Antioxidant Activity and Phytochemical Screening of Sudanese *Solanum dubium* seeds in Combination with Sudanese Honey

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ABSTRACT

The present study was conducted to investigate the antioxidant and phytochemical screening of secondary metabolites of a combination of *Solanum dubium* L. powdered seeds and Sudanese honey. The seeds, honey and seeds and honey combination were extracted with 96% ethanol, methanol and water. The prepared extracts were tested for their free radical scavenging properties using 2, 2- diphyenyl-1- picrylhydrazyl (DPPH) and propyl gallate assays. The water extract of the seeds and honey combination showed the highest radical scavenging activity (RSA) 91±0.04%, according to the DPPH assay and the control propyl gallate gave RSA 93±0.01%. Phytochemical screening of the seed and honey combination, seed and honey extracts with 96% ethanol, methanol and water revealed variable concentrations of alkaloids, sterols, triterpene, flavonoids, coumarins, saponins and tannins. The results concluded that the seed and honey combination were a potential source of antioxidants for use in several conditions requiring these properties.

Keywords: antioxidant activity, phytochemical screening, *Solanum dubium*, seed, Honey, Sudan

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1. INTRODUCTION

In the light of recent scientific developments, the medicinal properties of plants have been investigated, throughout the world due to their potent pharmacological activities and economic viability. A great number of aromatic and medicinal plants contain compounds, exhibiting antioxidant property. The natural antioxidants are primarily plant phenolic compounds that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks **(**Pratt, 1990**)**. Many of these antioxidant compounds posses anti-inflammatory, antiatherosclerotic, antitumor, anticarcinogenic, antibacterial or antiviral activities to a greater or lesser extent **(**Sala *et al*, 2002**).** Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolic**s** are increasingly of interest in food industry, because they retard oxidative degradation of lipids and thereby improve the quality and nutritive value of food **(**Kahkonen *et al*, 1999**;** Rice *et al*, 1995**).**

The genus *Solanum* consists of over 2000 species distributed worldwide is the largest member of the Solanaceae and is one of the largest genera among all flowering plants (Olmstead; Palmer, 1997). The species of the family Solanaceae are medicinal herbs (Caicedo; Schaal, 2004) and contain unique alkaloids and other biochemical constituents used for the treatment of diverse ailments such as diabetes, cholera, bronchitis, high blood pressure and as laxatives (Daunay; Chadha, 2004).

The curative potential of honey is well documented in the 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 and was used by the ancient Greeks and Sumerians (Molan, 1995).

The present study was conducted to investigate the antioxidant activities and to identify the secondary metabolites of aqueous extract, ethanolic extract and methanolic extract of Sudanese *S. dubium* seed, honey and combination of *S. dubium* seed and honey.

2. MATERIALS AND METHODS

2.1 Plant material

The seeds of *S. dubium* were collected from central Sudan between January and February 2015. The plant was identified and authenticated at Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.

The cleaned and shade-dried plant seeds were powdered using a grinding machine; each ground sample was weighed and stored in a dry container at ambient temperature.

2.2 Collection of honey

The honey material used in the study is known as the Sunnut honey from *Acacia nilotica* L. (Mimosaceae). The combination was prepared by mixing ground 2g seeds with 500ml sunnut honey.

2.3 Preparation of 96% ethanol, methanol and water extracts

Five hundred grams of each sample of honey, *S. dubium* seeds and honey and *S. dubium* seeds combination was extracted by maceration with 96 % ethanol using shaker apparatus. Extraction was carried out for three days with daily filtration. The filtrates were combined and the solvent was evaporated under reduced pressure using a rotary evaporator. The same procedure was repeated with methanol.

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging assay

The DPPH radical scavenging activity was determined according to Shimada *et al*., 1992 with some modification. In 96-wells plate, the test samples were allowed to react with 2, 2-diphenyl-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 µm. The extract was dissolved in DMSO (500µg/ml concentration), (5mg of extract dissolved in 1ml DMSO and then used 10 microliter) while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multi plate reader spectrophotometer (Thermo fisher scientific OY, Multi-scan spectrum). Percentage radical scavenging activity was determined in comparison with a DMSO treated control group and propyl gallate (PG). All tests and analysis were run in triplicate.

2.4.2 Statistical analysis:

All data were presented as means ± S.D. Statistical analysis of all the assay results was done using the Microsoft Excel program (2007).

2.5 Phytochemical Screening:

Phytochemical screening is of great importance in providing information about constituents found in the plant in term of their nature and range of occurrence. This information would enable us to correlate between the nature and range of occurrence of these chemicals and biological assays held to investigate a certain bioactivity of the mentioned plant. In this study the preliminary phytochemical screening for the active constituents was conducted using standard qualitative methods according to Martinez

and Valencia (1999), Sofowora (1993), Harborne (1984), Wall, *et al.* (1952), with many few modifications.

3. RESULTS AND DISCUSSION

The present study was conducted to investigate the antioxidant and phytochemical screening of secondary metabolites of a herbal drug used in Sudanese traditional medicine for treatment of Asthma. The herbal remedy is composed of a combination of *Solanum dubium* L. powdered seeds and Sudanese honey. The seeds, honey and seeds and honey combination were extracted with 96% ethanol to yield 17.54, 64 and 64.44 %, respectively (Table1). Pure methanol was used to extract the seeds, honey and seeds and honey combination to give 9.05%, 36.7% and 29.29% yield, respectively (Table 2). Water extract was used to extract the seeds to give 2.105 % yield (Table 3). The prepared extracts were tested for their free radical scavenging properties using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and propyl gallate assays. The water extract of the seed and honey combination gave the highest radical scavenging activity (RSA) (91±0.04%) and the honey aqueous extract revealed the lowest RSA (07±0.22%). The seed extract gave a good RSA (77±0.09%). The highest RSA of 96% ethanolic extracts was 83±0.1% of the seeds, followed by honey (16±0.07%) and seed and honey combination showed only 13±0.01%. The Methanolic extract of the seed gave the highest RSA (80±0.09%) and the seed and honey combination gave only 11±0.12% RSA for all extracts (Table4).

Phytochemical screening of the three samples showed variable concentrations of alkaloids, sterols and triterpenes, flavonoids, coumarins, saponins and tannins. (Table5, 6 and 7**).** The combination can be considered as a natural source of dietary antioxidants. Further work is required in order to isolate the active constituents of the combination and the seed responsible for the antioxidant activity.

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

Sample	Weight of Sample (g)	Yield (g)	Yield (%)
***S. dubium* seed**	500	87.69	17.54
Honey	500	320	64
Combination	500	322.22	64.44

Table (2): Yield percentage of methanolic extracts of *S. dubium* seed, honey and combination

Sample	Weight of Sample (g)	Yield (g)	Yield (%)
***S. dubium* seed**	500	45.25	9.05
Honey	500	183.5	36.7
Combination	500	146.45	29.29

Table (3): Yield percentage of water extracts of *S. dubium* seed, honey and combination

Sample	Weight of Sample (g)	Yield (g)	Yield (%)
***S. dubium* seed**	500	10.51	2.103
Honey	500	-	-
Combination	500	-	-

Table (4): Antioxidant activity of aqueous crude, 96% ethanolic and methanolic extracts of *S. dubium* seeds, honey and *S. dubium* seeds and honey combination

No	Sample	%RSA* ± SD (DPPH)		
		Aqueous	Ethanolic	Methanolic
1	Seed and honey combination	91 ± 0.04	13 ± 0.01	11 ± 0.12
2	Seed	77 ± 0.09	83 ± 0.01	80 ± 0.09
3	Honey	07 ± 0.22	16 ± 0.07	10 ± 0.08
4	*Control (PG)	93 ± 0.01		

Key: RSA* = Radicals scavenging activity ***Control = PG** = Propyl Gallate

Table (5): Preliminary phytochemical screening of 96% ethanolic extracts of *S. dubuim* seed, honey and *S. dubuim* seed and honey combination

No.	Secondary metabolites	Ethanolic extracts		
		S. dubium seed	Honey	*S. dubium* seed and honey
1	Alkaloids	+++	+	+
2	Sterols	++	++	++
3	Triterpene	++	+	+
4	Flavonoids	+	+	+
5	Coumarins	+	+	+
6	Saponins	+	+	+
7	Tannins	+++	+++	+
8	Anthroquine	-	-	-

(-) = Absent (+) = Low concentration (++): Moderate concentration (+++): High concentration.

Table (6): Preliminary phytochemical Screening of methanolic extracts of *S. dubuim* seed, honey and *S. dubuim* seed and honey combination

No.	Secondary metabolites	Methanolic extracts		
		S. dubium seed	Honey	*S. dubium* seed and honey
1	Alkaloids	++	+	++
2	Sterols	++	+	+
3	Triterpene	+	+	+
4	Flavonoids	++	+	+
5	Coumarins	+	++	+
6	Saponins	+	+	+
7	Tannins	+	+	+
8	Anthroquine	-	-	-

(-) = Absent (+) = Low concentration (++): Moderate concentration (+++): High concentration

Table (7): Preliminary phytochemical Screening of water extract of *S. dubuim* seed, honey and *S. dubuim* seed and honey combination

No.	Secondary metabolites	Extracts		
		S. dubium seed	Honey	*S. dubium* seed and honey
1	Alkaloids	+++	++	++
2	Sterols	+++	++	++
3	Triterpene	++	+	+
4	Flavonoids	++	+	+
5	Coumarins	++	++	+
6	Saponins	+	+	+
7	Tannins	+++	+	++
8	Anthroquine	-	-	-

(-) = Absent (+) = Low concentration (++): Moderate concentration (+++): High concentration.

CONCLUSION

Antioxidant activity and phytochemical screening studies of water extract, 96% ethanolic extract, methanolic extract of *S. dubium* seed, *S. dubium* seed and honey combination and honey recommended that these extracts could be used as easily available formulations of natural antioxidants, which can be used as a supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes, inflammation, etc., diabetes swelling. Further studies are needed on the isolation and elucidation of their chemical structures and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

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