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RESEARCH ARTICLE
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IN VIVO AND IN VITRO SINGLE AND SYNERGETIC EFFECTS OF FOENICULUM VULGARE, CICHORIUM INTYBUS AND SOLANUMN NIGRUM ON HEPATOPROTECTIVE AND ANTIOXIDANT HOMEOSTASIS

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Abstract
The foundation of holistic medicine, which is quickly gaining traction globally and steadily establishing itself in significant healthcare systems, is herbal medicine. Medicinal plants or traditional Indian medicines are a vital source for novel pharmaceuticals. The Apiaceae family plant Foeniculum vulgare, commonly referred to as fennel, has a number of health advantages, including lowering blood pressure, decreasing water retention, enhancing vision, and easing the symptoms of acne, constipation, arthritis, coughing, gingival sores, and more.A member of the Solanaceae family, Solanum nigrum, often known as Makoh, is used to cure a range of illnesses. It is a useful herbal remedy for fever, discomfort, and mouth ulcers. It is also an effective treatment for asthma. It is also the primary component of a lot of prepared medications. It cures a range of liver issues, is helpful in treating urinary tract infections, and possesses strong hepatoprotective and anti-inflammatory qualities. The absorbance at 517 nm was then measured. A low reaction mixture absorbance indicates a high amount of radical scavenging activity.The antioxidant activity of ascorbic acid was also investigated as a point of reference. The solution without any plant extract was used as a control. Each test was administered three times.

Keywords: Foeniculum vulgare, Solanum nigrum, Cichorium intybus, herbal medicine, conventional.

INTRODUCTION
Herbs and herbal preparations with active plant elements are referred to as herbal medicine when they are used to improve disease prevention, treatment, or efficacy. Since ancient times, people have utilized herbal medicines and supplements, which are derived to offer the greatest possible health benefits to people (Bent et al.2008). A growing number of individuals are now turning to herbal medicines and related products for a variety of ailments, and their use is spreading quickly throughout the world (Ekoret al.2014). Up to five billion people are thought to use herbal items and phytonutrients as a primary source of healthcare and a traditional medical practice in both developed nations like Canada and developing nations like India and Pakistan (Ahmad et al.2006).One important source of novel pharmaceutical drugs is traditional Indian medicines or medicinal plants. It is essential that people use herbal remedies in the traditional manner.
According to the WHO, the use of herbal remedies for a range of medical conditions is expanding

globally (Howes et al.2020).It makes use of the plant's seeds, stems, roots, and leaves, among other elements. barks, berries, or flowers for therapeutic uses in illness prevention or therapy (Sofowora et al.2013). Cinchona bark, for instance, has a variety of chemical components, including cinchonine, quinine, and quinidine.Quinidine is used to treat cardiac arrhythmias, while quinine is used to treat malaria. The goal of herbal therapy is often to result in a long-lasting improvement in health. Numerous benefits of plant extracts, including antioxidant, hepatoprotective, cardioprotective, vasodilator, anti-inflammatory, antibacterial, anticonvulsant, and antipyretic properties, have been demonstrated (Tabassumet al.2019). Herbal products and traditional medicine have been used extensively to treat a wide range of health issues in many developing nations, including Pakistan, India, the United States, and Canada. Numerous herbal systems, including Ayurveda, Unani, Siddha, and homeopathy, have their origins in India (Mukherjee et al.2006). The traditional medical system uses plants as a source of pharmaceuticals for therapeutic purposes. The Unani medical system is one example of such a system. The ancient traditional medical systems of Egypt, Iraq, Persia, India, Syria, and China are the source of this well-known traditional medical system (Saadet al.2011). The father of Unani medicine, Hippocrates, is credited with developing the theories that are used in the Unani medical system (Parveenet al.2022). It addresses the person as a whole and not as a collection of separate components and operates on the basis of the four humors: blood, phlegm, yellow bile, and black bile. The pharmacological and therapeutic applications of chicory, nightshade, and fennel will be examined in this study.

Foeniculum vulgare is the scientific name for this member of the Apiaceae family. Foeniculum vulgare is a biennial fragrant and medicinal plant that is also referred to as fennel in English, saunf in Hindi, and badyan in Urdu (Saddiqiet al.2011). This herb is delicious and has a variety of medical uses, including stomachic and carminative effects. Carbohydrates and proteins are the most plentiful macronutrients, whereas fats and reduced sugars are the least abundant. The highest moisture level is found in stems and leaves, whereas the lowest moisture content is found in inflorescences. Fenchone, ketone, estragole, phenolic ether, and anethole are the principal volatile oils. The liquid oil has a light yellow color (Tangpaoet al.2022).

The fruit's pharmacological impact is attributed to its essential oils, which are said to diminish in content as the fruit ages. Chicoric acid is the primary chemical component of Cichoriumintybus. The primary percentage is made up of derivatives of aliphatic chemicals, and the minor fraction is made up of terpenoids. Flavonoids, essential oils, cichorinamethoxycoumarins, and saccharides are all present in the blooms. Octaane, hexadecane, pentadecanone, and n-nonadecane are among the primary volatile ingredients (Nwaforet al.2017). Many researchers report that the concentration of insulin is 68%, with other chemicals making up 3%, cellulose 5%, sucrose 14%, and protein 6%. Fatty acids, volatile oil, alkaloids, triterpenes, latex tannins, saponins, and flavonoids are all present in chicory root extract. Cychorins A, B, and C are among the benzoisochromenes that have been extracted from the roots. The roots also include choline, pectin, fatty acids, α lactucerol, sugar, cichorin, fixed oils, and tannins. Chicory seeds have a high crude protein content and are rich in important amino acids such lysine, leucine, methionine, phenylalanine, and isoleucine (Das et al.2016). In addition, the seeds have stearic acid, palmitic acid, emollient oil, saturated and unsaturated fatty acids, and important linoleic acid. Chicory seeds also contain calcium, magnesium, potassium, selenium, and zinc (Alamgiret al.2018).

MATERIAL AND METHODS

Plant Material

We gathered fruit from Solanum nigrum, seeds from Foeniculum vulgare, leaves and roots from Cichoriumintybus, and fruit from the local market in Faisalabad, Pakistan. After being examined by a botanist from the Department of Botany at GC University in Pakistan and dried in the shade, the plant samples were pulverized using an electric grinder.

Preparation of Crude Extracts
An electric grinder was used to reduce the adulterant-free plant material to a coarse powder. The triple maceration procedure was used to extract the coarse powder material. It entailed soaking sealed amber glass vials in 10% aqueous methanol at 25 °C and shaking them three times a day for a week. The material was passed through two layers of muslin cloth after the maceration process, where the granulated powder was absorbed to remove any remaining plant material. Whatman-1 filter paper was then used to filter the filtrate.

The selection procedure was maintained in a sealed amber glass jar. The extraction procedure outlined above was .The filtrates were mixed following the completion of these three macerations twice on separate days.The filtrate was evaporated at 37 °C under decreased pressure using a Rotavapor (BUCHI labrotechnik AG, model 9230, Switzerland) rotary evaporator. It had a vacuum pump and a recirculation chiller attached to it. The moisture content of the dark green crude extract was eliminated using the process of freeze-drying. After being cooled to -4 °C, the dry extract was poured into an amber glass bottle.

Phytochemical analysis:
The Phenol test
The test was conducted using the methodology outlined by Sofowora (1993). There were two milliliters of extract in a beaker. Then, two milliliters of ferric chloride solution was added. There was a strong blue-green solution that indicated phenols were present.

Test for Carbohydrates
The test was conducted using the methodology outlined by Sofowora (1993).Three milliliters of aqueous extract were utilized for ingesting.After introduction, it was mixed with two milliliters of Molisch reagent. Carefully pour two milliliters of powerful sulfuric acid down the test tube's side. A successful test was indicated when the two layers' center phase changed to an opaque reddish violet color.

Terpenoid Examination
The Salkowski test was run using the methodology described in Edeoga et al. (2005). Two milliliters of chloroform and five milliliters of the aqueous extract were mixed together. Then, a layer was created using 3 cc of pure sulfuric acid. The interface's reddish-brown coloring suggested terpenoids were present.

Test for Saponins
The approach from Edeoga et al. (2005) was used to conduct the test. A water bath was filled with two grams of the powdered sample that had been cooked in 20 milliliters of distilled water. The mixture was then filtered after that. To create a stable, long-lasting foam, ten milliliters of the filtrate and five milliliters of distilled water were quickly combined and swirled. An emulsion developed when three drops of olive oil were added to the foam and vigorously stirred, suggesting the presence of saponins.

Test for Flavonoids
The test was administered using the methodology suggested by Harborne (2005). A 1 g powder sample was placed in a steam bath with 10 cc of ethyl acetate and cooked for 5 minutes at 40–50 °C.The filtrate was treated with one milliliter of diluted ammonia. A golden tint denotes good flavonoid performance.

Test for Alkaloids

For the examination, the Harborne method (2005) was utilized. 5 ml of methanol and 5 ml of 2 N hydrochloric acid were used to extract 1 g of the material. Meyer and Wagner reagents were then used to treat the filtrate. The turbidity values of the samples are positive.

Determination of total phenolic contents (TPC)

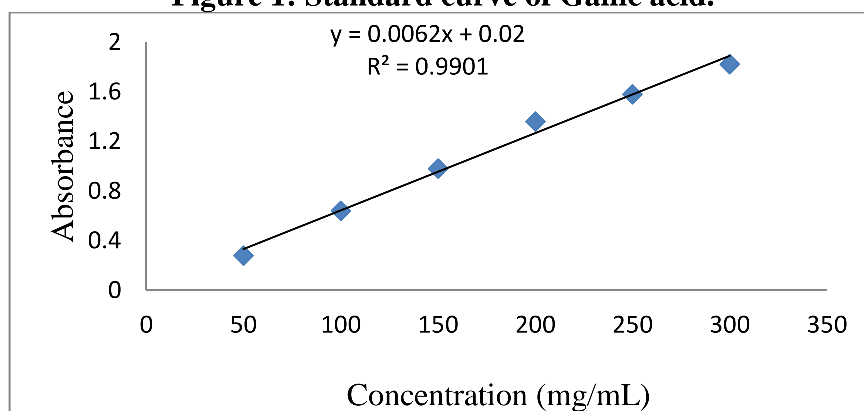
The total phenolic content of the sample was determined using the Folin-Ciocalteu technique as stated by Nazet al. (2016).

The calibration curve was created using different concentrations of gallic acid. Four milliliters of 20% sodium carbonate, five milliliters of diluted Folin-Ciocalteu reagent, one milliliter of gallic acid solution, and 0.01–0.02, 0.03–0.04, 0.05–0.06, 0.07–0.8, 0.09, and 0.10 mg/ml in methanol were combined.After an hour, the absorbance was measured at 765 nm, and the absorbance as a function of concentration was plotted to create the calibration curve.

One milliliter of sample extract (0.001 g/ml) was combined with the previously mentioned reagent, and an hour later, the absorbance of the resulting blue complex was measured at 765 nm. For each, three decisions were made. The quantification process was based on gallic acid as the standard.The following formula was used to determine the total phenolic component content in plant extracts, expressed in gallic acid equivalents (GAE).

T is equivalent to CxV/M.
As opposed to
T is the milligrams GAE/gram of plant extract, or the overall concentration of phenolic components.
C is the concentration of gallic acid, calculated using the calibration curve and expressed in milligrams per milliliter.
V is the milliliters of extract volume.
M is the weight in grams of the plant extract.

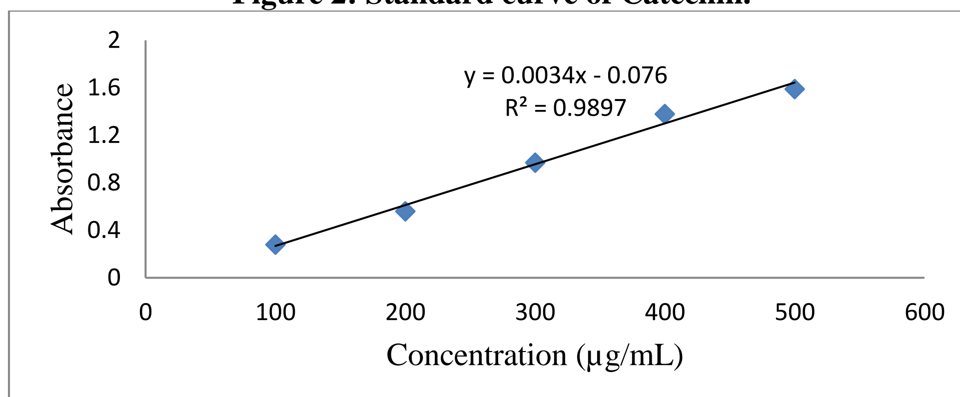
Figure 1: Standard curve of Gallic acid.



Total flavonoids contents

The total flavonoid content of the sample was calculated using the procedure outlined by Rehman et al. (2013).0.5 ml of the sample, 2 ml of distilled water, and 0.15 ml of 5% NaNO2 solution were combined to create the combination. And for six minutes, it was incubated. Incubation of the combination was continued for six more minutes after the addition of 0.15 ml of a 10% AlCl3 solution.After that, a 4% NaOH solution was introduced. The reaction mixture was well stirred until 5 milliliters were obtained after adding methanol. Once incubating for 15 minutes, the absorbance of the reaction mixture was measured at 510 nm. The total flavonoid content (TFC) of the extracts was expressed as µg of catechin equivalents per milliliter of plant extract, based on the catechin linear regression curve.

Figure 2: Standard curve of Catechin.



Catalase activity (CAT):

Using the Mohebbi et al. (2012) approach, catalase activity was measured. Using catalase enzymes, hydrogen peroxide (H 2 O 2) was broken down in this approach as a substrate. A UV spectrophotometer (UV-1601, Shimadzu, Germany) was utilized to find it; the absorbance decrease was computed over a five-minute period at 240 nm. The activity's outcomes were reported in terms of µM of H2O2 consumed/min/mg of protein.To calculate the catalase (CAT) value, the amount of H2O2 that the enzyme absorbed and converted into H2O and O2 was measured. CAT activity was tested using the same enzyme extract that was used to measure TSP. An ELISA plate was utilized to record the absorbance at 240 nm after 100 µl of the enzyme extract and 100 µl of H2O2 (5.9 mM) were applied to a well plate.

Superoxidase dismutase:

Superoxide dismutase (SOD) activity was measured by measuring the rate at which SOD enzymes inhibited nitrobluetetrazolium (NBT) photoreduction. 50 mM sodium phosphate buffer at pH 7.6, 50 mM sodium carbonate, 0.1 mM EDTA, 50 µM NBT, 12 mM L-methionine, 10 µL riboflavin, and 100 µL of crude extract make up the reaction mixture utilized for this, with the volume measured in milliliters. Conversely, the reaction that was being controlled was conducted without the use of crude extract. To achieve SOD activity, the following reaction mixture was created for 15 minutes at room temperature under white light. After incubating for 15 minutes, absorbance was measured at 560 nm using a spectrophotometer. The quantity of the enzyme that stopped nitro blue tetrazolium (NBT) from being photochemically reduced was used to measure superoxide dismutase (SOD) activity. The reaction mixture consisted of 200 µL of Triton distilled water, 500 µL of potassium phosphate buffer (pH 5), 100 µL of enzyme extract produced with TSP, and 200 µL of methionine. Following a 15-minute exposure to UV radiation and the addition of 100 µl of riboflavin, an ELISA plate was used to assess absorbance at 560 nm.

POD

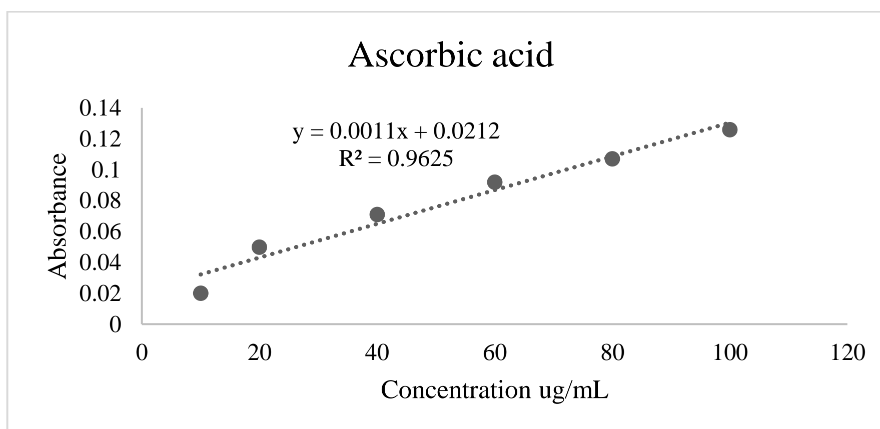
Guaiacol oxidation requires a certain amount of enzyme, which is measured by peroxidase activity, or POD. Both POD activity and TSP were measured using the same enzyme extract. 100 µL H2O2 (40 mM) + 100 µL guaiacol (20 mM) + 800 µL potassium phosphate buffer (pH 5) make up the reaction mixture. Using an ELISA plate, absorbance was measured at 470 nm following the addition of 100 µl of enzyme extract and 100 µl of reaction mixture.

DPPH Scavenging Activity

The antioxidant capacity of the sample was assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging experiment, in accordance with the recommendations given by Shahid et al. (2014). For this procedure, 3 mL of sample must be mixed with 1 mL of newly made 0.004% DPPH in methanol solution. After that, the combined solution is exposed to darkness for half an hour.

Next, we took a reading of the absorbance at 517 nm. A low absorbance of the reaction mixture indicates high radical scavenging activity. The antioxidant activity of ascorbic acid was also investigated as a point of comparison. The solution devoid of plant extract was utilized as a reference. Every test was run three times. To calculate the percentage inhibition of DPPH radical samples, the following formula was utilized.

DPPH inhibition (%) is calculated as follows: Blank absorbance (A0) - Sample absorbance (A1) * 100 Where Sample absorbance, or A1. A0 is the blank's absorbance. A positive control was ascorbic acid.



The outcomes of various plant extracts, including those from nightshades (Solanum nigrum), chicory (Cichoriumintybus), and fennel (Foeniculum vulgare), are shown here. The essential oils in fennel seeds encourage the secretion of digestive fluids and enzymes that facilitate better digestion. It also possesses anti-inflammatory and antispasmodic properties. Ingredients high in fiber found in fennel seeds lower the risk of heart disease. (Khan et al.2022).

Chicory contains various nutrients as good source of zinc, magnesium, calcium, folic acid, potassium, vitamin A, vitamin K, and vitamin E. The seeds of chicory plants contain saturated and unsaturated fatty acids. Chicory plant reduces the risk of cardiac disease, reduce anxiety, prevent cancer, act as anti- inflammatory, and also use as body weight loss

The berries or leaves of bittersweet nightshade are toxic, however the stem of black nightshade has anti-inflammatory properties. The stem is applied topically to treat dermatitis, acne, and wound healing. Rich in calcium, vitamin C, and vitamin A are nightshades. They help build stronger teeth and bones, lower inflammation, boost immunity, and prevent night blindness. Iron from nightshades also helps to build immunity. hemoglobin protein, which is responsible for carrying oxygen in the blood

DPH

Diphenhydramine, or DPH, is a sedative with antihistamine properties. It is used to treat tremors in Parkinson's disease, common cold symptoms, sleeplessness, and other allergies. It can be used topically, injected intramuscularly, injected into a vein, and taken orally are among the several methods of administration. The effects often continue up to seven hours after a dose and reach their peak around two hours later. Additionally, stomatitis and poor coordination are adverse consequences of DPH. For women who are nursing or pregnant, it is not advised. It functions by preventing some of histamine's actions, which are what give it its sedative and antihistamine properties. It also has delirious effects and is a potent anticholinergic.

DPPH

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) is a frequently used, quick, affordable, and straightforward method for assessing a compound's capacity and antioxidant activity. It functions as a hydrogen donor or free radical scavenger. It is used to measure antioxidants in liquid and solid materials in complex biological systems. This assay for antioxidants is based on the electron transfer that occurs when a violet solution in ethanol is heated. Antioxidant molecules eliminate free radicals, which are stable at ambient temperature and result in a colorless ethanol solution. When analyzing multiple products at once, the DPPH assay offers a quick and simple method for spectrophotometric evaluation of antioxidants.

The following table shows the results of DPPH in different plant extacts
CC for Chicory Chicory (Cichoriumintybus)
F for fennel (Foeniculum vulgare)
H for hepton
S forBlack Nightshade(Solanum nigrum),

Sample	Blank Ab	Sample Ab
CC	1.991	0.982
F	1.991	0.859
H	1.991	0.744
S	1.991	0.631

Percentage Inhibition of DPPH

The concentration of the oxidant (radical) and antioxidant, the ratios of the reagents, the temperature, the duration of the incubation period, the solvent used, and the presence of water, hydrogen, and metal in the measuring device are some of the variables that affect the range, which is 0 to 100%. The calculation involves dividing the quantity of enzyme units per milliliter by the milligrams of protein that the enzyme contains per milliliter. Units per milligram of protein is how the result is expressed. The amount of protein in a preparation of an enzyme is called apparent purity.

The percentage inhibition of DPPH is shown in below table

Percentage inhibition of DPPH
50.678
56.856
62.632
68.307

POD (Peroxidase)

Peroxidase is a hemecontaing enzyme, oxidize the various substrates by using hydrogen peroxide, and prevent excess accumulation of hydrogen peroxide that generated in normal metabolism or under stress condition. Plants and certain animal cells contain peroxidase,this enzyme catalyses many organic compounds by oxidation of hydrogen peroxide such as amines, benzidine, phenols, and hydroquinone**.** Peroxidases are class of oxidase enzyme that transfer oxygen from a donor peroxide ,such as hydrogen peroxide to the substrate. (Francozet al.2015).
The following table shows the results of peroxidases in different plant extacts

CC for Chicory Chicory (Cichoriumintybus)
F for fennel (Foeniculum vulgare)
H for hepton
S forBlack Nightshade(Solanum nigrum),

Sample	POD Absorbance	Final POD(U/mg protein)
CC	0.327	7.500
F	0.421	9.656
H	0.254	5.826
S	0.369	8.463

CAT

Hepatic catalase, an important antioxidant enzyme, uses non-radical hydrogen peroxide (ROS) as a substrate. Catalase neutralizes acidic environments by dissolving hydrogen peroxide and keeps the molecule at the proper concentration inside the cell, which is required for cell signaling pathways. The enzyme catalase is found in all aerobic organisms. Catalase breaks down two molecules of hydrogen peroxide into one oxygen molecule and two water molecules in two steps. The first step in the reaction pathway is the formation of an oxyferryl species with a porphyrin radical when a hydrogen peroxide molecule is reduced. Reaction component I is reduced by a redox reaction in which two electrons are transferred from an electron donor: the second hydrogen peroxide molecule. This produces the free enzyme, oxygen, and water in the second step. Catalase keeps harmful chemical accumulation from occurring and shields tissues and cellular organelles from harm caused by peroxide.

Calculate CAT absorbance

To do the calculations: (Decrease in absorbance x 100/1) divided by protein amount in mg divided by time in min=units/mg protein/min.

The following table shows the CAT absorbance and final CAT of different plants

CAT absorbance	Final CAT (U/mg protein)
1.961	44.977
2.017	46.261
2.49	57.110
2.758	63.257

SOD

One type of antioxidant protein is superoxide dismutase. Superoxide anion dismutation into hydrogen peroxide is catalyzed by SOD. SOD leaves the kidney quickly and does not bind to cell membranes.Catalase and GSH peroxidase detoxified SOD to oxygen and water. Three types of SOD are present in humans: extracellular SOD (EC), manganese SOD (Mn), and copper/zinc (Cu/Zn). Copper/zinc-SOD functions as an antioxidant and lowers inflammation in colonial fabrics.Diabetes and its related issues can be brought on by oxidative stress, however antioxidants help the body lower its oxidant levels as they rise.

An essential component of the body's defense against oxidative stress is the antioxidant SOD. It is also an effective treatment for diseases like cancer, inflammatory disorders, cystic fibrosis, ischemia, aging, rheumatoid arthritis, neurodegenerative disorders, and diabetes that are caused by reactive oxygen species.

The final SOD shows in below table,

Final SOD (U/mg protein)
15.949
10.152
12.953
15.674

FRAP (Ferric reducing ability of plasma)

Reduced antioxidant power is also caused by this ferric ion. The O-phenanthroline-Fe(2+) complex's production is the basis for this technique, which is an antioxidant capacity test. Antioxidant potential of dietary supplements containing polyphenols is measured using this approach. Initially, J.J. Strain and Iris Benzie of the University of Ulster, Coleraine's Human Nutrition Research group conducted the FRAP experiment.

Procedure:

All aerobic organisms include the enzyme catalase. In two stages, two molecules of hydrogen peroxide are transformed into one oxygen molecule and two water molecules by catalase. When a hydrogen peroxide molecule is reduced, an oxyferryl species containing a porphyrin radical is formed, marking the beginning of the reaction pathway. A redox process in which two electrons are exchanged from an electron donor—the second hydrogen peroxide molecule reduces component I of the reaction. In the following stage, this generates water, oxygen, and free enzyme. Catalase shields cell tissues and organelles from peroxide-induced damage and stops toxic chemical accumulation. The method of Benzie and Strain (1999) was applied in order to ascertain the date seed extraction process's iron-reducing activity. To make the FRAP reagent, they were mixed together. In a 2 ml container, the extract was combined with 10 µl of freshly made FRAP reagent. After that, the absorbance at 593 nm was measured against the blank for ten minutes at room temperature. The standard curve was constructed using Trolox. Similar to Trolox, the result was expressed in milligrams per 100 grams of date seed based on dry weight (DW).
The outcomes of FRAP in various plant extracts are displayed in the following table.

CC for Chicory Chicory (Cichoriumintybus)
F for fennel (Foeniculum vulgare)
H for hepton
S forBlack Nightshade(Solanum nigrum),

FRAP					
Sample	**Ab Sample**				**FRAP (µM)**
CC	0.431	0.005	0.023	0.408	**81.6**
F	0.274	0.005	0.023	0.251	**50.2**
H	0.259	0.005	0.023	0.236	**47.2**
S	0.416	0.005	0.023	0.393	**78.6**

Total Flavonoids contents (TFC)

Flavonoids work as a unique UV filters and defensive antimicrobial compound. Flavonoids protect the plants from biotics and abiotic streeses. Important antioxidant components that deactivate free radicals and have the capacity to donate hydrogen atoms to them include total flavonoids and phenolic content.

Procedure

Four milliliters of distilled water were mixed with roughly one hundred microliters of extract. But after adding five mints, 10% aluminum chloride—roughly 0.3 ml—is added. 5% of 0.3 ml of sodium nitrite is added. Add 2 ml of 1 M sodium hydroxide to the liquid and stir for six mints. Right away, 3.3 milliliters of distilled water were added to the mixture to dilute it, and it was then stirred. At 510 nm, the absorbance was measured. Catechin, the calibration curve standard, was applied. The total flavonoid-containing extract was reported in milligrams (mg/g) of catechin equivalents per gram of sample. The overall flovonoid content of the sample extract is displayed in the following results.
CC for Chicory Chicory (Cichoriumintybus)
F for fennel (Foeniculum vulgare)

H for hepton
S forBlack Nightshade(Solanum nigrum),

TFC			
Sample	**Absorbance**	**µg catechin equivalents per mL**	
CC	0.941	0.898	**236.3157895**
F	0.529	0.486	**127.8947368**
H	0.658	0.615	**161.8421053**
S	0.882	0.839	**220.7894737**

ABTS antioxidant assay:
ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) antioxidant assay is based on the ability of antioxidants to scavenge ABTS radical cations. Which are produced when ABTS reacts with a potent oxidant, like potassium persulfate.

Combine the ABTS radical cation solution with the sample solutions: To a microplate containing 980 µL of diluted ABTS radical cation solution, add 20 µL of each sample solution. For blank samples, add 80% methanol in place of the sample solution. Give the reaction mixture an incubator.The microplate requires to be placed at room temperature in the dark for six minutes. After incubation, measure the absorbance of the reaction mixture at 734 nm using a microplate reader. Use the following formula to determine each sample's antioxidant activity:
Antioxidant activity is calculated as follows: [(Control absorbance – Sample absorbance) / Control absorbance]

100Which Case Control Sample absorbance refers to the absorbance of the reaction mixture in the presence of a sample, while absorbance is the absorbance of the reaction mixture in the absence of a sample. It is possible to compare the antioxidant activity of several samples by using the antioxidant activity percentage, which is represented or figure out how much of an antioxidant component is present in a sample.

Antioxidant capacity is most commonly determined using tests that employ the radical cations of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Cation-based radicals of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) are the most widely used assays to evaluate antioxidant capacity; nevertheless, these assays are frequently employed in conjunction with tests based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals.

The ABTS material is supposed to be colorless, however it typically has a light greenish tint. It appears that little amounts are to blame.
Although the substance containing ABTS is often pale greenish in hue, it is intended to be colorless. This seems to be caused by ABTS traces of contaminants. This is demonstrated indirectly by the fact that when ascorbate or any other antioxidant chemical is added to the pale greenish solution that results from the native ABTS dissolving in water, it instantly turns colorless.

The following table shows the results of ABTS in different plant extacts
CC for Chicory Chicory (Cichoriumintybus)
F for fennel (Foeniculum vulgare)
H for hepton
S forBlack Nightshade(Solanum nigrum),

ABTS					
Sample	Ab Control	Ab Sample	%age Inhibition		
CC	0.881	0.45	0.431	0.48922	**48.922**
F	0.881	0.406	0.475	0.53916	**53.916**
H	0.881	0.619	0.262	0.29739	**29.739**
S	0.881	0.398	0.483	0.54824	**54.824**

TOS(Total Oxidant Status)
Total antioxidant capacity is a commonly used assay to assess the antioxidant response to free radicals, which are present in a variety of illnesses, as well as the amount of antioxidants in biological samples. Serum TAC typically ranges from 300 to 460 µmol/L in healthy persons.

The following table show the TOS in sample of different plants extract such as
CC for Chicory Chicory (Cichoriumintybus)
F for fennel (Foeniculum vulgare)
H for hepton
S forBlack Nightshade(Solanum nigrum),

TOS					
Sample	Ab Sample	Ab Blank	Trolox	Trolox Con	mM
CC	1.982	0.05	0.67	0.3	0.935
F	1.254	0.05	0.67	0.3	0.583
H	1.07	0.05	0.67	0.3	0.494
S	2.751	0.05	0.67	0.3	1.307

Total Phenolic Contents (TPC)
The Folin-Cicocalteu (FC) method, which is a well-established technique that oxidizes phenolic compounds using reagent, is another name for total phenolic contents. When the reaction product is detected at 760 nm with a blue color intensity that correlates the sample with the phenolic content, it is blue in color to lower the FC reagent. To extract the phenolics the main method is organic solvent extraction .To detect the presence of total phenolics content different chemical procedures are used while chromatographics and spectrophotometric techniques are used to identify and qualify the total phenolic compound.

The following table shows the TPC in sample of different plants extract such as
CC for Chicory Chicory (Cichoriumintybus)
F for fennel (Foeniculum vulgare)
H for hepton
S forBlack Nightshade(Solanum nigrum),

Total Phenolic Contents (TPC)					
Sample	Sample Ab	Blank ab	mgGAE/g		
CC	1.831	0.511	1.32	264.000	**264.000**
F	1.779	0.511	1.268	253.600	**253.600**
H	1.468	0.511	0.957	191.400	**191.400**
S	1.973	0.511	1.462	292.400	**292.400**

Discussion
All over the world, liver disease is one of the leading causes of death and has a severe effect on many families. The cost of current medicines is high, and they also have a lot of negative side effects. The

current research aimed to identify natural treatments as alternative medicines with little to no side effects for liver illness (Seeff et al.2015).In this study, the hepatoprotective properties of three medicinal plants (Foeniculumvulgari, Cichoriumintybus, and Solaraumnigrum) were studied in a variety of groups.

Hepatoprotective effect of these three selected plants might be because flavonoids, which come from plants and act as antioxidants. The phytochemical components of these therapeutic plants were examined, namely their total phenolic content (TPC) and total flavonoid content (TFC)(Othman et al.2014).Hydrogen peroxide test, which is DPPH, and reducing power were used to examine the antioxidant properties of particular medicinal plants.The cytotoxicity of selected medicinal plants was analyzed individually using a variety of toxicological tests including hemolytic activity, thrombolytic, mutagenicity and DNA Damage Prevention test (Yasiret al.2022).Biochemical profiles of selected medicinal plants were obtained from liquid chromatography (HPLC).

The use of herbs is the cornerstone of holistic medicine, an increasingly popular field that is spreading rapidly throughout the world and progressively taking over major healthcare systems. (Chanda et al.2009). An essential source for cutting-edge medications is traditional Indian medicine or medicinal herbs.Foeniculum vulgare, often known as fennel, is a plant in the Apiaceae family (Sanandia et al.2024).It offers several health benefits, such as lowering blood pressure, improving vision, and relieving symptoms of acne, constipation, arthritis, coughing, gingival ulcers, and more.Solanum nigrum, commonly known as Makoh, is a member of the Solanaceae family and is used to treat a variety of ailments. It is an effective herbal treatment for discomfort, fever, and mouth ulcers.

Furthermore, it works well as an asthma therapy. It serves as the main ingredient in several prepared pharmaceuticals as well. It is effective in treating a variety of liver diseases, has potent hepatoprotective and anti-inflammatory properties, and helps with urinary tract infections (Lam et al.2016).The absorbance at 517 nm was then measured. A low reaction mixture absorbance indicates a high amount of radical scavenging activity (Ak et al.2008).The antioxidant activity of ascorbic acid was examined as well as a point of recommendation. Three attempts were made at each test. The solution without any plant extract was used as a control.

Conclusion

Hepatoprotective effect of these three selected plants (Foeniculumvulgari, Cichoriumintybus and Solaraumnigrum) might be because flavonoids, Phenolic contents which are natural antioxidants that were analyzed. This study showed that these plants that treat a variety of diseases such as liver diseases, had potent hepatoprotective and anti-inflammatory properties, and also helped in urinary tract infections.

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