PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *VITEX NEGUNDO LINN* AND *VITEX AGNUS CASTUS LINN*

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ABSTRACT:
Medicinal plants are used for treatment because they have certain properties, including synergistic actions. The plants used in traditional medicine contain a vast variety of compounds and those compounds are used to treat chronic and infectious diseases. The active compounds in most parts of the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. The present study revealed that the various alcoholic and aqueous extract of leaf of V*itex negundo linn* and *Vitex agnus castus linn* contained the phytochemical constituents such as flavonoids, tannins, phenolics, sterols, carbohydrates, alkaloids and terpenoids which are the secondary metabolites of medicinal plant. Maximum amount of protein contents are found in the ethanol extract of V*itex negundo linn* and *Vitex agnus castus linn* and minimum amount of saponins are found in the acetyl and aqueous extracts of V*itex negundo linn* and *Vitex agnus castus linn*. Maximum zone of inhibition of was found in chloroform extracts of V*itex negundo linn* against *Escherichia coli* whereas in *Vitex agnus castus linn,* the maximum zone of inhibition was found in ethanolic extract against *Salmonella typhi. Candida parapsilosis* exhibited antifungal activity in all the extracts of both the plant species. No activity was seen in the chloroform and water extract of both the plant species against *Aspergillus niger*.

Keywords: V*itex negundo linn , Vitex agnus castus linn, Escherichia coli, Candida parapsilosis, Aspergillus niger*

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INTRODUCTION

India is rich in natural medicinal wealth, which is the birth place of Ayurveda. It has contributed to its maximum to the world of medicine. Medicinal plants are a precious natural resource, as they provide raw material for pharmaceutical industry, modern and traditional forms of medicine and generate employment and income in addition to conservation of bio-diversity and traditional knowledge (Balick and Mendelshon, 1992; Lambert et al., 1997).

The genus Vitex belongs to the family Lamiaceae, which is mint family of flowering plants with 236 genera and more than 7000 species. It comprises of 250 known species (Boisser, 1888; Rechinger, 1987) distributed in Tropics and Sub Tropics. *Vitex negundo* is native to tropical Eastern and Southern Africa and Asia. It is widely cultivated and naturalized elsewhere. *Vitex*

negundo are commonly found near bodies of water, recently disturbed land, grasslands, and mixed open forests.

Vitex negundo is generally known as Negundo in India. It is also known as the five-leaved chaste tree, is a large aromatic shrub with quadrangular, densely whitish, tomentose branch lets. It is widely used in folk medicine, particularly in South and Southeast Asia. It belongs to family Verbanaceae and is found throughout India. *V. negundo* has been used for various medicinal purposes in Ayurveda and Unani systems of medicine. In Indian traditional medicine system, *Vitex negundo Linn* is referred as sarvaroganivarani- for all diseases (Ladda *et al.*, 2012). *V. negundo a*nd *Vitex agnus –castus* contains number of phytochemicals or bioactive constituents which find ample use in the pharmaceutical industry.

Micro organism i.e. viruses, bacteria and parasites are present every- where among which some are beneficial and some are harmful to us (World Health Organization, Regional Office of South East Asia, 2016). Antibiotics are the medicine that inhibits the growth or destroys the microorganisms. The bacteria which have the genetic ability to transmit and acquire resistance to drugs are used as therapeutic agents (Nascimento). They have also indicated the association of microorganism with some diseases (Agnivesha Charaka *et al*., 2016). In general, the leaves of *Vitex negundo* are reported to possess pesticidal, antifungal and antibacterial properties.

To address this lacuna, the present study was carried out for qualitative phytochemical analysis Ash content, invitro antibacterial and antifungal activities of leaf of *Vitex negundo* and *Vitex agnus –castus* using various alcoholic (chloroform, ethanol, acetone and aqueous) extracts.

Scientific classification: *(Vitex negundo Linn.)*

Kingdom	**Planteae**
Sub kingdom	Tracheobionta
Super division	Spermatophyte
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Asteridea
Order	Lamilales
Family	Verbenaceae
Genus	Vitex
Species	negundo

Scientific classification *(Vitex agnus – causts)*

Kingdom	**Planteae**
Sub kingdom	Tracheobionta
Super division	Spermatophyte
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Asteridea
Order	Lamilales
Family	Verbenaceae
Genus	Vitex
Species	agnus-causts

VERNACULAR NAMES

Telugu : Vaavili
Tamil : Nirkundi, Vellai-nochi
Hindi : Shivari, Nirgundi
Malayalam : Vellanocchi, Indranee, Karunacci
Kannada : Nkkilu, Lakkigida, Nekka, Nakkigida
Punjab : Shwari
Assam : Aslok
Bengal : Nirgundi, Nishinda
English : Five leaved chaste tree
Gujarati : Nagod
Marathi : Nirgundi
Punjabi : Sambhalu, Banna
Sanskrit : Nirgundi

MATERIALS AND METHODS

2.1 Collection of plant materials

The fresh leaves of *Vitex negundo linn* (Plate 1) and *Vitex agnus - causts* (Plate 2) were collected in the month of December 2021 from Kundal, Kanya kumari District, Tamil Nadu, India. Leaves were subjected for washing under the tap water to remove adherent soil, dirt etc for 2-3 times and finally followed by ethanol. Washed and then allowed to shade dry at room temperature for 7 days. Finally leaves of *Vitex negundo linn* and *Vitex aguns* –

causts linn plants were powdered individually to make a coarse powder with mixer grinder. That powder was packed in locked polythene bags, labelled and stored in the tight container for further study .

Plate 1

Leaves of Vitex *negundo linn*

Plate 2

Leaves of *Vitex negundo linn.*

2.2 Preparation of extract

2.2.1 Ethanol extract

20 grams of *Vitex negundo Linn* and *Vitex agnus – caustus linn* powder was extracted with 200 ml of ethanol and filtered with Whatman. No 1 filter paper. Filtered extract was kept at room temperature for elimination of ethanol; 2.29 gram extract was collected and it was

diluted in 10ml ethanol. This extract was stored in a refrigerator for further use and called as the mother solution .

2.2.2 Chloroform extract

The chloroform extract of dried leaves was made in chloroform. About 1.5gm leaf powder were taken and mixed in 15ml of chloroform with continuous stirring. The extract was filtered by Whatman filter paper.

2.2.3 Acetone extract

About 1.5gm plant leaf powder were taken and mixed in 20ml of acetone with stirring. The extract was filtered by whatman filter paper.

2.2.4 Aqueous extract

About 5 grams of plant leaf powder were taken and mixed in 50ml of distilled water and heated for 10 minutes with continuous stirring.

The extract was allowed to cool at room temperature and then filtered by Whatman filter paper.

2.3 Preliminary Phytochemical Analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the various alcoholic and aqueous extract of *Vitex negundo* and *Vitex agnus-castus*. Phytochemical examinations were carried out for all the extracts as per the standard methods.

2.3.1 Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a. Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloid.

b. Hager's Test

Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

2.3.2 Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Benedict's Test

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

b) Fehling's Test

Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

2.3.3 Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

2.3.4 Detection of saponins

Foam Test

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of the presence of saponin.

2.3.5 Detection of tannins

Gelatin Test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

2.3.6 Detection of flavonoids

a) Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, dilute acid, indicates the presence of flavonoids.

b**) Lead acetate Test**

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

2.3.7 Detection of proteins

a) Xanthoproteic Test

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin Test

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid (Roopashree *et al.*, 2008), (Obasi *et al*., 2010) and (Audu *et al*., 2007).

2.3.8 Delection of steroids

Salkowski Test

Extracts were treated with few drops of chloroform and concentrated. sulphuric acid. Formation of bluish red to cherry colour in chloroform layer green fluorescence acid layer indicates the presence of steroids. (Harborne *et al*., 1998).

2.4 Ash test:

Percentage of ash values were performed according to the official methods prescribed in Indian Pharmacopeia, 1996 and the WHO guidelines on quality control methods for medicinal plant. ***Vitex*** was analyzed for total ash, water insoluble ash, acid insoluble ash which gives an idea about amount of organic and inorganic constituents present in the samples.

2.5 Microorganism

Bacteria such as *Escherichia coli* (ATCC 25922), *Salmonella typhi* MTCC 3216, *Bacillus subtilis* MTCC 7164, *Staphylococcus epidermidis* MTCC and fungal such as *Candida parapsilosis, Aspergillus niger* were used for the study.

Standardization of Inoculums

Each culture of the isolates was standardized by culturing on nutrient agar for 24hrs at 37°C. The overnight culture were diluted in normal saline (0.5 w/v) until turbidity marched with 0.5 McFarland standard to give a mean of 3.3*106 Cfu/ml(Deeni *et al*., 1991). Standard disc diffusion method(Murray *et al*., 1995; Zavala *et al*., 1997) was employed to screen for antimicrobial activity of the plant extracts. Muller Hilton agar plates were used for the inoculation of organism. The test organisms were streaked evenly on the surface of the agar plates with the use of sterilized wire loop. With the aid of sterile pair of forceps, impregnated paper discs containing the extracts of materials at different concentrations 3000µg/discs, 2000 µg /discs, 1000 µg /disc and 100 µg /disc were arranged radially and pressed slightly and firmly to the inoculated agar surface to ensure even contact. The plates were incubated at 35°C for 24hrs. The degree of sensitivity was determined by measuring the diameter in millimeter of the visible zone of inhibition of the microbial growth produced by the diffusion of the extracts

2.4.2 Preparation of inoculums

At least four morphologically similar colonies from an agar medium are touched with a wire loop and growth is transferred to a test tube containing 1.5ml of sterile broth. The tubes are incubated for 2 hours at 35°C to37°C to produce a bacterial suspension of moderate turbidity. The density of the suspension is standardized by dilution with sterile saline or both to a density equivalent to the Barium sulphate standard, 5 McFarland units. Before use, the standard should be shaken vigorously.

2.5.3 Inoculation

Plates are inoculated within 15 minutes of preparation of the suspension so that the density does not change. A sterile cotton –wool swab is dipped into the suspension and surplus removed by rotation of the swab against the slide of the tube above the fluid level. The medium is inoculated by even streaking of the swab over the entire surface of the plate in three directions.

2.5.4 Anibiotics discs

After the inoculums has dried, single disc is applied with forceps, a sharp needle or a dispenser and pressed gently to ensure even contact with the medium. When fastidious organisms are to be tested, touch multiple colonies with a loop and cross streak the appropriate plate for uniform distribution.

Not more than six discs can be accommodated on an 85mm circular plate and twelve are easily accommodated on a 135-mm circular plate. Discs should be stored at+4ºC in sealed containers with a desiccant and should be allowed to come to temperature before the containers are opened. Discs should be used before the expiry date on the label. If antimicrobial solution prepared in laboratory are being used, proceed as follows,

1. Pick up a 2mm loopful of the standard antibiotic solution and lower carefully onto a paper disc which, when moistened will adhere to the loop.
2. Place the moistened discs on the surface of inoculated plate in the appropriately labeled segment.
3. Repeat for each antimicrobial agent to be used, placing the impregnated discs in their respectively labeled segments.

NOTE: Take care to avoid inadvertent "contamination" of other discs in the Petri dish with the antibiotic solution.

2.5.5 Incubation

Plates are incubated for 16 to 18 hours at 35 to 37°C aerobically. The diameters of zones ware measured to the nearest millimeter with vernier calipers (preferably) or a thin transparent millimeter scale.

RESULTS

3.1 Phytochemical Analysis

The present study revealed that the various alcoholic and aqueous extract of leaf of *Vitex negundo Linn* and *Vitex agnus - castus Linn* contained the phytochemical constituents such as flavonoids, tannins, phenolics, sterols, carbohydrates, alkaloids and terpenoids are secondary metabolites of medicinal plant that serve as defense mechanism against different infectious diseases. The phytochemical constituents of the selected medicinal plant investigated are summarized in table 1. The ethanol extract of *Vitex negundo linn* showed the presence of phytochemical constituents like Carbohydrate, Glycoside, Tannins, Saponnin, Steriods and Terpenoids which could be responsible for the observed antimicrobial property. The ethanol extract of *Vitex agnus- causts* showed the presence of phytochemical constituent like carbhohydrate, glycoside, tannins, saponin, steroids and terpenoids.

The Chloroform extract *Vitex nigundo linn* showed the presence of phytochemical constituents like Carbohydrate, Saponin, Tannins, Protein, Steroids. The Chloroform extract *Vitex agnus-causts linn* showed the presence of phytochemical constituents like alkaloids, flavonoids and protein.

The Acetone extract of *Vitex nigundo linn* showed the presence of phytochemical constituents like carbohydrate, glycoside, flavonoids, tannins, protein, steroids. The acetone extract *Vitex agnus-causts linn* showed the presence of phytochemical constituents like Carbohydrate, Glycoside, flavonoids, tannin, protein and steroids.

The Aqueous extract of *Vitex nigundo linn* showed the presence of phytochemical constituents like alkaloids, glycoside, tannin, flavanoids, protein, steroids and protein which could be responsible for the observed antimicrobial property. The aqueous extract *Vitex agnus-causts linn* showed the presence of phytochemical constituents like alkaloids, carbohydrate, tannin, flavonoids, protein and steriods.

3.2 Ash Content

The results of ash content in the leaves of *Vitex nigundo linn* and *Vitex agnus-causts linn* are shown in Table.2. The total ash content, which is an index of mineral content was 0.975 in *Vitex negundo linn* and 0.950 in *Vitex agnus-causts linn. Vitex negundo linn* has 0.852% of Acid insoluble ash whereas has 0.865% in *Vitex agnus-causts linn*. Similarly the amount of water soluble ash in *Vitex negundo linn* was 0.080% and 0.075% was found in *Vitex agnus-causts linn.*

3.3 Antibacterial activity

The crude extracts (Ethanol, Chloroform) of *Vitex negundo linn* and *Vitex agnus – castus linn* were screened for antibacterial activities against human pathogens.

Antibacterial activities of two medicinal plants species were assessed in table 2 and 3 and figure 1 and 2, display that control revealed the highest significant antibacterial activity in both the species maximum zone of inhibition was found in the chloroform extract of V*itex negundo* against *Eschericia coli* where as the maximum zone of inhibition was found in the ethanolic ecxtract of *Vitex agnus castus* maximum zone of inhibition was found in both the (ethanol and chloroform) extract of *Vitex negundo* against S*taphylococcus* but in *Vitex agnus - castus* the minimum inhibition was note in chloroform extract against S*almonella typhi*. No activity was seen in any of the *Vitex negundo* extracts against S*almonella typhi* and *Bacillus subtilis*. When we compare both the species, *Vitex agnus castus* has more antibacterial activity than *Vitex negundo* .

3.4 Antifungal activity :

The effect of antifungal activity is shown in table 4 and 5 and figure 3 and 4. Two pathogans namely *Candida parapsilosis* and *aspergillus niger* were used in the study. When compared among the pathogens used, *Candida parapsilosis* exhibited antifungal activity in all the extracts of both the plants species. No activity was seen in the chloroform and water extract of both the plants species against *Aspergillus niger*. The control has showed effective activity in both the plant species against the pathogens *Candida parapsilosis* and *Aspergillus niger*.

Table 1: Preliminary qualitative phytochemical analysis of *Vitex negundo* and *Vitex agnus – castus* leaves

Compounds	Ethanol extract		Chloroform extract		Acetone extract		Aqueous extract	
	Vitex negundo	***Vitex agnus***	***Vitex negundo***	***Vitex agnus***	***Vitex negundo***	***Vitex agnus***	***Vitex negundo***	***Vitex angnus***
Alkaloids	+	+	-	+	-	-	+	+
Carbohydrates	+	+	+	-	+	+	-	+
Glycosides	+	+	-	-	+	+	+	+
Saponins	+	+	+	-	-	-	-	-
Tannins	+	+	+	-	+	+	+	+

Flavonoids	+	+	-	+	+	+	+	+
Protein	+	+	+	+	+	+	+	+
Steriods	+	+	+	-	+	+	-	+

+ present

-Absent

Table 2: Ash content in *Vitex negundo* Linn and *Vitex agnus – castus*

S.No	Parameters	***Vitex negundo* Linn**	***Vitex agnus – castus*.**
1	Total Ash	0.975	0.950
2	Acid insoluble ash	0.852	0.865
3	Water soluble ash	0.080	0.075

Table: 3 Effect of antibacterial activity *of Vitex negundo* Linn.

Pathogen used	**Solvent used**			
	Ethanol	**Chloroform**	**Water**	**Control**
Eschericia coli	14mm	22mm	19	23mm
Salmonella typhi	-	-	-	23mm
S*taphylococcus*	8mm	8mm	-	30mm
B.Subilis	-	-	-	27mm

Table: 4 Effect of antibacterial activity of *Vitex agnus – castus*.

	Solvent used			
	Ethanol	**Chloroform**	**Water**	**Control**
Eschericia coli	14mm	10mm	-	23mm
Salmonella typhi	15mm	8mm	-	23mm
S*taphylococcus*	9mm	-	-	28mm
B.Subilis	-	10mm	-	28mm

Table : 5 Effect of antifungal ativity of *Vitex negundo Linn*.

Pathogen used	**Solvent used**			
	Ethanol	**Chloroform**	**Water**	**Control**

Candida parapsilosis	12mm	15mm	17mm	17mm
Aspergillus niger	7mm	-	-	13mm

Table : 6 Effect of antifungal activity of *Vitex agnus – castus Linn*.

Pathogen used	**Solvent used**			
	Ethanol	**Chloroform**	**Water**	**Control**
Candida parapsilosis	10mm	12mm	13mm	9mm
Aspergillus niger	7mm	-	-	14mm

Fig: 1 Antibacterial activity of *V.negundo Linn.*

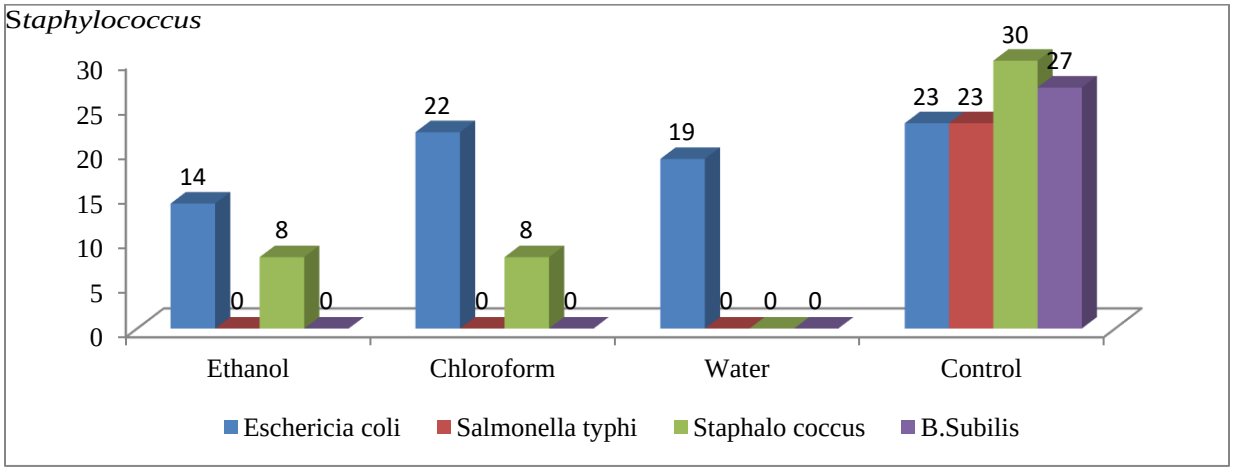


Fig : 2 Antibacterial Activity of *V.agnus-castus.*

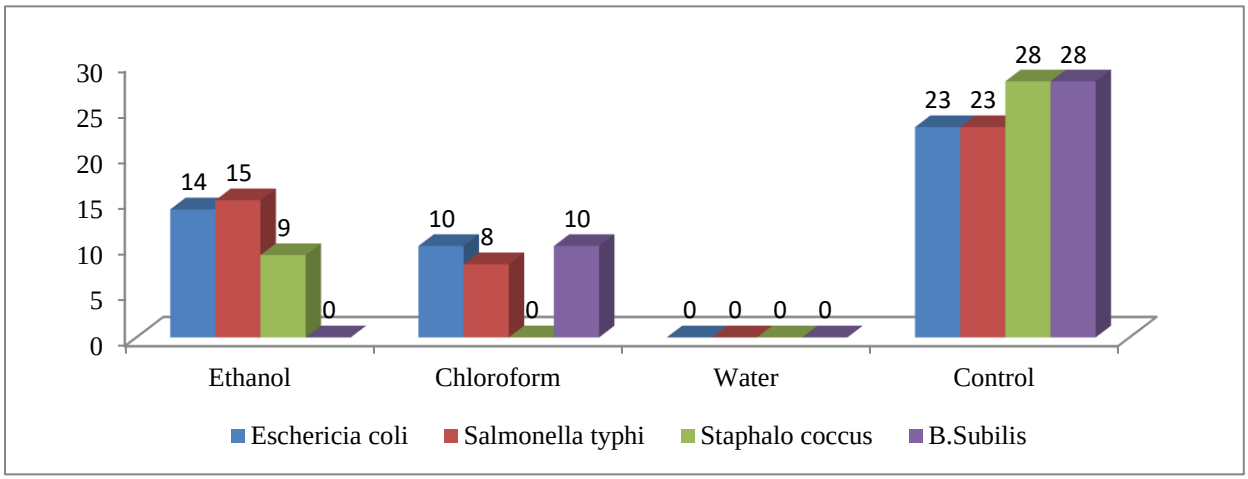


Fig:3 Antifungal activity of *V.negundo*

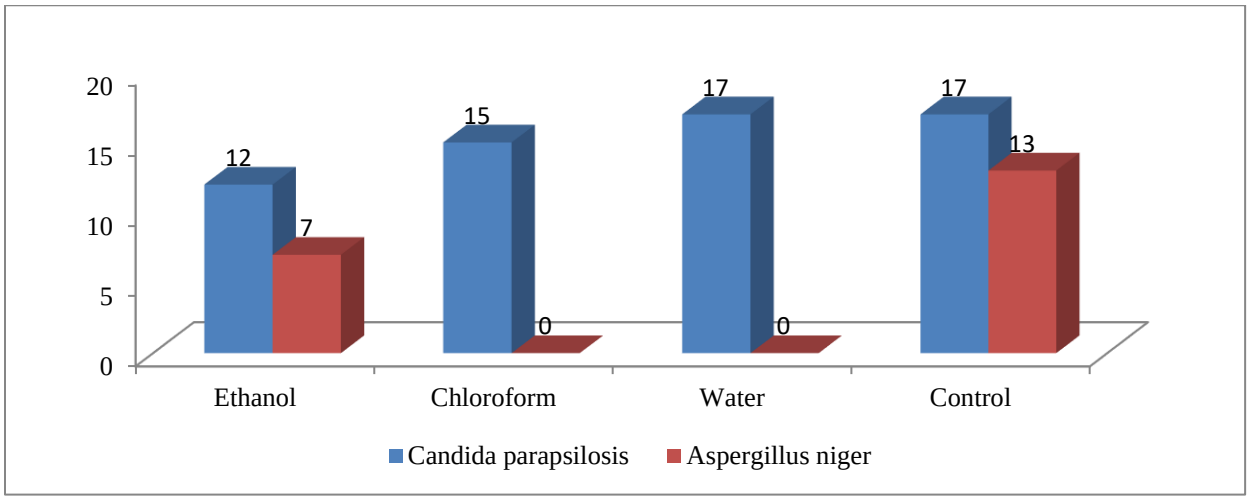
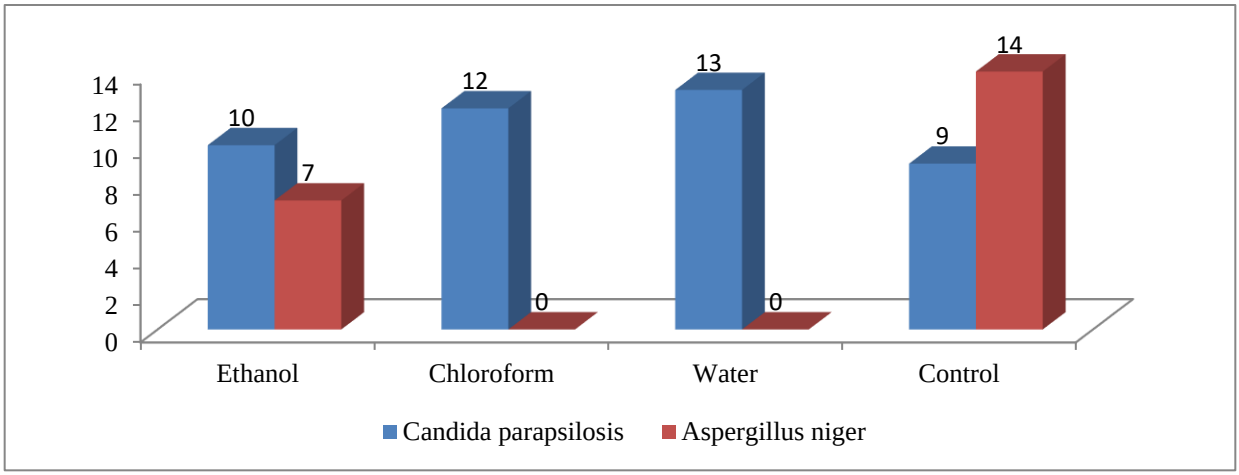


Fig:4 Antifungal activity of *V.agnus-castus.*



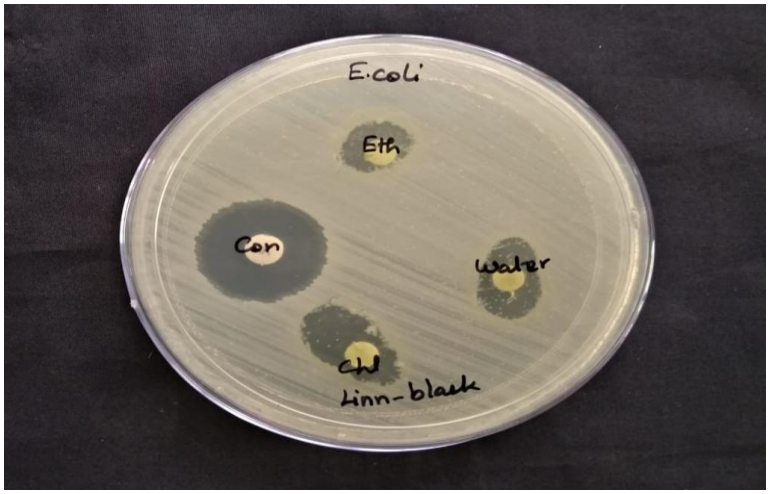


Plate 5 : Sensitive pattern of *E. coli* against ethanol, chloroform, water extract of (*Vitex negundo*)

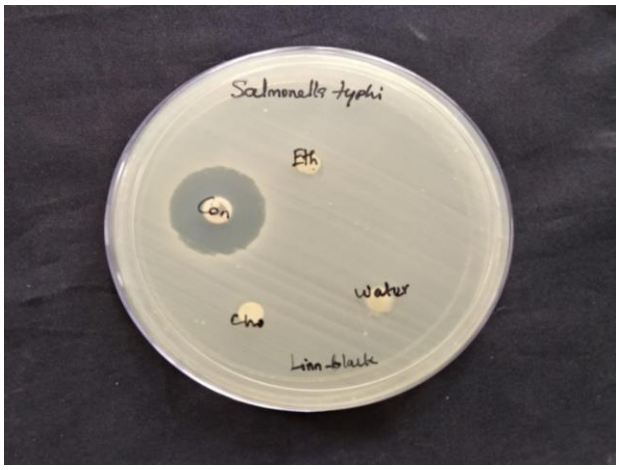


Plate 6: Sensitive pattern of *Salmonella typhi* against ethanol, chloroform, water extract of (*Vitex negundo*)

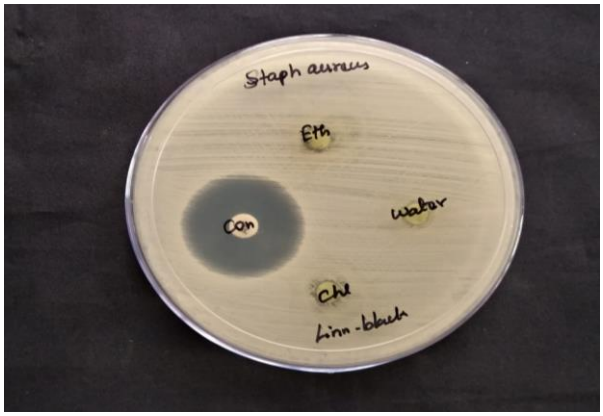


Plate 6: Sensitive pattern of S*taphylococcus* against ethanol, chloroform, water extract of (*Vitex negundo*)

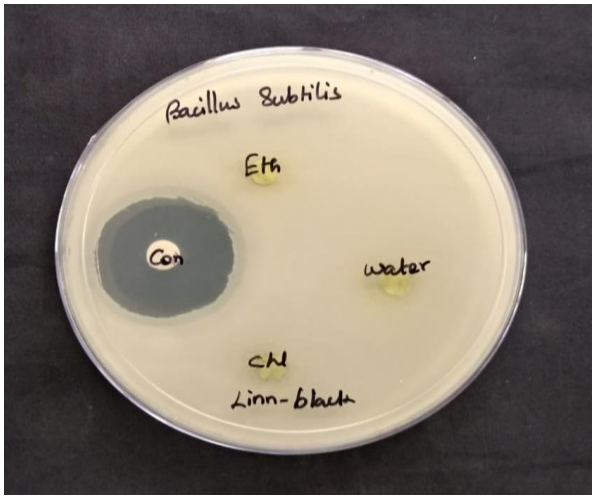


Plate 7: Sensitive pattern of Bacillus subtilis against ethanol, chloroform, water extract of (*Vitex negundo*)

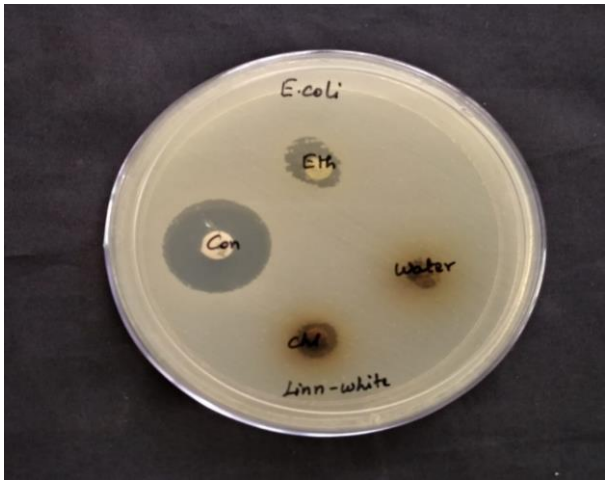


Plate 8 : Sensitive pattern of *E. coli* against ethanol, chloroform, water extract of (*Vitex agnus - castus*)

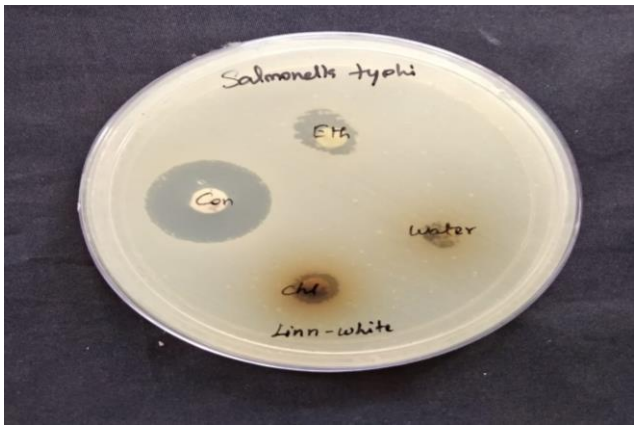


Plate 9: Sensitive pattern of *Salmonella typhi* against ethanol, chloroform, water extract of (*Vitex agnus - castus*)

Plate 10: Sensitive pattern of *Staph aureus* against ethanol, chloroform, water extract of (*Vitex agnus - castus*)

Plate 11: Sensitive pattern of *Bacillus subtilis* against ethanol, chloroform, water extract of (*Vitex agnus - castus*)

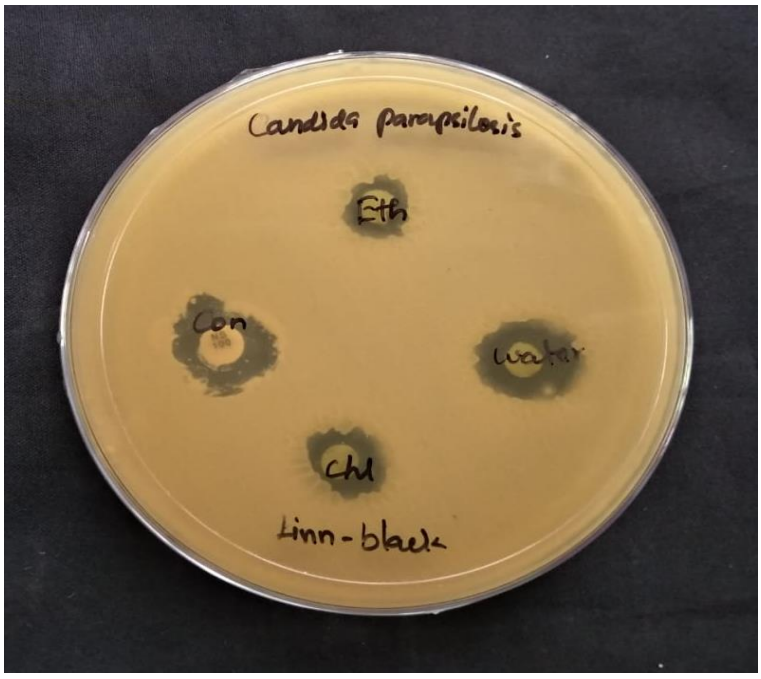


Plate 12: Sensitive pattern of *Candida parapsilosis* against ethanol, chloroform, water extract of (*Vitex negundo*)

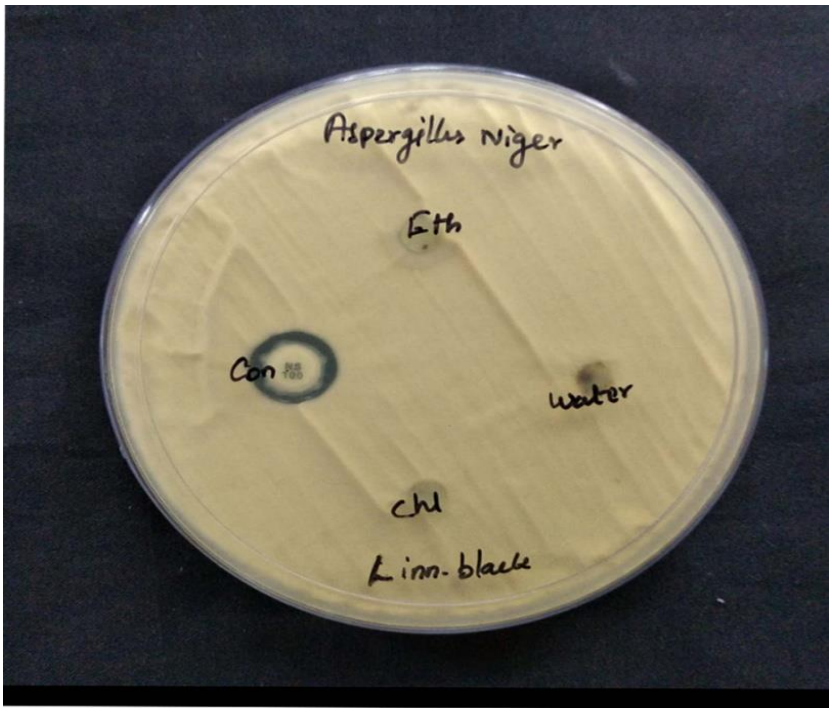


Plate 13: Sensitive pattern of *Aspergillus niger* against ethanol, chloroform, water extract of (*Vitex negundo*)

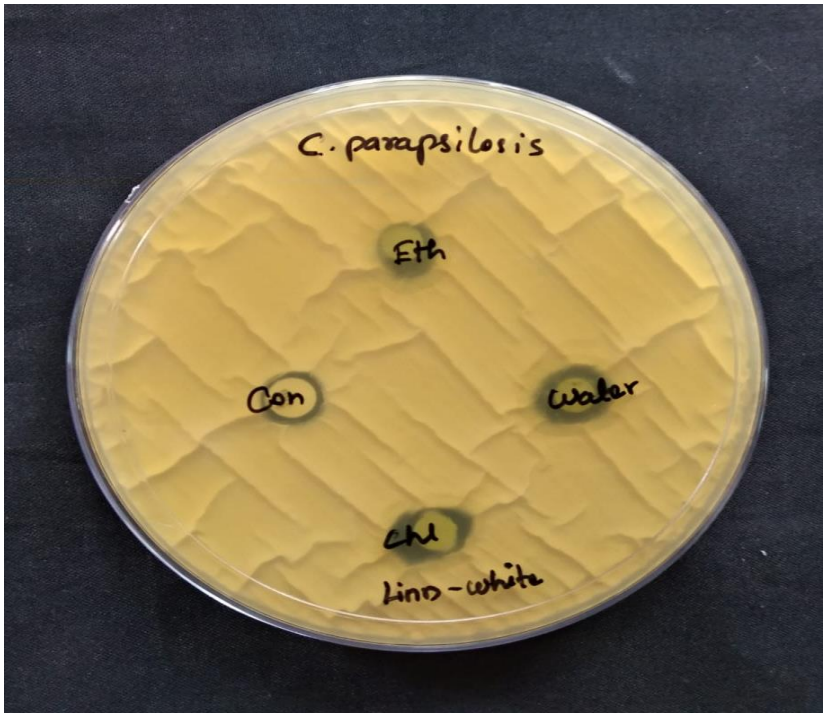


Plate 14: Sensitive pattern of *Candida parapsilosis* against ethanol, chloroform, water extract of (*Vitex agnus)*

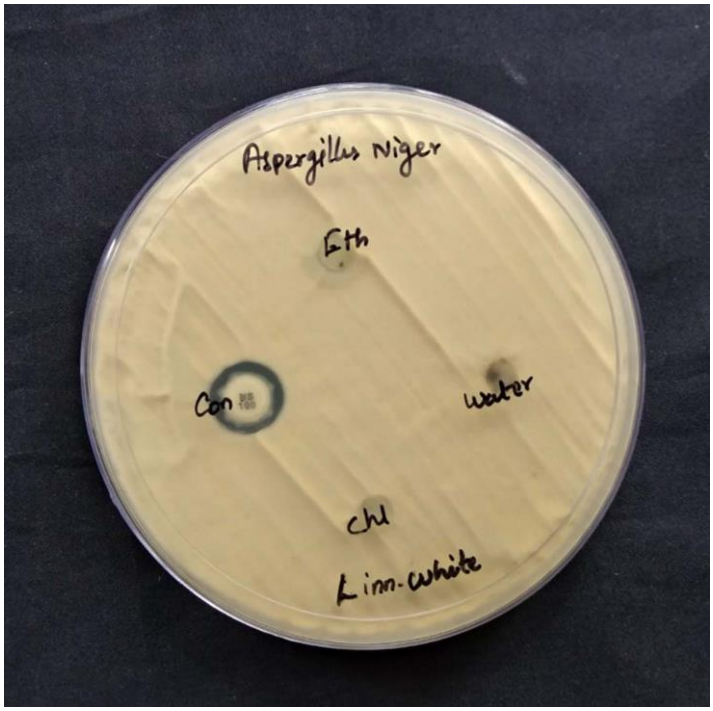


Plate 15: Sensitive pattern of *Aspergillus niger* against ethanol, chloroform, water extract of (*Vitex agnus - castus*)

DISCUSSION

In recent decades many researches are interested in medicinal plants for evolution of phyto chemicals such as flavonoids and tannins which have received more attention of human diseases (Ladda *et al.,* 2014). *Vitex negundo* is one of the important plant which have wide application in traditional systems of medicines. All parts of the plant, from root to fruits, possess a multitude of plytochemicals as secondary metabolites which import variety of medicinal uses to the plant. V*itex negundo* is an aromatic plant which is confirmed by the presence of alkanes, amines, primary and secondary amines and aromatic compounds (Corlett, 2012).

Detection of hydroxyl groups is an indication of presence of flavonoids, alcoholic and phenolic compounds (Kumar *et al.,* 2017). The presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, saponin and tannins in ethanol extract of V*itex negundo* and *V. agnus leaves* (Mani *et al.,* 2013). The present study also recorded the same phytochemicals.

It has been suggested that the antimicrobial activity of the plant is mainly due to the presence of essential flavonoids, alkaloids, tanins saponins and others natural polyphenolic compounds or three hydroxyl groups in plant extracts(Ramkumar *et al.,* 2004, Soetan, 2006). In the present

study we conclude that Vitex negundo leaves and stem have potential to act as a source of useful drugs because of the presence of various phytochemical constituents such as Reducing sugar, phenol, saponins, steroid and coumarin. These constituents seem to have the potential to act as a source of useful drugs and also to improve the health status of consumers as a result of the presence of various compounds that play a vital role for good health.

The antimicrobial activity of the plant extract is strengthened in the presence of antioxidant compounds (Ricardo *et al.,* 2011). It has been suggested that the antimicrobial activity of the plant is mainly due to the presence of essential flavonoids, alkaloids, tanins saponins and others natural polyphenolic compounds or three hydroxyl groups in plant extracts. (Ramkumar *et al.,* 2004, Soetan, 2006).

There results are similar to the antibacterial activity on *V. negundo* and *V. agnus.* The antibacterial showed activity on *V. agnus* against *E.coli, salmonella typhi, staphylococcus, B. subtilis* our result is similar to the antifungal activity on *V. agnus* against *C. parapsilosis, A. niger* (Samy *et al.,* 1998). Two pathogans namly *candida paraprilosis* and *A. niger* were used in the study when compared among the pathogens used, *candida parapsilisis* exhibited antifungal activity in all the extracts of both species are *V. negundo* and *V. agnus.*

CONCLUSION

It is very necessary to introduce new and biologically safe and active drugs eco-friend in nature and effective as antimicrobial agents. Usually medicinal plants contain several phyto chemical compounds, which are very much necessay to control the growth of the micro organism .*Vitex negundo* is one of the important plant which have wide applications in traditional systems of medicines. All parts of the plant, from root to fruits, possess a multitude of phytochemicals as secondary metabolites; nishindaside, mussaenosidic acids, vitedoin, negundin and vitexin are some important bioactive agents which impart a variety of medicinal uses to the plant is effective against bacterial and fungal activities.

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