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PRELIMINARY PHYTOCHEMICAL SCREENING OF POLYHERBAL FORMULATION (*ECHINOPS ECHINATUS, TABERNAEMONTANA DIVARICATA, AND PORTULACA QUADRIFIDA*)

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Abstract:
Need of the study: *Phytochemical screening is important because it helps to identify bioactive compounds in plants that can be used to develop drugs or dietary supplements for humans as well as for animals.*
Objectives: *Now a day's research on herbal medicinal plants is increasingly published in many journals because of their no side effects or minimum side effects. The valuable medicinal properties of different plants are due to presence of several constituents i.e. Saponins, tannins, alkaloids, phenols, glyco-alkaloids, flavonoids, sesquiterpenes lactones, terpenoides etc. Therefore, the main objective of this study is to identify the chemical constituents present in selected herbal plants. (i.e, Echinops echinatus, Tabernaemontana divaricata, and Portulaca quadrifida) by performing chemical tests.*
Methods: *Selected three traditionally used herbs from India (i.e, Echinops echinatus, Tabernaemontana divaricata, and Portulaca quadrifida) were collected from local field of Gulbarga (Karnataka) India. The methanolic extract was prepared as per literature, and various phytochemical screening was done.*
Results: *Secondary metabolites like Saponins, Phenolic compounds and Flavonoids are present abundantly in methanolic extract than in other extracts.*
Conclusion: *Our studies suggests that the selected polyherbal plants contains medicinally important secondary metabolites which has disease protective properties. This study will help into the development of some stable, biologically active compounds which can be employed in the formulation of many pharmacological activities.*
Keywords: *Polyherbal, Metabolites, Phytochemical screening.*

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INTRODUCTION:

Medicinal plants are gifts of nature used to cure number of human diseases. To promote the proper use and to determine their potential as sources for new drugs, it is essential to study the details of the medicinal plants[1].

From the ancient time, the belief has been that the plants contain some biologically active compounds with therapeutic properties useful for treatment of various ailments, including asthma, gastro-intestinal problems, skin disorders, respiratory and urinary complications, hepatic and cardiovascular disease etc. The medicinal value of these plants signifies a great potential for the discovery and development of new pharmaceuticals due to its chemical substances that produce a positive physiological action on the human body[2]. Different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds are the deposited areas of phytochemical and are often seen as pigmented molecules in the outer layer of plant tissue[3]. Medicinally important plants having its pharmacological benefits due to accumulation of bioactive phytochemicals in the plant tissue considered as primary and secondary metabolites. Primary metabolites as organic compounds that comprises of glucose, starch, polysaccharide, protein, lipids and nucleic acid which are helpful for growth and development of the human body. Plants produce secondary metabolites which include alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils etc[2,4].

The phytochemical screening of the plants with medicinal value is of great importance and has significance in pharmaceutical companies and research institutes as well for the production of the drugs for healing different ailments. The present investigation deals with the screening of phytoconstituents of the leaves of *Echinops echinatus*, *Tabernaemontana divaricata*, and *Portulaca quadrifida*.

Echinops echinatus which belongs to the family *Asteraceae* (*Compositae*) commonly known as "Usnakantaka" is a xerophytic herbaceous plant found in India, Pakistan and Sri Lanka. It is an erect, rigid, pubescent, annual herb about 1 m in height. It has short, stout stems with branches widely spreading from the base. The leaves are alternately arranged, sessile, oblong, deeply pinnatified, Flowering occurs between December and January[5]. the plant shows a wide range of pharmacological activities such as antifungal, analgesic, diuretic, reproductive, hepatoprotective, antioxidant, anti-inflammatory.

Tabernaemontana divaricata belongs to the Apocynaceae family. The generic synonym of *Tabernaemontana divaricata* is *Ervatamia coronaria* and widely distributed in tropical countries as a garden plant. *Tabernaemontana divaricata* is a shrub or small tree, usually glabrous, found in the Konkan, North Kanara, Western Ghats in Malabar, throughout North India[6,7,8]. In traditional medicine, *Tabernaemontana divaricata* is used to treat various diseases such as diarrhea, abdominal tumors, arthralgia, asthma, epilepsy, eye infections, fever, fractures, headache, inflammation, leprosy, mania, edema, paralysis, piles, rabies, rheumatic pain, skin diseases, urinary disorders, strangury, toothache[9].

Portulaca quadrifida which belongs to the family *Portulacaceae* is the small diffused herb and commonly grown at river banks. It is available in both wild and cultivated plants. It is also used as Vegetable. The plant is sour, bitter, hot, alterative, laxative; causes biliousness and "Kapha;" cures low grade fever, asthma, cough, urinary discharges, inflammation; good for eye diseases, skin diseases and ulcers(Ayurveda) and also cures jaundice, cardiovascular diseases and gonorrhea. *Portulaca quadrifida* has been reported to possess Centrifugal activity against *Asphergillus Fumigates* and *Candida albicans*[10]

Table 1: Selected medicinal plants for their phytochemical activity

S. No	Botanical Name	Local Name (Kannada)	Family	Parts used for analysis
1.	***Echinops echinatus***	*Brahmadandi*	*Asteraceae*	Leaves
2.	***Tabernaemontana divaricata***	*Nandi Battalu*	Apocynaceae	Leaves
3.	***Portulaca quadrifida***	*Goni soppu*	*Portulacaceae*	Leaves

MATERIALS AND METHODS:

Collection of Plant Materials:

Fresh leaves of *Echinops echinatus*, *Tabernaemontana divaricata*, and *Portulaca quadrifida* were collected from the local field of Gulbarga and Hyderabad and authenticated by P. V. Prasanna, Scientist In-charge, Botanical Survey of India, Deccan Regional Centre, Plot No.366/1, Pillar No. 162, Attapur (V), Hyderguda (P.O), Hyderabad – 500048. Telangana State. India. (A voucher specimen no. BSI/DRC/22-23/Tech./1007).

The fresh leaves were washed under running tap water to remove dust and then air dried. The dried leaves were separately powdered in a grinder. The powdered material was weighed approximately 500g.

Extraction Procedure[11]

Successive Solvent Extraction: The selected crude drugs were subjected to systematic phytochemical screening by successively extracting with various organic solvents of increasing polarity and subjected to phytochemical investigation.

Figure 01: Soxhlet apparatus

Preparation of Plant Extracts:

Echinops echinatus, Tabernaemontana divaricata, and Portulaca quadrifida was collected and shade dried, powdered in a mixer and sieved, powder of required particle size was obtained (sieve no 40).

The 500gm of powder material was extracted in soxhlet using successive solvents of increasing polarity such as Petroleum ether, chloroform, ethanol and aqueous for 48 hours. The filtrates were collected and evaporated to dryness under reduced pressure using evaporator (Rotary flash). The extracts obtained was weighed from each solvent; its percentage was calculated in terms of air-dried weight of plants materials. The dried extracts were preserved at 4˚C in small sterilized containers, for further use.

1. Preparation of Petroleum ether extract:

The powder was packed in soxhlet apparatus and extracted with petroleum ether (60 – 80 ° C) for 48 hours. The extraction was transferred into the previously weight empty china dish and evaporated to a thick paste on water bath, maintained at 50°C to get the petroleum extract. The marc was air dried thoroughly to remove the solvent used previously before it was taken for further extraction with next solvent.

2. Preparation of Chloroform extract:

The air dried powder from the above process was extracted successively with Chloroform to get Chloroform extract. The marc was collected, dried and used for preparing further extraction with ethanol. The percentage yield was calculated and tabulated (Table 02).

3. Preparation of ethanol extract:

The dried marc from the above process was extracted successively with ethanol to get ethanolic extract. The marc was collected, dried and used for further investigation. The percentage yield was calculated and tabulated (Table 2).

4. Preparation of Aqueous extract:

500 gm of powder were taken in a beaker (1000ml) and macerated with 800ml of distilled water and 10 ml of Chloroform (preservative) for 7 days shaking daily in a closed vessel. After this extraction, the marc was separated and subjected to concentrate at 50^0C on a water bath and a semi solid mass was obtained. The percentage yield was calculated and tabulated (Table 02). (These four extracts were stored in air tight container and refrigerator. All the extracts were examined for their colour and consistency)

Table 02: : Percentage yield of successive extract of *Echinops echinatus, Tabernaemontana divaricata, and Portulaca quadrifida.*

S.No.	EXTRACTS		NATUREOF EXTRACTS	COLOUR	WEIGHT (GM)	PERCENTAGE YIELD
1	EE	Pet. Ether	All Extracts are in the state of semisolid	Dark Green	95.2	9.52
	TD			Dark Green	100.8	10.08
	PQ			Dark Green	110.5	11.05
2	EE	Chloroform		Dark Green	110.79	11.07
	TD			Dark Green	105.21	10.52
	PQ			Dark Green	112.10	11.21
3	EE	Ethanol		Dark Brown	280.19	28.01
	TD			Dark Brown	270.68	27.06
	PQ			Dark Brown	296	29.6
4	EE	Aqueous		Dark Brown	210.90	21.09
	TD			Dark Brown	205	20.5
	PQ			Dark Brown	218.30	21.83

Whereas,
EE = Echinops echinatus, TD = Tabernaemontana divaricata, and PQ = Portulaca quadrifida

Preliminary phytochemical screening: The extracts thus obtained were subjected to preliminary phytochemical screening following the standard protocols [12-18]

Test for carbohydrates
To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for tannins
To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins
To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for flavonoids
To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for alkaloids
To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones
To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for glycosides
To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for cardiac glycosides
To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

Test for terpenoids
To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

Test for triterpenoids

To 1.5ml of extract, 1ml of Libemann–Buchard Reagent (acetic anhydride + concentrated sulphuric acid) was added Formation of blue green color indicates presence of triterpenoids.

Test for phenols
To 1ml of the extract, 2ml of distilled water followed

by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

Test for coumarin

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

Steroids and Phytosterols

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of Phytosterols.

Phlobatannins

To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

Anthraquinones:

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones

RESULTS:

In the present investigation, phytochemical screenings were performed in three different plants with four different solvents and comparison was made with each other.

(+ indicates presence, - indicates absence of phytochemical constituents)

Table 03: Preliminary Phytochemical analysis of successive extract of *Echinops echinatus, Tabernaemontana divaricata, and Portulaca quadrifida.*

S.No.	Phytochemical Constituents	Extract of Pet. ether			Extract of Chloroform			Extract of Methanol			Extract of Aqueous		
		EE	TD	PQ	EE	TD	PQ	EE	TD	PQ	EE	TD	PQ
01	Alkaloids	_	_	_	_	_	_	+	+	+	_	+	+
02	Flavonoids	_	_	_	_	_	_	++	++	++	++	++	++
03	Saponins	_	_	_	_	_	_	+	+	+	++	++	++
04	Tannins	_	_	_	_	_	_	+	+	+	+	+	+
05	Phenols	_	_	_	_	_	_	+	+	+	++	++	++
06	Steroids	_	_	_	_	_	_	+	+	+	+	+	+
07	Glycosides	_	_	_	_	_	_	+	+	+	+	+	+
08	Carbohydrates	_	_	_	_	_	_	_	_	_	++	++	++
09	Amino Acids	_	_	_	_	_	_	+	+	+	+	+	+

Whereas,

+	=	Indicates presence of phytochemical
_	=	Indicates absence of phytochemicals
++	=	Shows high concentration
EE	=	*Echinops echinatus*
TD	=	*Tabernaemontana divaricata*
PD	=	*Portulaca quadrifida*

DISCUSSION AND SUMMARY:

Preliminary phytochemical analysis of successive extract of selected poly herbals namely *Echinops echinatus, Tabernaemontana divaricata, and Portulaca quadrifida* was conducted in which the results were as follows

Alkaloids were showing absent in petroleum ether and chloroform extract where as it was present in ethanolic extract and aqueous extract.

- Flavanoids were showing absent in petroleum ether and chloroform extract where as it was present in ethanolic extract and aqueous extract.
- Saponins were absent in petroleum ether and chloroform extract where as it was present in ethanolic extract and aqueous extract.
- Phenolic were showing absent in petroleum ether and chloroform extract where as it was present in ethanolic extract and aqueous extract.
- Glycosides were showing absent in petroleum ether, chloroform extract and aqueous extract where as it was present in ethanolic extract.
- Carbohydrates were showing absent in petroleum ether, chloroform extract and ethanolic extract where as it was present in aqueous extract.
- Amino acids were showing absent in petroleum ether, chloroform extract and aqueous extract where as it was present in ethanolic extract

REFERENCES:

1. Paresh J, Chand S. Antibacterial activity of the crude methanol extract of Flower (Lythraceae). Brazilion Journal of microbiology, 2007; 38: 204-207.
2. Phytochemical and Pharmacological Properties of Medicinal Plants from Uzbekistan: A Review. Dilfuza Egamberdieva. Journal of Medicinally Active Plants. Volume 5 Issue 2 Vol 5 Issues 1-4.
3. Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry. Mamta Saxena. Volume 1 Issue 6.
4. Medicinal plants: Future source of new drugs. Arvind Kumar Shakya. International Journal of Herbal Medicine 2016; 4(4): 59- 64.
5. Maurya, *et al*. Echinops echinatus Roxb. plant review. Pharmacognosy Reviews. 2015; 9 (18): 149 - 154.
6. Ridge JA, Glisson BS, Lango MN, Feigenverg S. Head and Neck tumors. In: Pazdur R, Bagman LD, Camphausen KA, Hoskins WJ, editors. *Cancer Management.* 12th ed. New York: Multideciplinary approach Medical, Surgical and Radiation and Oncology, The Oncology Group. 2010. [Google Scholar].
7. Nadkarni KM. I. Bombay: Popular Book Depot; 1954. Indian Materia Medica. 516–8. [Google Scholar].
8. Sharma P, Mehta PM. Varansi: The Chowkhamba Vidyabhawan. Dravyaguna Vignyan. Part II and III. 1969: 586. [Google Scholar].
9. Henriques AT, Melo AA, Moreno PR, Ene LL, Henriques JA, Schapoval EE. *Ervatamia coronaria*: Chemical constituents and some pharmacological activities. *J Ethnopharmacol*. 1996; 50: 19–25. [PubMed] [Google Scholar].
10. Hoffman BR, Alas D, Blanco K, Wiederhold N, Lewi, RE and Williams L, Screening of antibacterial and antifungal activities of ten Pharmaceutical Biology, 2004; 42 (1): 13-17.
11. B. S. Nayak *et al*. Influence of Helium-Neon Laser Photo stimulation on Excision Wound Healing in Wistar Rats. On Line Journal of Biological Sciences 7 (2): 89-92, 2007.
12. Carbohydrates: Sofowora A medicinal plant and Traditional Medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd; Screening Plants for Bioactive Agents. 1993, 134-156.
13. Tannin: Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall, New York, 1973, 279. 3rd Edition.
14. Saponins: Smolenski SJ, Silinis H, Farnswoth NR. Alkaloids screening. V. Lloydia, 37: 506-536. Kapoor LD, Singh A, Kapoor SL and Shrivastava SN (1969). Survey of Indian medicinal plants for saponins, alkaloids and flavonoids. Lloydia, 1974; 32:297-302.
15. Sonali Jana, Shekhawat GS. Flavonoids, Cardiac glycosides, Terpenoids, Phlobatannins, Anthraquinones: Phytochemical analysis and antibacterial screening of *in vivo* and *in vitro* extracts of Indian medicinal herbs: *Anethum graveolens*, Research Journal of medicinal plants. 2010; 4(4):206-212. ISSN 1819-3455.
16. Alkaloids GA, Ayoola HAB, Coker SA, Adesegun AA, Adepoju-Bello K, Obaweya EC. Ezennia, Atangbayila, Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in South western Nigeria, Tropical Journal of Pharmaceutical Research, 2008; 7(3):1019-1024.
17. Sureshkumar CA, Varadharajan R, Muthumani P, Meera R, Devi P, Kameswari B. Quinones, Phenols, Coumarins:

Pharmacognostic and Preliminary Phytochemical Investigations on the stem of *Saccharum spontaneum*. J Pharm Sci & Res. 2009; 1(3):129-136.

18. Manasboxi, Rajesh Y, Rajakumar V, Praveen B, Mangamma K. Glycosides, Proteins: Extraction, phytochemical screening and *in-vitro* evaluation of anti- oxidant properties of *Commicarpus chinensis* (aqueous leaf extract), International Journal of Pharma and Bio Sciences. 2010; 1(4):. ISSN 0975-6299