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Phytochemical and Antimicrobial Activities of the Soapnut Saponin and its Derivatives from the *Sapindus mukurossi*

D. Rama Sekhara Reddy*, D. Mallika, G. Damodar Reddy and Y.L.N. Murthy
Department of Organic Chemistry, Andhra University, Visakhapatnam-530 003, India
E-mail: dachuru@gmail.com

The phytochemical study of the soapnuts of *Sapindus mukurossi* revealed the presence of saponins from water extract. The saponins and its derivatives are identified based on their melting points, IR, ^{1}H and ^{13}C NMR spectral data, as saponin, saponin acetate, saponin methyl ester, saponin methyl ester acetate, hederagenin. The antimicrobial activities of the saponin and its derivatives were investigated. The saponin and its derivatives were tested for their antibacterial activities against gram positive, gram negative organisms and fungi.

Key Words: *Sapindus mukurossi*, Saponins and its derivatives, Antimicrobial activity.

INTRODUCTION

Soapnut is the fruit of trees belonging to the genus *Sapindus* of about 12 species of shrubs and trees in the family Sapindaceae[1]. Soapnuts are the fruits derived from *Sapindus mukurossi*. *S. mukurossi* is distributed throughout India. The dried fruit is the most valuable part. Its fleshy pericarp contains saponins in high yields and it is a good substitute for washing soaps, detergents and also for quality shampoos. It is used for treating diseases like common cold, pimples, epilepsy, nausea and acts as expectorant and antihelmenthic in small doses.

Sarin and Bari[2] isolated the saponin from *S. mukurossi*. Row and Rukmini isolated the saponins of *S. mukurossi*[3] and *S. emerginatus*[4], named mukurosside and emergentoside. Chirva *et al.*[5] reported the isolation and structure elucidation of several saponins of hederagenin including sapindosides from the Japanese crude drug "Enmei-hi". Recently the saponins of *S. mukurossi* were reported to show molluscidal effects against golden apple snails[6]. From the foregoing studies, it is suggested that the saponins of soapnuts constitute a readily and abundantly available natural source having potential for drug development. With this aim the isolation of the saponin, identification of the sapogenin part of the saponin, preparation of derivatives of the saponin and to study their antimicrobial activities were undertaken.

EXPERIMENTAL

The soapnuts of *S. mukurossi* were taken, dried the pericarp and it was extracted with hot water. The procedure used as reported by Row and Rukmini[3]. The water extract was concentrated and the saponin was precipitated from the concentrate by the addition of solid $(NH_4)_2SO_4$, dried at 70 °C in an oven for 6 h and finally under

vacuum for 6 h at 60 °C. The saponin (S-01) was obtained as pale brown powder in 17 % yield, m.p. 156 °C. The saponin showed a single spot (R_f: 0.32) in butanol:acetic acid:water (4:1:5) on Whatman No. 1 filter paper and the spots developed by sodium meta periodate in alkali solution.

Isolation of saponin methyl ester (S-02): A solution of the saponin (28 g) in MeOH (70 mL) was treated with an ethereal solution of diazomethane (100 mL containing 10 g of CH_2N_2) and left overnight in a refrigerator. Evaporation of the solvent gave the methyl ester as a gum. This was dried in vacuum at 60 ºC, a pale brown powder was obtained with m.p. 149 ºC and R_f: 0.36 in butanol:acetic acid: water (4:1:5).

Isolation of saponin acetate (S-03): The saponin (2 g) was acetylated with acetic anhydride (5 mL) in pyridine (5 mL) for 12 h at room temperature. Water (20 mL) was then added to the contents when the acetate comes out as a white solid (4 g) with m.p. 118 ºC and R_f: 0.76 (9:1 $CHCl_3$:MeOH).

Isolation of saponin methyl ester acetate (S-04): The above methyl ester (7.72 g) was treated with acetic anhydride (10 mL) and pyridine (5 mL) for 12 h at room temperature. Water (100 mL) was then added when the sapoin methyl ester acetate come out as a white solid (14 g) with m.p. 109 ºC and R_f: 0.86 (9:1 $CHCl_3$: MeOH).

Isolation of hederegenin (S-05): The saponin (10 g) was hydrolyzed with 80 % methanolic benzene 200 mL and 2 N HCl (250 mL) under reflux for 6 h. Two layers separated out and a colourless crystalline solid separated at the top minor layer. This layer (30 mL) was separated and filtered to give the sapogenin as colourless shining microprisms (1.23 g) with m.p.315 ºC and R_f: 0.41 (9:1 $CHCl_3$: MeOH).

IR spectra were taken on Thermo Nicolet Avatar-320 instrument in KBr or $CHCl_3$. ^{1}H NMR spectra were taken at 300 MHz in a Jeol JNM-FX-300 spectrometer and chemical shifts are given as δ (ppm) with tetramethyl silane (TMS) as an internal standard. ^{13}C NMR spectra were taken at 75 MHz with a Jeol JNM-FX-300 spectrometer in pyridine d_5 using TMS as an internal standard and chemical shifts are given as δ (ppm). TLC was done in $CHCl_3$:MeOH system and spots were developed with methanolic H_2SO_4 spray.

Characterization of compounds

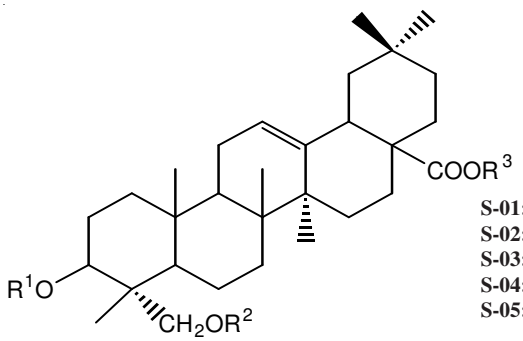
Saponin (S-01): It was crystallized from MeOH as light brown prisms, m.p. 156 ºC. Its IR spectrum exhibit the bands at ν_{max}: 3445, 3191, 1731, 1664, 1371, 1247, 1079, 1029 and 817 cm^{-1}. The ^{1}H NMR (300 MHz, D_2O) showed peaks at δ 5.41 d, 4.80 bs, 4.21 d, 3.33 t, 2.14 m, 1.73 s, 1.66 s, 1.29 s, 1.15 s, 0.9 s, 7.5 s and ^{13}C NMR (75 MHz, D_2O): δ 101.7, 75.7, 71.8, 70.3, 69.0, 68.3, 60.7, 20.4, 16.8, 16.5, 15.7 and 14.1. From the above data it was confirmed as saponin (Fig. 1).

Saponin methylester (S-02): It was crystallized from MeOH as pale brown prisms, melting point 149 ºC. Its IR spectrum exhibit the bands at ν_{max}: 3420 (-OH), 1661 (C=C), 1524 (ester) cm^{-1}. From the above data it was confirmed as saponin methyl ester (Fig. 1).

Saponin acetate (S-03): It was crystallized from MeOH as pale yellopw prisms, melting point 118 ºC. Its IR spectrum exhibits the bands at ν_{max}: 1743, 1372, 1230 cm^{-1}. ^1H NMR (300 MHz, D_2O) showed peaks at δ 5.471 d, 4.80 bs, 4.4B d, 4.29d, 1.39 s, 1.33 s, 1.09 s, 0.99 s, 0.96 s, 0.93 s and ^{13}CN MR (75 MHz, D_2O): δ 170.75, 150.3, 124.4, 104.9, 99.6, 84.3, 83.2, 78.0, 77.3, 21.35, 18.43, 17.61, 15.10, 13.74. From the above data it was confirmed as saponin acetate (Fig. 1).

Saponin methyl ester acetate (S-04): It was crystallized from MeOH as pale yellow prisms, m.p. 109 ºC. Its IR spectrum exhibit the bands at ν_{max}: 1743 (esters), 1661 (C=C) cm^{-1}. From the above data it was confirmed as saponin methyl ester acetate (Fig. 1).

Hederagenin (S-05): It was obtained from MeOH as colourless shining micro prisms, m.p. 315 ºC. Its IR spectrum exhibits the bands at ν_{max}: 3633 (OH), 3404 (Acid OH), 1727 (C=O) cm^{-1}. ^1H NMR (300 MHz, d_5-pyridine) showed peaks at δ 5.51 t (H-12), 4.2 bs (H-3), 3.73 d (H-23), 3.20 d (H-23), 1.19 s, 1.15 s, 1.06 s, 1.02 s, 0.99 s, 0.96 s and ^{13}C NMR (75 MHz, d_5-pyridine): C1-C30, δ 38.5, 27.3, 73.2, 42.6, 48.3, 18.3, 32.7, 39.5, 47.9, 37.0, 23.5, 122.3, 144.0, 41.9, 28.1, 23.4, 46.4, 41.7, 46.2, 30.7, 34.0, 33.1, 67.6, 12.8, 15.7, 17.2, 26.6, 179.9, 32.9, 25.9. These NMR data were found to be identical with data reported for hederagenin (S-05)[7,8].



S-01: R^1 = sugars; $R^2 = R^3 = H$
S-02: R^1 = sugars; R^2 = H; $R^3 = CH_3$
S-03: R^1 = acetylated sugars; $R^2 = COCH_3$; R^3 = H
S-04: R^1 = acetylated sugars; $R^2 = COCH_3$; $R^3 = CH_3$
S-05: $R^1 = R^2 = R^3 = H$

Fig. 1. Chemical structure of isolated compound and its derivatives from *Sapindus mukurossi*

Antibacterial activity: The bacterial strains obtained from Department of Pharmacy, Andhra University, Visakhapatnam. The water extracted compound saponin and its derivatives were obtained from *Sapindus mukurossi* and screened for their antibacterial activity against *B. pumilis, B. subtilis, E. coli* and *Proteus vulgaris*. The compounds were dissolved in DMSO to determine the activity. Cup plate agar diffusion method[9] was used to determine the zone of inhibition of the extracts. For comparison the penicillin (100 µg/mL) was used as a standard.

Solutions of the test compound at a concentration of 1000 µg/mL were prepared and 50 µL of each solution was placed in the cups by means of a sterile micropipette. The plates thus prepared were left for 1 h at room temperature for diffusion. After incubation for 24 h at 31 °C they were examined for the respective zone of inhibition.

Antifungal activity: The fungal strains obtained from Department of Pharmacy, Andhra University, Visakhapatnam. The saponins and their derivatives tested for antifungal activity against *Aspergillus niger* and *Rhizopus oryzae*. The antifungal activity was tested in the same way as antibacterial activity by using the fungal organisms and for comparison the ketoconazole (100 µg/mL).

RESULTS AND DISCUSSION

Chemical analysis of *Sapindus mukurossi* soapnuts revealed the presence of saponins. No attempts have been made to identify the no. of sugar units present in the saponin (S-01). Thus the saponin isolated in the present study was a hederagenin saponin. The saponin was converted into its methyl ester, its acetate, methyl ester acetate and the hydrolysis product. The saponin and its derivatives were tested for their antibacterial activities against four bacteria and the results were shown in Table-1. In the case of antifungal activity against two fungi and the results were shown in Table-2.

TABLE-1
ANTIBACTERIAL ACTIVITY OF EXTRACTS OF THE *S. mukurossi* SOAPNUTS
(All the compounds were tested at 1000 µg/mL concentration)

S. No.	Name of compound	Test organisms and zone of inhibition (mm)			
		Gram positive bacteria		Gram negative bacteria	
		B.P.	B.S.	E.C.	P.V.
S-01	Saponin (1000 µg/mL)	0	12	0	18
S-02	Saponin methyl ester (1000 µg/mL)	14	18	20	16
S-03	Saponin acetate (1000 mg/mL)	0	0	9	0
S-04	Saponin methyl ester acetate (1000 mg/mL)	0	0	13	0
S-05	Hederagenin (1000 mg/mL)	0	0	9	0
S-06	Pencillin (1 µg/mL)	15	14	14	14

Cup diameter = 6 mm; B.P. = *Bacillus pumilis*; B.S. = *Bacillus subtilis*;
E.C. = *Escherichia coli*; P.V. = *Proteus vulgaris*

TABLE-2
ANTIBACTERIAL ACTIVITY OF EXTRACTS OF THE *S. mukurossi* SOAPNUTS

S. No.	Extract and standards	Test organisms and zone of inhibition (mm)	
		Aspergillus niger	*Rhizopus oryzae*
1.	Chloroform extract (300 mg/mL)	–	–
2.	Chloroform extract (300 mg/mL)	–	–
3.	Methanolic extract (100 mg/mL)	13	11
4.	Methanolic extract (300 mg/mL)	14	14
5.	Ampicillin (1 µg/mL)	18	18

Cup diameter = 6 mm

In conclusion present results have shown that the soapnut saponin and its derivatives of *Sapindus mukurossi* possess antibacterial and antifungal activity. The soapnut saponins seem to have a promising value for the development of potent

phytomedicine for microbes. Further comprehensive pharmacological investigations are needed to elucidate the exact mechanism of the antimicrobial effect of *S. mukurossi*. The saponin showed antibacterial activity against *Bacillus subtilis*, *Proteus vulgaris* and antifungal activity against *Rhizopus oryzae*. The saponin methyl ester showed activity against all organisms tested. The saponin acetate and the saponin methyl ester acetate showed identical activity and showed antibacterial activity against *P. vulgaris* and antifungal activity against *R. oryzae*. All the compounds were tested at 1000 µg/mL concentration. The present investigation showed that the saponin and its derivatives have antimicrobial activities.

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