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EVALUATION OF TOTAL POLYPHENOLIC CONTENT AND FREE RADICAL SCAVENGING ACTIVITY OF SUCESSIVE EXTRACTS OF *PIMPENELLA TIRUPATHANSIS & AMORPHOPHALLUS PAEONIIFOLIUS.*

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ABSTRACT

An increasing demand for natural additives has shifted the attention from synthetic to natural antioxidants. As vegetables are found to be good source of antioxidants and the present study is to examine the potential & antimicrobial activity of extracts of leaves of ***Pimpenella tirupathansis*** *with corns of* ***Amorphophallus paeoniifolius.*** Antioxidant potential of leaves of ***Pimpenella & corns of Amorphophallus*** were studied by using method like DPPH and reducing power. The aqueous extracts of Amorphophallus showed maximum scavenging activity of DPPH followed by reducing power respectively when compared with Pimpenella & Amorphophallus. Total phenols were found to be 174 (Pimpenella) and 231.39 (Amorphophallus) mg/g Gallic acid equivalent /g of dry material. These results suggest that phenolic and flavonoids in the leaves proved substantial antioxidant activity.

KEYWORDS: *Pimpenella tirupathansis, Amorphophallus paeoniifolius,* Pimpenella & Amorphophallus.

1. INTRODUCTON

Pimpenella tirupathansis (Apiaceae) is distributed in the forest of Tirupati in Andhra Pradesh commonly known as Adavi kothimera. It is used for the treatment of Asthma, Aphrodisiac, Skin diseases, Bladder distress & Hepatoprotectiv.[2]

Amorphophallus paeoniifolius (Araceae) is distributed throughout Telengana & Andhra Pradesh regions commonly known as Kandagadda.Corms are used as thermogenic, irritant, anti-inflammatory, digestive, stomachic, anthelmintic, tumors, colic, constipation, and anemia.[3]

Table no: 1; Plant sources

SL.NO	NAME	BIOLOGICAL SOURCE	FAMILY	COMMON NAME
1	Pimpenella	*Pimpenella tirupathansis*	Apiaceae	Adavi kothimera
2	Amorphophallus	*Amorphophallus paeoniiofolius*	Araceae	Kandagadda

Free radicals have been implicated to causation of ailments such as liver cirrhosis, atherosclerosis, and cancer, diabetes etc.[4] Reactive oxygen species such as super oxide anions (O_2), hydroxyl radicals (OH) and nitric oxide (NO) inactivate enzymes and damage important cellular components causing injury.[5] Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals. Although leaving system possess several natural defense 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 vitamin C and E, Carotenoids, flavonoids, phenols and tannins etc. and thus can be utilized to scavenge the excess free radicals from the body.

2. MATERIALS AND METHODS

2.1. Collection and authentication of plant

Amorphophyllus Poeniophyllus was collected from Ankapoor village in Telengana state &***Pimpenella tirupathansis*** was collected from seshachalam forest from Tirupati and Identification has been done by Prof.K.Madhava cheety, Department of Botany, Sri Venkateshwara University, Tirupati, India.

2.2. Preparation of extracts

The plants were procured; 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, 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.[6]

2.3. Total phenolic content

Total phenolic content was determined by Begum Method[7]. Estimation of total phenolic content was done for chloroform, ethanol and water extracts and Gallic acid was used as standard. 1ml of different concentration (5, 10,15,20,25 *u*g/ml) of different extracts were mixed with 1ml 95 % ethanol, 5ml of distilled water and 0.5 ml of 50 % Folin-Ciocalteu reagent. The mixture was incubated for 1hr 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,[8] (2000) was used to determine the total antioxidant capacity of the extracts. The tubes containing 0.2 ml of the extracts (100-500 ug/ml), 1.8 ml of distilled water and 2 ml of phosphomolybdium reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95^{o} C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695nm.The anti-oxidant capacity was expressed as ascorbic acid equivalent (AAE).

2.5. Assessment of anti-oxidant activity

The assessment of anti-oxidant activity was done through various in-vitro assays. The free radical scavenging activity of three extracts of Amorphaphallus & Pimpenella ***Poeniophyllus*** and L-ascorbic acid (vitamin C) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH and % inhibition was calculated. 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*u*g/ml to 100 *u*g/ml and 1ml solution of DPPH 0.1mM (0.39mg in 10ml methanol) was added to different extracts.[9] An equal volume of ethanol and DPPH was added to control. Ascorbic acid was used as standard for comparison. After 20min of incubation in dark, absorbance was measured at 517nm and percentage of inhibition was calculated.

$$\text{Inhibition (\%)} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

2.7. Reducing power assay

The reducing powers of neutraceutical herbs were determined according to Oyaizu[10] (1986). Each extracts were prepared in different concentrations ranging from 20 *u*g/ml to 100 *u*g/ml and 1ml of each in distilled water were mixed with phosphate buffer (2.5ml, 2M,pH 6.6) and potassium ferric cyanide (2.5ml); the mixture was incubated at 50^0C for 20 min. A portion (2.5ml) of trichloroacetic acid (TCA, 10%) was added to the mixture, which was then centrifuged at 1500 RPM for 10min.The upper layer of solution (2.5ml) was mixed with distill water (2.5ml) and $FeCl_3$ (0.5ml, 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 1mg sample is equivalent to reducing power of 1 mMol ascorbic acid.[10]

2.8. Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1. Phytochemical investigation

Preliminary Phytochemical screening of *Pimpenella tirupathansis & Amorphophyllus poeniophyllus* was carried out to reveal the different primary and secondary metabolites. Petroleum ether (PEE) extracts showed the presence of steroids. Ethanolic (ETH) and Water (WTR) extract showed the presence of glycosides, phenols, carbohydrates, flavonoids and saponins.

3.2. Total phenolic content

Phenolic compounds are a class of antioxidant agents, which act as free radical terminators.[11] Total phenols were measured by Folin Ciocalteu reagent in terms of Gallic acid equivalent. The total phenolic in WTR extracts of ***Pimpenella tirupathansis, Amorphophyllus poeniophyllus*** 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.[12] According to our study, the high contents of this Phytochemical in aqueous extract of ***Pimpenella tirupathansis, Amorphophyllus poeniophyllus*** 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 517nm due to color of methanolic solution of DPPH. Body also contains many free radicals, which assumed same as DPPH.[13] Decrease in absorbance of mixture indicates the radical scavenging activity, which is measured in terms of IC_{50}. All the three plant extracts were subjected for DPPH scavenging activity to know the antioxidant potentials of different extracts of the plants. Amorphophallus showed maximum activity against DPPH free radical when compared to Pimpenella (Table & Fig no: 2, 4, 6 and 8).

3.3.2. Reducing power

The reduction of Fe^{3+} ions can be assayed by this reducing model for antioxidants. All the extracts were subjected for reducing activity. Water extract of Amorphophyllus showed significant reducing activity when compared to that of other water extractsPimpenella. The comparative study helped to know the reducing power of all the extracts of the plant. (Table & Fig no: 3, 5, 7 and 9).

3.3.3. 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, Klebsella, Pseudomonas, Escherichia coli.

Preparation was nutrient broth, sub-culture, agar media was done as per standard procedure. Gentamycin was employed as reference standard.(Table no: 10).

All this extracts were tested at a concentration of 500, 1000, 1500 ug/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 compound, control and reference standards (0.1mL) were added separately in the cups and Petri dishes were subsequently incubated at 37 $\pm 1^0$ C for 24 h for the antibacterial activity.[14]

Table no: 4; DPPH radical scavenging activity: *Pimpenella tirupathansis*

SR.NO	CONCENTRATION (ug/ml)	PEE	ETH	WTR	VIT-C	IC50
1	50	59.40±0.46	76.34±0.35	83.92±0.89	92.38±0.82	-
2	100	73.17±0.75	88.06±0.88	92.3±0.32	96.78±0.66	-
3	150	81.78±0.62	93.88±0.84	105.66±0.22	110.76±0.38	58
4	200	94.14±0.87	96.07±0.54	115.24±0.42	120.72±0.11	52
5	250	102.84±0.77	99.28±1.00	125.92±0.16	135.28±0.56	45

PEE: pet.Ether, ETH: ethanol, WTR: water, VIT-C: standard

***Values are mean ±SD, n=3**

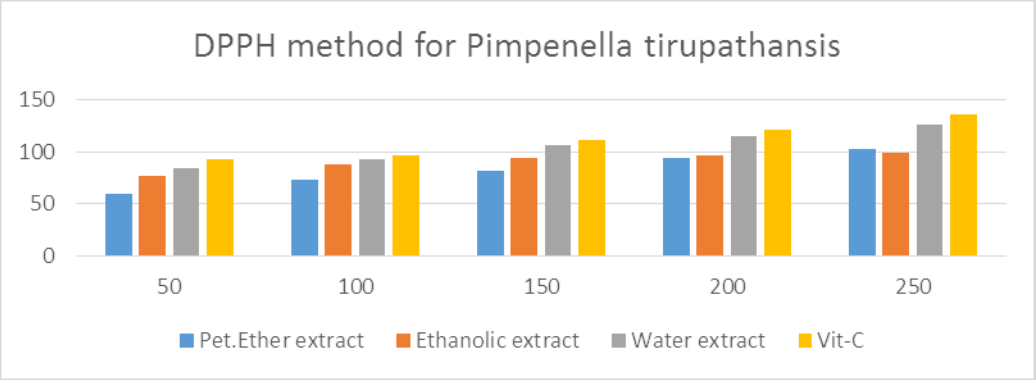

Table no: 5; Reducing power: *Pimpenella tirupathansis*

SR.NO	CONCENTRATION (ug/ml)	PEE	ETH	WTR	VIT-C
1	50	0.03±0.02	0.05±0.052	0.223±0.002	1.092±0.012
2	100	0.076±0.04	0.095±0.002	0.383±0.0029	1.208±0.0112
3	150	0.114±0.029	0.142±0.003	0.6±0.008	1.319±0.004
4	200	0.152±0.018	0.267±0.0019	0.768±0.004	1.439±0.0038
5	250	0.233±0.003	0.333±0.006	0.96±0.0015	1.501±0.0074

PEE: pet.Ether, ETH: ethanol, WTR: water, VIT-C: standard

***Values are mean ±SD, n=3**

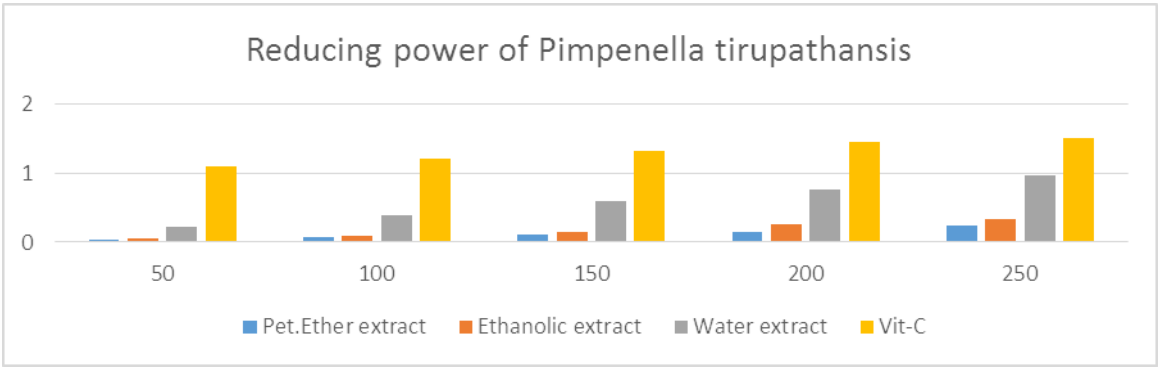

Table no: 6; DPPH radical scavenging activity: *Amorphophallus paeoniifolius*

SR.NO	CONCENTRATION (ug/ml)	PEE	ETH	WTR	VIT-C	IC50
1	50	34.25±0.090	65.4±0.17	86.26±0.30	92.38±0.52	-
2	100	44.01±0.06	72.5±0.28	93.02±0.35	96.78±0.70	-
3	150	50.07±0.66	77.1±0.27	112.21±0.56	110.76±0.84	52
4	200	51.24±0.24	82.03±0.10	117.09±0.87	120.72±0.14	48
5	250	61.1±0.54	90.7±0.41	128.61±0.22	135.28±0.12	43

PEE: pet.Ether, ETH: ethanol, WTR: water, VIT-C: standard

***Values are mean ±SD, n=3**

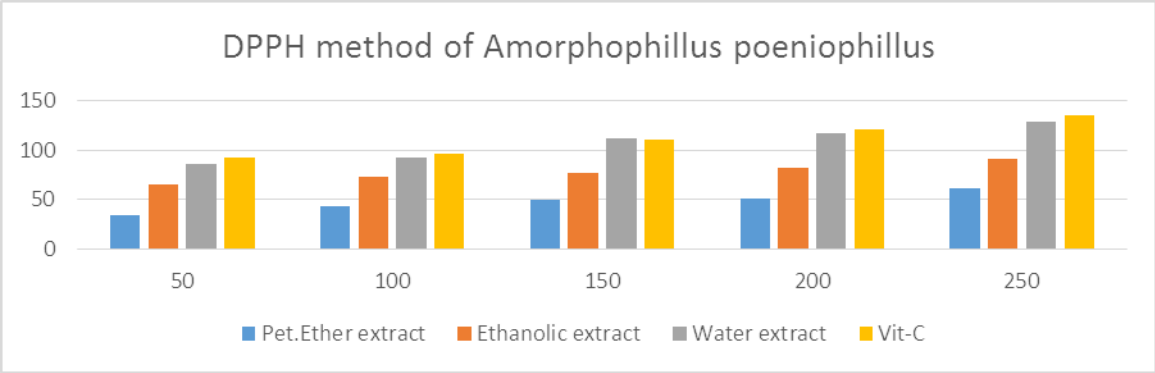

Table no: 7; Reducing power: *Amorphophallus paeoniifolius*

SR.NO	CONCENTRATION (ug/ml)	PEE	ETH	WTR	VIT-C
1	50	0.018±0.003	0.07±0.012	0.2±0.003	1.092±0.018
2	100	0.07±0.001	0.16±0.0112	0.6±0.006	1.208±0.0031
3	150	0.12±0.0056	0.29±0.004	0.81±0.0015	1.319±0.002
4	200	0.14±0.0027	0.38±0.0038	1.09±0.003	1.439±0.004
5	250	0.168±0.0028	0.45±0.0074	1.11±0.0028	1.501±0.0047

PEE: pet.Ether, ETH: ethanol, WTR: water, VIT-C: standard

***Values are mean ±SD, n=3**

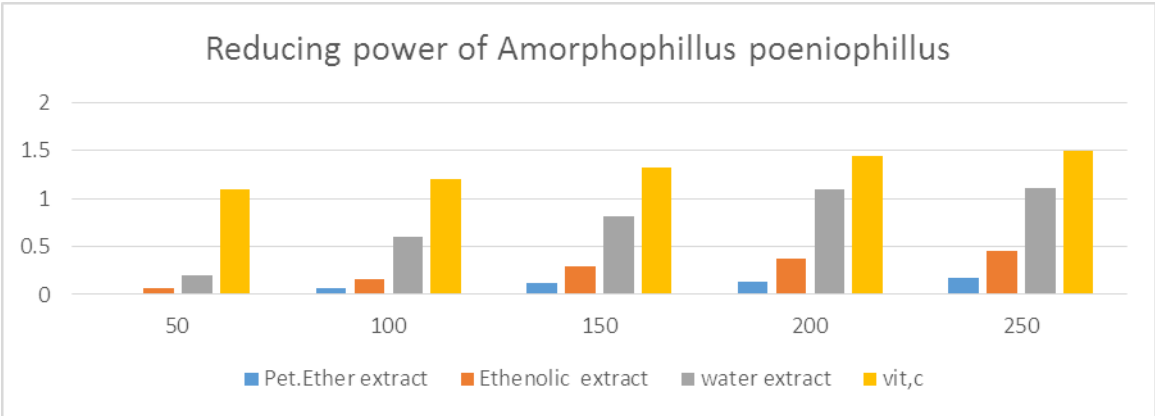

Table no: 8; Comparison of antioxidant activity of *Pimpenella tirupathansis, Amorphophyllus poeniophyllus by DPPH Method*.

SR.NO	CONCENTRATION (ug/ml)	WTR-PT	WTR AP	VIT-C
1	50	83.92±0.89	86.26±0.30	92.38±0.82
2	100	92.3±0.32	93.02±0.35	96.78±0.66
3	150	105.66±0.22	112.21±0.56	110.76±0.38
4	200	115.24±0.42	117.09±0.87	120.72±0.11
5	250	125.92±0.16	128.61±0.22	135.28±0.56

PEE: pet.Ether, ETH: ethanol, WTR: water, VIT-C: standard *Values are mean ±SD, n=3

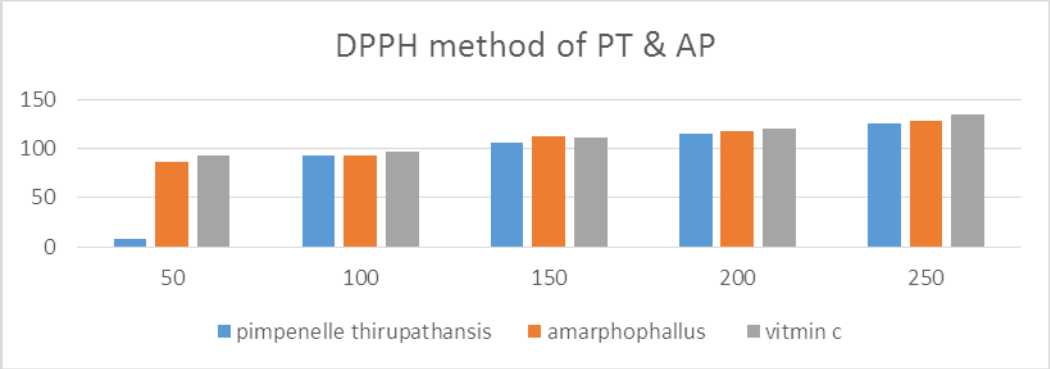

Table no: 9; Reducing method for Pimpenella tirupathansis, Amorphophyllus poeniophyllus.

SR.NO	Concentration (ug/ml)	WTR-PT	WTR-AP	VIT-C
1	50	0.223±0.002	0.2±0.003	1.092±0.012
2	100	0.383±0.0029	0.6±0.006	1.208±0.0112
3	150	0.6±0.008	0.81±0.0015	1.319±0.004
4	200	0.768±0.004	1.09±0.003	1.439±0.0038
5	250	0.96±0.0015	1.11±0.0028	1.501±0.0074

PEE: pet.Ether, ETH: ethanol, WTR: water, VIT-C: standard

***Values are mean ±SD, n=3**

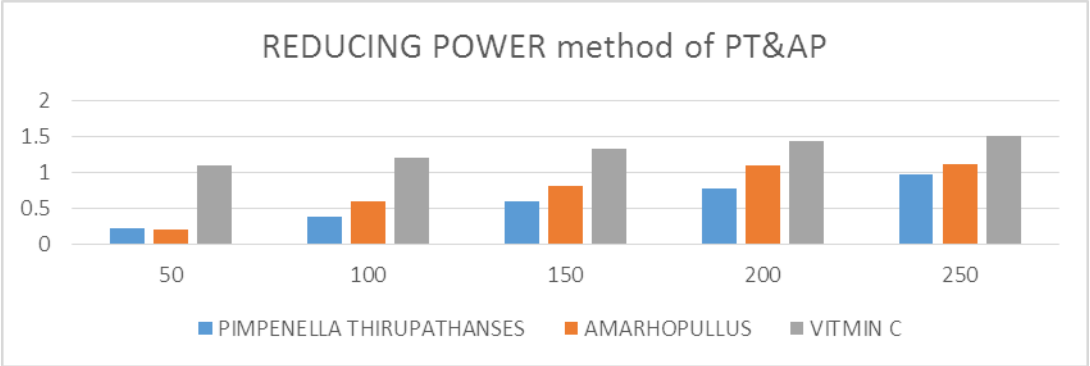

Table no: 10; Anti-microbial studies of Pimpenella& Amorphophallus

Plant	Extract	Bacillus subtilis			Staphylococcus aureus			Klebsiella pneumonia			Escherichia coli		
Concentration in µg		50	100	200	50	100	200	50	100	200	50	100	200
Pimpenella tirupathansis	PEE	9.4	10.2	12.6	R	12.8	13.1	9.6	13.4	14.7	R	R	11.3
	MEE	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
Amorphophyllus poeniophyllus	PEE	8.42	9.2	10.6	13	14	15	R	R	R	R	R	R
	MEE	14.7	15.9	17.3	12.9	14.2	15.7	15.3	17.8	19.6	11.0	12.8	15.5
	Aqueous	R	10.0	11.6	13	14.6	15.2	12	13.5	14.3	R	11.3	12.7
Control (DMF)		R	R	R	R	R	R	R	R	R	R	R	R
Streptomycin		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-8mm, Standard drug-Streptomycin (antibacterial),R-Resistance, DMF-Dimethyl Formamide, Reading indicates the zone of inhibition in mm (millimeters)

Conflicts of interests

Authors are interested in screening the Hepatoprotective activity of edible plants viz Amorphophallus & Pimpenella.

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