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Gas chromatography / mass spectrometry (GC-MS) analysis of *Jatropha curcas* latex and its antimicrobial activity on clinical isolates

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Abstract

The increasing rate of resistance to antibiotics has led to endless search for new drugs of natural origin with antimicrobial activities. The work was aimed at evaluating the phyto-constituents of *J. curcas* latex and its antibacterial activities against some selected clinical bacterial isolates. Gas chromatography / mass spectrometry (Gc-ms) analysis of latex of *J. curcas* phyto-constituents was done and tested for antibacterial activity. Antibacterial activity of the latex was determined on some selected clinical bacterial isolates using cup plate agar diffusion bioassay method. The main constituents include: alpha-benzamido-2-hydroxycinnamic acid, Pentanoic acid, 3-methyl-, N,N-dimethylaminoethanol, (2E,4E)-N-isobutyltetradeca-2,4-dienamide, Oxime-, methoxy-phenyl-, 1-(4-methoxy-3-methylphenyl)-2-methylpropan-2-amine, Maltol-dodecanoic acid, methyl ester, Theobromine, Hexadecanoic acid, Methyl ester, Methyl stearate, 1,1,2-trimethyl-3,8,9-trioxo-bicyclo[4.2.1]nonane. Antibacterial activity of latex on clinical isolates of *Escherichia coli*, *Bacillus* spp, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Citrobacter* spp and *Salmonella typhi* showed the latex had relatively high activity against all these bacteria except *Citrobacter* spp and *Salmonella typhi*. Latex of *J. curcas* had considerable percentage of therapeutically useful phyto-constituents and activity against most clinical bacteria isolates used in this work.

Keywords: *Jatropha*; Phyto-constituents; Gas-chromatography; Antibacterial activity; *K. pneumonia*; *P. aeruginosa*.

1. Introduction

The increasing rate of resistance to antibiotics has led to endless search for new drugs of natural origin with antimicrobial activities. Plants and their secondary metabolites (alkaloids, terpenoids and phenolic compounds) are prospective antimicrobial agents that can be useful in eliminating the crisis of antibiotic resistance [1].

Medicinal plants have immensely supported human health as being utilized as explorative compounds of drugs [2], for treatment of different ailments. The majority of rural populace of Nigeria and other parts of Africa have been using medical plants in the primary health care [3]. The World Health Organization [4] reported that more than 80% of the world's population especially developing nations depend on medicinal plants as means of medicines for primary healthcare.

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Jatropha curcas belongs to the family *Euphorbiaceae* and is mostly cultivated for large scale biodiesel production from its seed [5, 6]. It is employed in traditional medicine for the treatment of many ailments in subtropical and semi-arid regions [2]. Some parts of the plant; the seed, leaf and latex have many uses in treating infectious diseases [7]. The latex is used in treating wound and as blood anticoagulant [8], the leaf has insecticidal and antimicrobial activity [9, 10].

1.1. Statement of Problem

The increasing rate of resistance to antibiotics has led to endless search for new drugs of natural origin with antimicrobial activities [1].

1.2. Aim

Gas Chromatography-mass spectroscopy phyto-constituents analysis and antibacterial activities of *Jatropha curcas* latex.

1.3. Objectives

- To determine the phytochemical properties of *Jatropha curcas* latex
- To determine the antimicrobial activities of the latex against some selected pathogens.

2. Methodology

2.1. Collection of samples

Fresh crude latex was aseptically obtained from the stem of *Jatropha curcas* by incision using sterile surgical knife and collected into sterile sample bottles. The milky latex was stored air tight in the refrigerator at 4 °C and kept for further experiment.

2.2. Collection of test organisms

Seven bacterial isolates from clinical sources: *Escherichia coli*, *Bacillus* spp., *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Citrobacter* spp. and *Salmonella typhi* were collected from the department of medical microbiology laboratory, University College Hospital (UCH), Ibadan. The isolates were then sub-cultured on plates of nutrient agar and incubated for 24 hours at 37±2 °C. Culture obtained for each bacterium was used for further work while stock cultures were also made from this.

2.3. Gas chromatography- mass spectrometry analysis (Gc-ms) of latex samples

The compounds in the latex of *Jatropha curcas* were quantitatively measured by Gc-ms based on the method described by [11] with some modification. The Gc-ms system used was a Shimadzu QP2010PLUS system. Six microliters were analyzed on a BPX-5 SGE ultra-low bleed 5% phenyl polydimethylsiloxane capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Split less injection was performed with a purge time of 1.0 minutes. The carrier gas was Helium at a flow rate of 1 ml min⁻¹. The column temperature was maintained at 50 °C for 3 min, then programmed at 5 °C min⁻¹ to 80 °C and then at 10 °C min⁻¹ to 340 °C. The inlet and detector temperatures were 250 °C and 340 °C, respectively, and the solvent delay was 4 minutes.

The identification of the peaks was based on computer matching of the mass spectra with the National Institute of Standards and Technology (NIST 14.L) library and by direct comparison with published data.

2.4. Sensitivity test using agar well diffusion method

Plates of sterile nutrient agar were prepared and the test organisms stored in slants were aseptically picked with sterile inoculating loop and sub-cultured on the nutrient agar plates by spread plate method and incubated for 24 hours at 37 °C. A colony of each isolate was aseptically re-inoculated into McCartney bottles containing 10mls of sterile water. Following the MacFarland standard of 0.5 [12], a sterile swab stick was placed in each suspension and used in spreading each pathogenic isolate on plates of Mueller Hinton Agar (MHA) for sensitivity tests using the Agar Well Diffusion method [13]. A sterile 8 mm cork-borer, was used in boring wells on the agar and a micropipette was used in dispensing 100 µL of the latex into labeled wells, 60 µL of standard Streptomycin was used as positive control [14].

3. Results and discussion

3.1. Phyto-constituents of *Jatropha curcas* latex

Gas Chromatography-Mass Spectrometer analysis of *Jatropha curcas* latex showed the presence of 31 components, with twelve major phytochemicals (Table 1), the typical total ion chromatograms (TIC) is displayed in Figure 1.

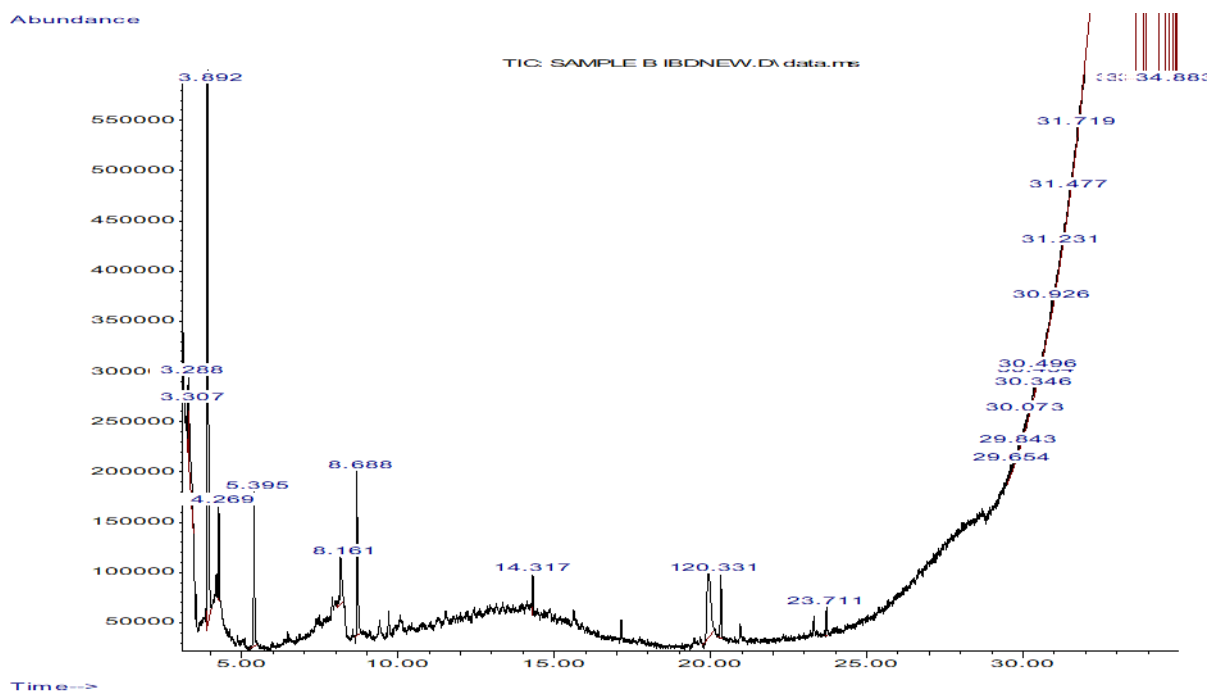
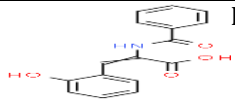
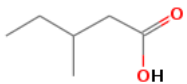
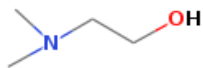
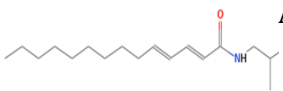
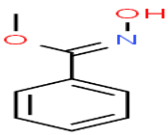
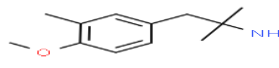
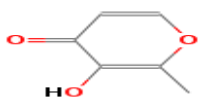
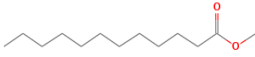
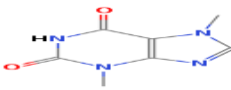
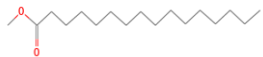

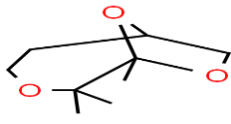


Figure 1 Showing typical GC-MS Total ionic chromatogram (TIC) of hexane/methane (1:1) *Jatropha curcas* latex.

Table 1 The major phyto-constituents of extract of *Jatropha curcas* latex

SN	PHYTOCHEMICAL NAME	M.M	F.M	Chemical structure	Chemical nature	Biological Activity**
1	alpha.-Benzamido-2-hydroxycinnamic acid	283	C ₁₆ H ₁₃ NO ₄		Phenolic acids	Flavors, antioxidant, anti-inflammatory,
2	Pentanoic acid, 3-methyl-	116	C ₆ H ₁₂ O		Fatty Acids	Flavor, Antispasmodic, Hypotensive, Sedative
3	N,N-Dimethylaminoethanol	89	C ₄ H ₁₁ NO		Tertiary amine with Hydroxy group	Improve Skin Health and Cognitive Function
4	(2E,4E)-N-Isobutyltetradeca-2,4-dienamide	279	C ₁₈ H ₃₃ NO		Alkaloid	Antimycobacteria,

5	Oxime-, methoxy-phenyl-	151	C ₈ H ₉ NO ₂			Antimicrobial and Antioxidant
6	1-(4-Methoxy-3-methylphenyl)-2-methylpropan-2-amine	193	C ₁₂ H ₁₉ NO			Unknown Activity
7	Maltol	126	C ₆ H ₆ O ₃		unsaturated cyclic compd	Antioxidant, Antimicrobial Sweetener, Cancer-Preventive, Pesticide
8	Dodecanoic acid, methyl ester	214	C ₁₃ H ₂₆ O ₂		Lauric acid, methyl ester (Fatty Acid Methyl ester) (FAME)	Antibacterial, Antiviral, Hypercholesterolemic, Candidicide.
9	Theobromine	180	C ₇ H ₈ N ₄ O ₂		Primary alkaloid	Antiasthmatic, Arteriodilator, Diuretic, Myocardiotonic, Pesticide.
10	Hexadecanoic acid, methyl ester	270	C ₁₇ H ₃₄ O ₂		Palmitic acid, methyl ester (FAME)	Hypercholesterolemic, Hemolytic, Flavor, Antioxidant, Antimicrobial
11	Methyl stearate	298	C ₁₉ H ₃₈ O ₂		Stearic acid, methyl ester (FAME)	Antimicrobial, Antioxidant
12	1,1,2-Trimethyl-3,8,9-trioxa-bicyclo [4.2.1]nonane	172	C ₉ H ₁₆ O ₃		Unknown probably new chemical compound	Unknown

Source: Dr .Duke's phytochemical and Ethnobotanical databases [Online database] [15].M.M=; F.M =. KEY: **M.M- Molecular mass, **F.M**. - formula, **S.N**. - Serial number.

3.2. Antimicrobial activity

The antibacterial potential of the *Jatropha curcas* latex samples against some selected clinical isolates is shown in Table 2. The antibacterial activity of the latex from *Jatropha curcas* against some pathogens ranged from 4.0-8.0 mm. The susceptibility of the pathogen is in the ascending order of *K. pneumonia* which is > *E. coli*, ≥ *P. aeruginosa* > *S. aureus* > *Bacillus* spp. while *Citrobacter* spp. and *Salmonella typhi* were not susceptible to the latex of *Jatropha curcas*.

Table 2 Antibacterial activity of *Jatropha curcas* latex on some selected clinical isolates

S/N	Organism	Zone of Inhibition (mm)
1	<i>Escherichia coli</i>	6.0
2	<i>Bacillus spp</i>	4.0
3	<i>Klebsiella pneumonia</i>	8.0
4	<i>Pseudomonas aeruginosa</i>	6.0
5	<i>Staphylococcus aureus</i>	5.0
6	<i>Citrobacter spp</i>	-
7	<i>Salmonella typhi</i>	-

S/N = serial number; mm= millimeter.

4. Discussion

The increasing antibiotics resistance as being the major challenge to the scientist lately, and use of natural agents to combat it has also been explored. Hence, this study was one of such, using *Jatropha curcas* latex as an agent in prospecting for possible new drug(s).

The results of the phytochemical analysis using Gc-ms showed that *Jatropha curcas* latex extract of hexane/methane contained twelve major constituents- alpha-Benzamido-2-hydroxycinnamic acid, Pentanoic acid, 3-methyl-, N,N-Dimethylaminoethanol, (2E,4E)-N-Isobutyltetradeca-2,4-dienamide, Oxime-, methoxy-phenyl-, 1-(4-Methoxy-3-methylphenyl)-2-methylpropan-2-amine, Maltol, Dodecanoic acid, methyl ester, Theobromine, Hexadecanoic acid, methyl ester, Methyl stearate, 1,1,2-Trimethyl-3,8,9-trioxa-bicyclo [4.2.1]nonane. The bioactive components range from being phenolic acids, fatty acids, fatty- acids methyl ester, alkaloid, tertiary amines and flavonoids in nature, which have antimicrobial properties and may confer the antimicrobial effect as shown by the result. Pentanoic acid, 3-methyl-hexadecanoic acid, methyl-ester are considered as flavonoids [15], and flavonoids have also been reported to have antimicrobial properties [16, 17].

The effects of the *Jatropha curcas* latex on the test organisms showed variation on the zones of inhibition produced and but more pronounced on Gram-negative than the Gram- positive test organisms. This variation may be due to nature and genetic property of the organism under tested, such as the cell- wall, spore formation and encapsulation which have been reported to aid resistance to microorganism [18, 19].

The above results indicated that the *Jatropha curcas* latex exhibited antimicrobial properties, and justifying scientifically their traditional use as medicinal plant. And further suggested that the latex contain bio-actives which are effective against some strains of bacteria tested as reported by Arekemase [20].

The latex extract was active against *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Thus agreed with report of Ekundayo *et al.* [21]. Some of the common health condition caused by *Klebsiella pneumonia* and *Pseudomonas aeruginosa* are pneumonia and complication in surgical wound sites, urinary tract, and lower biliary tract infection [22, 23]. The inhibition of *S. aureus* by the latex extract emphasizes the traditional use of the *J. curcas* in wound healing, scabies and other skin infections [24]

Some of the phytochemicals like Dodecanoic acid, methyl ester, Hexadecanoic acid, methyl ester, Methyl stearate are fatty-acids methyl ester and have been reported to have antimicrobial property [25].

In this study, 1,1,2-Trimethyl-3,8,9-trioxa-bicyclo [4.2.1]nonane and 1-(4-Methoxy-3-methylphenyl)-2-methylpropan-2-amine were found to likely be new and/ or unknown substance with unknown biological activity which may have contributory antimicrobial effect on test organisms.

5. Conclusion

Jatropha curcas extract had antimicrobial effect on the clinical isolates utilized in this work. The test organisms are of biomedical interest. Future work may include further purification of *Jatropha curcas* latex and tested on these test organisms for possible drug development against infections caused by these microorganisms.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest of any kind on this publication.

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