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Antioxidant capacity of *Pimpinella wallichiana*

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Abstract
Pimpinella wallichiana from to the Apiaceae family and has good biological applications. The chemical constitute of *Pimpinella wallichiana* shows applications certain Ayurveda formulations. The present study is identification of phytochemicals and Finding the antioxidant activity of leaves and stem extract. Determination of Antioxidant activity Using DPPH Method and phytochemical study using known methods. Our experimental results of the present indicate all extract of the leaves and stem shows best antioxidant activity. Phytochemical analysis of leaves was carried out which are useful to decide medicinal applications of plants as well different extract shows good antioxidant activity.

Keywords: *Pimpinella wallichiana*, antioxidant, phytochemical, sterols, reducing sugars, alkaloids

Introduction
Antioxidants are highly important components of daily life. Prooxidants are generated in nature due to toxic chemicals, oxygen radicle and various free radicles. The free radicles are highly reactive they easily react with the various biological molecules' lipid, protein, DNA, causing cellular/tissue damage on the other hand these affect the cell very badly to compensate its effect we require certain scavengers called as antioxidants.
Most of the oxidants are the natural origin occur in fruit and vegetable [1, 7]. Mostly the Antioxidant are phenolic, Ascorbic acid, carotenoids, vitamins, and phenolic are known as these are natural antioxidants known as hydrophilic antioxidants, while simple antioxidants are water insoluble and bioorgin known as lipophilic antioxidants [8, 11] Free radicles are causing the damage to the cell. Most of these ozone, cosmic radiation, UV-light, electromagnetic radiation, cigarette smoke and low wavelength electromagnetic radiations these are various sources which may causes various effect. Another way of general methods is from the Nitrogen derived (RNS), Oxygen derived (ROS) or peroxyl radicals (ROO) or hydroxyl radicals (OH), superoxide anion (O_2), hydrogen peroxide (H_2O_2) these are sources. Many times, nitric oxide (NO), Dinitrogen trioxide (N_2O_3), peroxy Nitrogen dioxide (NO_2), nitrite anion (ONOO), are nitrogen sources of free radicals [12, 13]. Now days these a many of the cells have good or very good prooxidant they are balance antioxidant and maintain the strength. Most of the prooxidants increases the oxygen percentage which leads to increase in the free radicles in such case we have to when levels of antioxidants are diminished. If percentage of the prooxidant is increased such state is called 'oxidative stress' which cause the cell damage. In these days natural or herbal antioxidants [14, 17] are employed they are very good antioxidant activity. They are alternative antioxidant.

Plant description
The plants occur in various region of Maharashtra it mostly occurs in the Sahyadri region. The plant is erect herbs, 40-75 cm tall, it has very long stem simple below, often reddish-purple tinged, branched above; branches terete, faintly these have stem and striate, these are global, young they are pubical and they are good. plant have leaves like pinnately 3-foliolate; the petioles 6-9 cm very long, better sheathing, very sparsely ciliate on margins; petiolules 0.6-2.5 cm long, they have glabrous or so very secondary leaflets of lateral leaflets sessile, very upper leaves often cut into numerous, acquire linear segments or reduced to sheaths. The flowers such as white, in terminal compound umbels, primary rays 10-15, 1.0-3.8 cm long, these are slender, peduncles in nature.
Plant is analyzed with respect antioxidant activity; Extraction of plant material is carried using the conventional method such as reflux in different solvents. These Extract are tested for the antioxidant properties. They have antioxidant activity was measured chemicals DPPH (2, 2-diphenyl-1-picrylhydrazyl). Most of the plant shows low to very good antioxidant property.

Materials and Methods
Chemicals and Reagents
All chemicals used were of analytical and molecular grade. These are purchased from the S.D. Fine and Loba chemicals.

Collection of Plant Materials
Plant Materiual *Pimpinella wallichiana* were collected from Raigad region and other sayhadri region of the Maharashtra India. The Plant was identified by Dr. B. K. Auti from the Radhabai Mahila Mahavidyalya Ahmednagar, Stored in Department of chemistry Karmaveer Bhaurao Patil College Vashi.

Experimental

The Plant material the aerial parts (stems and leaves) of Pimpinella wallichiana were collected. This Plant material was kept for drying at room temperature, after complete drying ground to a fine powder using a blender. The extract were prepared in different solvents extraction, this is carried using Soxhlet extraction with using solvents of various polarity such as hexane, dichloromethane, ethyl acetate and water-ethanol (1-6 V/V).

Phytochemical [18-19] testing carried given protocol and then then other biological activity carried using the correct protocol.

Detection of Sterol

Polyterpenes [20-22] Using LIEBERMANN reagent test help identifying these compounds, result it in the formation of the blue green colour it indicates presence of the steroid while pink colour not formed hence terpens are absent.

Detection of Reducing Sugars

Reducing sugars were identified in given by the Fehling reagent, and and confirmed by Tollens reagent test. To carry Fehling tests, 5-6 ml of extract are added to 5-6 ml of Fehling's solution it forms red brick after min of heating bath at 70 °C indicates a positive reaction. Tollens test consisted of adding 5 ml of extract to 5-6 ml of the Tollens reagent result in formation of silver mirror.

Detection of Alkaloids

Alkaloids [23-26] was characterized from (reagent iodo-iodized) Bouchardat reagent and (reagent iodobismuthate of potassium). Dragendorff 6 to 7 ml of each solution were evaporated to dryness. The residue is taken up in 6-7 mL alcohol at 60°. Orange colour formation of alkoloid.

2.2.4 Detection of Proteins

The proteins [27, 29] were identified in the extracts by the biuret reaction. To small amount of extract with dissolved in 2 ml of 20% aqueous NaOH in given a test tube add 2 -3 drops of an aqueous part of CuSO4 to 2%. Formations of purple colour indicate presence of protein.

Detection of Coumarins

In two test tubes, take 2 ml ethanolic solution obtained from each residue during extraction. In one of test tubes, the add 0.5 ml of 10% NaOH, heat both test tube heated in a water bath until boiling. Cool both the test-tube each test tube 4 ml of distilled water. If the liquid from the test tube in which was added the alkaline solution is transparent or more transparent compared to the control test tube liquid (without alkaline solution), it forms faint yellow solution indicate presence of coumarin [30].

Detection of Tannins

Finding catechic tannins [31] are the detected reagent Stiasny. 5-6 Ml of each extract were evaporated to dryness using oven. Add 10-15 mL of reagent Stiasny the residue, place water bath at 80° to 90^{0}C for 20-30 min. large flakes like precipitate not formed then tannins, are absent. If tannins are absent then no Gallic tannins.

Detection of Flavonoids

0.5 -1 mL of plant extract was solvent petroleum ether shake Fatty material can be easily removed. To residue put 10-20 mL of 80% ethanol and filtered. Take 5 mL of filtrate of the ad small amount of ammonia the aqueous filtrate of each plant extract followed by the addition of concentrated H_2SO_4, it gives the flavonoids [30, 32].

Detection of Saponosides

To find saponnins [30, 33], in a a test tube, 8-10 ml aqueous total extract. The tube was shaken for 10-15 s and allowed to stand for 12-15 min. A height of persistent foam greater than 1 to 2 cm indicated the presence of saponnins are recorded.

Determination of total phenolic content

This [30, 34, 38] is determined by the Folin-Ciocalteu method given by Lister and Wilson [12] 0.5 -1 mL of sample solution was mixed with 2.5-4 mL of Folin - Ciocalteu reagent water 1:10, or 1-15 then of 4 mL of Na_2CO_3 (7.5 %, w/v). The mixture was incubated in a water bath at 40-45°C for 30 min and the absorbance was measured at 765 nm using a spectrophotometer or UV-Vis spectrophotometer against blank sample. The standard Gallic acid is range of concentrations (0-200 mg/L).

Determination of flavonoids content

The Flavonoid present in the given plant extract measured colorimetric method [30].

0.25-0.50 mL was mix in test tube containing 1.25 mL of distilled water. To this add Sodium nitrite solution 5%, 0.075 mL was added to the mixture and maintained for 5-6 min. 0.15-0.30 mL of 10% aluminum chloride was after 6-10 min, 0.5 -0.8 mL of 1 Molar sodium hydroxide was finally added 0.275 mL of distilled water. The absorbance of the mixture at 510 nm was measured immediately in comparison to a standard curve prepared by quercetin.

Antioxidant Activity (AA)

2.5. Free radical scavenging activity (DPPH)

Radical scavenging activity of the various plant extracts was measured by 1-1-diphenyl-2-picryl-hydrazil (DPPH) [39], this is measured at 517 nm, the standard used id ascorbic acid and BHT results (Given in observation table one)

DPPH Scavenging Test

Quantitative measurement of radical scavenging [40] property was carried out in a universal bottle. The reaction mixture contained 50 µL of test samples (or 80% MeOH as blank) and 5 mL of a 0.004% (w/v) solution of DPPH in methanol. Using given standard protocol.

Table 1: Antioxidant Activity of Leaves Extract

Conc. Mg/ml	BHT	Ethanol	Water	Methanol
0.05	47.1	38.60	27.53	46.47
0.1	48.91	28.64	28.53	50.90
0.2	52.24	46.24	38.50	54.80
0.3	59.57	52.12	46.00	30.90

Table 2: Antioxidant Activity of Stem Extract

Conc. Mg/ml	BHT	Ethanol	Water	Methanol
0.05	47.1	32.69	25.35	23.56
0.1	48.91	27.78	23.35	32.67
0.2	52.24	29.89	21.56	23.68

Results and Discussion

Phytochemicals in *Pimpinella wallichiana* the proper test were performed qualitative tests were performed using the given protocol. The results showed that the *Pimpinella wallichiana* extract have good antioxidant activity.it also contain the phytochemicals such as flavonoids, alkaloids, quinines, phenols, carbohydrates and terpenoids they also contain the flavonoids Antioxidant property due to the chelating property. The aqueous extract of plant shows better antioxidant activity.

Pimpinella wallichiana is very medicinal plant of the family Apiaceae. Various extract of the leaves and stem can show the better antioxidant activity. The results reveal the presence of alkaloids, flavonoids, steroids, terpenoids, saponins, cardiacglycosides, and reducing sugars in the juice or extract can be studied using the perfect protocol.

Conclusion

The various results of the present study demonstrate that aqueous extract of leaves and stem of Pimpinella wallichiana contained the phytochemicals such as flavonoids, terpenoids, alkaloids, phenols, quinines, and carbohydrate. The extract was found to possess promising Antioxidant activity against several bacterial species. It appears that the use of plant extract of leaves ad stem as an antioxidant agent would be suitable for the development of cost-effective, safe and efficient novel drugs active against several pathogenic multidrug-resistant microorganisms in the future. It could be used as a natural antimicrobial and also represents a useful therapeutic supplement.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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