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ISOLATION, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF SEED EXTRACT OF *JATROPHA GOSSYPIFOLIA*

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ABSTRACT
Powdered seed of J. gossypifolia was macerated using methanol as extracting solvent. The seed extract of Jatropha gossypifolia was subjected to phytochemical and antimicrobial investigation using standard screening procedures. The phytochemical studies revealed the presence of some secondary metabolites such as alkaloids, saponins, tannins. There was no activity against the bacteria (Gram positive and negative organisms at 2.5-100mg/ml). The seed extract showed significant antifungal activity. The spectroscopic analysis (1D and 2DNMR) of the colourless oil gave 9-acetoxynerolidol.

Keywords: Extraction, characterization, Jatropha gossipyfolia, antimicrobial evaluation

INTRODUCTION

Medicinal plants have long serve as useful ingredients for the treatment of diseases in both developing and developed countries. About 80 % of the World's populations rely mainly on traditional medicines (WHO, 1993). Tremendous and intensive efforts have been made to discover new antimicrobial compounds from natural products. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds (Tomokoto *et al.*, 2002). Micro-organisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs (Sieradski *et al.*, 1991), which ultimately leads to treatment failure and complications of disease conditions. In order to overcome this ugly trend, the need to develop new and safe antimicrobial agents cannot be over emphasized. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al.*, 1999).

Jatropha gossypifolia plant has been used ethnomedically for the treatment of various disease conditions such as cough, tuberculosis, bacterial infections and cancerous growths (Aiyelaagbe *et al.*, 2007). Detailed description of the plant is reported in several literatures (Curshes, 1999, Biswanath, 1995, 1996; Bebawi *et al.*, 2007). The seed of the plant is used in traditional medicine for the treatment of bacterial infections. However, no literature exists on the phytochemical composition and antimicrobial activity of the seed extract. This study was aimed at investigating the phytochemical and anti- microbial activity of the seed extract of the plant with a view to justifying the ethno medicinal potential of the plant.

MATERIALS AND METHODS

General experimental conditions

Solvents were distilled and dried using standard procedures. TLC was carried out on silica gel 60 GF_{254} (Merck) with detection by UV light (λ = 254 nm) and/or by charring with 10% vanillin sulfuric acid in methanol. Silica gel 60 (70-230 mesh) (Merck) was used for column chromatography. Specific rotations were determined with a Gyromat HP (Dr. Kernchen). IR spectra were recorded with a Nicolet 205 FT-IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra (300.13 MHz) were recorded on Bruker instruments AC 250 and ARX 300, with $CHCl_3$-d_6 as solvent. The calibration of spectra was carried out on the solvent signals (δ (1H) = 7.25; δ (^{13}C) = 77.0). The ^{1}H- and ^{13}C-NMR signals were assigned by DEPT and two-dimensional ^{1}H,^{1}H COSY and ^{13}C,1H correlation spectra (HETCOR). The mass spectra were recorded on an AMD 402/3 spectrometer (AMD Intectra GmbH).

Collection and Identification of plant sample

Fresh *Jatropha gossypifolia* seeds were obtained from Ivbiaro, Owan East. Edo State, Nigeria. The plant was authenticated by Prof M.I. Idu of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

Extraction of Plant Material

Thirty gram (30g) of powdered seed plant material was macerated in 200ml of 80 % methanol for 72 hours at room temperature with occasional stirring. The extract was filtered using Whatman No. 1 filter paper; the filtrate was concentrated in vacuo at 40^{o}C and stored at -4^{o}C until further applications.

Phytochemical Screening

The powdered seed extract was subjected to phytochemical screening using established standard procedures, testing for the presence of alkaloids, tannins, saponins and flavonoids, steroids, (Trease and Evans, 1989; Harbone, 1973; Sofowora, 1993).

Isolation and characterization of compound 1

The air-dried seeds (1.2kg) were extracted with methanol in a soxhlet apparatus. The extract was filtered and then concentrated in vacuo to afford a syrupy gum (252g). The crude extract was partitioned with heptane, chloroform and ethylacetate. The chloroform fraction was concentrated to dryness using a rotary evaporator at 40^{o}C and reduced pressure. The fraction 58g was adsorbed onto silica gel eluted with heptane-EtOAc mixtures (100, 70%, and 30%) to afford 150 fractions. Fractions 6-19 contained one spot (compound **1,** 6mg) with similar R_f value. Compound **1** (colourless oil): 9-acetoxynerolidol, 6 mg. IR cm-^{1}: 3450, 3080, 2980-2860, 1740, 1680, 1455, 1380, 1250, 1040, 926, 840, MS; m/z (rel. int.): 28O[M]$^{+}$ (3), 238 (15),220 (5), 138 (12), 127 (23),93 (10),85(100),71 (12),68 (il), 55(16),43 (63),41 (23), ^{13}CNMR and ^{1}HNMR (Table 3).

ANTIMICROBIAL ACTIVITY

Test isolates

The microorganisms used for the antimicrobial activity evaluation were obtained from the Pharmaceutical Microbiology laboratory, Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria. The following strains of bacteria were used: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATTC27853, *Staphylococus aureus* ATCC 25923 (Oxoid, England), *Streptococcus spp, B.subtilis, K.pneumoniae* and *S. typhii*. The yeast strain used in this study was *Candida albicans* ATCC 10231 (Oxoid, England).

Antimicrobial Sensitivity testing

Antimicrobial activity of the crude methanolic extract of plant was determined by the broth dilution method (Van and Vlietinck, 1991). The anti-microbial activity testing of the seed extract of *Jatropha gossypifolia* was carried out to ascertain the antimicrobial activity. One (1 g) and 0.1g of crude extract of *Jatropha gossypifolia* seed was dissolved in 10 mls of distilled water each to make 100mg/ml and 10mg/ml stock solution respectively. Five (5 mls) each of the sterilized nutrient broth was poured into 64 different test tubes. The 64 test tubes were labeled according to the test organisms and the dilutions made from the stock solutions made from the 100 mg/ml and 10mg/ml of the crude extract. For each broth in the test tubes, the four concentrations each of the double fold dilutions made from 100 mg/ml and 10 mg/ml of the stock solutions were added and one loop- full (0.05 ml) of the organism each were also inoculated in each concentration of the crude extract so that there is 5 mls broth plus 0.25ml of the 100 mg/ml dilution plus 0.05 ml of the microbiological organism, etc. Before adding 0.25 ml of the crude extract dilution, 0.25 ml of the broth was withdrawn and then replaced with the 0.25 ml of the drug. This was repeated for all the concentrations respectively. After each inoculation, the wire loop was sterilized to red hot in a flame before using it for another organism. The mixture in the 64 test tubes was incubated for 24 hours at a temperature of 37°C. Sterilized and acidified nutrient agar in petri-dishes was labeled according to the labels on the test tubes. One loop-full (0.05 ml) of each mixture in the test tube was inoculated on the solidified nutrient agar in the petri-dishes as labeled, after which the petri-dishes were incubated for 24 hours at a temperature of 37°C. Minimum inhibitory concentration was determined by absence of growth (turbidity) in test tubes after 24 hours. The test tubes that had no growth were further screened for minimum bactericidal concentration (MBC) by subculture from acidified nutrient agar plates and the test tubes of the particular organism that had no growth. Incubation for 24hrs at 37°C was carried out.

RESULTS AND DISCUSSION

The phytochemical analysis of the seed extract of *Jatropha gossypifolia* (as shown in Table 1) revealed the presence of saponins, alkaloids, tannins, steriods and volatile oils. Flavonoids, cardiac glycosides, anthraquinones were absent. The presence of these compounds has been reported in the leaf and root parts of the plant (Biswanath *et al*, 1996). There phytochemical compounds are known to play inportant roles in bioactivity of medicinal plants. The values of medicinal plants lay in these phytochemical compounds and as such produce definite physiological actions on the human body. Flavonoids which are part of the phytochemical constituents of seed of *Jatropha gossypifolia*, exhibit a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals, thus health promoting in action. Flavonoids also exhibit anti-inflammatory, anti-allergic effects, analgesic and antioxidant properties. These observations support the usefulness of *Jatropha gossypifolia* in folklore remedies for the treatment of various infections. Saponin was also present in Jatropha seed extract and has supported the usefulness of this plant in managing inflammations and also in immune boosting.

The heptane: ethylacetate fraction 70 % gave a pure compound **1**, an ester of a nerolidol. The molecular ion of the compound appeared in the mass spectrum at *m/z* 280. Thus the molecular formula was $C_{17}H_{28}O_3$. Its ^{1}HNMR showed characteristic signals of a nerolidol skeleton. The IR spectrum of compound **1** exhibited absorption bands for an acetoxyl group (1740, 1250 cm-^{1}), double bonds (1650, 926cm-^{1}) and a hydroxyl group (3450cm-^{1}). The presence of an acetoxy group was confirmed at 1.89 (s, 3H). Signals at δ 5.03 (*dd*, J = 1.2, 11.00 Hz), 5.20 (*dd*, J = 1.2, 17.0 Hz) and 5.90 (*dd*, J = 10.4, 15.2 Hz, H-2) are consistent with those of a vinyl group. The proton NMR also showed the presence of a terminal double bond at position (carbon 1) 5.20 (*dd,* J = 1.2, 17.0).

The ^{13}CNMR (Table 2) revealed the presence of seventeen carbon atoms, two terminal methyl groups, three methylene, three methines and three quaternary carbons. The remaining carbons assigned to the acetoxy moiety. The ^{13}CNMR spectrum was similar to that of nerolidol and a farnesane skeleton (Bohlmann and Zdero, 1980).

The difference was the additional presence of the acetoxy group at position 9 of the chain. The compound was therefore named as 9-acetoxynerolidol. Plant originated antimicrobial drugs are of great importance in view of the undesirable side effects of orthodox antibiotics and multi-drug resistance (Ahmad and Beg, 2001). The determination of the antimicrobial activity of the seed extract of *J. gossypifolia* by broth dilution method (Table 2) showed that the plant extract tested exhibited no antibacterial effect against the five tested bacteria, *E. coli, P. aeruginosa, S. aerus, B. subtilis, Streptococcus* spp*, K. Pneumoniae* and *S. typhii*. This means that the extract had no effect on these organisms.

The results of the study showed that the extract was inactive against *Candida albicans* at 2.5 mg/ml but demonstrated strong effect at 5 mg/ml. This revealed that the seed extract of *J. gossypifolia* possessed potent antifungal activity. *Jatropha gossypifolia* seed extract successfully inhibited the growth of fungi (*Candida albicans*) used in this study, hence it has antifungal activity. In addition, using minimum inhibitory concentration value, the inhibition is dose related, the higher the concentration the more the inhibition. The inhibitory effect of the medicinal plant on the *C.albicans* could be due to the presence of the above phytochemical components present in the extract.

The result of the study showed for the first time the antimicrobial evaluation of the seed extract of *J. gossipyfolia* which could be a potential antifungal agent. The antimicrobial activity of the isolated compound was not tested due to small quantity of the sample. Future studies will be geared towards evaluating the detailed antifungal activity of the plant extract, isolation and characterization, synthesis and screening of 9-acetoxynerolidol.

Table 1: Phytochemical screening of the powdered seeds of *J. gossypifolia*

Secondary metabolites	Components
Alkaloids	+
Saponins	+
Tannins	++
Flavonoids	-
Steriods	+
Cardiac glycosides	-
Anthraquinones	-
Volatile oils	+

Table 2: Antimicrobial activity of the seed extract of *J. gossypifolia*

Organisms	*Concentrations of seed extracts (mg/ml)*							
	2.5	5	10	20	25	50	100	200
E.coli	+	+	+	+	+	+	+	+
P.aeruginosa	+	+	+	+	+	+	+	+
S. aerus	+	+	+	+	+	+	+	+
B.subtilis	+	+	+	+	+	+	+	+
C.albicans	+	-	-	-	-	-	-	-
Streptococcus spp	+	+	+	+	+	+	+	+
K.pneumoniae	+	+	+	+	+	+	+	+
S. typhii	+	+	+	+	+	+	+	+

+; presence of growth -; absence of growth

Table 3: ^{1}HNMR and ^{13}CNMR of the compound ($CHCl_3$)

CARBON No	^{13}CNMR	^{1}HNMR	Multiplicities (DEPT)
1	112.5	5.20 (*dd,* J = 1.2, 17.0), 5.03(*dd*, J = 1.2, 11.0)	CH
2	143.4	5.90 (*dd*, J = 10.4, 15.2)	CH
3	73.2	-	C
4	42.0	1.52 *m*	CH_2
5	22.7	2.2 *m*	CH_2
6	128.0	5.16 *t*	CH
7	135.8	-	C
8	45.3	2.31 (*dd*, J = 7.5, 12.8)	CH_2
9	70.7	5.62 *m*	CH
10	122.9	5.09 *m*	CH
11	131.2	-	C
12	25.5	1.71 *s*	CH_3
13	27.8	1.30 *s*	CH_3
14	16.8	1.59 *s*	CH_3
15	18.2	1.70 *s*	CH_3
$CH_3\underline{CO}$	171.4	-	CH_3
$\underline{CH_3}CO$	20.2	1.89 *s*	CH_3

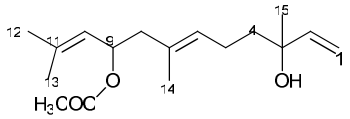

Figure 1: Proposed structure of the isolated compound

CONCLUSION

In this study, chromatographic characterization led to the isolation of an aliphatic acetoxy compound (similar to nerolidol) with fungi static activity (i.e. it inhibits the growth of fungi) but without antibacterial activity.

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