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Antibacterial Effect of Some Medicinal Plants against Staphylococcus aureus and Pseudomonas aeruginosa

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ABSTRACT

Multidrug resistant microorganisms are globally becoming a major confrontment because of illogical use of antibiotics and this played a good role in investigation about the antibacterial compounds in plants. Thus, the present study investigate for the antibacterial effect of alcoholic extracts of Curcuma longa L. rhizomes , CommiphoramyrrhaL. gums and Ginkgo biloba L. leaves products against Staphylococcus aureus and Pseudomonas aeruginosa. The plants samples extracted by soxhlet with methanol and fractionation with and four solution (chloroform, hexane, water and ethyl acetate) were used for investigation about antibacterial activity by disc diffusion method. The results showed that methanolic alcohol extract and fractions of C. longa L. rhizomes , C. myrrha L. gums showed biological activity against P. aeruginosa and S. aureus bacteria, but methanolic alcohol extract and fractions of G. biloba L. leaves product didn't show any activity as antibacterial substance. It can be concluded that the presence of secondary metabolites as flavonoids, alkaloids, tannins, glycosides and saponins in the plants under study would be marked a good anti-bacterial effect.

Keywords-- Curcuma longa , Commiphoramyrrha , Ginkgo biloba , Anti-bacterial effect.

I. INTRODUCTION

Antibiotics were used to treat human disease caused by bacteria , but The increasing of contagious agents are becoming more resistant to commercial antimicrobial compounds[1]. Multidrug resistant microorganisms are globally becoming a major confrontment because of illogical use of antibiotics and this played a good role in investigation about the antibacterial compounds in plants [2]. The use of plant's parts (seeds, berries, roots, leaves, bark, or flowers) for the raputical and biological purposes termed as Herbal medicine or phytomedicine[3]. Plants as Curcuma longa L. rhizomes , Commiphoramyrrha L. and Ginkgo biloba L. used in traditional medicine in many areas in the world in ancient times till now [4].

Curcuma name most probably is derived from the Arabic word "kurkum" which means yellow and also termed "Indian saffron" and that is matching with the rhizome and flowers [5]. Many studies illustrated that yellow pigment extracted from turmeric rhizomes exhibit strong antioxidant , antimicrobial activities [6]. In addition that turmeric extract inhibited the production of fungi toxins (aflatoxins) [7].

Ancient Egyptians used myrrha in embalming fluids and the ancient Chines used myrrha as a medicinal agent as well as in foods and drinks as a flavoring agent, in perfumes and other cosmetics as a fragrance [8] and a high potential antibacterial effect like their effect against urinary tract infection [9], also Myrrh shows numerous beneficial properties including antifungal, antiseptic and expectorant [10].

G. bilobawhich this found now in the wild small area in China , that it was found in the garden of holy place of worship and prayer [11] and become popular as an ornamental tree in parks, gardens and city streets in many states for instance Northern America [12]. Ginkgo leaf extract has shown the potential role in the prevention of Alzheimer's disease (AD) and treatment of neurodegenerative diseases like stress, memory loss, tinnitus [13].

The aim of present study to detect the antibacterial effect of alcoholic extracts of (Curcuma longa L. rhizomes , Commiphoramyrrha L. gums and Ginkgo biloba L. leaves products against Staphylococcus aureus and Pseudomonas aeruginosa.

II. METHODS

Soxhlet was used to extract plants samples by adding 25 gms of desiccated plants powder in filter paper and mixed with 250 ml of solvent (methanol 95%) for 24 hours. Then filtration and all extracts were concentrated by removing the solvents by using rotary evaporater. Then the dried extracts mixed in separating funnel with 1:1 rate of water and chloroform solution and shaking well. The results showed two layers , the lower layer (chloroform) mixed immediately in separating with 1:1 rate of chloroform and hexane solution , the results showed two layers , the upper layer(hexane) and the lower layer(chloroform).

The upper layer (water) mixed immediately in separatory funnel with 1:1 rate of water and ethyl acetate solution , the results showed two layers , the upper layer(ethyl acetate) and the lower layer(water). All the layers were dried and stored until used [14]. Then , the methanolic extract and four fractions (chloroform layer , hexane layer , water layer and ethyl acetate layer) were used for investigation about antibacterial activity.

Screening for Antibacterial Activity

It was carried out according to disc diffusion method[15] where the plates of Muller – Hinton agar media was inoculated with bacterial study with a sterile swabs. Sterile paper discs made from (Whatman No. 1) filter paper were soaked in the plants extracts with concentration (1000 µg/ml) , then allowed to desiccate beneath the laminar flow cabinet for a night. In the company of sterile forceps, the plant solutions discs were positioned on the inoculated plate and pushed gently into agar. Each plant extract was assayed in triplicate. The control discs were soaked with DMSO which provide a negative control. Antibiotic disc (four) used for comparison. The inoculated plates were incubated at 37°C for 18 - 24 h. By the measurement of the zone inhibition width in the region neighboring to discs with millimeters (mm) , the antimicrobial effects was construed.

Determination of Minimum Inhibitory Concentration (MIC) of Plants Extracts

Determination of the minimum inhibitory concentration (MIC)[16] as the (MIC) to the plant solution(extracts and fractions) could be find out to study bacteria (S. aureusand P. aeruginosa) with different concentrations of (40 , 20 , 10 , 5 and 2.5 µg/ml and these different concentrations could be got by placing (1 ml) of the solutions (plants extracts and fractions) containing the concentrations (80 , 40 , 20 , 10 and 5 µg/ml) were represented in varying test tubes, next we added (1 ml) from sterile media (nutrient broth) and followed by inserting a loopful bacterial growth on nutrient broth attenuated to reach the turbidity of 0.5 McFarland. The control tube included pure sterile nutrient broth media was inoculated with bacteria (S. aureusand P. aeruginosa) merely. All the cultures subsequently incubated for 24 hours next to 37°C. Finally, the bacterial development in the tubes were investigated with turbidity monitor –measuring by eyes.

III. RESULTS AND DISCUSSION

Table (1) illustrated the antibiotic sensitivity test of P. aeruginosa and S. aureusbacteria.The study of bacterial antibiotic sensitivity determines the treatment of bacterial infections and its complications for successful outcome, and thus to avert the appearance of resistant strains[17].

The results (table 2) showed thatC. longa has high antibacterial activity against P. aeruginosa and the topmost inhibition zone diameter was (31 mm) with hexane fraction solution and limited antimicrobial activity of Curcuma rhizomes was observed with methanolic extract (10mm) , but the water and ethyl acetate fractions did not show any antibacterial activity and its antibacterial activity against S. aureus was observed with all solutions , but is high in ethyl acetate fraction (23mm).

C.myrrha is highly effective antibacterial activity against S. aureusIsolates with all solutions and topmost inhibition zone diameter was (60 mm) with hexane fraction and less antimicrobial activity of C. myrrha was observed withP.aeruginosa. The minimum inhibitory concentration (MIC) values of methanol extract and fractions of oleo-gum resins of myrrh showed that the highest values were obtained in hexane fraction for P. aeruginosa and lowest MIC values for the bacterium S. aureus and this is significant when comport to the minimum inhibitory concentration (MIC) values of methanol extract and fractions of C. longa which show that the highest values are obtained in methanol extract and chloroform fraction for S. aureus and lowest MIC values for the bacteriumP.aeruginosa with hexane fraction.

The positive results of C. longa and C. myrrha solutions samples may be because that methanolic extracts and fractions contain some anti-microorganisms effective compounds such as the flavonoids, alkaloids, tannins, glycosides and saponins [18]. The occurrence of phenolic compounds in the plants suggests that these plants may be anti-microbial agent [19].The distinction in the inhibition among the gram positive and gram negative bacteria is due to the cell wall and cell membrane compositions especially the thickness of the mucous layer surrounding the cell wall [20]. The negative results could be because the plant active constituents which may be found in low amount in the samples of crude extract to be effective as antibacterial agents , or in spite of the active constituents presence in the samples,there is antagonistic effect for the antibacterial effect of them by another compounds in the samples[21].

G. bilobasolutions (extract and fractions) did not show any inhibition to bacterial growth for both bacteria (S. aureus and P. aeruginosa) and this result is somewhat in agreement with Boonkaew and Camper ,(2005) study [22]. The result may be return to the plant content of bilobalide (which consider as anti-microorganisms effective compound) in our study might have been too low to display activity against bacteria S. aureus and P. aeruginosa or the interaction of bilobalide with many other phytochemical

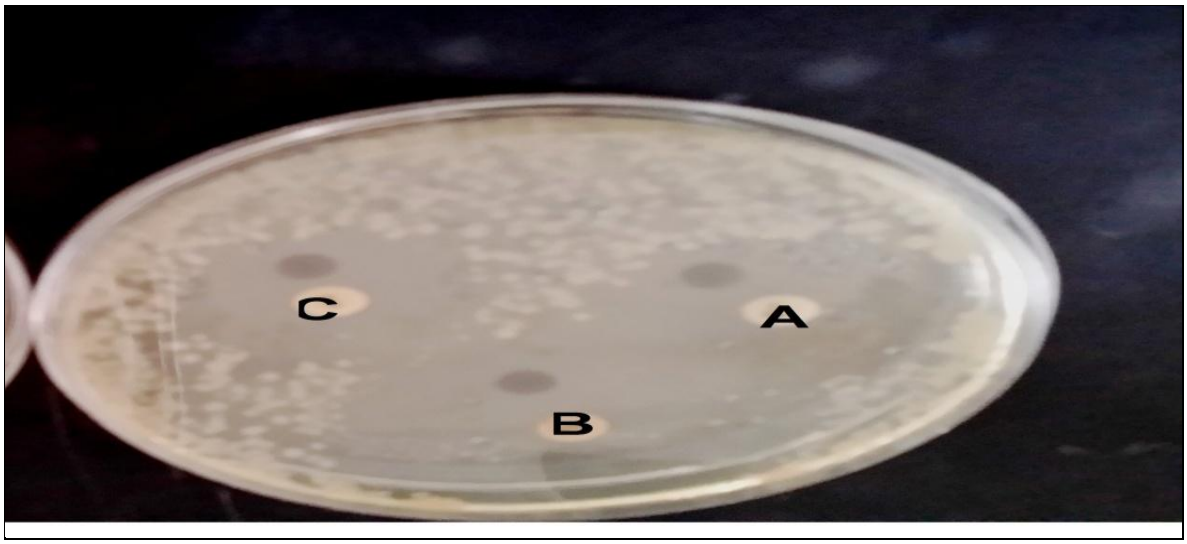
compounds in the same plant which lead to conclude the no activity against study bacteria [23].

Table (1): P. aeruginosa and S. aureus sensitivity test to some antibiotics.

Antibiotic name	Antibiotic symbol	Inhibition zone diameter (mm)	
		P. aeruginosa	S. aureus
Chloramphenicol 30 µg	C 30	22	23
Neomycin 30 µg	N 30	14	15
Doxycycline 30µg	DO 30	16	18
Amphotericin B 20	AMB 20	R	R

Table (2) : The antibacterial activity and MIC for C. longa rhizome and C. myrrha gum solutions on Staphylococcus aureus and Pseudomonas aeruginosa bacteria.

Plant solution (extract or fraction)	**Pseudomonas aeruginosa**				**Staphylococcus aureus**			
	Commiphoramyrrha		**Curcuma longa**		**Commiphoramyrrha**		**Curcuma longa**	
	mean of inhibition zone (mm)	MIC (µg/ml)	mean of inhibition zone (mm)	MIC (µg/ml)	mean of inhibition zone (mm)	MIC (µg/ml)	mean of inhibition zone (mm)	MIC (µg/ml)
Methanol extract	13	5	10	10	20	5	11	20
Chloroform fraction	12	5	13	10	25	2.5	10	20
Water fraction	15	5	5	0	27	2.5	17	10
Hexane fraction	13	10	31	2.5	60	2.5	16	10
Ethyl acetate fraction	12	5	5	0	5	0	23	5



Figure(1) : A. Antibacterial activity of C. myrrha water fraction on P. aeruginosa; B. Antibacterial activity of C. longa hexane fraction on P. aeruginosa. ; C. Antibiotic (Neomycin 30 µg).

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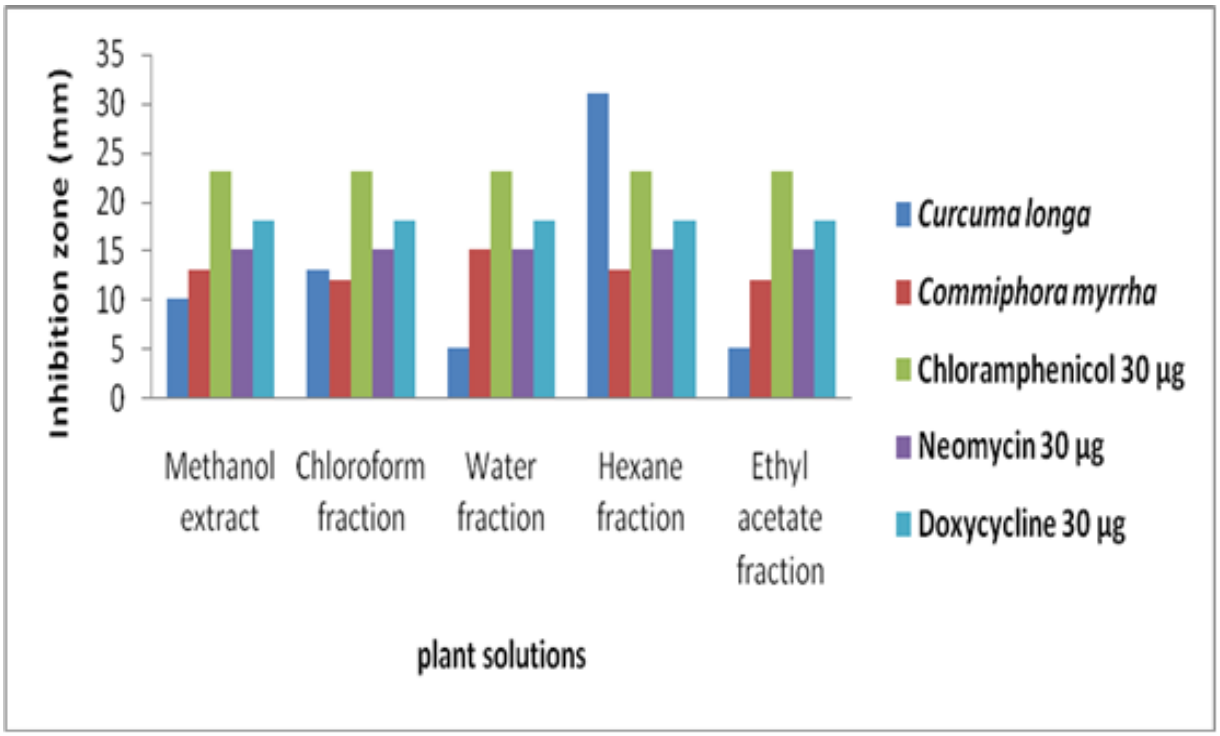

Figure (2): Antibacterial effects of (C. longa and C. myrrha) solutions on P. aeruginosa compared with antibiotics.

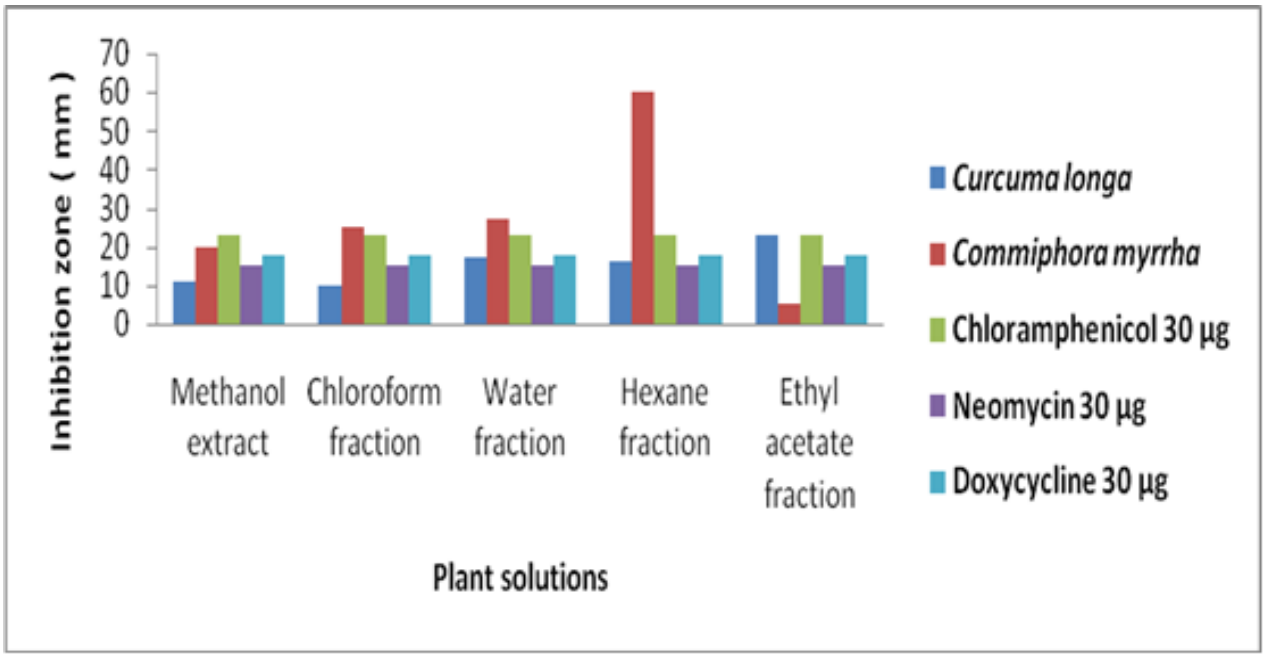

Figure (3): Antibacterial effects of (C. longa and C. myrrha) solutions on S. aureuscompared with antibiotics.

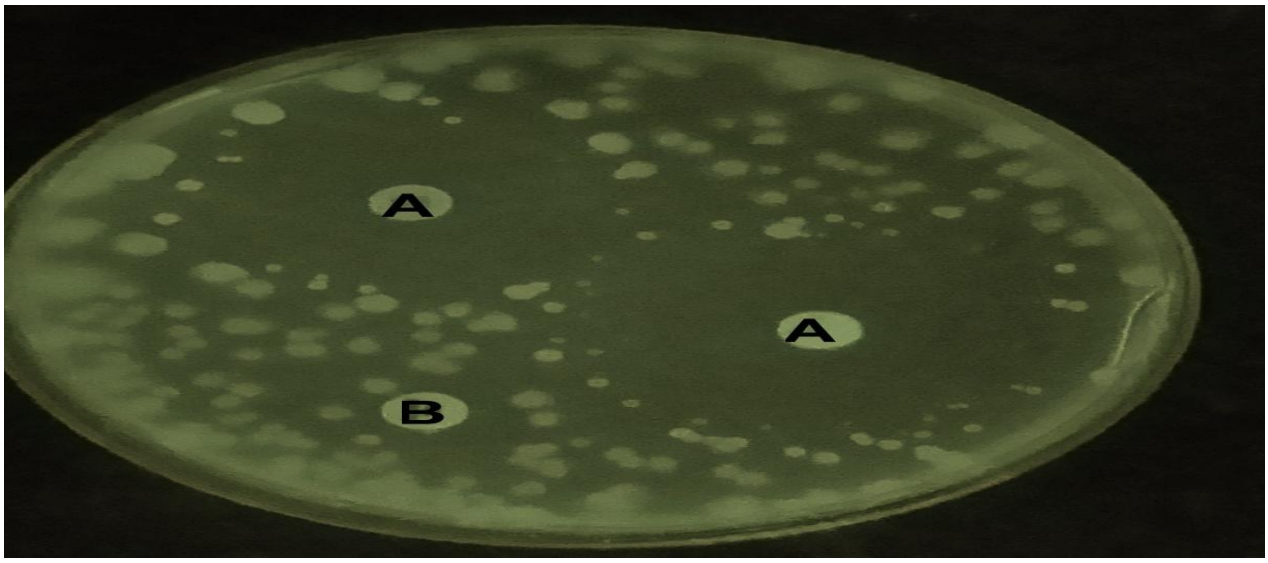

Figure (4) : A: Antibacterial activity of C. longa ethyl acetate fraction on Staphylococcus aureus. ; B: Antibiotic (AMB) disc.

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