WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 22, 1048-1064. Research Article ISSN 2277–7105

RESEARCH OF ANTI BACTERIAL ACTIVITY OF MELALEUCA VIMINALIS L.

Prajwal B. S.*, Suhas R. S., Manthan M., Nihas and Dr. Amal Joseph

Department of Pharmacognosy, Bharathi College of Pharmacy, Bharathinagara-571422.

Article Received on
26 October 2023,

Revised on 16 Nov. 2023,
Accepted on 06 Dec. 2023

DOI: 10.20959/wjpr202322-30576

***Corresponding Author**
Prajwal B. S.
Department of Pharmacognosy, Bharathi College of Pharmacy, Bharathinagara-571422.

ABSTRACT

Weeping bottlebrush, scientifically known as Melaleuca viminalis (formerly called Callistemon viminalis), belongs to the Myrtaceae family and is well-regarded for its therapeutic properties. This ornamental plant is celebrated for its multifaceted qualities, which encompass molluscicidal, antioxidant, antifungal, antibacterial, antiplatelet aggregation, allelopathic, anti-infective, anti-quorum sensing, and antihelminthic attributes. Additionally, it has been observed that these aesthetically pleasing plants possess outstanding insecticidal properties. The secondary metabolic products of this plant encompass a diverse range, including essential oils, pyrrole derivatives, monoterpenes, triterpenoids, phenolics, steroids, flavonoids, and steroidal glycosides. Previous research indicates that monoterpenes are the predominant constituents of C. viminalis and play a pivotal role in mediating the plant's various biological functions. This review aims to delve into the physicochemical composition, morphology, cultivation techniques, phytochemical constituents, and microscopic characteristics of Melaleuca viminalis, with the goal of harnessing its potential for the betterment of society.

KEYWORS: Callistemon viminalis, Phytoconstituents, Essential oil, Biological activity.

PLANT PROFILE

Figure 1: Image of *Melaleuca Viminalis.*

PLANT INTRODUCTION

Melaleuca viminalis, commonly known as weeping bottlebrush or creek bottlebrush, is a plant in the myrtle family myrtaceae and is endemic to New_South_WalesQueensland. (Some Australian state herbaria continue to use the name *Callistemon viminalis*). It is a multi-trunked, large shrub or tree with hard bark, often pendulous foliage and large numbers of bright red bottlebrush flowers in spring and summer. The genus Callistemon belongs to the family *Myrtaceae*, which consists of about 24 species characterized by cylindrical brush like flowers resembling the traditional bottlebrush. Considered antibacterial, antifungal, anthelmintic, hemostatic, diuretic. Studies have shown anthelmintic, anti-quorum sensing, insecticidal, anti-infective, antibacterial, molluscicidal, antioxidant, anticancer, anti-inflammatory, anti-platelet aggregation properties. Studies have the plant to be rich in phenolics, flavonoids, saponins, steroids, alkaloids, tannin, carbohydrates and protein compounds. Bottle brush blossoms were utilized as a natural energy beverage by Australia's indigenous inhabitants.

Vernacular Name of *Melaleuca viminalis*

English	weeping bottlebrush
Hindi	Cheel.
Finnish	Norjalamppuharja.
Afrikaans	Treur botteborsel
Maripuri	Barap lei

Taxonomy of *Melaleuca viminalis*

Kingdom	Plantae
Division	Tracheophytes
Sub Division	Angiosperms
Class	Eudicots
Sub Class	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	*Melaleuca*
Species	*M. viminalis*
Botanical name	*Melaleuca viminalis*
Comman name	*Bottlebrush*
Synonyms	*Metrosideros viminalis* Sol.ex Gaertn., *Callistemon viminalis* (Sol. ExGaertn.) G. Don

COLLECTION OF PLANT MATERIAL AND AUTHENTICATION

The plant *Melaleuca viminalis* belonging to the family *Myrtaceae* and is endemic to New South Wales, Queensland and Western Australia. For the present study, the fresh leaves of

Melaleuca viminalis were collected from natural habitat in and around Bharathinagara, Mandya District, Karnataka. The leaves were identified and authenticated by Botanist Dr. Thejesh Kumar, M P Msc, Ph.D.Co-ordinater, BharathiCollege of Post-Graduation and Research Centre, Bharathinagara, Maddur (Tq), Mandya (Dist), Karnataka state. The *Melaleuca viminalis* leaves were dried in shade the dried material was then reduced to coarse powder using a mechanical grinder. The resulting powder was then used for extraction by Soxhlet extraction method.

PREPARATION OF THE EXTRACT

The leaves of *Melaleuca viminalis* were dried under shade and crushed into coarse powder and subjected to extraction. Hot Soxhlet continuous process - dry coarse powder of the leaves of *Melaleuca viminalis* was extracted with 70% ethanol by using Soxhlet apparatus at a temperature 50-60 ^{0}C. Extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was concentrated by using rotary evaporator and stored in refrigerator until used.

PRELIMINARY PHYTOCHEMICAL SCREENING

1. Tests for Phenolic Compounds

- ***Ferric chloride test***

To 2-3 ml of extract, few drops of 5% FeCl3 solution was added a deep blue-black colour was observed.

- ***Dilute HNO_3 test***

To 2-3 ml of extract, few drops of Dilute HNO3 solution was added, a reddish to yellow colour was observed.

2. Tests for Flavonoids

- ***Lead Acetate solution test***

Test solution with few drops of lead acetate solution (10%) gives yellow precipitates.

- ***Alkaline reagent test***

Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour, which becomes colourless on addition of few drops of dilute acid.

3. Tests for carbohydrates

- ***Dragendroff's test***

To 1 ml of the extract, add 1 ml of Dragendroff's reagent (potassium bismuth iodide solution). An orange red precipitate indicates the presence of alkaloids.

- ***Wagner's test***

To 1 ml of the extract, add 1 ml of Wagner's reagent (iodine in potassium iodide solution). Formation of yellow precipitate indicates the presence of alkaloids.

4. Tests for Tannins

- ***Ferric Chloride test***

Test solution with few drops of ferric chloride solution shows intense black colour.

5. Tests for Alkaloids

- ***Dragendroff's test***

To 1 ml of the extract, 1 ml of Dragendroff's reagent (potassium bismuth iodide solution) was added. An orange red precipitate indicates the presence of alkaloids.

❖***Hager's test***

To 1 ml of the extract, 1 ml of Hager's reagent (saturated aqueous solution of picric acid) was added. A yellow-coloured precipitate indicates the presence of alkaloids.

- ***Wagner's test***

To 1 ml of the extract, 1 ml of Wagner's reagent (iodine in potassium iodide solution) was added. Formation of reddish-brown precipitate indicates the presence of alkaloids.

6. Tests for Steroids

Small quantity of extract is dissolved in 5 ml of chloroform separately. The above obtained chloroform solutions are subjected to Salkowski and Liebermann- Burchard tests.

- ***Salkowski test***

To the 1 ml of above prepared chloroform solution few drops of concentrated Sulphuric acid was added. Formation of brown ring indicates the presence of phytosterols.

- ***Liebermann-Burchard test***

The above prepared chloroform solutions are treated with few drops of concentrated sulphuric acid followed by 1 ml of acetic anhydride solution. A bluish green colour solution shows the presence of phytosterols.

7. Tests for Saponins

- ***Frothing Test*** 3 ml of the aqueous solution of the extract were mixed with 10 mL of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5 min, it was allowed to stand for 30 min and observed for honeycomb froth, which was indicative of the presence of saponins.

8. Tests for Protein

- ***Biuret test***

The organic sample is mixed with an equal volume of NaOH (10%) and 2 drops coppersulphate. The formation of blue precipitate indicates protein.

Determination of Antibacterial Activity of melaleuca viminalis

Preparation of melaleuca viminalis extracts Fresh samples of melaleuca viminalis were collected from its natural habitat in KM Doddi, Karnataka. The plant was washed under running tap water to remove any dirt and contaminant on the plant material. The plant then was dried using an electric oven at 80C until it reached constant weight at 5% moisture content. The dried plants were grounded into powder form. Three grams of herbs were extracted by using 200 ml of methanol, ethanol, and water as solvent. The extracts were collected and filtered to obtain clear crude extract solution. The extract were dried and stored.

Instruments and reagents used

Autoclave, laminar airflow cabinet, bacteriological incubator, beakers, petri plates, conical flasks, filter papers, ethanol, water, etc.

Preparation of agar plate

Potato Dextrose Agar(PDA) and nutrient agar were used in this study as medium for microbial growth. Potato Dextrose Agar and nutrient agar were prepared by adding 39 g of commercial PDA powder and 28 g of nutrient agar powder in 1 L distilled water. The mixture was dissolved and subsequently autoclaved for15 min at 121 C. The cooling media was poured into the petri dish and left hardened for 24 h before used.

Microorganisms used

SL NO	BACTERIAL STRAINS	GRAMSTRAINS
01	Escherichia coli	Gram negative
02	Bacillus subtilis	Gram positive

ANTI BACTERIAL ACTIVITY ASSAY

Susceptibly test was carried out by disc diffusion method suggested by national committee for clinical laboratory standard(NCCLS, 2000) with slight modification. The bacterial suspension was made by emulsifying the bacterial cultures in sterile saline to the correct cell density for the test which matches the turbidity of a McFarland 0.5 standard solution. The bacterial culture was then swabbed evenly across the entire surface of the media with a sterile swab after pressing firmly against the inside wall of the tube remove excess liquid. The cultures were allowed to soak into the medium for about 5min before placing the M.viminalis extracts on the agar. Three concentrations, 100 %, 70 % and 30 % of ethanol, methanol and water extract were prepared by diluting crude extract in sterile distilled water. Sterile distilled water was used a s control filter paper discs of 6mm diameter were placed on the agar. 1mL of extracts with different concentration was pipette on the filter paper discs. For each microbes, three replicates were done. The plates were incubated for 48 h at 37 °C in order to estimate the radial growth of strains and ratio of the inhibition zone was measured.

AGAR DISC DIFFUSION METHOD

The prepared leaves extracts of their 100 mg/ml concentration were tested for antibacterial activity by the disc diffusion method as described by Kirby, 1994. The two bacterial strains were individually streaked uniformly all over the Nutrient agar medium in 9 cm petri plate. This was allowed to solidify and holes of 5 mm width were made into the agar using sterile Pasteur pipettes. An amount of 100 mg/ml stock of crude plant extract was prepared by dissolving 100 mg of dried plant extract in 1 ml of ethanol. 100 µL of the stock extract was pipetted onto the holes to give a concentration of 10 mg per hole. 100 µL of 0.5 mg/ml ciprofloxacin was also pipetted into one of the holes to give a final concentration of 0.05 mg. This served as the positive control, while 100 µL of 10% ethanol was pipetted into one of the holes, which served as the negative control. The zone of bacterial growth inhibition was measured in mm and compared with ciprofloxacin. The plates were incubated at 37° C ± 1° C for two days to attain good growth.

Determination of Minimum Inhibitory Concentration for Bacteria

In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial drug that will inhibit the visible growth of a microorganism after overnight incubation. Each bacterium was cultivated on Mueller Hinton agar. Then, they were suspended on Mueller Hinton broth. Serial dilutions of the leaves extract containing broth medium were prepared. From the 50 µg/ml freshly prepared stock solution, four different concentrations 0µg/ml, 5µg/ml, 10µg/ml are 20µg/ml are prepared. Transfered them to four different test tubes (as T1, T2, T3, and T4) respectively. Each tube is added with respective bacterial species and then incubated overnight. Lastly, it was carried out an incubation at 37◦C for 48 hours. The growth or no-growth was considered by observation, and the MIC value was determined as the lowest extract concentration that avoids the bacterial growth. Each assay was repeated three times. After incubation based on turbidity MIC is calculated using the formula:

$$MIC = \frac{\text{(Lowest conc. Inhibit growth + Highest conc. allow growth)}}{2}$$

RESULTS

The ethanolic leaves extract of *Melaleuca viminalis* was subjected to phytochemical and pharmacological investigations. The results of the study are presented below.

PREPARATION OF EXTRACT

The ethanolic extract was obtained by extracting the dried course powder of *Melaleuca viminalis*plant with 70% of ethanol by Soxhlet extraction method. The physical characteristics are as follows,

Colour - Dark green

Odour –Pungent smell

Appearance -Powder

Calculation of % yield

36.03g of extract was obtained from 320g of the dried course powder of leaves*Melaleuca viminalis*.

Percentage yield = (practical yield/theoretical yield) × 100

= (36.03/ 320) × 100

= 11.34 %

Percentage yield of extract *Melaleuca viminalis*obtained = 11.34

Figure 3: Extraction of *Melaleuca viminalis* by soxhlation.

Solubility test of *Melaleuca viminalis*

SL NO	SOLVENTS	SOLUBILITY
1	WATER	INSOLUBLE
2	ETHANOL	PARTIALLY SOLUBLE
3	METHANOL	SOLUBLE
4	CHLOROFORM	SOLUBLE
5	ETHYL ACETATE	SOLUBLE
6	MSO (DIMETHYL SULFOXIDE)	SOLUBLE
7	HOT WATER	INSOLUBLE
8	TOLUENE	PARTIALLY SOLUBLE
9	ACETONE	PARTIALLY SOLUBLE
10	HEXANE	INSOLUBLE

Phytochemical investigation

SL NO	CHEMICALTEST	OBSERVATION
01	PHENOLIC	
	FERRIC CHLORIDE TEST	+
	DILUTE HNO3 TEST	+
02	FLAVONOIDS	
	LEAD ACETATE SOLUTION TEST	+
	ALKALINE REAGENT TEST	+
03	CARBOHYDRATES	
	DRAGENDROFF'S TEST	+
	WAGNER'S TEST	+
04	TANNINS	
	FERRIC CHLORIDE TEST	+
05	ALKALOIDS	
	DRAGENDROFF'S TEST	+
	HAGER'S TEST	+
	WAGNER'S TEST	+
06	STEROIDS	
	SALKOWSKI TEST	-
	LIEBERMANN BURCHARD TEST	-
07	SAPONINS	

	FROTHING TEST	-
08	PROTIEN	
	BIURET TEST	+
	XANTHOHYDRIN TEST	+

(+) REPRESENTS PRESENCE OF CHEMICAL CONSTITUENTS

(-) REPRESENTS ABSENCE OF CHEMICAL CONSTITUENTS

ANTI BACTERIAL ACTIVITY

Antibacterial activity of melaleuca viminalis leaves was done by agar disc diffusion method on bacterial strains like E. coli and B.subtillis which are gram negative and gram positive bacteria's respectively.

Measurement of zone of inhibition

After incubation, the diameter of clear zone of inhibition produced around was measured in mm and diameter of inhibition by the plant extract were compared with reference antibiotic. A Zone of Inhibition Test, also called a Kirby-Bauer Test, is a qualitative method used clinically to measure antibiotic resistance and industrially to test the ability of solids and textiles to inhibit microbial growth. Researchers who develop antimicrobial textiles, surfaces, and liquids use this test as a quick and easy way to measure and compare levels of inhibitory activity.

If the bacterial strain is susceptible to the antibacterial agent, then a zone of inhibition appears on the agar plate, such as on the agar plate on the left-hand side of the photo below. If it is resistant to the antibacterial agent, then no zone is evident, such as on the agar plate on the right-hand side of the photo below.

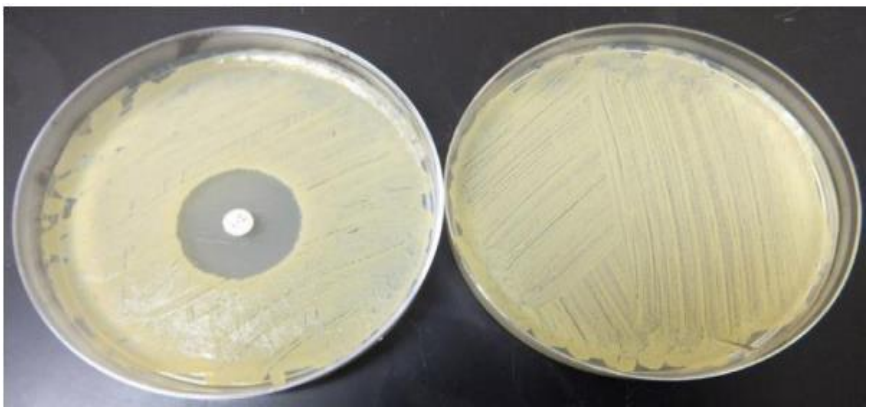

Figure 4: The Left Plate is of B.subtilis showing a zone of inhibition, Right Image is lawn growth of B. subtilis.

Zone of Inhibition Testing is a fast, qualitative means to measure the ability of an antimicrobial agent to inhibit the growth of microorganisms. In the world of antimicrobial substances/surfaces, the degree to which these materials are inhibitory can be of vital importance to the health of the consumer. This test is an outstanding qualitative way for manufacturers of antimicrobial surfaces/substances to be able to compare the inhibition levels of their products.

RESULTS

Extraction of bioactive compounds from plants is influenced by type of solvent used for extraction (Kim et al., 2009). In this study, three different types of solvents were used for extraction of M.viminalis. Extraction of M.viminalis by ethanol produced a greater yield (w/w) of extract 11.3%.

Concentration of extract

EXTRACT	100%	70%	30%
ETHANOL	8.4mm	5.1mm	2.4mm
METHANOL	5.4mm	4.2mm	-
WATER	3.3mm	2.0mm	-

Inhibition zone(mm)of E.coli by M.viminalis extract

The result of antimicrobial activity of M.viminalis extracts against E.coli are presented in Table 2. Ethanol extract showed the largest inhibition zone towards E.coli 8.4 mm and also the most effective extract against E.coli with 100 % concentration and at 70 % concentration of ethanol extract, the zone of inhibition was 5.1 mm. However, at the lowest concentration which is 30 % concentration, the zone of inhibition was only 2.4mm. Diameter of inhibition zone by methanol extract was slightly smaller against E.coli which was 5.4 mm at 100 % and 4.2 mm at 70 % concentration. Water extract was found to be less effective to inhibit the growth of E.coli which was 3.3 mm at concentration of 100 % and 2.0 mm at concentration of 70 %. No inhibition was found at concentration of 30% of methanol and water extract.

Figure 5: Effect of M.viminalis extracts against E. coli at different concentration(A)100% (B)70% (C)30%.

The inhibition zone of ethanol, methanol and water extracts on E.coli at different concentrations are shown in Figure5.2. Ethanol extract was the most effective to inhibit the growth of E.coli followed by methanol extract but growth was less effective to be inhibited by water extract. In this present study, all M.viminalis extracts were also tested on B. subtilis. The results of antimicrobial activity are shown in Table 3. Concentration of ethanol extract at 100% was found to inhibit B. subtilis effectively 16.4mm followed by at 70% concentration 12.2mm. By reducing the concentration of ethanol extract to 30 %, the inhibition zone of bacteria also was recorded smaller at 6.4 mm diameter. For methanol extract, the zone of inhibition was 10.3 mm at 100 % and 10.1 mm at 70 % concentration but recorded 5.6 mm at 30 % concentration. B.subtilis was also found to be susceptible to water extract of M.viminalis which had 8.4mm.

Concentration of extract

EXTRACT	100%	70%	30%
ETHANOL	16.4mm	12.2mm	6.4mm
METHANOL	10.3mm	10.1mm	5.6mm
WATER	8.4mm	8.2mm	6.0mm

inhibition zone(mm) of B.subtilis by M.viminalis extract

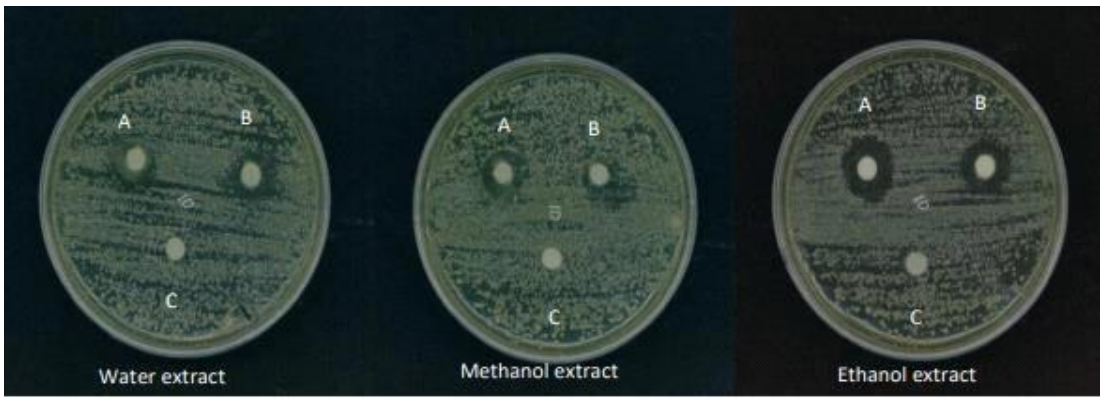

Figure 6: Effect of M.viminalis extracts against B.subtilis at different concentration(A)100% (B)70% (C)30%.

The difference of antimicrobial activity of M.viminalis extracts are shown in Figure 5.3 B.subtilis were susceptible to all type of extracts but less effective at the 30%concentration.

DISCUSSION

From this study, M.viminalis could be extracted either with ethanol, methanol or water. However, extraction by ethanol as solvent produced greater yield compared to methanol and water. This could be attributed to the increase polarity of the solvent from ethanol to water

which favours the extraction of non-polar solute such as plant oil (Abbasi et al., 2008). In other studies, many different solvents have been used for extraction of M.viminalis such as hexane, chloroform (Rattanakom and Yasurin, 2015) and petroleum ether (Dash et al., 2011). Ethanol was also the most effective solvent for the M.viminalis extraction as indicated by study from Taemchuayet al. (2009).

All the extracts of M.viminalis showed significant antibacterial activity against E.coli and B.subtilis at 100 %concentration. Both microbes were the most susceptible towards ethanol extracts followed by methanol and water extract. Both E.coli and B. subtilis showed no inhibition by distilled water which suggested no residual effect from the solvent. In this study, ethanol extracts showed maximum inhibitory effect which is similar with the study by Dashetal. (2011) that showed the ethanol extract of M.viminalis was very effective in inhibiting the growth of all the microorganisms like E.coli and B.subtilis.

Effectiveness of antimicrobial agent is influenced by solubility, volatility and polarity of compounds in plants(Stratford and Eklund, 2003). Triterpenes in M.viminalis are polar compounds which ionization of molecule combine with adsorption of poly phenols to bacterial membranes leads to inhibition of bacterial growth by disrupting the bacterial membranes (Kalita and Saikia, 2012).

B. subtilis which is a gram-positive bacterium was also found to be more susceptible towards M.viminalis extracts. This may due to gram-positive bacteria was more sensitive than gram-negatives (Singh et al., 2012). Compare to gram-positive bacteria, gram-negative bacteria has lower outer-membrane permeability that limits the entry of antimicrobial agents into the cells (Fidaleo et al., 2011) and different resistance mechanism such as target site modification and enzymatic inactivation (Vadlapudi etal., 2012) Antimicrobial activity of methanol extract and water extract at 100 % concentration against E.coli and B.subtilis showed little differences of inhibition zone as the 70 % concentration suggested the use of the extract at the less concentration but still giving significant inhibition of microbial growth. The results obtained in the present study indicated extracts of M.viminalis can be developed into broad spectrum of antibacterial and Essential oil from plants do have antimicrobial activity as proven by Ferdes and Ungureanu (2012) which have significant application against human pathogens, including those that cause enteric infections. They are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases (Hassan et al., 2004). According to Okoli and Iroegbu (2005), inhibition of microbes

by disc diffusion method is also influenced by concentration of extract, duration of exposure and microbes tested

SUMMARY

M.viminalis urban is a tropical medicinal plant, native to southeast Asian countries. It is an important medicinal plant with rejuvenating properties, used in Ayurveda for promoting vitality and life.

The extract of Melaleuca viminalis were found to be effective antibacterial agents against pathogens.

CONCLUSION

In conclusion, the ethanol extract of M.viminalis leaf has demonstrated promising antimicrobial properties. Increasing awareness, promotion and utilization of this fruit for public benefits are highly encouraged and identification of active phytoconstiuents in the extracts will serve as a natural cytotoxic agent against various cancers. Results of this study confirm that M.viminalis leaves possess antimicrobial activity. From the entire experiment, it can be concluded that cntella leaf have antibacterial activity. The antibacterial activity was strong enough to inhibit E. coli (Gram negative bacteria) and B. subtilis (Gram positive bacteria). This research indicates that M.viminalis leaves have potential natural antibacterial compound. Further research is suggested to study the application of antibacterial activity of M.viminalis leaf. This inhibitory effect was strain and concentration dependent; Gram positive bacteria were more susceptible to the effect of M.viminalis leaf compared to Gram negative. This effect could be attributed to the structure of gram negative bacterial cell wall that provides a level of intrinsic resistance to certain hydrophilic substances and thus preventing the penetration of active materials in ethanolic extracts into the bacterial cell. This could provide an explanation for our results. Inhibitory effects of acetone leaf extracts showed similar results to those of other studies.

The present work demonstrates the antimicrobial potential of M.viminalis leaf extract by using ethanol as solvent. The results indicate that ethanol and methanol are better than water for the extraction of the antibacterial properties of M.viminalis. The observed inhibition of Gram-positive bacteria, Bacillus subtilis suggests that M.viminalis possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using methanol or ethanol as the solvent. Comparisons with related data from the literature

indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of M.viminalis. On the basis of the present finding, M.viminalis leaf possesses the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections or diseases caused by B. subtilis.

REFERENCE

1. Srivastava S, Ahmad A, Syamsunder K, Aggarwal KK, Khanuja SPS. Essential Oil Composition of Callistemon viminalis Leaves from India. Flavour and Fragrance Journal, 2003; 18(5): 361-363.
2. Oyedeji OO, Lawal OA, Shode FO, Oyedeji AO. Chemical Composition and Antibacterial Activity of the Essential Oils of Callistemon Citrinus and Callistemon viminalis from South Africa. Molecules, 2009; 14(6): 1990-1998.
3. Abdelhady MI, Motaal AA, Beerhues L. Total Phenolic Content and Antioxidant Activity of Standardized Extracts from Leaves and Cell Cultures of Three Callistemon Species. American Journal of Plant Sciences, 2011; 2(6): 847.
4. Afrah JA. Studying of Antibacterial Effect for Leaves Extract of Callistemon viminalis in Vitro and Vivo (Urinary System) for rabbits. Journal of Kerbala University, 2012; 10(2): 246-254.
5. Salem MZ, Ali HM, El-Shanhorey NA, Abdelmegeed A. Evaluation of Extracts and Essential Oil from Callistemon viminalisLeaves: Antibacterial and Antioxidant Activities, Total Phenolic and Fl.
6. Garg S, Kasera H. Anthelmintic Activity of the Essential Oil of Callistemon viminalis. Fitoterapia, 1982; 53(5/6): 179-181.
7. Ghasemi V, Yazdi AK, Tavallaie FZ, Sendi JJ. Effect of essential oils from Callistemon viminalis and Ferula gummosa on Toxicity and on the Hemocyte Profile of Ephestia kuehniella (Lep.: Pyralidae). Archives of Phytopathology and Plant Protection, 2014; 47(3): 268-278.
8. Ji T. Traditional Chinese Medicine Pills for Treating Hemorrhoid. CN 101352524 A., 2009; 20090128.
9. Zubair M, Hassan S, Rizwan K, Rasool N, Riaz M, Ziaulhaq M, Defeo V. Antioxidant Potential and Oil Composition of Callistemon viminalis Leaves. Scientific World Jourrnal, 2013; 1-8.

10. Gohar AA, Maatooq GT, Gadara SR, Aboelmaaty WS, Elshazly AM. Molluscicidal Activity of the Methanol Extract of avonoid Contents. Asian Pacific Journal of Tropical Medicine, 2013; 6(10): 785- 791. Callistemon viminalis (Sol. Ex Gaertner) G. Don Ex Loudon Fruits, Bark and Leaves Against Biomphalaria Aexandrina Snails. Iranian Journal of Pharmaceutical Research, 2014; 13(2): 505-514.
11. Nath D, Das N, Das S. Bio-repellents for Land Leeches. Defence Science Journal, 2002; 52(1): 73-76.
12. Ndomo A, Tapondjou L, Ngamo L, Hance T. Insecticidal Activities of Essential Oil of Callistemon viminalis Applied as Fumigant and Powder Against Two Bruchids. Journal of Applied Entomology, 2010; 134(4): 333-341.
13. Eldib R, Elshenawy S. Phenolic Constituents and Biological Activities of the Aerial Parts of Callistemon viminalis (Sol. Ex Gaertner) G. Don ex Loudon. Bulletin of Faculty Pharmacy, 2008; 46(2): 223- 235.
14. Srivastava, S.; Ahmad, A.; Syamsunder, K.; Aggarwal, K.; Khanuja, S. Essential oil composition of Callistemon viminalisleaves from India. Flavour Frag. J., 2003; 18(5): 361-363.
15. Zubair, M.; Hassan, S.; Rizwan, K.; Rasool, N.; Riaz, M.; Ziaulhaq, M.; Defeo, V. Antioxidant potential and oil composition of Callistemon viminalis leaves. Sci. World. J., 2013; 1-8.
16. Sheat, W.G.; Schofield, G. Complete AZ of gardening in Australia, National Book Publishers, 1995.
17. Wheeler, G. Maintenance of a narrow host range by Oxyops vitiosa; A biological control agent of Melaleuca quinquenervia.Biochem. System. Ecolo., 2005; 33(4): 365-383.
18. Brophy, J.J.; Forster, P.I.; Goldsack, R.J.; Hibbert, D.B.; Punruckvong, A. Variation in Callistemon viminalis (Myrtaceae): New evidence from leaf essential oils. Austr. System. Bot., 1997; 10(1): 1-13.
19. Shinde, P.; Patil, P.; Bairagi, V. Pharmacognostic, phytochemical properties and antibacterial activity of Callistemon citrinusviminalis leaves and stems. Int. J. Pharm. Pharm. Sci., 2012; 4(4): 406-408.
20. Ruzin, S.E., Plant microtechnique and microscopy. Vol. 198.; Oxford University Press: New York, 1999.
21. Ruzin, S.E., Plant microtechnique and microscopy. Vol. 198.; Oxford University Press: New York, 1999.

22. Obrien, T.; Feder, N.; Mccully, M.E. Polychromatic staining of plant cell walls by toluidine blue. Protoplasma, 1964; 59(2): 368-373.
23. Simmons D, Parsons R. Variation in the Morphology and Volatile Leaf Oils of Eucalyptus yarraensis (Myrtaceae), a Disjunct Rare Species in Victoria. Australian Systematic Botany, 1999; 12(2): 157-167.
24. Quijanocelis C, Gaviria M, Vanegaslopez C, Pino JA. Chemical Composition and Antibacterial Activity of the Essential Oil of Callistemon viminalis (Gaertn.) G. Don Leaves from Colombia. Journal of Essential Oil-Bearing Plants, 2010; 13(6): 710-716.
25. USDA, N. The PLANTS Database, Version 3.5. National Plant Data Center, Baton Rouge, LA, 2004.
26. Sheat, W.G. Schofield, G. Complete AZ of gardening in Australia, National Book Publishers, 1995.
27. Cowan MM. Plant Products as Antimicrobial Agents. Clinical Microbial Research, 1999; 12: 564-582.
28. Rizwan K, Zubair M, Rasool N, Riaz M, Zia-Ul-Haq M, De Feo V. Phytochemical and Biological Studies of Agave Attenuata. International Journal of Molecular Sciences, 2012; 13: 6440-6451.
29. Wu JW, Li BL, Tang C, Ke CQ, Zhu NL, Qiu SX, Ye Y. Callistemonols A and B, Potent Antimicrobial Acylphloroglucinol Derivatives with Unusual Carbon Skeletons from Callistemon viminalis. Journal of Natural Products, 2019; 82: 1917–22.
30. Ahmad K, Athar F. Phytochemistry and Pharmacology of Callistemon viminali(Myrtaceae): A Review. Natural Products Journal, 2016; 7: 1–10.
31. Zubair M, Hassan S, Rizwan K, Rasool N, Riaz M, Zia-Ul-Haq M,et al. Antioxidant potential and oil composition of Callistemon viminalis leaves. Sci World J., 2013.
32. Abd AJ. Studying of antibacterial effect for leaves extract ofCallistemon viminalis in vitro and vivo (urinary system) for rabbits.J Kerbala Univ, 2012; 10(2): 646-654.
33. Saleem A, Nasir S, Rasool N, Bokhari TH, Rizwan K, Shahid M, et al. In vitro antimicrobial and haemolytic studies of Kalanchoepinnata and Callistemon viminalis. Int J Chem Biochem Sci., 2015; 7: 29-34.
34. Gohar, A.; Maatooq, G.; Gadara, S.; Aboelmaaty, W. One new pyrroline compound from Callistemon viminalis (Sol. Ex Gaertner) G. Don Ex Loudon. Nat. Prod. Rese., 2013; 27(13): 1179-1185.
35. Ernawita, S.F.S. Composition and antimicrobial activity of crude extracts and essential oil of Callistemon viminalis leaves. J.Biol. Edukasi., 2013; 1(2): 47-50.

36. Badawy, M.E.; Abdelgaleil, S.A. Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. Indu. Crops. Prod., 2014; 52: 776-782.