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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *LEPIDIUM SATIVUM LINN.* SEEDS

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ABSTRACT

The objective of this study is to investigate the phytochemical constituents. Phytochemical screening of seeds of *Lepidium sativum linn* (family-Brassicaceae) in different organic solvent revealed the presence of alkaloids, tannins, flavonoids. Antioxidant activities were evaluated by using *in-vitro* antioxidant assay models like phospho-molybdenum and reducing power assay. And hydrogen peroxide assay. The percentage of antioxidant activity by phospho-molybdenum assay was in the order methanol>ethanol>acetone. The reducing power assay was maximum shown in order acetone>methanol>ethanol and hydrogen peroxide assay was found in order acetone>ethanol>methanol. The results obtained in this study showed that the seeds of *lepidium sativum linn* have antioxidative properties which provide a basis for the traditional use of the plant.

Keywords: Lepidium sativum linn, Reducing power assay, H_2O_2 scavenging.

INTRODUCTION

Lepidium sativum linn (Garden cress) is an annual herb, belonging to Brassicaceae family. It is a fast-growing, edible plant botanically related to watercress and mustard and sharing their peppery, tangy flavor and aroma. Seeds, leaves and roots are economically important, however, the crop is mainly cultivated for seeds. *Lepidium sativum linn* in India and it is an important medicinal crop in India [1-3].

The seeds are useful both, internally as well as externally. The paste of seeds is applied in rheumatic joints to relieve the pain and swelling. The pulp of the seeds applied on the painful joints and on haematoma, effectively reduces the pain and swelling. The paste is salutary in the treatment of various skin diseases also.

Reactive oxygen species (ROS) are a class of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radical and hydrogen peroxide molecules are often generated as by-products of biological reactions or from exogenous factors. There is extensive evidence to involve ROS in the development of degenerative diseases. Evidence suggests that compounds especially from natural sources are capable of providing protection against free radicals. This has attracted a great deal of research interest in natural antioxidants. It is necessary to Screen out medicinal plants for their antioxidant potential [4-7].

MATERIALS AND METHODS

Plant Collection

Seeds of ***L.sativum linn*** were collected from Hubli, Karnataka and authenticated by Botanist, Bangalore University, Bangalore, Karnataka, India.

Chemicals and Reagents

All the chemicals and reagents used were of analytical grade and are purchased from Lancaster Research Lab, Chennai, India and Hi-media Lab, Mumbai, India.

EXTRACTION OF PLANT MATERIAL

Shade dried seed of *L.sativum linn* was powdered and about 15g of shade dried powder of seeds of *lepidium sativum linn* was successively extracted with

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ethanol, acetone, and methanol. The extract obtained was filtered, concentrated by evaporating at 100^0C in a water bath and dried [8-10].

PHYTOCHEMICAL ANALYSIS

The extracts were used for preliminary screening of phytochemicals such as alkaloids (Wagner's and Meyer's tests), saponins (foam and froth tests), (Acetone-water test), $FeCl_3$test, tannins (gelatin test), and flavonoids (Alkaline reagent and Lead acetate tests), the screening was done as per the standard method.

Test for alkaloids

- Dragendorff's test: 2mg of the test extract and 5ml of distilled water was added, 2M hydrochloric acid was added until an acid reaction occurs. To this 1mL of Dragendrof's reagent was added. Formation of orange red precipitate indicated the presence of alkaloids.
- Hager's test: 2mg of the test extract was taken in a test-tube, a few drops of Hager's reagent was added. Formation of yellow precipitate confirmed the presence of alkaloids.
- Wagner's test: 2mg of extract was acidified with 1.5%**v/v** of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids.
- Mayer's test: few drops of Mayer's reagent, 2mg of extract was added formation of white or pale yellow precipitation indicated the presence of alkaloids [11].

Test for flavonoids

- Ferric chloride test: test solution with few drops of ferric chloride solution shows intense green color
- Zinc hydrochloride acid reduction test: test solution with zinc dust and few drops of hydrochloric acid shows magenta red color
- Lead acetate solution test: test solution with few drops of lead acetate (10%) solution gives yellow precipitate [12].

Tests for saponins

- Foam test: to the extract solution, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. The formation of honeycomb like froth indicates the presence of saponins.

Test for steroids

- Salkowaski reaction: 2mg of dry test extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of the test tube. Formation of red colour indicated the presence of steroids

Test for tanins

- Ferric chloride test: 1-2ml of the extract, few drops of5%w/v $FeCl_3$ solution was added. A green colour indicates the presence of gallotanins; while brown colour indicates the presence of pseudotanins.
- Gelatine test: test solution when treated with gelatine solution gives white precipitate

Test for glycosides

- Keller-killiani test: the test solution was treated with few drops of ferric chloride solution and mixed. When conc. Sulphuric acid containing ferric chloride solution was added, it forms two layers, lower layer reddish brown and the upper layer acetic acid layer turns bluish green
- Bromine water test: test solution when dissolved in bromine water gives yellow precipitate

ANTIOXIDANT ACTIVITY

Determination of phospho-molybdenum assay

The antioxidant activity of all the extract was determined by the phospho-molybdenum Method as described by Prieto *et al* [13]. 0.3 ml of extract of different concentrations (100 to 500µg/ml) was mixedwith 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixturewas incubated at 95^0C for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nM using a spectrophotometer against blank. Distilled water (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.

REDUCING POWER ASSAY (IRON (III) TO IRON (II) REDUCTION)

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants

The Ferric reducing power method was applied with slight modifications of the method in which, 2.5 mL of extract solution of different concentrations (100 to 500 µg/mL) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 0.1%). This was incubated at 50^0C for 20 min. After the incubation, 2.5 mL of 10% trichloroacetic acid was added.2.5 mL of the reaction mixture was mixed with distilled water (2.5mL) and ferric chloride (0.5 mL, 0.1%). The absorbance was measured at 700 nM. Increasing absorbance of the reaction mixture indicates increasing reducing power. The same procedure was applied for ascorbic acid which acts as the standard. Increase in the absorbance indicates increase in reducing power.

H_2O_2 SCAVENGING ASSAY

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. It is widely used as a bleaching agent in the textile, paper and pulp industries. Human beings exposed to H_2O_2 indirectly via the environment are estimated as 0.28mg/kg/day with intake from leaf crops contributing most to this exposure. Hydrogen peroxide enters the human body through inhalation of vapour or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals

(OH˙) that can initiate lipid peroxidation and cause DNA damage [14-15].

A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (100-1000mg/ml) in distilled water is added to hydrogen peroxide and absorbance at 230 nM is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

% scavenged (H_2O_2) = (Abs control – Abs standard / Abs control) X 100

RESULT AND DISCUSSION

In this study of *In Vitro* antioxidant activity, preliminary phytochemical screening of the different fractions of seed extracts revealed that the presence of alkaloids, flavonoids, saponins, steroid, tannins and glycosides and the results are tabulated in Table 1.The results of the free radical scavenging potentials of different fractions tested by reducing power assay method Phospho-molybdenum assay and hydrogen peroxide assay are depicted in fig 1, 2 and 3.

The phospho-molybdate method is quantitative, since the total antioxidant capacity is expressed as ascorbic acid equivalents. The percentage of antioxidant activity was in the order methanol>ethanol>acetone.

In reducing power assay, acetone fraction seemed to have quite high reducing activity compared to methanol and ethanol. The reducing power assay of different extract is given in fig 2.

In hydrogen peroxide assay, acetone extract shows the highest scavenging activity followed by ethanol and methanol extract when compared to the standard ascorbic acid which is depicted in fig 3.

Table 1. Screening of Secondary metabolites

	Ethanol	Methanol	Acetone
Test for alkaloids			
Dragendorff's test	-	+	-
Hager's test	+	+	+
Mayer's test	+	+	+
Wagner's test	+	+	-
Test for flavonoids			
$FeCl_3$ test	-	+	+
Zinc test	-	-	-
Alkaline reagent test	+	+	+
Test for saponins			
Foam test	-	+	-
Test for tannins			
Gelatine test	-	+	-
Ferric chloride test	-	+	+
Test for steroids			
Salkowaski reaction	-	+	+
Test for glycosides			
Keller killiani test	-	+	+
Bromine water test	-	+	-

Fig 1. Different fractions tested by reducing power assay method

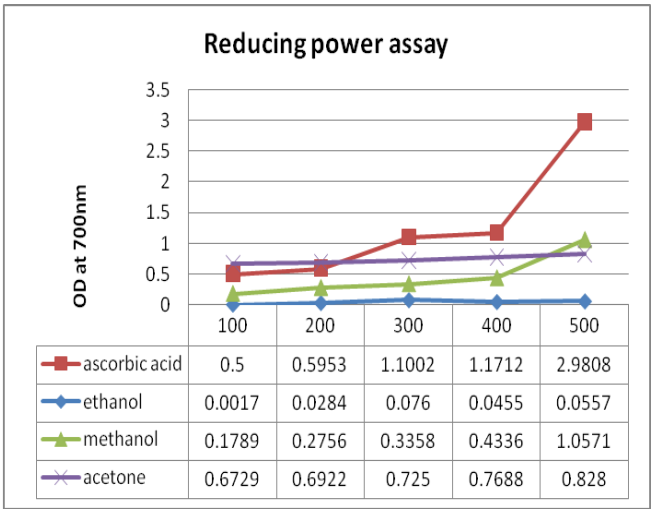


Fig 2. Different fractions tested by phospho-moly bdenum assay

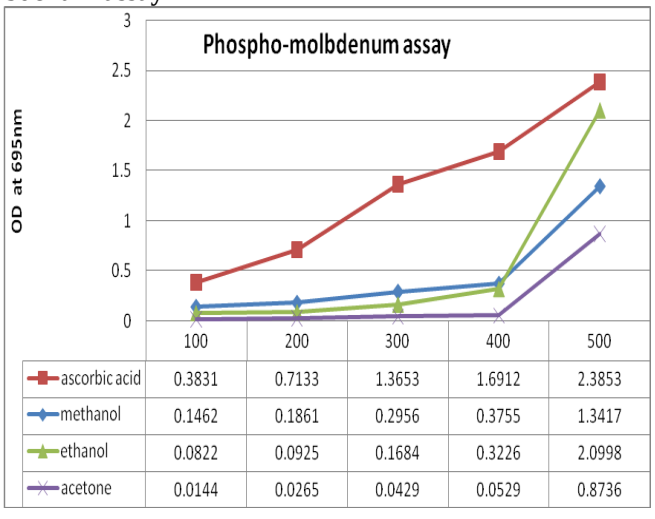
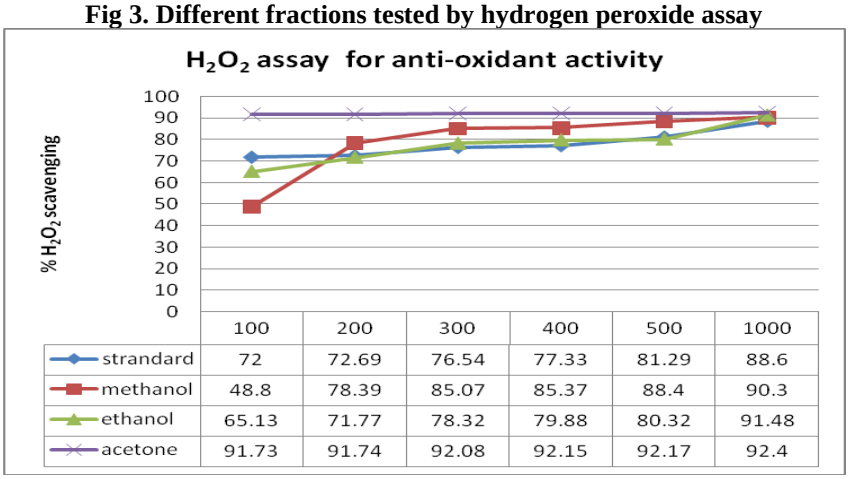


Fig 3. Different fractions tested by hydrogen peroxide assay



	100	200	300	400	500	1000
strandard	72	72.69	76.54	77.33	81.29	88.6
methanol	48.8	78.39	85.07	85.37	88.4	90.3
ethanol	65.13	71.77	78.32	79.88	80.32	91.48
acetone	91.73	91.74	92.08	92.15	92.17	92.4

CONCLUSION

Herbal drugs are being looked as very importance source for discovery of new agents for treating various ailments. *Lepidium sativum linn* showed good activity in savenging the free radicals. Further pharmacological and clinical studies are required to understand the actual efficacy of these herbal extracts

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