www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.2, pp 1068-1073, April-June 2010

Phytochemical investigation and Antipyretic activity of leaf extract of *Vitex negundo* Linn

J. Raama Murthy*[1], S. Venkataraman[1], R. Meera[1], Kiran S.Desmukh[1], N. Chidambaranathan[2], P.Devi[3]

[1]Department of Pharmaceutical Chemistry, [2]Department of Pharmacology, [3]Department of Pharmacognosy, K.M. College of Pharmacy, Uthangudi, Madurai – 625 107. TamilNadu, India

Corres.author: jraamamurthy@yahoo.co.in, meeraharsa@yahoo.com

Abstract: To compare the phytochemical constituents in the leaves and determine their antipyretic effect.The leaves of *Vitex negundo* collected from the Aritapatti village, Madurai district, Tamilnadu, were dried milled and extracted with petroleum ether and methanol. Phytochemical screening was carried out according to standard procedures. Antipyretic activity was determined by using yeast induced hyperpyrexia method. The activity was examined by treating different groups of male rabbit with single (2.5 g/kg) of oral doses of petroleum ether extract and methanol extract .Paracetamol (200 mg/kg) was used as positive standard in the study. 2ml of 1% CMC was used as control.Out of the both extract the methanol extract (250 mg/kg) significantly good antipyretic effect and the values are significant to $p< 0.01$.*Vitex negundo* leaf methanol extract possesss excellent antipyretic effect in rats.

Keywords: *Vitex negundo,* Antipyretic activity, Yeast induced pyrexia , Tannins, Coumarins.

Introduction

The problem of uncontrolled pain led early humans to seek remedies from any materials that they could lay their hands on. In recent times ,focus on plant research has increased and non steroidal anti inflammatory drugs constitute one of the most widely used classes of drugs. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. Herbal medicines are in line with nature ,with less hazardous reactions[1].

Vitex negundo Linn (Synonym : *Viteex incise* Linn, *Vitex incise Lam Var hetropylla.,* family : Verbanaceae). A large , aromatic shrub or sometimes a small slender tree, upto

4.5 m in height found throughout the greater part of India. Leaves possess anti-inflammatory, analgesic and antihistamine properties. Roots are used for leprosy, dyspepsia, rheumatism and piles. Bark is used as verminosis and opthalmopathy .Flowers are used in cholera. Fruit used as anthelmintic. The whole plant is used in inflammations, antiseptic, antipyretic and diuretic [2,3,4,5,6,7,8].

Earlier studies have shown that the plant possess anti-inflammatory and antihistamine[9], analgesic[10], antioxidant[11], antibacterial[12], CNS depressant[13], antifungal[14], snake venom neutralization[15], mosquito repellant activity[16], insecticidal[17], larvicidal efficacy[18], antinociceptive[19],antiandrogenic[20], hepatoprotective[21], antifertility[22], skin aging inhibitor[23] and anti dopaminergic[24] effects. Constituents previously isolated from the plant include eight lignans[25] (negundin A, negundin B, 6-hydroxy-4-(4-hydroxy -3 methoxy)-3- hydroxyl methyl -7 methoxy -3,4 di hydro 2 napathaldehyde, vitrofolal, (+) – iynoiresinol, (+) – iynoiresinol -3α-0-β-Dglucoside, (+)(-)(-) pinorecinol and (+) –diasyringaresinol, irridoid glycoside[26] (2-p-hydroxy benzoyl mussaenosidic acid), flavonones[27] (5,3' di hydroxyl -7,8,4' trimethoxy flavonone and (5,3' dihydroxy -6,7,4' trimethoxy flavonone), flavone[28] (vitexicarpin), β –sitosterol[29], essential oils[30] (α –pinene, linalool, terpinyl acetate,beta caryophyllene), non diterpene[31]

(vitedoin B), pentacyclic triterpenoids[32](beutinilic acid, ursolic acid) and flavanoid glycoside[33] (luteolin, agnuside, negundoside, iso-orientin).

At present, there was no known scientific study reported in available literature sources that has been carried out so far on antipyretic activity of the plant extract. Therefore, this study is aimed at exploring the plant, *Vitex negundo* for their therapeutic potentials in pyretic effect.

Materials and Methods

Collection and identification of plants

Vitex negundo plant materials were collected from Aritapatti village near Madurai district, in December 2008 and was identified by Dr.Stephen, Proffessor American college, Madurai, Tamilnadu; a voucher specimen has been deposited at the herbarium unit of the Department of Pharmacognosy, K.M.College of pharmacy, Madurai, Tamilnadu, India.

Extraction and phytochemical screening of plant

The powdered plant materials (500g) was extracted with petroleum ether at 40-60° C, by continous hot percolation using soxhlet apparatus. The extraction was carried out by using solvent of increasing polarity starting from petroleum ether and methanol respectively. The extraction was carried out for 72 hours. The petroleum ether extract was filtered and concentrated to dry mass by using vacumm distillation. A dark greenish brown residue was obtained. The marc left, after petroleum ether extraction was taken and then subsequently extracted with methanol for 72 hours. The methanolic extract was then filtered and concentrated to dry mass. A dark greenish residue was obtained .Phyto chemical screening was performed using standard procedures[34,35,36].

Preparation of column chromotography

Methanol extract obtained from the aerial parts of *Vitex negundo* was adsorbed on silica gel (60-120 mesh) for column chromatography. The slurry was air dried to remove any adsorbed moisture on surface and loaded on the top of the column of silica gel packed with disappearance or appearance of the existing /new spot, visualized on TLC. Various compounds isolated from the extract are listed below along with their spectral data.

Vitex negundo

Compound A (4', 5, 7, trihydroxy-3'-0-β glucornic acid-6" methyl ester, 1)

Elution of the column with benzene and hexane fraction (1:9) yielded dark greenish residue 30mg. It was recrystallized from benzene. R_f. 0.60 (chloroform :Ethylacetate) (8:2) mixture. M.P- 162^0c [37]. IR spectrum of compound A exhibited a band at 3422 (O-H Stretching), 2919 (C-H Stretching) , 2850 (C-H Stretching) , 1736 (C=O Stretching), 1462 (C-H Bending) , 1381 (C-H Bending), 737 (Bending out of plane), NMR ($CDCl_3$) (ppm): Spectrum showed signals of 1.246 (CH_2 proton attached to alkyl group), 2.4 (Olefinic protons desheilded by oxygen), 6.9 and 7.3 (Aromatic proton) .The UV-spectra showed peaks in the region-250, 280 and 410 nm. UV□ max at 250nm was observed in compound –A.

Compound B (Negundoside, 2)

Elution of the column fraction of benzene, chloroform (8:2) yielded 25mg of greenish brown semisolid residue. It was recrystallized from methanol. Rf: 0.80 (Chloroform: Ethylacetate) 8:2 mixture. M.P- 175^0c .I.R. Spectrum showed the bands at 3448 (O-H Stretching), 2922,2852 (C-H Stretching, 2371 and 2372 (Extended resonance), 1738 (C=O Stretching), 1460 (C-H Bending).The NMR-Signals ($CDCl_3$ □ppm) at 0.780-0.991 (CH_3 proton), 1.146-1.860 (CH_2 proton attached to alkyl group), 2.315-2.798 (CH_2 proton nearer to the carbonyl group, 7.253 (Aromatic proton).

Compound C (2'P-hydroxy benzoyl mussaenosidic acid)

This was obtained as reddish green in colour as semisolid from the fraction of methanol, ethylacetate (3:7) yielded 40 mgs of compound. Rf: 0.61, Chloroform; ethyl acetate(8:2) M.P-210-215 ^{0}C [38]. I.R Spectrum showed the bands at 3477cm^{-1} (OH-Stretching); 2926, 2893cm^{-1} (-C-H-stretching); 1701 cm^{-1} C=O-stretching; 1458 cm^{-1}C-H Bending, 669 Bending (Out of plane) HNMR Signals (□ppm) ($CDCl_3$) 0.824-0.993-R-CH_3 protons (methyl groups); 1.244-1.324 R-CH_2 protons attached to alkyl group; 1.407-1.937 (CH proton attached to C=C group), 2.030-2.296 (Desheilded olefinic proton near to oxygen), 3.660 (Vinyl proton which are non coupled), 7.255 (Aromatic proton). The UV-absorption spectra showed peaks at 230,280 and 330 nm.

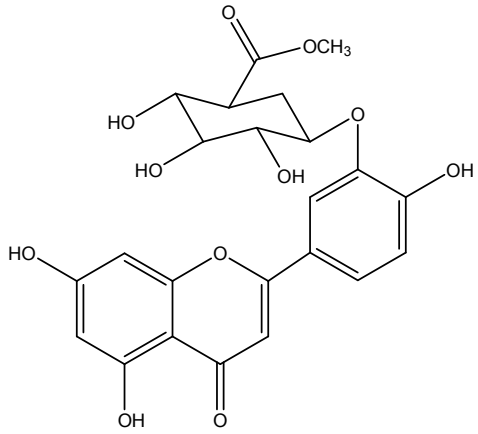
Compound D (Agnuside)

This was obtained as dark green in colour as semisolid from the fraction of chloroform , ethylacetate (9:1) yielded 45 mgs of compound. Rf 0.81, Chloroform; ethyl acetate(8:2) M.P-110-115 ^{0}C . I.R Spectrum showed the bands at 3448cm^{-1} (OH-Stretching); 2923, 2852cm^{-1} (-C-H-stretching), 1718, 1658 cm^{-1} C=O-stretching; 1460,1368cm^{-1}C-H Bending, 1267 cm^{-1} C-O Stretching and O-H Bending , 757cm^{-1} C-H Bending (Out of plane) HNMR Signals (□ppm) ($CDCl_3$) 1.244-R-CH_2 protons (methyl groups); 1.544-1.648 (C-H proton attached to C=C), 2.037-2.787 (CH_2 proton adjacent to carbonyl group like CH_2-C=O), 3.7-3.857 (CH_2 proton attached to C-O),7.257 (Aromatic proton).

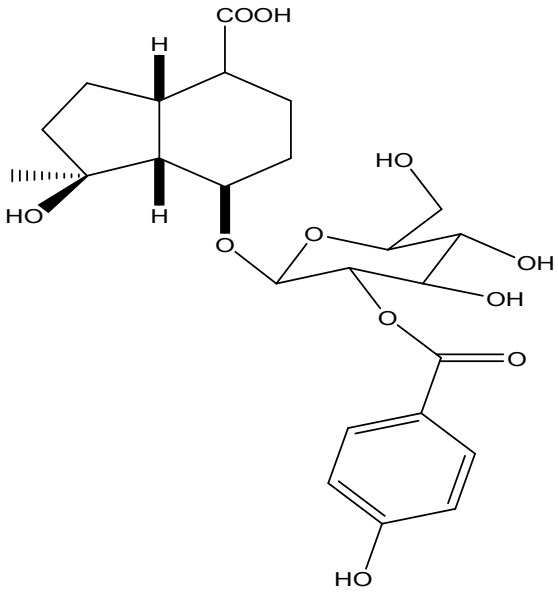
Phytchemical investigation of compounds

Vitexnegundo

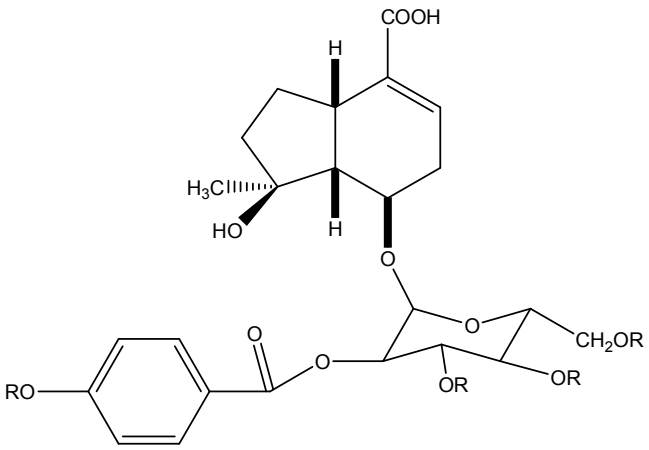
1. Compound A (4',5,7-trihydroxy 3'-o- β glucornic acid 6''methyl ester)



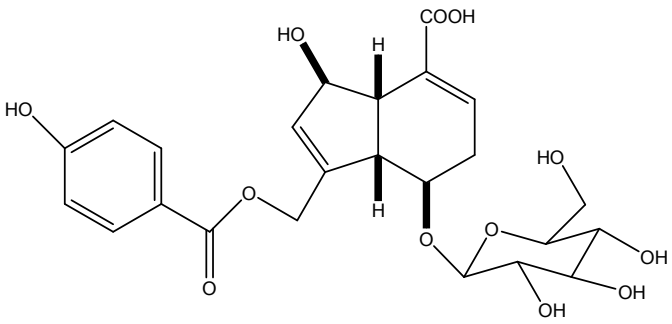
2.Compound B (Negundoside)



3.Compound C (2'p-hydroxy benzoyl mussaenosidic acid)



4.Compound D (agrruside)



Evaluation of Antipyretic activity[39,40,41,42]

The antipyretic activity of the petroleum ether and methanolic extract of *Vitex negundo* Linn was screened by using Yeast induced hyper pyrexia method. Newzeland strains of male rabbit weighing between 1000-1500 gm were selected and divided into 4 groups having three animals. They were maintained at a constant temperature of 24-25 ° C for 24 hrs. Pyrexia was induced by S.C injection of 2 ml of brewers yeast suspension in saline solution. After 12 hours of yeast injection the both extracts were suspended with 1% CMC and administered orally. Group 1 received 2 ml of 1% CMC. Group 2 received 200 mg/kg paracetamol administered through I.P. Group 3 received 2.5 g/kg of petroleum ether extract of *Vitex negundo* Linn. Group 4 received 2.5 g/kg of methanolic extract of *Vitex negundo* Linn. Rectal temperature were noted at 1 hr interval upto 4 hrs.

Statistical analysis[43]

The data were statistically analysed using one way ANOVA followed by Newman levels multiple range test for individual comparison of groups with control. 'P' values below 0.01 were considered as significant. Here all values of statistical analysis are expressed as mean ± SEM.

Results

Preliminary phytochemical screening of both petroleum ether and methanol extract revealed the presence of Tannins, Flavanoids, Flavones and Coumarins. The results of antipyretic parameters for preliminary phyto chemical screening of extracts are shown in Table 1.So far as an impact study on pyrexia induce by yeast is concerned, the drug also exhibits a significant decrease of body temperature and this decline is well comparable with the standard drug (Paracetamol) tested here. The animals show significant increase of rectal temperature after 12 h of administration of yeast. After administration of the drug on 12th of yeast induction a rise in body temperature is well visible in the animals. But one hour after administration of the plant extract (*Vitex negundo*) as well as the standard (Paracetamol) body temperature declines. This decline in case of *Vitex negundo* was not as sudden as paracetamol administration and therefore, can be considered more suitable.

Discussion

In general non steroidal anti inflammatory drugs produce their antipyretic action, through inhibition of prostaglandin synthesis within the hypothalamus[44,45]. Therefore it appears that antipyretic action of methanolic extract of *Vitex negundo* may be related to the inhibition of prostaglandin synthesis in hypothalamus. Fever may be a result of infection or one of the sequels of tissue damage, inflammation, graft infection or other diseases states. Antipyretics are drugs which reduce elevated body temperature .Regulation of body temperature requires a delicate balance between the production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevated and drugs like paracetamol don't influence body temperature when it is elevated by factors such as exercise or increase in ambient temperature[46]. The present study reveals that the leaf extract of *Vitex negundo* causes a significant antipyretic effect in yeast provoked elevation of body temperature. In the cases, the methanol extract caused a significant lowering of body temperature, with the effect being comparable to that of paracetamol. Thus the present pharmacological evidence provides support for the folkfore claim as an antipyretic agent. Flavonoids are known to target prostoglandins which are involved in the late phase of acute inflammation, pyrexia and pain perception[47]. Flavonoids reduces lipid peroxidation by preventing or slowing the onset of cell necrosis and by increasing the vascularity[48]. Hence the presence of flavanoids in the methanol extract of *Vitex negundo* may be contributory to its antipyretic activity.

Conclusion

The methanol extract of *Vitex negundo* has antipyretic effect supporting the ethno pharmacological use as antipyretics. This effect may be explored in the use of the plant in the management of some other diseases.

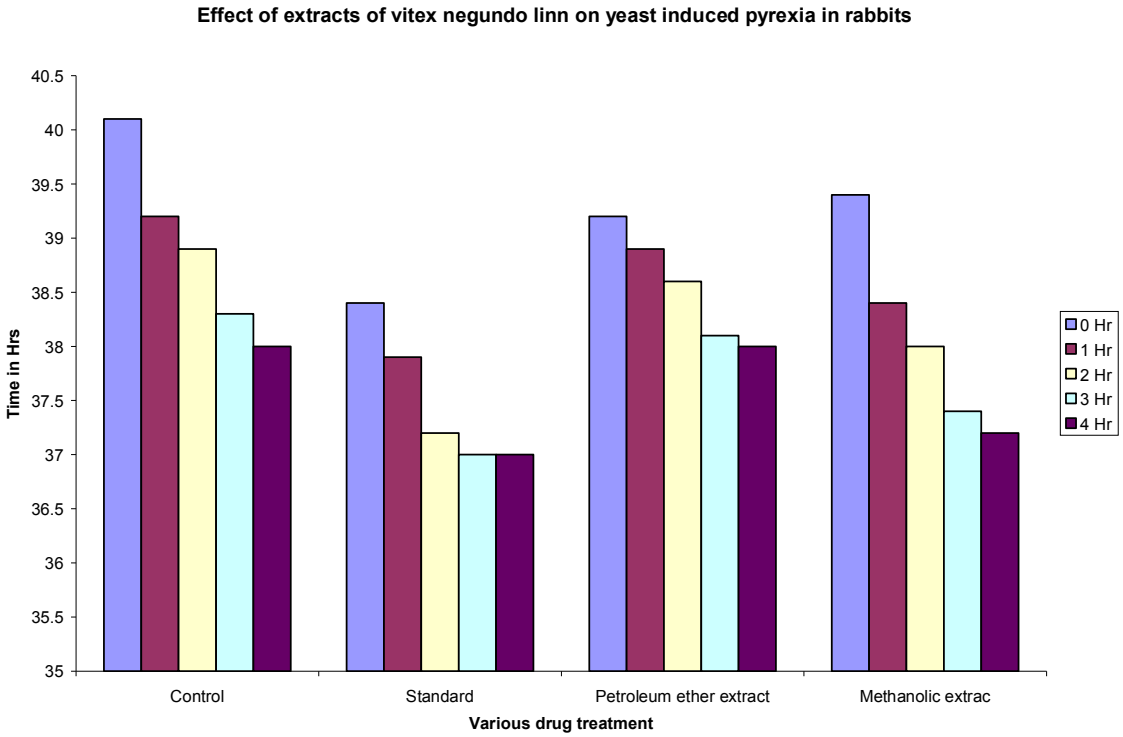
Table: Effect of leaf extracts of *Vitex negundo* Linn on yeast induced pyrexia in rabbits

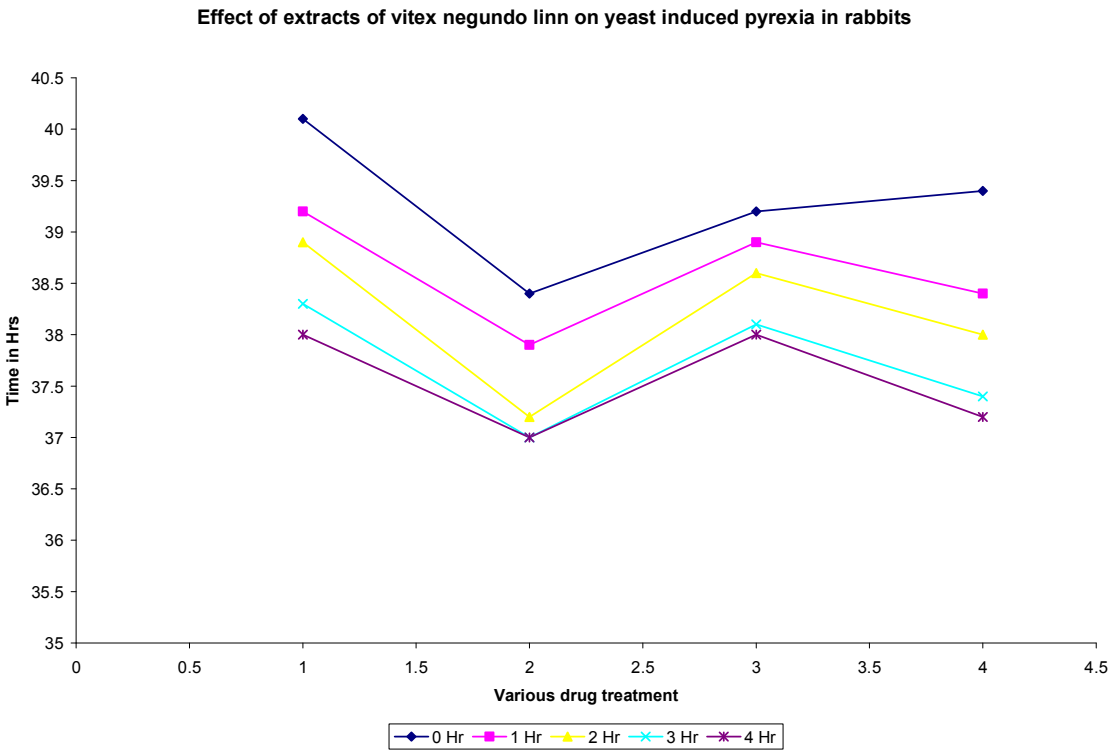
Treatment	Body wt.	Dose	Time in hrs				
			0hr	1hr	2hr	3hr	4hr
Group I	1-1.5Kg	2 ml of 1% CMC	40.1± 0.12	39.2± 0.23	38.9± 0.17	38.3± 0.14	38.00± 0..22
Group II	1-1.5Kg	200mg/Kg Paracetamol	38.4± 0.15	37.9± 0.26	37.2± 0.11	37.0± 0.36	37.00± 0.08
Group III	1-1.5Kg	2.5g/kg	39.2± 0.36	38.9± 0.10	38.6± 0.26	38.1± 0.15	38.00± 0.11
Group IV	1-1.5Kg	2.5g/Kg	39.4± 0.15	38.4± 0.26	38.0± 0.16	37.4± 0.15	**37.2± 022

Values are expressed as mean ± SEM

Values were find out by 7 using ONE way ANOVA followed by Newman levels multiple range test.

**- values are significantly different from control at ($P<0.01$)


Effect of extracts of vitex negundo linn on yeast induced pyrexia in rabbits
Time in Hrs
40.5
40
39.5
39
38.5
38
37.5
37
36.5
36
35.5
35
Control
Standard
Petroleum ether extract
Methanolic extrac
Various drug treatment
0 Hr
1 Hr
2 Hr
3 Hr
4 Hr


Effect of extracts of vitex negundo linn on yeast induced pyrexia in rabbits
Time in Hrs
40.5
40
39.5
39
38.5
38
37.5
37
36.5
36
35.5
35
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Various drug treatment
0 Hr
1 Hr
2 Hr
3 Hr
4 Hr

References

1. Ramarao AV, Gunjar MK. Drugs from plant resources an overview, Pharma times.1990; 22 : 19-27.
2. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products .2002; X: 522-524.
3. Varier. Indian Medicinal Plants .5: 387.
4. Dictionary of Indian Medicinal Plants.1992;
5. Kirthikar KR and Basu .Indian Medicinal Plants.1: 1937-1940.
6. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants.1956; 5: 257.
7. Flora of the Presidency of Madras Gamble. 2: 1101-1102.
8. Deni Bown. The Royal Horticultural Society, Encyclopedia of Herbs and their uses. 221-371.
9. Dharmasiri MG, Jayakody JR,*et al.* Journal of Ethnopharmacology.2003;2-3: 199-206.
10. Telang RS, Chatterjee S, *et al*. Indian Journal of Pharmacology. 1999;31: 363-366.
11. Om Prakash Tiwari, Yamini B, Tripathi. Food Chemistry. 2007;100: 1170-1176.
12. Perumalsamy R, Ignacimuthu S, *et al.* Journal of Ethnopharmacology.1998; 62: 173-182.
13. Ravishankar B, *et al.* Bull.Med.Ethnobot.Res. 1985; 6(1): 72-92.
14. Sanjay Guleria and Ashok Kumar. Journal of Cell and Molecular Biology.2006;95-98.
15. Alam MI, Gomes A. Journal of Ethnopharmacology. 2003;86: 75-80.
16. Hebblkar DS, Hebbalkar GD, *et al.* Indian Journal Med. Res. 1992;95:200-3.
17. Desmukh PB, *et al.* Pestisides. 1982; 16(2): 7-10.
18. Gupta RK, Tandon VR. Indian Journal of Physiol. Pharmacol.2005; 49(2): 163-70.
19. Bhatgava SK. Journal of Ethnopharma - cology.1989; 27(3): 327-339.
20. Avadhoot Y, Rana AC. Arch.Pharm.Res. 1991;14(1): 96-98.
21. Bhargava SK, *et al.* Phytother.Res. 1986; 20(2): 188-198.
22. Chiba Katsuyoshi, *et al.* Patent Abstract of Japan.2002;
23. Sadekar SS, Chauhan VB, *et al.* Journal of Natural Remedies.2007;6(2): 165-169.
24. Azar-ul-Haq, Malik A, Khan M.T,H. *et al.* Phytomedicine.2006;(13)255-260.
25. Sehgal CK, Taneja KL, Dhar, *et al.* Phytochemistry. 1982;21(2): 363-366.
26. Basudeb Achari, Uday S, Chawdhary, *et al.* Phytochemistry.1984;23(3):703-704.
27. Fredyc Diaz, Daniel chavez, *et al.* Journal of Natural Product.2003;66: 865-867.
28. Taralkar SV, Chattopadhay S, *et al.* Chem.Engg. Dept.,Dr. Babasaheb Ambedkar Tech.University,Lonere-402103.Rajgad,Maharashtra.
29. Pan JG, Xu ZL, Fan JF, *et al.* Zhongguo Zhong Yao Za Zhi. 1989;14(6):357-9,383.
30. Ono M, Nisidha Y, *et al.* J.Nat.Product. 2004;67(12): 2073-5.
31. Chandramu C, Manohar RD, *et al.* Phytother Res. 2003;17(2):129-134.
32. Elango V, Ambujavalli L. Fitoterapia. 1985; 56,300.
33. Davies LL, Raventos J, Walpole AL, Brit. J.Pharmacy.1946;1,246.
34. Sofowora A. Medicinal plants and Traditional Medicine in Africa. Spectrum books Ibadan.1993;150.
35. Trease GE, Evans WC. Pharmacognosy. 11th edn.Bailliere Tindall, London,1978;176-180.
36. Harbone JB, Phytochemical Methods. Chapman and Hall Ltd, London, UK.(1st eds). 1973;49-188.
37. Sathyamoorthy B, Prasoon Gupta et al. Bio organic and Medicinal chemistry Letters.2007; 17,239-242.
38. Sehgal CK, Taneja SC, Dhar KL. Phyto - chemistry.1982; 21(2),363-366.
39. Elango V, Ambujavalli L. Fitoterapia. 1985; 56,300
40. Davies LL, Raventos J, Walpole AL. Brit.J.Pharmacy.1946;1,246.
41. Pal PL, Nandy A. Indian Drugs. 1999; 36,106.
42. Brownlee G. J. Pharm. Pharmacol. 1937; 10,609.
43. Armitage P.Eds., In; Statistical Methods in Medical Research, Black Well Scientific Publication, London.1971;217.
44. Clark WO, Cumby HR. The antipyretic effect of indomethacian J.Physiol.1975;248: 625-38.
45. Zell R, Krupp P.In:Schorbaum E, Lomax P, Jacob J.eds:Temperature regulation and drug action, Basel, S.Karger. 1975;233-241.
46. Goodman and Gillman. The Pharmacological basis of therapeutics, Joel G. Hardman, Lee. Limbrid eds.9th ed,1996;620.
47. Rajnarayana K, Reddy MS, Chaluvadi MR. Bioflavonoids clarification, Pharmacological and biopharmacol.2001;33:2-16.
48. Manjunatha BK, Vidhya SM, Krishna V,Mankani KL. Wound healing activity of Leucas hirta, Ind.J.HARM.Sci. 2006; 60(3),380-384.

*****