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Research Article

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Evaluation of acute toxicity and antimicrobial effects of the bark extract of Bisham (*Commiphora gileadensis* L.)

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ABSTRACT

Bisham (Commiphora gileadensis L) is one of most well known plant used in Yemen as traditional medicine. The bark of plant was used in the treatment of burns and skin infection. This study aimed to investigate the antimicrobial activity, toxicity and LD_{50} of methanolic extract of bark of Commiphora gileadensis L, in addition to the phytochemical screening. The results showed that the methanolic extract of bark of plant showed an activity against Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Candia species. In addition the extract was safe in mice up to 5 g/Kg. the phytochemical screening showed the presence of flavonoids, phenol, saponin, steroids and amino acids, steroids and amino acids in plants.

Key words: *Commiphora gileadensis* L, antimicrobial activity, acute oral toxicity and Median lethal dose.

INTRODUCTION

Even though pharmacological industries have produced a number of new antimicrobial in the last three decades, resistance to these drugs by microorganisms has increased[1].The use of plant compounds for pharmaceutical purposes has gradually increased over all the world[2]. According to World Health Organization [3] medicinal plants would be the best common source to obtain a variety of traditional medication worldwide [4]. Also there is the worldwide green revolution which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs [5], and medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity [6].

Additionally, investigation of the antimicrobial properties of plants has brought attention to the opportunity of producing a natural and environment friendly source that could replace the synthetic antimicrobial compounds [7]. With the increase of bacterial resistance to antibiotics, there is considerable interest to investigate the antimicrobial effects of different extracts against a range of bacteria, to develop other classes of natural antimicrobials useful for the infection control[8]. Also, candida Invasive candidiasis has emerged as the commonest form of opportunistic mycoses throughout the world. Apart from its widespread occurrence, it is often acutely progressive, difficult to diagnose and associated with increased hospital stay and high mortality rates [9-13]. Treatment of this condition has become further complicated owing to the relative rise in the proportion of non-albicans Candida isolates, which often demonstrate intrinsic resistance towards specific antifungal agents [14-19].

Locally in Yemen *Commiphora gileadensis* L plant is known as Bisham, it grows in Hadramout. The bark of plant is used traditionally for the treatment of burns and skin infection. Therefore, such plant should be investigated to better understand their phytochemical properties, antimicrobial activity, acute oral toxicity and Median lethal dose (LD_{50}).

EXPERIMENTAL SECTION

Collection of the plant material

The bark of plant was collected from Hadhramout-Yemen. It was identified by Researcher; Ahmed Salim Batahir the head of the forests and grasslands, Public Authority for Agricultural Research and Extension. The sample of the bark was air-dried as to be done locally in Hadhramout.

Preparation of the crude methanol extract of bark of Commiphora gileadensis L

The air-dried bark of *Commiphora gileadensis* L (1500 g) was macerated in 4L of 99.9% of methanol for one week. The macerated barks were filtered and the filtrate was evaporated under reduced pressure using Rotary Evaporator. The Process was repeated for five times till complete extraction of bark[20, 21].

Fractionation of the methanol extract

The residue of total extract (100 g) was extracted using Petroleum ether (150 ml) in separating funnel for two times. The process was repeated for the residue from previous extraction using Chloroform, Ethyl Acetate, Methanol and Distilled water respectively (Satyajiy et al.,2006) .

Phytochemical screening

The Petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of bark of *Commiphora gileadensis L* were submitted to a preliminary screening, through chemical reactions, to detect the presence of the following classes: Flavonoids (Aluminium chloride and potassium hydroxide B Borntrager Test), Phenols and Tannins (ammonia Vapor and Aluminium chloride), Alkaloids (Dragendorff's Reagent), Steroids and Amino acid (Vanillin Reagent), Steroids and Triterpiene glycosides (Acetic Anhydrid sulfuric acid) and Saponin (Forth test).

Animals

Whit albino mice (25-30g) of both sexes obtained from the animal house of faculty of science, Sana'a University were used for determination of acute oral toxicity and median lethal dose. The animals were housed in polypropylene cages under controlled temperature (23 ± 2 °C) and light (light-dark cycle of 12 hours), and with food and water *adlibitum*. The mice were acclimated in the laboratory at least eight hours before the experiments. The experiments were approved by the Institutional Ethical Committee, Faculty of Medicine and Health Sciences, Sana`a University.

Acute oral toxicity and Median lethal dose (LD50) test

The acute oral toxicity and median lethal dose (LD50) of the total extract of bark of *Commiphora gileadensis* L was estimated in albino mice [22]. In a pilot experiment, five groups each of sex mice received the tested extracts dissolved in water at doses of 100, 1000, 2500, 4000, 5000 mg/kg b.wt, respectively. Animals were observed for 24 hours for signs of toxicity and number of deaths. Control animals were received the vehicle and kept under the same conditions without any treatments. Sign of toxicity and number of deaths per dose in 24 hours were recorded.

Antimicrobial activity using disc diffusion test

A modified agar diffusion method [23] was used to assess the antimicrobial activities of the total extract of bark of the plant against representatives of gram-positive bacteria (Staphylococcus aureus, and Staphylococcus Haemolyticus) Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, and Klebsilla Pneumoniae) and yeast (Candida species). Applying the agar diffusion method, cups were made using Pasture pipette using Sabouraud Dextrose agar, Mueller-Honton agar, and blood agar. Extracts were dissolved in distilled water at concentration of 200 mg/mL then 50µl (containing 10 mg of the extract under test) were aseptically added to the cups (10 mg/cup). Plates were incubated inverted at 37°C for 24-48 hr. After incubation, the inhibition zones were recorded in mm.

RESULTS AND DISCUSSION

Preliminary phytochemical Screening

The Preliminary chemical examination of extract of bark of *Commiphora gileadensis* L indicated the presence of flavonoids, phenols, tannins, steroids, amino acids, triterpiene glycosides and saponin Table (1).

The results showed that the flavonoid, phenol and tannins are mainly found in ethanolic and methanolic extracts, while steroids and amino acids are found in all extracts except petroleum ether extract. But the Triterpiene glycosides are found in all extracts except methanolic and aqueous extract .Moreover the alkaloids aren't found in all extracts, in contrast saponin is present in all extracts.

Acute oral toxicity and Median lethal dose (LD50) test

The results revealed that all the examined doses of Commiphoragileadensis L(up to 5000mg/kg b.wt) did not produce any demonstrable acute toxic effects or deaths in all groups of mice, except reversible reduction in motor activity that appeared in doses 2500 , 4000 , 5000 mg/kg **Table (2).**However, any tested compound that causes no adverse effect at a dose 5000 mg/kg will be considered as 'practically non-toxic [24].

From the above results, the methanolic extract of bark of *Commiphora gileadensis* L is considered safe or practically non-toxic.

Table (1): Preliminary phytochemical screening

NO	Chemical constituents	Chemical test	Extract				
			P	Ch	Et	M	A
1	Flavonoids	$AlCl_3$	-	-	+	+	-
		KOH B Borntrager Test	-	-	+	+	+
2	Phenols + Tannins	$FeCl_3$	-	-	+	+	-
		NH_3 Vapor	+	+	-	-	-
3	Alkaloids	Dragendorff's Reagent	-	-	-	-	-
4	Steroids + Amino acid	Vanillin Reagent	-	+	+	+	+
5	Steroids and Triterpiene glycosides	Acetic Anhydrid.H_2SO_4	+	+	+	_	_
6	Saponin	Forth test	+				

(+) present, (-) absent ,(P) petroleum ether , (Ch) Chloroform , (Et) Ethyl actate , (M) Methanol, (A) Aqueous.

Table 2: Effect of Methanolic Extract of bark of *Commiphora gileadensis* L on Toxicity signs

Parameters	Groups and behavior of animals					
	Control	100 mg/kg	1000 mg/kg	2500 mg/kg	4000 mg/kg	5000 mg/kg
Motor Activity	N	N	N	-	-	-
Aggressiveness	N	N	N	N	N	N
Reaction to noise	N	N	N	N	N	N
Reaction to pinch	N	N	N	N	N	N
State of tail	N	N	N	N	N	N
State of excrement	N	N	N	N	N	N
Clonic convulsion	N	N	N	N	N	N
Salivation	N	N	N	N	N	N
Mortality (Within 24hr.)	NM	NM	NM	NM	NM	NM

(N) Normal, (-) Reduced, (- -) profoundly reduced, (NM) No Mortality

Antimicrobial activity test

The results of the test showed that the total extract of bark of *Commiphora gileadensis* L has antimicrobial activity against Staphylococcus aureus, Pseudomonas aeruginosa, Klebsilla Pneumoniae, Candida species, but does not have antibacterial activity against Staphylococcus haemolyticus, *Escherichia coli* as shown in Table (3) .

In comparison to reference antibiotics(Ampicillin, Erythromycin, Gentamycin and Cefotaxime) the extract exhibited a highest activity against Staphylococcus aureus. But its activity against Klebsilla Pneumoniae is similar to Cefotaxime and lower than Levofloxacin and Gentamycin. However the antibacterial activity of extract showed weak activity against *Escherichia coli and* Staphylococcus haemolyticus in compared to reference antibiotics.

On the other hand the Candida species exhibited resistance to antifungal references (Itraconazole and Voriconazole) but it was sensitive to extract**.**

Table3- Results of the antimicrobial tests of the methanolic extract of investigated plants in agar diffusion assay

	Inhibition Zones (mm)[b] against					
	S.a.	*S.h.*	*E.c*	*P.a*	*K.p.*	*C.s.*
Extract	16	-	-	8	6	8
Ampicillin	10	16	4	-	-	
Levofloxacin	18	16	26	26	22	
Clindamycin	30	-	8	-	-	
Erythromycin	12	-	-	16	-	
Cefotaxime	14	18	4	14	6	
Gentamycin	14	6	14	18	14	
Itraconazole						-
Voriconazole						-

P.a.Pseudomonas aeruginosa , K.p.klebsiella pneumoniae ,C.s.candida species, S.a.Staphylococcus aureus ,S.h.Staphylococcus haemolyticus, E.c.Escherichia coli.

CONCLUSION

The results of the study demonstrate that the methanolic extract of bark of plant exhibited an activity against Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Candia species. in addition the extract was safe in mice up to 5 g/Kg. Phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound.

REFERENCES

[1] GGF Nascimento; J Locatelli; PC Freitas; GL Silva, *Braz. J. Microbiol*., **2000**, 31(4), 247-256.
[2] J Shri, ICMR. Bull., **2003**, 33,57-63. ACX Santos PRVO; TCB Tomassini, *Rev. Farm. Bioquím.*, **1995**, 31,35-38.
[4] WHO: National policy on traditional medicine and regulation of herbal medicines: Report of a global survey., **2005**.
[5] EM Williamson; DT Okpako; FJ Evans, Pharmacological methods in phytotherapy research (USA) **1996**.
[6] DS Fabricant; NR Farnsworth, *Environ. health persp.*, **2001**, 109(Suppl 1),69.
[7] L Zhu; YJ Tian; L Yang; JG Jiang, *EXCLI. J.*, **2001**, 10,62-68.
[8] IM Bakri; CWI Douglas, *Arch. Oral Biol.*, **2005**, 50(7),645-651.
[9] JA Abelson; T Moore; D Bruckner; J Deville; K Nielsen, *Pediatrics*., **2005**, 116(1),61-67.
[10] A Chakrabarty, Drug resistance in fungi-an emerging problem, In Regional Health Forum., **2011**, **2011**,97.
[11] VrR Aquino; LW Lunardi; LZ Goldani; AL Barth, *Braz. J. Infect. Dis*., **2005**, 9(5), 411-418.
[12] WH Sheng; JT Wang; MS Lin; SC Chang, *J. Formos. Med. Assoc.*, **2007**, 106(2),110-118.
[13] J Morgan; MI Meltzer; BD Plikaytis; AN Sofair; S Huie-White; S Wilcox; LH Harrison, EC Seaberg; RA Hajjeh; SM Teutsch, *Infect. Cont*., **2005**, 26(06),540-547.
[14] WE Trick; SK Fridkin; JR Edwards; RA Hajjeh; RP Gaynes, *Clin. Infect Dis.*, **2002**, 35(5),627-630.
[15] MA Pfaller; RN Jones; GV Doern; HS Sader; SA Messer; A Houston; S Coffman; RJ Hollis, *Antimicrob. Agents Ch*., **2000**, 44(3),747-751.
[16] MA Pfaller; DJ Diekema, *Clin. Microbiol. Infec.*, **2004**, 10(s1),11-23.
[17] V Krcmery; AJ Barnes, *J. Hosp. Infect.*, **2002**, 50(4), 43-260.
[18] YAL Chai; Y Wang; AL Khoo; FY Chan; C Chow; G Kumarasinghe; K Singh; PA Tambyah, *Med. Mycol.*, **2007**, 45(5),435-439.
[19] S Shivaprakasha; K Radhakrishnan; P Karim, *Indian J. Med. Microbiol.*, **2007**, 25(4),405.
[20] SS Handa; SPS Khanuja; G Longo; DD Rakesh; United Nations Industrial Development; E*arth Environ. Mar. Sci. Technol;* **2008**.
[21] HS Sukhdev; KSP Suman; L Gennaro; R Dutt, International center for science and high technology Trieste., **2008**,22-28.
[22] D Lorke, *Arch. Toxicol.*, **1983**, 54,275-287.
[23] A Bauer; W Kirby; WMM Sheriss; JC Sheriss; M Turck, *Am. J. Clin. Pathol.*, **1966**, 45,493–496.

[24]DJ Ecobichon. The basis of toxicity testing, 2nd edition, CRC press, Boca Raton FL, **1997**.