



Research Article

SCREENING OF ANTIBACTERIAL ACTIVITY, QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS IN VITEX TRIFOLIA

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ABSTRACT

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 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, Antibacterial activity, Zone of inhibition, Muller hinton agar.

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[17]. Vitex trifolia posses larvicidal, wound healing, anti-HIV, trypanocidal, anticancer activity[10]. 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 [15].

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 was 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.H₂O 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 FeCl₃ 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.H₂O and 5% ferric chloride solution and observed for formation of dark greenish color [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 powdered 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 evaporation 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.H₂O 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 (FeCl₃) in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm for 10 min [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 water bath to attain one-quarter of the original volume. Add concentrated ammonium hydroxide drop wise 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 505 nm 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 water bath. The solution was then evaporated into dryness over a water bath and weighed to a constant weight [1].

ANTIBACTERIAL ACTIVITY TEST

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 evaluated 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

Qualitative analysis of photochemicals in *Vitex trifoliata*

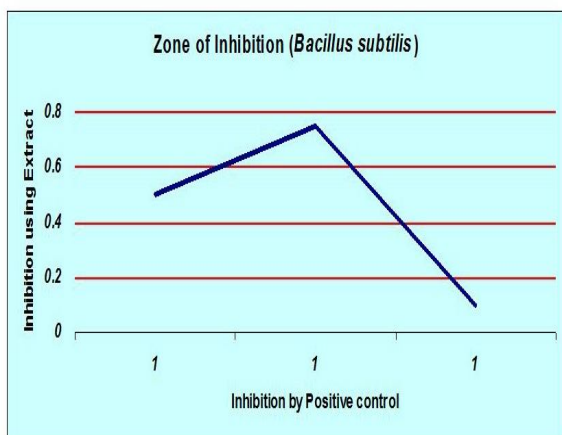
SOLVENTS	ALKALOIDS	FLAVONOIDS	PHENOLS	SAPONINS	STEROIDS	TANNINS	TERPENOIDS
Petroleum ether	-ve	-ve	-ve	+ve	+ve	-ve	-ve
Benzene	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Acetone	-ve	+ve	-ve	-ve	+ve	+ve	+ve
Ethanol	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Water	-ve	+ve	-ve	-ve	-ve	+ve	-ve

Positive (+ve) ; Negative(-ve)

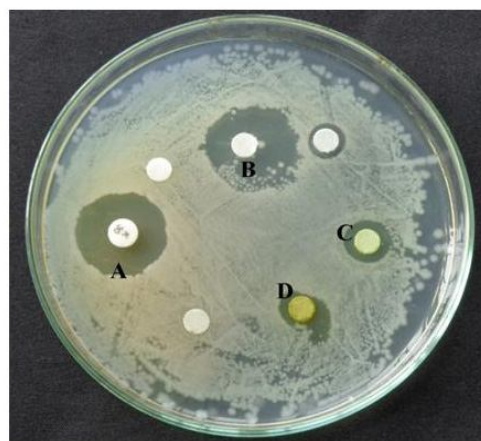
Quantitative analysis of phytochemicals in *Vitex trifolia* (weights in milligrams)

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	-

ZONE OF INHIBITION GRAPH (*Bacillus subtilis*)

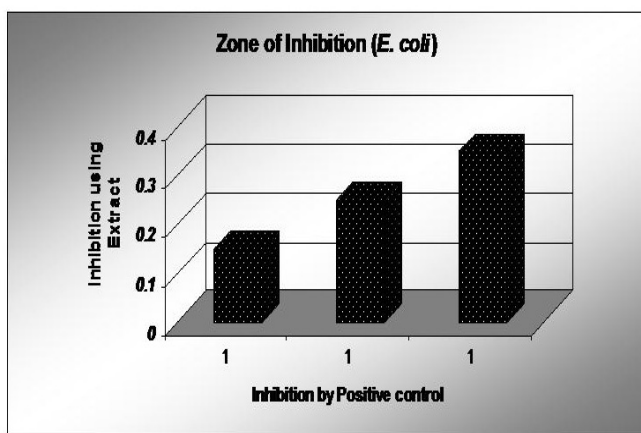


Inhibition in Positive control (Kanamycin) 1cm; Inhibition using solvent extract Acetone - 0.5cm;

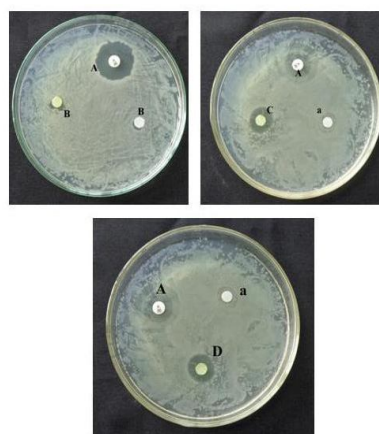


Antibacterial activity in *Bacillus subtilis* using solvent Extract. A. Positive control, B. Ethanol extract, C. Acetone extract, D. Dis H₂O

Zone of inhibition graph (*Escherichia coli*)



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



Antibacterial activity of solvent Extract in *E.coli*. A. Positive control, a. Petroleum ether, B. Acetone, C. Ethanol, D. Dis H₂O.

(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.H₂O extract of *V. trifolia* have shown the zone of inhibition at 0.35cm.

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