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Evaluation of antioxidant potential and antimicrobial activity of successive extracts of Pimpinella tirupatiensis

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ABSTRACT
An increasing demand for natural additives has shifted the attention from synthetic to natural antioxidants. As leafy vegetables are found to be good source of antioxidants and the present study is to examine the antioxidant potential and antimicrobial activity of leaf extracts of Pimpinella tirupatiensis.

Methods: Antioxidant potential of leaves of P. tirupatiensis was studied using different methods like DPPH, nitric oxide, hydrogen peroxide scavenging activity. Reducing power and antimicrobial activity was estimated by using both gram positive and gram negative microorganisms by using DMF as solvent.

Keywords:
Antioxidant potential
Antimicrobial activity
Leaf extracts
Pimpenella tirupatiensis

I. INTRODUCTION

Pimpenella tirupatiensis (Apiaceae) is distributed in the forest of Tirupati in Andhra Pradesh commonly known as adavi kothimeera (Forest Coriander). It is used for the treatment of External inflammation, Diuretic, treatment of bladder distress, Asthma, Aphrodisiac, Skin diseases, Ulcers, Blood disorders, Toothache and Hepatoprotective.[1] Free radicals have been implicated to the causation of ailments such as liver cirrhosis, atherosclerosis, cancer, diabetes etc.[2] Reactive oxygen species such as super oxide anions (O_2), hydroxyl radicals (OH) and nitric oxide (NO) inactivate the enzymes and damage important cellular components causing injury.[3] Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals. Although living system possesses several natural defence mechanisms, such as enzymes and antioxidants nutrients, which arrest the chain reaction of ROS initiation and production. Many plants often contains substantial amounts of antioxidants including vitamins C and E, carotenoids, flavonoids, phenols and tannins etc. and thus can be utilized to scavenge the excess free radicals from the body.

II. MATERIALS AND METHODS

2.1. Collection and authentification of plant

P. tirupatiensis was collected from Seshachalam forest from Tirupati & identification (Specimen voucher-1533) has been done by Prof. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India.

2.2. Preparation of extracts

The plant was procured, leaves were collected; dried and coarse powder was prepared. Successive extraction of dried coarse powder of leaves was carried out with solvents in increasing order of polarity viz. petroleum ether, benzene, chloroform, acetone, ethanol and then maceration with chloroform water. The solvents were evaporated under reduced pressure to get semisolid masses. The extracts were subjected to preliminary Phytochemical screening.[4]

2.3. Total phenolic content

Total phenolic content was determined by Begum Method.[5] Estimation of total phenolic content was done for chloroform, ethanol and water extracts and Gallic acid was used as standard. 1 ml of different concentration (5, 10, 15, 20, 25 µg/ml) of different extracts were mixed with 1 ml of 95% ethanol, 5 ml of distilled water and 0.5 ml of 50% Folin–Ciocalteu reagent. The mixture was incubated for 1 h in dark and absorbance was measured at 725 nm using UV–Visible spectrophotometer.

2.4. Determination of total antioxidant activity

The method described by Prieto[6] and was used to determine the total antioxidant capacity of

the extracts. The tubes containing 0.2 ml of the extracts (100–500 μg/ml), 1.8 ml of distilled water and 2 ml of phosphomolybdenum reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

2.5. Assessment of antioxidant activity

The assessment of antioxidant activity was done through various in-vitro assays. The free radical scavenging activity of six extracts of P. tirupatiensis and L-ascorbic acid (vitamin C) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, H_2O_2. Nitric acid was generated from sodium nitroprusside and measured by Griess reaction. The activity was further conformed by reducing power method.

2.6. DPPH radical scavenging activity

Each extracts were prepared in different concentrations ranging from 20 μg/ml to 100 μg/ml and 1 ml solution of DPPH 0.1 mM (0.39 mg in 10 ml methanol) was added to different extracts.[7] An equal volume of ethanol and DPPH was added to control. Ascorbic acid was used as standard for comparison. After 20 min of incubation in dark, absorbance was measured at 517 nm and percentage of inhibition was calculated. Inhibition (%)=Control−TestControl×100

2.7. Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction.[8] Sodium nitroprusside (5 mM) in PBS (phosphate buffer saline) was incubated with different concentrations (20–100 μg/ml) of the extracts, dissolved in phosphate buffer (0.25 M, pH 7.4) and the tubes were incubated at 25 °C for 5 h. Controls without the test compounds, but with equivalent amounts of buffer were conducted in identical manner. After 5 h 0.5 ml of Griess reagent (1% sulfanilamide, 2% O-phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance was measured at 546 nm.

2.8. Reducing power assay

The reducing powers of nutraceutical herbs were determined according to Oyaizu.[9] Each extracts were prepared in different concentrations ranging from 20 μg/ml to 100 μg/ml and 1 ml of each in distilled water were mixed with phosphate buffer (2.5 ml, 2 M, pH 6.6) and potassium ferric cyanide (2.5 ml); the mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of Trichloroacetic acid (TCA 10%) was added to the mixture, which was then centrifuged at 1500 RPM for 10 min. The upper layer of solution (2.5 ml) was mixed with distill water (2.5 ml) and $FeCl_3$ (0.5 ml of 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The reducing power was expressed as AAE means that reducing power of 1 mg sample is equivalent to reducing power of 1 mmol ascorbic acid.[10]

2.9. Determination of peroxide (H_2O_2) radical scavenging activity

Each extracts were prepared in different concentrations ranging from 20 μg/ml to 100 μg/ml in phosphate buffer saline (PBS) and was incubated with 0.6 ml of 4 mM H_2O_2 solution prepared in PBS for 10 min. The standard ascorbic acid was used as standard and absorbance was measured at 230 nm.[5]

2.10. Statistical analysis

Inhibition of concentration and total phenolic and antioxidant were determined by linear regression analysis method which was used to calculate IC_{50}. Results were expressed as mean ± SD (standard deviation) n = e.

2.11. Antimicrobial activity

Cup plate method was employed to study the preliminary antibacterial activity of different extracts i.e. pet-ether, chloroform, ethanol, water against two gram positive Bacillus subtilis, Staphylococcus aureus and four gram negative bacteria Salmonella, Klebsiella, Pseudomonas, Esc herichia coli.

Preparation of nutrient broth, sub-culture and agar media was done as per standard procedure. Streptomycin was employed as reference standard. All this extracts were tested at a concentration of 50, 100, 200 μg/ml and DMSO as control did not show any inhibition.

The cups of each 8 mm diameter were made by scooping out medium with a sterilized cork borer from Petri dish which was inoculated with the organisms. The solutions of each test

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compound, control and reference standards (0.1 ml) were added separately in the cups and Petri dishes were subsequently incubated at 37 ± 10 °C for 24 h for the antibacterial activity.[11]

III. RESULTS AND DISCUSSION

3.1. Phytochemical investigation

Preliminary Phytochemical screening of P. tirupatiensis was carried out to reveal the different primary and secondary metabolites. Petroleum ether (PEE) and benzene extracts showed the presence of steroids. Chloroform (CHE) extract showed the presence glycosides and phenols. Acetone (ACE), Ethanolic (ETH) and Water (WTR) extract showed the presence of carbohydrates, alkaloids, flavonoids, volatile oil and saponins.

3.2. Total phenolic content

Phenolic compounds are a class of antioxidant agents, which act as free radical terminators.[12] Total phenols were measured by Folin–Ciocalteu reagent in terms of Gallic acid equivalent. The total phenolic in ACE, MEE and WTR of P. tirupatiensis was found to be 150.16, 174 and 231.39 respectively. The compounds such as flavonoids and polyphenols, which contain hydroxyls, are responsible for the radical scavenging effect of plants.[13] According to our study, the high contents of this Phytochemical in aqueous extract of P. tirupatiensis can explain its high radical scavenging activity.

3.3. Antioxidant potential

3.3.1. DPPH radical scavenging activity

DPPH is a stable free radical at normal temperature. It shows specific absorbance at 517 nm due to color of methanolic solution of DPPH. Body also contains man free radicals, which assumed same as DPPH.[14] Decrease in absorbance of mixture indicates the radical scavenging activity (Table 1; Fig. 1).

Table 1. DPPH radical scavenging activity.

S. No	Extracts	Concentration (μg/ml) and % inhibition (SEM ± SD)*					IC_{50}
		20*	40*	60*	80*	100*	–
1	PEE	19.80 ± 0.46	24.39 ± 0.75	27.26 ± 0.62	31.38 ± 0.87	34.28 ± 0.77	–
2	CHE	30.45 ± 0.35	38.80 ± 0.88	40.25 ± 0.84	43.78 ± 0.54	45.46 ± 1.00	–
3	ACE	35.13 ± 0.89	38.42 ± 0.32	41.99 ± 0.22	45.52 ± 0.42	48 ± 0.16	–
4	ETH	38.17 ± 0.82	44.03 ± 0.66	46.94 ± 0.38	48.35 ± 0.11	49.64 ± 0.56	58
5	WTR	41.96 ± 0.90	46.15 ± 0.06	52.83 ± 0.66	57.62 ± 0.24	62.96 ± 0.54	52
6	Vit C	46.19 ± 0.17	48.39 ± 0.28	55.38 ± 0.27	60.36 ± 0.10	67.64 ± 0.41	45

PEE: pet. Ether, CHE: chloroform, ACE: acetone, ETH: ethanol, WTR: water, Vit C: standard.
*
Values are mean ± SD, n = 3.

3.3.2. Nitric oxide radical scavenging activity

Nitric oxide is a free radical produced in mammalian cells, which is mediator of many physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity.[14] Sodium nitroprusside generates nitric oxide radical in the presence of physiological buffer solution at 25 °C. Nitric oxide reacted with Griess reagent and diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine form color complex. Decrease in color intensity is directly proportional to nitric oxide radical scavenging, which is measured in terms of IC_{50} (Table 2; Fig. 2).

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Table 2. Nitric oxide scavenging activity of each extract.

S. No	Extracts	Concentration (μg/ml) and % inhibition*					IC_{50}
		20*	**40***	**60***	**80***	**100***	**–**
1	PEE	8.21 ± 0.30	10.54 ± 0.35	21.74 ± 0.56	24.58 ± 0.87	32.55 ± 0.22	–
2	CHE	21.51 ± 0.52	23.69 ± 0.70	24.62 ± 0.84	26.74 ± 0.14	28.64 ± 0.12	–
3	ACE	32.63 ± 0.26	38.36 ± 0.32	40.20 ± 0.08	42.52 ± 0.03	43.33 ± 0.29	–
4	ETH	42.39 ± 0.50	50.58 ± 0.11	52.26 ± 0.09	53.46 ± 0.18	56.85 ± 0.10	47
5	WATR	44.11 ± 0.90	47.45 ± 0.06	57.48 ± 0.47	59.62 ± 0.24	60.96 ± 0.34	38
6	Vit C	47.19 ± 0.12	54.26 ± 0.34	66.44 ± 0.27	77.40 ± 0.27	88.51 ± 0.27	34

PEE: pet. Ether, CHE: chloroform, ACE: acetone, ETH: ethanol, WTR: water, Vit C: standard.
*
Values are mean ± SD, n = 3.

3.3.3. Reducing power

The reduction of Fe^{3+} ions can be assed by this reducing model for antioxidants. All the extracts were subjected for reducing activity. Water extract showed significant reducing activity when compared to that of other extracts (Table 3; Fig. 3).

Table 3. Reducing power activity of Pimpenella tirupatiensis extracts.

S. No	Extracts	Concentration (μg/ml) and % inhibition*				
		20*	**40***	**60***	**80***	**100***
1	PEE	0.005 ± 0.02	0.006 ± 0.04	0.020 ± 0.029	0.030 ± 0.018	0.076 ± 0.003
2	CHE	−0.021 ± 0.052	−0.013 ± 0.002	−0.011 ± 0.0031	−0.008 ± 0.0019	−0.004 ± 0.006
3	ACE	−0.011 ± 0.002	−0.003 ± 0.0029	0.007 ± 0.0018	0.027 ± 0.004	0.057 ± 0.0015
4	ETH	−0.016 ± 0.003	−0.006 ± 0.0016	0.050 ± 0.0018	0.090 ± 0.0077	0.095 ± 0.003
5	WATR	0.0860 ± 0.003	0.179 ± 0.001	0.223 ± 0.0056	0.342 ± 0.0027	0.383 ± 0.0028
6	Vit C	1.092 ± 0.012	1.208 ± 0.0112	1.212 ± 0.004	1.439 ± 0.0038	1.501 ± 0.0074

PEE: pet. Ether, CHE: chloroform, ACE: acetone, ETH: ethanol, WTR: water, Vit C: standard.
*
Values are mean ± SD, n = 3.

3.3.4. Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiols (–SH) groups. Hydrogen peroxide crosses cell membrane and reacts with ferric and copper ions, which shows toxic effects. Extracts have the good hydrogen peroxide scavenging activity.[5] The total antioxidant capacity of the extracts was found to be 49; 68; 74 mg ascorbic acid equivalent at 500 μg/ml extracts concentration. The good antioxidant activity might be attributed to the presence of Phytochemicals like phenols and tannins (Table 4; Fig. 4).

Table 4. Hydrogen peroxide scavenging activity.

S. No	Extracts	Concentration (μg/ml) and % inhibition*				
		20*	**40***	**60***	**80***	**100***
1	PEE	−0.622 ± 0.001	−0.607 ± 0.0026	−0.553 ± 0.0029	−0.448 ± 0.0034	−0.424 ± 0.0039
2	CHE	−0.231 ± 0.0015	−0.135 ± 0.0039	−0.050 ± 0.0031	−0.043 ± 0.0010	0.029 ± 0.0012
3	ACE	−0.191 ± 0.002	−0.125 ± 0.003	0.025 ± 0.0018	0.073 ± 0.0144	0.125 ± 0.0021
4	ETH	−0.148 ± 0.004	−0.093 ± 0.001	0.054 ± 0.0027	0.096 ± 0.0077	0.145 ± 0.0032
5	WATR	−0.137 ± 0.002	−0.083 ± 0.0072	0.020 ± 0.001	0.171 ± 0.0038	0.203 ± 0.004
6	Vit C	0.040 ± 0.0013	0.050 ± 0.001	0.170 ± 0.002	0.334 ± 0.004	0.547 ± 0.007

PEE: pet. Ether, CHE: chloroform, ACE: acetone, ETH: ethanol, WTR: water, Vit C: standard.
*
Values are mean ± SD, n = 3.

3.3.5. Antimicrobial activity
The alcoholic and benzene extracts showed significant activity when compared with aqueous and pet-ether extracts (Table 5).

Table 5. Antimicrobial activities of Pimpenella tirupatiensis.

Sr. No	Antibacterial											
Empty Cell	Bacillus subtilis			Staphylococcus aureus			Klebsiella pneumonia			Escherichia coli		
Concentration in μg	**50**	**100**	**200**	**50**	**100**	**200**	**50**	**100**	**200**	**50**	**100**	**200**
Pet-ether	9.4	10.2	12.6	R	12.8	13.1	9.6	13.4	14.7	R	R	11.3
Benzene	14.7	15.9	17.3	12.9	14.2	15.7	15.3	17.8	19.6	11.0	12.8	15.5
Alcohol	15.5	18.6	20.9	12.8	14.1	14.9	12.0	12.7	14.2	14.4	15.9	18.2
Aqueous	R	9.3	11.1	13.6	15.2	17.0	12.8	14.2	15.7	R	12.9	15.2
Control (DMF)	R	R	8.5	R	R	R	R	8.3	8.5	R	R	R
Standard	16.7	19.1	22.3	13.9	15.8	17.6	16.5	19.5	21.9	16.9	18.6	21.2

Diameter of cup – 8 mm, Standard drug – Streptomycin (antibacterial), R – Resistance, DMF – Dimethyl Formamide, Reading indicates the zone of inhibition in mm (millimeters).

IV. CONCLUSION

An increasing demand for natural additives has shifted the attention from synthetic to natural antioxidants. As leafy vegetables are found to be good source of antioxidants and the present study is to examine the antioxidant potential and antimicrobial activity of leaf extracts of P. tirupatiensis. Many plants often contain substantial amounts of antioxidants including vitamins C and E, carotenoids, flavonoids, phenols and tannins etc. and thus can be utilized to scavenge the excess free radicals from the body.

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