Antibacterial Activity and Fatty Acid Composition of Sudanese Castor Bean (*Ricinus communis* L) Seed Oil

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The castor bean plant (*Ricinus communis* L) has very popular seed oil that is consumed in different applications around the world. In the present study, the seed oil of *Ricinus communis* grown in Sudan was investigated in respect to its antibacterial activity and fatty acid composition. The antibacterial activity of the oil was tested against six bacteria strains using the disc diffusion method, while its fatty acid composition was analyzed by Gas Chromatography Mass Spectrometry (GC-MS). The oil exhibited different degrees of antibacterial activity depending on the doses of the oil applied. The maximum zone of inhibition observed for each bacterium was as follows: *Staphylococcus aureus* (8.1 mm), *Enterococcus faecalis* (6.5 mm), *Bacillus subtilis* (6.2 mm), *Escherichia coli* (6.0 mm), *Pseudomonas aeruginosa* (5.5 mm) and *Salmonella typhi* (5.2 mm). The unsaturated fatty acid (UFA) content was 97.9% of the total fatty acid composition. Ricinoleic acid comprises over 85% while other fatty acids identified were linoleic (8.1%), oleic (4.3%), stearic (1.1%), palmitic (0.8%), and linolenic (0.4%). The findings from this study may add to the overall value of the industrial and medicinal potential of this plant.

Key words: *Ricinus communis*, Castor Oil, Antibacterial Activity, GC-MS, Fatty Acid.

Introduction

Castor bean plant (*Ricinus communis* L) belongs to the Euphorbiaceae family. It is grown extensively throughout tropical regions of the world with wide applications in industry and medicine (Jumat et al., 2010). It is one of the ten greatest oil crops in the world (Fenglan et al., 2012). Asia can be considered as the main producing area of castor oil and fat for

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Asia can be considered as the main producing area of castor oil and fat for medicinal and industrial uses. India is the world's largest exporter of castor oil followed by China and Thailand (Wang et al., 2013). Usually, the castor bean has been cultivated for the seeds and its respective valuable oil. The oil produced from castor plants is pale yellow, non drying, non-volatile, viscous and rich in ricinoleic acid. The presence of ricinoleic acid provides castor oil its unique properties and unusual versatility. Castor oil differs from other oils with its high acetyl or hydroxyl value and forms oil of comparable iodine value with a high viscosity and specific gravity (Felipe et al., 2013). Unlike other oils, it is mixable with alcohol, but only slightly soluble in petroleum ether at room temperature. Castor oil also has excellent emollient and lubricating properties as well as marked capability to wet and disperse dyes, pigments and fillers (Felipe et al., 2013).

The characteristic of castor oil and its antimicrobial activity from different countries such as Malaysia, Nigeria, India, Brazil and China had been reported. For instance, the determination of fatty acid composition and physicochemical properties of Malaysian castor bean oil obtained by solvent semi-continuous extraction (Soxhlet) method (Mann et al., 2012; Salimon et al., 2010); the analysis of variability for oil and fatty acid composition in 36 different Brazilian castor bean varieties (Da Silva et al., 1984) or the investigation of castor genetic resources to provide important information on current status of global castor collections, and its existent germplasm throughout the world (Anjani, 2012) had also been described. However, the characteristic and antibacterial activity of Sudanese castor oil has been found lacking in literature. Therefore, the objective of this study is to determine the most relevant physicochemical properties of crude Sudanese castor oil and its fatty acid composition, as well as the antibacterial activity against six pathogenic bacteria.

Materials and methods

Plant material and chemicals

Ricinus Communis seed was collected from Al Shwak, Al Qadarif state, Sudan (November, 2016). A voucher specimen (KI001) was deposited in the Medicinal & Aromatic Plants and Traditional Medicine Research Institute, Khartoum, Sudan. All chemicals used in this experiment were of analytical grade and were purchased from Sigma Chemical Co. (USA).

Microbial strains

The antibacterial activity of *R. communis* seed oil was investigated against six pathogenic bacteria; three *Gram*-positive: *Staphylococcus aureus* (ATCC1026), *Bacillus subtilis* (ATCC19659) and *Enterococcus faecalis* (ATCC29212); and three *Gram*-negative: *Salmonella typhi* (ATCC14038), *Pseudomonas aeruginosa* (ATCC15442) and *Escherichia coli* (ATCC10536). All bacteria strains were provided by Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan and maintained in Mueller-Hinton agar (Hopebio, China) and stored at -20 ºC.

Hot solvent semi-continuous (Soxhelt) extraction for seed oil

The seeds (10 g) of *R. Communis* were divided into the seeds coat and kernel parts. The seeds coat part (2.4 g) was discharged. The kernel part (7.6 g) was grinded into powder form using manual laboratory grinder and dried in air at 50 °C for three hours. The dried powder seeds (7.6 g) were extracted with hexane by solvent semi-continuous extraction method (Soxhlet) for six hours at 65 °C. The extract was concentrated by rotary evaporator under vacuum at 40 °C and further dried under open air. The oil content was calculated based on kernel weight and presented in v/w %.

Test concentrations and antibacterial activity

Stock solution of 1% oil was prepared by dissolving 100 mg of oil in 10 mL of solution solvent (9 mL H_2O + 1 mL Dimethyl sulphoxide (DMSO)), DMSO used to dissolve the oil in water. The stock solution was diluted to 50, 100 and 200 µg/mL. The different three test concentrations were assessed for their antibacterial activity against different strains of bacteria using disc diffusion method (Tuchilus et al., 2017; Al-Sehemi et al., 2016). Briefly, sterile 6 mm whatman No 1, filter paper disc was placed gently on MH agar freshly inoculated with bacteria, with the help of a sterile forceps to ensure complete contact with the agar surface, and oil was applied onto each paper disc, followed by incubated at 37 °C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of inhibitory zone in term of millimeters and recorded. Standard antibiotic Gentamycin (100 µl) was used as positive control while DMSO (10%) was used as negative control.

Preparation of fatty acid methyl esters (FAMEs) and GC-MS analysis

Fatty acid methyl esters (FAMEs) were prepared using sodium methoxide method (Zaha et al., 2016). Briefly, 4 mL of oil sample was mixed with methyl acetate in hexane and treated with sodium methoxide. The reaction was stopped after heating for short period by adding solution of oxalic acid in diethyl ether. From the reaction mixture sodium oxalate was precipitated by centrifuging the mixture at 4000 rpm for 10 min; then the aliquots of supernatant were injected for analysis. Composition analysis was performed by GC/MS (QP2010SE, system Shimadzu, Japan) with a DB-5 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The temperature began at 60 ºC for two min, increased at a rate of 4 ºC/min and was finally maintained at 200 ºC for 15 min. The total analysis time was 52 min, and the flow rate was 1.5 mL/min with helium as the carrier gas. The temperature of injector was maintained at 240 ºC and the mass range scanned was 3-500 *m/z* (Zaha et al., 2016).

Statistical analysis

All analytical determinations were achieved in triplicate (n=3). Values of each parameter are expressed as mean ± standard deviation (SD) and all the statistical analysis were performed using SPSS software (SPSS Statistical Version 22).

Result and discussion

Yield and antibacterial activity of Castor oil

Castor seed contain a relatively high yield of total lipid content; 48.5% which is in the same range as reported by Jumat et al. (2010) (35.7%-51.9%) for the African castor oil. The extracted castor oil inhibited the growth of all tested organisms. Among the *Gram*-positive bacteria, *S. aureus* was the most sensitive and *B. subtilis* was the least sensitive with zones of inhibition of 8.1 mm and 6.2 mm, respectively. Among the *Gram*-negative bacteria, *E. coli* was the most sensitive and *S. typhi* was the least sensitive with zones of inhibition of 6.0 mm and 5.2 mm, respectively. Generally, the oil was more effective on *Gram*-positive bacteria than *Gram*-negative as shown in Table 1. In a previous study on antibacterial activity of castor oil on some local clinical bacteria isolates. The authors reported that the oil inhibited the growth of *B. subtilis*, *S. aureus*, *P. aeruginosa*, *S. typhi* and *E. coli*. The Minimum Inhibitory Concentration (MIC) of the oil ranged from 0.723 to 9 µg/mL (Hashem et al., 2015). Similarly,

Rania et al. (2013) reported that, castor oil inhibited the growth of *E. coli* isolated from patients presented with recurrent urinary tract infections and the inhibition zone was 9.06 mm.

Table 1. Antibacterial activity of castor oil by disc diffusion method

	Zone of inhibition (mm)[a]					
Oil Con. (µg/mL)	*S. aureus*	*B. subtilis*	*E. faecalis*	*S. typhi*	*P.aeruginosa*	*E. coli*
50	6.2 ± 0.3	5.0 ± 0.3	5.6 ± 0.2	4.9 ± 0.3	4.8 ± 0.2	5.8 ± 0.2
100	7.4 ± 0.2	5.9 ± 0.2	6.0 ± 0.1	5.1 ± 0.2	4.9 ± 0.1	5.9 ± 0.1
200	8.1 ± 0.2	6.2 ± 0.2	6.5 ± 0.3	5.2 ± 0.3	5.5 ± 0.2	6.0 ± 0.4
Gentamycin (100 µl)	11.6 ± 0.3	14.1 ± 0.1	13.2 ± 0.2	12.9 ± 0.1	–	12.8 ± 0.2
DMSO (10%)	–	–	–	–	–	–

[a]Inhibition zone diameter; –: no antibacterial activity; values were means of three replicates

Fatty acid composition of castor oil

Table 2 and Figure 1 show the fatty acid composition of Sudanese castor oil. The ricinoleic acid which is the main component of the oil comprises over 85% of the total fatty acid composition. Other fatty acids identified were linoleic (8.1%), oleic (4.3%), stearic (1.1%) palmitic (0.8%), and linolenic (0.4%). The unsaturated fatty acids (UFA) content was 97.9% of the total fatty acid composition. This high amount of the unsaturated fatty acid makes the oil especially prone to oxidation, but which may have favorable industrial and medicinal implications. For example, ricinoleic acid (85.1%) was often used as a surface-active agent, plasticizer and lubricant additive in industry with increasing demands in the world (Huang et al., 2015). There for, it's expected that the demand for the raw material (Castor oil) will also increased.

Table 2: Fatty acids composition (%) of the Sudanese castor oil

No	Fatty acid	Percentage
1	Palmitic; C16:0	0.8
2	Stearic; C18:0	1.1
3	Oleic; C18:1	4.3
4	Linoleic; C18:2	8.1
5	Linolenic; C18:3	0.4
6	Ricinoleic; C18:1	85.1
7	Saturated fatty acids (SFA)	1.9
8	Unsaturated fatty acids (UFA)	97.9

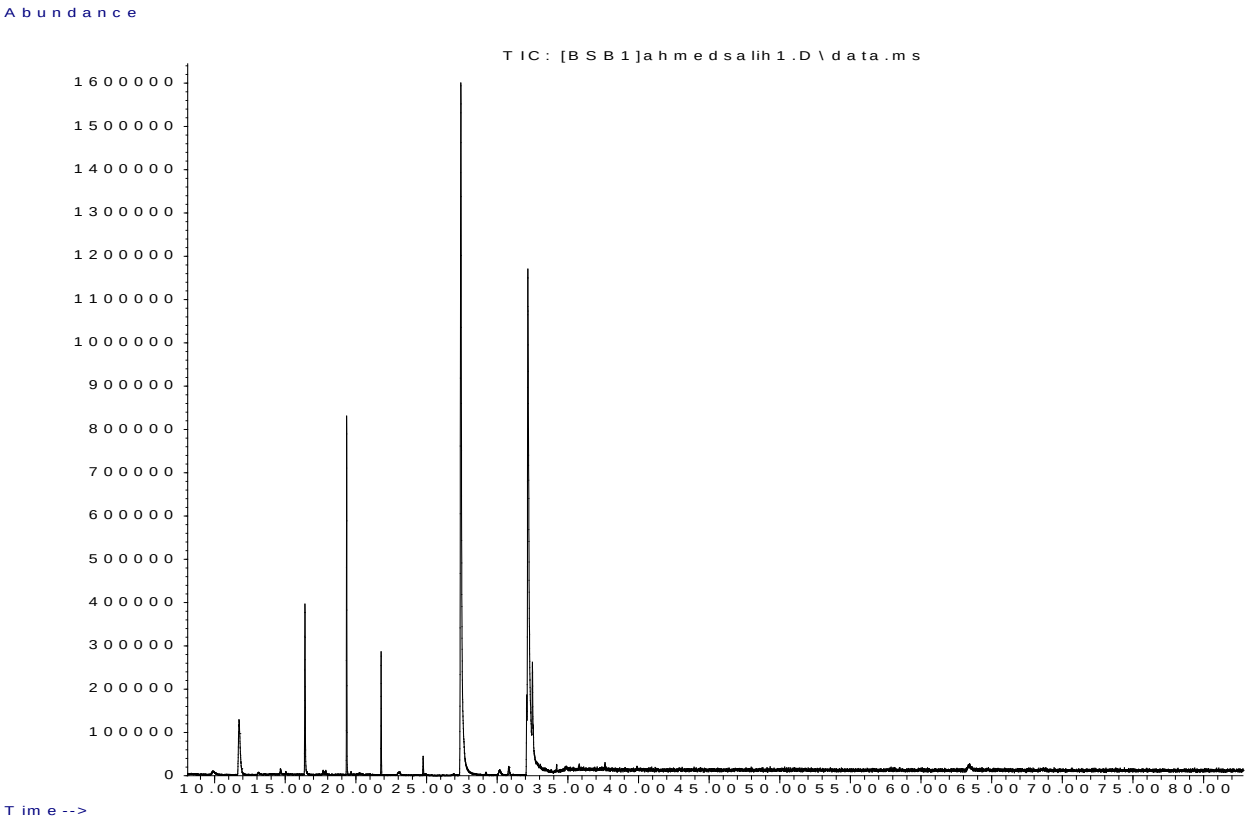

Figure 1: Fatty acid profile of Sudanese castor oil

Linoleic acid (8.1%) which is desirable for the potential industrial use of the oil as a drying oil. Previously, Jumat et al. (2010) reported the ricinoleic acid content from India and Brazil castor oils were 94.0% and 90.2%, respectively, which were higher than the analyzed sample. Low ricinoleic acid content of Sudanese castor oil was possibly due to the differences

in climatic conditions. The same authors also reported that fatty acid composition of the seed fat from Malaysian castor were ricinoleic (84.2), linoleic (7.3%), oleic (5.5%), palmitic (1.3%), stearic (1.2%) and linolenic (0.5%). However, in all cases the castor bean oil consists mainly of 12-hydroxy-9-octadecaenoic acid (ricinoleic acid). Therefore the presence of hydroxyl groups and double bonds makes the castor bean oil suitable for industrial application due to various chemical reactions that can be involved (Jumat et al., 2010; Mann et al., 2012).

Conclusion

The present study showed that Sudanese castor oil exhibited moderate antibacterial activity against both *Gram*-posative and *Gram*-negative bacteria tested compared with the commercial antibacterial. Furthermore, the fat of Sudanese *R. communis* L seed could be considered as a significant source of ricinoleic acid.

Acknowledgments

Authors gratefully acknowledge the botanist of the Medicinal & Aromatic Plants and Traditional Medicine Research Institute, National Centre for Research, Ministry of Higher Education and Scientific Research, Khartoum, Sudan for the plant identification.

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