Unveiling the potent antioxidant capabilities of essential oil derived from *Artemisia roxburghiana* leaves: A comprehensive exploration

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Abstract-Plants have potential due to their capability to synthesize an array of phytochemicals as secondary metabolites to defend themselves and respond on spot to a variety of environmental biotic or a biotic stresses. Such plants metabolites are used as drugs, medicines and also for many other industrial purposes including most attractive fragrances and nutraceuticals. Fragrance or flavour, the aroma behind is visualized by olfactory responses to the odorant molecules of different structures and forms. These molecules even with similar looking structures often express variation in quality of aroma because of electricity and stereocity Resultsing range of olfactive odors. De-stressing the world of its hectic lifestyles coupled with competitive ambience in all spheres is an area that aromaceuticals has occupied and thus has opened up a new horizon of aromatics research as well as commercial and industrial applications in near future taking lead in preventive healthcare scenario attracting global attention. Keeping in view of the above, the proposed study is aimed to carry out antioxidant potential form of essential oil from wild plant species namely *Artemisia roxburghiana* Wall. Ex Besser. Oil was extracted and studied for its physico-chemical characteristics and antioxidant potential.

Introduction

Essential oils, known for their strong aroma, are complex and volatile compounds produced naturally by aromatic plants as secondary metabolites. These oils are recognized as rich sources of biologically active compounds. Recent attention has focused on exploring the antimicrobial properties of extracts from essential oils hold significant importance as antioxidants, contributing to their widespread utilization in various fields[1-4] their antioxidant properties stem from a rich array of bioactive compounds, such as to copherols, phenols, and other volatile components. These compounds play a pivotal role in

neutralizing free radicals, unstable molecules that can cause cellular damage and contribute to various health issues, including aging and chronic diseases. As potent antioxidants, essential oils exhibit the ability to inhibit oxidative stress, a process associated with the imbalance between free radicals and the body's antioxidant defenses. By scavenging free radicals, essential oils help protect cells from damage, thereby potentially reducing the risk of oxidative stress-related conditions. Moreover, the antioxidant nature of essential oils extends their shelf life by preventing the oxidation of fats and oils in food products, thus serving as natural preservatives. This preservation quality makes them valuable in the food industry, offering a natural and health-conscious alternative to synthetic preservatives. The plant Artemisia roxburghiana wall ex: Besser, also known as Roxburgh's Wormwood is one of the least explored folk medicinal plants of the genus Artemisia[6-9]. The plant grows in the lower or mid-altitude of lower Himalayas from 500 to 4000 m. It is used as a folk medicine in various Asian countries for the treatment of malarial fever, skin disorders and intestinal wormsThe primary objective of this study is to evaluate the antioxidant potential of *A. roxburghiana* wall ex:Besser.

Material and Methods

Preparation of substrate

The aerial part of *Artemisia roxburghiana*was collected from Suwakholi (near Dhanaulti) at an altitude, latitude and longitude ranging from 2060 m, 30.45004º N, 78.15853º E - 2385 m, 30.44582º N , 78.19557º authenticated by Systematic Botany Division, Forest Research Institute, Dehradun, Uttarakhand, India. The leaves and the rhizomes were cut into small slices followed by oven drying at 60 °C - 80 °C for 48 hrs. The dried parts were then stored at room temperature and then used for the experiment.

Extraction of oil from leaves *Artemisia roxburghiana*

Fresh material was cut into small pieces and then subjected to hydro-distillation directly for 4 hours. The isolation of essential oil was carried out by using Clevenger assembly, according to standard method (Guenther, 1948). The extracted essential oils were dried using anhydrous sodium sulfate and stored in sealed vials at low temperature (4^0C) for further analysis. Quantitative determination and physicochemical analysis of essential oils viz. refractive index, saponification value, acid value, ester value, optical rotation, specific rotation and specific gravity etc, were carried out by following standard methods.

Studies of characteristics of extracted oil

(a) Physicochemical characteristics provide a base line for aptness of oil. These characteristics studied were colour, Specific gravity, Specific Viscosity, Refractive index, Optical Rotation **,** Acid number, Saponification number, Ester value, percentage of phenol and hydroxyl value

(b) **Specific gravity**

1. A cleaned and dried empty pycnometer was taken and weighed.
2. The pycnometer was filled upto the mark with double distilled water and weighed again.
3. The weight of water was recorded. After removing the water the pycnometer was dried in the oven and filled upto the mark with the essential oil under experimentation.

4. The weight of pycnometer with oil was calculated using the following formula.

$$\textbf{Specific gravity} = \frac{wt.\ of\ the\ oil}{wt.\ of\ an\ equal\ volume\ of\ water}$$

(c)**Specific Viscosity**

1. An Ostwald viscometer was cleaned and dried. Ten ml of 1% solution (in acetone) of the oil under experimentation was filled in the bulb of the viscometer.
2. The solution was sucked upto upper mark and the viscometer left as much.
3. The time taken by the solution to percolate down from the upper mark of the viscometer to the lower mark was recorded.
4. The process was repeated by filling the viscometer with pure acetone. The relative viscosity of the oil was calculated as per following formula:

$$\eta\textbf{Rel} = \frac{\textbf{Flow time of 1\%solution of essential oil in solvent}}{\textbf{Flow time of pure solvent}}$$

The value of relative viscosity thus obtained, was converted to specific viscosity by the formula as under:

$$\eta sp = \eta\ rel^{-1}$$

where,

ηsp = Specific viscosity

ηrel^{-1} = relative viscosity

(d)**Refractive index**

1. Abbe type of refractometer was used to determine the refractive indices of the oils.
2. The double prisms of the apparatus were cleaned with alcohol and one drop of the oil was placed between them.
3. The prisms were closed by tightening the screw heads and the refractometer was allowed to stand for few minutes to equate the temperature of the oils and the apparatus.
4. The alidade of the refractometer was moved backward of forward to get a broader line which was a band of colour.
5. A sharp colourless line was obtained by rotating the screw heads of the compensator. Finally, the line was adjusted in such a way that it fell on the point of intersection of the cross hairs.
6. The refractive index was head directly on the scales of the sector.

(e) **Acid number**

1. One ml of the essential oil was dissolved in 15 ml of 95 % ethanol in a conical flask.
2. Three drops of 1% phenolphthalein were added to the contents of the flask and it was titrated against 0.1 N sodium hydroxide solution.
3. The first appearance of pink colouration that did not fade within 10 second was considered as the end point.
4. Another set without oil was also run parallel to treatment and the difference in the amount of alkali used while titrating the treatment and the set without oil gave the amount of alkali consumed for determination of the acid number of the oil.
5. The acid number was calculated by the following formula :

$$\textbf{Acid number} = \frac{\textbf{Volume of 0.1 N Alkali consumed}}{\textbf{Weight of 1ml essential oil}}\ \textbf{X 5.61}$$

(f) **Saponification number**

1. One ml of the essential oil was taken into a 100 ml saponification flask. Ten ml of 0.5 N alcoholic sodium hydroxide solution was added to the flask and an air cooled glass condenser (1 meter in length and 1 cm in diameter) was attached to it. The mixture was refluxed for an hour on a water bath and then allowed to cool down to room temperature.
2. The contents were the titrated against 0.5 N aquous hydrochloric acid using 3 drops of 1% phenolpathalin solution as the indicator.
3. Another set, without oil was also run parallel to the treatment set and the

difference in the amount of acid consumed for the determination of saponification number of the oil, which was calculated by the following formula:

$$\textbf{Saponification number} = \frac{\textbf{Volume of 0.5 N Acid consumed}}{\textbf{Weight of 1ml essential oil}} \textbf{ X 28.05}$$

(g)Optical Rotation

1. Placed the 100mm polarimeter tube containing the oil or liquid under the examination in the trough of the instrument between the polarizer and analyzer.
2. Slowly turned the analyzer until both halves of the field, viewed through the telescope, show equal intensities of illumination.
3. At the proper setting, a small rotation to the right or to the left will immediately caused a pronounced inequality in the intensities of illumination of the two halves of the field.
4. Determined the direction of rotation. If the analyzer was turned counter-clockwise from the zero position to obtain the final reading, the rotation is leave and if clockwise, it was dextro.
5. After the direction of rotation had been established, carefully readjusted the analyzer until equal illumination of two halves of the field was obtained. Adjusted the eyepiece of the telescope to give clear, sharp lines between the two halves of the field.
6. Determined the rotation by means of protectors; read the degrees directly , and the minutes with the aid of either of two fixed Vernier's, the movable magnifying glasses will aid in obtaining greater accuracy.
7. A second reading should be taken, it should not differ by more than 5' from the previous reading.

Antioxidant Activity

DPPH Radical Scavenging Assay

Essential oil was tested spectrophotometrically for their ability to scavenge free radicals against stable DPPH (2,2-diphenyl-2-picrylhydrazyl). DPPH is reduced when it interacts with an antioxidant substance that can donate hydrogen. Using a UV visible light spectrophotometer, the transition from purple to yellow was measured at 517 nm. Fresh solution of DPPH in methanol was prepared each day. Before measuring absorption, the oil solutions and DPPH solution were combined in the ratio of 3:1 and kept in the dark for 30 minutes at room temperature. The experiment was carried out a total of three times. The following formula was used to determine the amount of radical scavenging activity:

(A_B-A_A/A_B)% Inhibition×100

Where, A_B = Absorption of the Blank Sample in this case

A_A is the tested sample's absorption.

Ferric Chloride Reducing Power Assay

A quick and easy screening technique for determining antioxidant potential is the assay for lowering power. According to Banerjee procedure (10), the reducing power of oil was assessed. Tannic acid and gallic acid were employed as a standard. The reagents used were 0.2 M Sodium phosphate buffer, pH 6.6, 1%w/v Ferricyanide, 10% w/v Trichloroacetic Acid, 0.1%w/v Ferric Chloride. Oil was combined with 5.0 ml of potassium ferricyanide and 5.0 ml of sodium phosphate buffer at concentrations ranging from 1.0 to 5.0 mg/ml of water. 5.0 ml of trichloroacetic acid were added after the mixture had been incubated at 50°C for 30 min. The mixture was then centrifuged at 980Xg for 10 min. at 50°C in a refrigerator-based centrifuge (VHM-17L Kokusan Denki, Tokyo, Japan). One ml of ferric chloride was added after the 5.0ml of the upper layer of the solutions were diluted with 5.0 ml of distilled water. Using a spectrophotometer (U-2001, Hitachi

Instruments Inc.), absorbance was measured at 700 nm.

Increase in reducing power (%) = (A_T-A_B/A_B) ×100

Where, A_B =Absorbance of the Blank Sample

A_T= Absorbance of Tested Sample.

Results

Results of the above studies are summarized in the **Table (1-3)** and discussed at appropriate place.

Table-1 Yield of extracted oil

Part of the plant used	Initially amount taken (in gm)	Oil obtained(in gm)
Leaves	900	3.4690

Table-2 Characteristics of Essential oil of*Artemisia roxburghiana*

Parameter studied	Value
Colour	Dark green
Specific gravity	0.924
Specific viscosity	0.110
Refractive index	1.553
Acid number	13.253
Saponification number	37.24

Table3:Antioxidant Potential of *Artemisia roxburghiana* Essential Oil

S.NO	Concentration(µg/ml) IC_{50} (µg/ml) in DPPH assay	Absorbance	% Antioxidant activity
1.	control	1.623	-
2.	Ascorbic acid IC_{50} (µg/ml) in DPPH assay	0.055	96.61
3.	Artemisia *roxburghiana* IC_{50} (µg/ml) in DPPH assay	0.273	78.03%
4	Artemisia *roxburghiana* µM (AAE)/100g dry mass in FRAP assay	0.787	60.98%

Discussion

The study focused on the preparation of essential oil from the aerial parts of Artemisia roxburghiana and subsequent characterization of its physicochemical properties. The plant material was collected from Suwakholi near Dhanaulti, and after thorough authentication, the leaves and rhizomes were sliced, oven-dried, and stored for further use.

The essential oil extraction process involved hydro-distillation using a Clevenger assembly. The obtained essential oils were then subjected to various analyses, including quantitative determination and physicochemical characterization. Parameters such as color, specific gravity, specific viscosity, refractive index, acid value, saponification value, ester value, and percentage of phenol were assessed using standard methods.

The specific gravity was determined by comparing the weight of the oil to that of an equal volume of water. Specific viscosity was evaluated using an Ostwald viscometer, and refractive index was measured using an Abbe refractometer. Acid number and saponification number were determined through titration methods. Optical rotation was assessed using a polarimeter. The Results indicated that the essential oil of Artemisia roxburghiana exhibited specific characteristics, including a dark green color, specific gravity of 0.924, specific viscosity of 0.110, refractive index of 1.553, acid number of 13.253, and saponification number of 37.24. Additionally, the antioxidant activity of the essential oil was assessed through DPPH radical scavenging and ferric chloride reducing power assays. The oil

demonstrated substantial antioxidant potential, as evidenced by its ability to scavenge DPPH radicals and exhibit reducing power comparable to the standard antioxidants, ascorbic acid.[9-13] The potential mechanisms of action underlying the relationship between antioxidant potential and essential oils are complex and multifaceted. Essential oils, derived from aromatic plants, have demonstrated significant antioxidant properties, and their effects can be attributed to various bioactive compounds present in these oils. Essential oils are rich in compounds like phenols, terpenes, and flavonoids, which have been shown to effectively scavenge free radicals. Free radicals are highly reactive molecules that can cause oxidative damage to cellular components. Antioxidant compounds in essential oils donate electrons to neutralize free radicals, thereby preventing cellular damage. Certain constituents of essential oils, such as phenolic compounds, are known for their ability to donate hydrogen atoms. This process inhibits the chain reaction of free radical formation by stabilizing and neutralizing radicals. It is crucial to note that the specific mechanisms can vary among different essential oils due to variations in their chemical composition. The synergistic effects of multiple compounds within the oils likely contribute to their overall antioxidant potential. Further research is needed to elucidate the specific pathways and interactions involved in the antioxidant activity of individual essential oils.

Conclusion

The study provided valuable insights into the physicochemical characteristics and antioxidant activity of the essential oil extracted from Artemisia roxburghiana, suggesting its potential applications in various industries, including pharmaceuticals and cosmetics. Further research could explore its specific bioactive components and therapeutic properties.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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