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Original Article

Antibacterial, Antifungal, Cytotoxic and Genotoxic Activities of Different Extracts of Arabic and Myrrh Gums

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ABSTRACT

Background: The Pharmaceutical companies are very interested in the discovery of new natural bioactive molecules with an impoortant effect and less toxicity, in order to replace old drugs, especially antibiotics which had lost their effectiveness following the spread of multi-resistant bacteria. This study was aimed to investigate, *in vitro*, some extracts (aqueous extracts, oily extracts and essential oils) of arabic and myrrh gums, plant exudates commonly used in folk medicine for treating several diseases. **Materials and Methods:** The antimicrobial activity against clinical bacterial and fungal strains was carried out using disk diffusion and broth dilution methods. Cytotoxic activity was mesured using the brine shrimp lethality bioassay determining the LC_{50} and genotoxic activity by the preincubation Ames Test using *Salmonella* strains TA100, TA98 and TA1535 treated with or without the metabolic activation (S9 fraction). **Results:** An interesting antimicrobial activity was demonstrated, especilly against Gram negative strains. Inhibition zones vary between 16 and 30 mm and MIC's values between 15.62 and 250 µg/ml. All the tested extracts exhibited a bactericidal activity. The arabic gum extracts showed no cytotoxic effect with LC_{50} > 100 µg/ml. Myrrh gum extracts showed a significant toxicity to the brine shrimp nauplii with LC_{50} < 100 µg/ml. Results of the Ames test indicated that all tested extracts did not possess genotoxic potential. **Conclusion:** Our study highlighted the antimicrobial potential of gums extracts, making them an excellent drug candidates against resistant pathogens.

Key words: Antibacterial activity, Antifungal activity, Arabic gum, Cytotoxicity, Genotoxicity, Myrrh gum.

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INTRODUCTION

Infectious deseases have accompanied human since time immemorial; they develop, when a trigger such as bacteria, virus, fungus, worm or protozoan enter the body or colonize skin.[1] When these microorganisms escape the immune system, we need to use antibiotics. However, the abuse of antibiotics has, unfortunately, led to the emergence of drug resistance and is currently a global challenge since the number of resistant strains has dramatically increased having a significant impact on the patients' morbidity, mortality and excess costs of care.[2,3] Therefore, a clear need to developping innovative antimicrobial agents with better pharmacological profiles and without side effect is required.[3]

In traditional medicine, diverse infectious diseases have been treated with a wide variety of plant/natural products. It has been scientifically demonstrated that plants contain secondary metabolites (alkaloids, resins, oleosins, steroids, tannis and terpenes etc.) to which biological properties are attributed.[4,5]

Edible gums known by different names: viz, Gond, Goond, Goond katira, Dinka, Gaund, Gondh are dried exudates of thorny trees and shrubs of *Fabaceae* family (*Acacia*, *Sterculia*, *Astragalus*, *Balanites*, *Buchanania*, *Anogeissus* species) and *Burseraceae* family (*Commiphora myrrha*).[6]

Arabic gum or Acacia gum is a dibble biopolymer obtained as exudates of mature trees of *Acacia senegal* and *Acacia seyal* which grow principally in the African region of Sahel in Sudan. The exudate is a natural polysaccharide hydrocolloid which was permitted as food additive, an emulsifier and stabilizer by JECFA/FAO (1999) and US Food and Drug Administration department.[7,8] It is reportedly used in pharmaceutical and cosmetics.[9] Moreover, it is used in medicine for its astringent properties to treat bleeding, bronchitis, diarrhea, gonorrhea, leprosy, lymphoid fever and upper respiratory and urinary tract infections and diabetes.[10] It exhibited antibacterial, anti-inflammatory, vasoconstrictor actions, antihypertensive, antispasmodic activities, inhibitory effect against hepatitis virus, cytotoxic activities and antioxidant activity.[11,12]

Myrrh gum, produced by *Commiphora myrrha*, a genus in the *Burseraceae* family is an aromatic gummy resin.[6,13] It contains 2 to 8% volatile oil that is composed of steroids, sterols and terpenes, 20 to 40% alcohol-soluble resin, and approximately 30 to 60% water-soluble gum, polysaccharides and terpenoids.[13,14] The main secondary metabolites isolated from the volatile oil were heerabolene, acadinene, elemol, eugenol, cuminaldehyde and numerous furanosesquiterpenes including furanodiene, furanodienone, curzerenone, lindestrene, commipherol and commipherin.[15]

Myrrh has several uses in many fields such us a component of perfumes, incense, and medicinals.[13] It exhibited antimicrobial, anti-inflammatory and anticancer activities.[16,17] It is an antiseptic, astringent, analgesic and antitussive and also aids wound healing and Wound care. Myrrh is employed to treat headaches, gout, throat disorders, and indigestion,[16] infectious diseases, mouth infections, paediatric coughs, and prevent the development of dental plaque.[13] Several compound found within myrrh exert local anaesthetic activity.[6]

The advantages of natural gums are their biocompatibility, availability at low cost, low toxicity and ecofrindliness.

In this study, we aimed to evaluate the antimicrobial activity against clinical bacterial and fungal strains, and the cytotoxic and the genotoxic potential of two gums extracts: Arabic gum and myrrh gum.

MATERIALS AND METHODS

Biological Material

The choice of the used gums, Arabic gum (*Acacia senegal L.*) and myrrh gum (*Commiphora myrrha*), was based on bibliographic review and an ethnopharmacological survey of the populations having knowledge of their use in traditional medicine. Gums were collected from an herbalist.

Bacterial and Fungal Strains

The antibacterial and antifungal activities of the different extracts were carried out against a total of 22 clinical strains (4 *Staphylococcus aureus* ; 4 *Escherichia coli* ; 4 *Klebsiella pneumonia,* 4 *Pseudomonas aeruginosa, 4 Serratia marcescensa, 2 Salmonella sp. and 2 Candida albicans*). The isolates were collected from public sanitary establishment (Wilaya of Skikda, Algeria), and were mainly isolated from pus, urine and vaginal swab.

Identification of the bacterial strains was made on cultural and biochemical characters (API Staph system, API 20E system and API 20NE system ; BioMérieux, France). These strains showed an important resistance to the most tested antibiotics (multiresistant clinical strains).

Two reference strains were used as controls: *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 (Pasteur Institute, Algiers).

Extraction Methods

Aquaous Estracts

A total of 50 g of each gum powder was macerated at room temperature in 100 ml of distilled water for 72 hr. The extraction was carried out and sterilised by filtration, stored in sterile bottles and kept in freezer at 4°C until further use for screening of biological activities.[18]

Oily Extracts

A total of 50 g of each gum powder was macerated at room temperature in 100 ml of sunflower oil (*Helianthus annuus*) and allowed to stand for 21 days. The extraction was carried out and sterilised by filtration, stored in sterile bottles and kept in freezer at 4°C until further use for screening of biological activities.[18]

Essentiel Oils

A total of 50 g of each pulverised gum sample was placed in hydro-distillation flasks and mixed with distilled water then submitted for 2 hr to hydrodistillation using a Clevenger-type apparatus according to the method recommended in the European Pharmacopeia (2002). The essential oil was dried over anhydrous sodium sulfate. It was filtered and stored in the refrigerator at 4°C until biological tests were performed.[19]

Several concentrations of the diffrent extracts were prepared: 1000 µg / ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 60.5 µg/ml, 31.25 µg/ml and 15.62 µg/ml.

Antibacterial and Antifungal Activities

Determination of Inhibition Zones Diameters

The tested gums extracts were screened for antimicrobial activity. Inhibition zones were determined using the Kirby Bauer disk diffusion method on Mueller-Hinton (MH) agar plates.[20] Petri plates were inoculated with a bacterial inoculum prepared in physiologically sterile water with an OD of about 0.08. Empty sterilized disks of 6 mm were placed on the plates and were impregnated with 30 µl of the different concentrations of the tested extracts. Petri plates were incubated at 37°C for 24 hr and the diameters of the inhibition zones were measured (mm).[20]

Minimal Inhibitory Concentration

MIC's values of tested gums extracts were determined by the Mueller Hinton broth dilution method.[20]

Bacterial inoculum with an OD_{625} about 0.08 was added to each tube containing extract dilutions ranging from 15.62 to 1000 µg/ml; a tube without gums extracts was used as control. The tubes were incubated at 37°C for 24 hr. The results were recorded according to the presence or absence of bacterial growth comparatively to the control.

Minimal Bactericidal Concentration

The Minimal Bactericidal Concentration (MBC) was carried out on nutritive agar by sub-culturing 0.1 ml from tubes showing no turbidity at MIC concentration of the tested gums extracts at 37°C for 24 hr.[20]

All tests were performed in duplicate, and experiment was performed in triplicate.

Cytotoxic Bioassay

Hatching the Shrimp

Gums extracts were screened for their cytotoxicity by the brine shrimp bioassay using Meyer protocol.[21] Brine shrimp eggs (about 50 mg) were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater. An uneven subdivision was made in the plastic dish. Eggs were scrattered into the large compartment, which was darkened while the minor compartment was illuminated. After 48 hr, the phototropic nauplii were collected from the lighted side.

Bioassay

To each vial containing 0.5 ml of tested extract and 4 ml of the artificial seawater, ten nauplii were transferred (30 shrimps/dilution); then, the volume was adjusted with seawater to 5 ml per vial. As nutrient source, a drop of dry yeast suspension (3 mg/5 ml artificial seawater) was added for the nauplii. After 24 hr, the number of survivors was counted.[21] The LC_{50} of the tested gums extracts was performed by Probit Analysis using the Prism 6 software.

Genotoxic Bioassay

The preincubation assay was performed as described by Mortelmans and Zeiger.[22-24] The TA100, TA98 and TA1535 strains were incubated at 37°C overnight in nutrient broth before each experiment.

In sterile tubes, we added in the following order, with stirring after each addition, 0.05 ml of the tested extract and 0.1 ml of the overnight culture of the *Salmonella* strain to a density of about 1-2 × 10^9 CFU/ml. The test was performed with and without metabolic activation by adding 0.5 ml of the S9 mix.

The mixture was incubated at 37°C for 20 min with gentle agitation. We added to each tube, 2 ml of molten top agar maintained at 40 to 43°C. The contents of test tubes were mixed and poured onto the surface of GM agar plates. When the top agar solidifies, the plates were incubated at 37°C for 48 h. The colonies were then counted and the results were expressed as the number of revertant colonies per plate.

For TA98 strain we used 4-NQO (2.5 µg/ml/plate) and for TA100 and TA1535 the sodium azide (5 µg/ml/plate) as positive controls. All tests were performed in duplicate, and experiment was performed in triplicate.

The average number of revertant colonies per plate and the standard deviation of three replicates for the different gums extracts were calculated for each individual plate. Compared with the negative and positive controls, positive results can be judged if the number of revertant colonies in any strain with or without metabolic activation has a dose-related increase. Otherwise, negative result was defined.

RESULTS

Antimicrobal Activity

Results indicated that the different extracts of the tested gums were effective (Table 1).

Regarding Arabic gum extracts, good results were obtained with the reference strains. In fact, the diameters of inhibition zones vary between 17 and 19 mm for *S. aureus* ATCC 25923 and MICs between 62.5 and 250 µg/ml. For *E. coli* ATCC 25922, the diameters of inhibition zones vary between 18 and 20 mm and the MICs between 31.25 and 125 µg/ml. Arabic gum essential oil was the most effective against these starins.

Results of myrrh gum extratcts were also very interresting with diameters varying between 18 and 24 mm and MICs between 15.62 and 125 µg/ml for *S. aureus* ATCC 25923. Concerning *E. coli* ATCC 25922, diameters of inhibition zones were ranged from 20 to 25 mm and MICs from 15.62 to 62.5 µg/ml. Gram-negative reference strain *E. coli* ATCC 25922 is more sensitive to these gums extracts than Gram-positif reference strain *S. aureus* ATCC 25923. It is also noted that essential oil of myrrh gum was more effective against reference strains compared to other tested extracts.

Otherwise, Gram-positive clinical strains *S. aureus*, expressed good results with the three Arabic gum extracts whith diameters of inhibition zones varying between 16 and 19 mm and MICs ranged from 62.5 to 250 µg/ml. The oily extract was most effective against *S. aureus* clinical strains with low MICs (62.5 and 125 µg/ml).

The three myrrh gum extracts were even more effective against *S. aureus* clinical strains showing diameters of inhibition zones ranging between 18 and 20 mm and low MICs values ranging from 31.25 to 62.5 µg/ml.

Regarding Gram-negative clinical strains, very interesting results were obtained with all extracts. The inhibition zones vary between 16 and 30 mm and the MICs values between 15.62 and 125 µg/ml.

K. pneumoniae, considered as resistant strains expressed, with all tested extracts, a very interesting result with important diameters of inhibition zones up to 30 mm and low MICs ranging from 15.62 to 31.25 µg/ml.

Concerning *Candida albicans* strains, very interessting results were obtained for the different extracts with inhibition zones varying between 20 and 22 mm and MICs values equal to 15.62 µg/ml.

Results with the three extracts of myrrh gum are more important those obtained with Arabic gum extracts.

Despite the fact that oily and aqueous macetations are simple and very inexpensive techniques, the obtained extracts from these two gums showed a very good antimicrobial activity, very close to that obtained with essential oils by hydrodistillation giving generally low yeld.

Table 1: Diameters of inhibition zones and MIC's values of the clinical strains against the tested extracts of the two gums.

Tested compounds / Clinical strains	Arabic gum						Myrrh gum					
	Aqueous extract		Oily extract		Essential oil		Aqueous extract		Oily extract		Essential oil	
	I.Z. (mm)	MIC (µg/ml)	I.Z. (mm)	MIC (µg/ml)	I.Z. (mm)	MIC (µg/ml)	I.Z. (mm)	MIC (µg/ml)	I.Z. (mm)	MIC (µg/ml)	I.Z. (mm)	MIC (µg/ml)
S. aureus ATCC 25923	17	250	19	125	18	62.5	18	62.5	19	125	24	15.62
E. coli ATCC 25922	19	125	18	62.5	20	31.25	20	62.5	22	31.25	25	15.62
S. aureus 1	17	250	18	62.5	16	250	18	62.5	19	31.25	19	62.5
S. aureus 2	16	250	18	62.5	17	250	21	31.25	18	62.5	19	62.5
S. aureus 3	17	250	17	125	17	250	20	31.25	18	60.5	20	31.25
S. aureus 4	18	125	19	62.5	16	250	21	31.25	19	31.25	21	31.25
E. coli 1	18	125	18	125	18	125	20	31.25	18	62.5	19	62.5
E. coli 2	18	125	18	125	16	250	22	15.62	20	31.25	20	31.25
E. coli 3	17	250	17	125	17	125	22	15.62	19	31.25	20	31.25
E. coli 4	19	62.5	17	125	17	125	20	31.25	19	31.25	19	62.5
K. pneumoniea 1	26	15.62	18	31.25	20	31.25	28	15.62	19	31.25	22	15.62
K. pneumoniea 2	24	15.62	19	31.25	21	15.62	27	15.62	19	31.25	23	15.62
K. pneumoniea 3	25	15.62	18	31.25	20	15.62	28	15.62	20	31.25	22	15.62
K. pneumoniea4	26	15.62	18	31.25	19	31.25	30	15.62	19	31.25	21	15.62
P. aeruginosa 1	18	31.25	17	125	16	250	20	31.25	21	15.62	19	31.25
P. aeruginosa 2	18	31.25	16	250	16	250	19	31.25	20	15.62	21	31.25
P. aeruginosa 3	17	31.25	17	125	17	125	20	31.25	20	15.62	20	31.25
P. aeruginosa 4	19	31.25	17	125	16	250	20	31.25	20	15.62	21	31.25
S. marcescens 1	22	15.62	20	15.62	23	15.62	24	15.62	23	15.62	25	15.62
S. marcescens 2	23	15.62	22	15.62	22	15.62	25	15.62	22	15.62	25	15.62
S. marcescens 3	24	15.62	23	15.62	22	15.62	25	15.62	20	31.25	23	15.62
S. marcescens 4	23	15.62	22	15.62	22	15.62	24	15.62	24	15.62	24	15.62
Salmonella* spp. *1	19	31.25	18	31.25	21	15.62	20	15.62	22	15.62	24	15.62
Salmonella* spp. *2	18	62.5	18	31.25	23	15.62	21	15.62	22	15.62	24	15.62
C. albicans 1	20	15.62	22	15.62	20	15.62	22	15.62	20	15.62	24	15.62
C. albicans 2	20	15.62	20	15.62	21	15.62	22	15.62	21	15.62	22	15.62

Table 2: Cytotoxic effect of the different extracts of Arabic and myrrh gums against Brine shrimp nauplii.

Tested extracts		Concentrations (µg/ml)	Initial number of nauplii	Total death			Pourcentage of lethality	LC_{50} (µg/ml)	Confidence interval
Arabic gum	Aqueous extract	1000	10	1	2	2	16.66	485.4	218.8 to 3067
		500	10	1	1	2	13.33		
		250	10	2	1	2	16.66		
		125	10	0	2	1	10.00		
		62.5	10	1	1	0	6.66		
		31.25	10	1	0	0	3.33		
		15.62	10	0	1	0	3.33		
	Oily extract	1000	10	2	2	3	23.33	388	224.9 to 834.9
		500	10	2	1	2	16.66		
		250	10	1	1	1	10.00		
		125	10	0	0	2	6.66		
		62.5	10	1	0	0	3.55		
		31.25	10	0	1	1	6.66		
		15.62	10	1	0	0	3.33		
	Essential oil	1000	10	1	1	2	13.33	1679	506.8 to 10600336
		500	10	2	1	0	10.00		
		250	10	1	1	0	6.66		
		125	10	2	0	1	10.00		
		62.5	10	1	1	0	6.66		
		31.25	10	1	0	0	3.33		
		15.62	10	0	0	0	0		
Myrrh gum	Aqueous extract	1000	10	5	4	6	50	27.9	6.884 to 57.35
		500	10	5	4	5	46.66		
		250	10	4	4	3	36.66		
		125	10	3	4	2	30		
		62.5	10	3	3	2	26.66		
		31.25	10	2	1	1	13.33		
		15.62	10	2	1	0	10		
	Oily extract	1000	10	6	5	4	50	43.37	17.48 to 92.53
		500	10	4	5	4	43.33		
		250	10	4	4	4	40		
		125	10	2	3	2	23.33		
		62.5	10	2	1	2	16.66		
		31.25	10	2	2	0	13.33		
		15.62	10	0	1	0	3.33		
	Essential oil	1000	10	7	7	6	66.66	26.89	??? to 69.83
		500	10	5	6	6	56.66		
		250	10	5	5	5	50		
		125	10	4	5	5	46.66		
		62.5	10	3	5	2	33.33		
		31.25	10	1	2	1	13.33		
		15.62	10	2	0	1	10		
	Control	/	10	0	0	1	3.33	/	/

Table 3: Enumeration of revertant colonies of *S. typhimurium* TA100, TA98 and TA1535 obtained with the oily extract of Arabic gum with and without metabolic activation (S9).

Ames bacterial strains / Concentrations (µg/ml)	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
1000	43 ± 2.33	55 ± 1.66	21 ± 1.66	34 ± 2.33	10 ± 2	17 ± 1.33
500	35 ± 1.66	53 ± 2	20 ± 0.66	33 ± 2	7 ± 1.66	17 ± 1
250	31 ± 0.33	43 ± 1.66	18 ± 1	30 ± 1.66	6 ± 1	13 ± 1.66
125	27 ± 2	40 ± 2.33	27 ± 1.66	30 ± 1	4 ± 1.33	11 ± 0.33
62.5	25 ± 1.66	33 ± 1.33	25 ± 2	22 ± 2	2 ± 1	7 ± 1.66
31.25	21 ± 1.33	29 ± 1	22 ± 1.33	20 ± 2.33	2 ± 0.33	5 ± 0.66
15.62	15 ± 1	22 ± 0.66	21 ± 0.33	19 ± 2	1 ± 0	4 ± 0.33

Table 4: Enumeration of revertant colonies of *S. typhimurium* TA100, TA98 and TA1535 obtained with the oily extract of myrrh gum, with and without metabolic activation (S9).

Ames bacterial strains / Concentrations (µg/ml)	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
1000	46± 2	51 ± 2.33	28 ± 2	30 ± 1	10 ± 2	15 ± 2
500	45 ± 2	47 ± 1.66	24 ± 1	30 ± 0.66	7 ± 1.66	15 ± 1
250	40 ± 1.33	45 ± 1	22 ± 2	27 ± 2	6 ± 1	11 ± 0.66
125	36 ± 0.66	43 ± 1.66	21 ± 0.33	23 ± 2	4 ± 1.33	8 ± 1
62.5	35 ± 1	40 ± 2	15 ± 1	19 ± 1.33	2 ± 1	5 ± 1.33
31.25	31 ± 2	40 ± 1.33	11 ± 1	16 ± 0.33	2 ± 0.33	3 ± 0.33
15.62	31 ± 1	38 ± 2	6 ± 1.33	15 ± 1.33	1 ± 0.33	2 ± 0.33

Table 5: Enumeration of revertant colonies of *S. typhimurium* TA100, TA98 and TA1535 obtained with oily extract of Arabic gum, with and without metabolic activation (S9).

Ames bacterial strains / Concentrations (µg/ml)	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
1000	66 ± 2	75 ± 2.33	35 ± 2	45 ± 1.33	17 ± 1.33	19 ± 1
500	61 ± 3	64 ± 1.66	31 ± 1.66	43 ± 2	15 ± 1	18 ± 0.33
250	56 ± 1.66	57 ± 2.33	28 ± 1	40 ± 1.66	12 ± 0.66	16 ± 1
125	52 ± 1.66	46 ± 3	23 ± 2	35 ± 2	10 ± 0.33	12 ± 1.33
62.5	45 ± 1	40 ± 1.33	15 ± 1.33	33 ± 1.33	5 ± 1	9 ± 0.66
31.25	42 ± 1.66	38 ± 1	10 ± 0.66	31 ± 1.66	2 ± 0.33	7 ± 1
15.62	37 ± 2.33	35 ± 2	6 ± 1.33	29 ± 1	2 ± 0	4 ± 0.33

Table 6: Enumeration of revertant colonies of *S. typhimurium* TA100, TA98 and TA1535 obtained with the oily extract of myrrh gum, with and without metabolic activation (S9).

Ames bacterial strains / Concentrations (µg/ml)	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
1000	48 ± 2	51 ± 1.66	44 ± 2.33	51 ± 2.33	10 ± 2	12 ± 1.66
500	43 ± 2.33	50 ± 2	41 ± 2	48 ± 3	9 ± 1.33	11 ± 1.33
250	40 ± 2.33	47 ± 2.33	36 ± 1.33	42 ± 2.66	5 ± 2	9 ± 1
125	37 ± 1	43 ± 1	32 ± 2	33 ± 1.66	4 ± 0.33	6 ± 2
62.5	31 ± 1.66	41 ± 1.33	25 ± 1.66	28 ± 2	3 ± 1	3 ± 1
31.25	25 ± 2.33	37 ± 1.66	19 ± 2	25 ± 1.33	3 ± 0.33	2 ± 0.33
15.62	20 ± 1	33 ± 2	15 ± 1.66	21 ± 1.66	1 ± 0.33	2 ± 0

Table 7: Enumeration of revertant colonies of *S. typhimurium* TA100, TA98 and TA1535 obtained with the essential oil of arabic gum, with and without metabolic activation (S9).

Ames bacterial strains / Concentrations (µg/ml)	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
1000	50 ± 2	70 ± 3	38 ± 1.66	42.33 ± 2.33	13 ± 2	15 ± 1.66
500	43 ± 2.33	64 ± 1.66	35 ± 1	38 ± 1.66	13 ± 1.33	14 ± 1.33
250	39 ± 2	57 ± 2.33	32 ± 1.33	36 ± 2	11 ± 1	10 ± 2
125	33 ± 2.33	46 ± 3	25 ± 2	31 ± 2.33	9 ± 2	8 ± 0.33
62.5	27 ± 2	40 ± 1.33	19 ± 2	26 ± 2	6 ± 1.66	5 ± 1.52
31.25	21 ± 1.33	36 ± 2	12 ± 1.66	22 ± 1	5 ± 2	3 ± 0.66
15.62	17 ± 2	33 ± 1.66	9 ± 2	13 ± 2.33	3 ± 0.33	1 ± 0.66

Table 8: Enumeration of revertant colonies of *S. typhimurium* TA100, TA98 and TA1535 obtained with the essential oil of myrrh gum, with and without metabolic activation (S9).

Ames bacterial strains / Concentrations (µg/ml)	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
1000	33 ± 1.33	48 ± 2.63	22 ± 2	37 ± 1.33	9 ± 2	11 ± 2
500	33± 2	45 ± 1.33	20 ± 1.33	30 ± 2	7 ± 2	10 ± 1.66
250	30 ± 1	41 ± 1	17 ± 1	25 ± 1.66	6 ± 1.66	10 ± 1
125	26 ± 1.33	33 ± 2	13 ± 2	21 ± 2	5 ± 1	8 ± 1.33
62.5	23 ± 2	25 ± 1.33	11 ± 1	19 ± 2.15	5 ± 0	4 ± 1
31.25	17 ± 0.33	18 ± 2	11 ± 1.33	18 ± 1	3 ± 0.33	1 ± 1.33
15.62	6 ± 1	15 ± 1	8 ± 1	10 ± 2	2 ± 0	1 ± 0

Both methanol solvent and sunflower oil were tested and no antibacterial activity was observed.

Regarding MCB test, no bacterial growth was observed with reference clinical strains with all the tested extracts. Therefore, extracts had a bactericidal activity.

Cytotoxicity Assessement

Experiment results of cytotoxic activity of Arabic gum extracts did not show any effect for all tested concentrations. The LC_{50} was equal to 485.4 µg/ml for the aqueous extract, 388 µg/ml for the oily extract and 1679 µg/ml for the essential oil, up to 100 µg/ml (Table 2).

The LC_{50} of the myrrh gum displayed potent cytotoxic activity against *Artemia salina* nauplii (Table 2), equal to 27.9 µg/ml for the aqueous extract, 43.37 µg/ml for the oily extract and 26.89 µg/ml for the essential oil. This result may help to serve as a basis for the development of cytotoxic agents for clinical applications such as the screening of the antitumour effect (anticancer activity).

Genotoxicity Assessement

Genotoxicity or Mutagenicity assessement whith Ames test using plate preincubation method indicated that the extracts of arabic and myrrh gums did not cause an increase in the number of histidine revertants colonies on *Salmonella* strains TA100, TA98 and TA1535 in the presence or absence of S9 Mix (Tables 3, 4, 5, 6, 7 and 8). The positive control chemicals 4-NQO, sodium azide produced the expected increase in mutation frequency while the bacteria grew normally with the negative control. All criteria for a valid study were met.

DISCUSSION

About 30% of the pharmaceuticals are prepared from plants worldwide. In our study, we investigated some biological properties of Arabic gum and myrrh gum. The antimicrobial results of the two tested gums showed very interesting activities which were essentially attributed to their secondary metabolites or due to the presence of saponin, saponin glycosides, volatile oil, hydrolysable tannin, triterpenoid, flavonoids, phenol, alkaloids and terpenes that are biologically active molecules. For the Arabic gum it is known that it contains high salt concentrations of Ca^{+2}, Mg^{+2} and K^{+2}, polysaccharides and many enzymes such as oxidases, peroxidases, and pectinases which can had antimicrobial properties.[23] Several authors reported that the activity of myrrh gum is mainly due to the presence of the following constituents: a-pinene, dipentene [= (±)-limonene], limonene, cuminaldehyde, cinnamic aldehyde, eugenol, m-cresol, heerabolene (probably tricyclic sesquiterpene), cadinene, a sesquiterpene, a bicyclic sesquiterpene ($C15H_{24}$), a tricyclic sesquiterpene ($C_{15}H_{24}$), formic acid, acetic acid, myrrholic acid ($C_{16}H_{21}O_3$. COOH) and palmitic acid.[24]

Because of their richness in biomolecules, several researchers were interested in these two gums. By testing arabic gum alcoholic and aqueous extracts, Dubey *et al.* (2015)[5] reported that *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *Pseudomonas aeroginosa*, *Proteus merabilis*, *Acinetobacter*, *Enterobacter*, *Klebsiella pneumoniae*,

Serratia spp., *E. coli*, *Salmonella typhi*, *Cerospora pongamae*, *Candida albicans* and *Aspergillus niger* were inhibited at elevated MICs values extended to 2560 µg/ml, contrary to our results, where the MICs values did not exceed 250 µg/ml with almost the same bacterial and fungal strains.

Another authors[25] tested the inhibitory effect of diethyl ether extract from Commiphora molmol (Myrrh) on *S. aureus*, *B. subtilis*, *E. coli* and *E. cloacae* and find diameters of inhibition zones varying between 6 and 13mm; no inhibitory effect was shown on *P. aeruginosa* and *S. marcescens*. The hexane extract showed minor inhibitory effect against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae* (4 to 6 mm). The water extract showed no inhibitory effect against all bacterial strains. Benyagoub *et al.* (2016)[26] also tested the antibacterial activity of the aquous extract and weak diameters of inhibition zones were obtained (8 and 9 mm) with *E. coli*, *Citrobacter sp.* and *S. aureus; P. aeruginosa* was resistant. These results were quite contrary to ours since our myrrh gum extracts prepared without the use of solvents, were more active on the same bacterial species with important inhibition zone diameters up to 30 mm. This could be due to the difference between the chemical composition of gums varying slightly depending on its origin, climate, harvest season, tree age, and processing conditions, such as spray dying.[27]

Regarding cytotoxicity evaluation of the different extracts of Arabic and myrrh gums based on the ability to kill cultured *Artemia salina* nauplii (brine shrimp) by mesearing the LC_{50}, no cytotoxic effect of Arabic gum extracts was showed. However, a high cytotoxic potential was demonstrated for the three myrrh gum extracts. These results are in line with Weli *et al.* (2014)[27] and El Alawi *et al.* (2018)[28] who showed that Arabic gum extracts are lacking cytotoxic activity. Many recent studies showed that arabic gum has the ability to prevent or treat the toxic manifestations of some common drugs such as indomethacin, aspirin, acetaminophen, and gentamicin as well as some chemotherapeutic drugs such as cyclophosphamide, doxorubicin, and cisplatin besides its potent prophylactic role in some chemical toxicity cases such as trichloroacetic acid, paraquat, and mercuric chloride.[28,29]

By assessing the genotoxic potential of the different tested extracts using the Ames test, absence of the latter has been showed. Few studies have been carried out and showed that these gums were not genotoxic.[30] El-Garawani *et al.* (2021)[31] suggested that Arabic gum exerted a protective role against TBX-induced oxidative stress and genotoxicity which may be attributed to its active metabolites.

Primary scientific study done by Qureshi *et al.* (1993),[32] aimed to assess cytotoxic and genotoxic effects of myrrha gum using normal and Ehrlich ascites carcinoma cell-bearing Swiss albino mice; results indicated that it had a significant cytotoxic potential and no genotoxic effect. Eventhough we did not use the same methods, similar results were obtained confimrming its use in cancer therapy.

Our results are very encouraging for the use of these two gums extracts in the treatment of several pathologies such as bacterial, and fungal infections and cancer without any fear of causing mutagenic, or even, carcinogenic effect.

CONCLUSION

From the present screening, it could be concluded that the Arabic and myrrh gums extracts exhibited potent antimicrobial activity especially against both Gram positive and Gram negative resistant bacteria and *Candida albicans* fungal strain and no genotoxic effect. Interstingly, aqueous and oily extracts, that were easy to prepare with inexpensive methods, showed a good antimicrobial activity. In addition to, cytotoxicity of myrrh gum makes it a potential anticancer candidate.

Results of this study supported the valuable use of Arabic and myrrh gums in traditional medicines for treatment of different deseases and infections.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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