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Antibacterial Efficacy Of *Casuarina Cunninghamiana* Extracts Against Some Pathogenic Bacteria

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ABSTRACT

*The objective of study prepare extract of Casuarina cunninghamiana leave, fruit and bark against bacteria Staphylococcus aureus and Escherichia coli . **Methods**: collect and prepare sample ,phytochemicals , identifying antibacterial activity. **Results**: The results indicated to effect of bacteria having higher value of inhibition zone of leaves compared with fruit and bark . Methanolic extract proceed both hot water and ethanolic extract in its effect and increased activity of extracts with increased concentration (500,250.125,62.5)mg/ml .*

***Conclusion:** Methanoic extracts proceed both hot aqueous and ethanolic extracts in its antibacterial activity against G+ve than G-ve .*

Keywords: ***Casuarina Cunninghamiana, Antibacterial Phytochemical , Crude Extract.***

Article Information

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INTRODUCTION

Medicinal plants are all higher plants that have been alleged to have medicinal properties, effects that relate to health and used as drugs [1] . Traditional medicine (TM) variously known as ethno medicine, folk medicine, native healing, or complementary and alternative medicine (CAM) ,recently defined as culture-bound method of healing that humans have used to cope and deal with various diseases that have threatened their existence and survival [2]. Casuarinas are rich in phytochemical which present in various parts of this forest crop and have pharmacologically active [3]. Extraction of Casuarinas parts for the phytochemical screening is based on the solvent, solvents play a major role in the analysis of the phytochemicals present in the extract, because it has great impact in rate of extraction, compounds to be extracted, toxicity of the solvent could also affect the bioassay process [4] There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram-negative microorganisms, and this results in that plants are a good bedrock for modern medicine to treat infectious diseasesp [5]. The plant is a source of biologically active compounds such as catechin , ellagic acid, gallic acid, quercetin and lupeol, which are antioxidants[6].

MATERIALS AND METHODS

Collection and preparation of samples

The plant Casuarina was collected two time from the gardens the Faculty of Medical Technology / Al-Furat Al-Awsat Technical University . Najaf City, during September 2016 and January 2017 after that cleaning and removal foreign materials.

Plant parts were separated as leave , fruit and bark then washed two times with tap water and once with distilled water After that, put under shade until dry. Each dried part was grinding by electrical grinder . The powdered parts were kept in plastic tubes in Refrigerator at 4C until the time of use [7].

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Aqueous Extracts (Cold And Hot]

Take 20 grams of finely powdered material and 200 ml of distilled water were refluxed in flask 500ml and the suspension was put on Magnetic stirrer plate for 48 hours at room temperature to prepare cold extract while hot water extract put in boiling water and put in magnetic stirrer plate for 48 hour at room temperature then filtered by multi- layer of muslin cloth then by filter paper type Whattman, No. 1, and dried in oven (40-30)°C. After that the extract kept in refrigerator until it has been used[8].

Alcoholic Extracts

Alcoholic extract which prepared by taking 20gm of powdered sample that was extracted in soxhlet by 200ml of alcohol solvent (Methanol, Ethanol) in flask 500 ml for 24hr then evaporated by oven (40-30)˚C until dried and the extract kept in refrigerated at 4˚C until used [7]

Phytochemical Screening

Chemical detection of the active components in plant extracts [9]

Biological Effect

Specimens Collection Of Bacteria

Staphylococcus aureus and Escherichia coli were obtained from Central Health Laboratory in AL-Najaf province. The bacteria were activated and sub cultured in nutrient agar and stored on nutrient agar slants at 4 °C**.**

Antibacterial Activity

It was carried out according to disc diffusion method, the plate of Muller – Hinton agar media was inoculated with Microorganisms (E.coli and S.aureus) with sterile swabs . 6mm sterile paper discs made from Whattman No. 1

were impregnated with plant extract with different concentration (500 , 250 ,125, 62.5) .By sterial forceps the discs were position on the inoculated plate and pushed gently into agar. Each plant extract was assayed in triplicate**.**Sterile paper discs loaded with DMSO were used as negative control .The discs were placed aseptically and distinctively onto the inoculated MHA plates. Agar plates were incubated at 37°C for 16-18 hours . After that, the inhibition zones was measured by ruler (mm) [10].

Statistical Analysis

The experiments were conducted and analyzed as factorial experiments with three replications using a completely Randomized Design (CRD) with two or three factor tested by Least Significant difference (L.S.D). The mean values were compared by using L.S.D tests at probability of 1% ($P \leq 0.01$) [11].

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening was done using color forming and precipitating chemical reagents on the leave , fruit and bark of *C. cunninghamiana* were shown in table (1) that high positive reaction with the used reagents was produced by leaves extract compared to fruits and bark , both fruit and bark were equal in the results.

Positive findings of leave , fruit and bark in alcoholic extracts and aqueous extracts agreed with the researchers [12] .The presence of phenols, tannins, steroids, alkaloids, glycosides, flavonoids and terpenoids in leave [13].

The main causes of presence or absence of phytoconstituents in plant extracts depend on solvent polarity, extraction efficiency increases by using different solvents to dissolve the different phytochemical compounds present in different plant parts [14].

Antibacterial Activity Of Plants Extracts

Disc diffusion method was used to determine the antibacterial effect of the crude extracts of leaves , fruit and bark of *C. cunninghamiana* .In table (2) showed the growth inhibition zone of bacteria increased as the concentration of extract increased . The susceptibility pattern to the extracts on *S. aureus* of methanol crude extract in leave expressed maximum inhibitory zone at concentration 500 mg/ml which was 41.66 mm but in low concentration 62.5 mg/ml was 28.33 mm . In fruit recorded the inhibition zone diameter to 26 mm in high concentration of methanol crude extract , but the low concentration was 18.66 mm . The inhibition

zone diameter of bark with methanol extract was 33.33– 24.33 mm in the high and low concentration of *S. aureus* .The results in table (3) showed bacteria *E.coli* maximum inhibitory zone concentration in methanolic extracts was 36mm but in low concentration was 25mm in leave , while in fruit 36.33mm – 18mm in the high and low concentration . On the other hand, the inhibition zone of bark was 30 mm in high concentration and 25mm in low concentration **.** The results was agreement with methanolic extract was most effective on both *S. aureus* and *E . coli* bacteria in contrast with hot aqueous and ethanol extract [15] this observed in figure (1) . [16] showed Similar results have been reported in previous studies .

Table (1): Preliminary phytochemical screening of *C. cunninghamiana* extracts leave , fruit and bark .

Part of plant / Reagents		Leaves				Fruits				Bark			
		Type Of Extract											
		C.W	H.W	E.	M.	C.A	H.A	E.	M.	C.A	H.A	E.	M.
Ferric Chloride	Phenol	+	+	+	+	_	+	+	+	_	+	+	+
Lead Acetate	Phenol	+	+	+	+	_	+	+	+	_	+	+	+
Dragendroff Reagents	Alkaloid	+	+	+	+	_	+	+	+	_	+	+	+
Mayer's Reagent	Alkaloid	+	+	+	+	_	+	+	+	_	+	+	+
Glycoside		_	+	+	+	_	+	+	+	_	+	+	+
Flavonides		_	+	+	+	_	+	+	+	_	+	+	+
Saponins		_	+	+	+	_	+	+	+	_	+	+	+
Tannins		_	+	+	+	_	+	+	+	_	+	+	+
Coumarins		_	+	+	+	_	+	+	+	_	+	+	+

M. =Methanol extract, E.= Ethanol extracts H.A. = Hot Water and C.A. = Cold Water .
(+)presence compound and (-) for absence.

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Table (2): Interaction between part of plant with type of extract and concentration in inhibition zone of crude extracts from (leaves , fruit and bark) of *C. cunninghamiana* on the growth of bacteria *S.aureus* .

Plant part	Type of extract	Concentration mg/ml				
		Control	62.5	125	250	500
Leave	H.W.	0	14.3	17	18.33	23
	M.	0	28.33	30	33.3	41.66
	E.	0	18.33	24	26.66	29
Fruit	H.W.	0	12	14.33	16	18
	M.	0	18.66	20	21.33	26
	E.	0	16.66	18.66	21.33	23
Bark	H.W.	0	13	14.66	17.33	18.66
	M.	0	24.33	26	28.66	33.33
	E.	0	17.33	17	18.33	20

L.S.D (0.01) = 2.260 , H. W. = Hot Water M.= Methanol extract . E=Ethanol extract.

Table (3): Interaction between part of plant with type of extract and concentration in inhibition zone of crude extracts from (leaves , fruit and bark) of *C. cunninghamiana* on the growth of bacteria *E. coli*

Plant Part	Type of extract	Concentration mg/ml				
		Control	62.5	125	250	500
Leave	H.W.	0	14.33	16	18.66	22.66
	M.	0	25	26.66	29	36
	E.	0	16	17.33	19.66	23.6
Fruit	H.W.	0	13.66	16	20	22
	M.	0	18	20	24.33	26.33

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	E.	0	16	19	22	24
Bark	H.W.	0	11.66	15	17	19
	M.	0	25	26	28	30
	E.	0	17.33	19	22	25

L.S.D (0.01) =1.521, H. W. = Hot Water M.= Methanol extract , E=Ethanol extract.

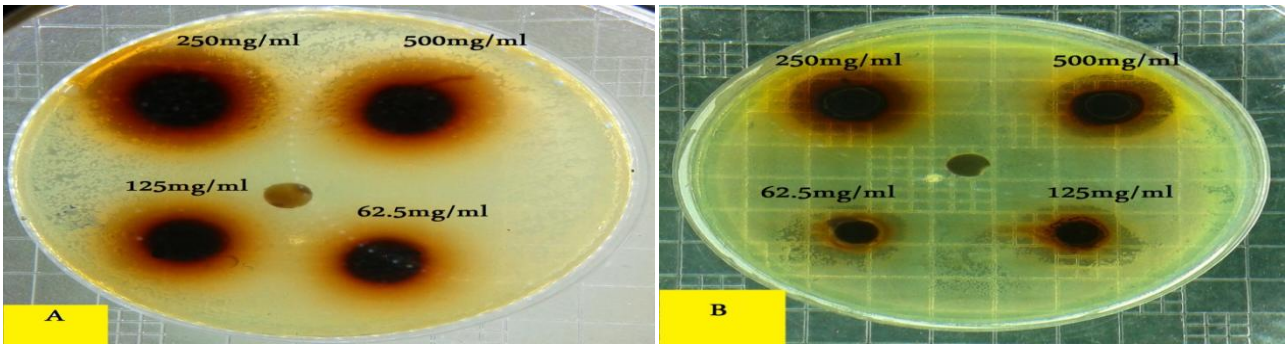

Figure (1) : Effect leave extract on bacteria A : *S.aureus* B :*E .coli* by concentration 500 , 250, 125 , 62.5 mg/ml .

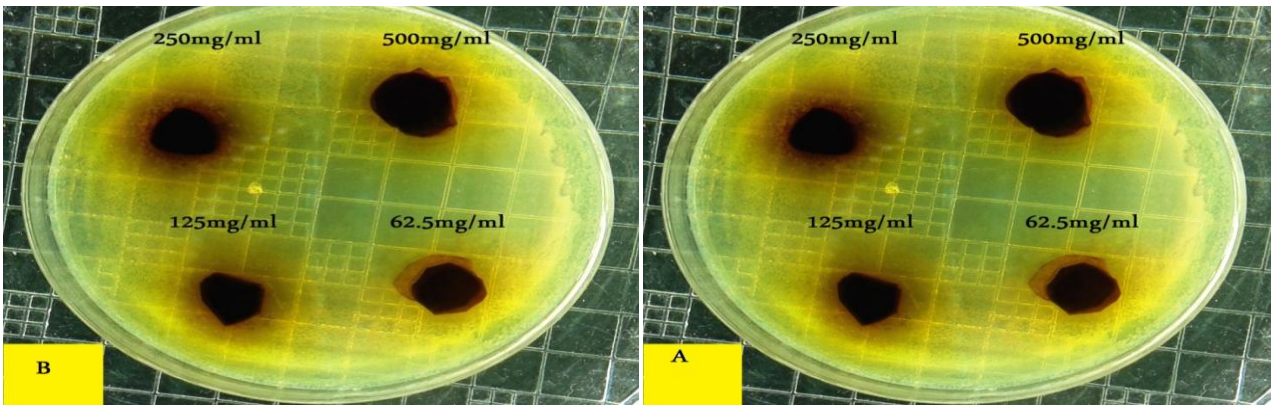

Figure (2) : Effect fruit extract on bacteria A : *S.aureus* B :*E .coli* by concentration 500 , 250, 125 , 62.5 mg/ml .

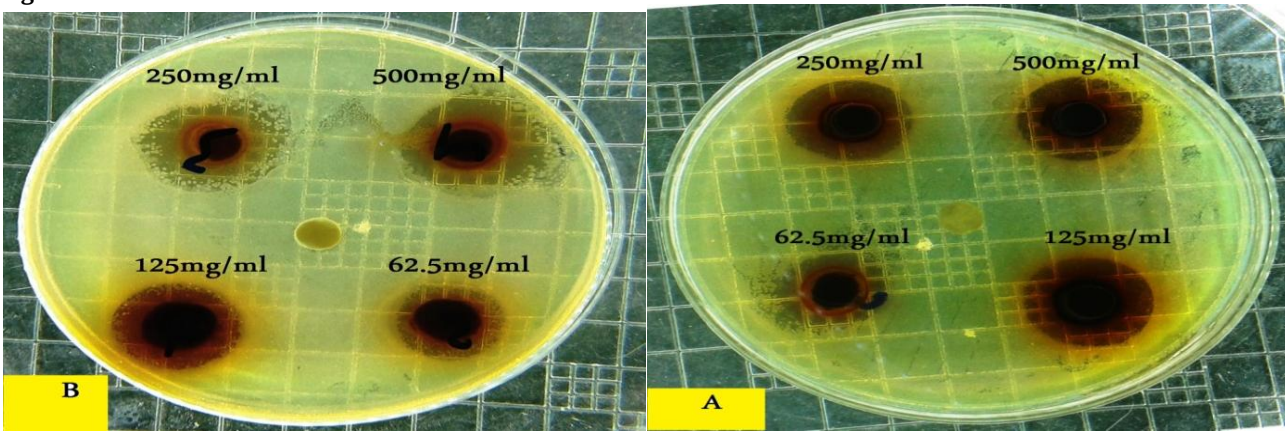

Figure (3) : Effect bark extract on bacteria A : *S.aureus* B :*E .coli* by concentration 500, 250,125,62.5mg

CONCLUSION

Parts of *C. cunninghamiana* including leave, fruit and bark vary in their constituent of phytochemicals including,: phenols , alkaloids, flavonoids, glycosides, tannins and triterpens **.** Methanolic, hot aqueous and ethanolic that was increase in inhibitory activity with increasing concentrations**.** Methanoic extracts proceed both hot aqueous and ethanolic extracts. Antibacterial activity against G+ve than G-ve.

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