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Research Article

COMARATIVE IN-VITRO ANTIOXIDANT AND PHYTOCHEMICAL SCREENING OF TRADITIONAL MEDICINAL PLANTS

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ABSTRACT

Lepidium sativum (Brassicaceae), *Ficus benghalensis (*(Moraceae)*) and Ficus religiosa* (Moraceae) is a popular Indian folk medicine for the screening of phytochemical and in-vitro antioxidant activity. The extract was used for study their phytochemical composition, total Phenolic content , Flavonoids contents, and in vitro antioxidant activities including 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric chloride scavenging, and phosphor-molybdenum scavenging activity. The phytochemical studies of the ethanolic extract of *Lepidium sativum* L. seeds have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids, amino acids and total Phenolic content (4.46±0.14 mg GAE/gm extract) and total flavonoid content (3.57±1.2 mg QE/gm). The IC50 values for scavenging DPPH, ferric chloride, phosphor-molybdenum were 18.46±0.27 µg/ml, 9.11±0.40µg/ml and 18.41±0.08µg/ml respectively and methanolic extraction of *Ficus benghalensis L* latex have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids, amino acids and total Phenolic content (2.76±0.84mg GAE/gm extract) and total flavonoid content (1.84±0.5 mg QE/gm). The IC50 values for scavenging DPPH, ferric chloride, phosphor-molybdenum were 28.63±0.16 µg/ml, 49.82 ±1.00µg/ml and 31.84±0.12 µg/ml respectively and methanolic extract of *Ficus religiosa* L. latex have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids, amino acids and total Phenolic content (3.15±0.32 mg GAE/gm extract) and total Flavonoids content (1.95±1.6 mg QE/gm). The IC50 values for scavenging DPPH, ferric chloride, phosphor-molybdenum were 31.75±0.12 µg/ml, 16.21±0.47 µg/ml and 18.35±0.48 µg/ml respectively. The results of present data was shown that the ethanolic extract of *Lepidium sativum* L. Seeds have contributed high potential in-vitro antioxidant activity then methanolic extract of *Ficus benghalensis* and *Ficus religiosa* L. Latex, due to lower IC50 values for scavenging of free radicals.

Keywords: Gallic acid: Querecetin: DPPH: TPTZ: Trolox.

INTRODUCTION

Free radical and Reactive oxygen species (ROS) like superoxide, hydroxyl radical peroxyl radical as well as non-radical species such as hydrogen peroxide (H2O2) (1). These free are derived from the normal metabolism or exogenous agent like chemical or medicine (2). Reactive species are act as oxidative damage of cellular tissue that is implicated as a possible factor in the etiology of several human diseases, including cancer, cardiovascular disease, and aging (3). *In vivo*; such reactive species is reduced by endogenous ant oxidative defences, so as to preserve optimal cellular function. In pathological conditions, however, the detoxifying mechanisms are often inadequate as excessive in finding antioxidant phytochemical, because they can inhibit the propagation of free radical reactions; protect the human body from diseases (4). The antioxidant property of Phenolic is mainly due to their redox properties. They act as reducing agents (free radical terminators), hydrogen donors, and singlet oxygen quenchers and metal chelators (5). The ethanolic extract of *Lepidium sativum* L. seeds contain volatile essential aromatic oils, active principle and fatty oils and carbohydrate, protein, fatty acid, Vitamin: β-carotene, used as aperients, diuretic, good anti inflammatory, demulcent, aphrodisiac, carminative, galactagogue, antiasthematic, antiscurbutic, and stimulant (6&7) and The methanolic extraction of *Ficus benghalensis L* latex. Contains glycoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitosterol-alpha-D-glucose, and meso-inositol have been isolated from the bark of the *Ficus benghalensis* (8). The fruit extracts exhibited antitumor activity in the potato disc bioassay (9). The leaves contain 9.63% crude protein, 26.84% crude fibres, 2.53% CaO, and 0.4% Phosphorous. It yields latex containing Caoytchoue (2.4%), Resin, Albumin, Cerin, sugar, and Malic acid. It is used in ayurveda for the treatment of diarrhea, dysentery, and piles (10) and as a hypoglycemic. (11&12) and the methanolic extract of *Ficus religiosa* L. latex. contain tannin, saponin gluanolacetate, *β* sitosterol, leucopelargonidin– 3 – O – *β* – D -glucopyranoside, leucopelargonidin – 3 – O – *α* –L - rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, *α*-amyrin acetate, leucoanthocyanidin, and leucoanthocyanin. [6] Some reported pharmacological activity of *F. religiosa* like fruit extracts exhibited antitumor activity in the potato disc bioassay. Aqueous extract was decreased the fasting blood glucose and exaggerated activity of superoxide dismutase SOD in streptozotocin induced type II diabetic rats, (13). anthelmintic activity of the methenolic extract. (14). Aqueous extract showed high antimicrobial activity against selected pathogenic like *B. subtilis* and P. *Aeruginosa* (15). Thus, the purpose of current study was investigate the in - vitro antioxidant potential of above mention plants extracts.

MATERIALS AND METHODS

Drug and Reagents

Folin-Ciocalteus's phenol reagent, sodium carbonate, Gallic acid (GA), Quercetin (QE), FeCl3, NaNO2, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid DPPH˙ (1,1-Diphenyl-2- TPTZ (2,4,6-Tripyridyl-s-triazine), Trolox, BHT (Butylated hydroxytoluene) were purchased from Sigma Chemical Company Ltd, and Sodium nitro preside (SNP), a-napthyl-ethylenediamine, potassium ferricyanide, trichloroacetic acid were purchased from Merck pvt. Ltd., India). All the chemicals used including the solvents, were of analytical grade.

Plant material

Lepidium sativum L. seeds were purchased from market of Mandsaur city (M.P., India). The plant was identified by Dr. H.S. Chattarjee (Ex professor of botany), P. G. College of Mandsaur, and M.P. And voucher specimen (BRNCP/L/02/2006) was submitted in department of Pharmacognosy; BRNCP, Mandsaur, M.P. and *Ficus benghalensis* L and *F. religiosa* plants were collected from village Pipariya, dist. Vadodara (G.P., India). The plant was identified by Dr. Nagar (Professor of botany), M.S. University vadodara (Gujarat) and voucher specimen (DPSV/F/01/2010) was submitted in department of Pharmacy, Sumandeep Vidyapeeth Vadodara, Gujarat.

Sample Preparation and Extraction

The trampled *Lepidium sativum* L. seeds were extracted by soxhlet apparatus using ethyl alcohol as a solvent. *Ficus benghalensis* L. and *F. religiosa* latex were extracted exhaustively by maceration process (24 hours) using methyl alcohol as a solvent. The extract was dried by rotator evaporator under reduced pressure. The extract was dried in rotator evaporator under reduced pressure.

Photochemical Screening

Standard phytochemical methods were used to test for the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyan genetic glycosides, amino acid & protein and Flavonoids (16, 17, 18, 19, 20, and 21).

Determination of total Phenolic content

The 100 mg pure Gallic acid was dissolved in 100 ml doubled distilled water then it was further dilution in µl to made five different concentration solutions such as 10µl, 20µl, 30 µl, 40µl, 50µl respectively. Then absorbance was taken for respective concentration of standard solution at 629 nm wavelength by U.V. spectrophotometer, and then standard curve was plotted with help of various concentration and absorbance. The extract was dissolved in doubled distilled water and was made up 100µl dilution and was added respective ingredient in above each step of procedure. Further absorbance was taken same as per standard at 629nm. (22)

Determination of total Flavonoid content

The 100 mg pure querecetin was dissolved in 100 ml doubled distilled water then further dilution in µl and was made five different concentration solutions such as 10µl, 20µl, 30 µl, 40µl, 50µl respectively. Then absorbance was taken of respective concentration of standard solution at 419 nm wavelength by U.V. spectrophotometer, then standard curve was plotted with help various concentration and absorbance. The 100 mg pure quercetin was dissolved in 100 ml doubled distilled water then further dilution in µl and was made five different concentration solutions such as 10µl, 20µl, 30 µl, 40µl, 50µl respectively. Then absorbance was taken of respective concentration of standard solution at 419 nm wavelength by U.V. spectrophotometer, then standard curve was plotted with help various concentration and absorbance. (23)

Antioxidant Activity

Determination of DPPH scavenging assay:

DPPH radical scavenging activity of ethanolic extract of *Lepidium sativum* seeds was determine according to the method reported by Blois [24]. An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was measured at 517 nm using UV-vis spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula.

% of inhibition = absorbance of control – absorbance of sample / absorbance of control ×100

Determination of Fecl3 scavenging Antioxidant assay (FSAA):

The ferric chloride scavenging assay was performed according to Benzie and Strain (25) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g CH3COONa.3H2O and 16 ml CH3COOH), pH 3.6, 10 mM TPTZ (2, 4, 6-Tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl3.6H2O solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml FeCl3.6H2O solution and then warmed at 37°C before using. The solutions of plant samples and trolox were formed in methanol (250 mg/mL). 10 mL of each of sample solution and BHT solution were taken in separate test tubes and 2990 mL of FSAA solution was added in each to make total volume up to 3 mL. The plant samples were allowed to react with FSAA solution in the dark for 30 min. Readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 595 nm. The FRAA values were determined as micromoles of trolox equivalents per mL of sample by computing with standard calibration curve constructed for different concentrations of trolox. Results were expressed in TE µg/mL.

Determination of phosphor- molybdenum scavenging assay:

The antioxidant activity of the ethanolic extract was determined by the phosphor-molybdenum Method as described by Prieto *et al*, (26). 0.3 ml of extract was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam) against blank. Methanol (0.3 ml) in place of extract was used as the blank. The total antioxidant capacity was expressed as the number of equivalents of Ascorbic acid (AAE).

RESULTS

The phytochemical studies of the ethanolic extract of *Lepidium sativum* L. seeds have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids, amino acids and total Phenolic content (4.46±0.14 mg GAE/gm extract) and total Flavonoids content (3.57±1.2 mg QE/gm). The IC50 values for scavenging DPPH, ferric chloride, phosphor-molybdenum were 18.46±0.27 µg/ml, 9.11±0.40µg/ml and 18.41±0.08µg/ml respectively (shown table 1,4 & fig.1, 2, &3).

Table 1: Phytochemical screening of ethanolic extract of *Lepidium sativum* seeds

S. No.	Name of Tests	Results
1.	Glycosides	+ve
	Cardiac glycosides	+ve
	Anthroquinone glycoside	+ve
	Cynogegenetic	+ve
	Flavonoids	+ve
	Coumarin glycoside	-ve
	Saponin glycoside	-ve
2.	*Alkaloids*	+ve
	Dragendraff's test	+ve
	Wagner test	+ve
	Hager test	+ve
	Mayer test	+ve
3.	Tannins and Phenolic Compound	+ve
4.	Proteins	+ve
	Sulpher contain aminoacid	+ve
	Biuret test	+ve
	Millions test	+ve
	Tyrosine	-ve
	Tryptophan	-ve
	Glycine	+ve
	Cysteine	+ve
	Glutamine	+ve
5.	Steroid	+ve
6.	Reducing glycoside	+ve
7.	Non reducing starch	-ve

Table 2: Phytochemical screening of the methanolic extraction of *Ficus benghalensis L* latex

S. No.	Name of Tests	Results
1.	Glycosides	+ve
	Cardiac glycosides	+ve
	Anthroquinone glycoside	+ve
	Cynogegenetic	-ve
	Flavonoids (shinoda test)	+ve
	Coumarin glycoside	-ve
	Saponin glycoside	-ve
2.	*Alkaloids*	+ve
	Dragendraff's test	+ve
	Wagner test	-ve
	Hager test	+ve
	Mayer test	-ve
3.	Tannins and Phenolic Compound	+ve
4.	Proteins	+ve
	Sulpher contain aminoacid	-ve
	Nihydrin test	+ve
	Biuret test	+ve
	Millions test	-ve
	Tyrosine	-ve
	Tryptophan	-ve
	Glycine	-ve
	Cysteine	-ve
	Glutamine	+ve
5.	Steroid	+ve
6.	Reducing glycoside	+ve

Table 3: Phytochemical screening of methanolic extract of *Ficus religiosa* L. Latex

S. No.	Tests	Results
1.	Glycosides	+ve
	Cardiac glycosides	+ve
	Anthroquinone glycoside	+ve
	Cynogegenetic	-ve
	Flavonoids (shinoda test)	+ve
	Coumarin glycoside	-ve
	Saponin glycoside	-ve
2.	*Alkaloids*	+ve
	Dragendraff's test	+ve
	Wagner test	-ve
	Hager test	+ve
	Mayer test	-ve
3.	Tannins and Phenolic Compound	+ve
4.	Proteins	+ve
	Sulpher contain aminoacid	-ve
	Nihydrin test	+ve
	Biuret test	+ve
	Millions test	+ve
	Tyrosine	+ve
	Tryptophan	-ve
	Glycine	-ve
	Cysteine	+ve
	Glutamine	-ve
5.	Steroid	+ve
6.	Reducing glycoside	+ve
7.	Non reducing sugar	+ve

Table 4: The total Phenolic and Flavonoids content of extract

Extract	Total Phenolic extract (mg GAE/gm)	Total Flavonoids content (mg QE/gm)
Ethanolic extract of *Lepidium sativum*	3.46±0.14	1.572±1.2
Methenolic extract of *Ficus benghalensis L*	276±0.84	1.84±0.5
Methanolic extract of *Ficus religiosa* L. Latex	3.15±0.32	1.95±1.6

The methanolic extraction of *Ficus benghalensis L* latex have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids, amino acids and total Phenolic content (2.76±0.84mg GAE/gm extract) and total Flavonoids content (1.84±0.5 mg QE/gm). The IC50 values for scavenging DPPH, ferric chloride, phosphor-molybdenum were 28.63±0.16 µg/ml, 49.82 ±1.00µg/ml and 31.84±0.12 µg/ml respectively. (Shown table 2, 4 & fig.4, 5, & 6).

The methanolic extract of *Ficus religiosa* L. latex have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids, amino acids and total Phenolic content (3.15±0.32 mg GAE/gm extract) and total Flavonoids content (1.95±1.6 mg QE/gm). The IC50 values for scavenging DPPH, ferric chloride, phosphor-molybdenum were 31.75±0.12 µg/ml, 16.21±0.47 µg/ml and 18.35±0.48 µg/ml respectively. (shown table 3,4 & fig.6, 7, & 8).

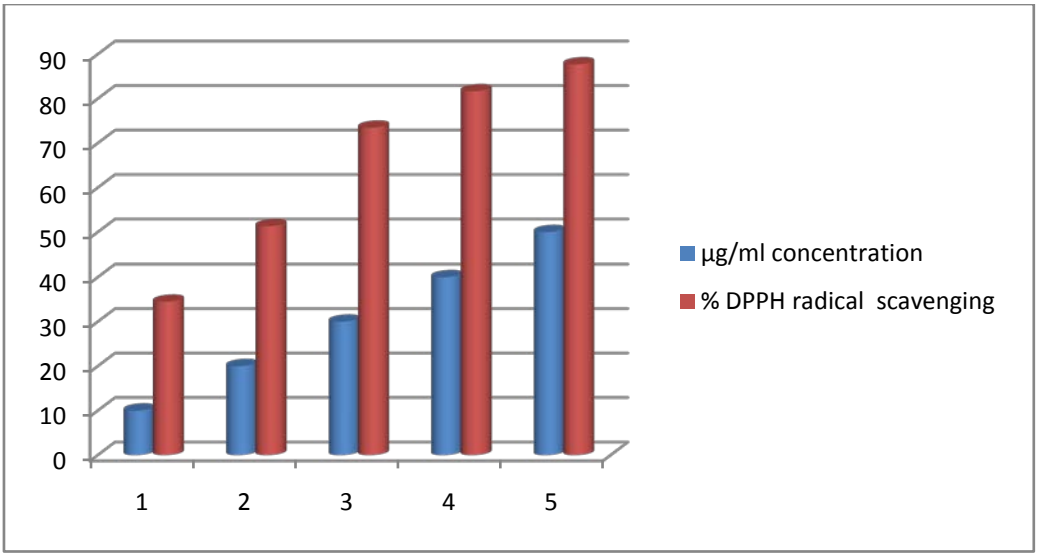

Fig. 1: % DPPH radical scavenging activity of ethanolic extract of *Lepidium sativum* seeds

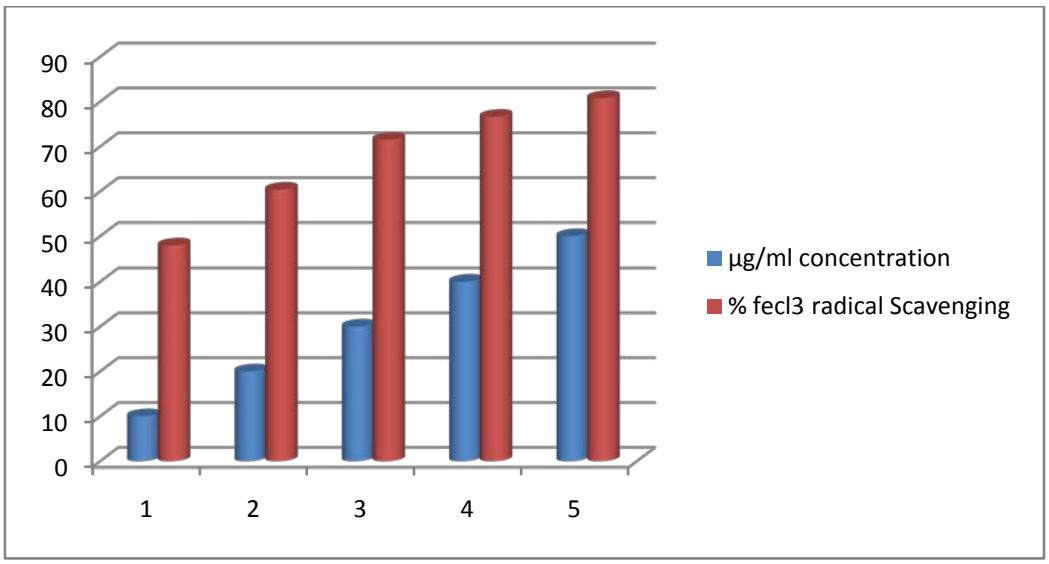

Fig. 2: % Fecl3 radical scavenging activity of ethanolic extract of *Lepidium sativum* seeds

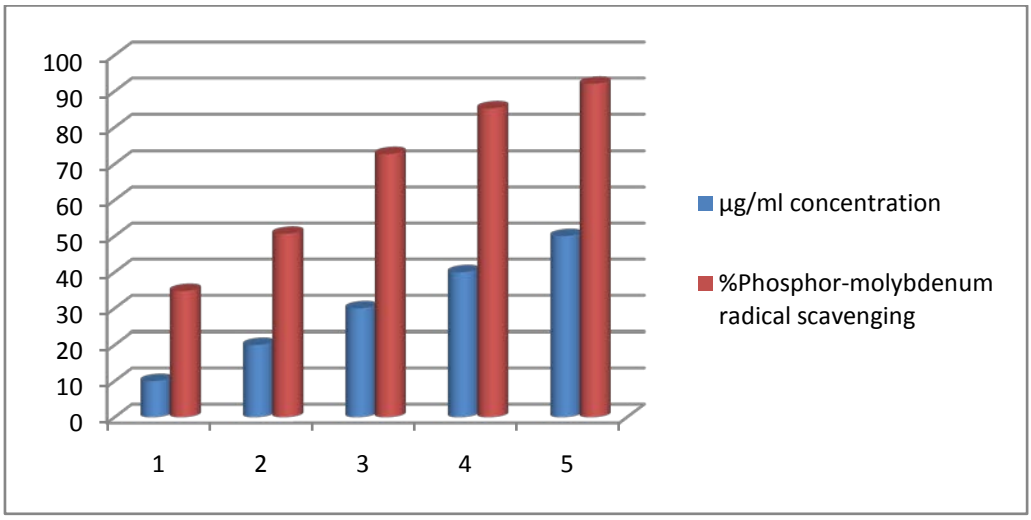

Fig. 3: % Phosphor-molybdenum radical scavenging activity of ethanolic extract of *Lepidium sativum* seeds

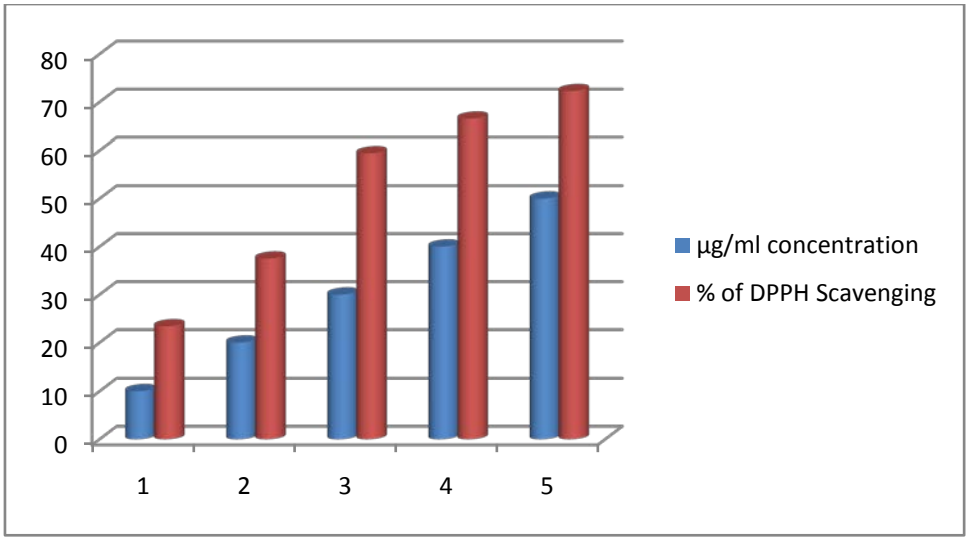

Fig. 4: %. DPPH radical scavenging activity of methanolic extraction of *Ficus benghalensis L* latex

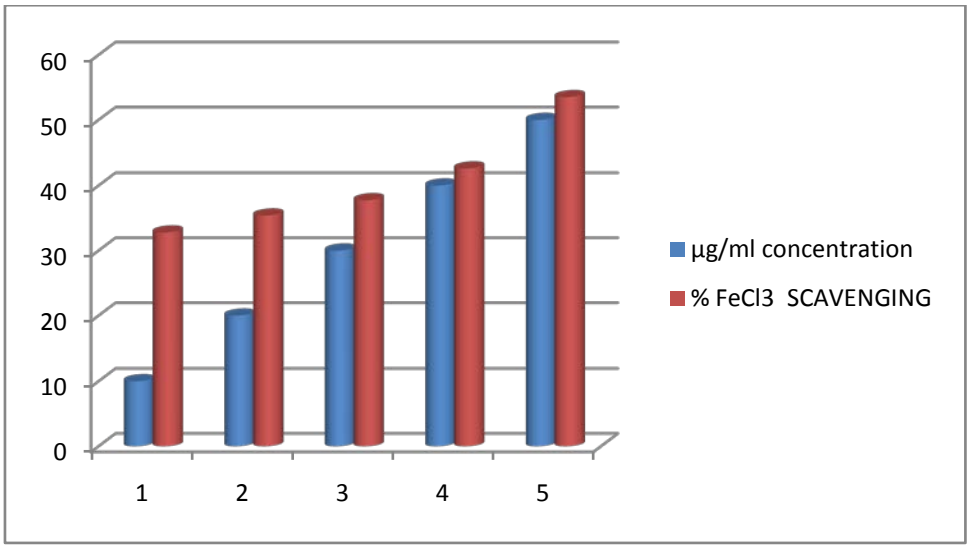

Fig. 5: % Fecl3 radical scavenging activity of methanolic extraction of *Ficus benghalensis L* latex

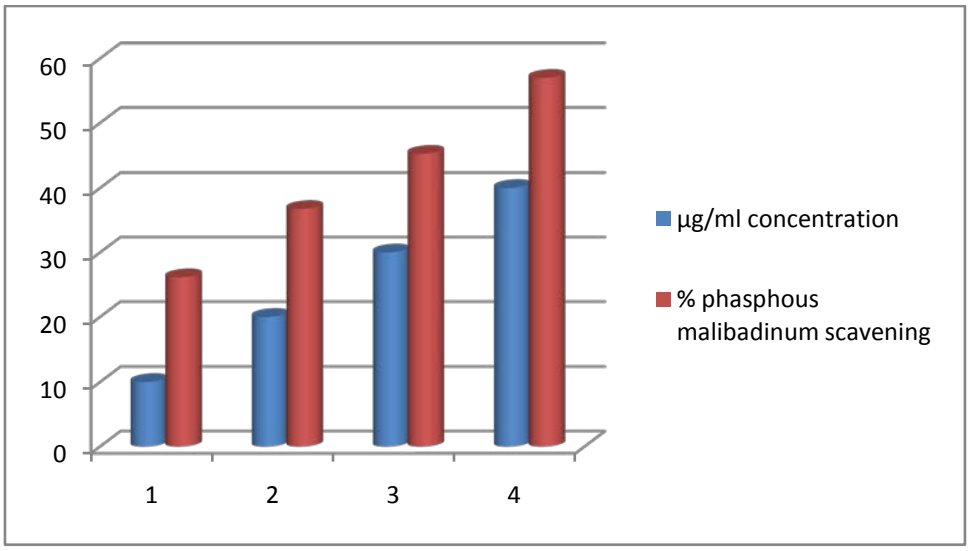

Fig. 6: % Phosphor-molybdenum radical scavenging activity of methanolic extraction of *Ficus benghalensis L* latex

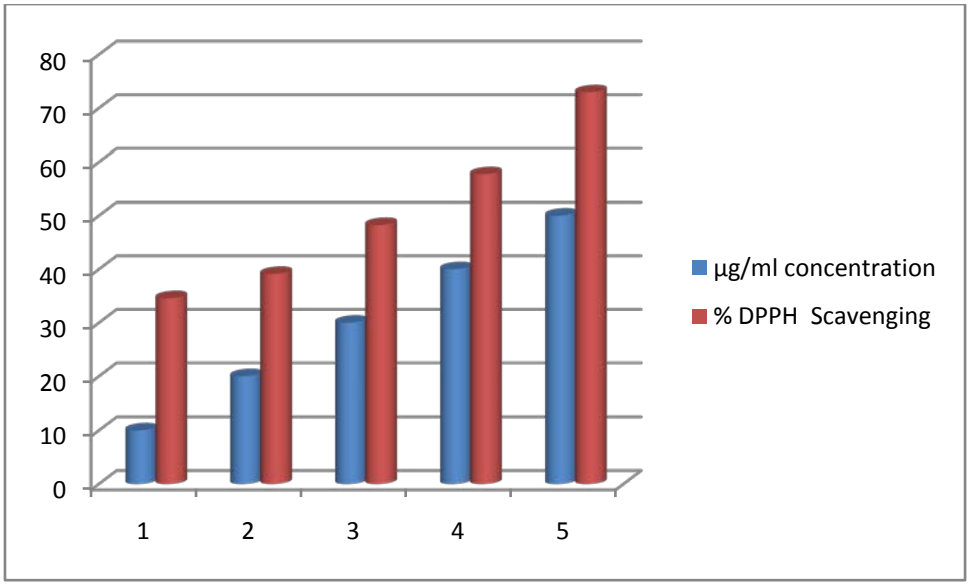

Fig. 7: % DPPH radical scavenging activity of methanolic extract of *Ficus religiosa* L. Latex

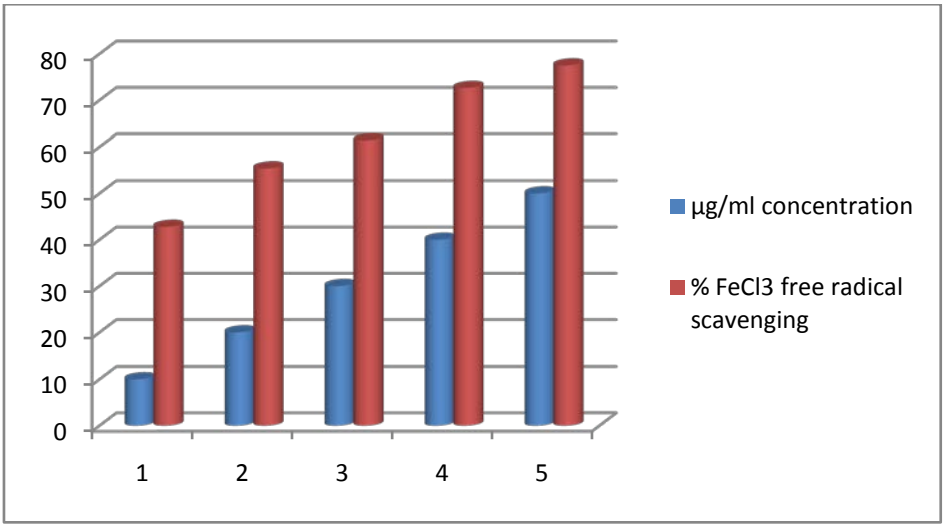

Fig. 8: % Fecl3 radical scavenging activity of methanolic extract of *Ficus religiosa* L. Latex

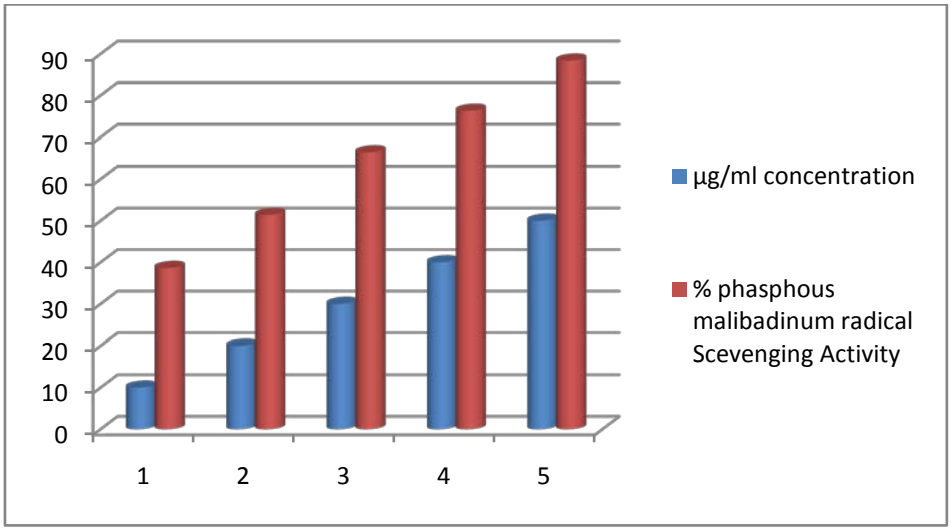

Fig. 9: % Phosphor-molybdenum radical scavenging activity of methanolic extract of *Ficus religiosa* L. Latex

DISCUSSION

The three methods were used for determine the antioxidant activity of the, ethanolic extract of *Lepidium sativum* L. Seeds, extract of *Ficus benghalensis and Ficus religiosa* latex Whereas DPPH free radical scavenging was considered a good in- vitro model widely used to assess antioxidant activity within the short time. DPPH was disappear on reduction by antioxidant compound or free radical spices to become stable diagnostic molecules resulting colour change from purple to yellow that can indicates hydrogen denoting ability of extract sample.(27 &28).

The IC50 values of ethanolic extract of *Lepidium sativum* L. Seeds for scavenging DPPH, ferric chloride, phosphor-molybdenum were 18.46±0.27 µg/ml, 9.11±0.40µg/ml and 18.41±0.08µg/ml respectively.

The IC50 values of extract of *Ficus benghalensis* latex for scavenging DPPH, ferric chloride, phosphor-molybdenum were 28.63±0.16 µg/ml, 49.82 ±1.00µg/ml and 31.84±0.12 µg/ml respectively.

The IC50 of *Ficus religiosa* latex values for scavenging DPPH, ferric chloride, phosphor-molybdenum were 31.75±0.12 µg/ml, 16.21±0.47 µg/ml and 18.35±0.48 µg/ml respectively.

All three method, those extract were shown lower IC50 Value that indicated that it have good in- vivo antioxidant potential due to present phytochemical study of the ethanolic extract have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids.

In the present study data was revealed that The IC50 values of ethanolic extract of *Lepidium sativum* L. Seeds was lower that the extract of *Ficus benghalensis and Ficus religiosa* latex. So that the ethanolic extract of *Lepidium sativum* L. Seeds have contributed high potential in-vitro antioxidant activity then methanolic extract of *Ficus benghalensis* and *Ficus religiosa* L. Latex, due to lower IC50 values for scavenging of free radicals .

CONCLUSION

Finally it was concluded that the ethanolic extract of *Lepidium sativum* L. have contributed high potential in-vitro antioxidant activity then methanolic extract of *Ficus benghalensis* and *Ficus religiosa* L. Latex.

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