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# THE LIPID CONTENT AND MOLLUSCICIDAL SAPONINS OF *TETRAPANAX PAPYRIFERUS* KOCH.

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**ABSTRACT**

The unsaponifiable part of the petroleum ether soluble fraction of the leaves of *Tetrapanax Papyriferus* was analysed by GLC to reveal the presence of a series of hydrocarbons from $C_{16}$ to $C_{30}$, cholesterol, campesterol, stigmasterol and β-sitosterol. Sixteen fatty acids were identified in the saponifiable part. The butanolic fraction was chromatographed on silica gel column and preparative TLC to afford three monodesmosidic triterpenoid saponins. The structures of these saponins have been established by FAB-MS, IR and $^1$HNMR as well as on the basis of acidic hydrolysis. The three isolated saponins showed high molluscicidal activity against *Biomphalaria alexandrina*, the intermediate host of *Schistosoma mansoni* in Egypt ($LC_{90}$ = 9, 14 and 22 ppm).

## INTRODUCTION

Schistosomiasis is among the most important health problems in many countries. There are great expectations in the potential control of this tropical parasitic disease, by interruption of its life cycle, by elemination of its snail vector using a safe, non-toxic and inexpensive molluscicides[1-3].

Plant molluscicides have been emerged as available alternative for this purpose[4-7]. Since the discovery of saponins as the potent molluscicidal principles in the fruits of *Phytolacca dodecandra*, several saponins and plant extracts have been evaluated for molluscicidal activity [8-12].

Plants of the family Araliaceae have been subject of considerable phytochemical investigations due to the therapeutic activity of many of these plants. Triterpenoid saponins of dammarane and oleanane skeletons are considered to be responsible for their biological activity [13-15].

In the course of our systematic screening studies of medicinal plants for molluscicidal activity[16-19], it was observed that the methanolic extract of *Tetrapanax papyriferus* (Araliaceae) showed an activity of 200 ppm within 24 hours against *Biomphalaria alexandriana*, the intermediate host of *Schistosoma mansoni* in Egypt. Meanwhile, screening the available literatures showed that nothing were reported on the lipid and molluscicidal saponin contents of *T. papyriferus* leaves. Therefore, the present work deals with the study of the lipid content and isolation of some molluscicidal saponins from this plant.

## EXPERIMENTAL

### General Experimental Procedures :

Melting points were uncorrected. The EI-MS were recorded on MAT-95 high-resolution mass specterometer using a direct inlet. FAB-MS were determined in a thioglycerol materix bombardment with Xenon gas. The $^1$HNMR were measured on a Varian 270 MHz Spectrometer using TMS as internal standard and chemical shifts are expressed in ppm. IR spectra were recorded on a Perekin-Elmer model FT-IR recording spectrophotometer.

TLC was performed using silica gel 60 $GF_{254}$. Spots were visualized by spraying with 40% $H_2SO_4$ followed by heating at 120 °C.PC of sugars were run on a Whatmann No. 1 paper using solvent system IV and detected with aniline-phthalate.

Column chromatography was performed using glass column (5 x 120 cm) using silica gel as stationary phase. For chromatographic studies the following systems were used :

I) $CHCl_3$ : MeOH : $H_2O$ (13 : 7 : 1)
II) $CHCl_3$ : MeOH : $H_2O$ (13 : 7 : 2)
III) $CHCl_3$ : MeOH (19 : 1)
IV) n-BuOH : AcOH : $H_2O$ (4 : 1 : 5)

GLC analysis of the unsaponifiable matter was carried out on a GCV Pye Unicam gas chromatograph equipped with a dual flame ionization detector and a dual channel recorder using a coiled glass column (2.8 m x 4 mm) packed with diatomite C (100-120 mesh) and coated with 1% OV-17. Oven temperature was programmed at 10°C/min from 70 - 270°C then run isothermally at 270 °C for 25 min. Nitrogen flow was 30ml/min. Detector and injector temperatures were generally 300 °C and 280 °C respectively. Hydrogen and air flow rates were 33 and 330 ml/min, respectively.

GLC analysis of the fatty acid methyl esters was performed adopting the previously mentioned conditions except the following :

A coiled glass column (1.5m x 4 mm) packed with 10% polyethylene glycol adiate (PEGA) on diatomite C (100 -120 mesh). Nitrogen was used as a carrier gas at a flow rate of (30 ml/min). The oven temperature was programmed at 8°C/min from 70 °C to 190 °C, then kept isothermal at 190 °C for 30 min.

**Plant Meterial :**

The leaves of *Tetrapanax papyriferus* Koch (Araliaceae) were collected in July 1995 from the Botanical Garden of El-Zohria, Cairo, Egypt. The plant was kindly authenticated by Eng. Badia H. Diwan (Agriculture Engineer of Orman Garden). The leaves were shade dried and powdered by electrical mill.

**Investigation of The Lipid Content :**

The air-dried leaves of *Tetrapanax papyriferus* (1.0 kg) were extracted with methanol (4 x 3.5 L.) at room temperature. The methanolic extract (70 g) was defatted with petroleum ether (60-80 °C) (3 x 200 ml) and then suspended in water and successively partitioned with $CHCl_3$ (4 x 250 ml), EtOAc (3 x 200 ml) and n-BuOH (4 x 200 ml) to yield 12, 3, 6 and 26 g respectively.

The petroleum ether extract (12 g) was saponified with methanolic potassium hydroxide (10%) for 8 hours. The unsaponifiable fraction (USM) and fatty acid methyl esters (FAME) were prepared (20-22). GLC analysis of USM and fatty acid methyl esters (FAME) of the leaves were carried out adopting the conditions mentioned before. The identification of USM and FAME were carried out by comparing their retention times with those of the available references . The results are listed in Tables 1 and 2 .

**Extraction and Isolation of Saponins :**

The butanolic fraction (26g) was chromatographed on silica gel column using a gradient $CHCl_3$-MeOH as eluent. Repeated column chromatograhy yielded three major fractions.Fractions eluted with $CHCl_3$:MeOH (70: 30), (40 : 60) and (10 : 90) contained saponins 1, 2 and 3 respectively .

**Saponin 1** was obtained by recrystallization from methanol whereas saponins 2 and 3 were purified by preparative TLC using solvent systems I and II respectively.

**Saponin 1**: was obtained as a white powder; mp 232-234°C, $R_f$ 0.36 (solvent system I). IR (KBr) $cm^{-1}$ ; 3415 (OH), 2927, 1695 (COOH), 1632, 1385, 1070, 1020, 785, 582. $^1$HNMR δ 0.78-1.23 (6 Me-groups), 1.59 (3H, s, Rha-Me), 4.82 (1H, d, Ara, H-1), 5.04 (1 H, s, Rha, H-1) and 5.16 (1H, s, H -12). FAB-MS (negatigve-mode); m/z 749 $(M-H)^-$, 603 $(M-H-Rha)^-$ and 471 $(M-H-Rha-Ara)^-$.

**Saponin 2**: white amorphous powder, mp 239-242 °C, $R_f$ 0.28 (solvent system I ). IR (KBr) $cm^{-1}$; 3412 (OH), 2932,1692 (COOH), 1635, 1385, 1075, 1000, 787, 581. $^1$HNMR δ 0.79-1.24 (6 Me-groups), 1.62 (3 H, d, Rha-Me), 4.84 (1 H, s,Ara, H-1), 4.98 (1H, d, Glc,H-1), 5.09 (1H, s, Rha, H-1) and 5.18 (1H, s, H-12). FAB-MS (negative-mode) m/z 911 $(M-H)^-$, 749 $(M-H-Glc)^-$, 603 $(M-H-Glc-Rha)^-$ and 471 $(M-H-Glc-Rha-Ara)^-$ .

**Saponin 3** : white powder, mp 232-233 °C, $R_f$ 0.25 (solvent system II). IR (KBr) $cm^{-1}$, 3400 (OH), 2931, 1690 (COOH), 1640, 1381, 1080, 781, 578. $^1$HNMR δ 0.76-1.21(7 methyl groups), 4.70 (1 H, d, GlcA, H-1), 4.80 (1H, d, Glc, H-1), 4.96 (1H, s, Glc, H-1) and 5.17 (1H, d, H-12). FAB-MS (negative-mode) m/z 955 $(M-H)^-$, 793 (M-H-Glc), 631 $(M-H-2 x Glc)^-$ , 617 $(M-H-Glc-GlA)^-$ and 455 $(M-H-2 x Glc-GlcA)^-$ .

**Acid Hydrolysis of Saponins 1-3 :**

Each saponin (30 mg) was refluxed with 7% $H_2SO_4$ in 50% aqueous ethanol for 6 hours. The reaction mixture was diluted with water and extracted with chloroform (4 x 100 ml). The chloroformic extract was evaporated to dryness to afford the aglycone part. Each aglycone was identified by direct comparison with authentic samples (mp, mmp, co-tlc using solvent system III) and through its mass fragmentation. Saponin 1 and 2 gave hederagenin as aglycone; mp 318-320 °C, MS; m/z 472 $(M^+)$, 454 $( M-H_2O )^+$, 426 $(M^+-HCOOH)^+$, 395 $(M^+-HCOOH- CH_2OH)^+$, 248, 233, 203, 189 and 169. Saponin 3 yield oleanolic acid , mp. 304-306 °C, MS; m/z 456 $(M^+)$, 438 $(M^+-H_2O)^+$, 423 $(M^+-CH_3-H_2O)^+$, 248, 207, 203, 189 and 133.

The aqueous layer was neutralized with barium carbonate and filtred and the filtrate was evaporated to dryness. The residue was extracted with pyridine. The residue after distilling off pyridine was dissolved in 10% isopropanol and compared with standard sugars on PC (solvent system IV).

**Test For Molluscicidal Activity :**

*Biomphalaria alexandrina* snails were collected from irrigation canals previously untreated with any molluscicide in Abou-Rawash ten Kilometers from Giza Governorate (Egypt) and kept in aquaria with a continuous changing of dechlorinated water for three weeks . The tests were carried out by placing ten snails in water with known concentrations of the tested material. Control groups of snails were placed in dechlorinated water. The exposure time was 24 hours folowed by 24 hours as recovery period. Statistical analysis of the data was done according to Litchfield and Wilcoxon method [23].

Table (1): GLC analysis of USM of lipid content of *Tetrapanax papyriferus* leaves.

| Peak No | $RR_t$ | Relative % | Authentic |
|---|---|---|---|
| 1 | 0.126 | 1.31 | n-Hexadecane |
| 2 | 0.183 | 3.38 | n-Heptadecane |
| 3 | 0.250 | 2.06 | n-Octadecane |
| 4 | 0.273 | 0.125 | n-Nonadecane |
| 5 | 0.285 | 3.83 | n-Eicosane |
| 6 | 0.313 | 0.88 | Unknown |
| 7 | 0.405 | 4.79 | n-Heneicosane |
| 8 | 0.434 | 2.11 | n-Docosane |
| 9 | 0.468 | 2.62 | n-Tricosane |
| 10 | 0.493 | 3.05 | n-Tetracosane |
| 11 | 0.558 | 20.65 | n-Hexacosane |
| 12 | 0.633 | 3.82 | n-Octacosane |
| 13 | 0.691 | 2.52 | n-Nonacosane |
| 14 | 0.729 | 0.69 | Squalene |
| 15 | 0.747 | 1.62 | n-Triacontane |
| 16 | 0.827 | 1.24 | Cholesterol |
| 17 | 0.840 | 2.65 | Campesterol |
| 18 | 0.869 | 16.38 | Stigmasterol |
| 19 | 1 | 24.26 | β-Sitosterol |

$RR_t$: Retention time relative to β-Sitosterol $R_t$ = 22.29

Table (2): GLC analysis of the fatty acid methyl esters of lipid content of *Tetrapanax ppyriferus* leaves.

| Peak No | $RR_t$ | Relative % | Authentic |
|---|---|---|---|
| 1 | 0.52 | 0.85 | Capric acid |
| 2 | 0.59 | 0.06 | Unknown |
| 3 | 0.60 | 0.07 | Hendecanoic acid |
| 4 | 0.69 | 0.82 | Lauric acid |
| 5 | 0.72 | 0.45 | Unknown |
| 6 | 0.73 | 0.22 | Lauroleic acid |
| 7 | 0.81 | 0.32 | Tridecanoic acid |
| 8 | 0.86 | 3.60 | Myristic acid |
| 9 | 0.90 | 1.92 | Myristolic acid |
| 10 | 0.91 | 1.62 | Unknown |
| 11 | 0.96 | 4.65 | Pentadecanoic acid |
| 12 | 1.00 | 35.60 | Palmitic acid |
| 13 | 1.08 | 11.75 | Palmitoleic acid |
| 14 | 1.14 | 2.08 | Margaric acid |
| 15 | 1.17 | 9.36 | Stearic acid |
| 16 | 1.20 | 4.82 | Oleic acid |
| 17 | 1.28 | 0.35 | Unknown |
| 18 | 1.43 | 8.20 | Linoleic acid |
| 19 | 1.62 | 12.02 | Arachidic acid |
| 20 | 1.76 | 0.34 | Behenic acid |

$RR_t$: Retention time relative to methyl palmitic acid $R_t$ = 14.32

## RESULTS AND DISCUSSION

The petroleum ether soluble fraction of the methanolic extract of *Tetrapanax papyriferus* leaves was saponified[20,21] with alc. KOH. Both the unsaponifiable matter (USM) and fatty acid methyl esters (FAME) were subjected to GLC analysis.

Results of GLC analysis of USM (Table 1) revealed that the major hydrocarbons was n-hexacosane (20.65%) and the lowest was -n-nonadecane (0.125 %).

The sterol mixture was represented by cholesterol, campesterol, stigmasterol and β-sitosterol where β-sitosterol recorded high percent (24.26 %). On the other hand, GLC analysis of the fatty acid methyl esters (Table 2) showed twenty components; Sixteen of them were identified. Palmitic acid was the dominent one (35.60%) and the lowest was hendecanoic acid (0.07%).

Column chromatography and preparative TLC of the butanolic fraction afforded three compounds. All the isolated compounds showed positive reactions to the Liebermann-Burchard and Molish tests as well as they formed a froth when shaken with water.

**Saponin 1**: was obtained as a white powder, mp 232-234 °C. The IR spectrum of this compound revealed the presence of hydroxyl group at 3415 $cm^{-1}$, free carboxylic group at 1695 $cm^{-1}$, double bond at 1632 $cm^{-1}$, and a characteristic[24,26] glycosidic linkage at 1070 -1020 $cm^{-1}$. The negative -ion FAB-MS spectrum of saponin 1 showed a deprotonated ion peak (M-H)⁻ at m/z 749 indicating its molecular weight as 750. Other fragments at m/z 603 (M-H- Rha)⁻ and m/z 471 (M-H-Rha-Ara)⁻ corresponded to the subsequent loss of a rhamnose moiety and an arabinose moiety from the deprotonated ion peak. This fragmentation pattern indicated clearly that rhamnose was the terminal sugar and the arabinose was the inner sugar [27-30]. Also, the $^1$HNMR spectrum showed two well resolved anomeric sugar protons at δ 5.04 and δ 4.82 for rhamnosyl and arabinosyl units respectively. The definite signals for six tertiary methyl groups of the aglycone appeared between δ 0.78 and 1.23, a characteristic of vinylic proton of H-12 at δ 5.16, a signal of secondary methyl group of rhamnosyl moiety appeared at δ 1.59 [30-33].

Hydrolysis of compound 1 under acidic condition afforded an aglycone which was identified as hederagenin by direct comparison with an authentic sample on TLC using solvent system III and by comparing its mass fragmentation m/z 472, 454,426,408, 395,248,233,203, 189 and 169 with published values [26,34]. The sugars obtained from the hydrolysates were identified as L-arabinose and L-rhamnose on paper chromatography by comparison with authentic samples using solvent system IV. On the other hand, the results of biological testing demonstrated that saponin 1 have high molluscicidal activity against Schistosomiasis-transmitting snails;

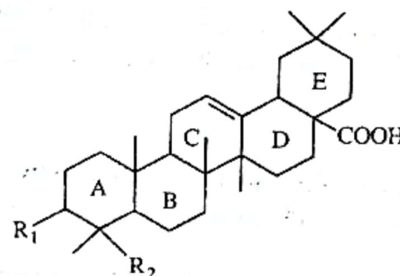
*Biomphalaria alexandrina* ($LC_{90}$ = 9 ppm) after an exposure 24 hours.

This result led to the hypothesis that saponin 1 is monodesmosidic triterpenoid saponin [6,35-39]. On the basis of the above studies, saponin 1 was characterized as 3-0- disaccharide of hederagenin possessing one arabinose unit and one rhamnose unit.

**Saponin 2:** was obtained as a white amorphous powder from methanol with mp 239-242 °C. Its IR spectrum showed absorption of hydroxyl groups at 3412 $cm^{-1}$, double bond at 1635 $cm^{-1}$ and glycosidic linkage at 1075-1000 $cm^{-1}$ [24-26]. The $^{1}$HNMR spectrum[30-33] of this compound revealed six tertiary methyl signals of the aglycone between δ 0.79-1.24, one olefinic proton signal of H-12 at δ 5.18 as well as three anomeric proton signals of sugar moiety δ 5.09, 4.98 and 4.84. On the other hand, negative -ion FAB-MS spectrum of this compound exhibited the deprotonated molecular ion peak at m/z 911 $(M-H)^-$ indicating its molecular weight as 912. Fragments at m/z 749 $(M-H-Glc)^-$, 603 $(M-H-Glc-Rha)^-$ and 471$(M-H-Glc-Rha-Ara)^-$ indicating that a glucose unit is terminal and it is linked to an inner rhamnose molecule which is linked to arabinose and the arabinose moiety is attached to the C-3 of hederagenin [27,28].

**Saponin 2** on acidic hydrolysis gave an aglycone which was identified as hederagenin by comparison with authentic sample. Three monosaccharides; D-glucose, L-rhamnose and L-arabinose were identified in the hydrolysis solution through comparison with authentic samples on PC (solvent system IV). Moreover, the high molluscicidal activity of this saponin as shown in Table 3 against *B. alexandrina* snails was $LC_{90}$ = 14 ppm. Thus, saponin 2 is monodesmosidic triterpenoid saponin [6, 53] and its structure was elucidated as 3-0-trisaccharide of hederagenin having glucose, rhamnose and arabinose as a linear chain.

**Saponin 3** was obtained as white powder with IR spectrum showing hydroxyl groups absorptions at 3400 $cm^{-1}$, carboxylic group at 1690 $cm^{-1}$, C=C absorptions at 1640 $cm^{-1}$ in addition to the characteristic glycosidic absorption at 1025-1080 $cm^{-1}$ [23,39]. The FAB-MS spectrum (negative-ion mode) of this compound exhibited a deprotonated molecular ion peak at m/z 955 $(M-H)^-$ indicating a molecular weight as 956. Other important fragments at m/z 793 $(M-H-Glc)^-$ and 631 $(M-H-2x\ Glc)^-$ corresponded to the simultaneous elemination of two glucose units from the deprotonated ion. Additional fragments at m/z 617 $(M-H-Glc-GlcA)^-$ and m/z 455 (M-H- 2x Glc-GlcA). This showed that the two glucose units are terminal and they are linked to a glucuronic acid which attached to oleanolic acid at position -3 of the aglycone[30,40,41]. The anomeric proton configurations of the sugars of this saponin were fully defined by $^{1}$HNMR spectral at δ 4.70, 4.80 and 4.96 for one glucuronic acid as well as two glucose units respectively [41-43]. Other signals of olefinic proton of H-12 appeared at δ 5.17, seven tertairy methyl groups of oleanolic acid appeared at δ 0.76-121 beside the appearing of the characteristic glycosidic linkage at 1025-1080 $cm^{-1}$ in IR spectrum [23-26]. Saponin 3 was hydrolysed with acid to yield D-glucose and D-glucuronic acid as sugar moeity by comparison with authentic sugars on PC using solvent system IV as well as an aglycone, mp. 304-306 °C which was identified as oleanolic acid by direct comparison with authentic sample on TLC (solvent system III) and from its mass fragmentation m/z 456, 438, 411, 248, 207, 203, 189 and 133 [26,44]. On the other hand, saponin 3 killed *B. alexandrina* snails within 24 hours at a concentration 22 ppm. Therefore, this compound was monodesmosidic triterpenoid saponin [6,35]. Thus the structure of saponin 3 was proved to be 3-0-trisaccharide of oleanolic acid where the sugar moiety contain two glucose units and one glucuronic acid as branched chain.




| Saponin | $R_1$ | $R_2$ |
|---|---|---|
| 1 | -Ara-Rha | $CH_2OH$ |
| 2 | -Ara-Rha-Glc | $CH_2OH$ |
| 3 | -GlcA<(Glc, Glc) | $CH_3$ |

GlcA = glucuronic acid, Glc = glucose,
Ara = arabinose, Rha = Rhamnose

**Table (3):** Comparative susceptibiltity of *B. alexandrina* snails to the methanol extract and isolated saponins from *T. papyriferus* after 24 hours exposure time.

| Material | $LC_{50}$ ppm | $LC_{90}$ ppm | S |
|---|---|---|---|
| Methanol extract | 165 (152.88 – 172.44) | 200 | 1.24 |
| Saponin 1 | 6 (5.156 - 7.99) | 9 | 1.36 |
| Saponin 2 | 11 (9.821 – 12.32) | 14 | 1.23 |
| Saponin 3 | 17 (15.178 - 19.04) | 22 | 1.22 |

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# دراسة للمحتوى الدهنى والصابونينات المبيدة لقواقع البلهارسيا فى نبات تيترابناكس بابيريفيرس

**مرتضى محمد السيد**

قسم الكيمياء العلاجية – معهد تيوذور بلهارس للابحاث – الجيزة – مصر

باجراء تفاعل التصبن على الجزء الذائب فى الاثير البترولى لاوراق نبات تيترابناكس بـابيريفيرس وباستخدام جهاز كروماتوجرافيا الغاز أمكن فحص المحتوى الدهنى للاوراق . ففى الجزء الغير متصبن أمكن التعرف على خليط من الهيدروكربونات والاستيرولات . ومن الجزء المتصبن أمكن التعرف على ستة عشر حمض دهنى .

ايضا تم اجراء فصل كروماتوجرافى للجزء الذائب فى البيوتانول باستخدام كروماتوجرافيا العمود والطبقة الرقيقة وأمكن فصل ثلاثة صابونينات وتم التعرف عليها بواسطة الاشعة تحت الحمراء والتردد النووى المغناطيسى وطيف الكتلة بالاضافة الى تحديد كل من الاجليكونات والسكريات المصاحبة لكل صابونين على حده .

وقد أظهرت الصابونينات الثلاثة فعالية عالية ضد قواقع بيموفلاريا الكسندرينا العائل الوسيط لطفيل البلهارسـيا المعويـة فـى مصر.