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Research Article

FREE RADICAL SCAVENGING ACTIVITIES, TOTAL PHENOLIC AND FLAVONOID CONTENT OF *LEPIDIUM SATIVUM* (LINN.)

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ABSTRACT

Background: Many oxidative stress related diseases are as a result of accumulation of free radicals in the body. A lot of research is going on worldwide directed towards finding natural antioxidants of plants origin.

Objective: The purpose of the present investigation is to evaluate the free radical scavenging activity of total phenolic and flavonoid compounds extracted from the seeds of *Lepidium sativum*.

Methods: Different *in vitro* chemical assays DPPH, superoxide radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging assays were used. Total phenols and flavonoids are also estimated in various extracts of seeds of *Lepidium sativum.*

Results and Discussion: The methanolic extract of *Lepidium sativum* showed significant free radical scavenging activity than that of standards studied. Similar results were not seen in other extracts. Higher amount of phenols and flavonoids were found in methanolic extract of seeds of *Lepidium sativum* than that of other extracts. The radical scavenging activity was found to be concentration dependent.

Conclusion: The results obtained from this study indicate that seeds of *Lepidium sativum* are a potential source of antioxidants and thus could prevent many radical diseases.

Keywords: *Lepidium sativum,* Free radical scavenging, Phenolics, Flavonoids.

INTRODUCTION

The role of free radicals in disease initiation cannot be overemphasized. Most free radicals such as, hydroxyl radical ($\cdot OH$), the superoxide radical (O^{2-}), lipid peroxide radicals and hydrogen peroxide (H_2O_2) are being implicated in many disease conditions. These include cancer, gastrointestinal inflammation, asthma, cataracts, cardiovascular disease, diabetes mellitus, liver disorder, periodontal disease, and other inflammatory processes. Free radicals produced from oxygen to form reactive oxygen species such as the singlet oxygen, superoxide, peroxyl, hydroxyl and peroxynitrite radicals are constantly produced within living cells for specific metabolic purposes[1]. Living cells have complex mechanisms that act as antioxidant systems to counteract the damaging effects of reactive species. Oxygen radicals induce oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing. However, there have been concerns about synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) because of their possible activity as promoters of carcinogenesis[2]. Recently, many natural and synthetic free radical scavengers and antioxidants have been employed in protecting biomolecules against free radical mediated damages. There is a growing interest towards natural antioxidants from herbal sources[3-5].

Lepidium sativum Linn. (Cruciferae) is commonly known as garden cress. It is a small, herbaceous annual plant, cultivated throughout India. The whole plant is administered in asthma and bleeding piles and the roots are used in secondary syphilis and tenesmus[6]. Seeds are considered to be aphrodisiac, galactogogue, emmenagogue and are used after boiling with milk to induce abortion. This plant has been studied pharmacologically for its antidiarrheal and antispasmodic[7], hypoglycaemic[8], laxative[9], antibacterial[10], antioxidant, contraceptive effects[11] and in inflammatory bowel disease[12]. It has been evaluated for its fracture-healing[13] and diuretic activities[14].

It has been documented to possess alkaloids, riboflavin, α-tocopherol, β-carotenes, β-sitosterol, ascorbic, linoleic, oleic, palmitic and stearic acids. It is considered a good source of mono-unsaturated fatty acids and L-arabinose[15]. Moreover, cucurbitacins and cardenolides have also been identified as plant constituents[16]. The present study was carried out to evaluate the free radical scavenging activity of total phenolic and flavonoid compounds extracted from the seeds of *Lepidium sativum* (Linn.).

MATERIALS AND METHODS

Chemicals

Chemicals nitroblue tetrazolium (NBT), 2,2-diphenyl-1- picrylhydrazyl (DPPH), naphthylethylene diamine dihydrochloride (NEDA) were purchased from Sigma, Germany, Catechol (Loba Chemie, Mumbai), sodium nitroprusside, vanillin and EDTA (S.D-fine chemicals, Mumbai). All reagents used in the present study were analytical grade.

Collection and identification of the Plant materials

The seeds of *Lepidium sativum* (Linn.) were collected from Tirunelveli District, Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India, Palayamkottai. The seeds were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The dried powder was extracted sequentially by hot continuous percolation method using Soxhlet apparatus[17], using different polarities of solvents like n-hexane, chloroform, ethyl acetate and methanol. The dried powder was packed in Soxhlet apparatus and successively extracted with n-hexane for 24h. Then the marc was subjected to chloroform extraction for 24h, ethyl acetate for 24h and then methanol for 24h. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by in vitro methods

DPPH photometric assay[18]

The effect of extract on DPPH radical was assayed using the method of Mensor *et al* (2001)[9]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1ml of the different concentrations of plant extract and allowed to react at room temperature for 30min. Methanol served as the blank and DPPH in methanol without the extract served as the control. After 30min, the absorbance was measured at 518nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard. Rutin was used as a standard.

Superoxide radical scavenging activity[19]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was detected by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne *et al* (1975)[10]. The assay mixture contained sample with 0.1ml of nitro blue tetrazolium (1.5mM NBT) solution, 0.2ml of EDTA (0.1M EDTA), 0.05ml riboflavin (0.12mM) and 2.55ml of phosphate buffer (0.067M phosphate buffer). The control tubes were also set up with DMSO instead of the sample. The reaction mixture was illuminated for 30min and the absorbance at 560nm was measured against the control samples. Quercetin was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Determination of nitric oxide radical scavenging activity[20]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by the method of Garrat (1964).The reaction mixture (3ml) containing 2ml of sodium nitroprusside (10mM), 0.5ml of phosphate buffer saline (1M) were incubated at 25ºC for 150min. After incubation, 0.5ml of the reaction mixture containing nitrite was pipetted and mixed with 1ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5min for completing diazotization. Then 1ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30min. Then the absorbance was measured at 540nm. Ascorbic acid was used as a standard.

Determination of hydroxyl radical scavenging activity[21]

This was assayed as described by Elizabeth and Rao (1990). The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-Ascorbic acid–EDTA–H_2O_2system (Fenton reaction). The reaction mixture contained 0.1ml deoxyribose (2.8mM), 0.1ml EDTA (0.1mM), 0.1ml H_2O_2 (1mM), 0.1ml ascorbic acid (0.1mM), 0.1ml KH_2PO_4-KOH buffer, pH 7.4 (20mM) and various concentrations of plant extract in a final volume of 1ml. The reaction mixture was incubated for 1hour at 37ºC. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated. Ascorbic acid was used as a standard.

Estimation of total phenol[22]

0.5ml of Folins phenol reagent and 2ml of sodium carbonate (20%) was added to different concentrations of extracts. The reaction mixture was kept in boiling water bath for 1min and the absorbance was measured at 650nm in a UV- spectrophotometer. Gallic acid was used as a standard.

Estimation of total flavonoids[23]

0.5ml of extract and 4ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) were added and kept in a boiling water bath for 15min. The absorbance was read at 360nm and a standard was run by using catechol (110µg/ml).

RESULTS AND DISCUSSION

DPPH radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH radical transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1–1-diphenyl–2–picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The reducing capacity of DPPH radical is determined by the decrease in its absorbance at 516nm induced by antioxidants. It is visually noticeable as a change in colour from purple to yellow. Hence DPPH is usually used as a substance to evaluate the antioxidant activity.

The percentage of DPPH radical scavenging activity of various extracts of *Lepidium sativum* are presented in Table 1. The n-hexane, chloroform, ethyl acetate and methanol extracts of *Lepidium sativum* exhibited a maximum DPPH radical scavenging activity of 32.62%, 40.28%, 69.94%, 76.83% respectively whereas rutin (standard) exhibited 79.84 % at 1000µg/ml. The IC_{50} values of the various extracts of *Lepidium sativum* and rutin were found to be 1340µg/ml, 1165µg/ml, 315µg/ml, 110µg/ml and 380µg/ml respectively. The above results clearly depict that the methanolic and ethyl acetate extract of *Lepidium sativum* showed significant DPPH radical scavenging activity than that of standard rutin. Similar results were not observed in other extracts.

Superoxide radical scavenging activity

Super oxide radical known to be very harmful to the cellular components. Superoxide free radical is formed by alkaline DMSO which reacts with nitro blue tetrazolium (NBT) to produce coloured diformazan. The percentage of superoxide anion radical scavenging activity of various extracts of *Lepidium sativum* are presented in Table 2. The n-hexane, chloroform, ethyl acetate and methanol extracts of *Lepidium sativum* exhibited a maximum superoxide anion radical scavenging activity of 61.87%, 68.44%, 78.98%, 95.93% respectively whereas, quercetin (standard) showed 98.13% scavenging activity at 1000µg/ml. The IC_{50} values of the n-hexane, chloroform, ethyl acetate and methanol extracts of *Lepidium sativum* and quercetin were found to be 895µg/ml, 485µg/ml, 215µg/ml, 85µg/ml and 80µg/ml respectively. The methanolic extract of *Lepidium sativum* showed significant superoxide anion radical scavenging activity than that of standard. Similar results were not seen with other extracts.

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside is measured by the Greiss reagent reduction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide.

The percentages of nitric oxide radical scavenging activity of various extracts of Lepidium sativum are presented in Table 3. The n-hexane, chloroform, ethyl acetate and methanol extracts of *Lepidium sativum* exhibited a maximum nitric oxide radical scavenging activity of 32.13%, 39.31%, %, 61.83%, 78.23% respectively whereas ascorbic acid (standard) exhibited 64.37% at 1000µg/ml. The IC_{50} values of the various extracts of *Lepidium sativum* and ascorbic acid were found to be 1415µg/ml, 1370µg/ml, 320µg/ml, 132µg/ml and 280µg/ml respectively.

Table 1: DPPH scavenging potential of various extract of *Lepidium sativum*

S. No.	Conc. (µg/ml)	% of activity (±SEM)* n-Hexane	Chloroform	Ethyl acetate	Methanol	Standard (Rutin)
1	125	16.93 ± 0.09	18.66 ± 0.13	37.07 ± 0.24	51.51 ± 0.03	17.75 ± 0.45
2	250	18.53 ± 0.82	32.23 ± 0.47	48.64 ± 0.01	59.53 ± 0.98	59.95 ±0.82
3	500	28.56 ± 0.01	36.93 ± 0.96	53.09 ± 0.04	72.73 ± 0.21	63.73 ±0.20
4	1000	32.62 ± 0.74	40.28 ± 0.56	69.94 ± 0.18	76.83 ± 0.78	79.84 ±0.19
IC_{50} (in µg/ml)		**1340**	**1165**	**315**	**110**	**380**

*All values are expressed as mean ± SEM for three determinations

Table 2: Superoxide anion radical scavenging potential of various extract of *Lepidium sativum*

S. No.	Conc. (µg/ml)	% of activity(±SEM)*				
		n-Hexane	Chloroform	Ethyl acetate	Methanol	Standard (Quercetin)
1	125	13.49 ± 0.09	28.37 ± 0.44	45.12 ± 0.04	64.43 ± 0.39	68.85 ± 0.66
2	250	16.69 ± 0.02	45.12 ± 0.78	56.64 ± 0.60	92.32 ± 0.18	74.81 ± 1.09
3	500	37.34 ± 0.90	51.20 ± 0.11	76.93 ± 0.98	93.72 ± 0.05	92.90 ± 0.11
4	1000	61.87 ± 0.94	68.44 ± 0.01	78.98 ± 0.12	95.93 ± 0.04	98.13 ± 1.92
IC_{50} (in µg/ml)		**895**	**485**	**215**	**85**	**80**

*All values are expressed as mean ± SEM for three determinations

Table 3: Nitric oxide anion radical scavenging potential of various extract of *Lepidium sativum*

S. No.	Conc. (µg/ml)	% of activity(±SEM)*				
		n-Hexane	Chloroform	Ethyl acetate	Methanol	Standard (Ascorbic acid)
1	125	16.15 ± 0.67	20.06 ± 0.03	40.01 ± 0.88	49.41 ± 0.08	26.87 ± 0.09
2	250	20.22 ± 0.87	31.48 ± 0.08	44.32 ± 0.05	64.32 ± 0.02	40.38 ± 0.08
3	500	29.36 ± 0.89	36.37 ± 0.29	56.58 ± 0.76	76.34 ± 0.21	71.64 ± 0.43
4	1000	32.13 ± 0.01	39.31 ± 0.56	61.83 ± 0.12	78.23 ± 0.11	75.23 ± 0.02
IC_{50} (in µg/ml)		**1415**	**1370**	**320**	**132**	**280**

*All values are expressed as mean ± SEM for three determinations

Hydroxyl radical scavenging activity

Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell[24]. Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water.

The percentage of hydroxyl radical scavenging activity of n-hexane, chloroform, ethyl acetate and methanol extracts of *Lepidium sativum* is presented in Table 4. The n-hexane, chloroform, ethyl acetate and methanol extracts of *Lepidium sativum* exhibited a maximum hydroxyl radical scavenging activity of 37.65%, 62.72%, 77.64%, 83.82% whereas ascorbic acid (standard) exhibited 75.23% at 1000µg/ml. The IC_{50} values of the various extracts of *Lepidium sativum* and ascorbic acid were found to be 1465µg/ml, 537µg/ml, 210µg/ml, 95µg/ml and 280µg/ml respectively. The above results clearly depict that the methanolic and ethyl acetate extracts of *Lepidium sativum* showed significant hydroxyl radical scavenging activity than that of standard ascorbic acid. Similar results were not seen with other extracts.

Table 4: Hydroxyl radical scavenging potential of various extract of *Lepidium sativum*

S. No.	Conc. (µg/ml)	% of activity(±SEM)*				
		n-Hexane	Chloroform	Ethyl acetate	Methanol	Standard (Ascorbic acid)
1	125	18.27 ± 0.03	26.77 ± 0.09	42.18 ± 0.04	54.23 ± 0.03	26.87 ± 0.09
2	250	28.58 ± 0.01	32.92 ± 0.06	52.46 ± 0.93	72.81 ± 0.31	40.38 ± 0.08
3	500	36.42 ± 0.92	46.71 ± 0.12	58.61 ± 0.22	79.21 ± 0.01	71.64 ± 0.43
4	1000	37.65 ± 0.63	62.72 ± 0.35	77.64 ± 0.02	83.82 ± 0.32	75.23 ± 0.02
IC_{50} (in µg/ml)		**1465**	**537**	**210**	**95**	**280**

*All values are expressed as mean ± SEM for three determinations

Total phenol

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups[25]. The phenolic compounds may contribute directly to antioxidative action[26]. The total phenolic content of n-hexane, chloroform, ethyl acetate and methanol extracts of seeds of *Lepidium sativum* is presented in Table 5. The methanolic extract of *Lepidium sativum* showed higher content of phenolic components than other extracts.

Table 5: The total Phenolic content of various extracts of *Lepidium sativum*

S. No.	Extracts	Total phenol content (mg/g of Gallic acid) (±SEM)*
1.	n-Hexane extract of *Lepidium sativum*	1.034 ± 0.078
2.	Chloroform extract of *Lepidium sativum*	1.923 ± 0.020
3.	Ethyl acetate extract of *Lepidium sativum*	2.834 ± 0.679
4.	Methanol extract of *Lepidium sativum*	8.651 ± 0.321

*All values are expressed as mean ± SEM for three determinations

Total flavonoids

The total flavonoid content of n-hexane, chloroform, ethyl acetate and methanol extracts of *Lepidium sativum* are summarized in Table 6. Flavonoids present in food of plant origin are also potential antioxidants[27],[28]. Higher content of flavonoids was found in methanolic extract of *Lepidium sativum* than in other extracts.

Table 6: The total flavonoid content of various extracts of *Lepidium sativum*

S. No.	Extracts	Total flavonoid content (mg/g of Catechol) (±SEM)*
1.	n-Hexane extract of *Lepidium sativum*	0.304 ± 0.987
2.	Chloroform extract of *Lepidium sativum*	0.966 ± 0.056
3.	Ethyl acetate extract of *Lepidium sativum*	2.362 ± 0.008
4.	Methanol extract of *Lepidium sativum*	4.023 ± 0.081

*All values are expressed as mean ± SEM for three determinations

CONCLUSION

The findings of the present study indicate that the methanolic extract of *Lepidium sativum* showed significant antioxidant activity by inhibiting DPPH and hydroxyl radical, super oxide anion scavenging, nitric oxide and hydrogen peroxide scavenging activities and the IC_{50} values were significantly greater than the reference standards studied. In addition, the methanolic extract found to contain a noticeable amount of total phenols and flavonoids which has a major role in controlling antioxidants. The methanolic extract of *Lepidium sativum* can be used as easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry. The antioxidant activity of the extract may be attributed to the polyphenolic compounds and isoflavonoids with potential application to reduce oxidative stress with health benefits. Therefore, further work needs to be performed on the isolation and identification of the antioxidant components in methanolic extract of *Lepidium sativum.*

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