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An *in Vitro* Antimicrobial Potential of Various Extracts of Commiphora Myrrha

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Abstract

Objectives The aim of this study was to evaluate the antimicrobial activity of various extracts obtained from the resin of medicinal plant *Commiphora myrrha* on standard microorganisms. ***Methods*** The agar well diffusion technique was followed to perform the antimicrobial activity of the candidate extracts against Gram-positive bacteria (*Bacillus subtilis* NCTC8236, *Staphylococcus aureus* ATCC 25923), Gram- negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 53657, *Proteus vulgaris* ATCC6380, *Pseudomonas aeruginosa* ATCC 27853), as well as, two fungal species (*Candida albicans* ATCC 7596; *Apergillus niger* ATCC 9763). ***Results:*** Methanolic and aqueous extracts of the resin of *C. myrrha* at concentration of 100 mg/ml was found to be more active against Gram- negative bacteria (*Proteus vulgaris* ATCC 6380; *Klebsiella pneumoniae* ATCC: 53657; *Escherichia coli* ATCC: 25922 and *Pseudomonas aeruginosa* ATCC: 27853) and Gram- positive bacteria (*Bacillus subitilis* NCTC: 8236), as well as, they showed high antifungal activity against (*Candida albicans* ATCC :7596 and *Aspergillus niger* ATCC:9765),while the chloroform extract of the resin showed moderate activity against Gram- positive and Gram- negative bacteria ,as well as against *Candida albicans* ATCC :7596,whereas the same extract revealed high antifungal activity against *Aspergillus niger* ATCC:9765. ***Conclusion*** Methanolic, chloroform and aqueous extracts of the resin of *C. myrrha* revealed that the selected plant had a significant potential effect capable to inhibit the growth of both bacterial and fungal standard species.

Keywords**:** *Commiphora myrrha*; antimicrobial activity; Methanol extract; chloroform extract; standard strains.

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Introduction

For a long time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies[1]. Currently a large and ever expanding global population base prefers the use of natural products in treating and preventing the attack of some illnesses. This has influenced many pharmaceutical companies to produce novel antimicrobial formulations extracted from plants or herbs. Virtually all cultures around the globe have relied historically, and continue to rely on medicinal plants for primary health care. There is currently a worldwide upsurge in the use of herbal preparations and the active ingredients isolated from medicinal plants in health care. Most of modern drugs were derived from natural sources, using either the natural substance or a synthesized version[2]. Some antibiotics have become almost obsolete because of the drug resistance and consequently new drugs must be sought for. Herbal treatment is one possible way to treat diseases caused by multidrug resistant bacteria. The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments. In the past few years, a number of studies have been conducted in different countries to prove such efficiency[3]. *C. myrrha* belonging to family *Burseraceae* is a shrub or small l tree (5 m tall); it is native to Arab countries, Northern Africa and Somalia[4, 5]. *C. myrrha* was used as a wine preservative, aromatic for funerals and insect repellents by the ancient Egyptians. Ancient Greek and Roman physicians used it to treat wounds and prescribed it as a digestive aid, menstruation promoter and analgesic[6]. It used as a remedy for numerous diseases, including intestinal disorders, wound infections and some helminths infections[7, 8]. It is used today as an aid to

nervous system disorders, rheumatic complaints, tooth decay, gum disease and as anticancer [9]. *C. myrrha* possesses secondary metabolites like flavonoids, alkaloids, tannins, glycosides, steroids, saponins, tannins and terpenoids. Bioactive compounds like flavonoids, glycosides are rich in methanolic extract[10, 11]. The antimicrobial activity and many applications including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies of *C. myrrha* resin extracts has been formed[12, 13]. The purpose of this study was to investigate the antimicrobial activity of various extracts obtained from the resin of *Commiphora myrrha* against standard microorganisms.

Materials and Methods

Plant Materials

Fresh resin of *C. myrrha* was purchased from Omdurman Local Market, Omdurman, Sudan. The laboratory work has been carried out at Microbiology Department, Medicinal and Aromatic Plants Research Institute (MAPRI).The resin was washed thoroughly three times with running water and once with distilled water and it was then air- dried under shade. Voucher specimens were deposited at the herbarium of the institute.

Preparation of Crude Extracts

Each of the coarsely powdered plant material (50 g) was exhaustively extracted with methanol and chloroform in Soxhlet apparatus. The extracts were filtered and evaporated under reduced pressure using a rotary evaporator until they become completely dry. The residues were stored at 4 ˚C for further need. Each residue was weighed and the yield percentage was determined and kept in refrigerator until used. For aqueous extract 100 g of powdered plant material was infused in 500 ml hot water for 4 hours then filtered through Whattman filter paper. The residue was

weighed and the yield percentage was determined and kept in refrigerator until used.

Test Microorganisms

Eight different standard strains examined in this study were obtained from National Collection of Type Culture (NCTC), Colindale, England and American Type Culture Collection (ATCC) Rockville, Maryland, USA. Those strains include Gram- positive bacteria (*Bacillus subtilis* NCTC8236, *Staphylococcus aureus* ATCC 25923), Gram- negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 53657, *Proteus vulgaris* ATCC6380, *Pseudomonas aeruginosa* ATCC 27853), as well as, two fungal species (*Candida albicans* ATCC 7596; *Apergillus niger* ATCC 9763).

Identification of Standard Strains

All examined strains were inoculated on blood agar and nutrient agar plates, incubated aerobically and the obtained growth were then purified by streaking on plates containing the appropriate selective and differential culture media, Mannitol salt agar and MacConkey's agar. Microscopic examination and biochemical tests of the purified microorganisms were done for identification and confirmation of these organisms. The biochemical tests that carried out include Fermentation tests, Methyl red tests, Voges- Proskauer test, Citrate utilization test, Indole production test, Hydrogen sulphide production test, Catalase test, Coagulase test, Oxidase test and Urease test[14].

Preparation Test Microorganisms

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 CFU/ ml. The suspension was stored in the refrigerator at 4° C till used. Each time a fresh stock suspension was prepared; all the above experimental

conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Testing of Extracts for Antimicrobial Activity

The cup-plate agar diffusion method[15] was adopted with some minor modifications to assess the antibacterial and antifungal activity of the prepared extracts. One ml of the standardized bacterial and fungal stock suspension 10^8 –10^9 CFU/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45 ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

Determination of minimum inhibitory concentration (MIC)

The principle of the agar plate dilution is the inhibition of growth on the surface of the agar by the plant extracts incorporated into the medium. Plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off into 4 segments. The organisms tested were growing in broth over night to contain 10^8 CFU/ml. Loop-full of diluted culture is spots with a standard loop that delivers 0.001 ml on the surface of segment. The endpoint (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth. Results are reported as the MIC in mg/ml.

Results

The average of the diameters of the growth inhibition zones produced by methanol, chloroform and aqueous extracts of the resin of *C. myrrha* are presented in Table 1. Table 2 and 3 showed on the other hand, the anti-

microbial activity of the reference chemotherapeutic agents on the standard bacterial and fungal strains tested. The results were interpreted as sensitive, intermediate and resistant. According to results that presented in Table 2 and 3 extract resulting in 15 mm or more growth inhibition zone are considered to be active and those resulting in less than 15 mm are inactive[14].

MIC of Resin Methanolic Extract of *C. myrrha* Against Standard Strains

The minimum inhibitory concentration for methanolic extract of the resin of *C. myrrha* exhibited various degrees of activity against the test microorganisms, it was 50 mg/ml for *Escherichia coli*, 25 mg/ml for *Pseudomonas aeruginosa* and *Proteus vulgaris*, 12.5 mg/ml for *Klebsiella pneumoniae*, whereas it was 6.25 mg/ml for *Bacillus subitilus*, *Candida albicans* and *Aspergillus niger* (Table 4).

Table 1: Antimicrobial activity of *C. myrrha* resin extracts against standard strains.

Solvent system	Yield %	Standard strains */MDIZ mm							
		E.c	***Ps.a***	***Kl.p***	***P.v***	***B.s***	***S.a***	***C.a***	***Asp.n***
Methanol	3.5	18.5	19.5	20.5	20.0	16.0	-	21.5	29.5
Chloroform	2	-	14.5	15.0	15.0	15.0	-	16.0	20.0
Aqoeous	2.4	17.5	16.5	17.0	16.0	14.0	-	17	18

Key: concentration used 100 mg/ml; Standard microorganisms (*E.c: Escherichia coli, Ps.a: Pseudomonas aeruginosa, Kl.p: Klebsiella pneumoniae, P.v: Proteus vulgaris, B.s:Bacillus subtilis, S.a: Staphylococcus aureus, C.a* :*Candida albicans* and *Asp.n* :*Apergillus niger* .
MDIZ: Mean diameter inhibition zone; (-) Not determined

Table 2: Antibacterial activity of reference antibiotics against standard strains.

Antibiotic	Conc.used (µg/ml)	Standard strains /MDIZ mm					
		E.c	***Ps.a***	***Kl.p***	***P.v***	***B.s***	***S.a***

Ampicillin	40	18	-	18	-	15	25
	20	16	-	15	-	14	20
	10	13	-	13	-	13	18
	5	-	-	12	-	12	15
Tetracyclin	40	24	16	27	16	23	31
	20	19	13	25	-	21	27
	10	-	12	21	-	20	25
	5	-	-	18	-	18	17

Table 3: Antifungal activity of reference antifungal drugs against standard strains.

Drug	Concentration mg /ml	Tested fungi	
		C.a	***Asp.n***
Nystatin	25	14	26
Clotrimazole	20	24	34

Table 4: M ICs of the resin methanol extract of *C. myrrha*.

Part used	MIC of Standard microorganisms mg/ml						
	E.c	***Ps.a***	***Kl.p***	***P.v***	***B.s***	***C.a***	***Asp.n***
Resin	50	25	12.5	25	6.25	6.25	6.25

Discussion

Methanol, chloroform and aqueous extracts of the resin of *C. myrrha* were investigated for their antimicrobial potential against eight standard microorganisms; two are Gram- positive bacteria (*Bacillus subtilis and Staphylococcus aureus*); four are Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus vulgaris*) and two fungal species (*Candida albicans* and *Aspergillus niger*). It is clear from Table 1 that the resin methanolic extract showed high activity against *Klebsiella pneumoniae* (20.5 mm), *Proteus vulgaris* (20 mm), *Pseudomonas aeruginosa* (19.5 mm), *Escherichia coli* (18.5 mm) and *Bacillus subtilis* (16 mm). This finding agreed in points with that

reported by Al-Daihan *et al*.[16] in Saudi Arabia, and Rahman *et al*.[17]. In our study, *Staphylococcus aureus* was not found to be sensitive to any one of the candidate extracts; this result is in agreement with the study of Al-Daihan *et al*.[16]. On the other hand, the same extract exhibited high antifungal activity against *Candida albicans* (21.5 mm) and *Aspergillus niger* (29.5 mm). This result is parallel to that study reported by Omer *et al*.[18].The chloroform extract of the resin of *C. myrrha* showed moderate activity against most of the tested bacterial strains and the mean diameter of inhibition zones were ranged from 14 mm to 15 mm for all tested bacteria, except *Escherichia coli* in which there is no activity for chloroform extract, while the same extract exhibited high antifungal activity towards *Candida albicans* (16 mm) and *Aspergillus niger* (20 mm). This finding is not agreed with those results of Masoud and Gouda[19]. The resin aqueous extract of *C. myrrha* showed high antimicrobial activity against most of the tested microorganisms and the mean diameter inhibition zones that obtained by the tested microorganisms were 17.5 mm, 16.5 mm, 17 mm, 16 mm and 14 mm for *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacillus subtilis*, respectively, whereas the antifungal activity of the same extract was 17 mm and 18 mm for *Candida albicans* and *Aspergillus niger*, respectively. These results are corresponded to that reported by Masoud and Gouda[19].

The comparison of observation given in Table 1, 2 and 3 demonstrated that the resin methanolic extract of *C. myrrha* showed high activity (20.5 mm) against *Klebsiella pneumoniae*, which is almost similar to the activity of 10 µg/ml Tetracycline and more than activity of 40 µg/ml Ampicillin. It also inhibits *Proteus vulgaris* (20 mm) which is higher than activity of 40 µg/ml Ampicillin. *Pseudomonas aeruginosa* being exhibited (19.5 mm) which is higher than activity of 40 µg/ml Tetracyclin. On the other side, the same

extract showed high activity against *Candida albicans* (25.5 mm) which is more than activity of 25 mg/ml Nystatin and less than activity of 20 mg/ml Clotrimazole, whereas it inhibit *Aspergillus niger* (29.5 mm) which is lower than activity of 20 mg/ml Clotrimazole and higher than activity of 25 mg/ml Nystatin. The chloroform extract of *C. myrrha* exhibited the least antimicrobial activity towards the tested microorganisms. It was about 15 mm for *Klebsiella pneumoniae, Proteus vulgaris and Bacillus subtilis* that is almost similar to the activity of 20 µg/ml Ampicillin for *Klebsiella pneumoniae*, less than activity of 40 µg/ml Tetracycline for *Proteus vulgaris* and less than activity of 40 µg/ml Ampicllin for *Bacillus subitilis*. Whereas the same extract showed high antifungal activity against *Aspergillus niger* (20 mm) which is less than activity of 25 mg/ml Nystatin. The aqueous extract of the same plant revealed moderate activity against *Escherichia coli* (17.5 mm) which is higher than activity of 20 µg/ml Ampicillin, and *Pseudomonas aeruginosa* (16.5 mm) which is more than activity of 40 µg/ml Tetracycline. On the other hand, the antifungal activity of the aqueous extract against the tested fungi was 17mm and 18 mm for *Candida albicans* and *Aspergillus niger* which is more than activity of 25 mg/ml Nystatin.

Conclusion and Recommendation

It was observed that all extracts obtained from *C. myrrha* found to be active against almost all of the tested organisms, except *Staphylococcus aureus* which was not affected by candidate extracts. Methanol extract of the resin of *C. myrrha* was found to be more active mainly against Gram-negative bacteria compared to the reset test extract. The most interesting finding

in our study is that *Klebsiella penumoniae* was inhibited by the three candidate extracts, as well as it was the

most sensitive organism compared with the rest microorganisms. In the present study *C. myrrha* resin extracts had a significant potential effect toward most of the tested organisms in spite of the difference in the solvent systems; this may be due to the highly antimicrobial ingredients of the resin. Further researches and investigations are required to elucidate the mechanism of action of *C. myrrha* resin as antimicrobial agent.

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