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Chemical and Biological Investigations on the Leaves of *Jatropha gossypefolia*

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Abstract

Two compounds were isolated from the petroleum ether crude extract of the dried leaves from *Jatropha gossypefolia*. Based on the spectral evidence, their structures were determined to be ***β*-sitosterol-3-*O*-*β*-D-glucopyranoside** and **stigmasterol**. The crude extract of petroleum ether, dichloromethane, carbon-tetrachloride, methanol extract were screened for their antimicrobial activity against a wide range of gram-positive bacteria, gram-negative bacteria and fungi by disc diffusion method and brine shrimp lethality bioassay. The crude dichloromethane extract showed mild to moderate inhibitory activity to microbial growth while the column fraction F-8 showed strongest cytotoxicity having LC_{50} 6.31 µg/ml.

Key words: *Jatropha gossypefolia,* Euphorbiaceae, *β*-sitosteol-3-o-*β*-D-glucopyranoside, stigmasterol, Brine shrimp lethality bioassay, Antimicrobial.

I. Introduction

Jatropha gossypifolia (Bengali name- Lal-verenda,Common name- Bellyache bush, Cotton-Leaf Physic Nut, pignut or fignut; Family- Eurphorbiaceae) is an Erect shrub or small tree to 4 m high, deciduous in dry conditions. Stems hairy, non-woody. Leaves initially purplish but green when mature, sticky, 5.5–14 cm long, 7.5–12.5 cm wide, rounded in outline, leaf stalks 4.5–11.5 cm long. Fruit an oblong capsule, initially green, ripening dark brown, mostly3 or 4 seeded. Seeds brown, slightly mottled. Roots fleshy and tuberous.[1] *The origin of J. gossypifolia in tropical central and South America and Caribbean islands.*[2] *Jatropha gossypifolia is now grown in all tropical countries and many sub-tropical regions of the world.*

J. gossypifolia is the common red species planted around houses, and is used as a therapeutic agent in different ways. The leaf decoction of *J.gossypifolia* is used for bathing wounds [3, 4, 5] reported that the leaf bath is used for sores, sprains, rash and bewitchment in Latin America and the Caribbean; the poultices are used for sores and pain in Trinidad.[4] The stem sap stops bleeding and itching of cuts and scratches.[4, 6] In Southern Nigeria, the extract from fresh leaf applied with crushed leaf is routinely used by herbalists and local people to stop bleeding from the skin and nose. stem latex used as a haemostatic agent [7] The young stem of the plant is used as toothbrush as well as to clean the tongue in the treatment thrush. The tuber of the plant grinded into a paste is also locally used in the treatment of hemorrhoids. this plant is also traditionally planted along slopes in Nigeria for controlling water erosion.[2]

Previous phytochemical investigations resulted in the isolation of gossypifan,[8] Jatrophenone,[9] cyclogossine B,[10] cyclogossine A, [10, 11] Jatrodien, [12] and isogadain [13] So far no details biological studies have been carried out on this plant. In this paper, the isolation and structure elucidation of the *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**1**) and stigmasterol (**2**) by using spectroscopic techniques and the preliminary antimicrobial and cytotoxic activities of the organic extractives are being reported.

II. Materials and Methods

General experimental procedure

The ^{1}H NMR spectra were recorded by using a Bruker DPX-400 (400 MHz) instrument. For NMR studies deuterated chloroform was used and the δ values for ^{1}H spectra were referenced to the residual nondeuterated solvent signals.

Plant Material. The leaves of *Jatropha gossypefolia* were collected from Jessore and identified by Sorder Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium. A voucher specimen has been deposited in Bangladesh National Herbarium (DACB accession no.- 34216) Dhaka, Bangladesh. The leaves were at first sun dried for five consecutive days. Finally the dried leaves were ground into a coarse powder using a grinding machine.

Extraction and Isolation. The powdered leaves (500 g) of *J. gossypifolia* was soaked in 2.5 L methanol for 7 days and filtered through a cotton plug followed by Whatman filter paper number 1.The extract was then concentrated by using a rotary evaporator. A portion of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method[14] into petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluable fractions. The concentrated crude petroleum ether extract (20gm) was subjected to column chromatography for fractionation on silica gel (Kieselgel 60, mesh 70-230) and eluted with gradients of petroleum ether/ethyl acetate, then ethyl acetate, after that gradients of ethyl acetate/methanol and finally with methanol to afford 28 fractions (each 100 mL). Fraction and fraction 20 washing with petroleum ether,mixture of petroleum ether with dichloromethane gave compound **1** (10 mg) and fraction 5 washing with n hexane gave compounds **2** (15 mg) respectively.

***β*-sitosterol-3-*O*-*β*-D-glucopyranoside (daucosterin) (1)** White powder; ^{1}H NMR (400 MHz, $CDCl_3$ + 2 drops CD_3OD) δ 5.33 (1H, *brs*, H-6), 4.37 (1H, *d*, *J* = 8.0 Hz, , H-6′β), 3.80 (1H, *m*, H-5′), 3.75 (1H, *m*, H-3), 0.88 (3H, *d*, *J* = 5.6 Hz, Me-21), 0.84 (3H, *s*, Me-19),0.82 (3H, *t*, *J* = 7.6 Hz, Me-29), 0.79 (3H, *d*, *J* = 7.6 Hz, Me-26), 0.77 (3H, *d*, *J* = 8.0 Hz, Me-27), 0.64 (3H, *s*, Me-18).^{13}C NMR (100 MHz, $CDCl_3$+2 drops CD_3OD): δ 36.02 (C-1), 29.47(C-2), 79.06(C-3), 39.62(C-4), 122.06(C-5), 138.2 (C-6), 33.81(C-7), 31.80(C-8), 50.04(C-9), 37.12 (C10), 20.92 (C-11), 38.57(C-12), 42.3(C-13), 56.62(C-14), 31.74 (C-15), 29.01(C-16), 55.92(C-17), 11.79(C-18), 19.63(C19), 36.12(C-20), 18.84(C-21), 39.62(C-22), 22.92(C-23), 45.72(C-24), 18.61(C-25), 28.11(C-26), 18.16(C-27), 24.15(C-28), 11.69(C-29), 100.95(C-1′ of glucose), 73.40(C-2′), 76.26(C-3′), 69.97(C-4′), 75.60 (C-5′), 61.72(C-6′).

Stigmasterol: ^{1}H NMR spectral data was identical to previously reported values.[23]

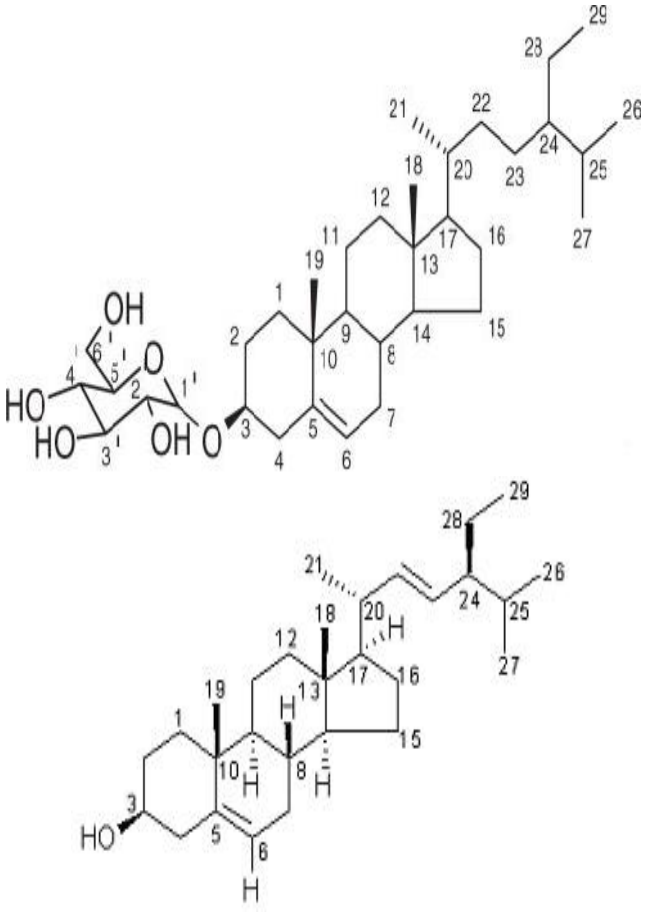
Brine Shrimp Lethality Bioassay. The antimicrobial activity of the extractives was determined by the disc diffusion method.[15,16] The samples were dissolved separately in specific volume of chloroform and applied to sterile discs at a concentration of 500 µg/disc and carefully dried to evaporate the residual solvent. For cytotoxic screening, DMSO solutions of fractions obtained from the crude extract were applied against Artemia salina in a 1-day in vivo assay.[17-19] For the experiment, 4 mg of each of the Kupchan fractions was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ml were obtained by serial dilution technique. The median lethal concentration (LC_{50}) of the test samples after 24 hours of exposure was obtained by plotting percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration).Here vincristine sulphate was used as a standard.

III. Results and Discussion

***β*-sitosterol-3-*O*-*β*-D-glucopyranoside (daucosterin)** and **stigmasterol** were isolated from the petroleum ether extract of the leaves of repeated chromatographic separation and purification over silica gel. The structure of the isolated compound was determined by ^{1}H NMR and ^{13}C NMR data analysis as well as by comparison with previously reported values.

The ^{1}H NMR spectrums (400 MHz, $CDCl_3$) of compound **1** showed two one-proton multiplets at δ 3.75 and δ 5.33 typical for H-3 and H-6 of a steroidal nucleus. The spectrum further revealed two singlets at δ 0.64 and δ 0.97 each integrating for three protons, assignable to two tertiary methyl groups at C-13 and C-10 respectively. The ^{1}H NMR spectrum also showed two doublets centered at δ 0.79 (*J* = 7.6 Hz) and 0.77 (*J* = 8.0 Hz) which could be attributed to two methyl group at C-25. The doublet at δ 0.88 (*J* = 5.6 Hz) was demonstrative of a methyl group at C-20. The doublet at δ 4.37 (*J* = 8.0 Hz) showed one proton for H-6′β and another multiplet at δ 3.80 showed one proton for H-5′.The ^{13}C NMR also contained resonances of 35 carbon atoms which were assigned after examination of the DEPT and HMQC Spectra as six methyl groups, 12 methylene, 14 methine and three quaternary carbon atoms. Particular signals at δ 100.95 (C-1′of glucose), 73.40 (C-2′), 76.26 (C-3′), 69.97 (C-4′), 75.60(C-5′) and 61.72 (C-6′) indicated the presence of a single monosaccharide moiety. These signals were in agreement with those obtained from the β-D-Glucose. Finally, the structure of was identified as ***β*-sitosterol-3-*O*-*β*-D-glucopyranoside** by comparing its reported ^{1}H NMR and ^{13}C NMR data.[20-22]

Compound **2** was identified as stigmasterol by comparison of their ^{1}H NMR spectral data with reported values[23] as well as by co-TLC with authentic samples.



1 **2**

Following the procedure of Meyer [17-19], the lethality of the crude petroleum ether, carbon-tetrachloride, methanol extract and two column fractions (F-5 & F-8) of petroleum ether extract were screened by brine shrimp lethality bioassay for probable cytotoxic activity. The LC_{50} obtained from the best-fit line slope were found to be 12.59 µg/mL, 12.60 µg/mL, 11.22 µg/mL,39.81 µg/mL 6.31 µg/mL for crude petroleum ether, carbon-tetrachloride,methanol extract and two column fractions (F-5 & F-8) of petroleum ether extract respectively (**Table 1**). In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by column fraction of **F-8** of petroleum ether

extract was significant.

Table. 1. LC_{50} data of *J. gossypifolia* extracts and vincristine sulfate.

Samples	LC_{50} (µg/ml)	Samples	LC_{50} (µg/ml)
VS	0.33	CCl_4	12.60
PE	12.59	F-5	39.81
ME	11.22	F-8	6.31

The values of **LC_{50}** are expressed in **µg/ml**. **VS:** vincristine sulphate (Std.); **PE**: Petroleum ether extract;**CCl_4**: Carbon tetrachloride, **ME**: Methanol extract **F-5 & F-8:** Column Fractions of petroleum ether extract.

The extractives of the *J.gossypifolia* when subjected to antimicrobial screening at 500µg/disc demonstrated mild inhibition of microbial growth (**Table 2**). The average zone of inhibition produced by the crude extract of petroleum ether, carbon-tetrachloride, dichloromethane extract and methanol extracts showed mild to moderate inhibitory activity to microbial growth and having the zone of inhibition of 10-15 mm each. On the other hand, some of the test organisms showed less activity to the extracts and column fractions of petroleum ether extract.

Table. 2. Antimicrobial activity of *Jatropha gossypifolia* extracts (500 µg/disc) and Kanamycin (30 µg/disc)

	Diameter of zone of inhibition (mm)				
	PE	**DCM**	**CCl_4**	**ME**	**Kanamycin**
Gram Positive					
Bacillus sereus	11	12	---	10	33
Bacillus megaterium	10	11	---	10	34
Bacillus subtilis	11	12	7	10	35
Staphylococcus aureus	11	15	8	11	34
Sarcina lutea	10	11	---	8	34
Gram Negative					
Pseudomonas aeruginosa	11	12	ND	10	35
Salmonella paratyphi	12	12	---	10	33
Shigella boydii	10	12	---	10	35
Vibrio mimicus	10	12	7	10	34
Shigella dysenteriae	10	11	8	11	34
Vibrio parahemolyticus	11	9	---	10	34
Salmonella typhi	11	13	7	11	34
Escherichia coli	11	12	---	ND	
Fungi					
Candida albicans	12	14	---	9	34
Aspergillus niger	12	12	7	8	34
Sacharomyces cerevacae	12	11	---	8	34

PE: Petroleum ether extract; **DCM**: Dichloromethane extract; **ME**: Methanol extract; **CCl_4**: Carbon tetrachloride extract; **NA**: No Activity.

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