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Original Article

Assessment of Antibacterial Efficacy of *Callistemon viminalis* (*Sol. ex Gaertn.*) *G. Don* against Some Isolates Obtained from Urinary Tract Infections

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Abstract

This study aimed to examine the antibacterial effects of constituents obtained from *Callistemon viminalis* leaves. This goal was achieved by using three organic solvents, namely Ethanol, Ethyl acetate, and Hexane to prevent the growth of the causative urinary tract infections isolates, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Proteus* sp. in Iraq. The *C. viminalis* fresh leaves collected from different regions of Hillah City, during March 2020, were classified according to the taxonomic features of Iraqi Flora. Extractions were completed by a method of digestion and then the stock solution of 200 mg/mL was prepared in 10% of Dimethylsulfoxide. A Millipore filter (0.22 µm) was used for the sterilization of all the extracts used in this study. Agar well diffusion method was utilized to test the antibacterial effects of the constituents separated from the dried leaves of *C. viminalis* against the urinary tract infection bacteria at three concentrations of 50, 100, and 200 mg/mL for each extracted constitute by the three different solvents. Dimethylsulfoxide 10% and the meropenem were utilized as the negative and positive controls. Constituents separated by ethanol solvent at 200 mg/mL exhibited significant supremacy ($P \leq 0.05$) over the meropenem against *Proteus* sp. isolate, and exhibited the same significant difference ($P \leq 0.05$), compared to the meropenem drug against *E. coli*. Constituents extracted by Ethyl acetate organic solvent at a concentration of 200 mg/mL exhibited a similarly significant effect ($P \leq 0.05$), compared to the meropenem against *Proteus* sp. isolate. However, the hexane extract was the least effective among the other solvents utilized in this study. The results of the current study revealed that constituents in the leaves of *C. viminalis* could be considered a valuable herbal remedy for controlling urinary tract infections pathogenic bacteria.

Keywords: Antibacterial activity, *Callistemon viminalis*, Urinary tract infections

1. Introduction

*Callistemon viminalis*is is a member of the Myrtaceae family, which belongs to the Plantae Kingdom and has several species (34 species). Some of the most famous species include *Metrosideros viminalis* (Sol. ex Gaertn.), *C. viminalis* (Sol. ex Gaertn.) G. Don, and *Melaleuca viminalis* (Sol.ex Gaertn.) Byrnes (1). The flowers are cylindrical and resemble bottlebrush. The common name of the *C. viminalis* is the weeping bottle brush. It is native to Australia and temperate regions. In its natural habitat, it grows in the form of shrubs or small trees reaching four meters in height (2-4).

Undoubtedly, urinary tract infections (UTIs) have become more dangerous due to bacterial resistance to antibiotics, which has led to the emergence of strains that are resistant to most antibiotics and cause multiple infections (5). The increasing use of antibiotics in various medical, industrial, and agricultural fields has

led to the emergence of a phenomenon called "multi-drug resistance" which refers to the resistance of bacteria to many classes of antibiotics. This increases the risk of infections and reduces the chances of recovery (6).

WHO (7) has reported that medicinal plants can meet community needs and provide primary healthcare in case of the aggravation of the problem of antibiotic resistance. This is due to their effective substances that are capable of making a difference and providing hope for the future. Secondary metabolites produced by medicinal plants can act as bacteriostatic and bactericidal against "multi-drug resistant" microorganisms and are regarded as a good precursor for the synthesis of new antibiotics and drugs for controlling infectious diseases, principally from the causative agent bacteria of UTIs (8-10).

Humans in all civilizations knew the importance of medicinal plants and were guided to the treatment of diseases by experience; hence, herbal medicine is the oldest known method of treatment. However, this study aimed to examine the antibacterial effects of constituents obtained from *C. viminalis* leaves by using three organic solvents, such as Ethanol, Ethyl acetate, and Hexane to prevent the growth of the UTI isolates in Iraq.

2. Materials and Methods

2.1. Materials of Plant

The fresh leaves of *C. viminalis* were collected from different regions of Hillah City, Iraq, in March 2020. The leaves were classified according to the taxonomic features (11), and plant materials were prepared and kept according to McClure (12).

2.2. *Callistemon viminalis* Components Extraction

The *C. viminalis* leaves were extracted by using three organic solvents, such as Ethanol, Ethyl acetate, and Hexane as previously described by Handa, Khanuja (13). The extractions were performed by a method of digestion according to Handa, Khanuja (13). The stock solution of 200 mg/mL was prepared in 10% of Dimethylsulfoxide. A Millipore filter (0.22 µm) was used for the sterilization of all the extracts used in this study. Afterward, the extracted samples were stored in the refrigerator at -20 °C (14).

2.3. Antibacterial efficacy

Agar well diffusion method was applied to evaluate the antibacterial effects of the *C. viminalis* dried leaves extracted by Ethanol, Ethyl acetate, and Hexane solvents at three different concentrations of 50, 100, and 200 mg/mL against the urinary tract infection bacterial isolates (15). A 6-mm diameter Cork borer was used to make wells in agars. Control negative was made by adding 10% Dimethyl sulfoxide to wells and meropenem antibiotic was used as a control positive treatment (Table 1).

Table 1. Concentration of meropenem and type of bacteria used in the study

Antibiotic	Abbreviation	(Disc/µg)	Isolates
Meropenem	MEM	10	*Escherichia coli*
			Pseudomonas aeruginosa
			Klebsiella pneumonieae
			Proteus sp.

2.4. Bacterial Pathogenic Isolates

Isolates of UTIs were obtained from Microbiology laboratories in different hospitals within the boundaries of the municipality of Hillah-Iraq (Table 2).

Table 2. Pathogenic Isolates and sources of isolates

No	Isolates	Source
1	*Escherichia coli*	Urinary tract infections
2	*Pseudomonas aeruginosa*	
3	*Klebsiella pneumonieae*	
4	*Proteus* sp.	

2.5. Statistical analysis

The experiments were based on a completely randomized design. An analysis of variance and least significant differences at $P \leq 0.05$ was performed by using SPSS software (version 16.0), and the results were expressed in the form of mean±standard deviation.

3. Results

The antibacterial effects of phytochemical complexes separated from *C. viminalis* leaves by using various sorts of organic solvents, such as Ethanol, Ethyl acetate, and Hexane, against urinary tract bacterial isolates, are represented in tables 3, 4, and 5. The outcomes displayed that the three organic solvents extracts of ethanolic, Ethyl acetate, and hexane of *C. viminalis* leaves exhibited significant decline ($P \leq 0.05$) in the growing of urinary tract bacteria, compared to the negative control. The antibacterial properties of *C. viminalis* were tested at a concentration of 50, 100, and 200 mg/mL, compared to dimethylsulfoxide (10%) as the negative control and meropenemas as a positive control.

The results showed that the inhibitory growth effects arise significantly ($P \leq 0.05$) at a concentration of 200 mg/mL for all the extracted materials by using all the solvents except for *K. pneumonia*. The *K. pneumonia* was not inhibited by the *C. viminalis* leaves extracted materials. The recorded data of the current study revealed that ethanolic *C. viminalis* extract was significantly superior to the meropenem when applied to *Proteus* sp. pathogenic bacteria with an inhibition diameter of 20±1.0, compared to 15±0.0 in the meropenem-treated group. The results also revealed that the constituents extracted by an ethanolic organic solvent at a concentration of 200 mg/mL exhibited similar influences on *E. coli*.

There were no significant changes ($P \leq 0.05$) between the *C. viminalis* extracts and the meropenem as the inhibitory belt reached up to 20±1.0 mm in diameter in the ethanolic *C. viminalis* crude extract, compared to the meropenem (20±0.0 mm) once utilized for the *E. coli* pathogen.

The *C. viminalis* also exhibited significant inhibition in the culture of *P. aeruginosa* isolates where the diameter of the inhibition increased to 15.33±0.57 mm at the concentration of 200 mg/mL, compared to the negative control. Nevertheless, this inhibition was not significant in comparison with the diameter of the inhibition produced by the meropenem antibiotic where the meropenem was significantly superior to the ethanolic plant extract when applied to *P. aeruginosa*. Moreover, *K. pneumonia* showed complete resistance to all concentrations used in this study (Table 3).

The results of the current study revealed that there were significant ($P \leq 0.05$) declines in UTIs bacterial isolates growth with the increasing concentrations of constituents obtained by Ethyl acetate organic solvent, compared to the negative control group as represented in table 4. In a similar direction, the results of the *Proteus* sp. isolate culture revealed that constituents extracted by Ethyl acetate solvent at a concentration of 200 mg/mL had lower inhibitory effects (14.66±0.57) compared with meropenemas (15±0.0) ($P \leq 0.05$). In contrast, *E. coli*, *P. aeruginosa*, and *K. pneumonia* exhibited complete resistance to the different concentrations of Ethyl acetate used in the study.

In addition, the hexane extract was the least effective among the solvents used in the study, as both *Proteus* sp. and *K. pneumonia* showed complete resistance to all concentrations. However, the meropenem antibiotic was significantly superior to the hexane extract although the inhibition diameter increased to 15±0.0 when applied to *E. coli* and *P. aeruginosa* (Table 5).

Table 3. An antibacterial of Ethanolic extract for *Callistemon viminalis* against the urinary tract infections (UTI) pathogenic bacteria

Treatment/mg/ml	UTIs bacteria			
	Escherichia coli	***Pseudomonas aeruginosa***	***Klebsiella pneumonieae***	***Proteus* sp.**
	Inhibition zone/m.m			
Control negative (D.M.S.O. 10%)	0±0.0	0±0.0	0±0.0	0±0.0
50.0	0±0.0	0±0.0	0±0.0	0±0.0
100.0	0±0.0	0±0.0	0±0.0	0±0.0
200.0	20±1.0	15.33±0.57	0±0.0	20±1.0
Control positive	20±0.0 Meropenem	20±0.0 Meropenem	20±0.0 Meropenem	15±0.0 Meropenem
Least significant difference	0.80	0.47	---	0.81

*Mean±standard deviation

Table 4. An antibacterial of Ethyl acetate extract for *Callistemon viminalis* against urinary tract infections (UTI) pathogenic bacteria

Treatment/mg/ml	UTI bacteria			
	Escherichia coli	***Pseudomonas aeruginosa***	***Klebsiella pneumonieae***	***Proteus* sp.**
	Inhibition zone/m. m			
Control negative (D.M.S.O. 10%)	0±0.0	0±0.0	0±0.0	0±0.0
50.0	0±0.0	0±0.0	0±0.0	0±0.0
100.0	0±0.0	0±0.0	0±0.0	12±0.0
200.0	0±0.0	0±0.0	0±0.0	14.66±0.57
Control positive	20±0.0 Meropenem	20±0.0 Meropenem	20±0.0 Meropenem	15±0.0 Meropenem
L.S.D	---	---	---	0.47

*Mean±standard deviation

Table 5. An antibacterial of Hexane extract for *Callistemon viminalis* against urinary tract infections (UTI) pathogenic bacteria

Treatment/mg/ml	UTI bacteria			
	Escherichia coli	***Pseudomonas aeruginosa***	***Klebsiella pneumonieae***	***Proteus* sp.**
	Inhibition zone/m.m			
Control negative (D.M.S.O. 10%)	0±0.0	0±0.0	0±0.0	0±0.0
50.0	0±0.0	0±0.0	0±0.0	0±0.0
100.0	0±0.0	0±0.0	0±0.0	0±0.0
200.0	15±0.0	15±0.0	0±0.0	0±0.0
Control positive	20±1.0 Meropenem	20±1.0 Meropenem	20±0.0 Meropenem	15±0.0 Meropenem
L.S.D	0.80	0.80	---	---

Mean±standard deviation

4. Discussion

Development of resistant pathogenic bacteria to drugs in humans, animals, and crops as well as the unwanted side effects of these drugs have encouraged scientists to investigate different medicinal plants to fight bacterial infections. In contrast to the chemical drugs and antibiotics, the medicinal plant is also characterized by being an integrated part of pharmacy that contains more than one effective substance which works synergistically with each other in treating the disease. All these combined factors and the possible synergistic effects have attracted great attention to the exploration of new safe plant-derived drugs, especially, in light of the high global poverty rates due to wars, suffocating economic crises, and high prices for chemical treatments.

There is no doubt that the effective compounds extracted from medicinal plants remain one of the important, if not the most important, sources in the fight against diseases, especially in light of the aggravation of the problem of the resistance of a microorganism to antibiotics. Medicinal plants are also less harmful in terms of side effects, compared to chemical drugs. Constituents are separated from different active parts of numerous medicinal plants, such as (*Lactuca serriola* leave, *Lepidium sativum* leaves, *Myrtus Communis* leaves, *Cassia senna* leaves, *Ricinus communis* leaves, *Cassia didymobotrya* leaves, *Melia azedarach* leaves, *Dianthus caryophyllus* flowers bud, and *Salvia hispanica* seeds), possess the ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples (16-24).

Hussein, Naji (25) reported that constituents separated from unicellular primitive plants, like *Chlorella Vulgaris* possess the ability of antibacterial counter to pathogenic bacteria. Kamal, Hussein (26) used phytochemical compounds separated from *Hibiscus sabdarifa* for controlling *E. coli* and *Proteus* sp. Kamal,

Al-Kaim (27) used constituents extracted from *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa*. AL-Masoodi, Hussein (28) used phytochemical compounds extracted from *Boswellia carteri* and *Curcuma longa* for controlling *Fusarium* sp. isolated from seeds of corn.

Hussain, Hussein (29) used terpenoids compounds extracted from *Carthamus tinctorius* L. against *Aspergillus* species isolated from stored medicinal plant seeds. Secondary metabolites represented by Alkaloids and Flavonoids compounds separated from *M. Communis* leaves are considered a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens (30).

Leaves of *C. viminalis* have an appreciable level of total phenolic and flavonoids content (31). Phytochemical compounds extracted by Methanol and Ethyl acetate solvents from the leaves of *C. viminalis* have antibacterial effects against pathogenic bacteria (32). Phytochemical compounds extracted from leaves of *C. viminalis* are used for Molluscicidal activity against Snails (33). Unlike *P. aeroginosa*, *S. aureus*, and *S. pyogenes*, Congener and Tormentic acid extracted from *C. viminalis* have antibacterial effects (34).

Essential oils extracted from *C. viminalis* have antibacterial effects against *Listeria monocytogenes* and antifungal activity against *Aspergillus flavus* (35). Essential oils also exhibited strong antibacterial activity against *S. faecalis*, *S. aureus*, *B. cereus*, and *S. macrcesens* (36). Lastly, the antibacterial effects of *C. viminalis* might belong to Constituents and their influence on the synthesis of proteins, DNA, RNA, polysaccharides, and disturbance in the permeability of cell membranes or preventing the efflux pump to work properly.

Based on the findings of the current study, the constituents found in the *C. viminalis* plant are very effective against microorganisms isolated from the urinary tract. Therefore, they can be recommended to be used against other pathogenic isolates as well as fungi and insects. Moreover, they can be used for the detection of these Constituents by using Gas chromatography and Mass spectrometry.

Constituents extracted from *C. viminalis* (Sol. ex Gaertn.) G. by utilizing Ethanol and Ethyl acetate are regarded as respectable sources for controlling isolates of Urinary Tract Infections.

Authors' Contribution

Study concept and design: S. H. R. and H. J. H.
Acquisition of data: S. A. K.
Analysis and interpretation of data: N. M. S. and H. J. H.
Drafting of the manuscript: S. A. K.
Critical revision of the manuscript for important intellectual content: H. J. H.
Statistical analysis: S. H. R. and H. J. H.
Administrative, technical, and material support: S. H. R. and H. J. H.

Conflict of Interest

The authors declare that they have no conflict of interest.

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