



Identification of chemical constituents and antioxidant activity of *Jatropha curcas* (L) seeds oil from Sudan

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Abstract

This study was aimed to extract seeds oil of *Jatropha curcas* and to identify the chemical constituents and to evaluate its potential antioxidant activity. The seeds were collected from South Kordofan state Sudan in December (2020). The extraction of seeds oil was carried out at Standards and Metrology Laboratories in (El- Obeid) and then analyzed at National Research Center and medical University for science and technology (Khartoum). 8 components of *Jatropha curcas* seeds oil extract were detected by Gas Chromatography-Mass spectroscopy (GC-MS) method. The results showed that the major components are: 9-octadecanoic acid methyl ester (39.24%); 9, 12-octadecanoic acid methyl ester (37.14%), hexadecanoic acid methyl ester (14.93%) and methyl stearate (8.08%). The antioxidant activity of the extracted oil was evaluated by using the standard 2, 2 diphenyl-1-picrylhydrazyl (DPPH) 0.5 ml. The antioxidant activity of the extracted oil was 19.94 ± 1.39 .

Keywords: *Jatropha curcas*, GC-MS, and antioxidant activity

Introduction

Plants are an essential part of human society since the civilization started. Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. In the last decades, various plant extracts have been the focus of great interest from researchers because they represent natural resources of new antibacterial agents with possibly novel mechanisms of action. The potential use of these products as an alternative for the treatment of several infectious diseases has been extensively screened. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [1]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterisation of their constituents. Systematic screening of them may result in the discovery of novel active compounds [2].

Jatropha curcas is considered to be the best sustainable source. It is drought resistant shrub belonging to the family of Euphorbiaceae [3]. It is a deciduous shrub that grows up to a height of 3-5 meters and with a productive life span of 50 years. It is a multipurpose shrub that grows throughout the arid, semi-arid tropical and subtropical regions of the world. *J. curcas* (L) has gained a world reputation as a plant that can be grown in wasteland and infertile land, which does not require much water, fertilizer and management, and has high oil yield [4-8]. The seeds of *J. curcas* are considered to be the good source of lipids. They contain approximately 20 to 39% of oil and this makes their use as an energy source for fuel production very attractive. In addition to this, the oil from its seeds has been found useful for manufacture of candles and soap, in cosmetic industry and also as for

medicinal purposes [3]. The present study is to investigate the antioxidant and chemical composition of *J. curcas*, hence determine its suitability as a source of oil for domestic, industrial and other purposes [2].

Materials and Methods Plant Material

Seeds of *Jatropha curcas* was collected in December (2020) from South Kordofan state Sudan. The plant was authenticated by a plant taxonomist at the Department of Botany Faculty of Science University of Kordofan Sudan. The Seeds of *Jatropha curcas* was shade dried and coarsely powered by hammer mill.

Instruments

GC-MS analysis was conducted on a Shimadzo GC-MSQP2010 Ultra instrument with a RTX-5MS column (30m length; 0.25mm diameter; 0.25 μ m thickness). And antioxidant activity was measured on spectrophotometer.

Methods

Oil Extraction

250g of dried seeds powder were macerated in 500ml mixture of ethanol and Chloroform (1:2) respectively at room temperature for three days and filtered after that left for slow solvent evaporation. The extract product used for further analysis.

Sample Preparation

2ml of the sample was mixed thoroughly with 7ml of alcoholic sodium hydroxide that was prepared by dissolving 2g in 100 ml methanol. 7 ml from alcoholic sulfuric acid (1ml H₂SO₄ to 100 ml methanol) was then added. The mixture was then shaken for 5 minutes. The content of the test tube was left to stand overnight. 1ml of super saturated sodium chloride was then added and the contents being shaken. 2ml of normal hexane was added and the contents were shaken thoroughly for three minutes. Then the n-hexane layer (the upper layer of the test tube) was taken

using disposable syringe. 5 μ l from the n-hexane extract was diluted with 5ml of diethyl ether. Then the mixture was filtered through syringe filter 0.45 μ m and dried with 1g of anhydrous sodium sulphate as drying agent and 1 μ l of the diluted sample was injected in the GC-MS instrument.

GC-MS Analysis

The qualitative and quantitative analysis of the sample was carried out by using GC-MS technique model (GCMS-QP2010-Ultra) from Japan's Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m \times 0.25 mm \times 0.25 μ m). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60 $^{\circ}$ C with rate 10 $^{\circ}$ C/min to 300 $^{\circ}$ C as final temperature degree with 3 minutes hold time, the injection port temperature was 300 $^{\circ}$ C, the ion source temperature was 200 $^{\circ}$ C and the interface temperature was 250 $^{\circ}$ C. The sample was analyzed by using scan mode in the range of m/z 40-500 counts to ratio and the total run time was 27 minutes. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patterns with those available in the library, the National Institute of Standards and Technology (NIST) [9].

Antioxidant Assay

The DPPH radical scavenging was determined according to the method of Shimada *et.al.* (1992), with some modification. In 96-wells plate, the test samples were allowed to react with 2.2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37 $^{\circ}$ C. The concentration of DPPH was kept as (300 μ M). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate [10].

Results and Discussion Results and Discussion Oil Extraction

The chemical properties of *Jatropha curcas* seeds oil was extracted by maceration method and the yield of extracted oil was 37.5%.

GC-MS Analysis of *Jatropha Curcas* Seeds Oil

GC-MS analysis of *Jatropha curcas* oil was conducted and the identification of the constituents was accomplished by comparison with the MS library (NIST).

Table 1: Constituents of *Jatropha curcas* seeds oil

No	Name	R. T	Area	Area%
1	9-Hexadecenoic acid, methyl ester, (Z)-	14.876	651405	0.35
2	Hexadecanoic acid, methyl ester	15.083	27525568	14.93
3	Heptadecanoic acid, methyl ester	16.063	106621	0.06
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.754	68466660	37.14
5	9-Octadecenoic acid (Z)-, methyl ester	16.818	72334368	39.24
6	Methyl stearate	16.990	14898110	8.08
7	cis-11-Eicosenoic acid, methyl ester	18.541	87957	0.05
8	Eicosanoic acid, methyl ester	18.738	271994	0.15

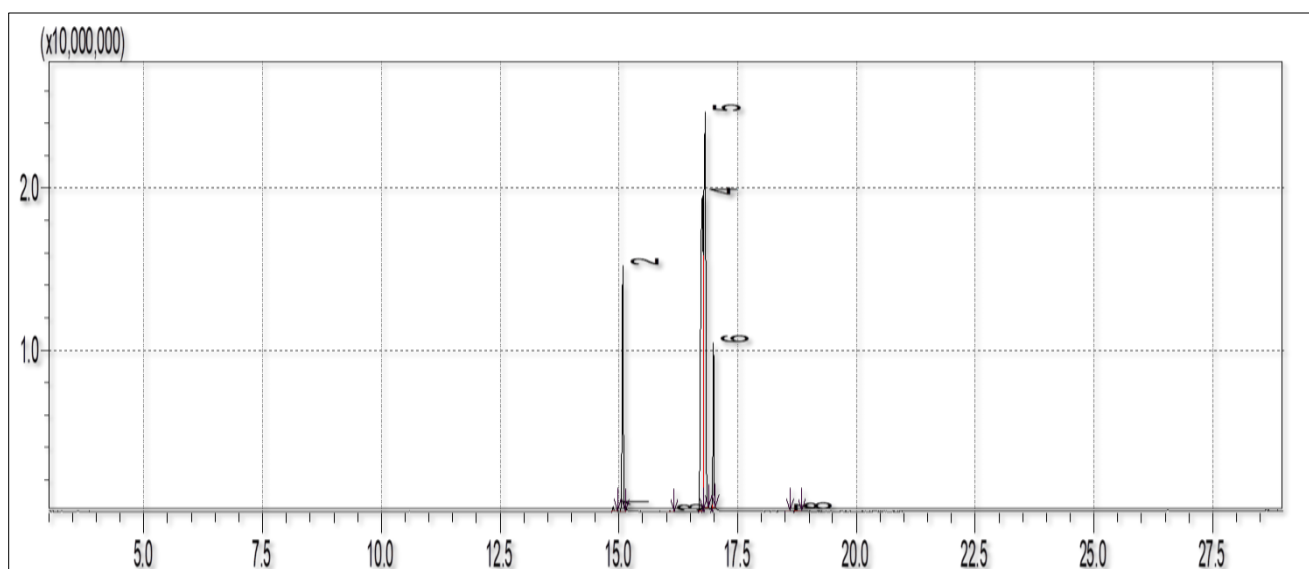


Fig 1: Chromatograms of *Jatropha Curcas* Seeds Oil

Constituents of Oil

The GC-MS analysis of the studied oil revealed the presence of 8 constituents as shown in (Table 1). The typical total ion of the following constituents were detected in the chromatogram as major constituents: The mass spectrum of

9-octadecanoic acid methyl ester (39.24%) is displayed in Fig. 5. The peak at m/z 296, which appeared at R.T. 16.818 corresponds to $M+[C_{19}H_{36}O_2]^+$. The signal at m/z 265 accounts for loss of a methoxyl function.

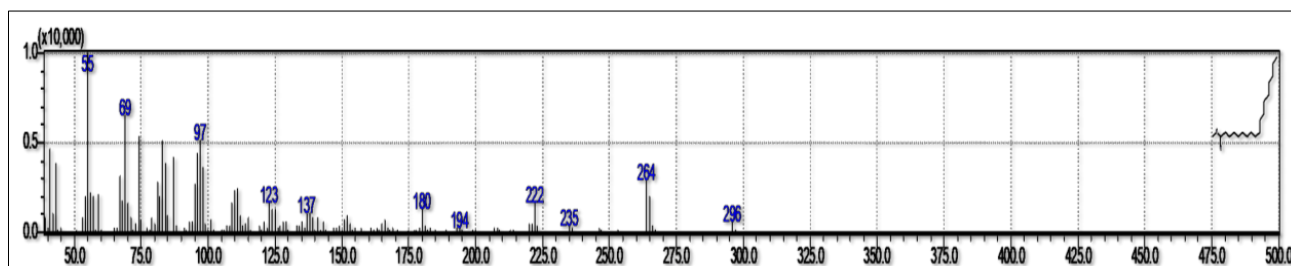


Fig 2: Mass spectrum of 9-Octadecanoic acid methyl ester.

The mass spectrum of 9,12-octadecanoic acid methyl ester (37.14%) is shown in Fig 2. The peak at m/z 294, which appeared at R.T. 16.754 in total ion chromatogram,

corresponds to $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z 263 corresponds to loss of a methoxyl function.

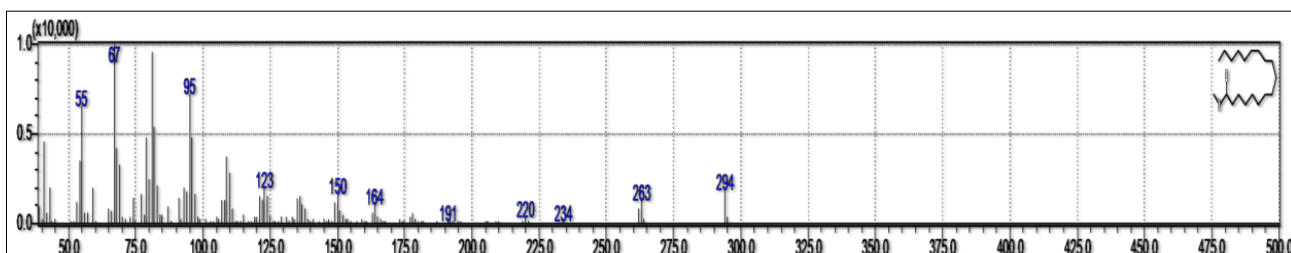


Fig 3: Mass spectrum of 9,12-Octadecanoic acid methyl ester

The Mass spectrum of hexadecanoic acid methyl ester (14.93%) is depicted in Fig.3. The signal at m/z 270 (R.T.

15.083) corresponds to $M^+[C_{17}H_{34}O_2]^+$. The signal at m/z 239 is due to loss of a methoxyl.

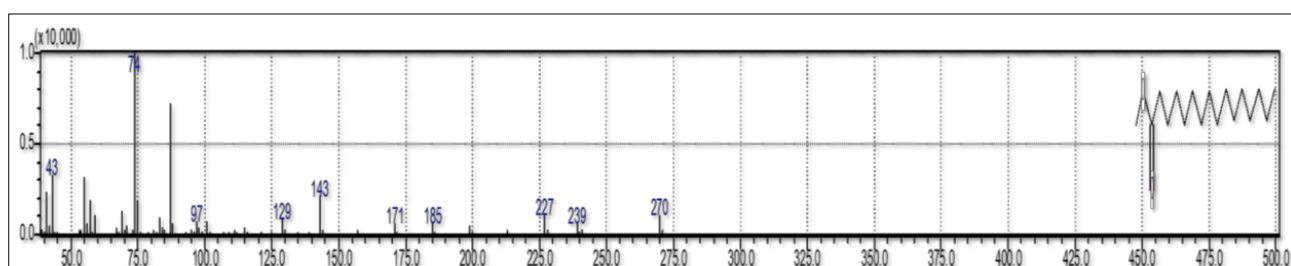


Fig 4: Mass spectrum of Hexadecanoic acid methyl ester.

The mass spectrum of methyl stearate (8.08%) is depicted in Fig.4. The signal at m/z 298 (R.T. 16.99) corresponds

to $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 corresponds to loss of a methoxyl.

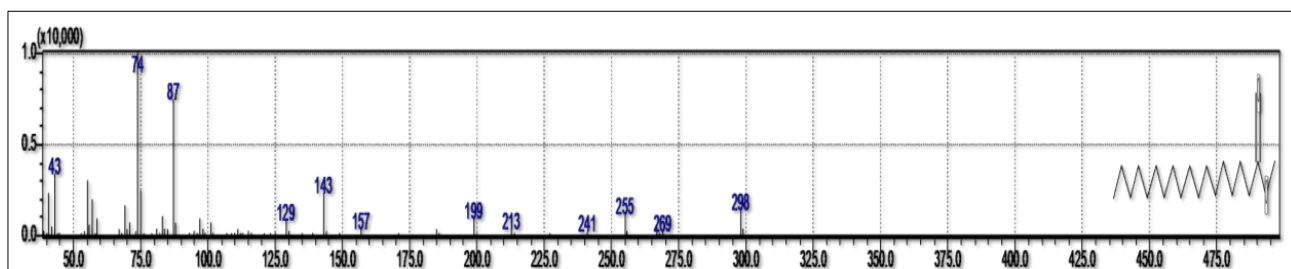


Fig 5: Mass spectrum of Methyl stearate.



Fig 6: *Jatropha curcas* seeds and oil

Antioxidant activity

The principle of antioxidant activity is their interaction to produce oxidative free radicals. The role of DPPH method is that the antioxidants react with the stable free radical. During the free radical reaction, DPPH 2,2Di

{(4-tert-octylphenyl)-1-picryl-hydrazyl} is converted into {2,2Di (4-tert-octylphenyl)-1-picryl-hydrazine} with color change. The rate of color change gradually decreases to indicate the scavenging potentials of the antioxidant sample.

Table 2: Antioxidant activity of *Jatropha curcas* seed oil

Ser. No	Sample Code	%RSA \pm SD (DPPH)
1	<i>Jatropha curcas</i> seeds oil	19.94 \pm 1.39
Standard	Propyl gallate	89.00 \pm 0.01

Conclusion

In the present investigation, the overall results from the GC-MS analysis of *Jatropha curcas* seeds oil showed 8 components the major components are: 9-octadecanoic acid methyl ester (39.24%); 9,12-octadecanoic acid methyl ester (37.14%), hexadecanoic acid methyl ester (14.93%) and methyl stearate (8.08%). And the seed oil has potential antioxidant activity.

Acknowledgment

Authors gratefully acknowledge the members of the Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan for their valuable help.

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