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Analyzing the antibacterial and antioxidant properties of Pimpinellatirupatiensis extracts in sequence

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ABSTRACT

*Background:*An increasing demand for natural additives has shifted the attentionfrom synthetic to natural antioxidants. As leafy vegetables are found to be good source of an- tioxidants 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 usingdifferent 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.

Results: The aqueous extracts showed maximum scavenging activity of DPPH followed by nitric oxide, hydrogen peroxide and reducing power respectively. Benzene and alcoholic extract showed maximum antimicrobial activity.

*Conclusion:*Substantial amounts of antioxidants including vitamins C and E, carotenoids, flavonoids, phenols and tannins etc. can be utilized to scavenge the excess free radicalsfrom the body.

*Keywords:*Antioxidant potential Antimicrobial activity Leaf extracts*Pimpenellatirupatiensis*

1. Introduction

Pimpenellatirupatiensis(Apiaceae)isdistributedintheforestof Tirupati in Andhra Pradesh commonly known as adavi kothimeera(ForestCoriander).Itisusedforthetreatment of External inflammation, Diuretic, treatment of bladder distress,Asthma,Aphrodisiac,Skindiseases,Ulcers,Blooddisorders, Toothache and Hepatoprotective.[1]Freeradicals have been implicated to the causation of ailments such asliver 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]Antioxidantsmayofferresistanceagainsttheoxidative

Table1eDPPHradicalscavengingactivity.

S. No	Extracts	Concentration(mg/ml)and%inhibition(SEMSD)*					IC_{50}
		20*	40*	60*	80*	100*	e
1	PEE	19.80 0.46	24.39 0.75	27.26 0.62	31.38 0.87	34.280.77	e
2	CHE	30.45 0.35	38.80 0.88	40.25 0.84	43.78 0.54	45.461.00	e
3	ACE	35.13 0.89	38.42 0.32	41.99 0.22	45.52 0.42	480.16	e
4	ETH	38.17 0.82	44.03 0.66	46.94 0.38	48.35 0.11	49.640.56	58
5	WTR	41.96 0.90	46.15 0.06	52.83 0.66	57.62 0.24	62.960.54	52
6	VitC	46.19 0.17	48.39 0.28	55.38 0.27	60.36 0.10	67.640.41	45

PEE:pet.Ether,CHE:chloroform,ACE:acetone,ETH:ethanol,WTR:water,VitC:standard.
*ValuesaremeanSD,*n*¼3.

stressbyscavengingthefreeradicals. Althoughlivingsystem possesses several natural defence mechanisms, such as en- zymes and antioxidants nutrients, which arrest the chain reaction of ROS initiation and production. Many plants often contains substantial amounts of antioxidants including vita- mins C and E, carotenoids, flavonoids, phenols and tannins etc. and thus can be utilized to scavenge the excess free rad- icals from the body. Total phenolic content was determined by BegumMethod.[5]Estimation of total phenolic content was done for chloro- form, ethanol and water extracts and Gallic acid was used as standard.1mlofdifferentconcentration(5,10,15,20,25mg/ ml)ofdifferentextractsweremixedwith1mlof95%ethanol, 5 ml of distilled water and 0.5 ml of 50%FolineCiocalteu reagent.Themixturewasincubatedfor1hindarkandabsorbancewasmeasuredat725nmusingUVeVisible

2. Materials and methods

Collectionandauthentificationofplant

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

Preparationofextracts

The plant was procured, leaves were collected; driedand 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]

Determinationoftotalantioxidantactivity

The method described by Prieto[6]and was used to determine the total antioxidant capacity of the extracts. The tubes con- taining 0.2 ml of the extracts (100e500mg/ml), 1.8 ml of distilled water and 2 ml of phosphomolybdenum reagent so- lution (0.6 Msulfuricacid, 28 mMsodiumphosphateand4 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 equiva- lent (AAE).

Assessmentofantioxidantactivity

The assessment of antioxidant activity was donethrough variousin-vitroassays.Thefreeradicalscavengingactivityof

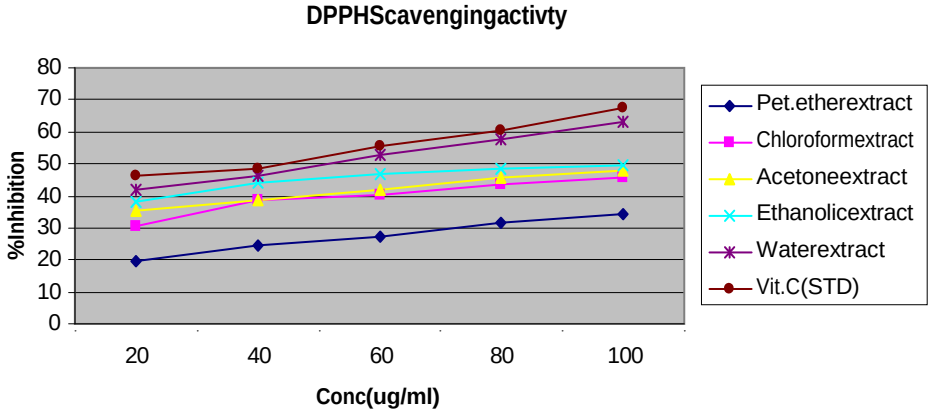

Fig.1eDPPHradicalscavengingactivity.

Table2eNitricoxidescavengingactivityofeachextract.

S. No	Extracts	Concentration(mg/ml)and%inhibition*					IC_{50}
		20*	40*	60*	80*	100*	e
1	PEE	8.21 0.30	10.54 0.35	21.74 0.56	24.58 0.87	32.550.22	e
2	CHE	21.51 0.52	23.69 0.70	24.62 0.84	26.74 0.14	28.640.12	e
3	ACE	32.63 0.26	38.36 0.32	40.20 0.08	42.52 0.03	43.330.29	e
4	ETH	42.39 0.50	50.58 0.11	52.26 0.09	53.46 0.18	56.850.10	47
5	WATR	44.11 0.90	47.45 0.06	57.48 0.47	59.62 0.24	60.960.34	38
6	VitC	47.19 0.12	54.26 0.34	66.44 0.27	77.40 0.27	88.510.27	34

PEE:pet.Ether,CHE:chloroform,ACE:acetone,ETH:ethanol,WTR:water,VitC: standard.
*ValuesaremeanSD,*n*¼ 3.

sixextractsof*P.tirupatiensis*and$_L$-ascorbicacid(vitaminC) was measured in terms of hydrogen donating or radical scavengingabilityusingthestableradicalDPPH,H_2O_2.Nitric acid was generated from sodium nitroprusside and measured by Griess reaction. The activity was further conformed by reducing power method.

DPPHradicalscavengingactivity

Each extracts were prepared in differentconcentrations rangingfrom20mg/mlto100mg/mland1mlsolutionofDPPH 0.1 mM (0.39 mg in 10 ml methanol) was added to different extracts.[7] AnequalvolumeofethanolandDPPHwasaddedto 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.

Control—Test

(1%sulfanilamide,2%*O*-phosphoricacidand0.1%naphthyl- ethylene diamine dihydrochloride) was added. The absor- bance was measured at 546 nm.

Reducingpowerassay

The reducing powers of nutraceutical herbs were determined accordingtoOyaizu.[9] Eachextractswerepreparedindifferent concentrationsrangingfrom20mg/mlto100mg/mland1mlof eachindistilledwaterweremixedwithphosphatebuffer (2.5ml,2M,pH6.6)andpotassiumferriccyanide(2.5ml);the mixture was incubated at 50 °C for 20min. 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 measuredat700nm.Increasedabsorbanceofthereaction

Control

mixtureindicatedincreasedreducingpower.Thereducing powerwasexpressedasAAEmeansthatreducingpowerof

Nitricoxideradicalscavengingactivity

Nitric oxide was generated from sodium nitroprussideand measuredbyGriessreaction.[8] Sodiumnitroprusside(5mM)in PBS (phosphate buffer saline) was incubated with different concentrations (20e100mg/ml) of the extracts, dissolved in phosphate buffer (0.25 M, pH 7.4) and the tubes were incu- bated at 25 °C for 5 h. Controls without the test compounds, butwithequivalentamountsofbufferwereconducted inidenticalmanner.After5h0.5mlofGriessreagent1mgsampleisequivalenttoreducingpowerof1mmol ascorbic acid.[10]

Determination of peroxide (H_2O_2) radical scavenging activity

Each extracts were prepared in different concentrations ranging from 20mg/ml to 100mg/ml in phosphate buffer saline (PBS) and was incubated with 0.6 ml of 4 mM H_2O_2 solutionpreparedinPBSfor10min.Thestandardascorbic

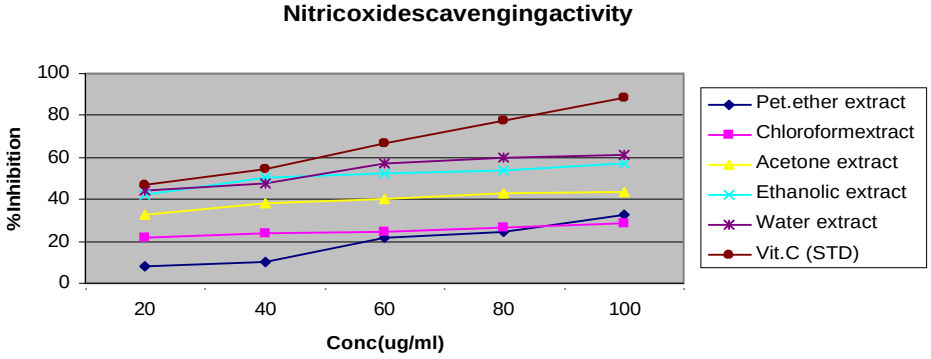

Fig.2eNitricoxideradicalscavengingactivity.

Table3eReducingpoweractivityof*Pimpenellatirupatiensis* extracts.

S. No	Extracts	Concentration(mg/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	0.086	0.003	0.179	0.001	0.223	0.0056	0.342	0.0027	0.383	0.0028
6	VitC	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,VitC:standard.
*ValuesaremeanSD,*n*=3.

acid was used as standard and absorbance was measured at 230 nm.[5]

Statistical analysis

Inhibition of concentration and total phenolic and antioxidant weredeterminedbylinearregressionanalysismethodwhich wasusedtocalculateIC_{50}.Resultswereexpressedas meanSD (standard deviation) *n* =*e*.

Antimicrobialactivity

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*Bacil- lus subtilis*,*Staphylococcus aureus*and four gram negative bacteria Salmonella, Klebsiella, Pseudomonas,*Escherichia coli.*

Preparation of nutrient broth, sub-culture and agar media was done as per standard procedure. Streptomycinwas employed as reference standard. All this extracts were tested ataconcentrationof50,100,200mg/mlandDMSOascontrol did not show any inhibition.

Thecupsofeach8mmdiameterweremadebyscooping outmediumwitha sterilizedcorkborer fromPetridishwhich was inoculated with the organisms. The solutions of each test compound, control and reference standards (0.1 ml) were added separately in the cups and Petri dishes were subse- quentlyincubatedat3710°Cfor24hfortheantibacterial activity.[11]

Results and discussion

Phytochemicalinvestigation

Preliminary Phytochemical screening of*P. tirupatiensis*was carried out to reveal the different primary and secondary metabolites.Petroleumether(PEE)andbenzeneextractsshowed thepresenceofsteroids.Chloroform(CHE)extractshowedthe presence glycosides and phenols. Acetone (ACE), Ethanolic (ETH)andWater(WTR)extractshowedthepresenceofcarbo- hydrates, alkaloids, flavonoids, volatile oil and saponins.

Totalphenoliccontent

Phenolic compounds are a class of antioxidant agents, which act as free radical terminators.[12]Total phenols were measured byFolineCiocalteureagentintermsofGallicacidequivalent. ThetotalphenolicinACE,MEEandWTRof*P.tirupatiensis*was found to be 150.16, 174 and 231.39 respectively. The com- pounds such as flavonoids and polyphenols, which contain hydroxyls, are responsible forthe radicalscavenging 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.

Antioxidantpotential

DPPHradicalscavengingactivity

DPPH is a stable free radical at normal temperature. It shows specificabsorbanceat517nmduetocolorofmethanolic

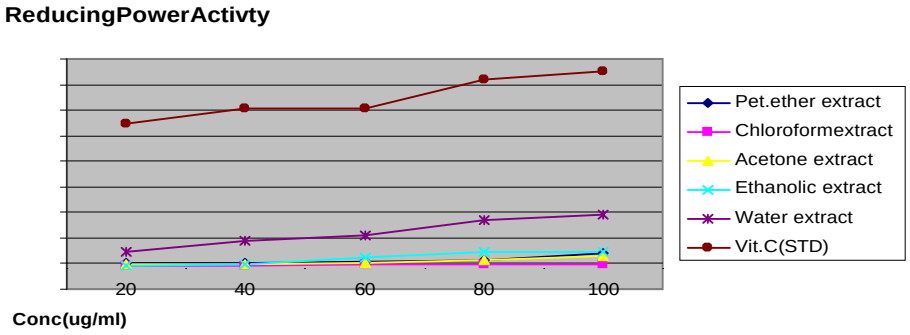

Fig.3eReducingpoweractivities.

Table4eHydrogenperoxidescavengingactivity.

S. No	Extracts	Concentration(mg/ml)and%inhibition*				
		20*	40*	60*	80*	100*
1	PEE	−0.6220.001	−0.607 0.0026	−0.553 0.0029	−0.448 0.0034	−0.4240.0039
2	CHE	−0.2310.0015	−0.135 0.0039	−0.050 0.0031	−0.043 0.0010	0.0290.0012
3	ACE	−0.1910.002	−0.125 0.003	0.025 0.0018	0.073 0.0144	0.1250.0021
4	ETH	−0.1480.004	−0.093 0.001	0.054 0.0027	0.096 0.0077	0.1450.0032
5	WATR	−0.1370.002	−0.083 0.0072	0.020 0.001	0.171 0.0038	0.2030.004
6	VitC	0.0400.0013	0.050 0.001	0.170 0.002	0.334 0.004	0.5470.007

PEE:pet.Ether,CHE:chloroform,ACE:acetone,ETH:ethanol,WTR:water,VitC: standard.
*ValuesaremeanSD,*n*=3.

solutionofDPPH.Bodyalsocontainsmanfreeradicals,which assumed same as DPPH.[14]Decrease in absorbance of mixture indicates the radical scavenging activity (Table 1; Fig. 1).

Nitricoxideradicalscavengingactivity

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 at25°C. Nitric oxide reacted with Griess reagent and diazoti- zation of nitrite with sulfanilamide and subsequentcoupling with naphthylethylene diamine form color com- plex.Decreaseincolorintensityisdirectlyproportionalto

thiols (eSH) groups. Hydrogen peroxide crosses cellmem- brane 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 be49;68;74mgascorbicacidequivalentat500mg/mlextracts concentration. The good antioxidant activity might be attrib- uted to the presence of Phytochemicals like phenols and tannins (Table 4; Fig. 4).

3.3.5.Antimicrobialactivity

The alcoholic and benzene extracts showed significant activ- ity when compared with aqueous and pet-ether extracts(Table 5).
nitricoxideradicalscavenging,whichismeasuredinterms
ofIC_{50}(Table2;Fig.2).

Reducingpower

Thereduction of Fe^{3+} ionscanbeassedbythisreducingmodel 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).

Hydrogenperoxidescavengingactivity

Hydrogen peroxide is a weak oxidizing agent and can inacti- vateafewenzymesdirectly, usually byoxidationofessential

3. Conclusion

An increasing demand for natural additives has shiftedthe attention from synthetic to natural antioxidants. As leafy vegetablesarefoundtobegoodsourceofantioxidantsandthe present study is to examine the antioxidant potential and antimicrobialactivityofleafextractsof*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.

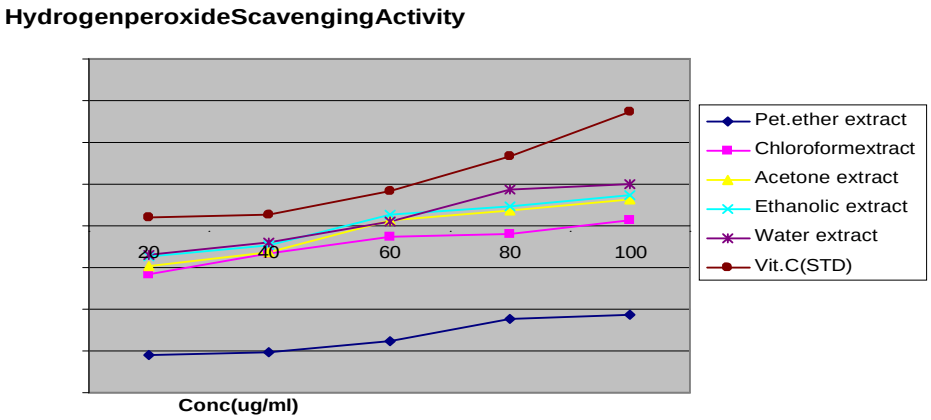

Fig.4eHydrogenperoxidescavengingactivity.

Table5eAntimicrobial activitiesof *Pimpenellatirupatiensis*.

Sr.No	Antibacterial											
	Bacillussubtilis			*Staphylococcusaureus*			*Klebsiellapneumonia*			*Escherichia coli*		
Concentrationinmg	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	
	RStandard	16.7	19.1	22.3	13.9	15.8	17.6	16.5	19.5	21.9	16.9	18.6
	21.2											

Diameterofcupe8mm,Standarddrugestreptomycin(antibacterial),ReResistance,DMFeDimethylFormamide,Readingindicatesthezone of inhibition in mm (millimeters).

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