AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Chemical composition of three medicinal plants use as traditional medicine in Manipur

Kh. Ujala Devi *[1], Th. Inaocha Devi [1], T. Chand [2]
1. Institutional Biotech Hub, Department of Botany, Presidency College, Motbung, Manipur, India.
2. Institutional Biotech Hub, Department of Botany, Waikhom Mani Girl's College, Thoubal, Manipur, India.

ABSTRACT

Leaves of Xylosma longifolium, Hyptis suaveolens and *Oreocnide integrifolia* collected from the Imphal East District, Manipur were analysed for phytochemical constituents. Leave extracts were prepared with methanol by Soxhlet apparatus. Alkaloid, phenol, flavonoid, tannin, terpenoid, steroid, saponin and glycosides were detected in the three samples. Glycosides was absent in *Xylosma longifolium and terpinoid in Oreocnide integrifolia.* Steroid and saponin were also not found in *Hyptis suaveolens.* Total phenol, flavonoid and tannin contents were determined by Folin-Ciocalteu, Aluminium chloride and Folin-Denis method respectively. The total phenol contents in terms of gallic acid equivalent were 57.38±2.563 mg/g, 65.00±0.475 mg/g and 50.87±0.035 mg/g of dry wt; total flavonoid contents in terms of quercetin equivalent were 36.70±0.676 mg/g, 49.24±0.521 mg/g and 32.78±1.753mg/g of dry wt. and total tannin contents in terms of tannic acid equivalent were 31.08±1.300 mg/g, 41.14±0.850 mg/g and 30.95±0.759 mg/g of dry wt. in the methanolicS leave extract of *X.longifolium, O.integrifolia and H.suaveolens* respectively .The order of total phenol , flavonoid and tennin contents of the samples were *Oriocnide integrefolia> Xylosma longifolium >Hyptis suaveolens.* It shows that *Oriocnide integrefolia* has the highest medicinal value than the two studied plants. The present study evidence that these plants are of therapeutic potential as a good number of bioactive chemical compounds have been confirmed.

Keywords: Methanol, Phenol, Flavonoid, Tannin, Phytochemicals, Soxhlet apparatus

*Corresponding Author Email: khujaladevi@gmail.com
Received 30 June 2017, Accepted 12 July 2017

Please cite this article as: Devi KU *et al.*, Chemical composition of three medicinal plants use as traditional medicine in Manipur. American Journal of PharmTech Research 2017.

INTRODUCTION

The commercially available synthetic chemical compounds are known to exhibit serious toxicity. Now-a-days, there has been focusing awareness about the importance of medicinal plants as no side effects and easily available. It is due to the presence of specific chemical compounds which are very useful for human health as well as curing of diseases. About 300 species of medicinal and aromatic plants are used world wide in the pharmaceutical, food, cosmetics and perfume industries [1]. A number of plant contains secondary metabolites such as alkaloid, steroid, tannin, glycoside, phenol, flavonoid, volatile oil, essential oil etc [2,3].

Alkaloids have been indicated as a starting material in the manufacture of steroidal drugs [4]. Flavonoids have therapeutic uses due to their strong anticancer activities, antioxidant and healing properties [5, 6]. Flavonoids can affect the functions of cells linked to inflammatory processes, acting on enzymes pathways involved in anti-inflammatory processes [7]. Phenolic compounds are widely distributed in the plant kingdom and presence of phenol is considered to be potentially toxic to the growth and development of pathogen [8].

Xylosma longifolium Clos. (Flacourtiaceae) is a medium sized tree, distributed in subtropical Himalaya, North-East India and East to West China. *Xylosma longifolium* is used in Assam, for intoxication and also exhibits antispasmodic, dysentery, restless and insomnia [9, 10]. In Manipur, the fresh leaf and stem bark extracts is used for curing ringworm, scabies, piles, stomach pains and acne in folk medicine [11].

The plant *Hyptis suaveolens*, a profuse under shrub plant which is commonly known as “Vilaiti tulsi” belongs to the family Lamiaceae and is an important aromatic medicinal plant. Almost all parts of this plant are being used in traditional medicine to treat various diseases. Both leaves and twigs are considered to exhibit antispasmodic activity and are used as source of anti-inflammatory, antioxidant activity, and anti-fertility agents and also as antiseptic burns, wounds, and various skin complaints [12-14]. The genus *Hyptis* possesses a diverse range of biological activities which have been attributed to essential oil obtained by hydrodistilation of the leaves of *Hyptis suaveolens* [15].

Oreocnide integrifolia is a small tree of family Ulmaceae. This plant is popular in north-eastern parts of India, especially in Manipur used as a folklore anti-diabetic therapeutant. Leaf is cooked-eaten for normal blood circulation. The roots smashed with ginger is taken to cure rashes and skin infections.

However, there is little information about the analysis of phytochemical properties in the three species from different sources of Manipur. Therefore, the present objectives focus on Phytochemical screening of leave and quantitative analysis of total phenol, flavonoid and tannin.

MATERIALS AND METHOD

Plant Material Collection: The present study included three wild plants which were *Xylosma longifolium, Oreoncide integrifolia* and *Hyptis suaveolens.* Fresh leaves collected from different places of Imphal East District. The collected plant species were used for both the qualitative and quantitative phytochemical analysis.

Preparation of Plant Extract:

The leaves of collected three wild medicinal plants were washed under running tap water to remove dust particles and rinsed again with distilled water. The plant samples were shaded, air dried for one week and evaporated water molecules under hot air oven at a temperature of 35^0C-40^0C for 2 days. The dried leaves were ground into powder form by using grinder and stored in polythene bags for chemical analysis.

Solvent Extraction:

Crude plant was prepared by Soxhlet extraction method. For methanolic extraction, each 20gm of powdered plant material was uniformly packed into thimble and extracted with 250ml of methanol separately at 65^0C. The process of extraction continues till the solvent in siphon tube of an extractor become colourless. The aliquots were cooled down and taken in colour amber bottles and kept in refrigerator for further analysis.

Phytochemical screening:

All the plant extracts were qualitatively tested by using standard method for the presence of chemical constituents such as alkaloids, phenols, tannin, flavonoids, siponin, glycosides, steroids and terpenoids[16-20].

Determination of Total Phenolic Content

The phenolic contents in the methanolic extract was estimated by Folin-Ciocalteu method with little modification as previously described [21-23]. 2.5 ml of 10% Folin-Ciocalteu reagent and 2ml of $NaCo_3$(2%W/V)were added to 0.5ml of the sample (3 replicates) of each plant extract solution(1mg/ml).The mixture was allowed to stand at 20^0C for 30 minutes and the absorbance of the developed colour was measured at 760 nm using UV-vis Spectrophotometer. Gallic acid was used as standard (1mg/ml) to calculate total phenolic content. All the tests were performed in

triplicates. Total phenolic content in the plant extract was expressed as Gallic acid equivalent (mg of Gallic acid equivalent/g of dry weight sample) and was calculated by the formula [24] .

T = CXV/M

Where, T = Total content of phenolic compound, mg/g of plant extract, in GAE;

C = Concentration of Gallic acid established from the Calibration curve, microgram/ml;

V = Volume of extract; M = Weight of methanolic plant extract, g.

Determination of Total Flavonoid Content

Aluminum Chloride spectrophotometric method was used to measure the flavonoid content of all plant extracts with slight modification [25-27]. 0.5ml of sample (3 replicates) of each plant extract solution (1mg/ml) was taken and it was added with 0.1ml of aluminum chloride (10%), 0. 1ml of potassium acetate(1M) and 2.8ml of distilled water to make up volume to 3.5ml.The reaction mixture was kept at room temperature for 30min.The absorbance was measured at 415nm using UV-vis Spectrophotometer against the suitable blank. Quercetin was used as standard (0.1mg/ml) to calculate total flavonoid content. The calibration curve was prepared using different concentrations of quercetin expressed in mg/gm dry weight.

Determination of Total Tannin Content

The total tannin contents in the methanolic extract was estimated by Folin-Denis method with little modification as previously described[28-30]. Tannic acid was used as standard (0.1mg/ml) to calculate total tannin content. 0.5ml of sample(1:100 dilution) of each plant extract solution(1mg/ml) was taken and it was added with 1.5ml of Folin-Denis reagent,5ml of $NaCo_2$(17%) and mix properly. The mixture was allowed to stand for 20min when a bluish-green colouration developed. The absorbances of the Tannic Acid Standard solutions as well as samples were read after colour development on a using UV-vis Spectrophotometer at a wavelength of 700nm against the suitable blank. Percentage tannin was calculated.

Statistical Analysis

The result of phenolic, flavonoid and tannin content in the plant extract were expressed as Mean± Standard Deviation (SD) by using Microsoft excel 2007.

RESULTS AND DISCUSSION

Phytochemical screening of three selected plants confirmed the presence of alkaloid, phenol, tannin, flavonoid, terpenoid , saponin, , steroid and glycosides (Table1). However, terpenoid is absent in *O. integrifolia* and glycosides in *X. longifolium.* Saponin and steroid are also absent in *H. suaveolens.* Presence of alkaloid, phenol, tannin flavonoid, steroid and absence of saponin were

reported in *H. suaveolens* [31]. The importance of alkaloid, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported [32]. Steroids have been reported to have antibacterial properties [33] and they are very important compounds due to their relationship with compounds such as sex hormones [34].

Table 1: Preliminary phytochemical constituents

Chemical Constituents	**Chemical tests**	***Xylosma longifolium***	***Oreocnide integrifolia***	***Hyptis suaveolens***
Alkaloid	Mayer,s test	+	+	+
Phenol and tannin	Braymer's test	+	++	+
Flavonoid	Shinoda test	+	+	++
Terpenoid	Salkawski's test	+	_	+
Saponins	Form test	+	+	_
Steroid	Salkawski test	+	+	_
Glycosides	Kellerkilan test	_	+	+

The total phenol, flavonoid and tannin contents are shown in Table 2. The standard graph for Gallic acid is represented in figure 1(y =0.007x-0.009, R^2=0.993).where, Y is absorbance at 760nm and X is concentration.The total phenolic content of the methanolic extract of *X. longifolium, O. integrifolia and H. suaveolens* were 57.38±2.563 mg/g, 65.00±0.475 mg/g, and 50.87±0.035 mg/g of gallic acid equivalent per gram of extract powder respectively. *O.integrifolia* recorded highest phenolic content and lowest in *H. suaveolens.* Some worker have reported the total phenol content in *X. longifolium* as 56.60±4.84 mg/g [35] and *H. suaveolens* as 74.56±1.33 mg/g [36]. In *X. longifolium* reported value is nearly similar with the present investigated value but in *H. suaveolens* reported value is higher than present investigated value.

Table 2: Total phenol, flavonoid and tannin contents in the methanolic extracts

Sample	**mg GA/g of extract X***	**mg Q/ g of extract X***	**mg TA/ g of extract X***
Xylosma longifolium	57.38±2.563	36.70±0.676	31.08±1.300
Oreocnide integrifolia	65.00±0.475	49.24±0.521	41.14±0.850
Hyptis suaveolens	50.87±0.035	32.78±1.753	30.95±0.759

GA= Gallic acid, Q= Quercetin, TA= Tannic acid, X* = mean ± standard deviation.

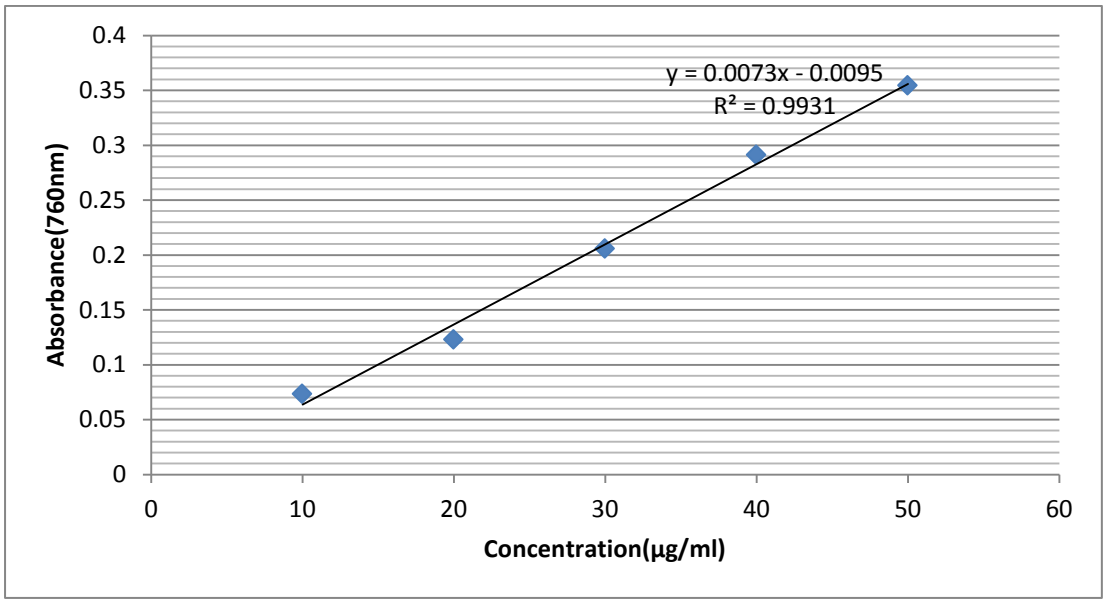

Figure 1: Gallic acid Standard curve

The total flavonoid content was measured by aluminium chloride method in term of quercetin equivalent. The standard curve equation(y=0.007x-0.035, R^2=0.997) is shown in figure 2. The total flavonoid content of *Xylosma longifolium*, *Oreocnide integrifolia* and *Hyptis suaveolens* were 36.70±0.676mg/g, 49.24±0.521mg/g and 32.78±1.753 mg/g. *O.integrifolia* was found highest flavonoid content then followed by *X. longifolium* and *H. suaveolens* .

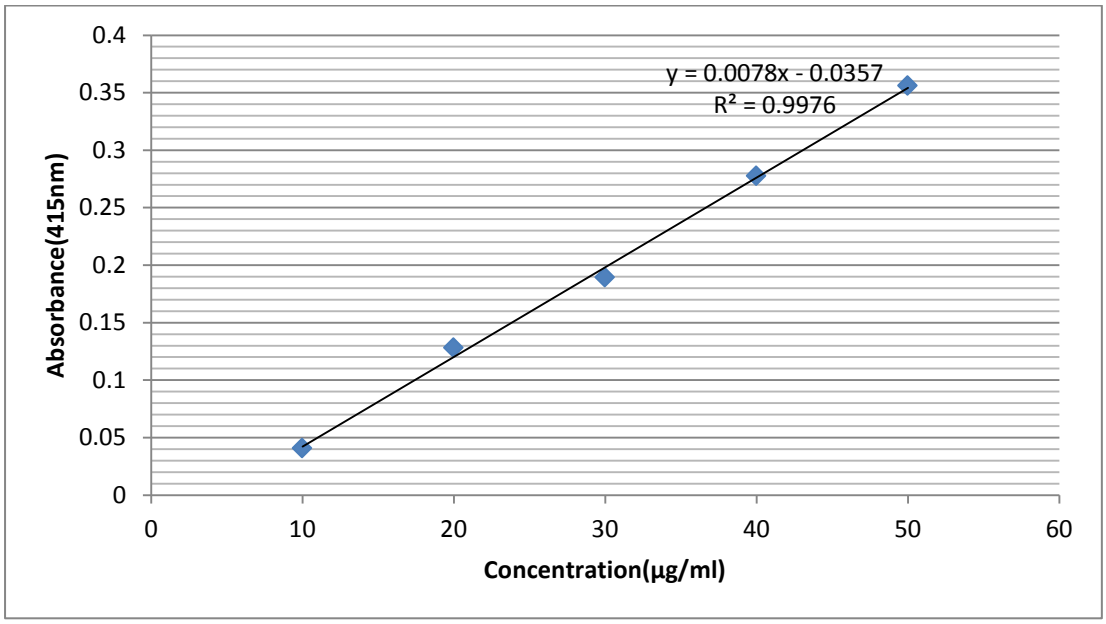

Figure 2: Quercetin Standard curve

The total tannin content of three selected wild plants were estimated by folin –denis technique in terms of tannic acid equivalent (the standard equation y =0.006x-0.002, R^2=0.986) figure 3.The total tannin content of *O. integrifolia*, *X. longifolium*, and *H. suaveolens* were 41.14±0.850 mg/g,

31.08±1.30 0mg/g and 30.95±0.759 mg/g of tannic acid equivalent per gram of extract powder respectively. The leaf extract of *O. integrifolia* occur the highest tannin content followed by *X. longifolium,* and *H. suaveolens*. Tannins have considered antimicrobial and antioxidant capacities (37).Flavonoids have been found to be antimicrobial substance against wide array of microorganisms in vitro [38]. *Oreocnide integrifolia* leave extract exhibits hypoglycemic and hypolipidemic potentials on diabetic rats [39]. *Hyptis suaveolens* have marked antioxidant and neuroprotective activities against oxidative stress-induced neurotoxicityn[36]. Phenolic compounds posses biological properties such as antiaging, anticarcinogen, anti-inflammation, cardiovascular protection etc [40]. Thus, from the present investigation medicinal properties of the three plants can be identified based on the chemical compounds present on them.

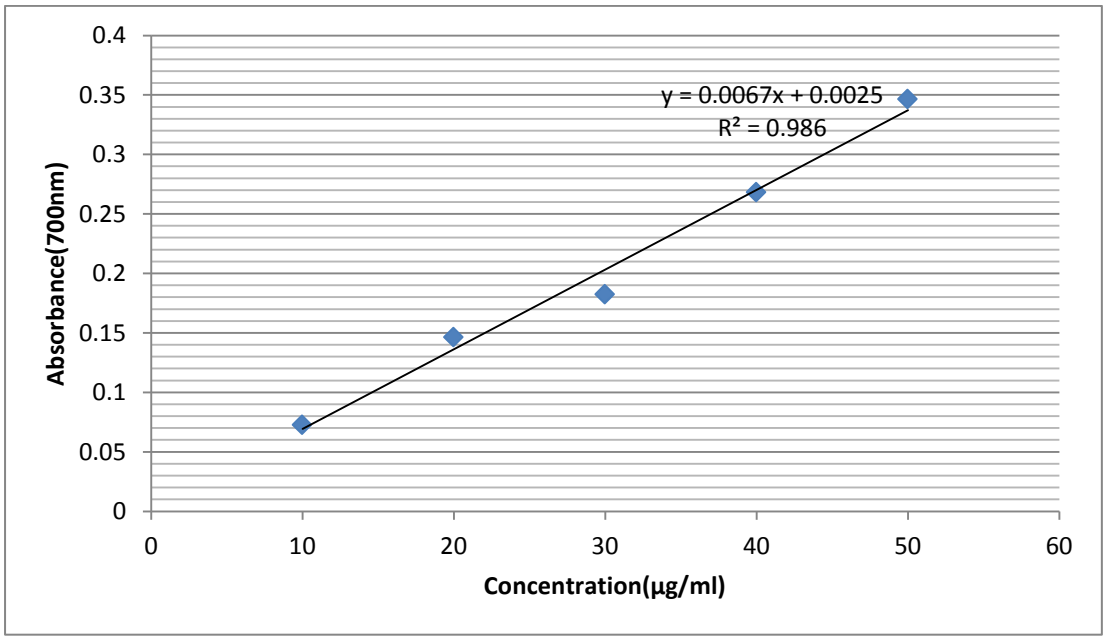

Figure 3: Tannic acid Standard curve

CONCLUSION

The present study determined the presence of alkaloid, phenol, tannins, flavonoid, terpenoid, saponin, steroid and glycosides except glycosides in *Xylosma longifolium and terpinoid in Oreocnide integrifolia.* Steroid and saponin were also not present in *Hyptis suaveolens.* The order of total phenol, flavonoid and tannin contents of the samples were *Oriocnide integrefolia >Xylosma longifolium > Hyptis suaveolens*. It proves that *Oriocnide integrefolia* has the highest therapeutic potential than others. The present study evidence that these plants are of therapeutic potential as a good number of bioactive chemical compounds have been confirmed. The investigated plants may be possess anti- inflammatory, anti-diabetic, antioxidant, antitumor and

antiviral activities. Therefore, these plants may be used to cure disease as traditional herbal medicine.

ACKNOWLEDGEMENTS

The authors highly acknowledged Department of Biotechnology (DBT), Government of India, and New Delhi for financial assistance to the Institutional level Biotech Hub, Department of Botany, Presidency College, Motbung, Manipur.

REFERENCE:

1. Robber JM, Speedie TV. Pharmacognosy, Pharmacobiotechnology. Williams and Wiikins, Batimore.1996;1-14.
2. Doss A. Preliminary phytochemical screening of some Indian medicinal plants. Anc Sci. Life. 2009; 29: 12-16.
3. Edoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnol. 2005; 4(7): 685-688.
4. Maxwell A, Seepersand M, Pingal R, Mootoo DR, Reynolds. WF3-Beta-amino spirosolane steroidal alkaloids from Solanum triste. J. Natl. Prod. 1995; 58:625-628.
5. Salah N, Miiler NJ, Pagange G, Tijbung L, Bolwell GP, Rice E, Evans C. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chai breaking antioxidant. 1995; 2: 339-346.
6. Okwu DE. Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. J Sustain Agric Environ. 2004; 6(1): 30-37.
7. Middleton JE, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalia cells: implications for inflammation, heart disease, and cancer. Pharmacological Review 2000; 52: 673-751.
8. Singh R, Sawhney SK. Advances in frontier areas of plant Biochemistry, Prentice Hall in India Private Ltd, New Delhi, 1988.
9. Khare CP. Indian medicinal plants: an illustrated dictionary. Springer-Verlag, Berlin Heidelberg, New York. 2007; 725.
10. Chadha HR. The wealth of India raw materials. Publication and information Directorate New Delhi, CSIR, XI. 2003; 11.
11. Sinha SC. Medicinal plants of Manipur.1996; 199.

12. Shirwaikar A, Shenoy R, Udupa AL, Shetty S.Wound healing property of ethnolic extract of leaves of *Hyptis suaveolens* with supportive role of antioxidant enzymes. Indian J Expt. Biol. 2003; 41: 238-241
13. Gavani U, Paarakh PM. Antioxidant activity of *Hyptis suaveolens* Piot. Int J Pharmacol. 2008; 4(3): 227-20029.
14. Shenoy C, Patil MB, Kumar R. Wound healing activity of *Hyptis suaveolens (L)* Poit (Lamiaceae). Int J Pharma Res. 2009;1(3):737-744.
15. Peerzada N. Chemical composition of the essential oil of *Hyptis suaveolens.* Molecules. 1992: 165-1687.
16. Yadav RNS, Munin A. Phytochemical analysis of some medicinal plants. Journal of phytology. 2011; 3(12): 10-14.
17. Aiyegroro OA, Okoh AI. Preliminary phytochemical screening and in vitro antioxidant activities of aquous extract of Helichrysum longifolium DC. BMC couple And Alt.Med. 2010; 10:21.
18. Harbone JB. Phytochemicals methods. Chapunan and Hall Ltd, London. 1973; 49 -188.
19. Andu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of Lophira lanceolata (O Chanaceae). Life Science Journal. 2007; 4(4): 75-79.
20. Manjulika Y, Sanjukta CH, Sharad KG, Geeta W. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. International Journal of pharmacy and pharmaceutical Science. 2014; 5: 539-542.
21. Barku VYA, Opoku Y, Owusu AE Mensh EF. Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract *Amaranthes Spinosu*. Asian Journal of Plant Science and Research 2013; 3(1): 69-74.
22. Lemino Kh, Ranjit L, Grihanjali P, Romabati N, Somorjit L, Bag GC. Comperative study of phytochemical constituents and total phenolic content in the extracts of three different species of Genus Hedychium. Internationl Journal of pharmatech Research. 2013; 5(2): 601-606.
23. Lincoln. Measurement of total phenolics and ABTS assay for antioxidant activity. Crop Reseach Institute Report. New Zeland, 2001.
24. Chakraborthy GS, Ghorpade PM. Free radical scavenging activity of *Abutilon indicum* (L)Sweet Stem extracts. Internationl Journal of Chemtech Research .2010; 2(1): 526-531.

25. Saravanakumar A, Venkateshwaran R, Vanitha J, Ganesh M, Vasudevan M, Sivakumar T. Evaluation of antibacterial activity,phenol and flavonoid contents of *Thespesia populnea* flower extracts. *Pak.j Pharm.Sci*.2009 ; 22(3) : 282-286.
26. Chang C, Yang M, Wen H, Chem J .Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal*.2002; 10: 178-182.
27. Nguyen Q, Eun J. Journal of Medicinal Plants Research.2011; 5(13): 2798-2811.
28. Soladoye MO, Chukwuma E. Quantitative phytochemical profile of the leaves of *Cissus populnea* Guill. And perr. (Vitaceae) -an important medicinal plant in central Nigeria. Scholars Research Library.2004; 4(1): 200-206.
29. Swain T. Tannins, Lignins. In:Herbivores: Their Interactions with plant metabolites. Rosenthal GA and D.H Janzen Eds. Academic press, New York, 1979.
30. Schanderi SH) In.Method in Food Analysis. Academic press, New York, 1970; 709.
31. Edeoga HO, Omosun G, Uche LC. Chemical composition of *Hyptis suaveolens* and *Ocimum gratissimum* from Nigeria. African J of Biotechnology. 2006; 5: 892-895.
32. Mensah JK, Okoli RI, Ohaju-Obodo JO, Eifediyi K . Aqueous extract of *Telfairia occidental*is leaves reduces blood sugar and increases haematological and reproductive indices in male rats. Afr. J. Biotechnol. 2008; 7: 2304-2309.
33. Raquel FE. Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. Biochemica et Biophysica Acta. 2007; 2500-2509.
34. Okwu DE. Evaluation of chemical composition of medicinal plants belonging to Euphorbiaceae. Pak. Vet. J. 2001; 14: 160-162.
35. Wangkheirakpam RD, Brojendro SS,Chingakham BS.Antioxidant and anti-dermatophytic properties leaf and stem bark of *Xylosma longifolia* .BMC Complementary and Alternative Medicine. 2013; 13: 155.
36. Ghaffari H, Ghassam BJ, Chandra SN, Ramachandra KK, Prakash HS: Antioxidant and Neuroprotective activities of *Hyptis suaveolens* against oxidative stress-induced Neurotoxicity. Cell Mol. Neurobiol. 2014; 34: 323-331. .
37. Reddy M.K, Gupta SK, Jacob M.R Khan,S.I; Ferreira,D. Antioxidant,antimalarial and antimicrobial activities of tannin rich fractions,ellagitannins and phenolic acids from *Punica granatum I .Planta Med.2007,73,461-467.*
38. Marjorie C. Plant products as antimicrobial agents. Clincal Microbiol Rev.1996; 12:564-582.

39. Ansarullah, Menaka T, Ravirajsinh J, Ranjitsinh D, Ramachandran AV. *Oreocnide integrifolia* exhibits hypoglycemic and Hypolipidemic potentials on Streptozotocin diabetic rats. Excli J. 2009; 8:97-106.
40. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci. 2007; 950-988.