KSV Journal of Pharmacy and Health Sciences

(Managed by K. B. Institute of Pharmaceutical Education and Research, Gandhinagar-382023, Gujarat, India)

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Research article

EVALUATION OF THE ANTI-OXIDANT ACTIVITY OF HERBAL ANTI-CANCER PLANTS

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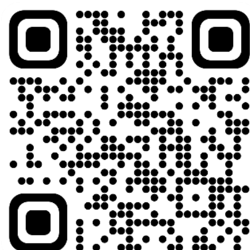
Abstract

Herbs have been used in both the olden days and the modern time because they contained natural ingredients and had minor side effects. Antioxidant drugs or additives are so multivalent in health benefits. In normal metabolism, there occurs oxidation leading to release of free radicals causing cell damage. The cell damage can lead to the higher diseases like diabetes, cancer, and obesity. Intake of antioxidants can help in preventing these diseases through relieving oxidative stress and minimizing the damage caused to cells by free radicals, which lead to better health and well-being. We selected four plants for the present study: *Brassica nigra* (seeds), *Mentha piperita* (fresh leaves), Glycyrrhiza glabra (stem), and *Cyperus rotundus* (rhizome). Four independent assays were done to evaluate antioxidant activity, which consisted of two scavenging assays and two chemical assays. Ascorbic acid was the standard. Results obtained showed that Brassica nigra and *Cyperus rotundus* have higher antioxidant activities than the other two plants.

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

Article Info: Received 18 Aug 2023; Review Completed 16 Sept 2023; Accepted 03 Oct 2023
Revised and resubmitted 01 Jul2024; Accepted 14 Aug2024



Cite this article as:
Dhru B, Thakkar J, Zaveri M. Anti-oxidant activity of some selected anti-cancer plants. KSV Journal of Pharmacy and Health Sciences 2024;1(1):1-7
Available from:
https://www.ksvjphs.com/index.php/journal/issue/current

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Introduction

Herbal medicines (HM) encompass a variety of plant-based substances, including herbs, herbal materials, preparations, and finished products, utilized for the prevention and treatment of ailments [1]. Research indicates that these medicines contain intricate chemical compounds responsible for their pharmacological effects, which may confer health benefits or toxicity [1]. Throughout history, herbal medicines have served both prophylactic and therapeutic purposes for a range of ailments [2-5]. Indeed, the medicinal use of herbs predates modern pharmacological treatments [6].

Oxidative stress plays a very important role in the development of many chronic diseases, with free radicals and reactive oxygen species concerned in conditions such as inflammatory disorders, asthma, diabetes, Alzheimer's and, Parkinson's diseases, cancers, atherosclerosis, and aging [7-8]. Antioxidants, broadly defined as substances that impede oxidative damage to molecules, including phenolic acids, polyphenols, and flavonoids, counteract free radicals and inhibit the oxidative processes associated with degenerative diseases [9-10].

This study focuses on investigating the antioxidant activity of four well-known plants, each renowned for its pharmacological properties. Four distinct assays were conducted to determine their antioxidant potential.

Plant Introduction:

Four herbal plants were selected such as. *Brassica nigra* (seed), *Mentha piperita* (fresh leaves), *Glycyrrhiza glabra* (stem) and *Cyperus rotundus* (rhizome).

Brassica nigra:

Brassica nigra, commonly known as Indian mustard which was belongs to the Cruciferae family. Traditionally, the leaves of Brassica nigra have been utilized in folk medicine as stimulants, diuretics, expectorants, and spices [11]. Allyl isothiocyanate is the primary pungent chemical component found in commercial Brassica nigra oils [12]. Presently, this isothiocyanate is recognized as a crucial cancer chemo preventive phytochemical, along with other potential health benefits [13-14].

Mentha piperita:

Mentha piperita, commonly known as mint, belongs to the genus of plants within the Lamiaceae family. Essential oils are the primary active components found in other Mentha species, including Mentha piperita [15]. These oils contain significant antioxidant constituents such as phenolic acids, flavones, and flavanones [16]. The essential oils of Mentha species have been utilized as insecticides [17]. Additionally, menthol and mint essential oils are employed in aromatherapy to clinically alleviate post-surgical nausea [18].

Glycyrrhiza glabra:

Glycyrrhiza glabra was commonly known as liquorice, has been extensively utilized in Ayurvedic medicine since ancient times. It has been employed for various purposes including treating dyspepsia, as an anti-inflammatory agent for allergic reactions, any many other uses [19-20]. The Glycyrrhiza roots were particularly valued for their demulcent and expectorant properties, often used to alleviate cough [21]. Some sources suggest that flavonoids derived from liquorice are among the most potent natural antioxidants currently known [22].

Cyperus rotundus:

Cyperus rotundas was belongs to the family Cyperaceae, which is the largest family among monocotyledons [23]. It has a long history of use as a traditional herbal medicine, employed for various purposes including analgesia, sedation, antispasmodic effects, treatment of malaria, stomach disorders, diarrhoea, pain, fever, dysentery, emmenagogue properties, and other intestinal issues [24-26]. Based on the literature review, *C. rotundas* have revealed the presence of phytoconstituents such as alkaloids, flavonoids, tannins, starch, glycosides, furochromones, monoterpenes, fatty oil, glycerol, linolenic acid, myristic acid, and stearic acid [27-30].

Materials and Methods

Preparation of extracts:

The herbal plant materials were sourced from the LVG (Lalubhai Vrijlal Gandhi) store in the required quantities. Each part of the herbal plant material used for extraction was precisely weighed at 50 grams, and 500 ml of water were added. The mixture was left to macerate overnight, and after 24 hours, the heated it and filtered. This process was repeated three times to ensure thorough extraction, and the filtrate was collected each time. The collected filtrate was then transferred to a porcelain dish and heated and obtain the extract. Similar way collects the extracts from four different plants. The aqueous extracts of the selected

herbal plants were subsequently air-dried and stored in airtight containers until needed. The residue obtained after drying was weighed and used for further investigation to assess antioxidant activity.

Chemical reagents and materials:
Analytical grade (AR) organic solvents were procured from S.D. Chemicals Private Limited in Mumbai, India.

Equipment used:
For analysis and measurements purpose here we have used UV spectrophotometer and ELISA (enzyme-linked immunoassay) models. To measure absorbance at specific wavelengths, the UV spectrophotometer was used for the quantification of various compounds/ reactions. For detecting and quantifying specific proteins or molecules in samples, relying on the binding of an enzyme-linked antibody to the target molecule, ELISA model was used. Above mentioned analytical methods were used for the assessment of the antioxidant activity and other biochemical parameters of the herbal extracts.

Methods:
"Reduction of 2, 2-Diphenyl Di picrylhydrazyl (DPPH) free radical" [31]:
Initially for the preparation of 0.1 milli mole DPPH solution, weigh 3.94 mg of DPPH, it was dissolved in 100 ml of ethanol solution. Then prepared solution was homogenized for 30 seconds after the addition of ethanol solution. Subsequently, at room temperature, the prepared homogenized solution. And then kept it for incubation in the dark room for half an hour to prevent further oxidation of the DPPH radical, which could interfere with the results.

For the assay, 96-well microplates were utilized, with markings corresponding to different concentrations. In the test solution, 100 µl of every extract at varying concentrations was added to their respective wells, then add 100 µl of the prepared DPPH solution in every well. In the blank solution, only 100 µl of the extracts were added. 100 µl of the 2,2-Diphenyl-1-picrylhydrazyl solution and 100 µl of EtOH were added in the well and it is considering as a control.

Ascorbic acid was employed as the standard for comparison. Absorbance measurements were taken at 517 nm using an ELISA reader, and the results were recorded. The Oxygen Radical Absorbance Capacity (ORAC) of the extracts was then calculated based on the obtained data.

2, 2'-azino bis3-ehtylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity [32]:
To prepare the ABTS solution (A), 0.038 grams of ABTS was dissolved in freshly prepared distilled water (10 ml). Additionally, 0.559 mg of ammonium persulfate was dissolved in distilled water (10ml) and prepare a 2.45 mM ammonium persulfate solution (B). Solution A and B were then mixed and stand in the dark at room temperature for 12-16 hours.

For the assay, 96-well microplates were used, with markings for different concentrations of all four extract. In the test solution, 100 µl of each extract at varying concentrations was added to their respective wells, followed by the addition of 60 µl of ABTS solution.

Blank solution: 100 µl of each extract at different concentrations were added in their respective wells without the ABTS solution.
Control solution: 60 µl of the ABTS and 40 µl of ethanol were added.

Absorbance measure at 734 nm using an ELISA reader, and the results were recorded. The ORAC of the extracts was calculated based on obtained data.

Hydrogen peroxide scavenging activity [33]:
In order to create a 2 mM hydrogen peroxide solution, 6.8 grams of potassium dihydrogen phosphate (KH_2PO_4) had to be dissolved in one litre of distilled water to create a phosphate buffer solution. NaOH was used to bring the solution's pH down to 7.4.

Following the preparation of the phosphate buffer solution, 68.02 mg of hydrogen peroxide was added to the phosphate buffer solution to create a 2 mM solution.

Three distinct doses of the extracts were employed for the assay: 1000 µg/ml, 500 µg/ml, and 250 µg/ml.

The hydrogen peroxide scavenging activity assay involved adding 1.8 µl of hydrogen peroxide, 1.2 µl of buffer, and 0.300 µl of each extract at each concentration to each test tube. The test tubes were then left for 10 minutes.

After that, absorbance were taken at 230 nm wavelength using a UV spectrophotometer. For the blank solution, 1.2 µl of buffer and 1.8 µl of hydrogen peroxide were mixed.

The percentage of hydrogen peroxide scavenging activity was calculated based on the absorbance readings. Finally, the Oxygen Radical Absorbance Capacity (ORAC) of the extracts was determined using the obtained data.

Nitric oxide scavenging activity [34]:
To generate nitric oxide from sodium nitroprusside in an aqueous solution at physiological pH, the Griess reaction was employed. First, a saline solution was prepared by dissolving 9 grams of NaCl in 700 ml of distilled water and then adjusting the volume to 1 litre. Next, a Phosphate-buffered saline (PBS) was prepared by adding 6.8 grams of potassium dihydrogen phosphate to 1 liter of the prepared saline solution and adjusting the pH to 7.4 using NaOH.

Subsequently, 2.98 grams of sodium nitroprusside was added to 1 litre of the saline phosphate buffer solution.

For the assay, a 96-well microplate was utilized and marking as test solution, blank solution, and control solution. In the test solution, 30 µl different concentrations of each extract was added to their respective markings, then add 30 µl of Na2[Fe(CN)5NO] (sodium nitroprusside) to each well.

In blank, a total of 130 µl of each extract was added to their respective concentrations.

Control solution preparation: 30 µl of each extract was added to their respective wells, then add 300 µl of Griess reagent (1% sulphanilamide, 2% H_3PO_4, and 1% naphthyl-ethylene diamine dihydro chloride).

At the wave length of 546 nm the absorbance of the formed chromophore was measured and the results were noted. Based on the obtained result data, the Oxygen Radical Absorbance Capacity (ORAC) of the extracts was calculated.

Result

The antioxidant activity was performed using various models for the evaluation of the aqueous extract of selected herbal plants. Antioxidant models such as:

- Reduction of 2,2, Diphenyl1picrylhydrazyl (DPPH) free radical.
- The assay for ABTS radical cation decolorization.
- Nitric oxide radical scavenging.
- Activity for scavenging hydrogen peroxide.

These assays were conducted to assess the ability of the herbal extracts to neutralize free radicals and exhibit antioxidant properties.

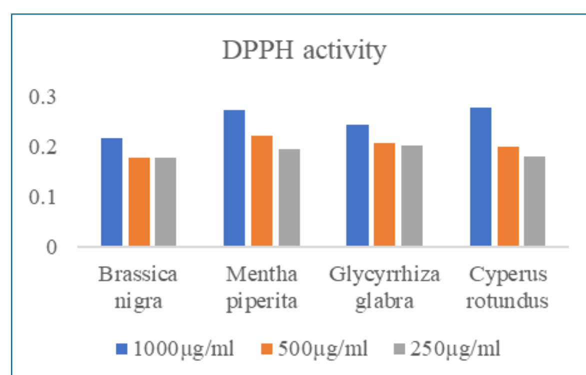
The antioxidant activity of aqueous extracts from selected herbal plants was assessed using various antioxidant models, including the reduction of 2,2, Diphenyl1picrylhydrazyl (DPPH) free radical, ABTS radical cation decolorization assay, scavenging of nitric oxide radical, and hydrogen peroxide.

The percentage yield of all the aqueous extracts was calculated as follows:

Brassica nigra: 8.36% (w/w)
Mentha piperita: 2.62% (w/w)
Glycyrrhiza glabra: 12.3% (w/w)
Cyperus rotundas: 6.52% (w/w)
Based above findings, it was noted that the highest yield was obtained from the aqueous extract of *Glycyrrhiza glabra*, indicating a substantial amount of extract was obtained in semi-solid/solid form from this particular selected plant.

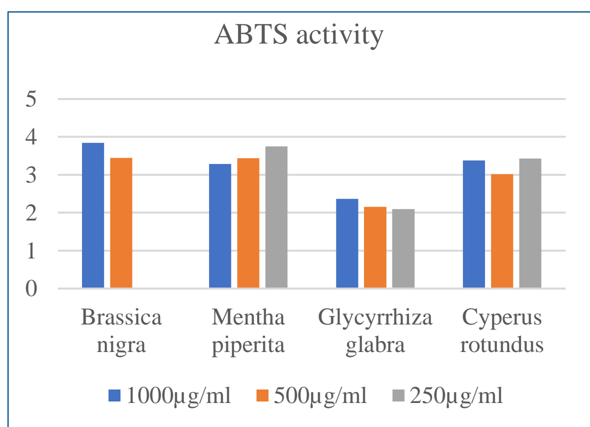
DPPH free radical scavenging assay:
The capacity of samples to scavenge DPPH radicals was evaluated across various concentrations. This results indicated a significant difference in the mean percentage scavenging between different concentrations of the aqueous extracts from selected herbal plants. Specifically, the scavenging activity of the aqueous extract of *Cyperus rotundas* was determined to be 41.40%. This value was obtained by utilizing the linear regression equation, as depicted in Figure 1.

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



ABTS Scavenging:
The dose-response curves for the ABTS scavenging activity of various aqueous extracts from selected herbal plants was shown in Figure 2. Then compared selected herbal plants with the ascorbic acid as reference standard. The aqueous extract of *Glycyrrhiza glabra* exhibited distinguished ABTS scavenging activity. Additionally, the Oxygen Radical Absorbance Capacity (ORAC) of the aqueous extract of *Glycyrrhiza glabra* was determined to be 43.81%, indicating its strong antioxidant potential.

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



Nitric Acid:
Figure 3 depicts the dose-response curves for the nitric oxide scavenging activity of various aqueous extracts from selected herbal plants, compared with the ascorbic acid as reference standard. Notably, in all the extracts, the aqueous extract of *Cyperus rotundas* demonstrated significant nitric oxide scavenging activity. As shown in Figure 3, the aqueous extract of Cyperus rotundas had an Oxygen Radical Absorbance Capacity (ORAC) of 1.30%. It is noteworthy that nitric oxide is associated with inflammation, cancer, and other pathological disorders in addition to reactive oxygen species. Consequently, Cyperus rotundas' capacity to scavenge nitric oxide may have therapeutic applications in the fight against such ailments.

Hydrogen peroxide:
The Hydrogen peroxide radical scavenging activity of various aqueous extracts from selected herbal plants was found to be dose-dependent. As shown in Figure 4, the scavenging activity of the aqueous extract of *Cyperus rotundas* was determined 53.0%, It indicates that *Cyperus rotundas* extract possesses a significant ability to neutralize hydrogen peroxide radicals. Which is a key aspect of its antioxidant properties.

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

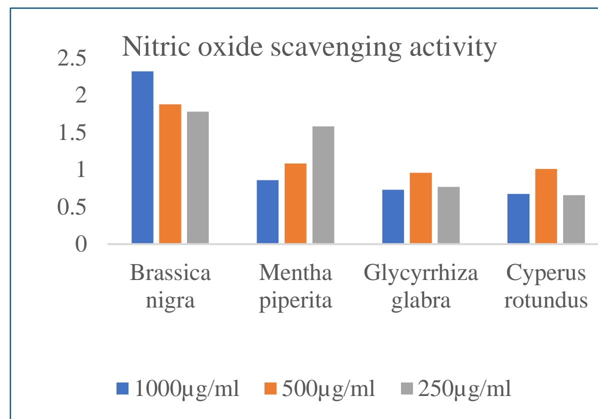
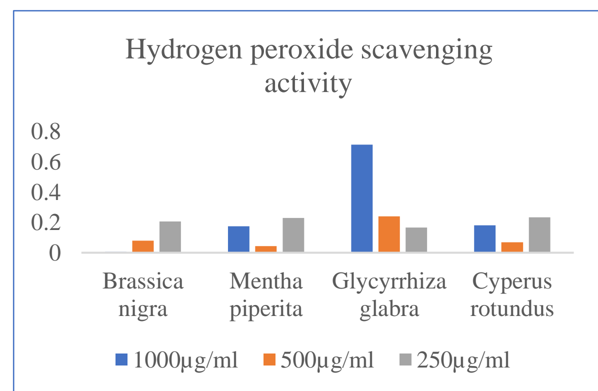


Figure 4: The scavenging ability of the different aqueous extracts on Hydrogen peroxide



Discussion

Based on our findings, here we concluded that *Cyperus rotundas* and *Brassica nigra* exhibited good antioxidant activities compared to *Glycyrrhiza glabra* and *Mentha piperita*. As per our result data base, the replacement of synthetic antioxidants with natural alternatives may hold significant value. Our study determines about the selected herbal plants possess potent free radical scavenging activity, prominence their potential as sources of natural antioxidants.

Further study should be done to assess the bioactivity of these plants using pure compounds, which could contribute to the development of potential pharmaceutical drugs. The findings of our results contribute to the existing knowledge of *Brassica nigra* and *Cyperus rotundas* and could be instrumental in the formulation of treatments for various categories of diseases.

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