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Mechanism of action of antioxidant, antibacterial, antimicrobial and other agents

ANTIBACTERIAL, ANTIDIABETICS AND LARVICIDAL ACTIVITY OF *Vitex negundo* AND *Ocimum basilicum* L. EXTRACTS

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ABSTRACT

Plants are one of the essential sources of medicines. About 150 aromatic plants are of the family, found in tropical areas and a few have got medicinal properties. Basil is one such plant used traditionally for a household remedy against human ailments from antiquity and is extensively used by the tribal people for common colds, headaches, abdomen disorders, inflammation, heart diseases, malaria, and anthelmintic. *Vitex negundo* belonging to the Verbenaceae family is an aromatic shrub, used to treat jaundice, toothache, wounds, asthma, eye pain, etc. Phytochemical analysis and antibacterial, antidiabetic and larvicidal activity were performed for the extracts of *Vitex negundo* and *Ocimum basilicum.* The treatment of various bacterial infections was investigated for *in-vitro* antimicrobial activity against pathogens namely *Staphylococcus aureus*, *Escherichia coli*, by disc diffusion method. Initially, the agar diffusion method led to results expressed within the dimension of the inhibition zone. Plant active compounds have anti-diabetic properties and can be used to treat diabetes mellitus. Inhibitory activity was measured using the spectrophotometric method. The α-amylase activity was measured at $\lambda = 540$ nm. The extracts of these plants were observed for larvicidal activity and the percentage of molarity was calculated. The leaf extract of *Ocimum basilicum* had the highest larval mortality when tested against *Aedes aegypti* larvae.

Keywords: *Vitex negundo*, *Ocimum basilicum*, antibacterial activity, antidiabetic activity, larvicidal activity.

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AIMS AND BACKGROUND

Mosquitoes are of the utmost importance in terms of public health as they can transmit various diseases including but not limited to malaria, filariasis, dengue, and Japanese encephalitis, resulting in the loss of millions of lives annually[1]. Herbs have been prized for their medicinal, flavouring, and aromatic qualities for centuries. The important advantage of medicinal plants in various treatments is that they are safe to consume as they cause very little or no side effects[2]. Plants have long been used by traditional healers to prevent or cure infectious diseases, and Western medicine is now attempting to replicate their effectiveness.

Plants are rich in a wide variety of primary metabolites such as protein, carbohydrate, lipid, carotenoid, etc. and secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids. These metabolites were identified to have antimicrobial properties and many pharmaceutical properties. The medicinal use of *Vitex negundo* has been used in Ayurveda, Unani, and Chinese traditional medicine systems, according to the Charaka Samhita, the most ancient and authoritative textbook. Different parts of the plant can cure different ailments and the therapeutic uses are dengue fever, puerperal fever, colic worms, diarrhea, splenic enlargement, irritable bladder, rheumatism, dyspepsia, liver disease, and hemoptysis are all symptoms of catarrhal dengue fever[3]. *Ocimum basilicum* L. is commonly known as sweet basil. It is a common herb, grown in several households with a wide range of therapeutic properties. Since the basil plant (*O. basilicum*) is commonly used as a daily household remedy and is cultivated in our plane areas as a garden ornamental plant. Its various sections, especially its leaves, seeds, and roots, are commonly used as daily household remedies and the therapeutic uses of *O. basilicum* are Leucoderma, toothache, earache, cures epistaxis, fever, cough and gout, Stomach complaints, Spermatorrhoea, blood dysentery, hematuria, inflammation, and congestion of kidney[4]. Malaria, filariasis, Japanese encephalitis, dengue hemorrhagic fever, yellow fever, and chikungunya are transmitted by mosquito species belonging to the genera Anopheles, Culex, and Aedes. Exploring floral biodiversity is one of the most successful alternative approaches to seeking a cleaner, more natural, and simple insecticide for mosquito control. Various plant extracts have presented many valuable, useful applications in recent years, ranging from trendy pharmaceuticals to insecticides. Dengue fever is endemic to Southeast Asia, and *Aedes aegypti* is thought to be a vector of the disease.

EXPERIMENTAL

PLANT EXTRACTION

The dried plant samples were extracted with different solvents and tested for various phytoconstituents. They were generally tested for the presence of carbohydrates, flavonoids, tannins, phenols, quinones, steroids by using standard procedures and

alkaloids, saponins, glycosides were found to be absent by using standard procedure and were recorded.

ANTIBACTERIAL ACTIVITY

The acetone leaf extract from the mother plant was used for the antibacterial study. Different concentrations (10, 20, 30, 40 and 200 µg/ml) of the concentrated acetone leaf extract were tested for antimicrobial activity against pathogenic bacterial strains such as *Staphylococcus aureus* and *Escherichia coli*[5]. Antibacterial activity was measured using the standard method of disc diffusion plates on agar. All bacterial strains were grown in Luria Bertani Broth medium for 24 h at 37°C and plated on LB agar for agar diffusion experiments. After a 24-hour incubation period at 37°C, inhibition diameters were measured[6].

ANTIDIABETIC ACTIVITY

The spectrophotometric assay determined the optical density (absorbance) and the data acquired were statistically treated using the calculation of averages[7,8].

The antidiabetic effect of various concentrations of plant extracts was examined by α- amylase inhibitory activity with slight modifications[9]. 0.25 ml of α-amylase solution were mixed with the plant extract in the test tube. After incubation for 1 h at room temperature, 0.5 ml of starch solution were added and reincubated for 5–10 min. The reaction was terminated by the addition of DNS (Dinitro salicylic acid) reagent and kept for a boiling water bath for 5 min at 100°C to stop the reaction. After cooling at room temperature, the sample was diluted with 5 ml of distilled water, and absorbance was recorded at 540 nm, and the same procedure was followed for all other plant extracts. The optical density was recorded at 540 nm against the blank. The percentage of α-amylase activity was calculated by using the following equation:

$$\text{inhibition \%} = (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}})/\text{Abs}_{\text{sample}} \times 100.$$

LARVICIDAL ACTIVITY

The acetone leaf extract was used for determination of the larvicidal activity. 1 ml of plant extract was taken and mixed in 4 dilutions of prepared distilled water (25, 50, 75 and 100 ml). Then 4 larvae were taken in different paper cups containing plant concentrations. The control was set up with methanol. Four replicates for each concentration and the control methanol were tested for larvicidal activity. The dead larvae and control were counted after 24 h of continuous exposure[10]. The percentage mortality at different concentrations was analysed and calculated by the following equation:

$$\text{percentage of mortality \%} = \text{number of dead larvae/number of larvae} \times 100.$$

RESULTS AND DISCUSSION

In the recent study, the properties of primary metabolites and various pharmacological activities of medicinally well-known plants *Vitex negundo* and *Ocimum basilicum* L. were studied using acetone extracts of pland samples (Fig. 1) and the results of the pharmacological observations may evidently support the long-term use of *Vitex negundo* and *Ocimum basilicum* L. as possessing antibacterial, antidiabetic and larvicidal activities[11].

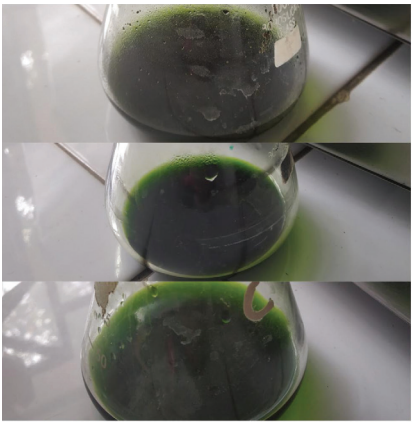

Fig. 1. Acetone plant extract

PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACT

The phytochemical screening was carried out on *Vitex negundo* and *Ocimum basilicum* L. The presence of secondary metabolites suggests that plants might have medicinal values. The results of the phytochemical analysis are shown in Table 1.

Table 1. Phytochemical analysis of *Vitex negundo* and *Ocimum basilicum* L. extracts

S. No	Secondary metabolism	*V. negundo* (acetone)	*V. negundo* (ethyl acetate)	*Ocimum basilicum* L. (acetone)	*Ocimum basilicum* L. (ethyl acetate)
1	Flavonoids	+	+	–	+
2	Phenol	–	+	–	–
3	Carbohydrates	+	+	+	+
4	Steroids	–	–	+	+
5	Glycosides	–	–	–	–
6	Quinones	+	+	–	–
7	Alkaloids	–	–	–	–
8	Saponins	+	–	–	–

Table 2. Phytochemical analysis of combination of *V. negundo* and *O. basilicum* L. plant extracts

S. No.	Secondary metabolism	Combination of *V. negundo* and *O. basilicum* L. (acetone)	Combination of *V. negundo* and *O. basilicum* L. (ethyl acetate)
1	Flavonoids	+	+
2	Phenol	–	+
3	Carbohydrates	–	–
4	Steroids	–	+
5	Glycosides	–	–
6	Quinones	–	+
7	Alkaloids	–	–
8	Saponins	+	–

It is seen from Table 1 that the components present in the extracts are carbohydrates, flavonoids, phenols, saponins, and steroids. Alkaloids, and glycosides were not detected in any of the plant extracts under study. The phytochemical analysis of combined plant extracts are tabulated in Table 2, which shows the presence of flavonoids predominantly in both acetone and ethyl acetate extracts. Combined extracts were found to be more biologically active than the individual extract[12].

ANTIBACTERIAL ACTIVITY – SENSITIVITY DISC PATTERN USING STANDARDS

The culture was incubated for 24 h and it was swabbed onto Luria-Bertani (LB) agar plates. Each plate was examined after 16 to 18 h of incubation. The resulting zones of inhibition were uniformly circular and there was a confluent lawn of growth. There were no individual colonies apparent indicating that the inoculum was not too light and dilution was right. The diameters of the zones of complete inhibition were measured by holding it back of the inverted plate, including the diameter of the disc. The Petri plate was illuminated with reflected light and held a few inches above a dark, non-reflecting background. Any discernible growth within the zone of inhibition is indicative of resistance to the respective antibiotic. The zone margin should be described as the area with no apparent, visible growth visible to the naked eye. Small colony growth that can only be seen with a magnifying lens at the edge of the inhibited growth zone is overlooked. *E. coli* strains isolated is Gram-negative, motile, opportunistic bacterium belonging to the family Enterobacteriaceae. This was found to be highly resistant to Amoxicillin[13,14]. The antibacterial activity of *Ocimum basilicum* and *Vitex negundo* is given in Tables 3 and 4 in which higher concentration of extract showed greater antibacterial activity against the pathogens[15,16].

Table 3. Antibacterial activity of *Ocimum basilicum* L. (zone of inhibition)

S. No	Concentration (µg/ml)	*E. coli* (mm)	*S. aureus* (mm)
1	10	9	7
2	20	6	8
3	30	10	14
4	40	8	12
5	200	40	32

Table 4. Antibacterial activity of *Vitex negundo* (zone of inhibition)

S. No	Concentration (µg/ml)	*E. coli* (mm)	*S. aureus*
1	10	2	–
2	20	5	–
3	30	6	–
4	40	8	–
5	200	23	–

ANTIBACTERIAL ACTIVITY – USING EXTRACTS

The antibacterial activity of combined extract showed promising results when compared to individual extracts of *V. negundo* and *O. basilicum.* Zone of inhibition was found to be higher for both the starins *E. coli* and *S. aureus* tested which is indicated in Table 5. Similar results were obtained for antibacterial activity against *Punica granatum* L. and *Areca nut* (P.A) combined extracts[17].

Table 5. Antibacterial activity of combination of *V. negundo* and *O. basilicum* extracts (zone of inhibition)

S. No	Concentration (µg/ml)	*E. coli* (mm)	*S. aureus* (mm)
1	10	–	6
2	20	7	7
3	30	9	8
4	40	8	10
5	200	24	22

ANTIDIABETIC ACTIVITY – α-AMYLASE INHIBITION

The antidiabetic activity of plant extract was evaluated in this study. The α-amylase inhibitory activities were observed in the sample extract[18,19]. The sample extract was used at various concentrations. In an *in vitro* analysis of α-amylase inhibition, all extracts display inhibition values ranging from 6 to 81%. 3 different samples were with inhibition values greater than 70% (Table 6). The α-amylase activities were inhibited by the *Vitex negundo* leaf extract. UV readings were taken and the readings were calculated and depicted in Fig. 2. Antidiabetic activity of the extracts was also studied for the leaf extracts of *Vitex doniana*[20]. Similar results were obtained for the extracts of *Ocimum basilicum* L. as indicated in Fig. 3 and Table 7.

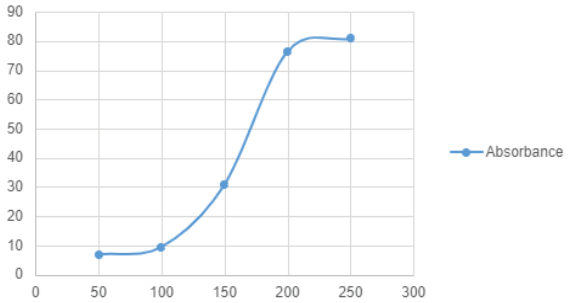

Fig. 2. Percentage inhibition of α-amylase enzyme – *Vitex negundo*

Table 6. *In vitro* antidiabetic activity of absorbance and Inhibition – *Vitex negundo*

S. No	Concentration (µg/ml)	Absorbance (450 nm)	Inhibition (%)
1	50	0.405	6.9
2	100	0.417	9.5
3	150	0.545	30.8
4	200	1.596	76.4
5	250	1.967	80.8

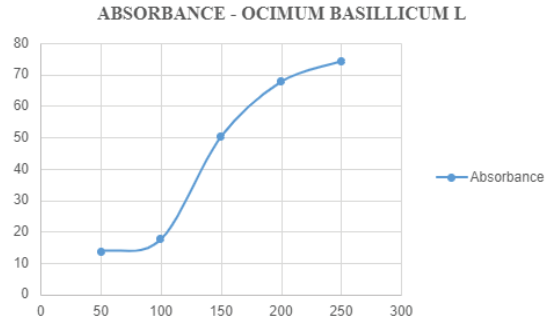

Fig. 3. Percentage inhibition of α-amylase enzyme – *Ocimum basilicum* L.

The α-amylase inhibitory activities of combined acetone extract of leaves of *O. basilicum* L. and *V. negundo* were evaluated. The combined extracts exhibited excellent α-amylase inhibitory activities when compared to individual extracts as indicated in Fig. 4 and Table 8. Antidaibetic potential of combined extracts of three different medicinal plants was studied which provided promising results[21].

Table 7. *In vitro* antidiabetic activity of absorbance and inhibition – *Ocimum basilicum*

S. No.	Concentration (µg/ml)	Absorbance (450 nm)	Inhibition (%)
1	50	0.437	13.7
2	100	0.457	17.5
3	150	0.757	50.2
4	200	1.168	67.7
5	250	1.470	74.3

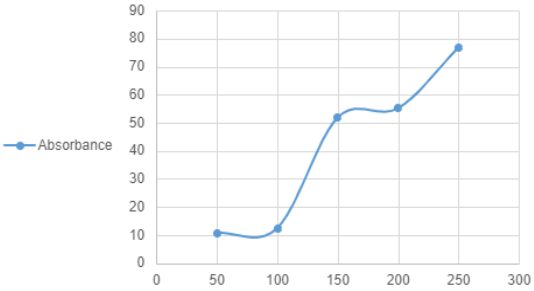

Fig. 4. Percentage inhibition of α-amylase enzyme – combination of *V. negundo* and *O. basilicum* L. plant extract

Table 8. Percentage inhibition of α-amylase enzyme for the combined extracts of *V. negundo* and *O. basilicum* L. plant extract

S. No	Concentration (µl)	Absorbance (450 nm)	Inhibition (%)
1	50	0.243	10.8
2	100	0.430	12.5
3	150	0.782	51.9
4	200	0.839	55.2
5	250	1.637	76.9

LARVICIDAL ACTIVITY

The larvicidal activity of the prepared sample (acetone extract) was tested at different concentrations (Tables 9 and 10). The 24-hours period had the highest mortality rate for the larvae. Against the larvae of *Aedes aegypti*, the leaf extract of *Ocimum basilicum* L. had the highest larvicidal mortality (Table 10). Similar work was performed by Sundararajan et al. in the form of nanoemulsion against mosquito larvae[22,23]. Five replicates of five larvae each were exposed to a total of 40 larvae. The humidity level in the experiments was held at 30°C. The mortality of the larvae was measured 24 h after they were put in paper cups. The dead mosquito larvae were assumed to be moribund. The preliminary screening method is a good way to assess the possible larvicidal behaviour of commonly used plants. All the extracts had moderate to low larvicidal effects; however, the acetone extract of *Ocimum basilicum* had the highest larval mortality.

Table 9. Larvicidal activity of extract from *Vitex negundo* plant – mortality

S. No	Concentration (ml/ml)	Number of larvae (exposed)	Number of larvae (dead)	Mortality (%)
1	1: 25	5	4	80
2	1:50	5	3	60
3	1:75	5	2	40
4	1:100	5	2	40

Table 10. Larvicidal activity of extract from *Ocimum basilicum* L. – mortality

S. No	Concentration (ml/ml)	Number of larvae (exposed)	Number of larvae (dead)	Mortality (%)
1	1: 25	5	4	80
2	1:50	5	4	80
3	1:75	5	3	60
4	1:100	5	2	40

CONCLUSIONS

The need for alternative strategies for the prevention and treatment of various infections and diseases caused by a bacterial organism is growing rapidly due to the resistance developed towards synthetic antibiotics. Traditional knowledge and historic literature on ethnomedicine provide another aspect of clues for discovering potential therapeutic agents that the plants *Vitex negundo* and *Ocimum basilicum* L. which are well-known plants and have been used as natural antifungal, antioxidant, anthelmintic, anti-hemorrhoidal and analgesic agents for years in Asian countries. Carbohydrates, flavonoids, phenols, saponins, and steroids were discovered in the phytochemical study. Alkaloids or glycosides were not found in any of the plant extracts studied. In both plants, *E. coli* produces more effects, but in the bacteria Staphylococcus aureus, there is no result in *Vitex negundo*. Diabetes mellitus is a complicated disease, and human physiology is much more complicated. All of the plant extracts have 70% inhibition values in antidiabetic activity. It has the highest value of 80.8% in *Vitex negundo*. Plant extracts, which are less harmful to human health, may play an important role in mosquito control. The results of larvicidal activity shows that the highest molarity was observed in *Ocimum basilicum* L. compared to *Vitex negundo*. It shows 80% of molarity in 1:25 and 1:50 concentrations. Therefore, the best results were seen in *Ocimum basilicum* L.

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