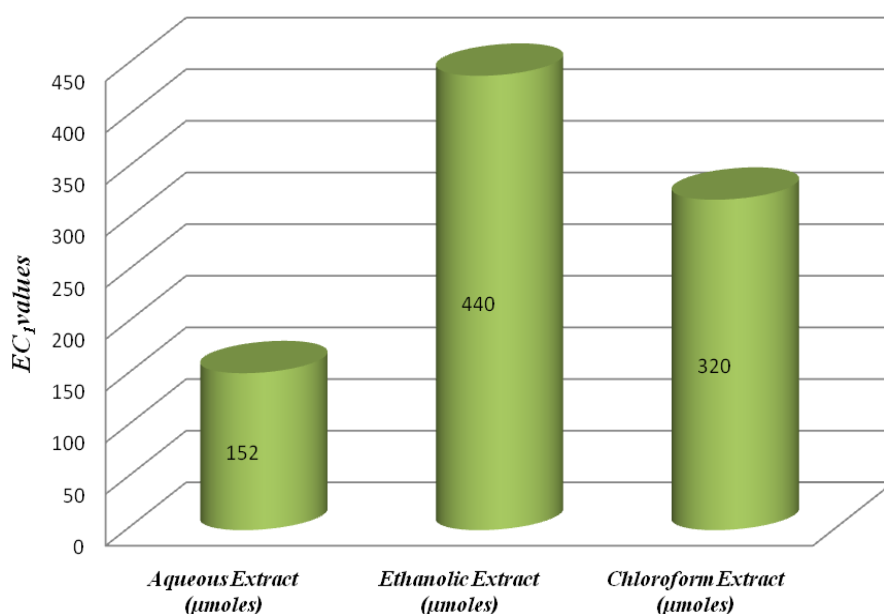


Figure 1: Different extracts of *Alpinia purpurata* in FRAP assay

Table 3: Quantitative Estimation of Phytoconstituents in Different Extracts of *Alpinia purpurata rhizome*

Parameters	Aqueous Extract %	Ethanol Extract %	Chloroform Extract %
Phenols	6.2 ± 0.09	9.5±0,10	6.37±0.09
Alkaloids	0.23±0.02	0.38±0.02	14.9±0.11
Flavonoids	0.61±0.02	0.85±0.02	0.79±0.05
Tannins	13.8±0.10	12.5±0.10	0.60±0.02

Values are mean ± SD of three samples in each column

of the rhizome of *Alpinia purpurata* showed potential free-radical scavenging activity. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured ethanol solutions. The use of DPPH provides an easy and rapid way to evaluate antioxidant activity. Results of DPPH reduction by extracts are shown in Table 1. The antioxidant activities of the individual compounds, present in the extracts may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features (Patt *et al.,* 1990). It is found that IC_{50} value of *Alpinia purpurata rhizome* is 90µg/ml which indicates the remarkable antioxidant activity of the ethanolic

The reaction may be monitored spectrophotometrically. This assay is often referred to as the Trolox equivalent antioxidant capacity (TEAC) assay. The reactivity of the various antioxidants tested was compared to that of Trolox, which is a water-soluble analog of vitamin E (Barclay *et al.,* 1985). Results of ABTS reduction by extracts are shown in Table 2.

FRAP Ferric reducing ability of plasma (FRAP, also *Ferric ion reducing antioxidant power*) is an antioxidant capacity assays which uses Trolox as a standard (Benzie and Strain, 1996). The FRAP assay is often used to measure the antioxidant capacity of foods, beverages and nutritional supplements containing polyphenols. It is reduced in the presence of an antioxidant molecule.

Use of FRAP provides an easy and rapid way to evaluate antioxidant activity. Results of FRAP reduction by extracts are shown (Concentration of 50 µm of aqueous, 152µm, ethanolic, 440µm and chloroform, 320µm) in figure-1. This result is evident for the majority of the ethanolic extract, hereby the greatest increase was observed. All tests were run on triplicate and mean values were used to calculate EC_1 values. EC_1 is defined as concentration of an antioxidant having a ferric reducing ability equivalent to that of 1mM ferrous salt.

The rhizome of *Alpinia purpurata* was carried out in different extracts in the present investigation, quantitative estimations were also carried out for Phenols, Flavonoids, Alkaloids, and Tannins. The results are given in table-3. Results of the quantitative estimation of Phenols and Tannins were more in aqueous, ethanolic, Alkaloids and Phenols were more in chloroform extract.

These variations were attributed to the structural factors of the individual antioxidants. With the present results it was difficult to reach any conclusion about the additive or synergistic contributions of individual antioxidants to the overall antioxidant activity of plant extracts since not all of their antioxidant components are known. It may also contain some other antioxidant components. On the other hand, *Alpinia purpurata rhizome* was found to contain a considerable amount of an antioxidant component which has an alkaloid, flavonoids with high phenolics content. This is confirmed by its ethanolic extract higher antioxidant activity than aqueous and chloroform extracts.

DISCUSSION

Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Victório *et al.,* 2009). Phenols, Flavonoids, Alkaloids, and Tannins contents were highly presented in the ethanolic extract which is compared with aqueous and chloroform extracts in the rhizome of *Alpinia purpurata*. Antihypertensive properties since alkaloids are known to be effective for these purposes (Parekh et al., 2007; Zeecheng, 1997). Phenols the aromatic compounds with hydroxyl group are widespread in plant kingdom. Phenols are said to offer resistance to diseases and pests in plants. Tannins are also known antimicrobial agents (Huang *et al.,* 2005; Parekh and Chandra, 2007). Flavonoids are also shown to inhibit microbes which are resistant to antibiotics (Sa´nchez-Moreno *et al.,* 1998; Willcox *et al.,* 2004). Antioxidants involved in the protection against tert-butylhydroperoxide (TBH), which leads to lipid peroxidation (Cragg *et al.,* 1997), are those available at the site of radical attack. They break the chain of oxidation by being preferentially oxidized by the attacking radical, thereby preventing oxidation of the adjacent fatty acid of cell membrane (Pereira *et al.,* 2003). A review pointed out that, today, approximately 60% of the anti-tumoral and anti-infective agents, either commercially available or in late stages of clinical trials are of natural origin (Sadipo *et al.,* 1991). Crucially the results of this study showed that antioxidant capacity is relatively stable in the ethanolic extract of *Alpinia purpurata rhizome*. The results of the three assays which were conducted are comparable in magnitude for ethanolic extract of *Alpinia purpurata rhizome*. There were some exceptions to this trend whereby particular ethanolic extract of *Alpinia purpurata* performed markedly better in one or more of the assays, ethanolic extract of *Alpinia purpurata* displayed the strongest radical scavenging capacity in both the DPPH• and ABTS•+ assays and also displayed the highest reducing capacity as measured by FRAP. A similar trend was observed for the ABTS•+ assay, in the DPPH• assay. There are several key differences between the extracts, which could account for these results. Differences were observed between the two radical scavenging assays (DPPH• and ABTS•+). Both assays measure the ability of all of the antioxidants in a sample to scavenge a preformed radical. This occurs because the mechanism involved in the reduction of DPPH free radicals is based on a scavenging activity. In this system, the structure (both planar and spatial) of the antioxidant compound, present in the extract, is important for its capacity of donating hydrogen ions. Compounds able to donate hydrogen are derived from the shikimate pathway, as for example, flavonoids (Tian and McLaughlin, 2000). These molecules are not produced by plants whose extracts display a very high IC_{50} in the DPPH test. Such plants are, in fact, very rich in compounds of the acetate pathway, like terpenoids and fatty acids, which are unable of scavenging the DPPH free radical, but are able to avoid oxidative damage of cell membranes (Association of Office Analytical Chemists, 1996; Das, 1987). The DPPH• radical is stable at formation, in fact in the current study the radical remained stable for a week or more (Brand-Williams, et al., 1995). Fundamental differences can be observed between the species of radical which is created in each of these methods and the methodologies by which they are executed. Firstly, the ABTS•+ assay is an electron transfer, end point assay whereby different antioxidant compounds donate one or two electrons to reduce the radical cation. Regardless of the donating potential of individual antioxidants they all have time to react fully giving an accurate measurement of total antioxidant capacity (TAC) at the end point of the assay (Cano *et al.,* 1998). The DPPH• assay is based on the normal hydrogen atom transfer (HAT) reaction that occurs between antioxidants and the peroxyl radical. Instead of peroxyl radicals, more stable and less transient nitrogen radicals are created, with which some antioxidants react more slowly than they would with the peroxyl radical in a biological system (Huang *et al.,*

2005). Additionally, the reaction then proceeds in a manner which bears more similarity to a single electron transfer (SET) reaction (Foti *et al.,* 2004). In the current study this does not seem to affect the TAC measurement of the ethanolic extract of *Alpinia purpurata rhizome*.

CONCLUSIONS

The ABTS, DPPH and FRAP assays gave comparable results for the antioxidant activity measured in aqueous, ethanolic and chloroform extracts of *Alpinia purpurata rhizome*. The FRAP technique showed high reproducibility, was simple, rapidly performed and showed the highest correlation with both ascorbic acid and total Phenols, Flavonoids, Alkaloids, and Tannins contents. Therefore, it would be an appropriate technique for determining antioxidant activity of extracts of *Alpinia purpurata rhizome*. Antioxidant activity measured in different extracts may also be estimated indirectly by using ascorbic acid or total phenolics since they showed high correlation with all assays. Antioxidant activity measured in aqueous extract of *Alpinia purpurata rhizome* was low compared to antioxidant activity measured in ethanolic and chloroform extracts. Ascorbic acid and phenolics are the major contributors to antioxidant activity in the extracts of *Alpinia purpurata rhizome* and the results confirm the use of the plant in traditional medicine. The most active extracts could be subjected to isolation of the therapeutic active components and it would be carried out for further pharmacological evaluation.

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REFERENCES

Andlauer, W., & Furst, P, 2003. Special characteristics of non-nutrient food constituents of plants-phytochemicals. Introductory lecture. International Journal for Vitamin and Nutrition Research, 73, 55–62.

Association of Office Analytical Chemists. Official Methods of Analysis, 15th ed. George Banta, Washington, DC. 1996.

Barclay, L. R. C.; Locke, S. J.; MacNeil, J. M, 1985. "Autoxidation in micelles - Synergism of Vitamin-C with Lipid-Soluble Vitamin-E and Water-Soluble Trolox", *Can. J. Chem.* 63 (2): 366–374.

Benzie IF and Strain JJ, Anal Biochem. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. 15; 239(1):70-6.

Benzie, I. F. F., & Strain, J. J. Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology, 1999: 299, 15–27.

Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem J 1973; 134:707–16.

Brand-Williams, W., Cuvelier, M. E., & Berset, C, 1995. Use of a free-radical method to evaluate antioxidant activity. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie, 28(1), 25−30.

Cameron GR, Milton RF and Allen JW. Lancent 1943; 179 – 180.

Cano, A., Hernandez-Ruiz, J., Garcia-Canovas, F., Acosta, M., & Arnao, M. B, 1998. An end-point method for estimation of the total antioxidant activity in plant material. Phytochemical Analysis, 9(4), 196−202.

Cragg GM, Newman DJ, Weiss RB. Coral reefs, forests, and thermal vents: the worldwide exploration of nature for novel antitumor agents. Semin Oncol 1997; 24:156–63.

Das U, 1987. Essential fatty acids, free radicals, limphokines and AIDS. J Assoc Physicians India; 35:611–2.

Dastmalchi, K., DamienDorman, H. J., Laakso, I., & Hiltunen, R, 2007. Chemical composition and antioxidative activity of Moldavian balm (Dracocephalum moldavica L.) extracts. Food Science and Technology, 40, 1655–1663.

Emmert, D.H., Kirchner, J.T., 1999. The role of vitamin E in the prevention of heart disease. Archives of Family Medicine 8, 537–542.

Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408:239–47.

Finley, J. W, 2003. The antioxidant responsive element (ARE) may explain the protective effects of cruciferous vegetables on cancer. Nutrition Reviews, 61, 250– 254.

Foti, M. C., Daquino, C., & Geraci, C, 2004. Electron-transfer reaction of cinnamic acids and their methyl esters with the DPPH center dot radical in alcoholic solutions. The Journal of Organic Chemistry, 69(7), 2309−2314.

Golden, T. R., Hinerfeld, D. A., & Melov, S, 2002. Oxidative stress and aging: Beyond correlation. Aging Cell, 1, 117–123.

Gordon MH. Dietary antioxidants in disease prevention. Nat Prod Rep 1996; 13:265–73.

Halliwell B., Murcia A.M., Butler J., Chiroco S., Aruoma O.I., 1995b. Free radicals and antioxidants in food

and *in vivo*: what they do and how they work. Crit. Rev. Food Sci. Nutr, 35, 7-20.

Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd. 1973; 49- 188.

Hercberg, S., Galan, P., Preziosi, P., 1999. Antioxidant vitamins and cardiovascular disease: Dr. Jekyll or Mr. Hyde,American Journal of Public Health 89, 289–291.

Huang, D., Ou, B., & Prior, R. L, 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 53(6), 1841−1856.

Huang, Dejian; Ou, Boxin; Prior, Donald L, 2005. "The Chemistry Behind Antioxidant Capacity Assays", *J. Agric. Food Chem.* 53 (6): 1841–1856,

Joyeux M, Moitier F, Fleurentin J, 1995. Screening of antiradical, antilipoperoxidant and hepatoprotective effects of nine plant extracts used in caribbean folk medicine. Phytother Res; 9: 228–30.

Kaur C, Kapoor HC. 2001. Antioxidants in fruits and vegetables - the millennium's health. Intl J Food Sci Tech 36:703–25

Lim, Y. Y., & Murtijaya, J. 2007. Antioxidant properties of Phyllanthus amarus extracts as affected by different drying methods. Food Science and Technology, 40, 1664–1669.

Linuma M, Tsuchiya H, Sato M, Yokoyama J, Ohyama M, Ohkawa Y,1994. J Pharmacol; 46(11): 892-895.

Malick CP and Sing MB. In; plant enzymology and histoenzymology, Kayan publishers, New Delhi, 1980; 286.

Ozgen, M., Reese, R. N., Tulio, A. Z., Scheerens, J. C., & Miller, A. R. (2006). Modified 2, 2- azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) methods. Journal of Agricultural and Food Chemistry, 54(4), 1151−1157.

Parekh J and S Chanda. J Microbiol 2007; 38: 204-207.

Patt, D.E. and B.J.F. Hudson, 1990. Natural antioxidants not exploited commercially. *In: food antioxidants.* Hudson B.J.F., Ed: Elsevier Applied Science: London, U. K. pp: 171-191.

Pereira MD, Herdeiro RS, Fernandes PN, Eleutherio ECA, Panek AD 2003. Targets of oxidative stress in yeast sod mutants. Biochim Biophys Acta; 1620:245–51.

Pulido R, Bravo, L and Saara-Calixto, F. 2000. Antioxidant activity of Dietary Polyphenols as determined by a modified ferric Reducing / Antioxidant power assay, J . Agric, Food. Chem, 48. 3396-3402.

Re, Roberta; Pellegrini, Nicoletta; Proteggente, Anna; Pannala, Ananth; Rice-Evans, Catherine 1999. "Antioxidant activity applying an improved ABTS radical cation decolorization assay", *Free Radical Biology and Medicine* 26 (9-10): 1231–1237,

Sa´nchez-Moreno, J. A., Larrauri & Saura-Calixto, F. 1998. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture, 76, 270–276.

Sadipo OA, Akanji MA, Kolawole FB, Odutuga AA. Bio Res Commun 1991;3: 171.

Schander SH. In : methods in food analysis, Academic press, New York, 1970; 709.

Silva C.G. Herdeiro R.S. Mathias C.J. Panek A.D. Silveira C.S. Rodrigues V.P. Renno M.N. Falcoa D.Q. Cerqueira D.M. Minto A.B.M. Nogueira F.L.P. Quaresma C.H. Silva J.F. Menezes M F.S. Eleutherio E.C.A, 2005. Evaluation of antioxidant activity of Brazilian plants. Pharmacological Research 52, 229–233

Swain, T., Hillis, W.E., 1959. The phenolic constituents of Prunus domestica I—the quantitative analysis of phenolic constituents. Journal of Science of Food and Agriculture 10, 63–68.

Tepe, B., & Sokmen, A. 2007. Screening of the antioxidative properties and total phenolic contents of three endemic Tanacetum subspecies from Turkish flora. Bioresource Technology, 98, 3076–3079.

Tian F, McLaughlin JL, 2000. Bioactive flavonoids from the black locust tree, *Robinia pseudoacacia*. Pharm Biol; 38:229–34.

Trease GE, Evans WC. Phamacognosy. Baillene Tindall, London 1982; 735- 738.

Victório CP, Kuster RM & Lage CLS, 2009. J Med Plants 11(2): 147-53

Walker, Richard B.; Everette, Jace D. 2009. "Comparative Reaction Rates of Various Antioxidants with ABTS Radical Cation", *J. Agric. Food Chem.* 57 (4): 1156–1161,

Willcox JK, Ash SL, Catignani GL, 2004. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr; 44:275–95.

Zee-cheng Rk, 1997. Plants Drugs Future; 22 (5): 515-530.