Antimicrobial Activity of Sudanese *Solanum dubium* seeds in Combination with Sudanese Honey

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ABSTRACT

The 96% ethanolic extract and Methanolic extract of Sudanese *Solanum dubium* seeds in combination with Sudanese honey (1:4), were tested against four standard bacteria: *Bacillus subtilis*; *Staphylococcus aureus*, *Escherichia coli*; *Salmonella typhi*, and two standard fungi: *Aspergillus niger* and *Candida albicans*, using the agar plate diffusion method. The tested 96% ethanolic extracts of seeds; honey and seeds and honey combination exerted significant antimicrobial activity of concentration of 100mg/ml ranging from 20 to 35mm zone of inhibition and intermediate to significant antifungal activity ranging from 14 to 25mm zone of inhibition. In conclusion the seeds of *S. dubium* and its combination with Sudanese honey proved to exert a potent *in vitro* antimicrobial activity.

Keywords: *In vitro*, antimicrobial activity, *Solanum dubium*, seeds, honey, combination.

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I. INTRODUCTION

Solanum dubium is a well known wild plant growing in most regions of Sudan belonging to the family Solanaceae and used in rural areas for milk coagulation. It is a bushy pubescent herb grown widely in northern, central and western Sudan along with other species such as *S. innacum*, *S. esculentum*, *S. macrocarpon* and *S. melongena* (Mohamed, *et al*., 2010). Research on *S. dubium* was focused mainly on obtaining Solanum crude enzyme from the seeds in pure form and commercial production of the enzyme for cheese making. (Mohamed, *et al*., 2010; El owni, *et al*., 2011). Solanaceous plants are known for their high alkaloidal content in all plant parts including the seeds, which are responsible for their antimicrobial activity in addition to other metabolites such as flavonoids and tannins (Daunay; Chadha, 2004).

Claims about traditional use of *S. dubium* seed in combination with honey for treatment of skin injuries and wounds, prompted us to investigate the antimicrobial activity of seed- honey combinations based on published data about the antimicrobial effect of a number of Sudanese honeys (Farouk, *et al.*, 1988; Molan, 1995).

The present study reports the results of antimicrobial activity assessment of *S. dubium* seed, honey and seed/ honey combination (1:4) against four standard Gram positive and Gram negative bacteria and two standard fungi.

II. MATERIALS AND METHODS

Collection of Plant and Identification

The plant was collected from Central Sudan between January and February 2015 and was identified and authenticated at Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.

Preparations of the *S. dubuim* seeds and honey Combination

Combination was prepared by mixing the ground seeds with honey (1:4) and stored in tied bottle untill used.

Preparation of 96% Ethanolic Extracts

Five hundred grams of each sample; *S. dubium* seeds, honey and *S. dubium* seeds and honey combination were extracted by maceration with 96 % ethanol using shaker apparatus. Extraction was carried out for three days with filtration. The filtrates were combined and the solvent was evaporated under reduced pressure using a rotary evaporator and the yield percentages were calculated. The same procedure was repeated with the solvent methanol. Extraction was carried out according to the method described by Sukhdev, *et al.*, (2008).

Test microorganisms

The extracts of honey, 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 hours and then used for the antimicrobial test.

***In vitro* testing of extracts for antimicrobial activity**

The cup-plate agar diffusion method described by Kavanagh, 1972, was used 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 was filled with 0.1 ml sample of the ethanol and methanol 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 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 and averaged. The mean values were tabulated.

Antifungal testing

The same method used for the antibacterial test was employed, and the growth media used in the case of fungi were 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*.

III. RESULTS AND DISCUSSION

The yield percentage of 96% ethanolic and methanolic extracts of *S. dubium* seeds; honey and seeds/ honey combination are reported in table (1). The ethanolic and methanolic extracts of *S. dubium* seeds, honey and seeds/honey combination were tested against four standard bacteria; two Gram positive: *Bacillus subtilis* and *Staphylococcus aureus* and two Gram negative *Escherichia coli* and *Salmonella typhi* and against two standard fungi: *Aspergillus niger* and *Candida albicans* using the agar plate diffusion method. Results are summarized in table (2and 3). The results of antibacterial and antifungal activity of reference antibiotics against standard microorganisms are reported in table (4).

The bacteria were more sensitive to the 96% ethanolic extracts of the three samples than the fungi. *S. aureus* gave the following MDIZ: 35mm for honey, 27.3mm for seeds and 25mm for seeds/honey combination. At the same concentration, *A. niger* gave 17.7mm for honey, 18mm for seeds and 15.7mm for combination.

The tested microorganisms showed similar results against the methanolic extracts of the three samples. *S. aureus* was the most sensitive to the seed extracts (27mm) and *S. typhi* was more sensitive to the seed/honey combination (28.5mm). *A. niger* gave 18mm against honey and seed extracts, while *C. albicans* was most sensitive to the honey extract (17mm).

The promising antimicrobial activity of *S. dubium* seed, honey and the seed/honey combination could be explained by the high content of steroidal alkaloids, flavonoids and tannins in Solanaceous plants reported in the literature (Daunay; Chadha, 2004).

Table (1): Yield percentage of 96% ethanolic and Methanolic extracts of *S. dubium* seeds, honey and combination

Sample	96%ethanol		Methanol	
	Yield (g)	Yield (%)	Yield (g)	Yield (%)
***S. dubium* seeds**	87.69	17.54	45.25	9.05
Honey	320	64	183.5	36.7
Seed/honey combination	322.22	64.44	146.45	29.29

Weight of sample: 5oog

Table (2): Antimicrobial activity of 96% ethanolic extract of honey, *S. dubium* seeds and *S. dubium* seeds and honey combination against the standard bacteria and fungi

Standard microorganisms	Zone of inhibition (100 mg/ml)		
	Honey	*S. dubium* seeds	*S. dubium* seeds and honey combination
Tested bacteria used			
Bacillus subtilis	**22.7**	**25**	**20.7**
Staphyococcus aureus	**35**	**27.3**	**25**
Escherichia coli	**20**	**26.7**	**27**
Salmonella typhi	**27.7**	**25.7**	**28.5**
Tested fungi used			
Aspergillus niger	**17.7**	**18**	**15.7**
Candida albicans	**17**	**18**	**14**

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

Table (3): Antimicrobial activity of methanolic extract of honey, *S. dubium* seeds and *S. dubium* seeds and honey combination against the standard bacteria and fungi

Standard microorganisms	Zone of inhibition (100 mg/ml)		
	Honey	*S. dubium* seeds	*S. dubium* seeds and honey combination
Tested bacteria used			
Bacillus subtilis	**22**	**25**	**20**
Staphyococcus aureus	**21**	**27**	**25**
Escherichia coli	**24.7**	**26**	**26**
Salmonella typhi	**27.7**	**25**	**28.5**
Tested fungi used			
Aspergillus niger	**18**	**18**	**16**
Candida albicans	**17**	**16**	**15**

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

Table (4): 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)			
		Bacillus subtilis	***Staphyococcus aureus***	***Escherichia coli***	***Salmonella typhi***
Ampicillin	**40**	15	25	-	-
	20	14	20	-	-
	10	13	18	-	-
	5	12	15	-	-
Gentamicin	**40**	29	35	32	18
	20	22	33	30	16

	10	20	30	17	12
	5	17	28	-	-
Tested fungi used (M.D.I.Z. mm)					
		Aspergillus niger		***Candida albicans***	
Clotrimazole	**40**	30		42	
	20	22		40	
	10	19		33	
	5	16		30	
Nystatin	**50**	28		17	
	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.

IV. CONCLUSION

The *S. dubium* seeds and honey showed various degrees of inhibitory activity against the microorganisms tested. The obtained results may justify the use of *S. dubium* seeds and honey as antimicrobial therapy in traditional medicine in Sudan and the neighboring countries. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

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