ORIGINAL RESEARCH PAPER

Botany

SCREENING OF PHYTOCHEMICAL CONSTITUENTS AND GC-MS STUDY OF VITEX NEGUNDO L., USED AS AN ANTI-TYPHOID PLANT IN SOME AREAS OF JHARKHAND

KEY WORDS: Phytochemical screening, Vitex negundo L., GC-MS analysis

Rima Julie Bhaunra* Department of Botany, Ranchi University, Ranchi, Jharkhand, India. *Corresponding Author

Ajay Kumar Srivastava Head, Department of Botany, St. Xavier's College, Ranchi-834001, Jharkhand, India.

ABSTRACT

Indigenous populations the world over, use plants as an important healthcare resource and remedies for different diseases. *Vitex negundo L.* (family: Verbenaceae) is a widely used plant in folk medicine. This study was aimed to perform the preliminary phytochemical and GC-MS analysis of *Vitex negundo L.* which is responsible for its pharmacological properties. The extract was subjected to qualitative phytochemical screening using standard procedures. Results show that eleven of twelve phytochemicals screened for were present. They are alkaloids, flavonoids, saponins, steroids, phenols, tannins, carbohydrates, glycosides, proteins, terpenoids and phlobatannins. The GC-MS analysis of the *Vitex negundo L.* leaves ethanol extract revealed that Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester was the major compounds, with an area of 31.42%. The diversity of phytochemicals found present suggests that *Vitex negundo L.* may serve as a source of useful drugs. Thus, our results encourage the potential use of *Vitex negundo L.* as a medicinal product.

INTRODUCTION

Green plants always fascinate us with their remarkable source of biochemical components. Our society is slowly progressing towards herbal formulations, which in practice are known to be extremely valuable against a large array of diseases. In the current pandemic scenario, sustainable green products particularly antiviral, antioxidant and antibacterial in nature are gaining worldwide fame in almost every walk of life (Kaur and Mahale, 2022). Presence of these compounds makes them commercially significant. Now a days, many pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost-effective medicines that are affordable to the common man (Raj and Rajeswari., 2016).

Vitex negundo L. belongs to the family of Verbenaceae and grows as small tree with thin grey bark and green leaves. *Vitex negundo L.*, commonly known as the Chinese chaste tree, five-leaved chaste tree, or horseshoe *vitex*. It is a large aromatic shrub, widely used in folk medicine, particularly in South and South Asia. The common name of *Vitex negundo L.* is Nirgundi, Nishinda, Samalu (Smrity *et al.*, 2019).

Figure 1: *Vitex negundo L.* leaves

Ethnobotanically, it is believed that *Vitex negundo L.* is used for the treatment of jaundice, wounds, body ache, toothache, asthma, eye pain and migraine (Kadir *et al.*, 2013; Halevy *et al.*, 1994; Sharma *et al.*, 2004; Hebbar *et al.*, 2004; Basavaraju *et al.*, 2009; Jain and Puri, 1984; Bhandary *et al.*, 1995). Leaf extracts of *Vitex negundo L.* possess anti-oxidant potential and antifungal activities previously reported by Tiwari and Tripathi, 2007; anthelminthic, dysmenorrheal, medication and pain suppressing activity, anti-hyperglycemic activity, anti-filarial, anti-bacterial and opposed plant activity (Sharma, 2005; Jadhav and Bhutani, 2005; Dharmasiri *et al.*, 2003; Raj *et al.*, 2008; Sathiamoorthy *et al.*, 2007; Samy *et al.*, 1998; Aswar *et al.*, 2009). Keeping this in view, the present study has been conducted to explore the preliminary phytochemical analysis of *Vitex negundo L.* which is responsible for its pharmacological properties and the chemical constituents were studied by GC-MS.

MATERIALS AND METHODS

Collection Of Plant Material

Leaves of *Vitex negundo L.* were collected from local area of Ranchi (Jharkhand). Plant material (leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water. Then the plant material was shed dried without any contamination for about 3 to 4 weeks. Dried plant material was ground using motor and pestle to obtain fine powder. The powdered samples was packed in air tight container and stored for phytochemical and biological studies.

Preparation Of Plant Extracts

10 g of sample was taken in a glass stoppered 250 ml flask. 100ml of methanol was added. the flask were shaken occasionally for 6 h and kept for 10 days with frequent shaking. The mixtures were decanted and filtered with the help of filter paper and thus obtained filtrates were kept in a beaker wrapping with aluminium foil containing small pores to facilitate the evaporation of the solvent. After complete evaporation of the solvent, semisolid extracts were obtained and stored in a refrigerator (Ingle *et al.*, 2017).

Preliminary Phytochemical Screening

The aqueous leaves extract was screened by different chemical tests to investigate the active compounds present in them (Raj and Rajeswari, 2016; Harborne, 1998; Anbalagan *et al.*, 2017; Balalakshitha and Kolanjinathan, 2021; Shaikh and Patil, 2020).

GC-MS analysis

The GC-MS identification was carried out to the ethanolic extract.

RESULTS AND DISCUSSION

Phytochemical analysis

Result obtained for qualitative screening of aqueous leaf extracts of *Vitex negundo L.* is presented in Table 1. Of the twelve phytochemicals screened for, eleven were found present, they are alkaloids, flavonoids, saponins, steroids, phenols, tannins, carbohydrate, glycosides, proteins, terpenoids and phlobatannins. Remarkably anthocyanins were not detected. These compounds have significant application against human pathogens, including those that cause enteric infections (El-Mahmood *et al.*, 2008).

Kaur and Mahale 2022; result reveals that alkaloids, iridoids, flavonoids, tannins were present but saponins and terpenoids were absent in the aqueous extraction.

Chitra *et al.,* 2009; reported that the preliminary phytochemical analysis carried out on the crude ethanol extract indicated the presence of alkaloids, glycosides, lignin, flavonoids, and saponins.

Paria *et al.,* 2012; also investigated the presence of various phytochemicals and found that leaf extracts contain different chemical constituents i.e. reducing sugar, tannins, phenols, sterols, saponins and flavonoids.

Table 1: Phytochemical Analysis Of Aqueous Leaf Extract Of *Vitex negundo L.*

S.No.	Phytoconstituents	Aqueous leaf extract
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Steroids	+
5	Phenols	+
6	Tannins	+
7	Carbohydrate	+
8	Glycosides	+
9	Proteins	+
10	Terpenoids	+
11	Phlobatannins	+
12	Anthocyanins	-

Keys; + =Positive/Present; - = Negative/Absent

GC-MS analysis

Total 45 compounds were identified and the detailed peak identification is shown in Figure 1. The retention time (RT), peak area (%), molecular formula, name of compound and molecular weight are given in Table 2.

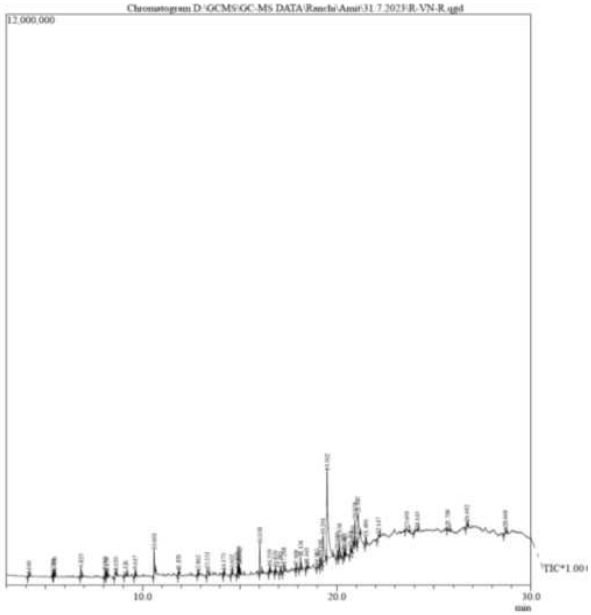

Figure 2: Chromatogram of *Vitex negundo L.* leaves by GC-MS

Table 2: Phytocomponents Identified In The Ethanolic Extract Of The Leaves Of *Vitex Negundo* L. by GC-MS

Peak	RT	Area %	Molecular formula	Name of the compound	Molecular weight
1	4.146	0.39	$C_{11}H_{24}$	Undecane	156
2	5.396	0.35	$C_{15}H_{18}N_2O_4$	Pyridine-3-carboxylic acid, 1-[(bicycle[4.1.0]heptane-7-carbony)amino]-6-oxo-1,6-dihydro-, methyl ester	290
3	5.456	0.64	$C_{12}H_{26}$	DODECANE	170
4	6.825	1.11	$C_{13}H_{28}$	TRIDECANE	184
5	8.066	0.93	$C_{16}H_{32}$	1-HEXADECENE	224
6	8.170	0.61	$C_{15}H_{32}$	PENTADECANE	212
7	8.620	1.57	$C_{15}H_{24}$	Caryophyllene	204
8	9.126	0.35	$C_{12}H_{22}O$	2-Octenal, 2-butyl-	182
9	9.617	0.78	$C_{14}H_{22}O$	Phenol,3,5-bis(1,1-dimethylethyl)-	206
10	10.601	4.02	$C_{10}H_{18}O_2$	Cyclooctanol, acetate	170
11	11.851	0.58	$C_{11}H_{20}O_3$	Cyclopentane-1-carboxylic acid, 2-hydroxy-1,2,3-trimethy-, ethyl ester	200
12	12.862	0.39	$C_{17}H_{34}$	1-Heptadecene	238
13	13.331	1.62	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296
14	14.173	0.64	$C_{20}H_{34}O$	Thunbergol	290
15	14.602	0.64	$C_{14}H_{22}O$	3-BUTEN-2-ONE, 3-METHYL-4-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)-	206
16	14.897	0.54	$C_{18}H_{36}O_2$	HEXADECANOIC ACID, ETHYL ESTER	284
17	14.944	0.79	$C_{20}H_{32}O_3$	2-(2,4-DITERT-PENTYLPHENOXY)BUTANOIC ACID	320
18	14.992	0.78	$C_{15}H_{26}O$	(7A-ISOPROPENYL-4,5-DIMETHYL-OCTAHYDRO-INDEN-4-YL)-METHANOL	222
19	16.038	4.04	$C_{20}H_{40}O$	Phytol	296
20	16.539	0.84	$C_{20}H_{38}O_2$	Ethyl Oleate	310
21	16.839	0.62	$C_{20}H_{36}O_2$	1-Naphthalenepropanol, .alpha.-ethenyldecahydro-2-hydroxy-.alpha.,2,5,5,8a-pentamethyl-,[1R-[.alpha.(R*),2.beta.,4a.beta.,8a.alpha.]]-	308
22	17.091	0.67	$C_{20}H_{36}O_2$	1-Naphthalenepropanol, .alpha.-ethenyldecahydro-2- hydroxy-.alpha.,2,5,5,8a-pentamethyl-,[1R-[.alpha.(R*),2.beta.,4a.beta.,8a.alpha.]]-	308
23	17.268	1.26	$C_{15}H_{26}O$	(-)-Globulol	222
24	17.909	0.39	$C_{10}H_8F_3NO_3$	acetamide,N-[4-(acetyloxy)phenyl]-2,2,2-trifluoro-	247
25	18.134	1.59	$C_{14}H_{20}O_3$	(1Ar-(1 aalpha,5abeta,9ar(*)))-5a,9,9-trimethyloctahydrobenzo(d)cycloprop(c)oxepin-2,4-dione	236
26	18.445	0.57	$C_{15}H_{26}O$	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	222
27	18.983	0.43	$C_{13}H_{24}O$	TETRAHYDROEDULAN C	196

28	19.146	1.66	$C_{10}H_9NO_2$	1H-INDOLE-3-ACETIC ACID	175
29	19.291	9.32	$C_{26}H_{52}O_2$	2-Ethylbutyric acid, eicosyl ester	396
30	19.502	31.42	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330
31	20.019	1.61	$C_{14}H_{22}O_3$	(E)-4-Hexenoic acid, 2-acetyl-2-(2-buten-1-yl)-,ethyl ester	238
32	20.138	2.65	$C_{20}H_{30}O_3$	Oxymesterone	318
33	20.363	0.51	$C_{14}H_{18}F_7O_2$	3-Heptafluorobutyryl-(-)-camphor	348
34	20.412	0.63	$C_{20}H_{32}$	ANTI,ANTI,ANTI-3,3,6,6,9,9,12,12-OCTAMETHYL-PENTACYCLO[9.1.0.0(2,4).0(5,7).0(8,10)]DODECANE	272
35	20.701	0.75	$C_{10}H_{19}NO_4$	Glycine, N-butoxycarbonyl-, propyl ester	217
36	20.830	1.18	$C_{13}H_{22}O$	3-BUTEN-2-OL, 4-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)-	194
37	20.938	3.98	$C_{22}H_{36}O_4$	3,4,10a(1H)-phenanthrenetriol, 2-ethenyldodecahydro-2,4b,8,8-tetramethyl-, 3-acetate, [2S-(2.alpha., 3.beta., 4.alpha., 4a.alpha., 4b.beta.,8a.alpha.,10a.beta.)]-	364
38	21.087	9.17	$C_{21}H_{42}O_4$	Octadecanoic acid,2,3-dihydroxypropyl ester	358
39	21.490	1.47	$C_{30}H_{48}O_2$	D:A-FRIEDOOLEANAN-28-AL, 3-OXO-	440
40	22.147	1.07	$C_{13}H_{24}O$	1H-INDEN-4-OL, OCTAHYDRO-3,3A,7,7-TETRAMETHYL-, (3.ALPHA.,3A.ALPHA.,4.BETA.,7A.ALPHA.)-	196
41	23.601	1.43	$C_{28}H_{48}O_2$	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman	416
42	24,145	1.19	$C_{30}H_{52}O$	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methylheptan-2-yl)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene	428
43	25.709	1.06	$C_{38}H_{74}O_3$	9-OCTADECEOIC ACID, 2-(OCTADECYCLOXY)ETHYL ESTER	578
44	26.692	1.68	$C_{29}H_{50}O$	.gamma.-Sitosterol	414
45	28.668	2.07	$C_{29}H_{48}O$	.gamma.-Sitostenone	412
		100.00			

There are 8 specific compounds out of 45 compounds which have shown greater percentage in terms of peak area, viz. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (31.42%), 2-Ethylbutyric acid, eicosyl ester (9.32%), Octadecanoic acid,2,3-dihydroxypropyl ester (9.17%), Phytol (4.04%), Cyclooctanol, acetate (4.02%), 3,4,10a (1H)-phenanthrenetriol, 2-ethenyldodecahydro-2, 4b, 8, 8-tetramethyl-, 3-acetate, [2S- (2.alpha., 3.beta., 4.alpha., 4a.alpha., 4b.beta., 8a.alpha., 10a.beta.)]- (3.98%), Oxymesterone (2.65%), and .gamma.-Sitostenone (2.07%) which is depicted in figure 2. Preetha *et al.,* 2021; revealed the presence of dodecanoic acid, coumarine-3-carbohydrazine, tetradecanoic acid. Trihydroxyisoflavone, hexadecanoic acid, 12-octadecenoic acid and 11-heneicosanone. Tawfeeq *et al.,* 2023; reported the presence of sabinene, pinene as monoterpenes, alpha-farnesene as sesquiterpene, terpenes, pregnan-3,11-diol-20-one and alpha-tocopherol from the hexane extract of *Vitex negundo L.* leaves. Simultaneously, Kumar *et al.,* 2010; revealed the presence of Benzoic acid 3-hydroxy, Ledol, 9,12,15-Octadecatrienoic acid,(Z,Z,Z)-, Vitamin E, 4HPyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Caryophyllene and hexadecanoic acid which might contribute to the medicinal property of the plant. Comparison of results with other available reports revealed that the secondary metabolites present in the shrub varies from place to place and environment to environment (Kumar *et al.,* 2010; Sahare *et al.,* 2008; Dhakal *et al.,* 2009).

CONCLUSION

Based on the result of the present study, it can be concluded that the aqueous extract of the *Vitex negundo L.* leaves extract shows the positive result for the alkaloids, flavonoids, saponins, steroids, phenols, tannins, carbohydrate, glycosides, proteins, terpenoids and phlobatannins. The GC-MS study also showed many phytochemicals Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 2-Ethylbutyric acid, eicosyl ester, Octadecanoic acid, 2,3-dihydroxypropyl ester, Phytol and Caryophyllene which contributes the activities like antimicrobial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory and other activites were present. As, a result of this study, it was concluded that the leaves extract of *Vitex negundo L.*can be used in the pharmaceutical industry to treat various diseases.

Acknowledgement

Authors are thankful to Department of Botany, St. Xavier's college Ranchi for providing laboratory facilities.

REFERENCES

1. Anbalagan, S., Sankareswaran, M., Moorthy, M., Elakkia, B., Fahamitha, E., (2017) Phytochemical analysis and antifungal activity of *Vitex negundo L.* leaf extracts against clinically isolated fungal pathogens. *Indian Journal of Applied Microbiology* 20(2): 119-125.
2. Aswar, P.B., Khadabadi, S.S., Kuchekar, B.S., Rajurkar, R.M., Saboo, S.S., Javarkar, R.D., (2009) In vitro evaluation of anti-bacterial and anti-fungal activity of *Vitex negundo L.* (Verbenaceae). Ethnobotanical Leaflets 13: 962-967.
3. Balalakshitha, M., Kolanjinathan, K., (2021) Phytochemical analysis of *Vitex negundo L.* by TLC, UV-VIS, FTIR techniques. *International Journal of Biology, Pharmacy and Allied Sciences* 10(11): 858-866.
4. Bameta, A., Sanwal, S., Ambwani, S., (2019) Phytochemical screening and antimicrobial activity of *Vitex negundo L.* leaf and stem extracts against bacterial and fungal pathogens. *Int. J. Curr. Microbiol. App. Sci.* 8(12): 1071-1081.
5. Basavaraju, R., Vennel, Raj J., Bhiravamurthy P. V., (2009) Medicinal plant resources of Puttaparthi mandal: Taxonomic overview and need for conservation. Ethnobot. Leaflets 13: 1382-1400.
6. Bhandary, M. J., Chandrashekar, K. R., Kaveriappa, K. M., (1995) Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, India. *J Ethnopharmacol* 47: 149-158.
7. Chitra, V., Shrinivas, Sharma., Nandu, Kayande.(2009) Evaluation of Anticancer Activity of *Vitex negundo L.* in Experimental Animals: An in Vitro and in Vivo Study. *Int. J. PharmTech Res.* 1(4): 1485-1489.
8. Dhakal, R.C., Rajbhandari, M., Kalauni, S.K., Awale, S., Gewali, M.B., (2009) Phytochemical constituents of the bark of *Vitex negundo* L. *Journal of Nepal Chemical Society* 23: 89-92.
9. Dharmasiri, M. G., Jayakody, J.R.A.C., Galhen, G., Liyanage, S.S.P., Ratnasooriyab, W.D., (2003) Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo L. Journal of Ethnopharmacology* 87: 199-206.
10. El-Mahmood, A.M., Doughari, J.H., Chanji, F.J., (2008) Invitro antibacterial activities of crude extracts of *Nauclea latifolia* and *Daniella oliveri*. *Sci. Res. Essay* 3(3): 102-105.
11. Halevy, A., Gold-Deutch, R., Negri, M., Lin, G., Shlamkovich, N., Evans, S., *et al.* (1994) Are elevated liver enzymes and bilirubin levels significant after laparoscopic cholecystectomy in the absence of bile duct injury ? *Ann Surg.* 219: 362-364.
12. Harborne, J.B.,(1998) Phytochemical method, A Guide to Modern technique of Plant Analysis. 3rd Edition, Chapman and Hall. New York:-198.
13. Hebbar, S. S., Harsha, V. H., Shripathi, V., Hegde, G. R., (2004) Ethnomedicine of Dharwad district in Karnataka, India-plants in oral health care. *J Ethnopharmacol*

94: 261-266.

14. Ingle, K.P., Deshmukh, A.G., Padole, D.A., Dudhare, M.S., Moharil, M.P., Khelurkar, V.C., (2017) Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry* 6(1): 32-36.
15. Jadhav, A. N., Bhutani, K. K., (2005) Ayurveda and gynecological disorders. *Journal of Ethnopharmacology* 97: 151-159.
16. Jain, S. P., Puri, H. S., (1984) Ethnomedicinal plants of Jaunsar-Bawar hills, Uttar Pradesh. *J. Ethnopharmacol* 12: 213-222.
17. Kadir, F. A., Kassim, N. M., Abdulla, M. A., Yehe, W. A., (2013) Hepatoprotective role of ethanolic extract of *Vitex negundo L.* in thioacetamide-induced liver

fibrosis in male rats. *Evid Based Complementary Altern Med.* 739850(9).
18. Kaur, M., Mahale, G. (2022) Phytochemical screening, antioxidant activities and quantification of compounds by HPLC of the leaf extracts from two varieties of *Vitex negundo L.* to explore their potential for textile uses. *The Pharma Innovation Journal* 11(3): 1665-1672.
19. Kumar, P.P., Kumaravel, S., Lalitha, C., (2010) Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo L.*. *African Journal of Biochemistry Research.* 4(7): 191-5.
20. Preetha, S.P., Kamalabai, R.A.S., Jayachandran.K.S.,(2021) Qualitative and quantitative phytochemical screening of*Vitex negundo L.* extract using chromatographic and spectroscopic studies. *Nat. Volatiles & Essent. Oils* 8(4): 11949-11961.
21. Raj, P.V., Chandrasekhar, H.R., Vijayan, P., Dhanaraj, S.A., Rao, J.V., Nitesh, K., (2008) In vitro and in vivo hepatoprotective effect of *Vitex negundo L.* leaves. *Pharmacology Online* 3: 281-295.
22. Raj, S., Rajeswari, L., (2016) Preliminary phytochemical screening of *Vitex negundo L.*, Linn. *Journal of Advances in Biological Science* 3(1,2): 11-14.
23. Sahare, K.N., Anandhraman, V., Meshram, V.G., Meshram, S.U., Reddy, M.V., Tumane, P.M., Goswami, K.,(2008) Anti-microfilarial activity of methanolic extract of *Vitex negundo L.* and *Aegle marmelos* and their phytochemical analysis. *Indian Journal of Experimental Biology* 46: 128-131.
24. Samy, R.P., Ignacimuthu S., Sen, A., (1998) Screening of 34 Indian medicinal plants for antibacterial properties. *Journal of Ethno pharmacology* 62: 173-182.
25. Sathiamoorthy, B., Gupta, P., Kumar, M., Chaturvedi, A.K., Shukla, P.K., Maurya, R., (2007) New antifungal glycoside from *Vitex negundo L. Bioorganic and Medical Chemistry Letters* 17: 239-242.
26. Shaikh, J.R., Patil, M.K., (2020) Qualitative tests for preliminary phytochemical screening : An overview. *International Journal of Chemical Studies* 8(2): 603-608.
27. Sharma, P., Chauhan, N., Lal, B., (2004) Observations on the traditional phytotherapy among the inhabitants of Parvati valley in Western Himalaya, India. *J. Ethnopharmacol* 92: 167-176.
28. Sharma, P.V., (2005) Caraka Samhita Chaukhamba Orientalia.
29. Smrity, K. K., Sultana, S., Hassan, A., Hossain, L., (2019) Medicinal activity of *Vitex negundo* L. (Family: Lamiaceae) leaves extract: Assessment of phytochemical and pharmacological properties. *Journal of Pharmacognosy and Phytochemistry* 8(3): 3571-3575.
30. Tawfeeq, T.A., Tawfeeq, A.A., Eldalawy, R., Ibraheem, S.K., (2023) Phytochemical analysis, GCMS identification, and estimation of antioxidant activity of Iraqi *Vitex negundo* L. *Journal of Medicinal and Chemical Sciences* 6: 876-883.
31. Tiwari, O. P., Tripathi, Y. B., (2007) Antioxidant properties of different fractions of *Vitex negundo* Linn. *Food Chemistry* 100: 1170-1176.