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Potential in-Vitro Antioxidant Activity of Polyherbal Drugs

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Abstract: ***The aim & objective of the research is to evaluate the potential antioxidant activity of herbal drugs such as Gymnema Sylvestre (Leaves), Trigonella Foenum-Gr (Leaves), Tinospora Cardifolia (Stems), Azadirachata Indica (Leaves), Cinnamomum Zeylanicum (Stem Barks), Syzygium Jambolana (Fruits) & Nardostachys Jatamansi (Roots) which are well-known medicinal plants available throughout India and they are commonly used for the treatment of various diseases. All the herbal drugs were collected from herbal shop (Grovel Drugs & Chemicals Pvt Ltd. Hyderabad). The quality of the finished product was evaluated as per the World Health Organization's guidelines for the quality control of herbal materials. The quality testing parameters of the herbal drugs were within the limits. The herbal drugs were subjected for extraction with ethanol (90%) by cold maceration & soxhelation method to get ethanolic extracts. The ethanolic extracts of herbal drugs were evaluated for its antioxidant activity. Screening of preliminary phytochemical investigation of herbal extracts shows the presence of large amounts of phenolic, tannins & flavonoid compounds which exhibits the highest antioxidant and free radical scavenging and also inhibited lipid peroxidation. A positive correlation was observed between phenolic content and free radical (DPPH and OH) scavenging efficiencies and lipid peroxidation inhibition activity. All the herbal extracts shows promising effect of antioxidant activity and it may be used as an alternative remedy for the treatment of diabetes mellitus.***

Keywords: Herbal drugs, Antioxidant, Flavonoids, Ethanol, etc.

1. Introduction

Correspondingly to conventional medicines, the indications of folk HMs are diverse, being employed for the treatment of a wide range of diseases. The indications spread from simple health conditions such as cold, pain, surface wounds to serious conditions such as psychosis, diabetes, malaria, sickle cell disease, tuberculosis, cancer, hypertension, infertility, and so on. In certain communities, HM is a major component of the primary healthcare. Indeed, up to 80% of the rural world population uses herbal-based traditional medicines for most of their healthcare[1].

Oxidative stress is the major driving factor responsible for the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes[2]. The condition is brought by excessive generation of free oxygen and nitrogen species or their inefficient quenching in the cell. Free oxygen and nitrogen species are unstable molecules that are present in the environment (exogenous) and are also generated in the body (endogenous) during the normal aerobic metabolic processes in the body[3]. Exogenous sources of free radicals include cigarette smoke, exposure to ozone, ionizing radiation such as X-rays, and drugs among others. On the other hand, endogenous sources of free radicals include the electron transfer chain reactions in the mitochondria, xanthine oxidase pathway, during disease states such as inflammation, ischemia and reperfusion injury[4]. The body possesses a complex antioxidant defense system, comprising of enzymatic and nonenzymatic pathways, which in the normal physiologic state, maintain a steady equilibrium between prooxidants and antioxidants, thereby ensuring well-being[2]. &e enzymatic antioxidants comprise the catalase, glutathione peroxidase, and superoxide dismutase. Conversely, nonenzymatic antioxidants employed by the body include the bilirubin, uric acid, and lactoferrin among others. However, during disease states, the endogenous antioxidant systems are overwhelmed, leading to accumulation of excessive free radicals, which inturn cause oxidative stress-associated damage to cellular machinery, as implicated in various diseases[5].

Conventionally, oxidative stress is managed using various synthetic antioxidant compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). Despite their usage, these synthetic antioxidant compounds have been associated with undesirable effects[6]. For instance, BHT and BHA cause hepatotoxicity and have been demonstrated to be carcinogenic. Additionally, synthetic antioxidants are inaccessible, unaffordable, and labile, thus limiting their utilization[4]. Therefore, due to the profound consequences of oxidative stress and the drawbacks of synthetic antioxidants, the need for alternative antioxidants, which are safer, easily accessible, and potent, are warranted[7], hence the current study. Considering the available alternative and complementary strategies, medicinal plants stand a better chance of providing potent, safer, affordable, and easily accessible therapies for oxidative stress-related maladies[8]. Medicinal plants contain various secondary metabolites, which have demonstrated a wide spectrum of pharmacologic activities. Antioxidants properties of plants have been demonstrated to play a protective role in the body against diseases, since their consumption lowers the risk of cancer, heart disease, hypertension, dementia, and stroke [9]. The major groups of phytochemicals that contribute to antioxidant capacity of plants include polyphenols and vitamins (A, C, and E). Phenolic compounds of plants are

hydroxylated derivatives of benzoic acid and cinnamic acids, which possess antioxidant and anticarcinogenic effects[4]. & they include phenols, flavonoids, coumarins, tannins, and anthocyanidins. & theese phytoactive complexes are important in plant defense mechanisms against biotic and abiotic stresses[10]. When plants or plant products rich in these phytoactive principles are consumed, they are deemed to confer the same beneficial effects to humans[9]. For instance, flavonoids have for long been recognized to possess anti-inflammatory, antiallergic, antiviral, immunomodulatory, antiaging, and antiproliferative properties[11]. The search for better alternatives to synthetic antioxidants has triggered a significant research interest on dietary and medicinal plants that can inhibit, reverse or ameliorate diseases caused by oxidative stress [4, 11]. In this present study, we investigated the in vitro antioxidant activity of the ethanolic extracts of different parts of medicinal herbal drugs belonging to different families.

2. Materials and Methods

Table 1: List of herbal drugs

S. no	Botanical Name	Common name	Parts used
1	*Gymnema Sylvestre*	Gurmar	Leaves
2	*Trigonella Foenum-Gr*	Fenugreek, Methi	Seeds
3	*Tinospora Cardifolia*	Guduchi	Stems
4	*Azadirachata Indica*	Neem	Leaves
5	*Cinnamomum Zeylanicum*	Cinnamon	Barks
6	*Syzygium Jambolana*	Jamun	Fruits
7	*Nardostachys jatamansi*	Jatamansi, Spikenard	Roots

2.1 Preparation of herbal extracts[12, 13]

All herbal powdered drugs were taken (Table 1) and sieved using mesh no.85. Powdered herbal drugs were sterilized using UV radiation at 60°C. The Herbal drugs were homogenized using mortar and pestle and stored in air tight container for further use.

2.2 Extraction of herbal extracts[12, 13]:

All the selected herbal drugs were separately extracted by using cold maceration using 90% ethanol. The extract was evaporated using Rota flash evaporator under reduced pressure and low temperature and then on a water bath. The obtained marc is then air dried.

The individual air dried marc were subjected for extraction with ethanol (90%) with the help of Soxhlet apparatus at 50ºC. The solvent was evaporated using Rota flash evaporator under reduced pressure. The dried extracts were preserved in refrigerator (4 ºC) for further use. The percentage yield, colour and consistency of the extracts were recorded.

Table 2: List of ethanolic extracts of herbal drugs

S. no	Herbal drugs	Ethanolic extracts
1	*Gymnema Sylvestre*	EEGS
2	*Trigonella Foenum-Gr*	EETF
3	*Tinospora Cardifolia*	EETC
4	*Azadirachata Indica*	EEAI
5	*Cinnamomum Zeylanicum*	EECZ
6	*Syzygium Jambolana*	EESJ
7	*Nardostachys jatamansi*	EENJ

2.3 Preliminary phytochemical investigation of herbal extracts

Preliminary phytochemical investigations of all extracts:
The various extracts of herbal drugs obtained were screened for its chemical constituents with the following tests[14, 15].

2.3.1Test for Alkaloids
The small portions Fraction was stirred separately with a few drops of dilute HCl, filtered and filtrates were subjected for the following tests.

a) Dragendorff's Test
Filtrates were treated with dragendorff's reagent (Potassium Bismuth iodide).
Formation of orange-brown precipitate indicates the presence of alkaloids.

b) Mayer's Test
Filtrates were treated with Mayer's reagent (Potassium mercuric iodide).
Formation of cream precipitate indicates the presence of alkaloids.

c) Wagner's Test
Filtrates weretreated with Wagner's reagent (Potassium iodide).
Formation of reddish-brown precipitate indicates the presence of alkaloids.

d) Hager's Test
Filtrates were treated with Hager's reagent (saturated solution of picric acid).
Formation of Yellow precipitate indicates the presence of alkaloids.

2.3.2 Test for Glycosides
a) Modified Borntrager's test (For Anthraquinone glycosides)
About 0.1 g of extract/fraction was boiled with dilute hydrochloric acid for 2 minutes and few drops of ferric chloride solution were added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia was added to the benzene extract and shaken well. Formation of pink colour indicates the presence of anthraquinone glycosides.

b) Test for Cardiac Glycosides (Keller-killiani test)
About 1 g of the extract/fraction was boiled with 10 ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10 ml of water and 5 drops of the solution of lead sub-acetated were added and filtered, evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid. To this 2 ml of ferric chloride solution was added from the side of the test tube carefully and observed. Formation of reddish colour ring at the junction indicates the presence of cardiac glycosides.

c) Legal's Test (For Cardinolides)
Extract/fraction was dissolved in pyridine. Add sodium-nitroprusside solution to it. Formation of pink or red colour indicates the presence of cardiac glycosides.

2.3.3 Test For Saponins

1) About 0.5 g of the extract/fraction was boiled gently for 2 minutes with 20 ml of water and filtered while hot and allowed to cool.5 ml of the filtrate was taken, dilute with water and shaken vigorously. Frothing occurred indicating the presence of saponins.
2) **Liebermann Burchard's Test**
   To the extract/fraction, a mixture of acetic anhydride and concentrate sulphuric acid (19: 1) was dissolved in suitable anhydride solvent; development of violet purple colour, indicting the presence of steroidal-saponins.

2.3.4 Test for Tannins

1) To the extract/fraction of the powdered drug, few drop of ferric chloride was added. Bluish colour indicates the presence of tannins.

Test for Flavonoids

a) Shinoda test
A little amount of extract/fraction was dissolved in alcohol and filtered. The test solution obtained as above was treated with magnesium ribbon and few drops of concentrated hydrochloric acid. Boil for few minutes, characteristic magentacolor is indicates the presence of flavonoids.

b) Alkali test
To the alcoholic solutionof extract/fraction, 10% potassium hydroxide solution was added. Formation of intense yellow colour indicates the presence of flavonoids. This yellow colour becomes colourless on addition of dilute acid.

c) Acid test
To the alcoholic solution of extract/fraction, few drops of sulphuric acid is added. Change indicates presence of flavonoids.

d) Lead acetate test
The alcoholic solution of extract/fraction was treated with few drops of lead acetate solution. Formation of yellow coloured precipitate indicates the presence of flavonoids.

2.3.5 Test For Steroids

a) LiebermannBurchard's Test
The extract/fractionwas dissolved in chloroform and filtered. Glacial acetic acid, one drop of conc. Sulphuric acid was added to the solution. Rose to violet or blue to green color indicates the presence of steroids.

b) Salkowski's Test
To the solution of sample, conc. sulphuric acid was added. Red color indicates the presence of steroids.

2.3.6 Test For Terpenoids

a) Salkowski's Test
Few mg of extract/fraction was added into chloroform and conc. sulphuric acid was added. The yellowish white colour slowly becomes red, indicating the presence of triterpenoids.

b) Liebermann Storch Morawski Test
Few mg of extract/fraction was dissolved in few ml in acetic anhydride and few drops of sulphuric acid were added. Red or blue color produced which indicating the presence of triterpenoids.

c) Hirschorn Test
Extracts were treated with trichloroacetic acid and warmed. Red to yellow colour produced which indicates the presence of triterpenoids.

2.3.7 Tests for Carbohydrates

Extract were dissolved separately in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2ml of concentrated sulphuric acid was added carefully along with the side of the test tubes. Formation of violet ring at the junction indicates the presence of carbohydrates.

b) Benedict's Test
Filtrates were treated with Benedict's reagent and heated in water bath. Formation of orange red precipitates indicates the presence of reducing sugars.

c) Fehling's Test
Filtrates were hydrolyzed with dilute HCl, then neutralized with alkali and heated with Fehling's A and Fehling's B solutions. Formation of red precipitates indicates the presence of reducing sugars.

d) Barfoed's Test
Filtrates were treated with Barfoed's reagent and heated on a water bath. Formation of red precipitate indicates the presence of monosaccharides.

2.3.8 Test for Phenol: About 0.5 g of each of the studied plant extracts was boiled in 5 ml of 70% ethanol in a water bath for 5 minutes and then filtered through Whatman filter paper No.1. After cooling, 5 drops of 5% ferric chloride were added and mixed. The appearance of a green precipitate indicates the presence of phenols in the sample.

2.3.9 Test for Saponins: About 2 g of each of the studied plant extracts was weighed and dissolved in 5 ml of distilled water. Thereafter, aliquots of 2 ml were taken from each plant extract solution, stirred for 30 seconds, and briskly agitated. &e setups were then allowed to settle for 15 minutes. &e presence of frothing, which persists for over 15 minutes, is an indication of the presence of saponins in the tested sample.

2.3.10 Test for Volatile oils:

Sudan red III test: Thin section of sample + Sudan red III solution gives red color

Tincture alkane test: Thin section of sample + tincture alkane solution gives red color.

2.4 Determination of In-vitro antioxidant activities of ethanolic herbal extracts

2.4.1 DPPH Radical Scavenging assay[16]

Free radical scavenging ability of the herbal extracts was tested by DPPH radical scavenging assay described by described by Sreejayan et al. To the 1 ml of various concentrations of herbal extracts, 1 ml of solution of DPPH 0.1 mM (0.39 mg in 10 ml methanol) was added to the test tube. An equal amount of methanol and DPPH was added to the control. After 20 minutes of incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate. The percent scavenging was

calculated using the formula:
% radical scavenging activity = (A0− A1) /A0 × 100

Where, A0 is the absorbance of the control, and A1 is the absorbance of the extracts/standard. The % of inhibition was plotted against concentration and the graph IC50 was calculated. The experiment was repeated three times at each concentration.

2.4.2 ABTS radical scavenging assay[17]
Free radical scavenging activity of herbal extracts was determined by ABTS radical cation decolorization assay described by Vaijanathappa et al. In a 96-welled microtitre plate, 40 µL of the herbal extracts, 200 µL of ethanol and 30 µL of ABTS solution were added. This was performed in triplicate. The plate was then incubated at 37ºC for 20 min after which the absorbance was measured at 700 nm using an ELISA plate reader. Sample blank and control were also taken. The percent scavenging was calculated using the formula:
% radical scavenging activity = (A0− A1) /A0 × 100
Where, A0 is the absorbance of the control, and A1 is the absorbance of the extracts/standard. The % of inhibition was plotted against concentration and the graph IC50 was calculated. The experiment was repeated three times at each concentration.

2.4.3 Ferric Reducing Antioxidant Power Assay[18].
The reducing power of the herbal extracts was determined according to the method described by Oyaizu et al with some modifications. Briefly, five different concentrations of ethanolic extracts (20, 40, 60, 80, and 100 µg/ml) and L-ascorbic acid at same concentrations were mixed with 2 ml phosphate buffer (0.2 M, pH 6.6) and 2 ml of 1% potassium ferricyanide (K3Fe (CN) 6). The mixture was incubated at 50°C for 20 minutes. &en, 2 ml of 10% trichloroacetic acid (TCA) was added and the mixture was centrifuged at 1000 revolutions per minute (rpm) for 10 min. The supernatant (2 ml) was aspirated and mixed with 2 ml of distilled water and 1 ml of 0.1% ferric chloride (FeCl3). In each case, the experiment was performed in triplicate. Afterward, the absorbances were measured spectrophotometrically at 700nm using a UV-vis spectrophotometer and recorded. The concentrations of each extracts able to yield an absorbance value of 0.5 were determined from the graph of absorbance at 700nm against extract concentrations and considered as the median effective concentration (EC50).

2.4.4 Hydroxyl Radical Scavenging Activity[19].
The hydroxyl radical scavenging activity was performed as per the method described by Klein et al. with minor modifications. The reaction mixture was constituted by adding 2.4 ml of phosphate buffer (pH 7.8) into test tubes. To the same test tubes, 90 μl of 1mM of 1, 10 phenanthroline, 150 μl of 0.1mM hydrogen peroxide, 60 μl of 1mM iron (III) chloride and 1.5 ml of the Phytexponent and the standard at different concentrations were added except in the controls, followed by incubation at room temperature for 5 minutes. The increase in absorbance at 560nm was measured, and radical scavenging activity was calculated using the following formula.
% radical scavenging activity = (A0− A1) /A0 × 100
Where, A0 is the absorbance of the control, and A1 is the absorbance of the extracts/standard. The % of inhibition was plotted against concentration and the graph IC50 was calculated. The experiment was repeated three times at each concentration.

2.4.5 Determination of Total Phenolic Contents[20].
The total phenolic content of the extracts was measured according to the Folin-Ciocalteu method adapted from Do et al with some modifications. Briefly, the extract (1 ml) was mixed with 2 ml of Folin-Ciocalteu reagent, which was prepared by dilution with distilled water in a ratio of 1: 10 v/v, after which 1 ml of 20% sodium carbonate (Na2CO3) was added. The mixture was shaken for 20 seconds and incubated at 40°C for 30 minutes. Absorbance was measured at 765 nm. Gallic acid was used for the generation of the standard curve. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram (g) of the studied extracts. The experiment was repeated in triplicates.

2.4.6 Determination of Total Flavonoid Contents[21].
The total flavonoid content of the herbal extracts was evaluated through a technique described by Park et al. In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of NaNO2 (0.5 M), and 0.15 ml of AlCl3·6H2O (0.3 M) were mixed. After 5 minutes, 1 ml of NaOH (1 M) was added and mixed well, and the absorbance was measured against the reagent blank at 510 nm. &e standard curve for total flavonoids prepared using quercetin standard solution (0–100 mg/l). The total flavonoids were expressed as milligrams of quercetin equivalents per g of sample. The experiment was repeated thrice.

3. Results and discussion

3.1 Percentage yield, colour and consistency of herbal extracts

Table 3: Percentage yield, colour and consistency of herbal extracts

S. No.	Extracts	Colour in day light and consistency	% Yield
1	EEGS	Semisolid dark brown	18.17
2	EETF	Semisolid Golden brown	15.55
3	EETC	Semisolid Light Yellow	32.98
4	EEAI	Semisolid greenish brown	29.67
5	EECZ	Semisolid brown	11.89
6	EESJ	Semisolid light brown	11.24
7	EENJ	Semisolid dark brown	28.56

3.2 Preliminary phytochemical investigation of herbal extracts

Table 4: Preliminary phytochemical constituents present in ethanolic herbal extracts.

S. no	Phyto Constituents	EEGS	EETF	EETC	EECZ	EESJ	EENJ	EEAI
1	Alkaloids	+	+	+	+	+	+	+
2	Glycosides	+	+	+	+	+	+	+
3	Flavonoids	+	+	+	+	+	+	+
4	Tannins	-	-	+	+	+	+	+
5	Steriods	-	-	-	-	-	+	-
6	Terpenoids	+	+	+	+	+	+	+
7	Carbohydrates	-	-	-	-	-	-	-
8	Proteins	-	-	-	-	-	-	-
9	Phenols	+	+	+	_	_	+	_
10	Saponins	+	_	+	-	-	+	_
11	Volatile Oils	_	+	_	+	_	_	+
12	Gums	_	_	_	_	_	_	_

+ indicates positive,-indicates negative result

3.3 Determination of In-vitro antioxidant activities of herbal extracts

3.3.1 DPPH Radical scavenging activity

The free radical scavenging activity of the ethanolic extracts of *Gymnema sylvestre*, *Trigonella foenum-graceum*, *Tinospora cardifolia*, *Azadirachata indica*, *Cinnamonumzeylanicum*, *Syzygium jambolana* and Jatamansi. Among all the extractives, Jatamansi possessed the highest activity. At a concentration of 100 µg/mL, the free radical scavenging activity of *Gymnema sylvestre*, *Trigonella foenum-graceum*, *Tinospora cardifolia*, *Azadirachata indica*, *Cinnamonumzeylanicum*, *Syzygium jambolana* and Jatamansi was found to be 87.6±0.87, 89.4±0.89, 91.61±0.91, 93.2±0.93, 95.3±0.95, 97.4±0.97, 100.2±1.02%, respectively. The IC50 of ethanolic extracts of *Gymnema sylvestre*, *Trigonella foenum-graceum*, *Tinospora cardifolia*, *Azadirachata indica*, *Cinnamonum zeylanicum*, *Syzygium jambolana* and Jatamansi was found to be 5±0.05, 7±0.07, 48.5±0.48, 60.4±0.60, 86.5±0.86, 9.2±0.09, 100±1.25µg/mL, respectively.

Table 5: Determination of DPPH radical scavenging activity

Concentration (µg/mL)	EEGS	EETF	EETC	EEAI	EECZ	EESJ	EENJ
20	9.21±0.09	11.67±0.11	18.68±0.18	25.74±0.25	35.66±0.35	39.48±0.39	48.96±0.48
40	20.11±0.20	28.18±0.28	38.11±0.38	33.11±0.33	44.12±0.44	42.11±0.42	60.11±0.60
60	33.67±0.33	45.64±0.45	51.27±0.51	64.32±0.64	75.11±0.75	58.81±0.58	70.11±0.70
80	63.78±0.63	68.97±0.68	70.12±0.70	81.23±0.81	85.64±0.85	75.12±0.75	90.25±0.90
100	87.6±0.87	89.4±0.89	91.61±0.91	93.2±0.93	95.3±0.95	97.4±0.97	100.2±1.02

The values are expressed as mean ± SEM. Values with the same uppercase superscript letter within the same row and those with the same lowercase subscript letter within the same column are not significantly different ($P > 0.05$, one-way ANOVA followed by Tukey's test).

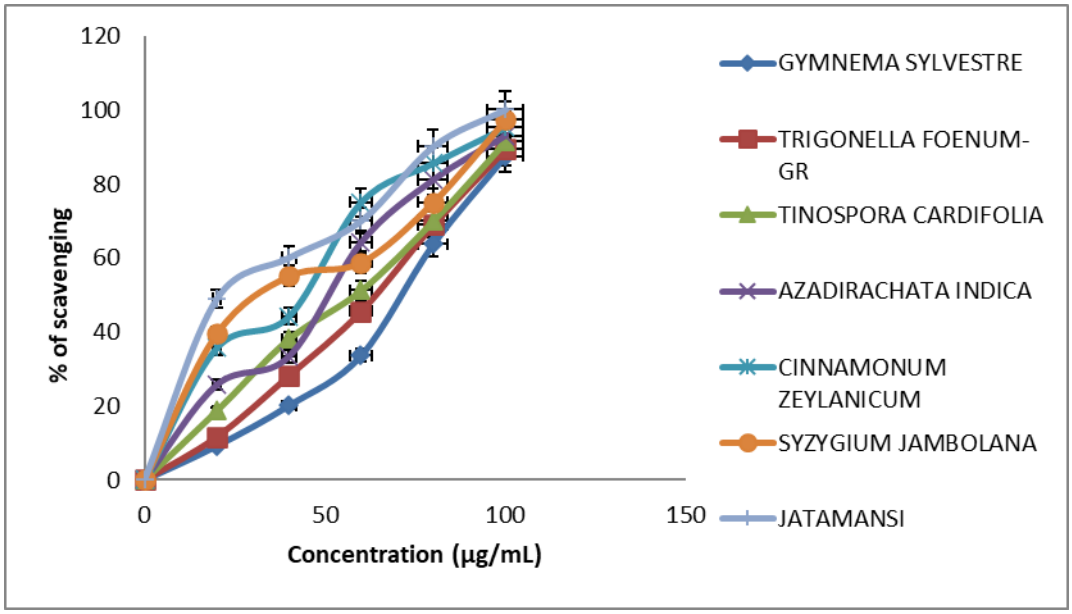

Figure 1: Histograph of determination of DPPH radical scavenging activity

Table 6: IC_{50} of ethanolic extracts of plant materials

S. No	Name of the Sample	$IC_{50\ \mu g/mL)}$
1	EEGS	5±0.05
2	EEGS	7±0.07
3	EEGS	48.5±0.48
4	EEGS	60.4±0.60
5	EEGS	86.5±0.86
6	EEGS	9.2±0.09
7	EEGS	100±1.25

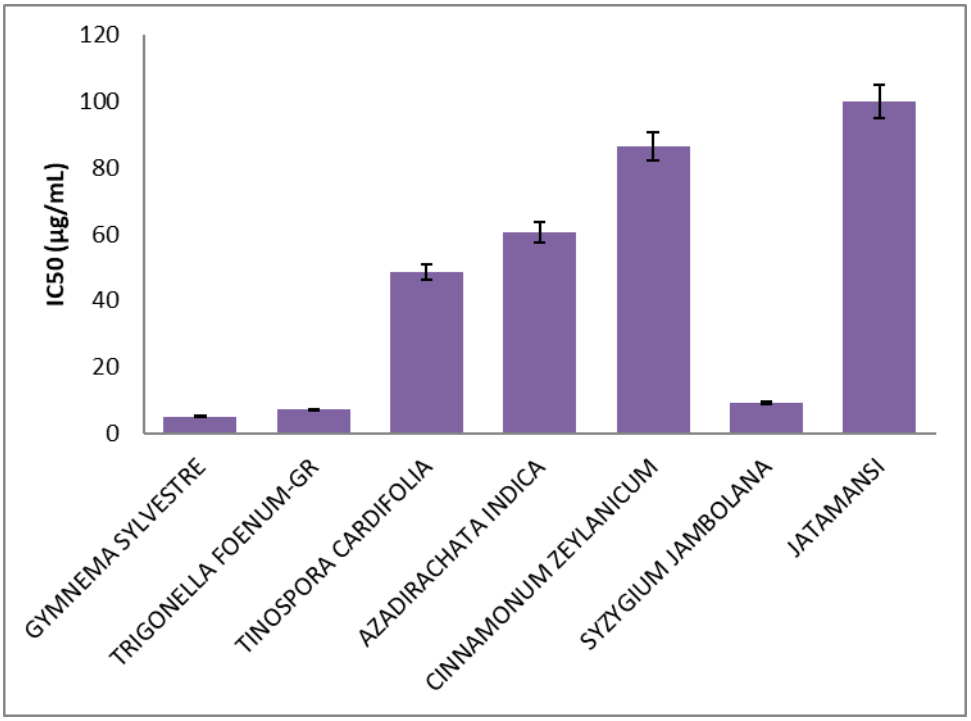

Figure 2: Histograph of IC_{50} of ethanolic extracts of plant materials

3.3.2 ABTS radical scavenging assay

The free radical scavenging activity of the ethanolic extracts of *Gymnema sylvestre*, *Trigonella foenum-graceum*, *Tinospora cardifolia*, *Azadirachata indica*, *Cinnamonumzeylanicum*, *Syzygium jambolana* and Jatamansi. Among all the extractives, Jatamansi possessed the highest activity. At a concentration of 100 µg/mL, the scavenging activity of *Gymnema sylvestre*, *Trigonella foenum-graceum*, *Tinospora cardifolia*, *Azadirachata indica*, *Cinnamonum zeylanicum*, *Syzygium jambolana* and Jatamansi are found to be 90.41±0.90, 92.36±0.92, 94.67±0.94, 91.29±0.91, 87.66±0.87, 89.45±0.89, 98.23±0.98% respectively.

Table 7: Determination of **ABTS radical scavenging assay** activity

Concentration (µg/mL)	EEGS	EETF	EETC	EEAI	EECZ	EESJ	EENJ
20	11.67±0.11	14.97±0.14	17.84±0.17	21.32±0.21	23.97±0.23	28.85±0.28	36.49±0.36
40	22.41±0.22	28.18±0.28	23.66±0.23	33.45±0.33	44.12±0.44	55.12±0.55	60.11±0.60
60	48.66±0.48	48.66±0.48	50.12±0.50	64.32±0.64	75.11±0.75	58.81±0.58	70.11±0.70
80	75.23±0.75	85.45±0.85	60.98±0.60	81.23±0.81	85.64±0.85	75.12±0.75	90.25±0.90
100	90.41±0.90	92.36±0.92	94.67±0.94	91.29±0.91	87.66±0.87	89.45±0.89	98.23±0.98

The values are expressed as mean ± SEM. Values with the same uppercase superscript letter within the same row and those with the same lowercase subscript letter within the same column are not significantly different ($P > 0.05$, one-way ANOVA followed by Tukey's test).

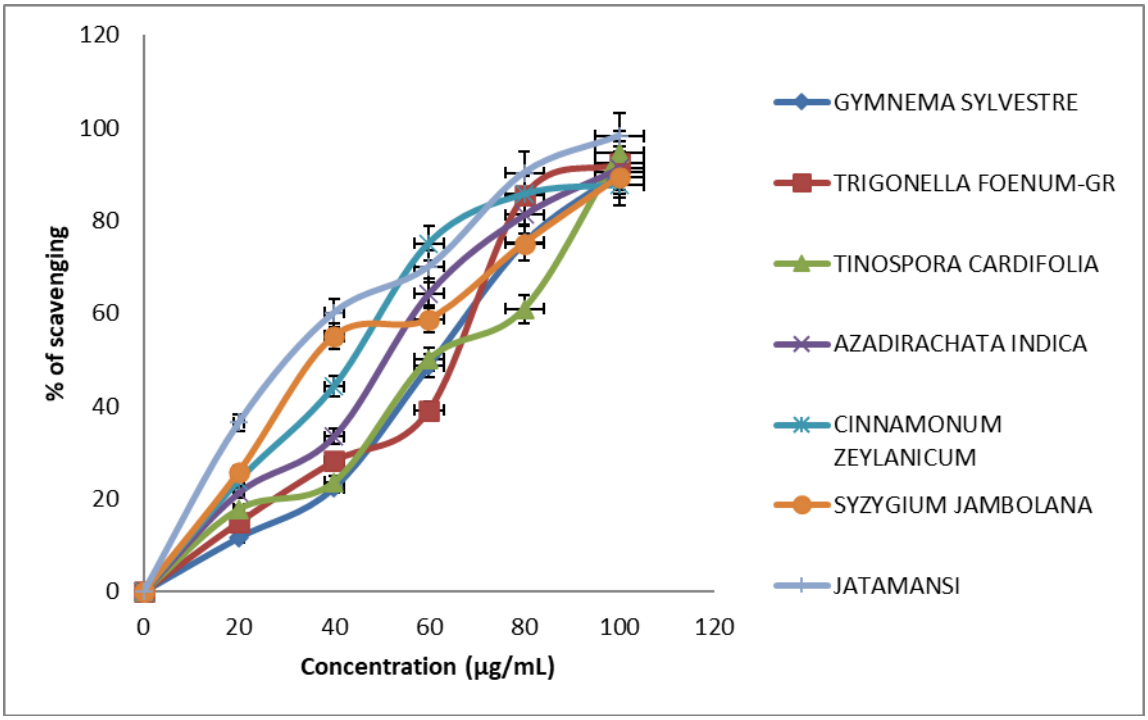

Figure 3: Histograph of determination of ABTS radical scavenging assay activity

3.3.3 Ferric Reducing Antioxidant Power Assay

The ethanolic extracts of medicinal plants exhibited remarkable concentration-dependent increases in absorbance values at a wavelength of 700 nm (Table). At all the tested concentrations, the ethanolic extract of Jatamansi produced significantly higher absorbance values compared with the absorbance generated by remaining extracts ($P < 0.05$). However, the standard (L-ascorbic acid) demonstrated significantly higher absorbances than those obtained for the three studied plant extracts ($P < 0.05$; Table). Furthermore, the half effective concentrations (EC50) of the studied plant extracts required to produce an absorbance value of 0.5 were determined in this study. It was demonstrated that the EC50 value for L-ascorbic acid was lower than the EC50 values of all the studied plant extracts.

Table 8: Determination of ferric reducing antioxidant power activities of ethanolic extracts

Concentration (µg/mL)	L-Ascorbic acid	EEGS	EETF	EETC	EEAI	EECZ	EESJ	EENJ
20	1.84±0.01	1.04±0.01	1.02±0.01	1.08±0.01	1.32±0.01	1.12±0.01	1.24±0.01	1.66±0.01
40	1.98±0.02	1.17±0.02	1.20±0.02	1.33±0.02	1.44±0.02	1.16±0.02	1.68±0.02	1.86±0.02
60	2.26±0.01	1.54±0.01	1.67±0.01	1.58±0.01	1.75±0.01	1.67±0.01	1.94±0.01	1.97±0.01
80	2.78±0.02	1.92±0.02	1.88±0.02	1.76±0.02	1.86±0.02	1.82±0.02	2.08±0.02	2.42±0.02
100	2.98±0.01	2.06±0.01	1.99±0.01	1.83±0.01	2.02±0.01	2.12±0.01	2.52±0.01	2.66±0.01
EC 50 Values	0.15	0.21	0.21	0.21	0.18	0.19	0.17	0.17

The values are expressed as mean ± SEM. Values with the same uppercase superscript letter within the same row and those with the same lowercase subscript letter within the same column are not significantly different ($P > 0.05$, one-way ANOVA followed by Tukey's test).

3.3.4 Hydroxyl Radical Scavenging Activity

In Vitro Hydroxyl Radical Scavenging Activities of ethanolic Extracts of herbal drugs exhibited remarkable in vitro hydroxyl radical scavenging activities (Table 4). At all the tested concentrations, the ethanolic extracts of jatamansi exhibits significantly higher hydroxyl radical scavenging activities than those of the ethanolic extracts ($P < 0.05$; Table). However, at all the tested concentrations, the in vitro hydroxyl radical scavenging activities of L-ascorbic acid were significantly higher than those of the ethanolic extracts ($P < 0.05$; Table). In this study, the concentration of the studied plant extracts capable of scavenging 50% of the hydroxyl radicals (IC50) was also determined.

Table 9: Determination of hydroxyl radical scavenging activity of ethanolic extracts

Concentration (µg/mL)	L-Ascorbic acid	EEGS	EETF	EETC	EEAI	EECZ	EESJ	EENJ
20	87.46±1.20	66.56±0.21	80.56±0.21	64.56±0.21	64.26±0.21	78.32±0.31	63.56±0.21	82.56±0.22
40	81.45±0.63	56.42±0.19	72.42±0.17	50.42±0.19	60.12±0.17	74.12±0.17	52.42±0.19	76.42±0.20
60	75.32±1.04	46.14±0.12	69.14±0.22	42.14±0.12	38.14±0.11	68.14±0.22	46.14±0.12	72.14±0.26
80	64.29±0.24	42.26±0.18	56.26±0.38	32.26±0.18	32.16±0.14	58.16±0.38	30.26±0.18	58.26±0.38
100	53.14±0.46	32.38±0.40	43.38±0.44	28.38±0.40	28.38±0.40	45.18±0.44	24.38±0.40	45.38±0.44
EC 50 Values	1.06	0.14	0.48	0.14	0.16	0.48	0.16	0.50

The values are expressed as mean ± SEM. Values with the same uppercase superscript letter within the same row and those with the same lowercase subscript letter within the same column are not significantly different (P > 0.05, one-way ANOVA followed by Tukey's test).

3.3.5 Determination of Total Phenolic Contents & Total Flavonoid Contents

Determination of total phenolic and flavonoid contents of ethanolic extracts of herbal plants was performed in this study. The results showed that the ethanolic extract of Jatamansi had a significantly higher total phenolic content (41.80±0.07 mg of GAE/g) than the phenolic content of other ethanolic extracts (P < 0.05; Table). On the other hand, analysis of the total flavonoid content, in ethanolic extracts of the studied plant extracts, showed that jatamansi contained significantly higher total flavonoids (38.98±0.05 of QE/g) compared with the total flavonoid content of other ethanolic extracts (P < 0.05; Table 9)

Table 10: Determination of total phenolic contents & total flavonoid contents of ethanolic extracts

Extracts	TPC	TFC
EEGS	28.62±0.06	25.12±0.05
EETF	30.06±0.03	27.03±0.05
EETC	37.21±0.02	34.21±0.03
EEAI	36.24±0.06	30.46±0.04
EECZ	38.02±0.05	33.12±0.05
EESJ	27.54±0.07	22.04±0.06
EENJ	41.80±0.07	38.98±0.05

TPC, total phenolic content; mgGAE/g, milligrams gallic acid equivalent per gram of sample extracts. TFC, total flavonoid content; mg QE/g, milligrams of quercetin equivalent per gram of sample extracts. Values are expressed as mean ± SEM. Means with different superscript letters are significantly different by one way ANOVA followed by Tukey's test.

3.3.6 Lipid peroxidation assay (TBARS)

The free radical scavenging activity of the ethanolic extracts of *Gymnema sylvestre, Trigonella foenum-graceum, Tinospora cardifolia, Azadirachata indica, Cinnamonumzeylanicum, Syzygium jambolana* and Jatamansi. At a concentration of 100 µg/mL, the scavenging activity of *Gymnema sylvestre, Trigonella foenum-graceum, Tinospora cardifolia, Azadirachata indica, Cinnamonum zeylanicum, Syzygium jambolana* and Jatamansi are found to be 90.41±0.90, 92.36±0.92, 89.14±0.94, 94.67±0.91, 91.22±0.87, 90.41±0.89, 99.45±0.98%, respectively. A positive correlation of lipid peroxidation inhibition with free radical (DPPH and OH) scavenging activities was observed.

Table 11: Determination of lipid peroxidation assay (TBARS)

Concentration (µg/mL)	EEGS	EETF	EETC	EEAI	EECZ	EESJ	EENJ
20	14.97±0.11	21.18±0.14	23.97±0.17	21.32±0.21	23.97±0.23	29.45±0.28	48.11±0.36
40	22.41±0.22	28.18±0.28	23.66±0.23	33.45±0.33	44.12±0.44	55.12±0.55	60.11±0.60
60	48.66±0.48	48.66±0.48	50.12±0.50	64.32±0.64	75.11±0.75	58.81±0.58	70.11±0.70
80	75.23±0.75	85.45±0.85	60.98±0.60	81.23±0.81	85.64±0.85	75.12±0.75	90.25±0.90
100	90.41±0.90	92.36±0.92	89.14±0.94	94.67±0.91	91.22±0.87	90.41±0.89	99.45±0.98

The values are expressed as mean ± SEM. Values with the same uppercase superscript letter within the same row and those with the same lowercase subscript letter within the same column are not significantly different (P > 0.05, one-way ANOVA followed by Tukey's test).

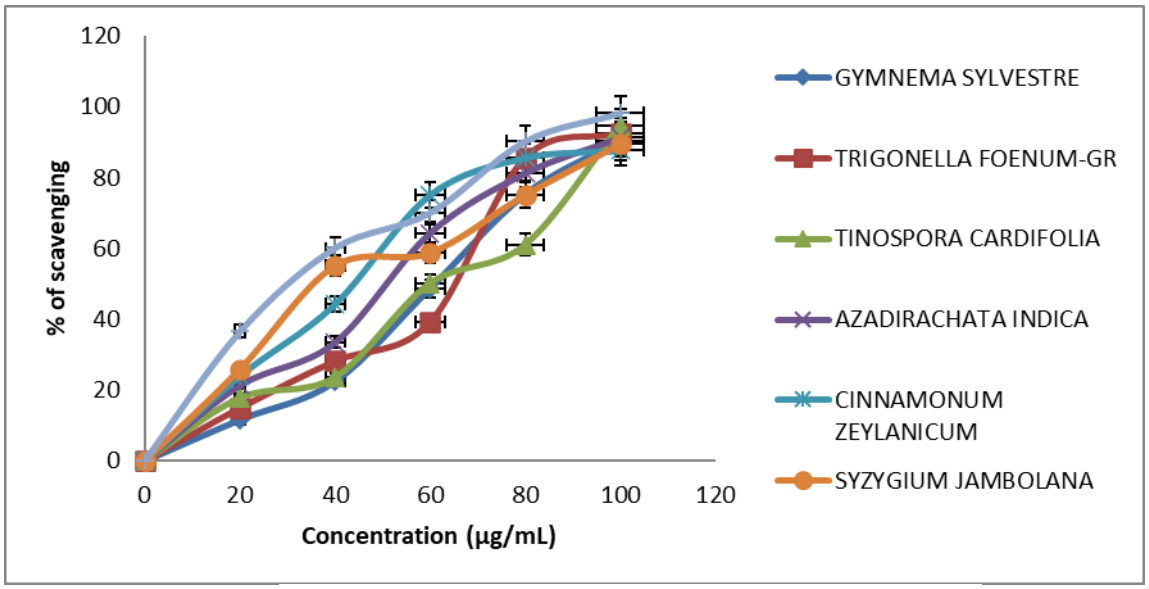

Figure 4: Histograph of determination of lipid peroxidation assay (TBARS)

4. Conclusion

From the above obtained results, it was concluded that the ethanolic extracts of various etanolic herbal extracts of *Gymnema sylvestre, Trigonella foenum-graceum, Tinospora cardifolia, Azadirachata indica, Cinnamonum zeylanicum, Syzygium jambolana* and Jatamansi have shown appreciable antioxidant activity. With reference to other ethanolic herbal extracts, Jatamansi exhibits the potential antioxidant capacity and antioxidant-associated phytochemicals. Further studies that aimed to isolating and characterizing the pure phytoactive principles for enhancement are recommended. Toxicity studies should be performed to determine their safety & efficacy.

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