

In-vitro Studies on Phytochemical Screening of selected plant species of Vignan's University campus, Vadlamudi, Andhra Pradesh

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Abstract

The present work will focus on the importance of some of the selected plant species *Mimosops elangi*, *Araucaria heterophylla*, *Callistemon lanceolatus*, *Capparis brevispina*, *Cassytha filiformis*, *Solidago chinensis* and preliminary phytochemical analysis. Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. In the present study, chief phytoconstituents of the six selected medicinal plants of different families were identified in order to relate their presence with bioactivities of the plants [1,2]. Adequate amount of extracts of plants were prepared by successive extraction and prepared with organic solvents such as distilled water, acetone, ethanol, methanol, n-hexane and petroleum ether. All plant extracts were subjected against the selected plant samples for their phytochemical screening. Out of these six plant samples (aqueous/methanol), 13 various phytochemical components were analyzed. All the data is recorded in this paper. The results are mostly in conformity with the medicinal uses and they are discussed.

Key words: *In-vitro* studies, phytochemical screening, plant species, Vignan's University

INTRODUCTION:

Vignan's University (VU) (formerly Vignan's Engineering College is a premier institution affiliated to Jawaharlal Nehru Technological University in Andhra Pradesh). It is having the splendid avenue, imposing buildings and sprawling playgrounds, and the verdure in and around the campus. The college is a virtual haven of rural quiet and idyllic beauty. Since its inception in 1997, VU has been striving to promote high quality standards in technical education & research for the aspirants of Engineering Studies [4].

The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [2]. Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well [1]. Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Numerous research groups have also reported such studies throughout the world [4,8]. Thus, the present study deals with the screening based on phytochemical tests of six medicinal plants viz., species *Mimosops elangi*, *Araucaria heterophylla*, *Callistemon lanceolatus*, *Capparis brevispina*, *Cassytha filiformis*, *Solidago chinensis* for identifying their chemical constituents. All these plants possess different bioactivities which were later correlated with the presence of some specific phytoconstituents[1,5].

TOPOGRAPHY:

Vignan's University is located in the serene environs of Vadlamudi on the Guntur -Tenali highway, about 14 km

from Guntur and 11 km from Tenali. The nearest railway station Tenali is located on Chennai-Kolkata trunk line[4].

Brief enumeration and description of habit & habitat of selected Medicinal plant species for their phytochemical screening:



Scientific Name: *Mimosops elengi* L. (SAPOTACEAE)
Ln.: Madhupushpa, Bullet wood, Indian Medlar, Spanish cherry

Mimosops elengi L.

Distribution & uses

A medium-sized evergreen tree found in tropical forests in South Asia, Southeast Asia and northern Australia. The bark, flowers, fruits, and seeds of *Bakula* are used in Ayurvedic medicine in which it is purported to be astringent, cooling, anthelmintic, tonic, and febrifuge. It is mainly used for dental ailments such as bleeding gums, pyorrhea, dental caries, and loose teeth[1,4].



Scientific Name: *Araucaria heterophylla* (Salisb.)Franco
(ARAUCARIACEAE)

Ln.: Monkey's puzzle, Norfolk pine

Araucaria heterophylla (Salisb.)Franco

Distribution & uses

It is sometimes called a **star pine, triangle tree** or **living Christmas tree**, due to its symmetrical shape as a sapling. It is endemic to Norfolk Island, a small island in the Pacific Ocean between New Zealand and New Caledonia. Resin isolated from stem exudates showed anti-ulcerogenic activity, resin and isolated compounds showed variable cytotoxic activities against breast (MCF7) and colon (HCT116) cancer cell lines[20,22]. Its leaves has the potential for a cheap and efficient biosorbent for toxic hexavalent chromium removal from natural and wastewaters[4,8].



Scientific Name: *Capparis brevispina* DC., Prodr.
(CAPPARACEAE)

Ln.: Wild Caper Bush, Aridonda

Capparis brevispina DC. Prodr.

Distribution & uses

These plants are shrubs or lianas and are collectively known as caper shrubs or caperbushes. *Capparis* species occur over a wide range of habitat in the subtropical and tropical zones. Indian Caper is a shrub armed with thorny stipules. plant used as stomachic, tonic, appetizer; removes kapha and vata, cures fever, tumours, inflammation, wound healing and antimicrobial, analgesic, anthelmintic and hepatoprotective activities[9,10,23,26].



Scientific Name: *Callistemon lanceolatus* R.Br.
(MYRTACEAE)

Ln.: Lemon Bottle brush

Callistemon lanceolatus R.Br.

Distribution & uses

Tree. The entire genus is endemic to Australia but widely cultivated in many other regions and naturalized in scattered locations. *Callistemon* species have commonly been referred to as bottlebrushes because of their cylindrical, brush like flowers resembling a traditional bottle brush[4,14]. They are mostly found in the more temperate regions of Australia, especially along the east coast and typically favour moist conditions so when planted in gardens thrive on regular watering[3,5]. Some species are drought-resistant and some are used in ornamental landscaping elsewhere in the world.



Scientific Name: *Cassytha filiformis* L. (LAURACEAE)

Ln.: Dodder laurel, Love vine

Cassytha filiformis L.

Distribution & uses

Cassytha filiformis, common name love-vine, is a species of obligate parasitic vine. The species has a pantropical distribution encompassing the Americas, Indo-Malaya, Australasia, Polynesia and East Africa In the Caribbean region, it is one of several plants known as Love Vine because it has a reputation as an aphrodisiac. Plant as tonic, alterative, in bilious affections, chronic dysentery[20,29]. It was also reported as a beneficial medicine against the gonorrhoea, kidney ailments and as the diuretic[8,14].



Scientific Name: *Wedelia chinensis* (Osbeck.) Merr.
(COMPOSITAE)

= *Solidago chinensis* Osbeck

Ln.: Chinese Wedelia, Birimagari

Wedelia chinensis (Osbeck.) Merr.

Distribution & uses:

It is scabrous procumbent perennial soft herb with high camphor like odour. Is procumbent, perennial herb found in wet places in Uttar Pradesh, Assam, Andhra Pradesh and along the coastal areas. It is found in plains district of Madras presidency, China and Japan. The species is widely used in traditional medicine, and investigated for use in contemporary medicine[1,15]. Generally whole plant is used for treating disease. Its various parts have been used as folklore medicine for treating various ailments like hepatoprotective efficacy, cholagogue, jaundice, diarrhoea, cough, cephalahagia, diphtheria and pertussis etc[4,8,18].

MATERIALS AND METHODS

Vignan's University has campus with a good number of plants. It includes landscaping gardens, exotic elements and natural forest elements, includes rare and endemic categories of trees, shrubs, herbaceous members, climbers and a good number of medicinal plant species like *Mimosops elangi*, *Araucaria heterophylla*, *Callistemon lanceolatus*, *Capparis brevispina*, *Cassytha filiformis*, *Solidago chinensis*. An inventory experimental study were conducted on selected most promising plant species which are having utilization of domestic, commercial samples.

The present work was conducted in Department of Biotechnology, Biochemistry laboratory of Vignan's University, Vadlamudi to determine the Phytochemical composition of the above mentioned plant samples by the presence of tannins, flavonoids, terpenoids, saponins, steroids, phlobatannins, carbohydrates, glycosides, coumarins, alkaloids, proteins, emodins, anthraquinones, anthocyanins and leucoanthocyanins was done by the standard procedures prescribed by Bhattacharya (1956), Chhabra et.al (1984) and Harborne (1973, 1977)[6,7,12,13].

Collection and preservation of samples:

The leaves and bark was collected from the plants, washed under the tap water to remove the dust. Washed samples are cut into very small pieces which are shade dried for 10-15 days at room temperature to eliminate surface moisture. Dried leaves were grinded in an electric grinder to obtain powder which was then kept in plastic/paper bags for further use. Extracts of sample are prepared by taking of 20 gr. of sample in 200 ml. methanol. Each sample is tested for

13 components Results are given in Table-1.

TABLE:1
RESULTS OF PHYTOCHEMICAL SCREENING OF SOME SELECTED MEDICINAL PLANTS:

S.No.	Phytochemical constituents	Plant samples					
		<i>Mimosops elangi</i>	<i>Araucaria heterophylla</i>	<i>Callistemon lanceolatus</i>	<i>Capparis brevispina</i>	<i>Cassytha filiformis</i>	<i>Wedelia chinensis</i>
1	<i>Tannins</i>	+	+	+	+	+	+
2	<i>Flavonoids</i>	+	+	+	+	+	+
3	<i>Terpenoids</i>	+	+	-	+	+	+
4	<i>Saponins</i>	-	-	-	-	+	-
5	<i>Phlobatannins</i>	-	-	-	-	-	+
6	<i>Steroids</i>	-	-	-	-	+	-
7	<i>Carbohydrates</i>	+	+	-	+	+	-
8	<i>Glycosides</i>	+	+	-	+	+	-
9	<i>Alkaloids</i>	-	+	-	+	+	-
10	<i>Protiens</i>	+	-	-	-	+	-
11	<i>Anthraquinones</i>	-	-	-	-	-	-
12	<i>Anthocyanins</i>	-	-	-	-	-	-
13	<i>Leuco-Anthocyanins</i>	-	-	-	-	-	-

Preparation of Plant samples for Phytochemical tests

Screening of the mentioned six selected medicinal plants for various phytochemical constituents were carried out using standard methods [9-11] as described here in the text.

1. Tannins (*Braymer's Test*)
2ml extract + 2ml H₂O + 2-3 drops FeCl₃ (5%)
Green precipitate
2. Flavonoids
1ml extract + 1ml Pb(OAc)₄ (10%)
Yellow coloration
3. Terpenoids
2ml extract + 2ml (CH₃CO)₂O + 2-3 drops conc.H₂SO₄
Deep red coloration
4. Saponins (*Foam Test*)
(a) 5ml extract + 5ml H₂O + heat Froth appears
(b) 5ml extract + Olive oil (few drops) Emulsion forms
5. Steroids (*Salkowski Test*)
2ml extract + 2ml CHCl₃ + 2ml H₂SO₄ (conc.)
Reddish brown ring at the junction
6. Phlobatannins (*Precipitate Test*)
2ml extract + 2ml HCl (1%) + heat
Red precipitate
7. Carbohydrates (*Molisch's Test*)
2ml extract + 10ml H₂O + 2 drops Ethanolic α -naphthol (20%) + 2ml H₂SO₄ (conc.)
Reddish violet ring at the junction
8. Glycosides (*Liebermann's Test*)
2ml extract + 2ml CHCl₃ + 2ml CH₃COOH Violet to Blue to Green coloration
Coumarins 2ml extract + 3ml NaOH (10%) Yellow coloration
9. Alkaloids (*Hager's Test*)
2ml extract + few drops of Hager's reagent
Yellow precipitate
10. Proteins (*Xanthoproteic Test*)
1ml extract + 1ml H₂SO₄(conc.) White precipitate
Emodins 2ml extract + 2ml NH₄OH + 3ml Benzene
Red coloration
11. Anthraquinones (*Borntrager's Test*)
3ml extract + 3ml Benzene + 5ml NH₃(10%)
Pink, Violet or Red coloration in ammonical layer
12. Anthocyanins
2ml extract + 2ml HCl (2N) + NH₃
Pinkish red to bluish violet coloration
13. Leucoanthocyanins
Turns 5ml extract + 5ml Isoamyl alcohol
Organic layer into Red

RESULTS & DISCUSSION

The data shown in the table-1, reveals the different plant species screening of aqueous/methanol extracts of different parts of seven medicinal plants viz., *Mimusops elangi*, *Araucaria heterophylla*, *Callistemon lanceolatus*, *Capparis brevispina*, *Cassipoula filiformis*, *Solidago chinensis* based on phytochemical tests. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes[1,8,24]. The observations and inferences made in the phytochemical tests are presented as follows:

Results of different tests performed on the six medicinal plants:

Tannins: A green precipitate was observed in all the extracts indicating thereby the presence of tannins in all six medicinal plants analysed.

Flavonoids: A yellow coloration was also observed in all the extracts indicating thereby the presence of flavonoids in all six medicinal plants screened

Terpenoids: A deep red color was observed in five extracts out of six except *Callistemon lanceolatus*

Saponins: Persistent frothing on warming the extract of *Cassipoula filliformis* indicated the presence of saponins in this plant only. The same extract with few drops of olive oil formed a soluble emulsion, confirming the presence of saponins.

Steroids: A reddish brown ring at the interface was observed only with the extract of *cassipoula filliformis* out of six screened plants indicating the presence of steroids only in this plant

Phlobatannins: Presence of a red precipitate in *Solidago chinensis* root juice only was taken as an evidence for the presence of phlobatannins in this.

Carbohydrates: Red violet ring appeared at the junction in most of the extracts was confirmed by the presence of carbohydrates except *Araucaria heterophylla* and *Solidago chinensis*.

Glycosides: Similarly, a color change from violet to blue to green confirming the presence of glycosides was also observed in all other extracts except *Araucaria heterophylla* and *Solidago chinensis*

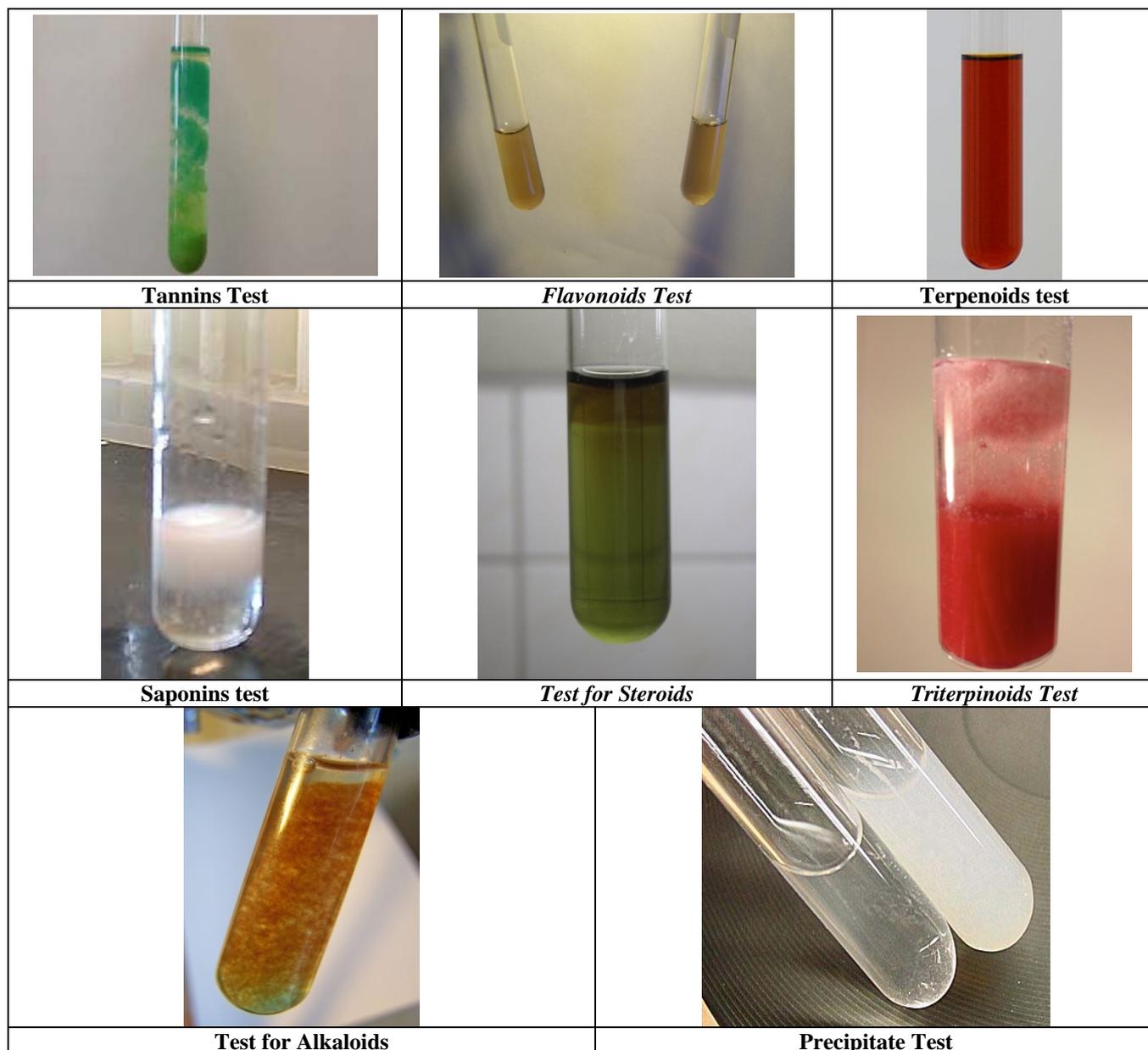
Alkaloids: A yellow precipitate was observed in three extracts confirming thereby the presence of alkaloids. Surprisingly, this time *Mimusops elangi* were also devoid of alkaloids in addition to *Callistemon lanceolatus* and *Solidago chinensis*

Proteins: White precipitate formation which turns yellow on boiling was only observed in the extract of *Cassipoula filliformis* and *Mimusops elangi* showing thereby the presence of proteins and confirming there by the absence of proteins in rest of the extracts.

Anthraquinones: Absence of a pink, violet or red coloration in ammoniacal layer indicated the absence of free anthraquinones in all the six extracts.

Anthocyanins: The absence of pink-red to blue-violet coloration indicated the absence of anthocyanins in all the six extracts.

Leucoanthocyanins: Absence of red color in organic layer indicated the absence of leucoanthocyanins in all the six extracts.



These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities etc [3,10,11]. All the six selected medicinal plants for screening were found to possess tannins. Tannins have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes. Flavonoids are also present in all six selected medicinal plants as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [17,18,25]. It also helps in managing diabetes induced oxidative stress. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer [26,27,28].

Screening of six selected medicinal plants clearly reveals that the maximum classes of phytoconstituents are present

in *Cassytha filliformis* extracts express positive response for highest number of phytochemical components (i.e. 9 components), the species like *Mimosops elengi*, *Araucaria heterophylla*, *Capparis brevispina* extracts were also shown the second highest number of phytochemical components (i.e. 6 components), the plant samples like *Wedelia chinensis* & *Callistemon lanceolatus* were shown the meager number of components when compare with previous plant samples i.e. the extracts of *Cassytha filliformis*, *Mimosops elengi*, *Araucaria heterophylla*, *Capparis brevispina*. The plant species (extracts) were subjected to various phytochemical screening tests were shown a good number of diversity while bearing the (higher – lower number of components), since these plants have been used in the treatment of different ailments, the medicinal roles of these plants could be related to such identified bioactive compounds [15,17]. Hence, the above mentioned plant extract/s could be explored for its highest

therapeutic efficacy by pharmaceutical companies in order to develop safe drugs for various ailments[27]. The quantitative analyses of these phytochemical components will be an interesting area for further study. The presence of certain class phytochemical components for their pharmaceutical utilization for exploration and evaluation of new drugs and formulations.

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REFERENCES

1. Anonymous- *The Wealth of India (Raw Materials)*. CSIR, New Delhi, India. Vol. 1-11. 1948-76.
2. Bharath Kumar, R & B.Suryanarayana- A Study of Phytochemical composition of a few Tribal Medicinal Plants from Sriharikota, A.P. Presented at Indian National Congress 3 – 7 Jan. Pune.(Pub.in International E- Journal *Ethnobotanical Leaflets* 13: 281-92. 2009.)
3. R.Bharath Kumar, S.Asha, Sravani, P, Y Kiranmayee, S Narasimha M and VS Reddy-*In-vitro Experimental Studies on Selected Natural Gums and Resins for Their Antimicrobial Activity*. January - February 2014 *Research Journal of Pharmaceutical, Biological and Chemical Sciences*.2014.,5(1) 154-172
4. Bharath Kumar,R., S.Asha & B. Sarath Babu-A note on Phytodiversity and Phytochemistry of important Plant species of Vignan University Campus, Vadlamudi, Andhra Pradesh. *International Journal of Pharma and Bio Science*.2014,Vol. 5(1): (B) 373 – 386
5. Bharath Kumar,R., S.Asha, J. Sravanthi, G.V.S.L. Manasa & B.Sravan Kumar- *In-vitro* experimental studies of selected biopesticides & their effect on selected plant pathogens. *Int.J.Pharm. & Life Sci. (IJPLS)*,Vol.4(9):Sep:2013,2931-2944.
6. Bhattacharya, S.C. -Constituents of *Centella asiatica* Linn. Examination of the Ceylonese Variety. 1956 a *J. Indian. Chem. Soc.* 33 : 599-586.
7. Chhabra, S.C., F.C.Viso and E.N.Mshiu- Phytochemical screening of Tanzanian Medicinal plants 1. -1984, *J. Ethno pharmacology* 11 : 157-179.
8. Chopra, I.C. Chopra & B.S. Verma- *Supplement to glossary of Indian medicinal plants*. CSIR, New Delhi, 1969.
9. Chopra, I.C. Chopra, K.L. Handa & L.d. Kapoor. *Chopra's Indigenous drugs of India*. U.N. Dhur & Sons Pvt. Ltd., Calcutta, 1958.
10. Dasgupta S, Parmar A, Patel H. Preliminary phytochemical studies of *Kalanchoe Gastonis- bonnieri*. *Int J Pharm Bio Sci* 2013; 4:550-557.
11. Doss A. Preliminary phytochemical screening of some Indian medicinal plants. *Anc Sci Life* 2009; 29:12-16.
12. Harborne JB. *Phytochemical Methods*. Chapman and hall Ltd., London: U.K., 1973, 49-188.
13. Harborne, J.B. -*Phytochemical methods: A guide to Modern Techniques of plant Analysis*. 1 - 278 P. Chapman and Hall, London. 1984.
14. Hemadri, K. -*Medicinal Plants from Andhra Pradesh*. Telugu Akademy, Hyderabad., 1979.
15. Kavitha R, Premalakshmi V. Phytochemical analysis of ethanolic extract of leaves of *Clitoria ternatea* L. *Int J Pharm Bio Sci* 2013; 4:236-242.
16. Kharat SS, Kumkar PB, Siddhesh RR, Sonawane KS. Qualitative phytochemical screening of *Gnidia glauca* (Fresen) Gilg. Plant extract. *Int J Pharm Bio Sci* 2013; 4:144-148.
17. Kumari SPK, Sridevi V, Lakshmi MVVC. Studies on Phytochemical screening of aqueous extract collected from fertilizers affected two medicinal plants. *J Chem Bio Phy Sci* 2012; 2:1326-1332.
18. Negi JS, Singh P, Rawat B. Chemical constituents and biological importance of *Swertia*: a review. *Curr Res Chem* 2011; 3:1-15.
19. Ojala,T, Remes S, Haansuu P, Vuorela H, Hiltunen R, Haatela K, et al.-Anti-microbial activity of some coumarin containing herbal plants growing in Finland. *J Ethnopharmacol* 2000; 73:299-305.
20. Okwu DE. Phytochemicals and vitamin content of indigenous spices of southeastern Nigeria. *J Sustain Agric Environ* 2004; 6:30-37.
21. Pandey P, Mehta R, Upadhyay R. Physico-chemical and preliminary phytochemical screening of *Psoralea corylifolia*. *Arch Appl Sci Res* 2013; 5:261-265.
22. Rabi T, Bishayee A. Terpenoids and breast cancer chemoprevention. *Breast Cancer Res Treat* 2009; 115:223-239.
23. Raphael E. Phytochemical constituents of some leaves extract of *Aloe vera* and *Azadirachta indica* plant species.*Global Adv Res J Environ Sci Toxicol* 2012;1:14-17.
24. Rio DA, Obdulio BG, Casfillo J, Marin FG and Ortuno A. Uses and properties of citrus flavonoids. *J Agric Food Chem* 1997; 45:4505-4515.
25. Salah N, Miler NJ, Pagange G, Tijburg L, Bolwell GP, Rice E, et.al.- Polyphenolic flavonoids as scavenger of aqueous phase radicals as chain breaking antioxidant. *Arch Biochem Broph* 1995; 2:339-46.
26. Satyanarayana T,Anjana A. Mathews,Vijetha P- Phytochemical and Pharmacological Review of Some Indian Capparis Species-*Pharmacognosy Reviews [Phcog Rev.] -Supplement Vol 2, Issue 4, Jul-Dec, 2008 Page 36-45*
27. Setchell KD, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. *J Nutr* 1999; 29:758-767.
28. Shah BA, Qazi GN, Taneja SC. Boswellic acids: a group of medicinally important compounds. *Nat Prod Rep* 2009; 26:72-89.
29. S. Mythili, S. Gajalakshmi, A. Sathiavelu, T. B. Sridharan-Pharmacological Activities of *Cassipha filiformis* : A Review - *Asian Journal of Plant Science and Research*, 2011, - 1 (1): 77-83