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

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ABSTRACT

Plants are the basic source of knowledge of modern medicine. Almost all the parts of the plant, namely leaves, flowers, fruits, bark, roots, stem and seeds are known to have various medicinal properties. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials, anticancer agents and antioxidants. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. 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 *Amaranthus tricolor& Pimpenella tirupathansis with corns of Amorphophallus paeoniifolius*. Antioxidant potential of leaves of *Amaranthus tricolor, 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 & Amaranthus*. Total phenols were found to be 150.16 (Amaranthus); 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 ofmaranthus tricolor, Pimpenella tirupathansis, Amorphophyllus poeniophyllus. The results obtained from phytochemical screening, antioxidant and antimicrobial analysis of *Amaranthus tricolor, Pimpenella tirupathansis, Amorphophyllus paeoniifolius* indicate these plants as “natural herbal sources” which can be used in pharmaceutical industry.

KEYWORDS: ***Amaranthus tricolor, Pimpenella tirupathansis, Amorphophyllus paeoniifolius, polyphenolic activity, anti-microbial activity, plant extracts***

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INTRODUCTON

Amaranthus tricolor (Amaranthaceae) is distributed throughout Andhra Pradesh& Telangana regions .The synonyms of *Amaranthus tricol*or includes *A.mangostanus, A.gangeticus, A.orientalis, and A.trestis.* It is used for the treatment of External inflammation, Diuretic, Treatment of bladder distress, Astringent, Haemorrhage, Dysentery, Piles, Blood disorders, Toothache and Hepatoprotective. *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 & Hepatoprotective[1]. *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[2].

Table 1
Plant sources

Sl.no	Name	Biological source	Family	Common name
1	Amaranthus	*Amaranthus tricolor*	Amaranthaceae	Thotakura
2	Pimpenella	*Pimpenella tirupathansis*	Apiaceae	Adavi kothimera
3	Amorphophallus	*Amorphophallus paeoniiofolius*	Araceae	Kandagadda

Free radicals have been implicated to causation of ailments such as liver cirrhosis, atherosclerosis, and cancer, diabetes etc[3]. 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[4]. 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.

MATERIALS AND METHODS

Collection and authentication of plant

Amaranthus tricolor& Amorphophyllus Poeniophyllus were collected from Ankapoor village in Telangana state and *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.

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[5].

Total phenolic content

Total phenolic content was determined by Begum Method[6]. 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 f 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.

Determination of total antioxidant activity

The method described by Prieto[7], (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).

Assessment of anti-oxidant activity

The assessment of anti-oxidant activity was done through various in-vitro assays. The free radical scavenging activity of six extracts of *Amaranthus tricolor* 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.

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[8]. 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.

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

Reducing power assay

The reducing powers of neutraceutical herbs were determined according to Oyaizu[9] (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[9].

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.

RESULTS AND DISCUSSION

Phytochemical investigation

Preliminary Phytochemical screening of *Amaranthus tricolor, Pimpenella tirupathansis* and *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.

Total phenolic content

Phenolic compounds are a class of antioxidant agents, which act as free radical terminators[10]. Total phenols were measured by Folin Ciocalteu reagent in terms of Gallic acid equivalent. The total phenolic in WTR extracts of *Amaranthus tricolor, Pimpenella tirupathansis* and *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[11]. According to our study, the high contents of this Phytochemical in aqueous extract of *Amaranthus tricolor,Pimpenella tirupathansis* and *Amorphophyllus poeniophyllus* can explain its high radical scavenging activity.

Antioxidant potential

DPPH Radical scavenging activity

DPPH is a stable free radical at normal temperature. It shows specific absorbance at 517nm due to colour of methanolic solution of DPPH. Body also contains many free radicals, which assumed same as DPPH. 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* & *Amaranthus* (Table 2, 3, 4, 5 and Figure 1, 2, 3, 4).

Table 2

DPPH radical scavenging activity: Amaranthus tricolor

Sr.no	Concentration (ug/ml)	Pet.etr extract	Eth-extract	Wtr-extract	Vit-C	IC50
1	50	64.95±1.38	73.26±1.07	90.47±1.58	92.38±0.82	-
2	100	71.1±1.07	77.01±0.87	91.8±1.27	96.78±0.66	-
3	150	74.85±1.58	82.21±0.78	92.88±1.72	110.76±0.38	47
4	200	78.66±1.98	86.09±0.81	93.45±0.59	120.72±0.11	38
5	250	83.69±1.57	91.61±0.69	94.21±0.72	135.28±0.56	34

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

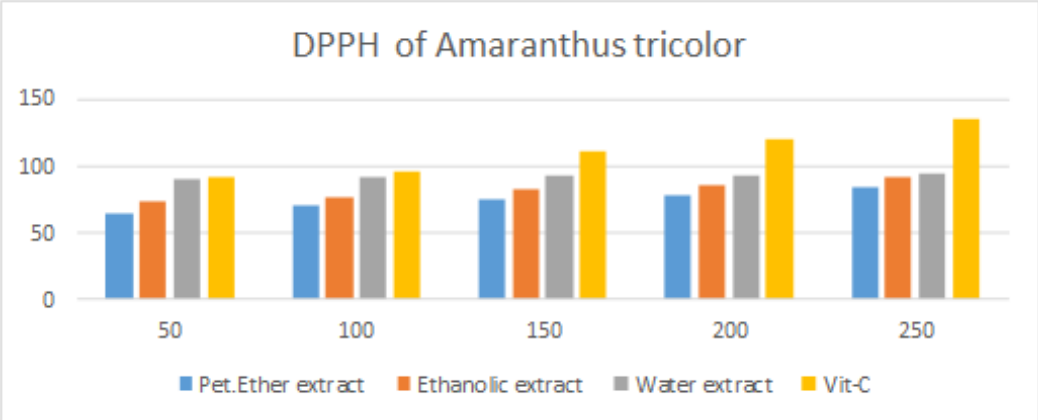

Figure 1

DPPH radical scavenging activity: Amaranthus tricolor

Table 3
DPPH radical scavenging activity: Pimpenella tirupathansis

Sr.no	Concentration (ug/ml)	PE extract	ETH-extract	WTR-extract	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

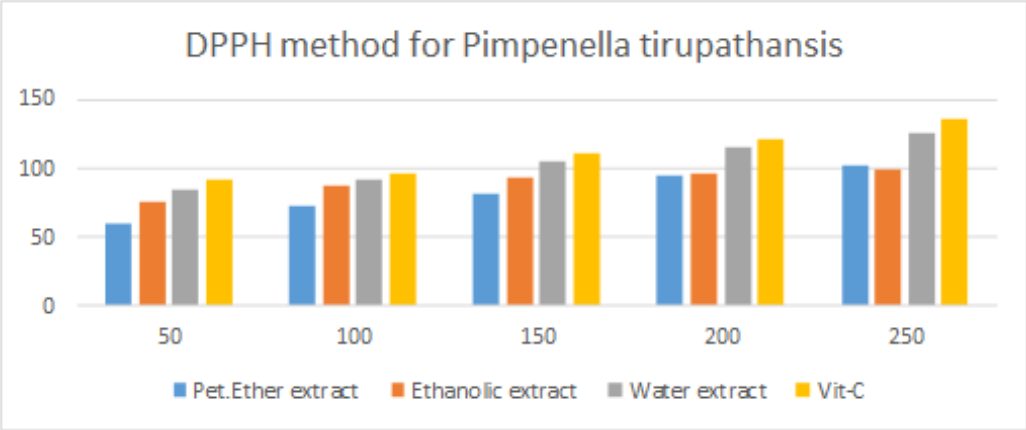

Figure 2
DPPH radical scavenging activity: Pimpenella tirupathansis

Table 4
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

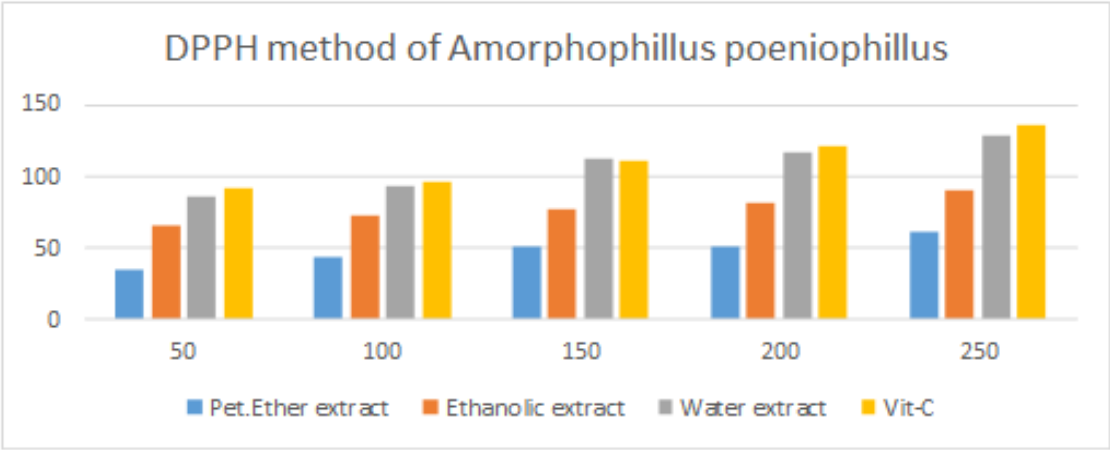

Figure 3
DPPH radical scavenging activity: Amorphophallus paeoniifolius

Table 5
Comparison of antioxidant activity of Amaranthus tricolor, Pimpenella tirupathansis, Amorphophyllus poeniophyllus by DPPH Method.

SR.NO	Concentration (ug/ml)	WTR-AT	WTR-PT	WTR AP	VIT-C
1	50	90.47±1.58	83.92±0.89	86.26±0.30	92.38±0.82
2	100	91.8±1.27	92.3±0.32	93.02±0.35	96.78±0.66
3	150	92.88±1.72	105.66±0.22	112.21±0.56	110.76±0.38
4	200	93.45±0.59	115.24±0.42	117.09±0.87	120.72±0.11
5	250	94.21±0.72	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

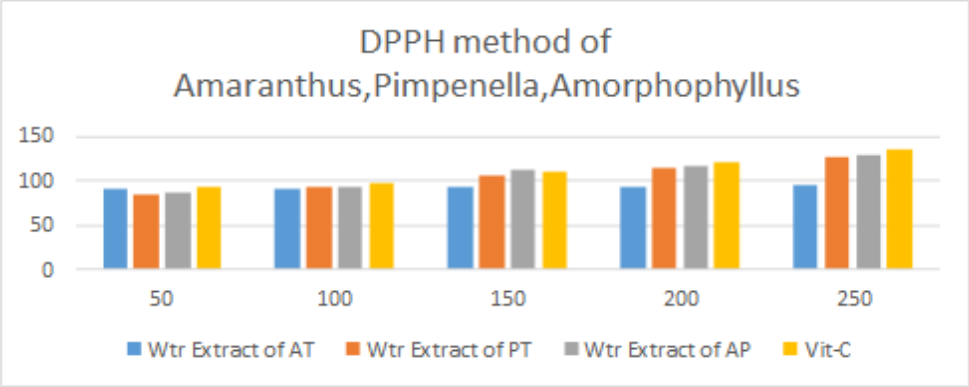

Figure 4
Comparison of antioxidant activity of Amaranthus tricolor, Pimpenella tirupathansis, Amorphophyllus poeniophyllus by DPPH Method.

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 of Amorphophyllus showed significant reducing activity when compared to that of other water extracts Pimpenella & Amaranthus tricolor. The comparative study helped to know the reducing power of all the extracts of the plant (Table 6, 7, 8, 9 and Figure 5, 6, 7, 8).

Table 6
Reducing power: Amaranthus tricolor

Sr.no	Concentration (ug/ml)	PET.ETR extract	ETH-extract	WTR-extract	VIT-C
1	50	0.165±0.003	0.032±0.0035	0.193±0.0056	1.092±0.012
2	100	0.063±0.0052	0.086±0.007	0.348±0.0084	1.208±0.0112
3	150	0.0945±0.0026	0.129±0.0032	0.522±0.008	1.319±0.004
4	200	0.126±0.005	0.172±0.0011	0.696±0.0047	1.439±0.0038
5	250	0.1575±0.0012	0.215±0.006	0.87±0.009	1.501±0.0074

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

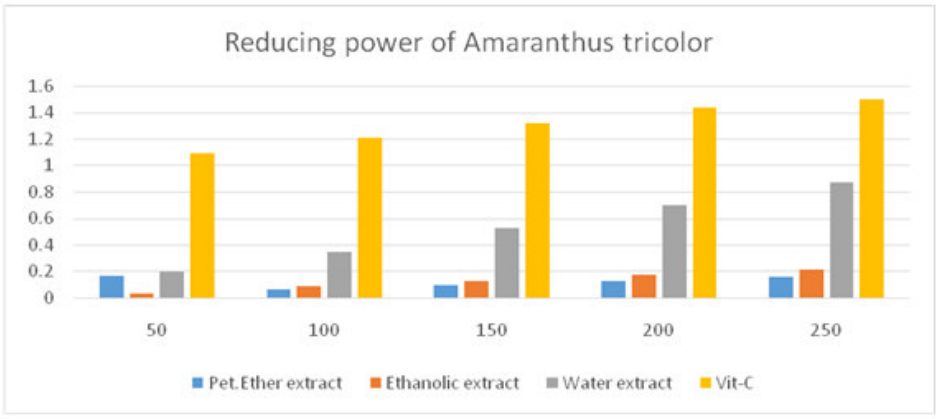

Figure 5
Reducing power: Amaranthus tricolor

Table 7
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

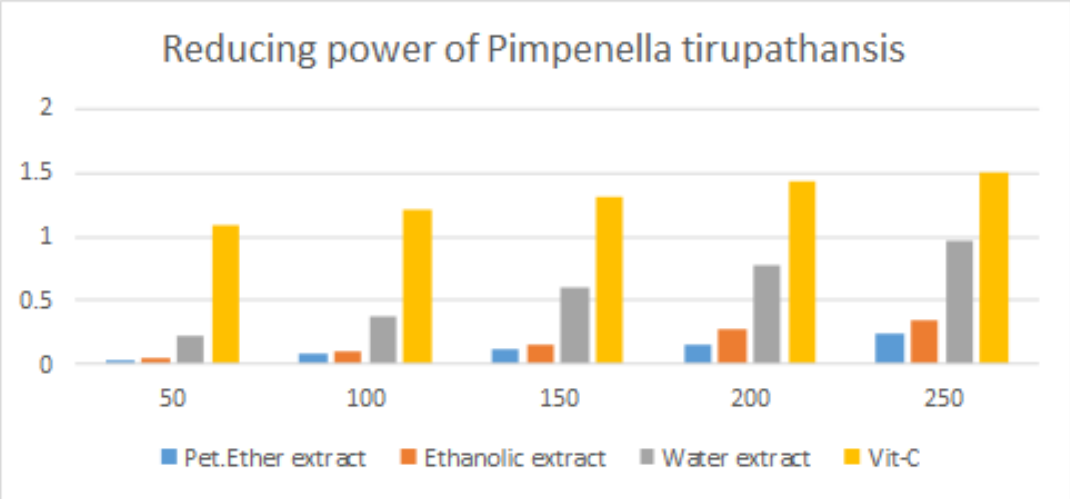

Figure 6
Reducing power: Pimpenella tirupathansis

Table 8
Reducing power: Amorphophallus paeoniifolius

Sr.no	Con (ug/ml)	PET.ETR extract	ETH-extract	WTR-extract	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

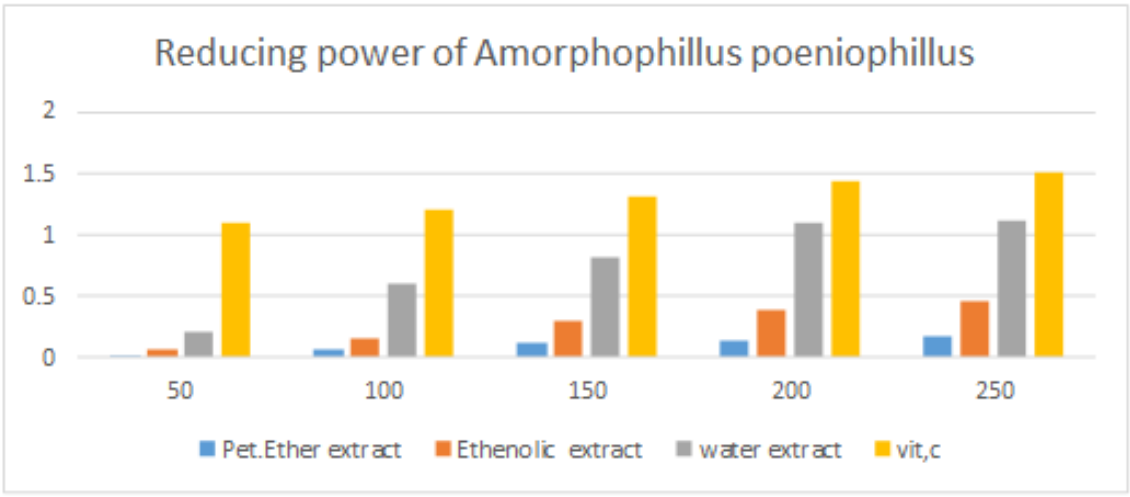

Figure 7
Reducing power: Amorphophallus paeoniifolius

Table 9
Comparison of reducing power for Amaranthus tricolor, Pimpenella tirupathansis, Amorphophyllus poeniophyllus.

Sr.no	Concentration (ug/ml)	WTR-AT	WTR-PT	WTR-AP	VIT-C
1	50	0.193±0.0056	0.223±0.002	0.2±0.003	1.092±0.012
2	100	0.348±0.0084	0.383±0.0029	0.6±0.006	1.208±0.0112
3	150	0.522±0.008	0.6±0.008	0.81±0.0015	1.319±0.004
4	200	0.696±0.0047	0.768±0.004	1.09±0.003	1.439±0.0038
5	250	0.87±0.009	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

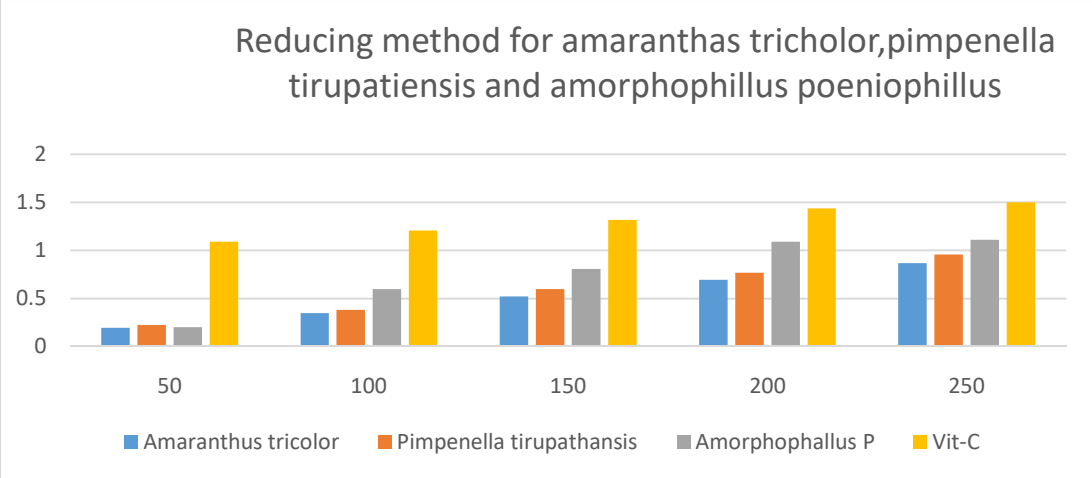

Figure 8
Comparison of reducing power for Amaranthus tricolor, Pimpenella tirupathansis, Amorphophyllus poeniophyllus.

Antimicrobial activity

The alcoholic extracts of Pimpenella tirupathansis showed significant activity against microorganisms when compared with alcoholic extracts of Amorphophallus which are followed by Pet-ether extracts of both the plant extracts. There was no antimicrobial activity for Amaranthus tricolor (Table 10).

Table 10
Anti-microbial studies of Amaranthus, 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
Amaranthus tricolor	PEE	R	R	R	R	R	R	R	R	R	R	R	R
	MEE	R	R	R	R	R	R	R	R	R	R	R	R
	Aqueous	R	R	R	R	R	R	R	R	R	R	R	R
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)

CONCLUSION

Thus, the present study strongly establishes the medicinal properties of all the three plants studied and scientifically validates folkloric use of this plant as a remedy for various infections. The results obtained from phytochemical screening, antioxidant and antimicrobial analysis of *Amaranthus tricolor, Pimpenella tirupathansis, Amorphophyllus paeoniifolius i*ndicate these plants as "natural herbal sources" which can be used in pharmaceutical industry.

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CONFLICTS OF INTEREST

Conflict of interest declared none.

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