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Phytochemical and Biological Investigations of *Carica papaya*

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Abstract

β-sitosterol is isolated from petroleum ether extract of the leaves of Carica papaya (Family: Caricaceae). The crude extract of methanol, petroleum ether, carbon-tetrachloride, dichloromethane extract and six column fractions (F-6, F-8, F-13, F-17, F-21& F-24) of petroleum ether 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 four crude extracts and six column fraction of petroleum ether showed significant activity against four of the test organisms such as *B. subtilis, S. aureus*, *E. coli and S. typhi* while the petroleum ether extract showed strongest cytotoxicity having LC_{50} 1.41µg/mL.

Key words: *Carica papaya,* Caricaceae, β-sitosterol, Brine shrimp lethality bioassay, Antimicrobial.

I. Introduction

Carica papaya (Common name- papaya, papaw, fruta bomba, lechosa, melon tree; Family- Caricaceae) is an evergreen big shrub or small tree for the tropical or subtropical landscape.

The origin of *C.* papaya in tropical America, it is likely that *C. papaya* originates from the lowlands of eastern Central America, from Mexico to Panama [2].Its seeds were distributed to the Carribean and south-east Asia during Spanish exploration in the 16th Century, from where it spread rapidly to India, the Pacific and Africa [3]. Papaya is now grown in all tropical countries and many sub-tropical regions of the world.

The juice is used for warts, cancers, tumors, corns, and indurations of the skin. Sinapisms prepared from the root are also said to help tumors of the uterus. Green fruit said to be ecbolic. Vermifugal seeds said to quench thirst. Leaves poulticed onto nervous pains and elephantoid growths.In Asia, the latex is smeared on the mouth of the uterus as ecbolic. The root infusion is used for syphilis in Africa. Leaf smoked for asthma relief in various remote areas. Javanese believe that eating papaya prevents rheumatism. Dietary papaya does reduce urine acidity in humans. Flowers have been used for jaundice. Experimentally papaya is hypoglycemic. Inner bark used for sore teeth. Latex used in psoriasis, ringworm, and prescribed for the removal of cancerous growths in Cuba[1,5]. Latex used locally as antiseptic. Seeds considered alexeritic, abortifacient, counter-irritant, emmenagogue, and anthelmintic. Infusion of roots said to remove urine concretions. Young leaves, and to lesser degree, other parts, contain carpain, an active bitter alkaloid, which has a depressing action on heart. Plant is strong amoebicide. Latex, used as dyspepsia cure, is applied externally to burns and scalds [6]. Fruit and seed extracts have pronounced bactericidal activity against *Staphylococcus aureus, Bacillus cereus, Escherischia coli, Pseudomonas aeruginosa, and Shigella flexneri* [7].

There are about Six genera and 34 species of Caraca papaya. Previous phytochemical investigations resulted in the isolation of protocatechuic acid, p-coumaric acid, caffeic acid, 5,7-Dimethoxy coumarin, chlorogenic acid, kaempferol, quercetin[9], benzylglucosinolate,cyanogenic-glucosides, phenyl propanoids[10],papain,caricain, chymo-papain, lycine endopeptidase [11] and alkaloide- carpaine,c arpasemine [12] 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 **(1)** 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 fresh leaves of Carica papaya was collected from Botanical Garden, Dhaka, in the month of February, 2007. It was identified by Boshra Khan, Senior Scientific officer, Bangladesh National Herbarium, Dhaka. A voucher specimen has been deposited in the Bangladesh National Herbarium, Dhaka (DACB Accession No. 32,765), for the collection. 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 stem bark (533 g) of *C. papaya* was soaked in 2.5 L methanol for 15 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 [13] into petroleum ether, carbon tetrachloride and dichloromethane. The petroleum ether soluble fraction(3.5gm) was fractionated by column chromatography (CC) over silica gel (70-230 mesh) using petroleum ether, dichloromethane and methanol mixtures of increasing polarities to give 30 fractions, collecting each 100 ml. Fraction 18 upon washing with petroleum ether gave compounds **1**.

***β*-sitosterol:** white crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.51(1H, m,H-3),5.34 (1H, m, H-6), 1.00 (3H, s, 10-CH_3), 0.67 (3H,s, 13-CH_3), 0.91 (3H, d , J = 6.8, 20-CH_3), 0.81 (3H, d , J = 7.6, 25-CH_3), 0.82(3H, d , J = 7.6, 25-CH_3).

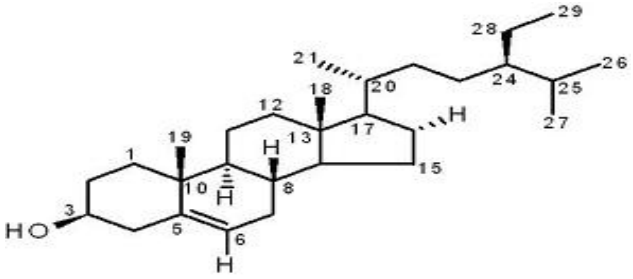
Bioassays

The antimicrobial activity of the extractives was determined by the disc diffusion method.[14,15] 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 cytotoxicity screening, DMSO solutions of fractions obtained from the crude extract were applied against Artemia salina in a 1-day in vivo assay.[16-18] 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 was isolated from the dichloromethane soluble fractions of a 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 data analysis as well as by comparison with previously reported values[19, 20]

The ^{1}H NMR spectrums (400 MHz, $CDCl_3$) of *β*-sitosterol showed two one-proton multiplets at δ 3.51 and δ 5.33 typical for H-3 and H-6 of a steroidal nucleus. The spectrum further revealed two singlets at δ 0.67 and δ 1.00 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 0.81(*J* = 7.6 Hz) and 0.83 (*J* = 8.0 Hz) which could be attributed to two methyl group at C-25. The doublet at δ 0.92 (*J* = 6.0 Hz) was demonstrative of a methyl group at C-20.



***β*-sitosterol**

These NMR spectral features are characteristics of a steroidal carbon skeleton of *β*-sitosterol. Finally, the structure of was identified as *β*-sitosterol by comparing its reported ^{1}H NMR data[19,20].

Following the procedure of Meyer[16-18], the lethality of the crude petroleum ether, carbon-tetrachloride, dichloromethane, methanol extract and six column fractions (F-6, F-8, F-13, F-17, F-21& F-24) 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 1.41 µg/mL, 1.65 µg/mL, 1.78 µg/mL,4.68 µg/mL, 1.50µg/mL, 2.99 µg/mL, 2.51 µg/mL, 3.98 µg/mL, 1.58 µg/mL and 3.55 µg/mL for crude petroleum ether, carbon-tetrachloride, dichloromethane, methanol extract and six column fractions (F-6, F-8, F-13, F-17, F-21& F-24) of petroleum ether extract respectively(**Table 1**). In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by petroleum ether extract was significant.

Table. 1. LC_{50} data of *Carica papaya* extrats and vincristine sulfate

Samples	LC_{50} (µg/ml)	Samples	LC_{50} (µg/ml)
VS	0.33	F-8	2.99
ME	4.68	F-13	2.51
PE	1.41	F-17	3.98
CCl_4	1.65	F-21	1.58
DCM	1.78	F-24	3.55
F-6	1.50		

The values of **LC_{50}** are expressed in **µg/ml**. **VS:** vincristine sulphate (Std.); **PE**: Petroleum ether extract; **DCM**: Dichloromethnae extract; **CCl_4**: Carbon tetrachloride,**ME**: Methanol extract **F-6, F-8, F-13, F-17, F-21& F-24:** Column Fractions of petroleum ether extract.

The extractives of the *C. papaya* 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, methanol and six column fractions (F-6, F-8, F-13, F-17, F-21& F-24) of petroleum ether extract was 7-17 mm. The crude extract of petroleum ether, carbon-tetrachloride, dichloromethane extract, methanol and six column fractions (F-6, F-8, F-13, F-17, F-21& F-24) of petroleum ether

extract showed significant activity against four of the test organisms such as *B. subtilis, S. aureus* , *E. coli and S. typhi* and having the zone of inhibition of 14-17 mm each. On the other hand, others test organisms showed less activity to the extracts and column fractions of petroleum ether extract.

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

	Diameter of zone of inhibition (mm)										
	PE	**CCl_4**	**DCM**	**ME**	**F-6**	**F-8**	**F-13**	**F-17**	**F-21**	**F-24**	**Kanamycin**
Gram Positive											
B. cereus	7	8	8	7	---	8	---	7	9	7	33
B. megaterium	8	7	9	7	8	---	8	8	8	---	33
B. subtilis	16	16	16	12	14	15	15	17	14	16	32
S. aureus	16	14	15	16	13	17	14	18	15	16	33
Sarcina lutea	7	7	9	8	8	---	7	8	7	7	35
Gram Negative											
E. coli	14	15	15	16	16	16	13	16	14	17	35
S. paratyphi	8	8	9	7	7	8	7	11	7	8	32
S. typhi	16	15	14	17	16	14	17	15	14	14	31
S. boydii	7	8	9	---	7	---	8	9	8	9	33
S. dysenteriae	8	7	---	7	---	8	9	7	9	8	34
V. mimicus	7	8	9	9	8	7	---	9	7	---	32
V.parahemolyticus	---	7	7	7	8	7	7	9	8	8	32
Fungi											
C .albicans	8	9	8	9	8	9	10	10	8	9	33
A. niger	9	9	9	8	9	9	8	10	8	8	34
S. cerevacae	7	7	7	---	---	7	7	9	7	7	34

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

The results of antimicrobial and cytotoxicity screening were found to be consistent with the folk uses of Carica papaya by local people.

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