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Phenolics and antioxidant potentials of some medicinally important arid zone plants

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Abstract
Petrolium ether, dichloromethane, ethyl acetate, methanol and aqueous extracts of six medicinally important arid zone plants of India, *viz. Gisekiapharn aceoides, Lepidagathis trinervis, Mollugo nudicaulis, Polycarpea corymbosa, Sericostoma pauciflorum* and *Trianthem adecandra* belonging to different families were investigated. Total levels of phenolics, flavonoids and antioxidant potentials were analyzed using Folin-Ciocalteau method, $AlCl_3$spectrophotometic method, DPPH and FRAP methods respectively. Among all the test extracts, total phenolics was found to be higher in ethyl acetate fractions of *S. pauciflorum* (123.26 ± 0.75 mg GAE/g extract), total flavonoids in ethyl acetate fraction of *M. nudicaulis* (30.05 ±1.04 mg QE/g extract), while the lowest IC_{50} (5.5 µg/ml with % inhibition of 96.22 and 96.17 µg/ml respectively) in ethyl acetate and methanol fractions of *L. trinervis.* However, in FRAP method the maximum absorbance was found to be in ethyl acetate extract of *L. trinervis*.

Keywords: Arid zone medicinal plants, phenolics, flavonoids, antioxidant potentials

1. Introduction
Metabolic processes in human body produce highly reactive free radicals which are capable of oxidizing biomolecules resulting in cell death. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process [1]. Antioxidants are the substances that significantly delay or prevent oxidation of biomolecules. Plants are the potent source of natural antioxidants which usually belong to polyphenol group.
Polyphenols are large group of biologically active compounds, *viz*. caffeic acid [2], chlorogonic acid [3] and several others. Flavonoids is a class of phenolic compounds widely distributed in plants having many medicinal functions, such as diuretic, laxative, antispasmodic, antihypertensive and anti-inflammatory action [4]. The antioxidant effect of phenolics and flavonoids has been of interest for the considerable time, as the oxidative damage is an explanation for the pathology of many diseases. Plants such as herbs have been used in folk medicine for centuries in most of the cultures throughout the world. Since long time *Gisekiapharna ceoides* L., *Lepidagathis trinervis* Nees., *Mollugo nudicaulis* Lam., *Polycarpea corymbosa* Lam., *Sericostoma pauciflorum* Stocks. ex Wight and *Trianthema decandra* Linn. were used as local medicines. *Gisekiapharn aceoides* is used in female diseases, defective semen, destroys fat and malfunctioning of sex organs. From *G. pharnaceoides*, tannin-like principles α- and β- gisekia and glycosides such as triacontane, myristone, tetracosanol, dotriacontane and alkaloids, resins, cardiac glycosides [5-8]; *L. trinervis* ashes used to cure eczema [9] and show the presence of immunosuppressive tryptophan-derived alkaloid [10].
M. nudicaulis used in whooping cough and boil suppuration. On chemical investigation mollugo flavonolo sides, eight saponins, cynogenic glycosides were isolated [11, 12]. Likewise, *P. corymbosa* used to cure venomous bites of reptiles, jaundice and inflammatory swellings [13, 14]. *P. corymbosa* some sterols such as α-1 barrigenol, camelliagenin A and stigmasterol have beenisolated [15]; *S. pauciflorum* used in dehydration and acidity [16]. On chemical investigation fernane, hopane and other type of triterpenoids have been isolated [17-19]. While *T. decandra* commonly used in hepatitis, asthama, suppression of the menses, inflammation of testicles and relieve one-sided headache by using the leaf juice [20, 21].
However, till date no study has been conducted on total levels of phenolics and flavonoids of these plants. *In vitro* antioxidant capacity of dichloromethane and methanol extract has been conducted. The present investigation levels of total phenolics, flavonoids and antioxidant activity of further sub fractioned extracts has been carried out.

2. Materials and Methods
2.1 Reagents
Chemicals and reagents were purchased from SIGMA ALDRICH Chemicals CO. and Merck.

2.2 Plant material
Each of the selected six plant materials was collected from the field locally during the months of July - October, 2020. The botanical identity was confirmed by Herbarium, Department of Botany, University of Rajasthan, Jaipur. Voucher specimens of the plants have been deposited at the Herbarium and Laboratory for further reference.

2.3 Extraction and biochemical estimation
The plant materials were air-dried, powdered and Soxhlet extracted in pet. ether, dichloromethane, ethyl acetate, methanol and water in succession (3×72h). Each of the resultant extract was filtered, concentrated and used for further studies.

For the estimation of total phenolics [22], 50 mg of the extract was dissolved in 1 ml of 80% ethanol, to which 1 ml of Folin-Ciocalteau reagent (1:2 ratio with distilled water) was added and placed at room temperature for 5 min. To this, 2 ml of 20% Na_2CO_3 was then added and the total volume made upto 25 ml using distilled water, shaken and kept on water bath at (50 ^{0}C, 1 min). Optical density (OD) was measured at 750 nm (Pharmaspec UV- Vis spectrophotometer by Shimadzu).A standard calibration curve of gallic acid (10 - 500 mg/l) was prepared and total phenolics in the extacts were expressed in mg of gallic acid equivalents (mg GAE /g of extract). All determinations were carried out in triplicate and stastically analyzed.

Total flavonoids were estimated by $AlCl_3$spectrophotometic method [23]. Total 20 mg of the extract dissolved in 1 ml of absolute alcohol, 4 ml of distilled water was added followed by 0.3 ml of 5% $NaNO_2$. After 5 min, 0.3 ml of 10% $AlCl_3$was mixed to the solution. On 6th min of incubation, 2 ml of 1M NaOH was added and the volume was raised to 10 ml with distilled water. Later, optical density was measured 510 nm. Similarly, a standard curve of quercetin (0-100 mg/l) was prepared using. The total flavonoids were expressed as mg of quercetin equivalents (QE/g) of extract and stastically analyzed.

2.4 Antioxidant Potentials using DPPH method
The scavenging activity was measured by the method of DPPH reduction assay [24]. 0.1 mM solution of 2, 2-diphenyl-1-picryl-hydrazil (DPPH) in methanol was prepared; 2.5 ml of this solution was added in 2.5 ml methanol at different concentration (10 – 80 µl). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. The optical density was measured at 517 nm and the capability to scavenge the DPPH radical was calculated using following equation:

$$\% \text{ Inhibition} = [(A_0-A_1/A_0) \times 100]$$

(where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of given extracts). All results were taken in triplicate and mean values were taken.

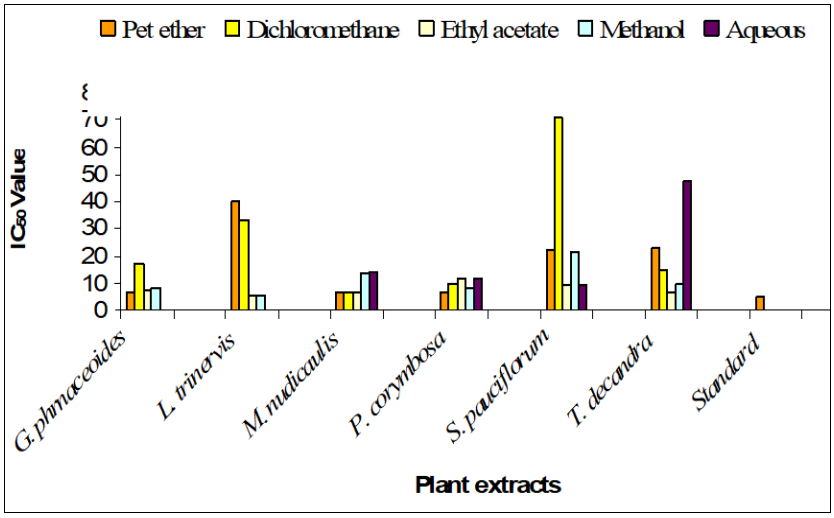

Fig 1: Histogram showing comparison of IC_{50} value between different tested extracts

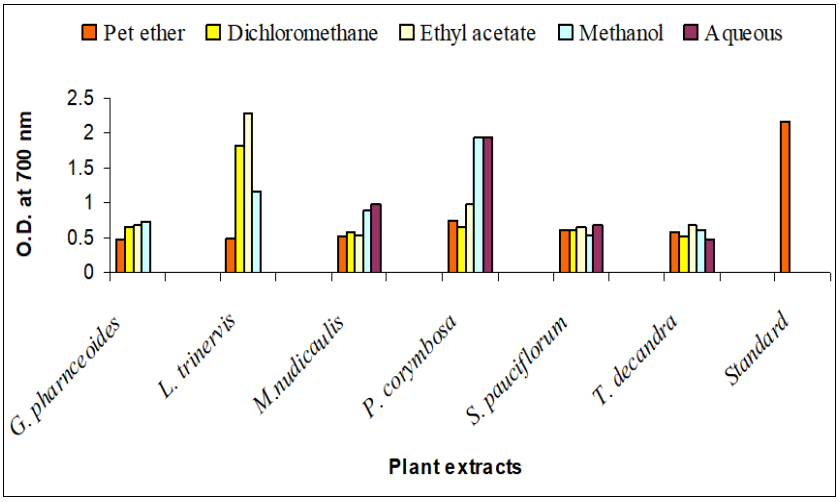

Fig 2: Histogram showing absorption on FRAP method.

Table 1: Plant species, family, local name, key constituents and biological activities of plants.

	Plant species & family	Local name	Traditional uses	Key constituents & biological activity
1.	*Gisekia pharnaceoides* L.; Aizoaceae	Sareli-morang	Female diseases, defective semen, destroys fat, malfunctioning of sex organs Plant extract for killing roundworms [17]	Tannins α- and β- gisekia are anthelmintic [6]; glycosides such as triacontane, myristone, tetracosanol, dotriacontane and acetyl glycosides [7]; alkaloids, resins, cardiac glycosides [8]
2.	*Lepidagathis trinervis* Nees.; Acanthaceae	Harancharo	Ashes of plant used to cure Eczema [28]	L. *trinervis* shows anticancer activity in leukemia and hypotensive effect [29]; biologically active triterpenoids saponin isolated from L. *hyaline* leaves [29]; immunosuppressive tryptophan-derived alkaloid from L. *cristata* [11]
3.	*Mollugo nudicaulis* Lam.; Ficoidaceae	Parppadagam	Pectoral used in whooping Cough, Leaves used in boil Suppuration [13]	Mollugo flavonolo sides, eight saponins, cynogenic glycosides isolated from *M. nudicaulis* [10, 12]; triterpene isolated from*M. Spergula* [30]; flavon glycosides (mollugogenol A, B and C) isolated from *M. pentaphylla* with splasmolytic and antifertility activity [31, 32].
4.	*Polycarpea corymbosa* Lam.; Caryophyllaceae	Machechi	As remedy for venomous bites from reptiles; jaundice and on inflammatory swellings [21]	Some sterol such as α-1 barrigenol, camelliagenin A and stigmasterol were isolated [15]
5.	*Sericostoma pauciflorum* Stocks. ex Wight; Boraginaceae	Karvas	Dehydration, acidity [13]	Fernane, hopane and other type of triterpenoids were isolated from the plant [17-19]
6.	*Trianthema decandra* Linn.; Ficoidaceae	Gadabani	Hepatitis, asthama, and suppression of the menses; inflammation of testicles. juice of leaves used to relieve headache [14]	Antioxidant activity of roots were evaluated in rats [21].

Table 2: Yield**,** total phenolics and flavonoids of selected plant extracts

S. No.	Plants species	Type of extract	Yield (%)	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
1.	*G. pharnaceoides*	Pet ether	1.41	63.93 ± 0.29	5.33 ± 0.21
		Dichloromethane	0.13	62.1 ± 0.2	7.41 ± 029
		Ethyl acetate	0.16	101.43 ± 0.53	10.41 ± 0.17
		Methanol	9.93	117.16 ± 0.44	10.06 ± 0.28
		Aqueous	-	-	-
2.	*L. trinervis*	Pet ether	2.05	75.33 ± 1.36	13.2 ± 1.06
		Dichloromethane	1.20	109.00 ± 1.22	27.52 ± 0.57
		Ethyl acetate	0.81	110.5 ± 1.07	29.04 ± 0.75
		Methanol	9.15	106.33 ± 0.62	26.6 ± 0.86
		Aqueous	-	-	-
3.	*M. nudicaulis*	Pet ether	1.98	43.33 ± 0.34	17.05 ± 1.75
		Dichloromethane	3.79	43.67 ± 0.84	21.75 ± 0.86
		Ethyl acetate	1.35	72.36 ± 0.25	30.05 ± 1.04
		Methanol	0.33	75.9 ± 0.26	10.00 ± 1.00
		Aqueous	1.81	74.33 ± 0.62	10.01 ± 0.56
4.	*P. corymbosa*	Pet ether	0.01	68.4 ± 0.37	10.14 ± 1.75
		Dichloromethane	0.46	66.26 ± 0.53	12.45 ± 0.86
		Ethyl acetate	0.15	82.3 ± 0.39	24.64 ± 1.08
		Methanol	2062	96.96 ± 0.26	17.25 ± 0.64
		Aqueous	0.20	89.96 ± 0.36	13.2 ± 0.54
5.	*S. pauciflorum*	Pet ether	1061	31.5 ± 1.67	12.30 ± 0.26
		Dichloromethane	0.37	57.4 ± 0.35	21.33 ± 0.21
		Ethyl acetate	0.02	123.26 ± 0.75	29.00 ± 0.21
		Methanol	1071	84.93 ± 0.15	8.41 ± 0.29
		Aqueous	1083	104 ± 0.44	15.25 ± 0.09
6.	*T. decandra*	Pet ether	0.60	79.2 ± 0.46	17.9 ± 0.30
		Dichloromethane	0.86	77.16 ± 0.43	20.5 ± 0.28
		Ethyl acetate	0.21	89.06 ± 0.36	24.41 ± 0.39
		Methanol	4.14	116.46 ± 0.44	7.00 ± 0.38
		Aqueous	2.05	81.06 ± 0.56	11.7 ± 0.52

[aa] Values expressed are mean ± standard deviation of three experiments

Table 3: Antioxidant activity of selected plant extracts

S. No.	Plants species	Type of extract	IC_{50}* (µg/ml)	% inhibition (µg/ml)				
				10	20	40	60	80
1.	*G. pharnaceoides*	Pet ether	7.0	76.12	78.95	80.42	81.87	87.65
		Dichloromethane	17.5	28.97	68.82	71.20	73.57	76.60
		Ethyl acetate	7.5	71.85	74.30	81.37	82.45	85.92
		Methanol	8.0	69.42	71.70	77.87	82.30	83.45
		Aqueous	-	-	-	-	-	-
2.	*L. trinervis*	Pet ether	40	27.37	30.35	34.72	38.45	48.77
		Dichloromethane	33.5	39.06	47.82	64.22	65.65	81.50
		Ethyl acetate	5.5	80.38	87.97	92.32	94.30	96.22
		Methanol	5.5	80.32	89.10	95.47	95.85	96.17
		Aqueous	-	-	-	-	-	-
3.	*M. nudicaulis*	Pet ether	6.5	76.20	79.30	81.55	82.92	87.50
		Dichloromethane	6.5	77.97	79.72	85.50	86.42	90.82
		Ethyl acetate	6.5	7775	79.48	81.25	83.67	89.07
		Methanol	13.5	45.92	53.00	54.07	56.35	67.10
		Aqueous	14	45.47	4.717	47.75	48.72	53.65
4.	*P. corymbosa*	Pet ether	6.5	80.40	81.70	85.35	88.37	92.95
		Dichloromethane	10.0	83.00	85.37	90.32	90.92	94.40
		Ethyl acetate	11.0	54.55	66.45	67.70	69.02	93.60
		Methanol	8.0	83.00	85.37	90.32	90.92	94.40
		Aqueous	11.5	55.00	63.47	74.87	81.61	94.07
5.	*S. pauciflorum*	Pet ether	22.0	32.05	34.42	36.30	39.30	45.80
		Dichloromethane	71.0	37.65	41.67	46.50	49.80	65.47
		Ethyl acetate	9.5	57.69	61.71	68.75	71.97	94.40
		Methanol	21.5	40.88	56.12	81.97	85.45	94.87
		Aqueous	9.5	42.50	60.20	77.17	81.75	94.52
6.	*T. decandra*	Pet ether	22.5	46.95	54.75	64.15	65.95	74.47
		Dichloromethane	14.5	40.57	41.92	44.12	46.72	54.05
		Ethyl acetate	7.0	77.05	78.32	80.30	81.75	88.80
		Methanol	10.0	57.07	65.82	71.20	78.45	90.56
		Aqueous	47.5	50.25	54.10	56.50	58.65	64.92

Values expressed are mean ± standard deviation of three experiments
*Concentration that causes 50% reduction in absorbance

2.5 Antioxidant Potentials using FRAP method

Ferrus ion Reducing Potentials (FRAP) was assayed on method described by Yen & Chen, 1995[25]. Different concentrations of extracts (62.5 - 1000 µg/l) were prepared in 1 ml of ethanol followed by 2.5 ml each of phosphate buffer (0.2 M, pH 6.6) and 1% $K_3Fe(CN)_6$. The mixture was incubated at 50ºC for 20 min and 2.5 ml trichloro acetic acid (10%) was added. Out of these, 2.5 ml of the mixture was taken out and mixed with 2.5 ml distilled water and 0.5 ml of $FeCl_3$ (0.1%) and the optical density was measured at 700 nm.

3. Results

Total levels of phenolics were found to be higher in ethyl acetate extract of *S. pauciflorum* (123.26 ± 0.75 mg GAE/g) followed by methanol extract of *G. pharnaceoides* (117.16 ± 0.44mg GAE/g). In case of total flavonoids, among all the plant ethyl acetate extractof *M. nudicaulis* showed higher levels (30.05 ± 1.04 mgQE/g) followed by dichloromethane extract of *L. trinervis* (27.52 ± 0.57 mg QE/g).

Among all the test fractions, lowest IC_{50} value was found in *L. trinervis* ethyl acetate fraction (5.5 µg/ml), with higher levels of phenolics (110.5 ± 1.07 mg GAE/g extract), total flavonoids (29.04 ± 0.75 mg QE/g) as well as maximum absorbance in FRAP histogram (Fig 2). % Inhibition of ethyl acetate and methanol fractions (80 µg/ml) was found to be 96.22 and 96.17 respectively (Table 3).

In *G. pharnaceoides*, pet. ether, ethyl acetate and methanol fractions showed IC_{50}value (7, 7.5, 8 µg/ml and % inhibition 87.64, 85.92and 83.45 respectively). In FRAP method, degree of absorbance was found higher in methanol > ethyl acetate > pet ether (Fig. 2), whereas, total phenolic levels of ethyl acetate and methanol extracts were 101.43 ± 0.53 and 117.16 ± 0.44 mg GAE/ g. Total flavonoids were also higher (10.41 ± 0.17 and 10.06 ± 0.28 mg QE / g) in ethyl acetate and methanol extracts respectively (Table 2).

In *M. nudicaulis*, pet. ether, DCM and ethyl acetate fraction demonstrated lowest IC_{50} value (6 µg/ml in all the cases) with higher % inhibition 87.50, 90.82 and 89.07 respectively (Table 2). Similarly, flavonoid contents were 17.05 ± 1.75, 21.75 ± 0.86 and 30.05 ± 1.04 mg QE/g extract respectively (Table 2), whereas in FRAP method, more total phenolics containing fractions such as methanol and water (75.9 ± 0.26 and 74.33 ± 0.62 mg GAE/g extract) showed maximum OD (Table 2; Fig. 3).

In *P. corymbosa*, lowest IC_{50} was of pet. ether fraction (6.5 µg/ml) followed by methanol (8 µg/ml) > ethyl acetate (10 µg/ml) > water (11.5µg/ml). In FRAP method, absorption values were found to be in order of water >methanol > ethyl acetate (Fig. 3). Total phenolics were highest in methanol extract (75.9 ± 0.26 mg GAE/g) > water (74.33 ± 0.62 mg GAE/g extract) > ethyl acetate (72.36 ± 0.25 mg GAE/g extract) and the total flavonoids values were 17.25 ± 0.64, 13.2 ± 0.54 and 24.64 ± 1.08mg QE/g extract respectively (Table 2).

In *S. pauciflorum*, lowest IC_{50} value was of ethyl acetate and water extract (9.5 µg/ml) and % inhibition was 94.40 and 94.52 respectively (Table 3). Likewise FRAP method also showed highest absorption in ethyl acetate and water fractions (Fig. 3). Their total phenolics were 123.26 ± 0.75 mg and 104 ± 0.44 mg GAE/g while total flavonoids were 29.00 ± 0.34 and 15.25 ± 0.09 mg QE/g extract respectively.

Similarly in *T. decandra*, ethyl acetate and methanol fractions

showed lowest IC_{50} value (7 and 10 µg/ml respectively). Maximum absorbance in FRAP method was demonstrated by ethyl acetate and methanol fraction (Fig. 3) and their phenolic contents had highest values 89.06 ± 0.0.36mg GAE/g extract and 116.46 ± 0.44 mg GAE/g respectively. Interestingly, total flavonoids of these two extracts were found to be 24.41 ± 0.39 and 7.00 ± 0.38 mg QE/g.

4. Discussion

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant molecules by hydrogen donation process decreased the absorption of DPPH radical. It is visually noticed by changing in the color purple to yellow. Further it is also established that lower the IC_{50} values, more will be the antioxidant capacity.

Likewise, the reducing capacity of a compound may also serve a significant indicator of its potential antioxidant activity, which prevents chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, hydrogen abstraction, reductive capacity and radical scavenging effects [26]. In FRAP method, higher absorbance of the reaction mixture indicated greater reducing power.

This evaluation supports the statement that polyphenolics and flavonoids compound(s) have an inhibitory effect on mutagenesis and carcinogenesis in human. It is known that polyphenols and flavonoids in particular, exhibit significant antioxidant activity and helpful in the treatment of carcinogenesis and other age related diseases [27].

L. trinervis shows anticancer activity against L_{1210} lymphoid leukemia in mice and hypotensive effect on experimental animal [29]; biologically active triterpenoidsaponin isolated from L. *hyaline* leaves (Yadava 2001); immunosuppressive tryptophan-derived alkaloid from *L. cristata* (Ravikanth *et al.* 2003). It is estimated about 80% of the world population still depends directly on the plant based remedies for their health care. Present paper will be helpful to investigate the potential aspect of these rare medicinal plant of the production of antioxidant compounds for therapeutic purposes.

5. Conclusion

Through the results of the research and their discussion several points were concluded, the most important of which are:

- The plant under investigation were used as folklore medicine since ancient times, but the antioxidant potentials were not identified.
- The studied medicinal plant belongs to different families so the chemical groups responsible for investigation may belongs to different categories. All of the investigated plant demonstrate antioxidant potentials but the methanolic extract possess maximum antioxidant, phenolic and flavonoid content.

6. Future suggestion

The results of this study shown the importance of traditional medicinal plants in the field of pharmaceutical industry. As these plants were reported endangered, new aspect of antioxidant potentials may help in the preservation of these plants.

6.1 Furthermore

a) Analysis of active ingredient, separation, isolation of each component will be conducted of *L. trinervis* which demonstrate highest antioxidant potentials.
b) Further work will be conducted for exploration of extract in other fields (antimicrobial and antiviral properties) also.
c) Importance of the work will help in preservation of the plant species.

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