A comparative evaluation of pharmacological properties of Turmeric rhizome and *Vinca rosea*

P Annathai[1],MRuksar Banu[2], J Rammya[3], A K Ramya[4], S Gopalakrishnan[5*]

[1]Research Associate, Veridian Micro Lab Pvt Ltd, Kelambakkam, Chennai, India
[2,3]Research Associate, Veridian Micro Lab Pvt Ltd, Kelambakkam, Chennai, India
[4]Research Scholar, AarupadaiVeedu Medical College,Vinayaga Mission Research Foundation, Pondicherry, India
[5]Director, Veridian Micro Lab Pvt Ltd, Kelambakkam, Chennai, India*

Abstract

Introduction

In India, Ayurveda is an ancient system to treat several dreadful diseases and contagious illness. It has been found that plant consists of bioactive components which have more pharmacological property. These bioactive compound are used in various fields like fragrance, food, pharmaceutical, agriculture, veterinary etc. As per WHO (World Health Organization), one third of the population using our ancient folk medicine to treat various disease. It also encourages National Health Program to use Indian ayurvedic treatment which are less expensive and also do not cause any side effect. Secondary metabolites like alkaloids, steroids, cyanogenic glycosides, flavonoids, saponins and terpenoids which are commonly present in medicinal herbs have the ability to protect themselves from various environmental stresses.

Vinca rosea

Vina rosea is an important green traditional plant which belongs to Apocynacae family. They grow upto 1-3.5 cm. They are commonly known as Madagascar periwinkle (or) Lchnera rosea. The flower looks in different colours such as white, dark pink to dark red in the center. The size of the lorolla is about 2-5 diameter with 5 petals. The fruits are 2-4cm long and 3mm broad. Vinca is commonly cultivated for two classes of active compounds and for decoration.

Botanical Classification

BOTANICAL NAME(S)-Vinca rosea(Catharanthus Roseus)

FAMILY -Apocynaceae

KINGDOM -Plantae

DIVISION -Magnoliopsida(flowering plant)

ORDER -Gentianales

FAMILY -Apocynaceae

GENUS -Catharanthus

SPECIES -Catharanthus rosea

Turmeric

Turmeric is flowering plant which is used for cooking. In Indian ayurvedic medicine turmeric plays an important role to cure all type of diseases. It is also called as haridra, nisha etc. The commonly found compound in turmeric is curcumin which is approved by WHO (World Health Organization) as food addictive. Turmeric has a aromatic rhizomes which is highly branched, cylindrical and yellow to orange in color. The leaf shaped like a blade in two rows. It is usually range between 76 to 115cm rarely upto 230cm. This plant reaches upto 1m(3ft).

Botanical classification

KINGDOM -Plantae

ORDER -Zingiberates

FAMILY -Zingiberaceae

GENUS -Curcuma

SPECIES -Curcuma Longa

Materials Methods

Collection and Processing of Sample

The fresh rhizomes were collected from the organic market. The rhizomes were then sun dried. The dried rhizomes are blend in the mixer. The vino rosea plant and fresh flowers were collected from the garden.

Sequential Extraction for Turmeric Rhizome

In 10g of dried turmeric powder was added to 100ml of hexane and kept in the Magnetic stirrer at 60^0 C for 1 hour. After 1 hr, the solvent treated material was kept for maceration in room temperature for 24 hrs. Next day it was filtered and the residue was again treated with 100ml of chloroform and again kept in the magnetic stirrer at 60^0 C for 1 hour. After 1 hr, the solvent treated

material was kept for maceration in room temperature for 24 hrs. Same process was carried out sequentially for Ethyl acetate, carbinol and water.

Solvent Extraction for Vinca Rosea flowers

In **Vinca Rosea** plant, the fresh flower was used for the extraction. 5g of flower taken in two separate flasks 50 ml of methanol was added in one flask and in one flask and in another 50 ml of water was added. Both the flasks were kept in the Magnetic stirrer at 60^0 C for 1 hour. After 1 hr, the solvent treated material was kept for maceration in room temperature for 24 hrs. Next day it was filtered and the crude were concentrated through distillation. the concentrated crude was stored for further studies.

Identification of bioactive compound by Thin Layer Chromatography

TLC is used to detect number of compounds present in the crude. The silica gel was used as a stationary phase and solvents like hexane/ethyl acetate 1:1, hexane/ ethyl acetate 7:3 and hexane/ethyl acetate 3:7 was used as mobile phase The efficiency of compound extraction can be identified using different ratio of mobile phase. The retention factor of the sample is calculated by the formula given below.

RF= Distance travelled by sample/ Distance travelled by solvent.

Phytochemical Analysis

Phytochemical screenings were performed with different extracts of selected plants and primary screening for presence of different secondary metabolites in the plant extracts were performed and the results were recorded during the present investigation

Test for carbohydrates

2ml of plant extract was taken. 1mL of Molisch's reagent and few drops of concentrated Sulphuric acid were added. Presence of purple or reddish colour. This indicates the presence of carbohydrates.

Test for tannins:

1ml of plant extract was taken. 2mL of 5% ferric chloride was added. Presence of dark blue (or) greenish black. This shows the presence of tannins.

Test for saponins:

2ml of plant extract was taken. 2mL of distilled water was added. It was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer foam. This shows the presence of saponins.

Test for flavonoids:

2ml of plant extract was taken. 1mL of 2N sodium hydroxide was added. Presence of yellow colour. This indicates the presence of flavonoids.

Test for alkaloids:

2ml of plant extract was taken. 2mL of conc. Hydrochloric acid was added. A few drops of Mayer's reagent were added. Presence of green colour (or) white precipitate. This indicates the presence of alkaloids.

Test for Quinones:

1ml of plant extract was taken. 1mL of concentrated Sulphuric acid was added. Presence of red colour. This indicates the presence of Quinones.

Test for glycosides:

2ml of plant extract was taken. 3mL of chloroform and 10% ammonia solution was added. Pink colour was formed. This indicates the presence of glycosides.

Test for cardiac glycerides:

0.5ml of plant extract was taken. 2mL of glacial acetic acid and few drops of 5% ferric chloride were added. It was under layered with 1mL of concentrated Sulphuric acid. Brown ring was formed at the interface. This indicates the presence of cardiac glycerides.

Test for terpenoids:

0.5ml of plant extract was taken. 2mL of chloroform was added and concentrated Sulphuric acid was added. Red brown colour was formed at the interface. This indicates the presence of terpenoids.

Test for phenols:

1ml of plant extract was taken. 2mL of distilled water was added. A few drops of 10% ferric chloride were added. Blue (or) green colour was formed. This indicates the presence of phenols.

Test for coumarins:

1ml of plant extract was taken. 1mL of 10% sodium hydroxide was added. Yellow color was formed. This indicates the presence of coumarins.

Test for proteins and amino acid by Ninhydrin test:

2ml of plant extract was taken. A few drops of .2% Ninhydrin were added. It was heated for 5minutes. Blue color was formed. This shows the presence of proteins.

Test for steroids and phytosteroids:

1ml of plant extract was taken. Equal volume of chloroform was added. A few drops of concentrated Sulphuric acid were added. Brown ring was formed. This indicates the presence of steroids and appearance of bluish brown ring indicated the presence of steroids.

Test for phylobatannins:

1ml of plant extract was taken. A few drops of 2% HCl were added. Red color precipitate was formed. This indicates the presence of phylobatannins.

Test for Anthraquinones:

1mL of plant extract was taken. A few drops of 10% ammonia solution were added. Pink colour precipitate was formed. This indicates the presence of Anthraquinones.

Antibacterial Activity

Antibacterial activity of turmeric and vinca rosea was observed by agar well diffusion method. The extracts obtained from sequential extraction of turmeric rhizome and extract of vinca flower were tested against four strains of human pathogenic bacteria which were isolated from clinical sample like urine, sputum. The test cultures are *Escherichia coli, Staphylococcus sps, Klebsiella sps, Salmonella sps.* The test culture swabbed over the surface of the Muller Hinton Agar plate. The wells were prepared using agar puncher on the culture swabbed plates. Each well loaded with different extracts from vinca and turmeric as well as standard antibiotics like tetracycline used as positive control and DMSO used for dissolving the crude extract used as negative control. After inoculation , the plates were incubated for 24 hrs at 37^0 C. Next day the plates were observed and measured the zone formation around each well and recorded. The zone of inhibition obtained above 6 mm indicates the effective antimicrobial activity of the bioactive compound.

ResultandDiscussion

The turmeric powder was sequentially treated with solvents like Hexane: Chloroform: Ethyl Acetate: Methanol : Water. Likewise, methanol and water were used to treat fresh flower of vinca. The yield obtained by different solvents were measured.

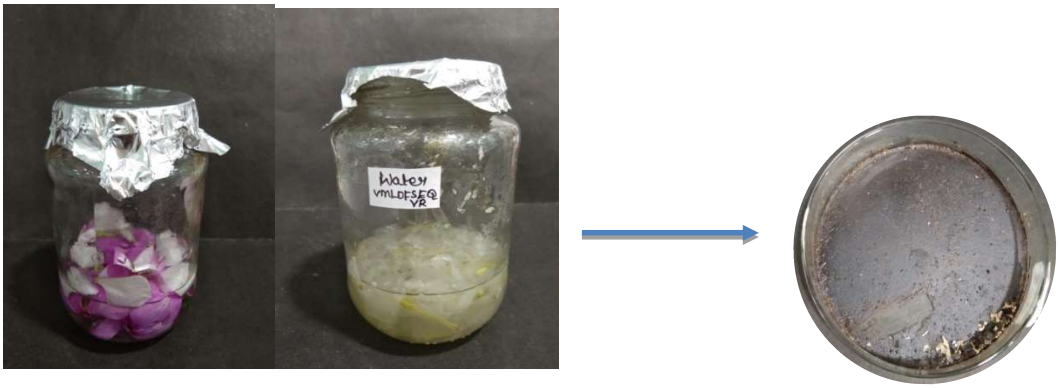


Figure.1 Extraction of Bioactive Compound from flower of Vinca rosea

Figure.2 Extraction of Bioactive Compound from Turmeric Rhizome

Table 1. Yield of crude obtained from different solvents from turmeric rhizome and Vinca rosea

Sl No	Solvent	Yield of extract from Turmeric(mg)	Yield of extract from Vinca rosea(mg)
1	Hexane	0.25	-
2	Chloroform	0.21	-
3	Ethyl Acetate	0.49	-
4	Methanol	0.18	0.14
5	Water	0.2	0.26

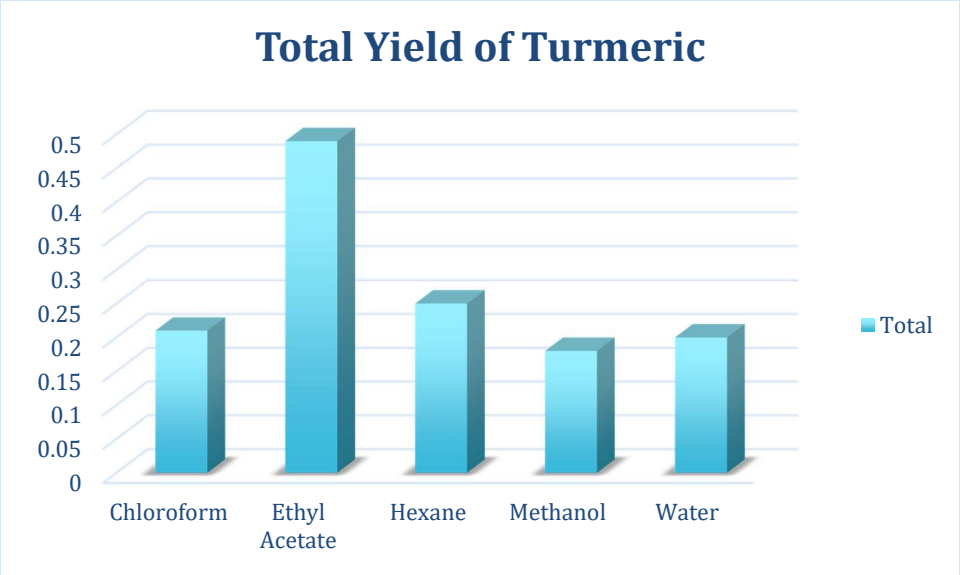


Figure 3. Shows yield of crude from various solvent treatment of Turmeric Rhizome

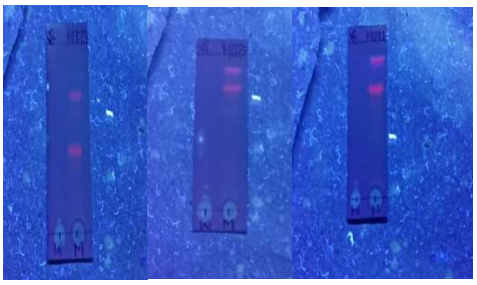
The crude obtained were analyzed for bioactive compound by Thin layer Chromatography. Each crude was analyzed using different ratio of mobile phase. The following are

Table 2 show Rf value of each bioactive compound from different ratio of mobile phase used for different extracts

Sample Name	Solvent Used for Extraction	Mobile Phase	Ratio	Rf Value
VMLOF_SEQ-T	Hexane	Hexane/ethyl acetate	1:1	0.534,0.88,0.74,0.41,0.23,0.11,0.02
VMLOF_SEQ-T	Chloroform	Hexane/ethyl acetate	1:1	0.511,0.651,0.81,0.93,0.96,0.58,0.46,0.23,0.02
VMLOF_SEQ-T	Ethyl Acetate	Hexane/ethyl acetate	1:1	0.23,0.58,0.65,0.74
VMLOF_SEQ-T	Methanol	Hexane/ethyl acetate	1:1	0.11
VMLOF_SEQ-T	Water	Hexane/ethyl acetate	1:1	0.02
VMLOF_SEQ-T	Hexane	Hexane/ethyl acetate	3:7	0.55,0.875,0.02,0.5,0.75
VMLOF_SEQ-T	Chloroform	Hexane/ethyl acetate	3:7	0.65,0.725,0.85,0.02,0.95,0.87

VMLOF_SEQ-T	Ethyl Acetate	Hexane/ethyl acetate	3:7	0.02,0.5,0.75,0.95
VMLOF_SEQ-T	Methanol	Hexane/ethyl acetate	3:7	0.02
VMLOF_SEQ-T	Water	Hexane/ethyl acetate	3:7	-
VMLOF_SEQ-T	Hexane	Hexane/ethyl acetate	7:3	0.2,0.375,0.525,0.825,0.2,0.12,0.25,0.45,0.87
VMLOF_SEQ-T	Chloroform	Hexane/ethyl acetate	7:3	0.075,0.125,0.225,0.375,0.02,0.2,0.5,0.62,0.87
VMLOF_SEQ-T	Ethyl Acetate	Hexane/ethyl acetate	7:3	0.02,0.12,0.25,0.75,0.87
VMLOF_SEQ-T	Methanol	Hexane/ethyl acetate	7:3	0.02
VMLOF_SEQ-T	Water	Hexane/ethyl acetate	7:3	-
VMLOF_SEQ-VR	Methanol	Hexane/ethyl acetate	1:1	0.825,0.925
VMLOF_SEQ-VR	Water	Hexane/ethyl acetate	1:1	0.02
VMLOF_SEQ-VR	Methanol	Hexane/ethyl acetate	7:3	0.85,0.925
VMLOF_SEQ-VR	Water	Hexane/ethyl acetate	7:3	0.02,0.375,0.45,0.5
VMLOF_SEQ-VR	Methanol	Hexane/ethyl acetate	3:7	0.85
VMLOF_SEQ-VR	Water	Hexane/ethyl acetate	3:7	0.02

In turmeric,the hexane extract and chloroform extract showed more number of bands,when mobile phase used as Hexane: Ethyl acetate(7:3).In Vinca, the methanol extract showed more bands, when hexane : ethyl acetate (1:1; 7:3) ratio.



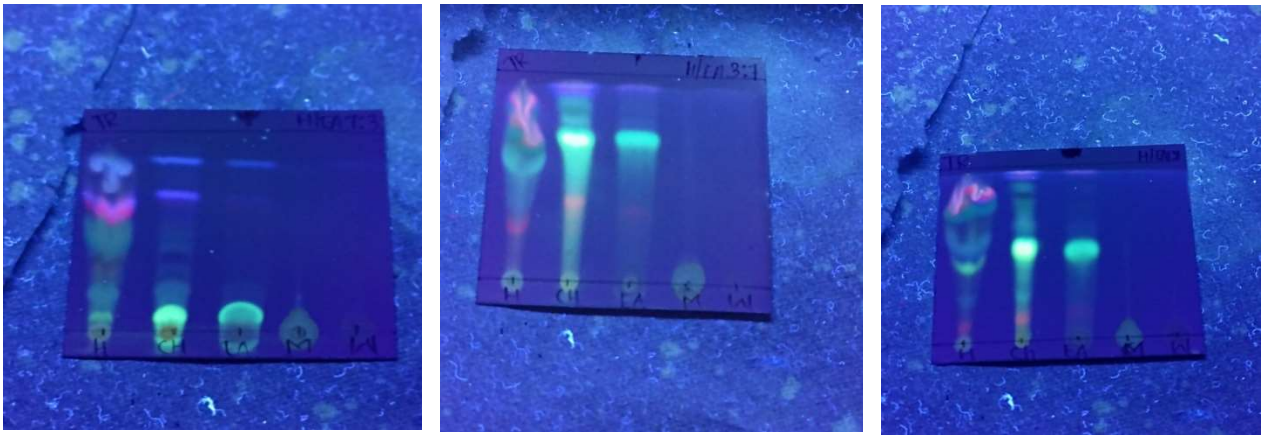


Figure 4 shows separation of bioactive compound in TLC under UV Light

Phytochemical Analysis

Table 3 Phytochemical Analysis of *Vinca and Turmeric*

Tests	Inference	Methanol		Chloroform		Water		Hexane	
		Vinca	Turmeric	Vinca	Turmeric	Vinca	Turmeric	Vinca	Turmeric
Phenol	Presence of Blue or Green colour	+	+	+	+	-	-	-	-
Flavonoid	Presence of Yellow colour	+	+	+	-	+	-	+	-
Quinones	Presence of red colour	+	-	+	-	-	-	-	-
Tannins	Presence of dark blue or greenish black	+	+	+	+	+	-	-	-
Saponins	Formation of 1 layer of foam	+	-	+	-	-	-	-	-
Coumarins	Presence of yellow colour	+	+	+	-	+	-	-	-
Phylotannins	Formation of red colour precipitate	-	-	-	-	-	-	-	-
Anthraquinones	Formation of pink colour precipitate	-	-	-	-	-	-	-	-
Cardiac glycosides	Formation of blue ring	+	-	-	-	+	-	-	-
Carbohydrates	Presence of purple or reddish colour	-	-	-	-	-	-	-	-

Alkaloids	Formation of white or green colour precipitate	+	+	+	+	+	-	+	-
Glycosides	Formation of pink colour	+	-	-	+	-	-	-	-
Terpenoids	Formation of red colour	-	-	-	-	-	-	+	-
Steroids& phytosteroids	Formation of bluish ring	+	-	+	-	+	-	+	-

Figure 5 shows antibacterial activity of different solvent extract of Turmeric

Table 5 shows zone of inhibition against test culture by Different extracts of Turmeric

Test cultures	Positive	Negative	Hexane	Chloroform	Ethyl acetate	Methanol
E coli	**10mm**	**0 mm**	**0 mm**	**3 mm**	**14mm**	**0 mm**
Staphylococcus sps	**20mm**	**0 mm**	**0 mm**	**0 mm**	**8mm**	**2 mm**
Klebsiella sps	**18mm**	**0 mm**	**0 mm**	**4 mm**	**12 mm**	**0 mm**
Pseudomonas sps	**8mm**	**0 mm**	**0 mm**	**0 mm**	**6mm**	**5 mm**

Table 6 shows zone of inhibition against test culture by Different extracts of vinca flower

Test cultures	Positive	Negative	Water	Methanol
E coli	**10mm**	**0 mm**	**0 mm**	**0 mm**
Staphylococcus sps	**20mm**	**0 mm**	**0 mm**	**8mm**
Klebsiella sps	**18mm**	**0 mm**	**0 mm**	**2mm**
Pseudomonas sps	**8mm**	**0 mm**	**5mm**	**0 mm**

In antimicrobial assay, the ethyl acetate extractof turmeric showed positive result with high zone of inhibition among all the test organism the chloroform extract and water extract also showed positive result with less zone of inhibition. But in Vinca, the zone of inhibition observed and recorded in methanol extract, with low antimicrobial activity against *Staphylococcus* and *Klebsiella sps.*

Conclusion

Reference

1. Salem M E, Fatimh M M, Fouzy A. HPhytochemical Study and In Vitro Antibacterial Activity of Two Traditional Medicinal Plants (Vinca Rosea and Vinca Difformis) from Libya. *Open Acc J of Toxicol*. **2019**; 4(1): 555626. DOI: 10.19080/OAJT.2019.04.555626
2. Jayaraj, AngelinJebamalar Et Al. "Evaluation Of Antimicrobial Activity And Phytochemicals Analysis Of Whole Plant Extract Of Vinca Rosea." *Asian Journal Of Pharmaceutical And Clinical Research*.**2019**; N. Pag.
3. Sayantani Chanda, T.V. Ramachandra. Phytochemical and Pharmacological Importance of Turmeric (Curcuma longa): A Review. Research & Reviews: *A Journal of Pharmacology*. **2019**; 9(1): 16–23p.
4. K.K. Jae, J. Cheorun, J.H. Han, J.P. Hyun, J.K. Young and W.B. Myung. Color improvement by irradiation of Curcuma aromatica extract for industrial application. *Radiation Physics and Chemistry*.**2006**; 75 (3): 449-452.
5. Leena KP, Sreelakshmi KS. Phytochemical screening and in vitro cytotoxicity studies of Mussaendafrondosa Linn leaves. *Res J Pharm Tech***2017**;10:4227-30.
6. Sreesha NN, Alexeyena V, Meenu B, Greeshma R, Neeraja ED. Comparative evaluation of Coriandrum sativum Linn. And Apius graveolens for antimicrobial activity. *Res J Pharm Technol***2017**;10:541-4.

7. Velayudhan KC. Ethnobotany of Turmeric Indian Journal of Traditional Knowledge. **2012**;11(4):607–614.
8. Ravikant Vishwakarma, Vishal Prajapati,Rajesh Kumar Yadav, Neda Fatima, Vishesh Singh and Manish Kumar Maurya,. "A Herbal Drug of Vinca: Used As a Anticancer Agent" *International Journal of Current Research*, **2019**;11, (10), 7979-7982.
9. V. Vats, S. P. Yadav, and J. K. Grover, "Ethanolic extract of Ocimumsanctumleaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydratemetabolism in rats,"*Journal of Ethnopharmacology* , **2004**; 90(1), 155–160.
10. Manpreet kaur, Vinca sources. Macroscopical character and uses, Article. Erdogrul Antibacterial activities of some plant extract used in folk medicine. *Pharm. Biol.***2002**; 40:269- 273.
11. Plants of the World Online, Kew Science, Kew Gardens, Royal Botanic Gardens, Kew, England. **2018**; Retrieved 26 March 2018.
12. C. A. Jaleel, R. Gopi, G. M. A. Lakshmanan, and R. Panneerselvam, "Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in Catharanthus roseus (L.) G. Don," *Plant Science*, **2006**;171, 2, 271–276.
13. V.S.a, Surwase&Laddha, Kirti & Kale, Ravindra & Hashmic, Syed &Lokhande, Siddharth.Extraction and isolation of turmerone from turmeric. *Electronic Journal of Environmental, Agricultural and Food Chemistry.***2013**; 10. 2173-2179.
14. Sofowara. A . 'Medicinal plants and traditional medicinal in Africa. Screening plants for bioactive agents'.1993; 134- 156.
15. Harborne JB. 1973. 'A guide to modern techniques of plant analysis'. Phytochemical methods. Vol 3rd Ed; 279.
16. Kapoor SL and Shrivastsava SN. Lloyida. 'Survey of Indian Medicinal plants for saponins, alkaloids, flavonoids'.**1969**; 32; 297- 302.
17. Smolenski SJ, Silinis. H , Farnsworth. N R.. 'Lloydia. Alkaloids screening'. **1969**;37, 506- 536.
18. Devika. R and Justin Kovilpillai. 2012. 'Phytochemical screening studies of bioactive compounds of *TAGETES ERECTA'.International Journal of pharma and Biosciences*.**2012**;3, 596- 602.
19. Sonali jana and Shekhawat G.S. (2010). 'Phytochemical analysis and antibacterial screening of in vivo and in vitro extracts of Indian medicinal herbs'. *Journal of Medicinal Plants*. **2010;**4,206-212.
20. Ayoola, G. A., Coker, H., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C., & To, A. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*,**2008;7**.

21. Suresh kumar. C.A, Varadharajan. R ,Muthumani. P , Meera. R , Devi. P and Kameswari. B. 'Pharmacognostic and preliminary phytochemical investigations on the stem of *Saccharum spontaneum'*. *Journal of pharma science and Research.***2009**; 1, 129- 136.
22. Satheesh kumarbhandary, Suchetha kumara. N, Vadisha. S , Bhat sharmila. K P, Mahesh Prasad bekal. 'Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds'. *Nitte University Journal of Health science*.**2012**; 2, 34- 38.
23. Vikrant Arya, Narendar Thakur, Kashyap. CP.2012. 'Preliminary phytochemical analysis of the extracts of *Psidium*leaves'. *Journal of pharmacognosy and phytochemistry*.**2012**;4, 1- 4.
24. Sabri Fatima zohra, Belarbimerieum, Sabri samri, Alsayadimuneer. MS. 'Phtyochemical screening and identification of some compounds from *Mallow*'. *Journal of Natural products and Plant resource*. **2012**;2, 512- 516.
25. Ashok kumar, Jha. kK, Dinesh kumar, Abhirvaagarwal and Akhil Gupta. 2012. 'Preliminary phytochemical analysis of leaf and bark (Mixture) extract of *ficusinfectoria* plant'. *The pharma innovation*. **2012**;1, 71-76.
26. Andu. sA, Mohammed. I, Kavita. A. 'Phytochemical screening of the leaves of *Laphira lanceolata* (Ochanaceae)'. *Life Science Journal*.**2007**;4, 75- 79.
27. Gulluce, M., Sahin, F., Sokmen, M. Ü. N. E. V. V. E. R., Ozer, H., Daferera, D., Sokmen, A. T. A. L. A. Y. &Ozkan, H. İ. C. A. B. İ. Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. longifolia. *Food chemistry*, **2007**;103,4, 1449-1456.
28. Daniyan SY, Mahammad HB (2008) Evaluation of the antimicrobial activities and phytochemical properties of extracts of Tamaridus indica against some diseases causing bacteria *Afri J of Biotic*, **2008**;7,14, 2451- 2453.