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Phytochemical and antimicrobial investigation of *Luffa cylindrica*

[Investigacion fitoquimica y antimicrobiana de *Luffa cylindrica*]

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Abstract

3-hydroxy-1-methylene-2,3,4,4-tetrahydroxynapthalene-2-carbaldehyde (1), 22,23-dihydroxy spinasterol (2) were isolated from petroleum ether extrct of the fruits of Luffa cylindrical. The structures of the isolated compounds were elucidated by extensive spectroscopic studies including IR and high field NMR analysis.Petroleum ether extract (i.e. crude extract) exhibited mild to moderate antimicrobial activity. This is the first report of isolation of compound of 1 – 2 from this species.

KEYWORDS: *Luffa cylindrica*, Cucurbitaceae, 3-hydroxy-1-methylene-2,3,4,4-tetrahydroxynapthalene-2-carbaldehyde; 22,23-dihydroxy spinasterol

Resumen

3-hydroxi-1-metilen-2,3,4,4-tetrahidroxinaftalen-2-carbaldehido (1), 22,23-dihidroxispinasterol (2) fueron aislados de un extracto en eter de petróleo de la fruta de *Luffa cylindrica*. Las estructuras de estos compuestos fueron deducidas por estudios espectroscópicos incluyendo IR y RMN de alto campo. El extracto crudo en éter de petróleo mostró actividad antimicrobiana de leve a moderada. El presente estudio representa el primer reporte de estos compuestos en *L cylindrica*.

PALABRAS CLAVE: *Luffa cylindrica*, Cucurbitaceae, 3-hidroxi-1-metilen-2,3,4,4-tetrahidroxinaftalen-2-carbaldehido; 22,23-dihidroxiespinasterol.

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INTRODUCTION

Luffa cylindrica is one of the important medicinal plants of the family of Cucurbitaceae. Locally it is known as Dhundul. The Cucurbitaceae species is found in Bangladesh (Schilling *et al*.1990 and Hamid *et al*.1995). The botanical features of the genus Luffa and the order cucurbitals have been recorded. The fruit of *Luffa cylindrica* is used for the treatment of skin disease. It is also good for the liver, lungs, heart and stomach; having a cooling effect on the body (Kirtikar *et al*.). In Chinese medicine, the inner skeleton of the dried fruit is used to treat pain in the muscle and joints, chest and abdomen. It is also used as a edible vegetable.It is a good source of vitamin C, vitamin K, and potassium, also providing dietary fiber, vitamin A, vitamin B6, thiamin, folate, pantothenic acid, magnesium, phosphorus, copper, and manganese (Duke at al. 2002 and Leung *et al*. 1996).

Sterols, saponins, terpinoids, flavonoids, fatty acids, amino acids, phenolic types of compounds and antioxidants (Qizhen Du *et al.*, 2006) have been isolated by different workers from the *Luffa cylindrica*. More investigation and thorough study of this plant may open a new chapter towards the significant role of the plant for the human well-beings. With this end in view, phytochemical studies of the fruit *Luffa cylindrica* have been undertaken. The fruit powder of *Luffa cylindrica* was extracted with petroleum ether and ethyl acetate exhaustively and separately in the study. Here petroleum ether extract was used as a research extract. A voucher specimen for this collection has been maintained in Bangladesh National Herbarium (DACB 15350), Dhaka, Bangladesh.

MATERIALS AND METHODS

The fruits Dhundol (*Luffa cylindrica*) were collected from Jattrabari Kacha Bazar, Dhaka, Bangladesh. The collected fruits were cleaned with water. The fruits were then cut into small pieces and dried in the direct sunlight. Then they were ground to yield powder and the powder was preserved in thimbals for extraction. The powder (800 g) in four thimballs was extracted in a Soxhlet apparatus with petroleum ether (40º - 70º C) and ethyl acetate (EtOAC) sequentially. The extracts were filtered and concentrated to a small volume. This process changes the extracts into dark green liquid.

Then the PE extract was kept undisturbed for a week. No solid was found and then the petroleum ether extract was concentrated in vacuum and was subjected to VLC for separation of different compounds. The eluates from the VLC of PE extracts were monitored by TLC (Stahl, 1996). Fractions of the similar TLC behavior were combined together and were designated as T-1(1-3), T-2(4-10), T-3(11-43) T-4 (44-81), T-5 (82-121), T-6 (122-159), T-7 (160-221), T-8 (222-230).

Fractions T-2, T-4, T-5, T-7, T-8 were analyzed but no satisfactory result was obtained. Fraction T-1 and T-3 were yellow in color, and then fraction T-1 & T-3 were mixed. Upon standing undisturbed at room temperature a white solid appeared. The TLC study in solvent 100% petroleum ether showed the presence of a violet spot on spraying with vanillin-sulphuric acid spray. Its R_f value was 0.90 in solvent 100% petroleum ether and the amount was ~5 mg. The white solid compound was designated as compound-1.The melting point of this compound is 120º C. Its IR, ^{1}H-NMR spectrum was taken and revealed that it was a fat-like compound. Based on the IR, ^{1}H-NMR spectral data, the tentative structure of the compound-1 was determined.

Upon standing the fraction T-6 undisturbed at room temperature for several weeks, crystal appeared at the body of the conical flask. The crystal was white in colour. Then the liquid was removed from the conical flask carefully and the crystals were dissolved in petroleum ether. Its TLC pattern (in solvent 25% EtOAc in petroleum ether) showed a bright violet spot on spraying with vanillin–sulphuric acid reagent followed by heating at 110º C in an oven. Its R_f value was 0.70 in solvent 100% petroleum ether+10 drops EA. The white solid compound obtained upon evaporation of petroleum ether was designated as compound-2. Its IR and ^{1}H-NMR spectrum were taken. Based on the IR, ^{1}H-NMR spectral data, the tentative structure of the compound-2 was determined.

RESULTS AND DISCUSSION

Characterization of compound 1:

The compound-1 was isolated as white crystal. The TLC examination of this compound

(100% pet ether+10 drops ethyl acetate) showed brown coloured single spot in the iodine chamber. The spot turned into violate on spraying with vanillin-H_2SO_4 followed by heating in an oven at 110º C for about 10 minutes.

The analysis of IR spectrum of compound C-1 exhibited a tiny absorption peak at 3050 cm^{-1} was for C=C-H selection (Lambert *et al*). The two absorption peak at 1900 cm^{-1} and 2850 cm^{-1} were due to the presence of aliphatic C-H stretching for –CHO group. A broad peak of 1700 cm^{-1} was suggestive of a >C=O group. The lower frequency of >C=O group due to the >C=C< and –OH in its vicinity .The peak of 1450cm^{-1} was demonstrative of C-H bending of $–CH_2-$ group. The absorption band at 1280 cm^{-1} was due to the C-O stretching. The peak of 960 cm^{-1} 720 cm^{-1} were due to C=C and $–CH_2$ group respectively.

The ^{1}H-NMR (400 MHz, in $CDCl_3$) spectrum of the isolated compound -1 showed a singlet at δ value 9.75 ppm, which was indicative of the presence of aldehydic proton at C-2. The multiplet at δ value 7.3 ppm was indicative of the presence of aromatic proton. The singlet at δ value 7.25 ppm was due to presence of $CHCl_3$ proton with $CDCl_3$. The spectrum showed a singlet at δ value 5.33 ppm was due to the presence of olefinic protons of substituted methylene at C-1. The singlet at δ value 4.12 ppm was indicative of the H-C-O proton at C-3. Down field chemical shift at 4.1ppm and size –shape of the pick indicative of the position of hydroxyl proton at C-3. The triplet at δ value 2.3 (J = 8 Hz) was indicative of the presence of methine proton at C-2 and C-3. The peak at δ value 2.33 ppm and δ value 2.35 ppm was indicative of the presence of methylene proton at C-4.

Based on the IR, ^{1}H-NMR spectral data, the tentative structure of the compound-1 was proposed as 3-hydroxy-1-methylene-2,3,4,4-tetrahydroxy naphthalene -2-carbaldehyde.

Characterization of compound-2:

The compound-2 was isolated as white crystal. The TLC examination of this compound (100% pet ether+10 drops ethyl acetate) showed brown coloured single spot (Rf value was 0.70) in the iodine chamber. The spot turned into violate on spraying with vanillin-H_2SO_4 followed by heating in an oven at 110º C for about 10 minutes. The analysis of IR spectrum of compound-2 exhibited a broad peak at 3425 cm^{-1} was indicative of the presence of –OH group. The tiny absorption peak at 3000 cm^{-1} in the IR spectrum was indicative of the presence of -C=C-H selection (Lambert et al., 1987).The two absorption peaks at 2900 cm^{-1} and 2850 cm^{-1} were due to the presence of aliphatic C-H stretching for methyl group.The peak at 1600 cm^{-1} was suggestive of a >C=C< double bond in the compound skeleton. The peak at 1450cm^{-1} was demonstrative of C-H bending of methylene group and 1020 cm^{-1} was demonstrative of C=O stretching. The peak at 980 cm^{-1} in IR spectrum was indicative of the presence of steroidal carbon skeleton. (Finar. 1987). The presence of steroid type compound in the sample was also confirmed by the positive reaction of The Liebermann Burchard reaction and The Salkowski reaction. (Finar, 1987). The peak at 825 cm^{-1} in IR spectrum was suggestive of the presence of >C=C< between C-7 and C-8 (Itoh *et al* .1978). The peak at 1250 cm^{-1} was indicative of presence of $–CH(CH_3)_2$ group.

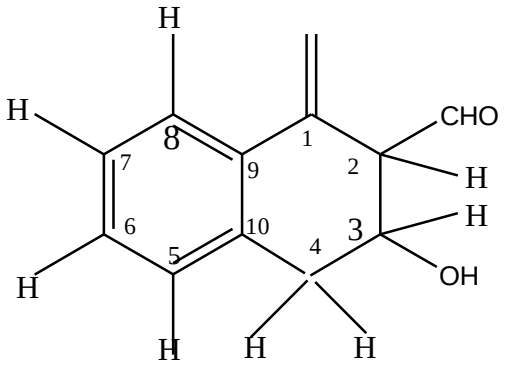

Figure 1: Compound-1 (3-hydroxy-1-methylene-2,3,4,4-tetrahydroxynapthalene-2-carbaldehyde).

The ^{1}H NMR (400 MHz, in CDCl3) spectrum of the isolated compound-2 had a sharp singlet at 0.504 ppm indicative of the three protons of methyl group at C-18. The singlet at 0.812 ppm was indicative of the presence of three protons of methyl group at C-10.

A broad singlet at δ value 5.14 ppm was due to the olefinic proton (7-H) at C-7. Its chemical shift together with its shape and size was indicative of double bond between C-7 and C-8 of a steroidal nucleus. (Rubinstein and Goad, 1974a). A sharp singlet at δ value 3.5 ppm in ^{1}H-NMR spectrum was indicative of the presence of axial proton at C-3 confirming the presence of -

OH at equatorial position. The multiplet at δ value 5.1ppm (J = 8 Hz) was demonstrative of the presence of olefinic proton at C-7.

The peak at δ value 3.5 ppm was due to the proton at C-23. The decreased intensity of the peak was due to influence of alkyl groups in its close proximity. The triplet at δ value 4.1 ppm (J = 8 Hz) was demonstrative the presence of oxygenated methine proton at C-23.

The peak at δ value 4.3 (J = 6 Hz) was indicative of the presence of proton at C-22. The doublet at δ value 1.0 (J = 8 Hz) was due to the presence of proton at 21-Me. The doublet at δ value 0.8 (J = 8 Hz) was due to the presence of proton at 26-Me.The triplet at δ value 0.9 (J = 8 Hz) was due to the presence of proton at 27- Me. The singlet at δ value 0.53 ppm and 0.78 ppm were due to the presence of proton at 18 -Me and 19-Me respectively.

Based on the IR, ^{1}H-NMR spectral data, the tentative structure of the compound-2 was proposed as 22, 23-dihydroxy spinasterol.

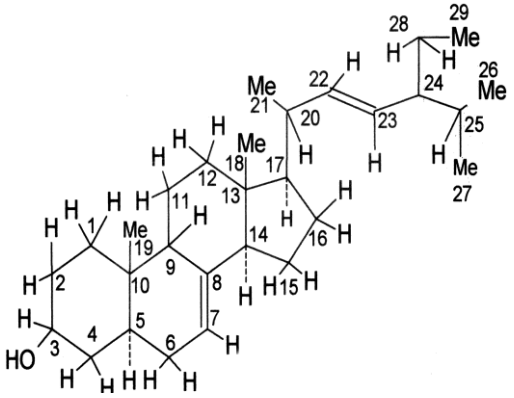

Figure -2: Compound-2 (22, 23-dihydroxy spinasterol).

Antimicrobial screening

Bacteria and fungi are responsible for many infectious diseases. The increasing clinical implications of drug resistant fungal and bacterial pathogens have lent additional urgency to antimicrobial drug research. The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the *in vitro* fungal and bacterial growth. The disc diffusion (Bayer *et al*., 1966) is a widely accepted *in vitro* investigation for preliminary screening of test agents which may possess antimicrobial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bacteriocidal activity can be made by this method.

The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both Gram positive and Gram-negative organisms were taken for the test and they are listed in the Table 1.

The following media is used normally to demonstrate the antimicrobial activity and to make subculture of the test organisms: Nutrient agar medium (Bacto peptone 0.5 g; Sodium chloride 0.5 g; Bacto yeast extracts 1.0 g; Bacto agar 2.0 g; Distilled water q.s. to 100 ml pH 7.2 ± 0.1 at 25º C), Nutrient broth medium (0.3 g Bacto beef extract, 0.5 g Bacto peptone, Distilled water q.s. to 100 ml, pH :7.2 ± 0.1 at 25º C); Muller – Hunton medium (Beef infusion 30 g, Casamino acid 1.75 g, Starch, 0.15 g, Bacto agar 1.70 g, Distilled water q.s. to 100 ml, pH 7.3 ± 0.2 at 25º C), Tryptic soya broth medium (TSB) (Bacto tryptone 1.7 g, Bacto soytone 0.3 g, Bacto dextrose 0.25 g, Sodium chloride 0.5 g, potassium hydrogen phosphate 0.25 gm, Distilled water q.s. to 100 ml, pH 7.3 □ 0.2 at 25º C

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. pH (at 25º C) was adjusted at 7.2 – 7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs. pressure/ sq. inch at 121º C for 20 minutes. The slants were used for making fresh culture of bacteria and fungi that were in turn used for sensitivity study.

Preparation of sample discs with test samples: Measured amount of each test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank

petridish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

Table 1: Antimicrobial activity of the Petroleum ether crude extract (PE) of the fruits of *Luffa cylindrica*,

Test bacteria and fungi	PE	Kanamycin
Gram positive bacteria		
Bacillus cereus (BTCC-19)	7 ± 0.50	28 ± 0.52
Bacillus megaterium (BTCC-18)	7 ± 0.53	25 ± 0.48
Bacillus subtilis	7 ± 0.54	27 ± 0.50
Staphylococcus aureus (BTCC-43)	7 ± 0.55	28 ± 0.51
Sarcina lutea (ATCC-9341)	8 ± 0.66	27 ± 0.49
Gram negative bacteria		
Escherichia coli (BTCC-172)	7 ± 0.45	27 ± 0.25
Salmonella paratyphi	7 ± 0.43	22 ± 0.31
Salmonella typhi	7 ± 0.46	25 ± 0.38
Shigella boydii	8 ± 0.52	25 ± 0.37
Shigella dysenteriae	7 ± 0.45	27 ± 0.22
Vibrio mimicus	6 ± 0.35	25 ± 0.36
Vibrio parahemolyticus	10 ± 0.65	25 ± 0.35
Fungi		
Candida albicans	7 ± 0.46	27 ± 0.25
Aspergillus niger	8 ± 0.53	25 ± 0.23
Sacharomyces cerevisiae	No activity	25 ± 0.22

PE tested at 500 and 30 µg/disc respectively. mm ± SEM.

Preparation of sample discs with test samples of *Luffa cylindrica:* Petroleum ether extract were tested for antimicrobial activity against a number of both gram positive and gram negative bacteria and fungi.

Test sample for crude extracts: The amount of sample per disc was 500 g.

Preparation and application of the test samples: the test sample was weighed accurately and calculated amounts of the solvents were added accordingly using micropipette to the dried samples to get desired concentrations. The test sample was applied to previously sterilized discs using adjustable micropipette under aseptic conditions.

The antimicrobial potency of the test agent is measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

RESULTS AND DISCUSSION

The crude extract was tested for antibacterial and antifungal activities against a number of Gram-positive bacteria, Gram-negative bacteria and fungi respectively. Standard disc of kanamycin (30 µg/disc) was used. Petroleum ether crude extract exhibited mild to moderate antimicrobial activity against most of the test organisms (Table 1).

The zone of inhibition produced by crude petroleum extract was found to be 7 – 10 mm at a concentration of 500 µg/disc. The crude petroleum extract was screened against 12 test bacteria and 3 fungi. This extract showed moderate activity against the all the test bacteria *Bacillus cereus* (BTCC-19), *Bacillus megaterium* (BTCC-18), *Bacillus subtilis, Staphylococcus aureus* (BTCC-43), *Sarcina lutea* (ATCC-9341), *Escherichia coli* (BTCC-172), *Salmonella typhi, Pseudomonas aeruginosa, Salmonella paratyphi, Shigella dysenteriae, Vibrio mimicus, Vibrio parahemolyticus* and the fungus *Candida albicans, Aspergillus niger, Aspergillus niger.* On the other hand, the fungi *Sacharomyces cerevisiae* was found to be resistant (Table 1).

CONCLUSION

The phytochemical study of the petroleum ether extractives of the *Luffa cylindrica* afforded two purified compounds, 3-hydroxy-1-methylene-2,3,4,4-tetrahydroxynapthalene-2-carbaldehyde (1), 22,23-dihydroxy spinasterol (2) (first reported compounds from this species) whose structures were established by extensive spectroscopic studies as well as comparison with published results. The bioactivities exhibited by different extractives of *L. cylindrica*. substantiate the folk uses of this plant species in various diseases.

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