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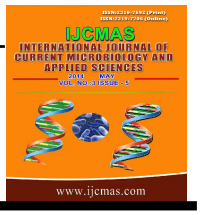

Original Research Article

Screening of Antibacterial Activity and Qualitative and Quantitative analysis of Phytochemicals in *vitex trifolia*

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A B S T R A C T

The present focus of the study was to evaluate the antibacterial activity and phytochemical analysis in the medically important plant *vitex trifolia* leaf extract by using solvents like aqueous, methanol, acetone, benzene, petroleum ether. The plant *vitex trifolia* is used for its phytochemical and pharmacological studies. In qualitative and quantitative analysis of *vitex trifolia* viz., alkaloids, saponin, tannin, phenols, terpenoids, flavonoids, steroids, were determined using standard methods. *Vitex trifolia* leaves were collected, air dried and soxhlet extracted by using standard method. These extracts were then tested for antimicrobial activity using disc diffusion method. *Vitex trifolia* showed highest antibacterial activity (IZ= 0.5cm in acetone extract, IZ = 0.75 in ethanol extract) against *Bacillus subtilis* and (IZ= 0.15cm in acetone extract, IZ = 0.25cm in ethanol extract, IZ = 0.35cm in aqueous extract) against *Escherichia coli*. Results concluded that the phytochemicals of leaves extract of *vitex trifolia* has the potential to act as a antibacterial agent.

Keywords

Vitex trifolia, Anti-bacterial activity, Zone of inhibition, Phyto-chemicals

Introduction

Vitex trifolia is found in tropical and sub-tropical regions, although *vitex* species may be found in temperate zones. *Vitex trifolia* belongs to genus *vitex* and family *Verbenaceae* there are approximately 270 known species of shrubs and trees (Manjunatha, et al., 2007). *Vitex trifolia* posses larvicidal, wound healing, anti-HIV, trypanocidal, anticancer activity (Ramasamy, et al., 2009). It is also used as a anti-bacterial, anti-inflammatory, antipyretic agent. They are also used as sedative for rheumatism, headache and common cold in some countries. *Vitex* species used in traditional medicine to

treat ailments like wounds, allergies, asthma and body pains (Thenmozhi, et al., 2013).

Materials and Methods

Colletion of plant materials

The fresh leaves of *vitex trifolia* were collected from well grown trees in Tamilnadu Agriculture University, Katuthotam, Thanjavur district, Tamilnadu. The fresh leaves were washed twice in ordinary water and in distilled water. The washed leaves were shade dried in room temperature for 15 to 20 days.

Preparation of extracts from leaves of *vitex trifolia*

Selected organic solvents were used for the extraction of phytochemical compounds from leaf materials of vitex trifolia. The shade dried leaves are finely powdered using blender[12]. 15gm of the powdered leaf were first extracted with the solvent petroleum ether for defatting the extract using soxhlet apparatus. The defatted powder were dried and extractions were carried out with the solvents petroleum ether benzene, acetone, ethanol and distilled water using soxhlet apparatus successfully[2]. Vaccum rotatory evapourator was used to evapourate solvents and the concentrated extracts were obtained. The concentrated extracts were subjected to qualitative and quantitative test for the identification of phytochemicals present in the leaf of vitex trifolia. The concentrated extracts were also used for the antibacterial test.[2]

Qualitative analysis of phytochemical in *vitex trifolia*

Saponin test: Take water extract and add 5ml of dis.H_2O in a test tube and it was vigorously shaken. Add few drops of olive oil. The stable foam formation was taken as an indication for the presence of saponin[3].

Tannin test: 2ml of water extract was treated with 0.1% of $FeCl_3$ solution and observed for blue black colouration for the presence of tannins[5].

Alkaloids test: 2ml of methanol extract was taken with 1%HCl, 1ml filtrate with 6 drops of Wagner's reagent (1.27gm of iodine, 2gm of potassium iodide and 100ml of water). Formation of brownish red precipitation confirms the presence of alkaloids[7].

Phenol test: 400ml of the crude acetone extract was treated with 5ml of dis.H_2O and 5% ferric chloride solution and observed for formation of dark greenish colour[3].

Flavonoids test: 3ml of crude chloroform extract was treated with few drops of 20%sodium hydroxide solution for the formation of intense yellow colour. Addition of dilute hydrochloric acid becomes colourless which indicates the presence of flavonoids[9].

Quantitative analysis of phytochemicals in *vitex trifolia*

Saponin test: The leaf sample was powered and 4gm of each were put into a conical flask and add 20% aqueous ethanol. The samples were heated using water bath for 4 hours at 55°C with continuous stirring. The mixture was filtered and it was re-extracted with another 200ml of 20% ethanol. The extracts were reduced to 40ml using water

bath at 90°C. The concentrate was transferred into separator funnel and add 20ml of diethyl ether and shaken vigorously. The ether layer was discarded while the aqueous layer was recovered. The purification process was repeated once. Add 60ml of n-butanol. The combined n-butanol extracts were washed two times using 9ml of 5% aqueous sodium chloride. The remaining solution was remained in hot water bath. After evapouration the samples were dried in the hot air oven to obtain the constant weight. The saponin content was calculated[7].

$$\text{Percentage of total saponin} = \frac{\text{Weight of residue} \times 100}{\text{Weight of the sample taken}}$$

Tannin test: The sample of 500mg was weighed into a 50ml plastic bottle. Add 50ml of dis.H_2O and shaken for 1hr using mechanical shaker. This was filtered in a volumetric flask of 50ml. From the filtered 5ml was pipetted out into a test tube and mixed with 2ml of 0.1M ($FeCl_3$) in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm for 10min[5].

Alkaloids test: 5gm of the dried powder of leaf sample was weighed into a 200ml of 10% acetic acid in ethanol and covered, the mixture was allowed to stand for 4hr. The mixture was filtered. The extract was concentrated on a hot waterbath to attain one-quarter of the original volume.

Add concentrated ammonium hydroxide dropwise to the extract until the precipitation was complete. The solution was allowed to settle in the bottom of the beaker. The precipitate was collected and washed using dilute ammonium hydroxide and then filtered. The residue formed was the alkaloid which was dried, weighed and percentage was calculated[5].

Phenol test using spectrophotometric method: The leaf sample was boiled with 50ml of ether for 15 min to extract the phenolic component. From the extract 5ml was pipetted into a 50ml flask and add 10ml of distilled water + 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol. The samples were marked and left to react for 30minutes for colour development. This was measured at 505nm using a spectrophotometer[3].

Flavonoid test: 10gm of leaf sample was repeated extracted with 100ml of 80% aqueous methanol at room temperature. The solution was filtered using Whatman filter paper No 42. The filtrate was transferred into a waterbath. The solution was then evapourated into dryness over a water bath and weighed to a constant weight[1].

Antibacterial test

Preparation of bacterial culture suspension

The bacterial cultures were incubated on a nutrient agar slant (stationary culture) for 48hours at 37°C. The stationary cultures were maintained by inoculation in Muller Hinton Agar(MHA) medium.

Antibacterial Activity test

Antibacterial activity was evaluvated by disc diffusion method[11] The tests were done using sterile disc [Whatmann no.1] of 6mm diameter which was loaded with 50µl of various crude solvent extracts (concentration of 100 mg/ml) on the muller hinton agar plate surface which was previously inoculated with 10ml of muller

hinton liquid medium with cultures *Bacillus subtilis* and *Escherichia coli* (inoculum size 1×10^9 CFU/ml).

Solvents without plant extract were treated as negative control. Kanomycin is used as the positive control. The plates were incubated in an incubator at 37°C for 24 hours[11].

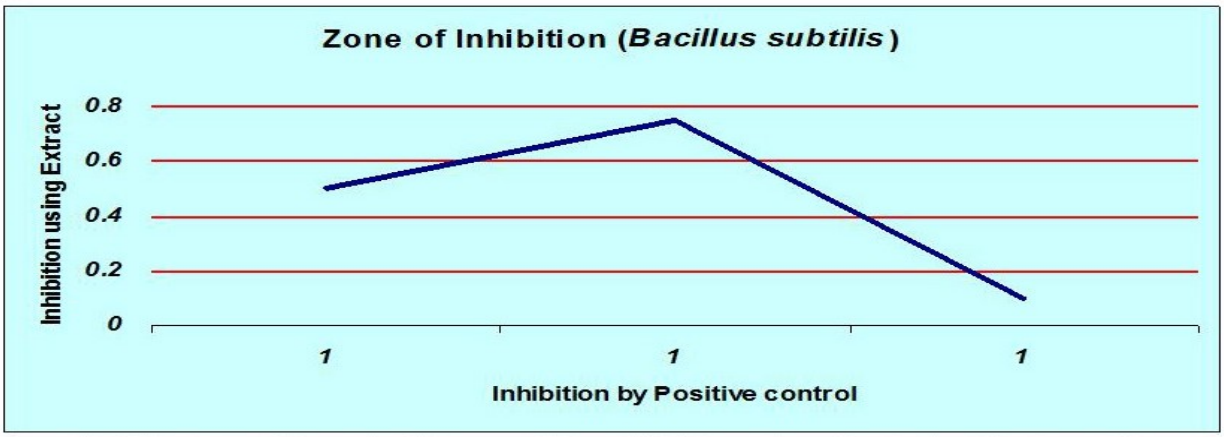
Results and Discussion

The qualitative and quantitative study of solvent extracts of *vitex trifolia* leaves revealed the presence of phytochemicals. Whereas, the petroleum ether extract showed the presence of saponin and steroids. Benzene extract showed the presence of alkaloids (0.25mg), flavonoids(0.367mg), phenol(0.30mg), steroids, tannins(0.231mg) and terpenoids(0.461mg). Acetone extract showed the presence of flavonoids(0.432mg), steroids, tannins(0.521mg) and terpinoids(0.321mg). Ethanol extract showed the presence of alkaloids, flavonoids(0.232mg), phenols(0.163mg), saponins(0.123mg), tannins(0.461mg) and terpenoids(0.361mg). Finally water extract showed the presence of flavonoids(0.423mg) and tannins(0.62mg).

The antibacterial activity of the different extracts was done by disc diffusion method. The extracts have shown different degrees of antibacterial activity. In *Bacillus subtilis* the acetone extract of *V trifolia* have shown the zone of inhibition at 0.5cm, the ethanol extract of *V.trifolia* have shown the zone of inhibition at 0.75cm and aqueous extract have shown the zone of inhibition at 0.1cm.

In *Escherichia coli* the acetone extract of *V.trifolia* showed the zone of inhibition at 0.15cm, the ethanol extract of *V.trifolia* have shown the zone of inhibition at 0.25cm, the Dis.H_2O extract of *V.trifolia* have shown the zone of inhibition at 0.35cm.

Chart.1 Antibacterial Activity Test **(**Zone of Inhibition Graph (*Bacillus subtilis*))



Inhibition in Positive control (Kanamycin) 1cm; Inhibition using solvent extract Acetone – 0.5cm; Ethanol – 0.75cm; Dis.H2O – 0.1cm

Table.1 Qualitative analysis of Phytochemicals in *Vitex trifolia*

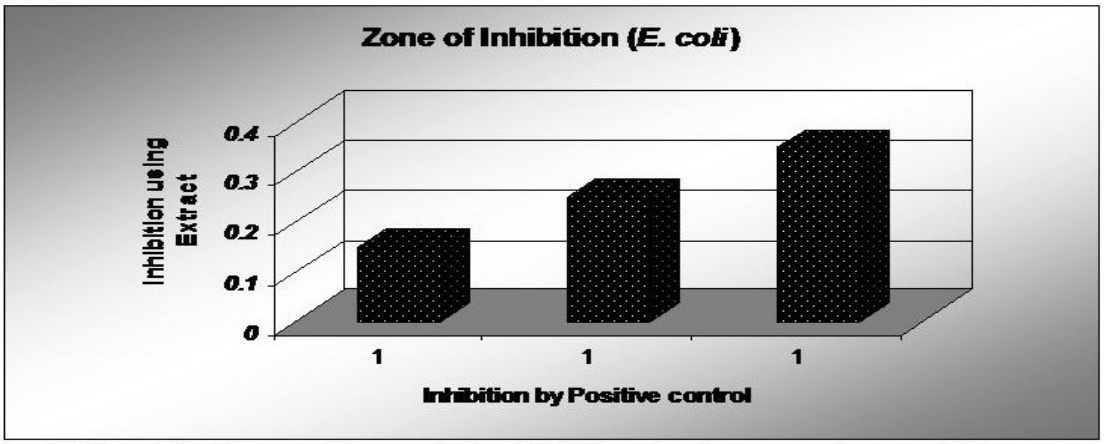
Solvents	Alkaloids	Flavonoids	Phenols	Saponins	Steroids	Tannins	Terpenoids
Petroleum ether	-ve	-ve	-ve	+ve	+ve	-ve	-ve
Benzene	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Acetone	-ve	+ve	-ve	-ve	+ve	+ve	+ve
Ethanol	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Water	-ve	+ve	-ve	-ve	-ve	+ve	-ve

POSITIVE(+ve) ; NEGATIVE(-ve)

Table.2 Quantitative Analysis of Phytochemicals in *Vitex trifolia* (weights in milligrams)

Solvents	Alkaloids	Flavonoids	Phenols	Saponins	Steroids	Tannins	Terpenoids
Petroleum ether	-	-	-	0.362	0.412	-	-
Benzene	0.25	0.367	0.30	-	-	0.23	0.461
Acetone	-	0.432	-	-	+ve	0.521	0.321
Ethanol	-	0.232	0.163	0.123	0.362	0.461	0.361
Water	-	0.423	-	-	-	0.62	-

Chart.2 Antibacterial Activity Test **(**Zone of Inhibition Graph (*E.coli*))



Inhibition in Positive control (Kanamycin) 1cm; Inhibition using solvent extract Acetone – 0.15cm; Ethanol – 0.25cm; Dis.H2O – 0.35cm

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References

1. Syed, M.M.S., Farhat, A.K., Syed, M.H.S., Kamran.A.C., Syed.M.S.S.P. and Muhammad.A.K.A. 2011. Evaluation of Phytochemicals and Antimicrobial Activity Of White and Blue Capitulum and Whole Plant of Silybum Marianum. World Applied Sciences Journal. 12(8): 1139-1144.
2. Murugan, M. and Mohan.V.R. 2012. Efficacy of Different Extracts of Vitex trifolia L. and Aristolochia indica L. for Potential Antibacterial Activity. Science Research Reporter. 2(1): 110-114.
3. Amin, M. M., Sawhney, S. S. and Jassal, M. M. S. 2013. Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. Wudpecker Journal Of Pharmacy and Pharmacology. 2(1): 001- 005.
4. Ganesh, N. S., Susheel, K.D., Nitin, S. and Jyotsana, S. 2011. Phytochemical Screening and Estimation of Total Phenolic Content in Aegle marmelos Seeds. International Journal of Pharmaceutical and Clinical Research. 3(2): 27-29.
5. Ajiboye, B. O., Ibukun, E. O., Edobor, G., Ojo, A. O. and Onikanni, S. A. 2013 Qualitative and quantitative analysis of phytochemicals in senecio biafrae leaf. Int.J.Inv.Pharm.Sci. 1(5): 428-432.
6. Anjali, S. and Sheetal, S. 2013. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. Journal of Pharmacognosy and phytochemistry. 2(4): 22-29.
7. Kumar, A. and Bhardwaj, A. 2012. Comparative, qualitative and quantitative chemolytic characterization among north Indian Tribulus terrestris. International research journal of pharmacy. 3(6): 2230-8407.
8. Manoj, K., Amit, K., Sukumar, D. and Sinha, M. P. 2013. Phytochemical screening and antioxidant potency of Adhatoda vasica and Vitex negundo. An

International Quarterly Journal of Life Sciences. 8(2): 727-730.

9. Solomon, C. U., Arukwe, U. I. and Onuoha, I. 2013. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala. Asian Journal of Plant Science and Research. 3(3): 10-13.
10. Ramasamy, A., Balasundaram, J., Biusan, K., Seevalen, B., Rajappan, M. and Raju, S. K. 2009. Effect of ethanol extract of flowers of Vitex trifolia Linn on CCL_4 induced hepatic injury in rats. Pak. J. Pharm. Sci. 22(4): 391-394.
11. Gautam, K. and Kumar, P. 2012. Evaluation of phytochemical and antimicrobial stidy of extracts of Vitex negundo Linn. Int. J. Drug Dev. & Res. 4(4): 192-199.
12. John, D. B. A., Herin, S. G. D. and Benjamin, J. R. K. P. 2013. Qualitative and quantitative analysis of phytochemicals in Marsilea minuta Linn. Int. J. Pharm. Bio. Sci. 4(1): 800-805.
13. Herin, S. G. D., John, D. B. A and Benjamin, J. R. K. P. Qualitative and quantitative analysis of phytochemicals in five Pteris species.
14. Esath, S. N., Sekar, C., Amuthara, P.,Syed, A. R.M. and Feroz, K. K. 2012. Evaluation of antibacterial activity of Morinda citrifolia, Vitex trifolia and Chromolaena odorata. African Journal of Pharmacy and Pharmacology. 6(11): 783-788.
15. Thenmozhi, S., Vibha, K., Dhanalakshmi, M., Manjuladevi, K. and Diwedi, S. 2013. Evaluation of Anthelmintic activity of Vitex trifolia Linn. leaves against Pheretima posthuma. International Journal of Pharmaceutical & Biological Archives. 4(5): 878 – 880.
16. Mona, A. M ., Allia, M. A., Manal, M. H. and Amal, M. S. 2012. Characterization of bioactive phytochemical from the leaves of Vitex trifolia. International Journal of Pharmaceutical Applications. 3(4): 419-428.
17. Manjunatha, B. K., Vidya, S. M., Krishna, V., Mankani, K. L., Singh, S. D. J. and Manohara, Y. N. 2007. Comparative evaluation of wound healing potency of Vitex trifolia L. and Vitex altissima L. Phytotherapy Research. 21(5): 457-461.
18. Ramasamy, A., Balasundaram, J., Biusan, K., Seevalen, B., Rajappan, M. and Raju, S. K. 2009. Effect of ethanol extract of flowers of Vitex trifolia Linn. on CCL_4induced hepatic injury in rats. Pak. J. Pharm. Sci. 22(4): 391-394.
19. Li, W. X., Cui, C. B., Cai, B., Wang, H. Y. and Yao, X. S. 2005. Flavonoids from Vitex trifolia Linn. inhibit cell cycle progression at G2/M phase and induce apoptosis in mammalian cancer cells. Journal of Asian Natural Products Research. 7(4): 615-626.
20. Wen-Xin Li, Cheng-Bin Cui, Bing Cai. and Xin-Sheng Yao. 2005. Labdane-type diterpenes as new cell cycle inhibitors and apoptosis inducers from Vitex trifolia Linn. Journal of Asian Natural Products Research. 7(2): 95-105.
21. Ramasamy, A., Balasundaram, J., Biusan, K., Seevalen, B., Rajappan, M., and Raju, S. K. 2009. Effect of ethanol e+xtract of flowers of Vitex trifolia Linn. on CCL_4induced hepatic injury in rats. Pak. J. Pharm. Sci. 22(4): 391-394.