



Full Length Research Article

ANTIMICROBIAL ACTIVITY OF AQUEOUS CRUDE EXTRACT OF SUDANESE *SOLANUM DUBIUM* SEEDS, SUDANESE SUNNUT HONEY AND COMBINATION *S. DUBIUM* SEEDS AND SUNNUT HONEY

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ABSTRACT

The purpose of the study was to investigate the *in-vitro* antimicrobial activity of aqueous crude extract, of Sudanese *Solanum dubium* seeds, Sudanese Sunnut honey, combination *S. dubium* seeds and Sunnut honey and combination *S. dubium* seeds and Sunnut honey fresh. The aqueous crude extract, of *S. dubium* seeds, combination *S. dubium* seeds and Sunnut honey, Sunnut honey and combination *S. dubium* seeds and Sunnut honey fresh were tested against four standard bacteria i.e.: two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram negative (*Escherichia coli* and *Salmonella typhi*) and against two standard fungi species i.e. (*Aspergillus niger* and *Candida albicans*) using the agar plate diffusion method. The combination *S. dubium* seeds and Sunnut honey showed high activity (28 and 27.5 mm) against Gram negative bacteria (*S. typhi* and *E. coli*) and showed high activity (26.5 and 26 mm) against Gram positive (*B. subtilis* and *S. aureus*), also showed moderate activity (21 and 17 mm) against fungi (*A. niger* and *C. albicans*). In conclusion: Combination *S. dubium* seeds and Sunnut honey showed great potential activities against antibacterial as well as antifungal activity than combination *S. dubium* seeds and Sunnut honey fresh, *S. dubium* seeds and honey. The results indicate that combination *S. dubium* seeds and Sunnut honey extract may recommend to use as herbal drug.

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INTRODUCTION

Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore, it is useful to investigate the potential of local plants against these disabling diseases (Amaral et al., 2006 and Koko et al., 2008).

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The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (Austin et al., 1999). Natural products of higher plants may give a new source of antimicrobial agents. There are many research groups that are now engaged in medicinal plants research (Samy et al., 1998 and Hamil et al., 2003 and Motsei et al., 2003). The genus *Solanum* L. consists of over 2000 species distributed worldwide is the largest in Solanaceae and is one of the largest among all flowering plants (Olmstead and Palmer, 1997). The species are medicinal herbs (Caicedo and

Schaal, 2004). *Solanum dubium* L, an indigenous plant in northern and central Sudan, is a woody herb with solid erect stem and green in colour, about 30 cm in height. The fruits are grouped in clusters with exile alternately bent to bring all clusters to one side of the stem or the branch. Unripened fruits are green and almost enclosed in spiny calyx, while the ripened ones are yellow. The seeds are dark brown in colour. (Salih, 1979 and Yousif *et al.*, 1996). Honey is a natural product which has an extensive history of traditional human medicinal use in many societies (Zumla and Lulat, 1989). The use of honey in treating wound and mild bacterial infections has long been known and practiced. Honey added to oral rehydration solution has been found to speed up recovery from vomiting and diarrhea in infants and children suffered from gastroenteritis (Abdulrahman *et al.*, 2010). Honey is widely available in most communities and although the mechanism of action of several of its properties remains obscure and thus there is a need for further investigation. The time has now come for conventional medicine to lift the blinds off this 'traditional remedy' and gives its due recognition. The curative potential of honey is well documented in almost all available worlds' oldest medical literatures and religious testaments. At the research level, honey is currently showing potential in minimizing cellular injuries of the skin and post-radiotherapies. Honey is widely used in traditional medical systems. It was used by the ancient Greeks and Sumerians (Molan, 1995). The present study was conducted to investigate the antimicrobial activity of aqueous extract of *S. dubium* seeds and combination *S. dubium* seeds and Sunnut honey, Sunnut honey alone and combination *S. dubium* seeds and Sunnut honey fresh.

MATERIALS AND METHODS

Plant Collection and Identification

The samples of *S. dubium* seeds were collected from the fields of the area Al-Halfaya, Khartoum North. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.

Preparation of Plant Material

The cleaned and shade-dried plant seeds were fine powdered using a grinding machine, each ground sample was weighed and then stored in a dry container at ambient temperature. Combination was done by mixing the ground seeds with Sunnut honey (1:4) and stored in tied bottle until used.

Extraction of Plant Material

500g of the powdered seeds were extracted in distilled water (1g to 250mls) for 24 hours, filtered and then evaporated to dryness at 60 C in an incubator. The yield was 2.1 % W/W. All desired concentrations were freshly prepared in normal saline prior to use.

Test microorganisms

The aqueous extract of Sunnut honey, *S. dubium* seeds and combination were tested against four bacterial species: two Gram-positive bacteria viz., *Bacillus subtilis* (NCTC 8236)

and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (NCTC 0650), and two fungal strains viz, *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596). The bacterial and fungal strains used in the study were obtained from the Department of Microbiology, of the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) and National Health Laboratory of Khartoum in Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test.

In vitro testing of extracts for antimicrobial activity

The cup-plate agar diffusion method described (Kavanagh, 1972) was used adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension (between 10^8 and 10^9 CFU/ml) was thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dish plates. Agars was left to set and in all of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Each cup were filled with 0.1 ml sample of the ethanol extracts using an automatic microlitre pipette, and thereafter the extracts were allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 18 h. 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 and averaged. The mean values were tabulated.

Antifungal Testing

The same method used for the antibacterial test was employed. However, the growth media used in case of fungi was Sabouraud dextrose agar instead of nutrient agar. The inoculated medium was incubated at 25°C for two days for *Candida albicans* and three days for *Aspergillus niger*.

RESULTS AND DISCUSSION

The aqueous crude extract of *S. dubium* seeds, combination *S. dubium* seeds and Sunnut honey, combination *S. dubium* seeds and Sunnut honey fresh and Sunnut honey alone were tested against four standard bacteria i.e.: two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram negative (*Escherichia coli* and *Salmonella S. typhi*) and against two standard fungi species i.e. (*Aspergillus niger* and *Candida albicans*) using the agar plate diffusion method. The crude extract of Sunnut honey showed high activity (20.7 and 20 mm) against Gram negative bacteria (*E. coli* and *S. typhi*) and showed high activity (21 and 18.7 mm) against Gram positive (*S. aureus* and *B. subtilis*). also showed moderate activity (14.7 and 14 mm) against fungi (*A. niger* and *C. albicans*), aqueous crude extract of *S. dubium* seeds showed high activity (25 and 24.7 mm) against Gram negative bacteria (*S. typhi* and *E. coli*) and showed high activity (20 and 18 mm) against Gram positive (*S. aureus* and *B. subtilis*). also showed moderate activity (18 and 18 mm) against fungi (*A. niger* and *C. albicans*), aqueous crude extract of *S. dubium* seeds and

Table 1. Antimicrobial activity of aqueous crude extract of Sunnut honey, *S. dubium* seeds, *S. dubium* seeds and Sunnut honey combination and *S. dubium* seeds and Sunnut honey fresh combination against the standard bacteria and fungi

Standard microorganisms	Zone of inhibition (100 mg/ml)			
	Sunnut honey	<i>S. dubium</i> seeds	<i>S. dubium</i> seeds and Sunnut honey combination	<i>S. dubium</i> seeds and Sunnut honey Fresh combination
Bacteria used				
<i>Bacillus subtilis</i>	18.7	18	26.5	20
<i>Staphyococcus aureus</i>	21	20	26	21
<i>Escherichia coli</i>	20.7	24.7	27.5	17
<i>Salmonella typhi</i>	20	25	28	22
Fungi used				
<i>Aspergillus niger</i>	14.7	18	21	25
<i>Candida albicans</i>	14	18	17	17

Key: Interpretation of results: MDIZ (mm): >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant.

Table 2. The antimicrobial activity of aqueous crude extract of Sunnut honey, *S. dubium* seeds, *S. dubium* seeds and Sunnut honey combination and *S. dubium* seeds and Sunnut honey fresh combination against the standard bacteria and fungi species

Name of crude extracts	Concentration (mg/ml)	Tested Bacteria				Tested Fungi	
		<i>Bacillus subtilis</i>	<i>Staphyococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>
		Mean Diameter of Growth Inhibition Zone (mm)					
Sunnut honey	100	18.7	21	20.7	20	14.7	14
	50	14	19.5	22	22.5	14.5	14
	25	14	15.7	21.7	21	14	10.7
	12.5	10.5	11.5	15.5	15	14	14
<i>S. dubium</i> seeds	100	18	20	24.7	25	18	18
	50	16.5	17	24.7	16.7	18	16.3
	25	14	16.3	18.7	19	15	15
	12.5	13	15.3	15	14	14	14
<i>S. dubium</i> seeds and Sunnut honey combination	100	26.5	26	27.5	28	21	17
	50	19	19.7	24	22.7	20	16.3
	25	16	16	22	16	16	15
	12.5	15	17	17	16	15	14
<i>S. dubium</i> seeds and Sunnut honey fresh combination	100	20	21	17	22	25	17
	50	18	21	25	22	17	17
	25	15	16	19.5	20.5	14.5	14
	12.5	15	16	16	15	14	14

Key: Interpretation of results: MDIZ (mm) : >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant.

Table 3. Antibacterial and antifungal activity of reference antibiotics against standard microorganisms

Drugs	Concentrations (µg/ml)	Standard microorganisms used			
		Gram positive		Gram negative	
		Tested bacteria used			
		Mean Diameter of Growth Inhibition Zone (mm)			
		<i>Bacillus subtilis</i>	<i>Staphyococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
Ampicillin	40	15	25	-	-
	20	14	20	-	-
	10	13	18	-	-
	5	12	15	-	-
	40	29	35	32	18
Gentamicin	20	22	33	30	16
	10	20	30	17	12
	5	17	28	-	-
Tested fungi used (M.D.I.Z. mm)		<i>Aspergillus niger</i>		<i>Candida albicans</i>	
Clotrimazole	40	30		42	
	20	22		40	
	10	19		33	
	5	16		30	
	50	28		17	
Nystatin	25	26		14	
	12.5	23		-	

Key: Interpretation of results: MDIZ (mm): >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant. (-): No inhibition.

Sunnut honey combination showed high activity (28 and 27.5 mm) against Gram negative bacteria (*S. typhi* and *E. coli*) and showed high activity (26.5 and 26 mm) against Gram positive (*B. subtilis* and *S. aureus*), also showed moderate activity (21 and 17 mm) against fungi (*A. niger* and *C. albicans*) and aqueous crude extract of *S. dubium* seeds and Sunnut honey fresh combination showed high activity (25 and 17 mm) against fungi (*A. niger*) and showed high activity (22) against Gram negative bacteria (*S. typhi* and *E. coli*) and showed high activity (21 and 20 mm) against Gram positive (*S. aureus* and *B. subtilis*), also showed moderate activity (17 mm) against Gram negative bacteria (*E. coli*) and fungi (*C. albicans*) (Table 1 and 2). Therefore this result showed that the extracts tested inhibited the growth of all microorganisms though the sensitivities of microorganisms varied. There are several reports stating that other *Solanum* species extracts exhibit antibacterial activity. *Solanum torvum* showed activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Wiert, et al., 2004), while *Solanum nigrum* was active against *Salmonella typhi* (Rani, and Khullar, 2004). *Solanum trilobatum* was able to reduce bacterial load in aquaculture system (Citarasu, et al., 2003) and *Solanum incanum* could inhibit the growth of *Staphylococcus aureus* (Kambizi and Afolayan, 2001). *Solanum trilobatum* showed activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *E. coli* (Doss et al., 2009) and Similarly, Suffredini et al., (2004). The result of minimum inhibition concentration (MDIZ) from Table (1-2) showed that 12.5 µg/ml was the lowest concentration at which all the tested microorganisms were inhibited a comparison of observation given in Tables (1-2), showed that the aqueous crude extract of honey, *S. dubium* seeds, *S. dubium* seeds and Sunnut honey combination and *S. dubium* seeds and Sunnut honey fresh combination inhibited all bacteria except *S. aureus* higher than 40 µg/ml Ampicillin and higher than 5 µg/ml Gentamicin. And showed that the aqueous crude extract of *S. dubium* seeds, *S. dubium* seeds and Sunnut honey combination and *S. dubium* seeds and Sunnut honey fresh combination inhibited *A. niger* with a higher than 5 µg/ml of Clotrimazole, and inhibited *C. albicans* at more than 50 µg/ml of Nystatin.

Conclusion

Based on these results, it can be concluded that, the extract of combination *S. dubium* seeds and Sunnut honey have great potential as antimicrobial compounds against the microorganisms tested. The obtained result suggests it's potential to be used as an alternative therapeutic agent for a number of medical conditions. Further studies are needed to isolate the active constituents of the plant responsible for the antimicrobial activity.

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