

PHYTOCHEMICAL EVALUATION AND ANTIOXIDANT SCREENING STUDIES OF *OCIMUM TENUIFLORUM* LINN SEEDS

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ABSTRACT

Objective: In the existing study, *Ocimum tenuiflorum* was assessed for its antioxidant activity. This activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The drug was identified and standardized on the basis of organoleptic, negligible characters and its quantitative physicochemical standards.

Methods: Preliminary phytochemical screening was also carried out to examine out the presence of various phytoconstituents. It revealed the presence of alkaloids, phytosterols, resin, flavonoids, tannins, diterpenes, and protein in the seed extract. DPPH method was done used for evaluating the antioxidant activity.

Results: It has been observed that different concentrations (100, 200, 300, 400, and 500 µg/mL) used had shown 82.1%, 83.4%, 86.6%, 89.1%, and 93.2% of cell inhibition, respectively.

Conclusion: Thus, the present study on pharmacognostic standardization, physicochemical evaluation and *in vitro* antioxidant activity of *O. tenuiflorum* seed would be useful to supplement information in regard to its *in vivo* evaluation for different pathological conditions.

Keywords: *Ocimum tenuiflorum*, Lamiaceae, Standardization, Phytochemical screening, Antioxidant.

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INTRODUCTION

Herbal plants or Folk medicines have been long used as a medication since classical times for the treatment of the majority of diseases. Folk plants used to enact a very significant role in world health [1,2]. For this World Health Organization (WHO) recognized the requirement of evenness and high content of herbal drugs [3]. WHO had fixed certain parameters for explaining the basic criteria for the evaluation of quality, safety and efficacy of herbal remedies. Since herbal drugs have gained momentum, so the standardization of herbal drugs is most desirable at this moment. Morphological characters such as organoleptic characters, arrangement of leaflets, venation, texture, surface characters, markings, and hardness of the plant materials.

Tulsi itself is originated from Sanskrit language which means “matchless one [4,5].” This is considered as popular basil and is placed at the top of herbs because it is excessively used to its economic, nutritional and medicinal properties. Volatile oil such as monoterpenes, sesquiterpenes, and aromatic constituents had been reported in the fixed oil obtained from the leaves of the plant [6]. Monoterpenes such as camphene, sabinene, and myrcene have been reported. Oxygen containing monoterpenes such as linalool. Borneol, terpin-4-ol was also extracted out from the oil of the leaf. Other sesquiterpene reported in the oil of leaf are α-copane, α-caryophyllene, etc., aromatic compounds such as estragole, thymol, and carvacrol were also present in the oil of the tulsi plant.

Various phytochemical presents in the tulsi plant are as follows:

Extract	Phytochemicals	Plant part
Fixed oil [7]	Linoleic acid, oleic acid, and stearic acid	Seeds of the plant

Essential oil [8-10]	Aromadendrene oxide, benzaldehyde, borneol, bornyl acetate, camphor, cubenol, d-limonene, eicosane, eucalyptol, eugenol, farnesene, limonene, n-butylbenzoate, ocimene, oleic acid, cadinene, humulene	Leaves
Mineral contents [11]	Vitamin C, Vitamin A, calcium, phosphorus	Whole plant
Alcoholic extracts [12]	Aesculetin, apgenin, caffeic acid, chlorogenic acid, circineol, isovitexin, luteolin, molludistin, orientin, protocatechuic acid, stigmasterol, ursolic acid, vallinin, viceni, vitexin, vlinin acid	Aerial parts

The oil extracted from the plant exhibit various biological activities such as antidiabetic [13], antihypotensive [14], anticonvulsant [15], antioxidant activity [16], anthelmintic [17], muscle relaxant [18], antibacterial, and anticarcinogenesis [19,20]. Other reported direct usage of the plant parts such as leaves for the healing of headache [21], eye disorders [22], healing power [23], anti-inflammatory [24], and skin disorders such as insect bites, pimples [25-27], and teeth disorder [28]. Even the tulsi plant is of huge advantage that it can be even used for the treatment of stress [29] and heart diseases [30].

Ethanollic extract of *Ocimum* species found to be potent in showing antimetastatic activity and in boosting antioxidant enzyme activity. Many herbs mentioned in Ayurveda for the treatment of tumors including cancer therapy and are also capable of preventing the side effects with the complete healing power [31,32]. Oxidative phosphorylation may cause the emergence of reactive oxygen species (ROS) that can destroy cellular DNA and other natural proteins. Higher amount of ROS may break double stranded DNA and causes the ageing

of the cell, cardiac disorders, mutation with in the cells, and cancerous cell growth [33,34].

METHODS

Collection and authentication of seeds of *Ocimum tenuiflorum* (Linn.)

The *O. tenuiflorum* (Linn.) seeds were procured from the Central Institute of Medicine and Aromatic Plant Lucknow near Kukrail Picnic Spot Lucknow. The Herbal plant was examined and authenticated by Dr. Amardeep Kaur, Lecturer in Botany at S.D. College Barnala. After authentication, plant materials were cleaned (hand picking and winnowing methods) and dried under shade at room temperature.

Macroscopic evaluation of seeds of *O. tenuiflorum* (Linn.)

The macroscopic characters such as expansion, appearance, shade, and flavor of seeds of *O. tenuiflorum* (Linn.) were determined [35].

Physicochemical evaluation of seeds of *O. tenuiflorum* (Linn.)

Determination of ash values of seeds of *O. tenuiflorum* (Linn.)

Residue left after the complete burning of the crude drug is known as ash. Determination of ash value is important for evaluating grade and contamination of the folk drug. It detects the fact of occurring inorganic matter such as metallic salts, ions, silica content, and earthy materials such as sand, pebbles, and minerals [36,37].

- Determination of total ash

Specifically weighed 3 g of drug dried in atmospheric air was retained in a pre-ignited and weighed silica crucible. The granules of drug were layered in the crucible and incinerated in the muffle furnace by slowly evaluating the temperature to make it complete white free from traces of carbon. Then, crucible was kept out in air to cool down, weighed, and the steps were recapitulated till a steady weight was obtained.

- Determination of acid insoluble ash

Total ash boiled with 25 mL of 2M HCl for 4-5 minutes. The matter which was not dissolved was deposited on ash less filter paper, followed by the washing with hot water. Transfer that insoluble ash to the preweighed silica crucible, incinerate it, cool it and measure its weighed. The steps were again followed till a fixed weight was obtained.

- Determination of water soluble ash

Total ash was bubbled with 25 mL of water for 4-5 minutes. Undissolved matter was gathered on ash less Whatman filter paper and rinse with little warm water. Place filter paper with insoluble ash in a crucible and allow to getting completely burn within 15 minutes at a temperature of 450°C and weighing it. Mass of undissolved matter was deducted from the weight of total ash obtained. The variation obtained represents water soluble ash.

Determination of solvent extractive values of seeds of *O. tenuiflorum* (Linn.)

- Determination of water soluble extractive value

About 5 g of powdered drug was taken in a conical flask, fitted with a cork, to that 100 mL of chloroform was added and shaken regularly for 6-7 hrs using an electrical shaker and then allow for maceration for next 18 hrs. Filtered it carefully and small amount of filtrate was heated to dryness at a temperature of 105°C in a small china dish. The extract was weighed, and percentage was calculated with reference to air dried drug.

- Determination of alcohol soluble extractive value

About 5 g of correctly measured air-dried powdered drug was macerated with 100 ml of 90% alcohol for 24 hrs. It was shaken constantly for 6 hrs in an electrical shaker and kept constant for next 18 hrs. Filter it carefully so that there should be any loss of alcohol and 30 mL of the filtrate was evaporated to dryness at a temperature 105°C in a tarred flat-bottomed shallow china dish. Weight of extract was taken, and percentage was calculated.

- Determination of ether soluble extractive value

About 5 g of weighed granular drug was placed in a conical flask fitted with a stopper. To that 100 mL of petroleum ether was added. Using electrical shaker, shake it for 6-7 hrs and keep them undisturbed for next 18 hrs for maceration. Filter it properly so that there should not be any loss of petroleum ether and evaporate it to dryness in a flat bottomed china dish. Weight of extract was taken, and percentage was calculated.

Determination of moisture content of seeds of *O. tenuiflorum* (Linn.)

Accurately weighed quantity of drug was taken into tarred crucible, crucible was covered, and crucible and the contents were weighed. Loaded silica crucible was placed in a hot air oven at 105°C, and the cover was removed and left in chamber. Sample was dried to a fixed weight. Moisture content of sample was calculated with reference to the air-dried drug.

Determination of foreign organic matter of seeds of *O. tenuiflorum* (Linn.)

Accurately weigh 10 g of sample and spread it equally on a white paper without overhanging. Inspect the sample using ×5 lens and separate out foreign matter, if found. Once separation was completed, weigh the matter and calculate percentage w/w of foreign organic matter.

Determination of swelling index of seeds of *O. tenuiflorum* (Linn.)

To evaluate the swelling behavior, 1 g of the seeds of *O. tenuiflorum* (Linn.) was taken in a 100 mL measuring cylinder with graduated readings in it. Initial volume occupied by seeds was noted. In addition to this, add 2 mL of alcohol for better dispersion and then add sufficient quantity of water to make up the volume up to 100 mL. The measuring cylinder was kept at a room temperature, and swollen mass of seeds was noted after 24 hrs.

Phytochemical screening of seeds of *O. tenuiflorum* (Linn.)

Various extracts, viz., hexane, chloroform, ethyl acetate, and methanol extracts were prepared by hot percolation process for preliminary phytochemical screening. Identification tests were carried out for these extracts to detect various phytoconstituents such as alkaloids (Mayer's test, Wagner's test, Dragendroff's test, and Hager's test), saponins (Foam test), tannins (lead acetate, ferric chloride test, and Potassium dichromate test), phytosterols (Salkowski test, Liebermann's test, and Liebermann-Burchard's test), phenols (ferric chloride test), proteins (Biuret test), flavonoids (Shinoda test, alkaline reagent test, and lead acetate test), triterpenoids (Liebermann-Burchard's test), carbohydrates (Molisch's test, Barfoed's test, and Fehling solution test) amino acids (Ninhydrin test, and Xanthoproteic test) in the seeds of *O. tenuiflorum* (Linn) [38].

Test for steroids

- a. Salkowski reaction

Few drops of the extract were taken in a test tube, to that 1-2 mL of chloroform was added, 2 mL of sulfuric acid was added by the side of the test tube. Shake it for 2-3 minutes and if there is the formation of red color that may indicate the presence of steroids in the given sample.

- b. Liebermann's test

To a few drops of extract in a test tube, 2 ml of acetic anhydride was added, and a gentle heating was given to the test tube. Then, cool down the content of the test tube. On addition of few drops of sulfuric acid by the side of the test tube, if there is the appearance of blue color that would indicate the presence of sterol in the sample.

- c. Liebermann-Burchard's reaction

Few drops of the extract were taken in test tube, to that 1 mL of chloroform was added, followed by addition of acetic anhydride. On addition of sulfuric acid to test tube, a transient color develops

from red to blue and finally to green. And that is the indication for the presence of sterol in the sample.

Test for alkaloids

Few mg of extract was taken separately in 5 ml of 1.5% v/v hydrochloric acid and filtered. These filtrates were then subjected to various tests for confirmation of the presence of alkaloidal content within the extracts.

- a. Mayer's test
To the filtrate, on addition of potassium mercuric iodide, if there is formation of cream colored precipitate that would reflect the presence of alkaloids in the sample.
- b. Wagner's test
On the treatment of filtrate with iodine in potassium iodide, if there is formation of brown/reddish precipitate that would depict the presence of alkaloids in the sample.
- c. Dragendorff's test
When filtrate was treated with a solution of potassium bismuth iodide, and if there is formation of red precipitate that would signify the presence of alkaloids in the sample.
- d. Hager's test
On treatment of filtrate with saturated picric acid solution, if there is formation of yellow colored precipitate that would display the presence of alkaloids in the sample.

Tests for tannins

The extract was taken in water, warmed, and filtered. Tests were carried out with the filtrate using following reagents to find whether the extract was found to be positive for tannin content or not.

- a. Ferric chloride reagent
To the filtrate, 5% w/v ferric chloride in 90% alcohol was added. If in the solution shows there is appearance of dark green or deep blue color that indicates the presence of tannins.
- b. Lead acetate test
To the filtrate, add 10% w/v solution of lead acetate, if there is formation of precipitate that would suggest the presence of tannins.
- c. Gelatin solution test
Solution of 1% gelatin in 10% sodium chloride was prepared. To this a few mL of filtrate was added, and if there was formation of a white precipitate that would reflect the presence of tannins in the given sample.
- d. Bromine water test
On addition of bromine to filtrate, if decolorization of bromine water occurs that would convey the presence of tannins in the given sample.

Test for flavonoids

- a. Shinoda test
Few mg of extract was dissolved in 5 mL of ethanol and to that 2-3 drops of hydrochloric acid was added. To the above 0.5 g of magnesium metal was also added. Development of pink or magenta color would manifested the flavonoids in the sample.
- b. Alkaline reagent test
Treat the extract with few drops of sodium hydroxide solution which leads to the formation of yellow color solution, which on addition of dilute acid, become colorless, reflects the existence of flavonoids.
- c. Lead acetate test
On treatment of the extract with lead acetate solution, if there is formation of a yellow color precipitate that would vision the presence of flavonoids in the sample.

Test for amino acids

- a. Ninhydrin test
To the extract add 0.25% w/v ninhydrin reagent and allow it to boil for 5-6 minutes. If there is formation of blue color precipitate that would suggest the presence of amino acid.

- b. Xanthoproteic test
When the extract was treated with few drops of concentrations nitric acid, and if there is formation of a yellow color precipitate that would evident the presence of proteins.
- c. Biuret test
When the equal volume of sodium hydroxide and copper sulfate was added to the extract, and there is appearance of pink or purple color that would imply the presence of amino acid in the sample.

Test for sugars

- Molisch's test
To the extract add small amount of water and add few drops of Molisch reagent to that add sulfuric acid by the side of the test tube, if there is appearance of brown ring at the interface of two liquids that would point out the presence of sugars in the sample.
- Barfoed's test
To the test sample add few drops of Barfoed's reagent and allow it to boil for some time. If by that time there is appearance of a red precipitate of cuprous oxide within that would declare the presence of monosaccharides.
- Fehling's solution test
Both the solution, i.e., Fehling's A and Fehling's B were added to the extract in equal amount, and the extract was allowed to boil for some time if there was formation of a red precipitate of cuprous oxide recommended the presence of reducing sugar.

Test for saponins

- Foam test
Few ml of extract was taken in the graduated measuring cylinder, dilute it with water and then carried agitation for 15 minutes. If there formation of 1 cm layer of foam that would indicate the presence of saponins.

Extraction of mucilage from seeds of *O. tenuiflorum* (Linn.)

The crushed powder of seeds was dissolved in distilled water. The inflated mass of seeds was spread on to a glass tray and was dried at 60°C. Allowed to pass that material from mesh #30. Then winnowing was done after that passes that material from mesh #60. The mucilage obtained at this point was weighed [39].

Organoleptic characters of mucilage extracted from seeds of *O. tenuiflorum* (Linn.)

Organoleptic characters of mucilage extracted from seeds of *O. tenuiflorum* (Linn.) such as appearance, color, odor, and taste were determined by standard methods.

Physicochemical parameters	Results
Appearance	Amorphous
Color	Brown
Odor	Odorless
Taste	Mucilaginous

Physicochemical evaluation of mucilage of seeds of *O. tenuiflorum* (Linn.)

For physicochemical evaluation of mucilage of seeds of *O. tenuiflorum* (Linn.) various parameters such as percentage yield, angle of repose, swelling index, moisture content, density, compressibility index, and solubility were determined.

- Percentage yield
The percentage yield can be calculated from the yield of the reaction. The actual mass which was obtained by the end of the reaction.
- Angle of repose
Funnel method was used to find the angle of repose. When the dried powder was allowed to fall down from the funnel, and the heap was formed, that is known as angle of repose.
- Swelling index
To evaluate the swelling behavior, 1 g of the seeds of *O. tenuiflorum* (Linn.) was taken in a 100 mL measuring cylinder

with graduated readings in it. Initial volume occupied by seeds was noted. In addition to this, add 2 mL of alcohol for better dispersion and then add sufficient quantity of water to make up the volume up to 100 mL. The measuring cylinder was kept at a room temperature, and swollen mass of seeds was noted after 24 hrs.

- **Moisture content**
Appropriate quantity of mucilage at 105°C was kept for 2 hr in hot air oven. For this measured quantity of drug was kept in a flask was shaken continuously. Filter out the material and allow it to dry at room temperature for complete evaporation of petroleum ether and oil was collected [39].
- **Density**
A pre-sieved and pre-weighed dried mucilage were poured into a graduated cylinder, and the volume covered by mucilage was recorded. The cylinder was tapped until the powder bed volume reached a minimum volume and the tapped volume was recorded.

Exaction of fixed oil of seeds of *O. tenuiflorum* (Linn.)

The *Ocimum* seeds were crushed and were kept in close contact with petroleum ether in a conical flask for 12 hrs. Using electrical shaker, the flask was shaken continuously. Filter out the material and allow it to dry at room temperature for complete evaporation of petroleum ether and oil was collected [39].

Physicochemical evaluation of fixed oil of seeds of *O. tenuiflorum* (Linn.)

Determination of lipid contents

1. Exactly weighed seeds of *O. tenuiflorum* (Linn.) were grounded in mortar and pestle and dried and were kept at room temperature.
2. The extract was added test tube, to this 3 ml of 2:1 mixture (methanol and chloroform) was added. The contents were smashed with a glass rod.
3. Allow them to mix properly using nutator, for 20 minutes.
4. Discard the upper aqueous layer.
5. The organic phase was collected and transferred to the new tube. The contents were dried using nitrogen.
6. The dried residue was dissolved in 1:1 (chloroform and methanol) mixture.
7. The chloroform and methanol solvents were removed, and the percentage yield of the oil was calculated [40].

Estimation of acid value

1. Accurately weighed oil was transferred into 250 ml conical flask.
2. 50 ml of neutralized alcohol solution was added to the oil solution.
3. This mixture was heated for 10 minutes using the heater to dissolve the content.
4. To the above solution, 1-2 drops of phenolphthalein solution were added as an indicator.
5. The above mixture was titrated against the KOH solution from the burette.
6. At the end point, pink color was appeared [41].

Calculation of acid value

$$\text{Acid value} = \frac{\text{Volume (ml) of KOH} \times \text{normality of KOH} \times \text{equation wt of KOH}}{\text{Weight of the oil sample}}$$

Equivalent weight of KOH = 56.11.

Determination of density of oil

Density is a basic physical property that can be used in correlation with other properties to learn both the light and heavy fractions of petroleum and petroleum products.

To know about the density or relative density of petroleum and its products is important for the conversion of measured volumes to volumes at the standard temperatures of 15°C [42].

Principle

When a small liquid sample was added into an oscillating sample tube and because of change in mass of tube, oscillating frequency gets changed. This was used along with calibration data for determination of density of the sample.

Procedure

1. Using a suitable syringe, few mL of sample was poured into the clean, dry sample tube of the digital density analyzer.
2. Sample tube was examined carefully using the illumination light. The tube should be kept free from bubbles and was filled above the suspension point on the right-hand side. The sample should be homogenous in nature.
3. On introduction of the sample into the tube illumination light should be turned off, otherwise, that would affect the measurement temperature.
4. Once the instrument start displaying constant value for density and t-value, the values for both should be recorded.

Determination of saponification value

1. Take 2 g of sample into a 200 ml conical flask.
2. 25 ml of potassium hydroxide ethanol solution was added to it, and the cooling tube was fixed to it.
3. The flask was heated at a constant temperature with continuous shaking so as to prevent the back flow of ethanol to the cooling tube.
4. After 30 minutes of heating, cool it down and then titrate it with 0.5 mol/L HCl.
5. Blank test (without sample following step 1-4 above) should be done 3 times to have a mean value [43].

Antioxidant activity of fixed oil of seeds of *O. tenuiflorum* (Linn.) 2,2-diphenyl-1-picrylhydrazyl (DPPH)

DPPH free radical scavenging activity

Measurement of antioxidant activity of any fixed oil or volatile oil was done in terms of hydrogen donating or ability to scavenge free radical using DPPH method. Mixture was consisting of 2 mL of oil at different concentration (100, 200, 300, 400, and 500 µg/mL) and 2 mL of DPPH (0.2 mM). These were vigorously shaken and incubate them in the dark at a room temperature for 30 minutes. When DPPH from the mixture react with an antioxidant constituent in the oil, that had donated hydrogen, reduction occurs, and that would cause decrease in absorbance at 517 nm in ultraviolet-visible spectrophotometer. Lower the value, greater was the antioxidant value of the oil [44].

RESULTS

Macroscopic evaluation of seeds of *O. tenuiflorum* (Linn.)

Results of macroscopic studies of seeds of *O. tenuiflorum* (Linn.) are tabulated in Table 1.

Physicochemical evaluation of seeds of *O. tenuiflorum* (Linn.)

Physicochemical parameters of seeds of *O. tenuiflorum* (Linn.) were determined, and results are shown in Table 2.

Phytochemical screening of seeds of *O. tenuiflorum* (Linn.)

The preliminary phytochemical screening of various extracts, viz., hexane, chloroform, ethyl acetate, and methanol extracts of seeds of *O. tenuiflorum* (Linn.) was carried out, and results are tabulated in Table 3.

Organoleptic characters of mucilage extracted from seeds of *O. tenuiflorum* (Linn.)

The results of organoleptic characters of mucilage of seeds of *O. tenuiflorum* (Linn.) are tabulated in Table 4.

Physicochemical evaluation of mucilage of seeds of *O. tenuiflorum* (Linn.)

The physicochemical parameters of mucilage extracted from seeds of *O. tenuiflorum* (Linn.) were determined and tabulated in Table 5.

Extraction of fixed oil from seeds of *O. tenuiflorum* (Linn.)

The fixed oil from crushed seeds of *O. tenuiflorum* (Linn.) was extracted using petroleum ether as solvent. The yield and color of fixed oil are given in Table 6.

Physicochemical evaluation of fixed oil of seeds of *O. tenuiflorum* (Linn.)

Physicochemical parameters of fixed oil of seeds of *O. tenuiflorum* (Linn.) were determined, and the results are tabulated in Table 7.

Antioxidant activity of fixed oil of seeds of *O. tenuiflorum* (Linn.)

The fixed oil of seeds of *O. tenuiflorum* (Linn.) was evaluated for antioxidant activity by DPPH method, and the results are tabulated in Table 8.

Table 1: Macroscopic evaluation of seeds of *O. tenuiflorum* (Linn.)

Parameters	Results
Condition	Fresh mature seeds
Color	Yellow with small black marks
Odor	Aromatic
Taste	Mucilaginous
Shape	Broadly ellipsoid
Size	1.2 mm long
Texture	Nearly smooth

Table 2: Physicochemical parameters of seeds of *O. tenuiflorum* (Linn.)

Parameter	Results
Ash value (% w/w)	
Total ash (% w/w)	8.8
Acid insoluble ash (% w/w)	0.4
Water soluble ash (% w/w)	3.9
Solvent extractive value (% w/w)	
Water soluble extractive value (% w/w)	3.6
Alcohol soluble extractive value (% w/w)	2.6
Ether soluble extractive value (% w/w)	3.9
Foreign matter (% w/w)	0.2
Swelling index	12
Moisture content (% w/w)	3.3

Table 3: Preliminary phytochemical screening of seeds of *O. tenuiflorum* (Linn.)

Constituents	Tests	Hexane	Chloroform	Ethyl acetate	Methanol
Phytosterols and Triterpenoids	Salkowaski test	+	-	-	-
	Liebermann test	+	-	-	-
	Liebermann-Burchard's test	+	-	-	-
Alkaloids	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
	Dragendorff's test	+	+	+	+
	Hager's test	+	+	+	+
Tannins	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
	Potassium dichromate test	-	-	+	+
Flavonoids	Shinoda test	-	-	+	+
	Alkaline reagent test	-	-	+	+
	Lead acetate test	-	-	+	+
Sugar	Molisch's test	-	-	-	+
	Barfoed's test	-	-	-	+
	Fehling's solution test	-	-	-	+
	Ninhydrin test	-	-	-	+
Protein and Amino acids	Xanthoproteic test	-	-	-	+
	Biuret test	-	-	-	+
	Foam test	-	-	-	-
Saponin		-	-	-	-
Fats and oil	Grease oil test	+	-	+	+
Mucilage	Ruthenium red	-	-	-	+

+: Present, -: Absent

DISCUSSIONS

The drugs derived from natural resources have a significant contribution in the traditional and modern system of medicines. Relevant steps have been taken by the World Health Organization WHO to carry out the research with the aim of finding new and effective medicinal agents from plants. The development of science of phytopharmaceuticals and the hopes for remedy in chronic diseases generated new enthusiasm in the research work to develop herbal medicines. Plants used in traditional medicines can serve as source of novel therapeutic agents directly or as model compounds for synthetic or semi-synthetic structural modifications.

Various parts of *O. tenuiflorum* (Linn.) (Lamiaceae) are being used traditionally for the treatment of several diseases, except for a few biological studies, not much work has been done to exploit the medicinal properties of *O. tenuiflorum* (Linn.) seeds.

Leaves of this plant are used for antipyretic, analgesic, antioxidant, antibacterial, antifungal, anti-inflammatory, and anticancer activity. Hence, we were interested to submit seeds of this plant to a detailed macroscopic, physicochemical, phytochemical, antioxidant, and anticancer studies.

O. tenuiflorum (Linn.) seeds were purchased from Central Institute of Medicinal and Aromatic Plants, Lucknow, India. The material was identified and authenticated by botanist Dr. Amandeep Kaur Lecture in Botany at S.D. College Barnala. For identification of plant material, macroscopic studies were conducted, and the results are tabulated in Table 1.

In the physicochemical studies, an attempt to standardize *O. tenuiflorum* (Linn.) seeds, several analytical studies were carried out.

Total ash value, acid insoluble ash value, and water soluble ash value were determined and were found to be 8.8% w/w, 0.4% w/w, and 3.9% w/w, respectively.

Water soluble extractive value, alcohol soluble extractive value, and ether soluble extractive value were determined and were found to be 3.6% w/w, 2.6% w/w, and 3.9% w/w, respectively.

Foreign matter was determined and was found to be 0.2% w/w.

Table 4: Organoleptic character of mucilage extracted from seeds of *O. tenuiflorum* (Linn.)

Physicochemical parameters	Results
Appearance	Amorphous
Color	Brown
Odor	Odorless
Taste	Mucilaginous

Table 5: Physicochemical evaluation of mucilage of seeds of *O. tenuiflorum* (Linn.)

Physicochemical parameters	Results
Percentage yield (% w/w)	2.5
Angle of repose	25°21'
Swelling index	15.2
Moisture content (% w/w)	4.5
Bulk density (g/cc)	0.3
Tapped density (g/cc)	0.33
Compressibility index (%)	20.56
Solubility	
Water	Soluble
Acetone	Insoluble
Methanol	Insoluble
Chloroform	Insoluble
Ether	Insoluble
Ruthenium red test	Pink color

Table 6: Yield and color of fixed oil of seeds of *O. tenuiflorum* (Linn.)

Plant material	Yield of fixed oil (% w/w)	Color
Seeds of <i>O. tenuiflorum</i> (Linn.)	08	Golden yellow color

Table 7: Physicochemical evaluation of fixed oil of seeds of *O. tenuiflorum* (Linn.)

Physicochemical parameters	Results
Lipid content (g/ml)	0.305
Acid value	123.42
Density of oil (g/cm ³)	0.936
Saponification value	56.12

Table 8: Antioxidant activity of fixed oil of seeds of *O. tenuiflorum* (Linn.)

Concentration of Vitamin C (µg/ml)	% of inhibition	Concentration of fixed oil (µg/ml)	% of inhibition
100	89.4	100	82.1
200	91.5	200	83.4
300	94.3	300	86.6
400	96.6	400	89.1
500	97.2	500	93.2

The moisture content in the seeds was determined by dry heat method in the oven and was found to be 3.3% w/w.

Swelling index was determined and was found to be 12.

For phytochemical screening, various extracts, viz., hexane, chloroform, ethyl acetate, and methanol were prepared by successive Soxhlet process, and the extracts were subjected to various chemical tests to detect the presence of various classes of phytoconstituents. The results

revealed the presence of carbohydrates, proteins, amino acids, phenols, tannins, phytosterols, triterpenoids, alkaloids, flavonoids, fixed oils, and mucilage in *O. tenuiflorum* (Linn.) seeds. The results are shown in Table 3.

O. tenuiflorum (Linn.) seed mucilage was isolated, and percentage yield was determined.

The organoleptic characters of mucilage of *O. tenuiflorum* (Linn.) seeds were determined. The obtained mucilage was an amorphous brown powder with mucilaginous taste. No characteristic odor was observed in the mucilage.

The physicochemical characters of mucilage of *O. tenuiflorum* (Linn.) were determined, and the results are given in Table 5.

The percentage yield was found to be 2.5. The angle of repose was determined and was found to be 25°21'. Mucilage was found to have good swelling potential with swelling index 12. The moisture content, bulk density, tapped density, and compressibility index of mucilage were determined and were found to be 4.5, 0.3, 0.33, and 20.56, respectively. The mucilage was found to be soluble in water and insoluble in ether, acetone, methanol, and chloroform. The results indicate that the obtained mucilage has good flow properties.

The fixed oil from the seeds of *O. tenuiflorum* (Linn.) was extracted with light petroleum ether (40-60°C), with the help of electrical shaker for about 12 hrs and the solvent was removed by rotary vacuum evaporator; 08% w/w golden yellow oil was obtained. The fixed oil evaluated for physicochemical properties.

Lipid content, acid value, density, and saponification value of the oil were determined, and the results are shown in Table 7.

Antioxidant activity of fixed oil of *O. tenuiflorum* (Linn.) seeds was evaluated by DPPH method. DPPH radical scavenging activity assay assessed the ability of the extracted oil to donate hydrogen or to scavenge free radical. DPPH radical is a stable free radical, and when it reacts with an antioxidant compound, which can donate hydrogen, it is reduced to di-phenyl acryl hydrazine. The change in color (i.e., from deep violet to light yellow) is measured spectrophotometrically.

The fixed oil, when mixed with DPPH, decolorized the color of DPPH solution due to hydrogen donating ability. Results revealed that fixed oil has strong antioxidant activity at all concentrations from 100 to 500 µg/ml, the results are shown in Table 8.

CONCLUSION

In this study, pharmacogonostical, physicochemical properties of mucilage, physicochemical properties, antioxidant, and anticancer activities of fixed oil of seeds of *O. tenuiflorum* (Linn.) were carried out, and from this study, the following conclusions can be made.

In pharmacogonostical studies, an analytical parameter such as ash values, extractive values, moisture content, and swelling index of *O. tenuiflorum* (Linn.) seeds was determined; these studies will be helpful for identification of the plant material. Various extract of *O. tenuiflorum* (Linn.) seeds, viz., hexane, ethyl acetate, chloroform, and methanol extracts were prepared and were subjected to phytochemical screening. The studies revealed the presence of phytosterols and triterpenoids, alkaloids, tannins, flavonoids, sugar, protein, amino acid, fat, and oils.

The mucilage from *O. tenuiflorum* (Linn.) seeds was extracted and the physicochemical parameters, viz., color, taste, odor, percentage yield, swelling index, density, compressibility index, and solubility of the extracted mucilage were determined. The various physicochemical properties show the ability of the mucilage as disintegrating and binding agent tablets. However, further study is required to establish

the mucilage of *O. tenuiflorum* (Linn.) seeds as an excipient in pharmaceutical formulations.

The extracted oil from crushed seeds using petroleum ether as a solvent was subjected for physicochemical evaluation. Lipid content, acid value, density of oil, and saponification value of fixed oil were determined. These physicochemical studies will help for the standardization and identification of fixed oil.

The antioxidant activity of the oil was estimated by DPPH method and the results that come out reveals that the fixed oil possesses the potent antioxidant activity.

The findings of the present work indicate that the mucilage of *O. tenuiflorum* (Linn.) seeds may be established as an excipient to prepare various formulations, and fixed oil has demonstrated significant antioxidant activity. However, further studies are required so that oil may be established as antioxidant drug.

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