World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

COMPARATIVE ANALYSIS OF METHANOL EXTRACT (LEAF) OF VITEX SPECIES USING CHEMICAL AND BIOLOGICAL PARAMETERS.

Dipti Ranjan Behera & Sunita Bhatnagar*

Medicinal and Aromatic Plants, Regional Plant Resource Centre, Ekamra Kannan, Bhubaneswar, India.

***Corresponding Author: Dr. Sunita Bhatnagar**
Medicinal and Aromatic Plants, Regional Plant Resource Centre, Ekamra Kannan, Bhubaneswar, India.

Article Received on 30/08/2023 **Article Revised on 20/09/2023** **Article Accepted on 10/10/2023**

ABSTRACT
Three members of family Lamiaceae and genus Vitex were compared; these were *Vitex agnus-castus*, *Vitex negundo* and *Vitex trifolia.* Methanolic extract of the three species was prepared and subjected to TLC analysis against 12 different solvents. Extract was also evaluated for cytotoxic activity using brine shrimp assay. Amongst the three species only *Vitex negundo* showed cytotoxic activity in 24 hrs. All the species were compared morphologically as well. Each and every parameter used depicted some variations in the three species. Results have been discussed.

KEY WORDS: *Vitex agnus-castus, Vitex negundo, Vitex trifolia,* brine shrimp assay, TLC, Methanol extract.

INTRODUCTION

Vitex genus belongs to family Lamiaceae consists of trees or shrubs widely distributed in tropics and warm temperate regions of both the hemispheres.[1] Fourteen species are available in India and founds mention in the Ayurvedic system of medicines.[2] Most common of the three species is *Vitex negundo* commonly known as nirgundi. Whole plant is used for medicinal purpose as anthelmintic, expectorant, carminative, anti pyretic, anti inflammatory and diuretic.[3] *Vitex agnus-castus* is a native of Mediterranean region commonly known as Monk's pepper, berries of the plant are considered tonic for male as well as female reproductive system.[4] *Vitex trifolia* is commonly known as three leaved chaste tree. Leaves and roots of the plant are used as astringent, expectorant, depurative and useful in vitiated conditions of vata.[5] In the present study all the three species were compared with one another using cytotoxic and TLC fingerprinting.

MATERIALS AND METHODS

Plant collection and preparation of Solvent extract
Fresh leaves of three different species of Vitex i.e, V.agnus-castus(V1), V. negundo(V2) nd V. trioflolia(V3) were collected from the medicinal germplasm garden of Regional plant resource center (RPRC), Bhubaneswar. Leaves were were washed in running tap water to remove dust and impurities. Morphological study of leaves was conducted, parameters studied were color, foliation, shape of margin and length of midrib. After that leaves were shade dried and made into powder using a mechanical grinder. Methanolic extract was prepared using cold percolation method.[6] After extraction the extract was concentrated by using Buchhi(R-200) Rotavapour under vacuum. Extract was stored in screw cap vials till further studies.

TLC analysis of methanolic extract of three species.
TLC plates were prepared on 7cm glass slides using silica gel GF254 (Acme research Laboratory, Bombay). Slides were washed with detergent and dried. Clean and dried slides were wiped with ethyl acetate for removing surface adherents. 3 gms of silica was taken in 20 ml of distilled water and slurry was prepared by constantly stirring, and finally was poured over the slides and slides were left undisturbed till the drying of silica layer. Slides were activated at 100 degree Celsius before running the TLC. A total of 12 solvents were used for TLC analysis and these solvents were as follows:
1) Ethyl acetate : Water: acetic acid(8:1:1)
2) Toluene : ethyl acetate(1:1)
3) Benzene
4) Benzene: Chloroform(1:1)
5) Butanol: acetic acid: water(4:1:5)
6) Chloroform
7) Toluene
8) Acetonitrile
9) Chloroform: acetic acid(9:1)
10) Ethyl acetate: chloroform(1:9)
11) Chloroform: hexane(3:2)
12) Ethyl acetate: benzene(9:1)

Spots were visualized either by naked eye, under UV at 365nm and 254nm or using Iodine vapour. Results were recorded and RF value of all the spots was calculated.
Retention factor= Distance moved by solute from origin/ Distance moved by solvent

Brine shrimp (Artemia salina) mortality assay
Cytotoxic activity study was carried out by brine shrimp lethality assay using standard protocols.[7] Brine shrimp (*Artemia salina*) eggs were hatched in artificial sea water, which was prepared using black salt 3.6 gm/ 200 ml distilled water. The eggs were incubated for 48 hours at temperature of about 28° C to get the desired growth of the larvae for biological evaluation. For each dose level 3 replicates were used. To each test tube of negative control, positive control and extracts, 20 numbers of brine shrimp and volume was made up to 10ml by adding salt water. Cytotoxic assay was carried out at three doses 200, 400 and 800µg/ml. Motility assessment of larvae was conducted at each hour up to four hours.

Motility readings were graded as below.
4+ = high motile
3+ = motile
2+ = sluggish
1+ = slow
Nil = no activity

RESULTS AND DISCUSSION

Three species of Vitex were found to be remarkably different from one another as can be observed in Table 1.*Vitex negundo* leaves possess unique feature with two different colors on the upper and lower surface, where as others are green on both sides. As the name suggested *Vitex trifolia* has been named as per the number of leaves three together. Margins of the two species were serrated while one had smooth. Similarly remarkable variations were observed in the length of midribs of leaves. Thus, on the basis of leaves itself three species can be distinguished.

Table 1. Morphological analysis of Vitex species.

Species/Parameter	***Vitex agnus castus***	***Vitex negundo***	***Vitex trifolia***
Color of leaves	Green on both sides	Purple on one side green on other	Green on both sides.
Number of leaves	5	5	3
Margins of leaves	Serrated	Smooth	Serrated
Size of midrib(middle)	9.88 ± 0.97	11.28 ± 0.35	8.7 ± 0.64
Size of midrib Left a	7.3 ± 1.0	8.04 ± 1.1	6.1 ±0.98
Size of midrib Right a	7.54 ± 0.99	7.88 ± 0.65	6.56 ± 0.72
Size of midrib Left b	2.4 ± 0.7	3.6 ± 0.6	NA
Size of midrib Right b	2.94 ± 0.56	3.4 ± 0.52	NA

Apart from morphological differences TLC fingerprinting also provides means to distinguish between the different species. In the present study a total of 12 solvents were used to see the chemical finger prints of the three Vitex species. As can be seen from Table 2, solvents 1, 2, 4, 8, 10 and 12 showed similar results in all the species hence not suitable for identification purpose of species, but can be used for the identification of genus. In remaining solvents(3, 5, 6, 7, 9 and11) fingerprints were different in the three species but some common points were also there. In two solvent s 2 and 11 all the species sowed varied number of bands but one or two bands were common in all. Thus study has provided insight in the chemical fingerprinting of the species. Bioassay results were not as promising as only one species *Vitex negundo* exhibited activity at a very high dose of 800 microgram/ml, rest of the species were inactive.

Table 2: TLC fingerprinting of three speices of Vitex.

Solvents	***Vitex agnus castus***		***Vitex negundo***		***Vitex trifolia***	
	RF values	No of bands	RF values	No of bands	RF values	No of bands
1. Ethyl acetate: water: Acetic acid(8:1:1)	0.21, 0.31, 0.65,0.71, 0.87, 0.90	6	0.21, 0.31, 0.65,0.71, 0.87, 0.90	6	0.21, 0.31, 0.65,0.71, 0.87, 0.90	6
2. Toluene : ethyl acetate(9:1)	0.08, 0.17,0.26,0.32	10	0.08, 0.17,0.26,0.32,	10	0.08, 0.17,0.26,0.32,	10

	,0.47,0.58,0.61, 0.7, 0.83,0.94		0.47,0.58,0.61, 0.7, 0.83,0.94		0.47,0.58,0.61, 0.7, 0.83,0.94	
3. Benzene	0.1, 0.14	2	0.8, 0.14, 0.18	3	0.14	1
4. Benzene: chloroform (1:1)	Reddish fluorescent streak at Rf 50		Reddish fluorescent streak at Rf 50		Reddish fluorescent streak at Rf 50	
5. Butanol: acetic acid: water(4:1:5)	A complete streak		0.86	1	0.86	1
6. Chloroform	0.076, 0.46	2	0.08, 0.36, 0.48	3	0.8, 0.48	2
7. Toluene	0.1, 0.14	2	0.1,0.14, 0.24, 0.32	4	0.1,0.14	2
8. Acetonitrile	0.32, 0.48	2	0.32,0.48	2	0.32,0.48	2
Chloroform : 9. acetic acid(9:1)	0.1,0.2, 0.34	3	0.1, 0.2	2	0.1, 0.2	2
10. Ethyl acetate: chloroform (1:9)	0.14, 0.56, 0.72, 0.84	4	0.14, 0.56, 0.72, 0.84	4	0.14, 0.56, 0.72, 0.84	4
11. Chloroform: hexane(3:2)	0.08, 0.14, 0.21, 0.35, 0.67	5	0.14, 0.35, 0.71	3	0.08, 0.14, 0.23, 0.67	4
12. Ethyl acetate: Benzene(9:11)	0.24, 0.42, 0.45	3	0.21, 0.36, 0.41	3	0.18, 0.28, 0.33	3

ACKNOWLEDGEMENTS

Authors acknowledge the Forest, Environment and Climate change Department, Government of Odisha for providing funding for the smooth conduct of entire work and also acknowledge Chief Executive, Regional Plant Resource Center, Bhubaneswar, Odisha for providing lab facilities.

REFERENCES

1. Das N, Salgueiro ACF, Choudhury DR, Mandal SK, Logesh R, Hassan MM, Devkota HP.(2022) Traditional uses, phytochemistry, and pharmacology of genus Vitex (Lamiaceae). Phytother Res., 36(2): 571-671. doi: 10.1002/ptr.7330.
2. Thakur U, Ashawat MS.(2023) Potential Phytoconstituents of Genus Vitex for the Management of Rheumatoid Arthritis: A Review. Curr Rheumatol Rev., 19(2): 134-150. doi: 10.2174/1573397118666220919120722.
3. Zheng CJ, Li HQ, Ren SC, Xu CL, Rahman K, Qin LP, Sun YH.(2015) Phytochemical and Pharmacological Profile of Vitex negundo. Phytother Res., 29(5): 633-47. doi: 10.1002/ptr.5303.
4. Van Die MD, Burger HG, Teede HJ, Bone KM.(2012) *Vitex agnus-castus* extracts for female reproductive disorders: a systematic review of clinical trials. Planta Med., 79(7): 562-75. doi: 10.1055/s-0032-1327831.
5. Yan CX, Wei YW, Li H, Xu K, Zhai RX, Meng DC, Fu XJ, Ren X. (2023). *Vitex rotundifolia* L. f. and *Vitex trifolia* L.: A review on their traditional medicine, phytochemistry, pharmacology. J Ethnopharmacol., 308: 116273. doi: 10.1016/j.jep.2023.116273.
6. Behera DR, Bhatnagar S. (2018). Assessment of macrofilaricidal activity of leaf extracts of Terminalia sp. against bovine filarial parasite Setaria cervi. J Infect Public Health., 11(5): 643-647. doi: 10.1016/j.jiph.2018.01.006.
7. Hong D, Ma Y, Song S, Li N, Wang J.(2020) Design, synthesis, brine shrimp lethality and cytotoxicity of some novel 17a-aza-D-homo-androster-17-one derivatives. Nat Prod Res., 35(21): 3985-3991.