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Research article

ANTI-OXIDANT ACTIVITY OF SOME SELECTED ANTI-CANCER PLANTS

Bhavita Dhru, Japan Thakkar, Maitreyi Zaveri*

Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India

Abstract

Nowadays and even back in time herbal medicines were used by people because of natural herbs used and because of the reduced side effects of the herbal medicine. Anti-oxidant substances or medicines can be useful in a plethora of ways. Let's try to understand this, during normal metabolism process, oxidation occurs and it releases free radicals which cause cell damage. Because of cell damage, there are certain diseases which put human health in danger such as, diabetes, cancer, obesity and many more. To reduce the risk of certain diseases there should be anti-oxidant intake which can reduce the oxidative stress and reduce the cell damage caused by the free radicals, which can improve the human health at last making human life healthier and happier. We select four plants i.e; *Brassica nigra* (seed), *Mentha piperita* (fresh leaves), *Glycyrrhiza glabra* (stem) and *Cyperus rotundus* (rhizome). There were four different assays performed for determination of anti-oxidant activity. Two scavenging activities were performed and two chemical assays were performed. We used Ascorbic acid as standard. It can be clearly seen that *Brassica Nigra* and *Cyperus Rotundus* were the two plants which give better anti-oxidant activities as compared to other two plants.

Key words: Antioxidant activity, *Brassica nigra* (seed), *Mentha piperita* (fresh leaves), *Glycyrrhiza glabra* (stem), *Cyperus rotundus* (rhizome).

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*Corresponding Author

Introduction

Herbal medicines (HM) include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations and are used especially for the prevention and treatment of diseases [1].

Scientific research has shown that Herbal medicines contain complex chemical compounds that are responsible for the pharmacological activities, which corresponds to health benefits and/or toxicity they elicit [1]. Herbal medicines have been used as prophylaxes for the passive maintenance of health as well as for radical treatment of varieties of mild to serious diseases [2-5]. The pharmacological treatment of disease began long ago with the use of herbs [6]. Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging [7-8].An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule [9]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases[10]. In this project we select four plants and find their Antioxidant activity. All four plants are well-known plants with their many pharmacological activities. There were four different assays performed for determination of anti-oxidant activity.

Plant Introduction:

We select four plants i.e.; *Brassica nigra* (seed), *Mentha piperita* (fresh leaves), *Glycyrrhiza glabra* (stem) and *Cyperus rotundus* (rhizome).

Brassica nigra:

Brassica juncea, also known as Indian mustard, Chinese mustard, oriental mustard, leaf mustard, or mustard green, is a species of mustard family of *Brassicaceae (Cruciferae)* plants The leaves are used in a range of folk medicines as stimulants, diuretics and expectorants as well as a spice [11]. The major pungent chemical constituent of such commercialized oils is Allyl isothiocyanate [12]**.** This isothiocyanate is now considered to be the most important cancer chemo-preventive phytochemical with other potential health benefits [13-14].

Mentha piperita:

Mentha which is also famous as mint is a genus of plants fit in the *Lamiaceae* family. The mentha species have essential oils as main active component [15]. Major antioxidants comprise Phenolic acids such as rosmarinic and caffeic acids, flavones such as luteolin derivatives, and flavanones such as eriocitrin derivatives [16]. Essential oils of mentha have been mainly used as insecticides [17]. Menthol and essential oils of mint are also used in aromatherapy to relieve post-surgical nausea clinically [18].

Glycyrrhiza glabra:

Glycyrrhiza glabra Linn is one of the most extensively used medicinal herb from the ancient medical history of Ayurveda. It is employed in dyspepsia as an anti-inflammatory agent during allergenic reactions, as contraceptive, laxative, anti-asthmatic, emmenagogue, galactagogue, antiviral agent in folk therapy [19-20]. Glycyrrhiza roots are useful for treating cough because of its demulcent and expectorant property [21]. Ju, H.S. reported that flavonoids from liquorice are currently the strongest natural antioxidants known [22].

Cyperus rotundus:

C. rotundus Linn belong to family – Cyperaceaeare, the largest family in the monocotyledons [23]. *C. rotundus* is a traditional herbal medicine used widely as analgesic, sedative, antispasmodic, antimalarial, stomach disorders and to relieve diarrhea, pain, fever, diarrhoea, dysentery, an emmenagogue and other intestinal problems [24-26]. Different phytochemical studies on *C. rotundus* revealed the presence of alkaloids, flavonoids, tannins, starch, glycosides, furochromones, monoterpenes, fatty oil, glycerol, linolenic, myristic and stearic acids [27-30].

Materials and Methods

Preparation of extracts: All the plant materials were collected from LVG (Lalubhai Vrijlal Gandhi) store of required quantity. Each part plant material used for extraction were accurately weighed 50gm and 500ml of water was added. This was kept for overnight for maceration, after 24 hours the solution was heated and then filtered. Repeat this three times and collect the filtrate. The filtrate was then poured in porcelain dish and heated to get the extract. Similarly, four extracts of four different plants were collected. The aqueous extract of selected plants was then air-dried and stored

in airtight container until usage. This residue was weight and used for further investigation for potential antioxidant activity.

Chemical reagents and materials:

All different organic solvents used for extraction were obtained from the S.D. Chemicals Private Limited (Mumbai, India), and were analytical grade. The other chemicals used were 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS), sodium nitroprusside, sulphanilamide, potassium superoxide, ortho-phosphoric acid, napthyl ethylene diamine dihydrochloride, potassium chloride, ferric chloride, ferrous sulphate, thio barbituric acid, trichloro acetic acid (TCA), nitroblue tetrazolium (NBT), dimethyl sulphoxide (DMSO), ethylene diamine tetra acetic acid (EDTA), ortho- phenanthroline and sodium hydroxide (NaOH).

Equipment used: UV spectrophotometer and ELISA (enzyme-linked immunoassay) model were used during the process.

Antioxidant Activity was determined by using 2, 2-Diphenyl dipicrylhydrazl (DPPH) activity, 2, 2'-azino bis3-ehtylbenzothiazoline-6-sulfonic acid (ABTS), scavenging of nitric oxide radical and Hydrogen peroxide.

Reduction of 2, 2-Diphenyl dipicrylhydrazl (DPPH) free radical 31:

First of all, 0.1mM DPPH solution was prepared by adding 3.94mg in 100ml of ethanol. After addition of ethanol, the solution was homogenized for 30 seconds. After homogenizing, the solution was incubated in dark for 30 minutes at room temperature. The reason for incubating the DPPH solution in dark is because the light would oxidize further the DPPH radical with solution to be determined interfering with your results. After that, 96 well micro plates were taken, markings were done according to different concentrations. In test, 100µl of each extract of different concentrations were added to their respective well, and 100µl of DPPH solution was added to each well. In blank, 100µl of extracts were only added to their respective well plates. In control, 100µl of DPPH solution, and 100µl of ethanol was added to their respective well plate. Ascorbic acid was used as the standard for comparison. Absorbance was taken at 517nm in ELISA and results were noted and calculate the Oxygen Radical Absorbance Capacity (ORAC) of the extracts.

2, 2'-azino bis3-ehtylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity 32:

In ABTS radical cation decolorization assay method, dissolve 0.038gm of ABTS in 10ml distilled water. After that 2.45mM ammonium per sulphate solution is prepared by adding 0.559mg in 10ml distilled water. After that both the solutions are mixed and allowed to stand in dark at room temperature for 12-16 hours. After that 96 well micro plates are taken, markings were done, of different concentrations of each extract. In test, 100µl of each extract of different concentrations were added to their respective well plate, and 60µl of ABTS solution was added to each well. In blank, 100µl of each extract of different concentrations was added to their respective well. In control, 60µl of ABTS solution, and 40µl of ethanol was added. Absorbance was taken at 734nm, and results were noted and calculate the Oxygen Radical Absorbance Capacity (ORAC) of the extracts.

Hydrogen peroxide scavenging activity 33:

Hydrogen peroxide 2mM was prepared by mixing with 50mM phosphate buffer. First of all, phosphate buffer was prepared by adding 6.8gm potassium dihydrogen phosphate to 1L distilled water, pH is adjusted with NaOH to make pH 7.4. After that, 2mM hydrogen peroxide was prepared by adding 68.02mg of hydrogen peroxide to previously prepared phosphate buffer solution. Furthermore, three different concentrations of the extracts were prepared of 1000µg/ml, 500µg/ml, and 250µg/ml. For performing the activity, 0.300µl of each extract of each concentration was taken in a test tube, then 1.2µl of buffer was added to each test tube, and 1.8µl of hydrogen peroxide was also added to each test tube. The test tubes were allowed to stand for 10 minutes, after that absorbance was taken in UV spectrophotometer, under 230nm wavelength. 1.2µl of buffer and 1.8µl of hydrogen peroxide was taken as blank. The results were noted and then % hydrogen peroxide activity was calculated and calculate the Oxygen Radical Absorbance Capacity (ORAC) of the extracts.

Nitric oxide scavenging activity34:

Nitric oxide, generated from sodium nitropruside in aqueous solution at physiological pH, interact with oxygen to produce by Griess reaction. Saline solution was prepared by adding 9gm NaCl in 700ml of distilled water and was made up to 1L. Then, phosphate buffer was prepared by adding 6.8gm potassium dihydrogen phosphate to 1L above prepared saline solution and pH was adjusted to 7.4 with the help of NaOH. Furthermore, 2.98gm of sodium nitroprusside was added to 1L above prepared saline phosphate buffer.

After that, 96 well microplate was taken, markings were done, as test, blank, and control. In test, 30µl of each extract of different concentrations were added to their respective markings, and 30µl of sodium nitroprusside was added to each well. In blank, total of 130µl of each extract was added to their respective markings according to their respective concentrations. In control, 30µl of each extract to their respective well, and 300µl of Griess reagent (1% sulphanilamide, 2% H3PO4 and 1% naphthylethylene diamine dihydro chloride) was added. The absorbance of the chromophore formed was observed at 546 nm. The results were noted and calculate the Oxygen Radical Absorbance Capacity (ORAC) of the extracts.

Result

The antioxidant activity of aqueous extract of selected plants were tested. Anti-oxidants models such as Reduction of 2, 2-Diphenyl dipicrylhydrazl (DPPH) free radical, ABTS radical cation decolorization assay, scavenging of nitric oxide radical and Hydrogen peroxide were performed.

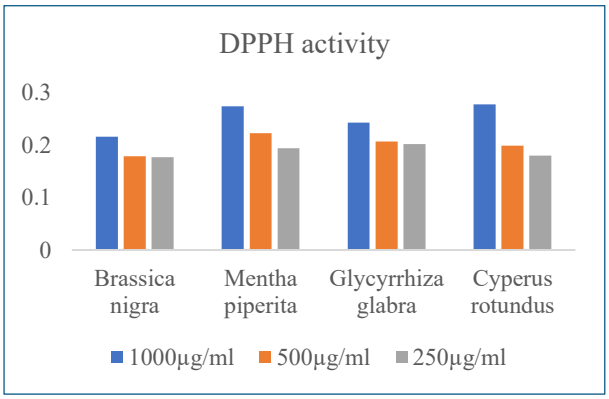
The % yield of all aqueous extract of Brassica nigra (8.36 %W/W), Mentha piperita (2.62 %W/W) Glycyrrhiza glabra (12.3 %W/W) Cyperus rotundus (6.52 %W/W). All the extracts were semi solid/solid form and highest yield were obtained from aqueous extract of Glycyrrhiza glabra.

Antioxidant activity:

DPPH free radical scavenging assay:

The capacity of samples to scavenge DPPH radical was estimated on the basis of their concentrations. The result indicates significant difference of mean percentage scavenging between different concentrations of the different aqueous extracts of selected plants. The scavenging activity of aqueous extract Cyperus rotundus 41.40% were obtained using the linear regression equation as shown in Fig. 1.

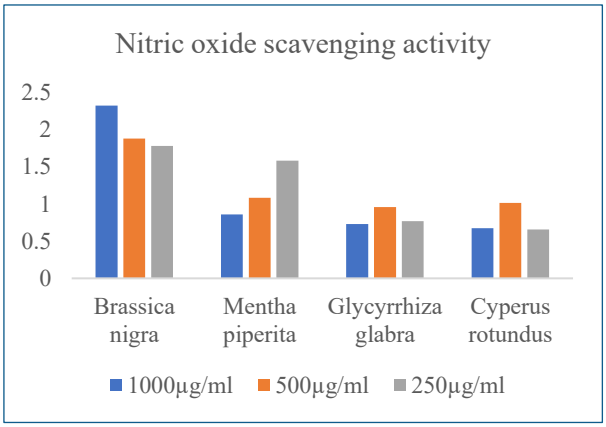
Figure 1: The scavenging ability of the different aqueous extracts on DPPH



ABTS Scavenging:

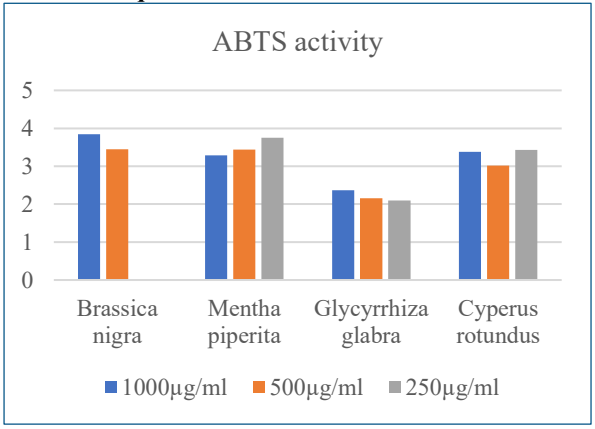
In figure 2, the dose response curve of ABTS Scavenging of the different aqueous extracts of selected plants were compared with reference standard ascorbic acid. Compared with the different extracts, aqueous extract of Glycyrrhiza glabra showed good ABTS Scavenging activity. Oxygen Radical Absorbance Capacity of aqueous extract of Glycyrrhiza glabra was 43.81%.

Figure 2: The scavenging ability of the different aqueous extracts on ABTS



Nitric Acid: The dose response curve of nitric acid of the different aqueous extracts of selected plants were compared with reference standard ascorbic acid as shown in figure 3. As compared to all the extracts aqueous extract of Cyprus rotundus showed good nitric oxide scavenging activity. Oxygen Radical Absorbance Capacity of aqueous extract of Cyprus rotundus was 1.30% as shown in Fig. 3. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological condition.

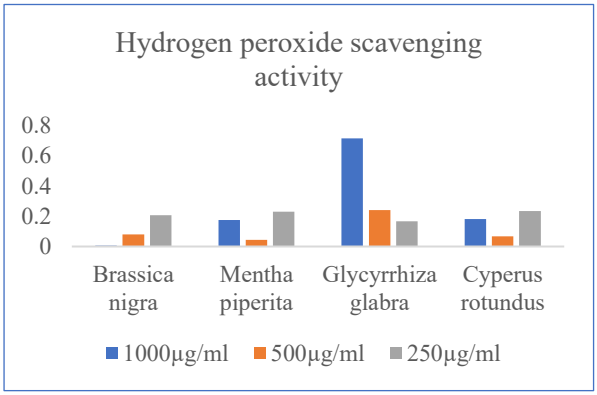
Figure 3: The scavenging ability of the different aqueous extracts on Nitric Acid



Hydrogen peroxide:

Hydrogen peroxide radical scavenging activity of the different aqueous extracts of selected plants were found to be dose dependent. The scavenging activity of aqueous extract of Cyperus rotundus was 53.0% as shown in Fig. 4.

Figure 4: The scavenging ability of the different aqueous extracts on Hydrogen peroxide



Discussion

Based on our result, it can be concluded that Brassica nigra and Cyperus rotundus are the two plants which has better anti-oxidant activities as compared to other G. glabra and M. piperita. The replacement of synthetic antioxidants with natural may be invaluable. In the present study analysis of free radical scavenging activity demonstrated that this plant can be the potent source of natural antioxidants. Therefore, further examinations be done to assess its bioactivity utilizing unadulterated compounds in developing potential pharmaceutical drugs. The present study adds to the existing knowledge of Brassica nigra and Cyperus rotundus and will be very useful for development of a formulation for treating various diseases.

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