International Journal of Advanced Scientific Research
ISSN: 2456-0421; Impact Factor: RJIF 5.32
Received: 03-11-2020; Accepted: 18-11-2020; Published: 04-12-2020
www.allscientificjournal.com
Volume 5; Issue 6; 2020; Page No. 105-107

Identification of antibacterial activity from *Ocimumbasilium* against various pathogenic bacteria

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Abstract
Nowadays, the medicinal plants are the good source of remedy for various infections and diseases. It has a potential antibacterial activity against various pathogenic bacteria. In this study, we investigate the antibacterial activity of *Ocimumbasilium* was determined for the various bacterial strains with aqueous, ethanol and acetone extracts. These studies present the ethanol extract has the maximum inhibitory effect for all the strains and the aqueous extract showed the high inhibitory effect on *Pseudomonas aeruginosa.* The highest zone of inhibition was recorded for the acetone extracts for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli.* These results concluded that the ethanol extract of *Ocimumbasillicum* has the potential antibacterial activity against pathogenic bacterial strains.

Keywords: *Ocimumbasillicum*, antibacterial activity, pathogenic bacterial strains

Introduction
Phytochemicals are used to describe the large number of secondary metabolic compounds found in plants. The different photoconstituents present in plants include alkaloids, flavanoids, glycosides, saponins and terpenoids and many compounds are having antibacterial activities. The major phytochemical compounds determine the biological properties of essential oils such as menthol and eugenol were considered outstanding compounds demonstrating an antibacterial potential [1]. It has strongest antimicrobial activity against all the tested bacteria and nine essential oils were examined for antimicrobial activity against reference and clinical strain of Salmonellaenteritis's [2]. The use of herbal products has been increased and it shows the good antibacterial activity [3]. The **e**ffects of ethanol extract of leaves sweet basil *Ocimumbasilicum* upon *Escherichia* coli *in vitro* were studied. At a ratio of 100 mg/ml, *Ocimumbasilicum* caused a marked increase in zone of inhibition of the *Escherichia* coli growth. The size of inhibition zones was different and substantially increased according to concentration of extract and again the growth was completely inhibited in the highest concentration [4].
The phytochemical analysis revealed the presence of various pharmaceutically active secondary metabolites like phenolic compounds, flavonoids, carbohydrates, glycosides and tannins.
Using the zone of inhibition as inhibitory parameter, the crude extract exhibited the best antibacterial activity among all the other solvent extracts but was lower than the standard drug ciprofloxacin [5]. The phytochemical screening of aqueous extract of *Ocimum*basillicum *L*. was done along with the evaluation of antibacterial activities of crude ethanolic, methanolic extracts and soxhlet essential oil against four bacterial strains. *Staphylococcusaureus*, *Escherichiacoli*, Bacillus subtitles and *Bacillus thuringiensis*. Phytochemical screening showed the presence of carbohydrates, tannins, coumarins and steroids. *Ocimumbasillicum* showed the antibacterial activates against all four strains by forming zone of inhibition [6]. Extract of *Ocimum basilicum* having strong antibacterial and antioxidant properties are widely used for medicinal purposes [7].
The methanol extracts of *Ocimumbasillicum* exhibited the antimicrobial activity against tested microorganisms and showed inhibition zones against strains of *Pseudomonas aeruginosa, Shigella sp., Listeria monocytogenes, Staphylococcus aureus* and two different strains of *Escherichia coli* [8]. Antibacterial activity of *Ocimumbasillicum* against various gram negative strains such as E.Coli, A. hydrophila and C. freundii are sensitive when compare with other strains [9]. *Ocimumbasillicum* showed antibacterial activity against Gram positive bacteria and Gram negative bacteria [10].

Material and Methods
Collection of Sample and preparation of plant extract
The leaves of *Ocimum basillicum* were collected from Perambalur region. The sample was dried under shadow and it was made powdered for extraction. The powdered form of sample was extracted with solvents such as aqueous, ethanol and acetone using soxhlet apparatus. These extracts were subjected for phytochemical analysis and identify the phytoconstituents.

Isolation of organisms
The bacterial identification was carried out by biochemical methods according to the standard microbiological techniques. The bacterial test strains such as *Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli* were used in this study.

Disc diffusion method
Agar disk diffusion method was used for screening of antibacterial activity of selected plant extracts. 20ml of

Mueller Hinton agar in each plate autoclaved and cooled at 45oC was poured into sterile petri plates and allowed to solidify completely.

The test isolates were prepared by evenly spreading the inoculum with help of a sterilized spreader or swabs on to the entire surface of the agar plate. After drying, the sterile filter paper discs were impregnated with the aqueous extracts and placed on the inoculated plates with the help of forceps.

The plates were left at ambient temperature for 30 minutes to allow exceed pre diffusion prior to incubation at 37oC for 24 hours.

After incubation the plates were observed for appearance of zone of inhibition around the discs.

Antibacterial activity was evaluated by measuring diameter of zones of inhibition (in millimeters) of bacterial growth.

HPLC

HPLC works on the principle that some molecules take longer than others to pass through a Chromatography column. This depends on the affinity of the molecule with the mobile phase (liquid or gas) and the stationary phase (solid or liquid). It has more affinity with the stationary phase take longer to pass through and vice versa. There are different types of column to separate molecules by different criteria.

Results and Discusion

HPLC Results

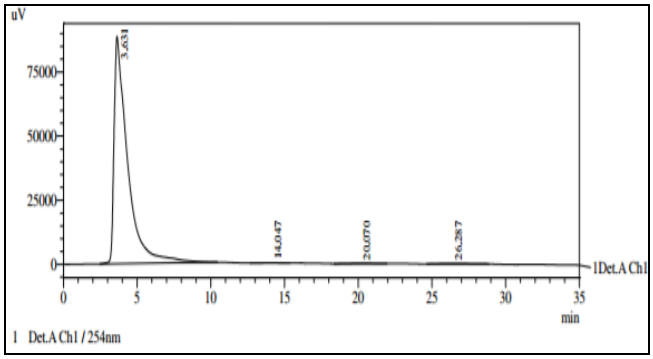


Fig 1

Table 1: Compounds determined by HPLC

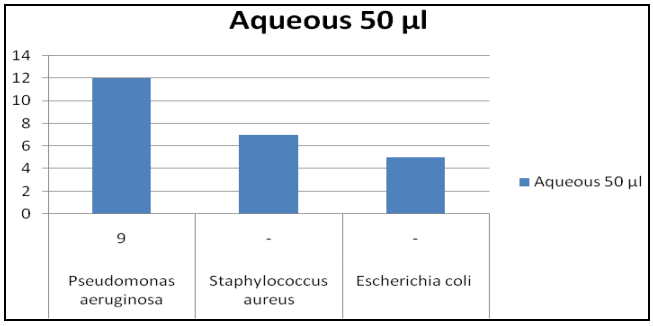
PeakTable

Detector A Ch1 254nm

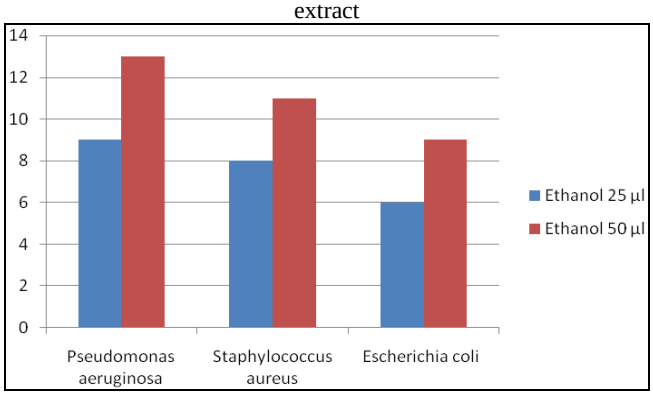
Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.631	5664336	88728	99.170	99.538
2	14.047	7765	92	0.136	0.104
3	20.070	13015	132	0.228	0.148
4	26.287	26636	188	0.466	0.211
Total		5711753	89139	100.000	100.000

Table 2: Antibacterial activity of aqueous, ethanol and acetone extracts of *Ocimumbasillicum*

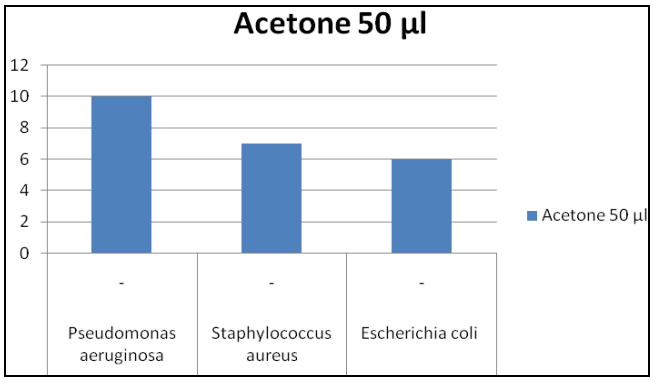
Organisms/extract	Aqueous		Ethanol		Acetone	
Concentration	25 µl	50 µl	25 µl	50 µl	25 µl	50 µl
Pseudomonas aeruginosa	9	12	9	13	-	10
Staphylococcus aureus	-	7	8	11	-	7
Escherichia coli	-	5	6	9	-	6



Graph 1: Zone of Inhibition for antibacterial activity in aqueous extract



Graph 2: Zone of Inhibition for antibacterial activity in ethanol extract



Graph 3: Zone of Inhibition for antibacterial activity in acetone extract

Antibacterial activity of *Ocimum basillicum* was determined and the bacterial inhibiting activity was identified. The antibacterial activity in aqueous, ethanol and acetone extracts against the isolated organisms were determined. The inhibition zones in diameter were identified and the results are showed in the table 2.

The activity of aqueous extracts shows the sensitivity to the organisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus and Escherichia coli* and if the concentration has increased it shows the high inhibitory effect. The ethanol extract shows the significant inhibitory activity against all organisms. The acetone extracts show the sensitivity to *Staphylococcus aureus* and resistance to *Pseudomonas aeruginosa and Escherichia coli.* In the acetone extract, if the concentration has increased the zone of inhibition has increased.

Conclusion

In this study, the antibacterial activity was determined for the various bacterial strains based on the aqueous, ethanol and acetone extracts. From these results, we observed that ethanol extract showed the maximum zone of inhibition on all the organisms when compare with aqueous and acetone extracts.

The aqueous extract showed the high inhibitory effect on *Pseudomonas aeruginosa* when compare with other organisms. If the concentration has increased, the acetone extract showed the high inhibitory effect against the organisms. From these observations, we concluded that the ethanol extract of *Ocimum basillicum* has the antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli.* In future, it could be used for further analysis and identify the antibacterial resistance against pathogenic organisms.

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