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Smooth muscle relaxant evaluation of *Jatropha Curcas* Linn (Euphorbiaceae) and isolation of triterpenes

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Summary: *Jatropha curcas* is a herbal preparation used in the tropics for the treatment of threatened abortion and related problems associated with pregnancy. The Stem bark of *Jatropha curcas* is used ethno medicinally in Nigeria especially in the eastern part of the country for the treatment of infertility and spontaneous abortion (miscarriage). The present study was undertaken in order to validate the folkloric claim, using scientific experimental procedures and bioassay guided fractionation. The crude powdered sample was subjected to phytochemical screening testing for the presence of alkaloids, tannins, saponins and carbohydrates. Chromatographic analysis (TLC and VLC) were carried out using various solvent systems. The effect of methanolic extracts on rat uterine contractions was studied in vitro, in 40ml organ baths containing physiological salt solution of De Jalon maintained at 37^{O} C, aerated with 95%O_2 and 5%CO_2 with an isometric transducer connected an UgoBasile recorder under a resting tension of 750mg. The result of the phytochemical screening revealed the presence of glycosides, tannins, saponins and alkaloids. The extract abolished significantly ($P<0.0001$) the spontaneous contraction of the uterus and reduced acetylcholine induced uterine contractions at a dose of 50mg/ml. The tocolytic effects indicate the presence of active principle(s) which would explain the ethno medicinal use of the stem bark of *Jatropha curcas* to treat spontaneous abortion.

Keywords*: Jatropha curcas*, Triterpene, Smooth muscle, Tocolytic effect

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INTRODUCTION

Medicinal plants have treated cultures around the world for generations and still is the primary form of treatment in many areas today. However, among the 250,000 – 500,000 species of plants on earth, only a relatively small percentage (1–10%) are used for food by humans and animals (Borris, 1996). It is possible that more serve medicinal purposes (Moerman, 1996).

The drugs of today's modern society are products of research and development by major pharmaceutical companies but among the most important raw materials researched and developed are naturally occurring materials from plants (Farnsworth and Soejarto, 1985). In developing countries, about 80% of the population rely on traditional medicine for their primary health care need and about 85% of these traditional medicine involves the use of plant extracts (Farnsworth, 1985).

Jatropha curcas Linnaeus (family: Euphorbiaceae) is a tree or large shrub growing up to 5m in height. Though native to America, the species is widely distributed in tropical Africa including Sudan, Senegal and Nigeria. A decoction of the leaves is used against cough and as antiseptic, latex used to dress wounds and ulcers, root decoction as mouthwash for bleeding gums and toothache (Duke and Ayensu, 1985). Branches are used as chewing stick and for the treatment of preterm labor in Nigeria (Isawumi, 1978). The latex has been demonstrated to have antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus pyogenes* and *Candida albicans*.(Thomas, 1989; Duke and Ayensu, 1985).
A methanol extract of the leaves afforded moderate protection for cultured human lymphoblastoid cells against the cytopathic effects of human immunodeficiency virus (Muaza *et al.*, 1995). Extract of the fruit has been shown to have pregnancy terminating effects in rats (Goonasekera *et al.*, 1995).

Based on the claimed folkloric use in the treatment of preterm labour, the present study is aimed at investigating the uterine smooth muscle relaxant effect of *Jatropha curcas* and also to isolate some chemical principles in the plant.

MATERIALS AND METHODS

Animal and tissue preparation

Female Wistar rats weighing between 160-200g were brought into oestrus by injecting 0.2mg/kg of stilbesterol in ethanol intra-peritoneneally 24 hours before the experiment. The rats were stunned by a blow on the head and decapitated. Uterine horns were removed, trimmed off of mesenteric fat and a strip of longitudinal uterine smooth muscle 1.0-1.5cm long was set in a 40ml organ bath containing De Jalons solution with the following chemical composition NaCl, 154mM/L $NaHCO_3$, 5.95mM/L, D-Glucose, 2.78mM/L KCl, 5.40mM/L,$CaCl_2.2H_2O$, 5.44mM/L. The preparation were bubbled continuously with 95%O_2 and 5% CO_2 and warmed to 37^OC. The spontaneous contractions of the uterus were recorded isometrically by means of force displacement transducer connected to amplifier and a multi-channel recorder. The preparation was allowed to equilibrate for 30 minutes (with changes in bath fluid every 10minutes) under a resting tension of 750mg. The effects of extract and Acetylcholine were investigated. The effect of extract on acetylcholine-induced contractions was also investigated.

Plant preparation

The stem bark of *Jatropha curcas* were collected in Okhoro area in New Benin, Benin City, Nigeria. The stem barks used for the project were sun dried and then crushed into a gritty powder with the aid of a mechanical grinder.

Phytochemical screening

The crude powdered plant material was subjected to phytochemical screening testing for the presence of secondary metabolites in accordance with established experimental procedures (Sofowora, 1980; Harborne, 1973; Trease and Evans, 2002).

Experimental Protocol

Column chromatography was carried out using column silica gel of 70-230 and 230-400 mesh size. Aluminium sheets pre-coated with silica gel 60 F_{254} (0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and visualized under UV light (254 and 366 nm) followed by ceric sulfate as spray reagent. Optical rotations were measured on a Jasco DIP-360 digital polarimeter. The UV spectra were recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm). IR spectra were recorded on Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). EIMS, HREIMS, FABMS and HRFABMS spectra were recorded on Jeol JMS-HX 110 spectrometer with data system. The ^{1}H-NMR spectra were recorded on Bruker AMX-400 and 100 MHz instruments using TMS as an internal reference. The chemical shifts are reported in ppm and coupling constants *(J)* in Hz.

Isolation

The stem bark powder of *J.curcas* (1kg) was extracted with (3X 7L) methanol by maceration at room temperature for 48 hours. The methanol extracts were concentrated in vacuum to give a residue (254g), which was partitioned between n-hexane, chloroform and ethylacetate. The chloroform soluble fraction (45.6g) was subjected to a silica gel Column chromatography eluted with solvents of increasing polarity chloroform-EtOAc to give 12 fractions (fr1-12). Fraction 2 was re-chromatographed to give a semi purified fraction which was purified on a silica gel CC to give a pure compound **1**. The ethylacetate soluble fraction (37.2g) was subjected to column chromatography on a 200-300mesh silica gel, eluted with gradient polarity to give 20 fractions. Fraction 1 gave needle like crystals which were purified and recrystallized in acetone to give compound **2**. The two compounds were subjected to spectroscopic analysis using IR, NMR and EIMS.

Compound **1**: Colourless needles from $CHCl_3$
MP: 305-306^oC
$[\alpha]_D^{25}$: + 78.9 (c = 0.07, $CHCl_3$)
IR (KBr) $\nu_{max}cm^{-1}$: 3400-2640, 1660 and 820, 1700

^{13}C-NMR ($CDCl_3$, 400MHz): δ 183.4 (C-28), 143.6 (C-13), 122.7 (C-12), 79.0 (C-3), 55.2 (C-5), 47.6 (C-9), 46.5 (C-17), 45.9 (C-19), 41.6 (C-14), 41.0 (C-18), 39.1(C-8), 38.7 (C-4), 38.4 (C-1), 37.1 (C-10), 33.8 (C-21), 33.0 (C-29), 32.6 (C-7), 32.4 (C-22), 30.6 (C-20), 28.1 (C-23), 27.7 (C-15), 27.2 (C-2), 25.9 (C-27), 23.5 (C-30), 23.4 (C-16), 18.3 (C-6), 17.1 (C-26), 15.6 (C-24) and 15.3 (C-25).

^{13}C-NMR ($CDCl_3$,100MHz): δ 183.4 (C-28), 143.6 (C-13), 122.7 (C-12), 79.0 (C-3), 55.2 (C-5), 47.6 (C-9), 46.5 (C-17), 45.9 (C-19), 41.6 (C-14), 41.0 (C-18), 39.1 (C-8), 38.7 (C-4), 38.4 (C-1), 37.1 (C-10), 33.8 (C-21), 33.0 (C-29), 32.6 (C-7), 32.4 ((C-22), 30.6 (C-20), 28.1 (C-23), 27.7 (C-15), 27.2 (C-2), 25.9 (C-27), 23.5 (C-30), 23.4 (C-11), 23.4 (C-16), 18.3 (C-6), 17.1 (C-26), 15.6 (C-24) and 15.3 (C-25).

EIMS *m/z*: 456 $[M]^+$ (4), 248 (98), 208 (12), 203(60) and 133 (53).
HREIMS *m/z:* 456.3610 (calcd. For $C_{30}H_{48}O_3$, 456.3603)

Statistical Analysis

All results were expressed as the mean of five experiments ± SEM. The statistical package used was SAS, 1994 Users guide, Version 8.2. SAS Institute

Inc., Cary, NC, USA. The statistical significance ($p < 0.05$) of differences between means was assessed by an analysis of variance (ANOVA) followed by Duncan's multiple range test.

RESULTS

The results of the phytochemical screening tests are shown in Table 1. The test revealed the presence of carbohydrate, saponins, tannins and alkaloids.

Table 1:
Phytochemical composition of the stem bark of J*atropha curcas*

Class of compounds	***Jatropha curcas***
Carbohydrate	+
Reducing Sugar	+
Saponins	+
Tannins	+
Alkaloids	+
Flavonoids	+

Table 2:
^{13}C-NMR ($CDCl_3$, 100MHz of compound **2**)

Carbon No	Multiplicity DEPT	^{13} CNMR δ
1	CH_2	38.6
2	CH_2	27.3
3	CH	78.9
4	C	38.4
5	CH	55.2
6	CH_2	18.2
7	CH_2	34.2
8	C	40.7
9	CH	50.3
10	C	37.1
11	CH_2	20.9
12	CH_2	25.0
13	CH	38.0
14	C	42.7
15	CH_2	27.4
16	CH_2	35.5
17	C	42.9
18	CH	48.2
19	CH	47.9
20	C	150.8
21	CH_2	29.8
22	CH_2	39.9
23	CH_3	27.9
24	CH_3	15.3
25	CH_3	16.1
26	CH_3	15.9
27	CH_3	14.5
28	CH_3	17.9
29	CH_2	109.3
30	CH_3	19.2

Figure 2 shows the log concentration response curve obtained from the various doses of acetylcholine administered in vitro. Significant contraction of the rat uterus was obtained with maximum response being produced at 1mg/ml. The crude stem bark extract of *Jatropha curcas* caused relaxation of the isolated rat uterus, the contractile response of acetylcholine was reduced by graded doses of extract as presented in figure 1. The effect of 0.2ml of 10mg/ml acetylcholine was completely blocked by extract. The response was brought back by higher doses of acetylcholine.

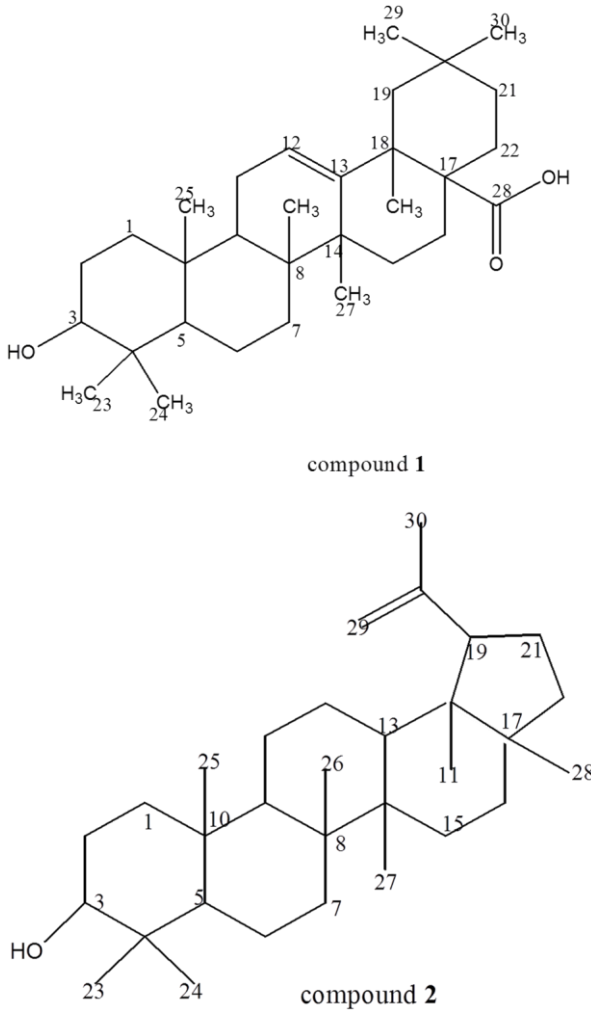

Figure 1:
Triterpenes isolated from the stem bark of *Jatropha curcas*

DISCUSSION

The observed inhibition of the contractions of the rat uterus may be due to the reduction or abolition of the spontaneous contraction. The crude extract of *J. curcas* has a relaxant effect on uterine smooth muscle, which is consistent with the ethnopharmacological use of the plant. This work is in disagreement with the oxytocic or pregnancy terminating studies of the plant (Goonasekera *et al.*,

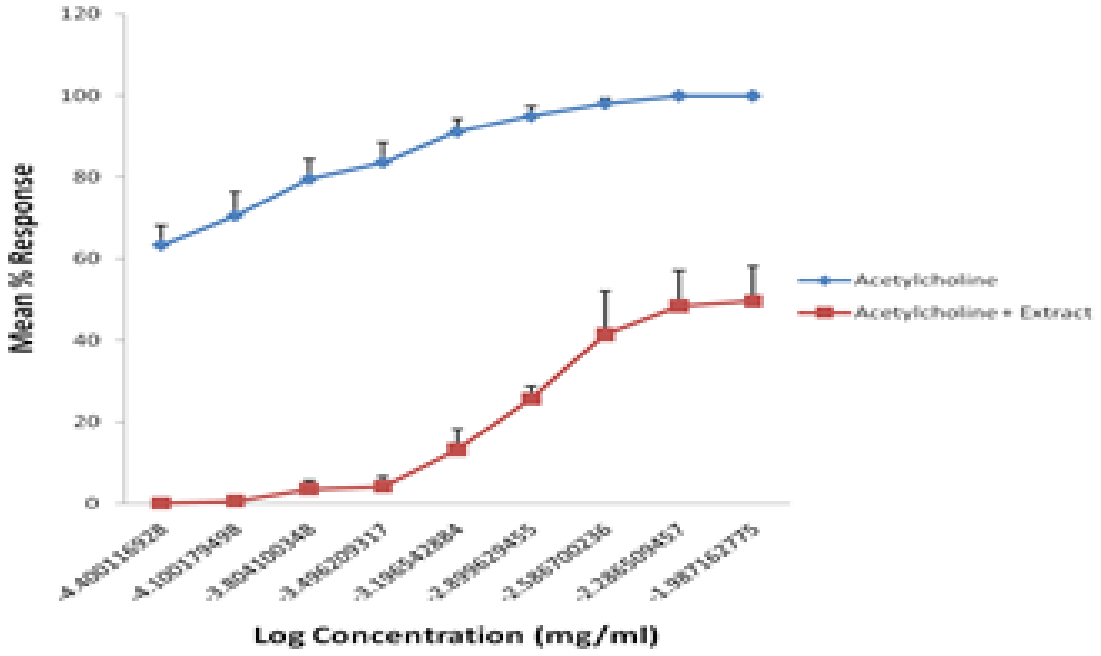

Figure 2:
Log concentration response curve of acetylcholine and acetylcholine + extract on the isolated diethylstilboesterol pretreated uterus of rats.

1995). The plant may possess both oxytocic and tocolytic activities at different doses. Some plants have been reported to possess tocolytic activity such as *Pyrenacantha staudtii* (Falodun *et al.*, 2006; Falodun *et al.*, 2005; Owolabi *et al.*, 2009). Further pharmacological investigation on the mechanism of action of the extract will be done in future studies.

Compound**1** was isolated from chloroform soluble fraction. The molecular formula was established as $C_{30}H_{48}O_3$ by HR mass spectrometry which showed $[M]^+$ peak at *m/z* 456.3610 (calcd. For $C_{30}H_{48}O_3$, 456.3603). The nature of oxygen in the compound was shown to be hydroxyl group as indicated by IR spectrum (3400cm^{-1}). The IR spectrum also exhibited absorption bands for double bond (1660 and 820cm^{-1}), carbonyl group (1700cm^{-1}). The mass spectrum showed characteristic fragmentation pattern of a sterol. The ^{1}H-NMR of the compound showed the signals for seven methyls as singlets at δ 0.89, 0.90, 0.97, 0.98, 1.03 and 1.12. The signal at δ 5.24 (1H, t, *J*=3.4 Hz) was due to the olefinic proton while the proton germinal to the hydroxyl group was observed at δ 3.60 (dd, *J*= 4.1, 9.9Hz). The ^{13}C-NMR assignments of various carbon atoms were substantiated by DEPT experiment. The spectra of compound revealed the presence of thirty carbon atoms. The DEPT experiment showed the presence of seven methyl, nine methylene, seven methine and six quaternary carbons. The comparison of the data reported in literature showed a closed resemblance to ursolic acid (2 methyl groups at position 20), Falodun *et al.*, 2009. The structure was identified as a triterpene called oleanolic acid.

Compound **2** was isolated from the ethyl acetate soluble fraction. The molecular formula was established by HR-EIMS (m/z 426.3855) as $C_{30}H_{50}O$). The IR spectrum showed absorption bands due to the hydroxyl (3400 cm^{-1}) and olefinic (1640cm^{-1}) functional groups. The ^{13}C-NMR assignments of the various carbons as shown in Table 2 were confirmed by the DEPT experiment, which revealed the presence of seven methyl groups, eleven methylenes and six methine carbons. The chemical structure of the compound was identified as lupeol by comparison with reported data.

Jatropha curcas extract therefore has tocolytic activity, it abolished the spontaneous contractions of the isolated rat uterus and reduced the contractile activity of acetylcholine and this could explain the ethno-medicinal use of this plant as herbal tocolytic.

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