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Phytochemical and Toxicological Studies of *Zygophyllum album* L.f.

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Abstract: Investigation of the chemical constituents of *Zygophyllum album* L.f. (Zygophyllaceae family) let to isolate three flavonoids via Kaempferol, Isorhamnetin and Quercetin-3-O-glucoside, one β-carboline alkaloids; Harmine, 16 n-alkanes (C_{12}-C_{32}), β-amyrin, stigmasterol and β-sitosterol and nine fatty acids. The stucctures of these compounds were established by Mass spectrometry (MS), Gas-liquid chromatography (GLC) and spectroscopic techniques, including Ultra-violet (UV), Infra-red (IR) and Nuclear Magnetic Resonance spectroscopic analysis (^{1}H NMR). The oral LD_{50}±standard error and their 95% fiducial limits of the total alcoholic extract were 5.9±0.25 and (5.59-6.23) g kg^{-1} bw, respectively. While, the intra peritoneal LD_{50}±standard error and their 95% fiducial limits of the extract were 2.60±0.15 and (2.44-2.77) g kg^{-1} bw, respectively. The total alcoholic extract of the plant could be highly toxic for rats. The extrapolated calculation to human revealed that, this plant could be considered as slightly toxic for man.

Key words: *Zygophyllum album*, Zygophyllaceae, kaempferol, isorhamnetin, quercetin-3-O-glucoside, β-carboline compounds

INTRODUCTION

Zygophyllum album L. belongs to Zygophyllaceae family, genus *Zygophyllum*. Nine species of *Zygophyllum* are recorded in Egypt (Täckholm, 1974).

This plant used in traditional medicine as a remedy for rheumatism, gout, asthma and as a diuretic. Some Bedouins used it as hay or added it to the dry ration. However, it was found to be toxic to the sheep and caused high mortality (Attia and Samar, 2004).

Previous investigation of *Zygophyllum album* L.f. revealed that the plant contains Zygophyllin, β-sitosterol-β-D-glucopyranoside, carbohydrates, tannins, lactones, proteins/amino acids, saponins, triterpene and flavonoid glycosides (Attia and Samar, 2004; Hani, 1995; Hassanean, 1993; Hassanean, 1993; Shoaib, 1957; El-Monayeri *et al*., 1981; El-Shourbagy and Kishk, 1975).

The aim of this study deals with the chemical study of the plant as regards their constituents particularly alkaloids and flavonoids as well as lipids. The acute toxicity of the total alcoholic extract of the plant was studied to determine the safety margin, qualitative features and quantitative assessment of toxic over dosage. This study was carried out using oral and intra peritoneal administration.

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MATERIALS AND METHODS

Plant Material

Fresh aerial parts of *Zygophyllum album* L.f. (Zygophyllaceae) were collected from Suez Canal region and Southern Sinai in April during the flowering stage. The identity was established by Prof. Dr. Moustafa Zaghloul, Prof. of Floriculture and Medicinal plants, Department of Horticulture, Faculty of Agriculture, Suez Canal University. A voucher specimen (Number AMYM-1003) has been deposited in the Herbarium Department of Botany, Faculty of Science, Suez Canal University, Ismailia, Egypt.

General Methods

Melting points were determined on Büchi 535 melting point apparatus. IR spectra were recorded on 1430 Ratio Recording, Perkin-Elmer, IR-data station Epson FX-86e, in KBr disks. UV spectra were obtained on lambda 4B UV/Vis spectrophotometer (Perkin-Elmer) in the region of 190-900 nm. EIMS (ionization voltage 70 ev) was measured on HP-Model, MS-5988. High resolution, Inlet type-DIP, final temperature 200°C and was measured on GC-MSQP 1000EX Schimädzu. ^{1}H NMR was carried out in d_6-dimethylsulphoxide, using Jeol JNM-EX 270 FT NMR system, operating at AMX 270 MHZ. The chemical shifts are given in ppm (δ), relative to TMS as internal standard and coupling constants are in Hz. The fractions obtained were subjected to gas-liquid chromatographic analysis (PYE UNICAM Series 304GC), using coiled glass column (2.8 m×0.4 mm I.D.), packed with Diatomite C (100-120 mesh) and coated with 1% OV-17, programmed at 10°C min^{-1} from 70 to 270°C, then isothermally at 270°C for 25 min, injector temperature and FID detector at 300°C and the nitrogen carrier gas at a flow rate of 30 mL min^{-1}. GLC of the methyl esters of the fatty acids was carried out by PYE UNICAM Series 304 Gas Chromatograph equipment with FID and SGE injector split mode, using capillary column (25 m×0.22 mm I.D., 0.2 μm thickness) packed with vitreous silica coated with FFAP (free fatty acid phase), programmed at 12°C min^{-1} from 70 to 190°C, injector temperature at 250°C and FID detector at 270°C and the flow rate of hydrogen is 41.0 cm sec^{-1}.

Extraction, Isolation and Characterization

The upper parts of the plant (leaves, flowers and stems) were air dried and ground altogether as a fine powder. The phytochemical screening and the proximate analysis included ash and moisture contents were performed in accordance with AOAC (1990).

Lipids

Air dried and powder aerial parts (1.5 kg) of the plant were extracted with petroleum ether (40-60°C) to yield 35 g of a dark green oily residue (lipid fraction). The marc was macerated with ethanol (80%) at room temperature till exhaustion. The resulting alcoholic extract was concentrated to obtain a crude residue (750 g). The obtained lipid fraction was treated with hot acetone to effort 8 g of acetone insoluble fraction and 18 g of acetone soluble fraction as oily material. Ten grams of the last fraction were saponified to yield 4.5 g of yellowish brown, semi-solid residue of unsaponifiable matter and 3.2 g of semi-solid residue of fatty acids. Three grams of the unsaponifiable matter were subjected on a neutral alumina column chromatography with petroleum ether and increasing the polarity with benzene.

Flavonoids

The flavonoids were isolated by treating the alcoholic extract of the plant with hot water, followed by extraction with chloroform ($CHCl_3$) then ethyl acetate, to effort 7.5 and 11.5 g, respectively.

Fractionation of the flavonoids was affected by PPC and sephadex LH-20 column chromatography. UV- absorption spectra of the isolated flavonoidal components Z-I, Z-II and Z-III were measured in methanol (MeOH) as well as in MeOH after the addition of shift reagents. Acid hydrolysis of the flavonoidal compound Z-III. Compound (Z-III) (25 mg) was refluxed with10 mL of 10% sulphuric acid for 3 h, the concentrated mixture was applied on PC (Attyia and Ashour, 2002).

Compound (Z-I)

(50 mg), R_f; [0.76, 0.08, BAW (4:1:5) and 15%AcOH respectively]. IR bands (KBr) ν_{max}; 3494, 1665, 1605, 1590, 1540, 1464, 1377, 1082 and 1029 cm^{-1}. UV λ_{max} nm (MeOH): 269.8, 319sh, 367.8. EIMS m/z (rel. Int.): 286 (100), 285 (33.56), 258 (5.57), 153(4.97), 152 (0.60), 121 (14.97), 93 (4.59).

Compound (Z-II)

(45 mg), yellow crystals, m.p. 302-303°C, R_f; [0.64, 0.05, BAW (4:1:5) and 15% AcOH, respectively]. IR bands (KBr) ν_{max}; 3402, 1660, 1600, 1587, 1515, 1450, 1091 and 1027 cm^{-1}. UV λ_{max} nm (MeOH): 276.6, 372. EIMS m/z (rel. Int.): 316 (100), 315 (23.60), 288 (2.70), 153 (5.70), 152 (4.30), 151 (4.40) and 123 (4.40). ^{1}H NMR (270 MHZ, d_6-DMSO): δ 6.15 (1H, d, H-6), 6.45 (1H, d, H-8), 7.65 (1H, d, H-6`), 7.8 (1H, s, H-2`), 6.9 (1H, d, H-5`), 3.85 (3H, s, OMe at C-3`), 12.5 (1H, s, OH C-3), 9.5 (1H, s, OH at C-7), 9.8 (1H, s, OH at C-4`), 10.8 (1H, s, OH at C-5) which are exchangeable.

Compound (Z-III)

(30 mg), R_f; [0.56, BAW (4:1:5)]. UV λ_{max} nm (MeOH): 255, 258, 300sh, 347, 356. PC analysis of Z-III residue after hydrolysis revealed the presence of glucose as a sugar moiety.

Alkaloids

The study of alkaloids of the investigated plant was carried out after defatting by percolating with ethanol at room temperature till exhaustion then concentrating and acidifying with HCl. The acidic solution was extracted with ether and then reduced with zinc dust. The solution was rendered alkaline with NH_4OH. The alkaloidal fraction was extracted with $CHCl_3$ to yield 1.76 g of yellowish brown residue which represented 0.12% total crude alkaloids of the dry plant material. Fractionation of the major alkaloidal constituent Z-IV was affected by PTLC using solvent $CHCl_3$-MeOH (85:15).

Compound (Z-IV)

(32 mg), R_f; [0.63, $CHCl_3$-MeOH (4:1:5)]. EIMS m/z (rel. Int.): 212 (100), 211 (6.13), 197 (29.18), 184 (2.35), 183 (7.43), 182 (2.26), 181(2.59), 155 (0.93), 154 (2.35), 127 (4.37). ^{1}H NMR (270 MHZ, d_6-DMSO): δ. 11.45 (1H, s, -NH), 8.25 (1H, d, H-3), 8.05 (1H, d, H-6), 7.8 (1H, d, H-4), 7.05 (1H, d, H-8), 6.8 (1H, q, H-5), 3.85 (3H, s, OMe at C-7) and 2.75 (3H, s, Me at C-1).

Toxicological Studies

Acute toxicity studies were performed on 128 mature albino male rats and nearly of the same age and weight (190-210 g), which were obtained from the animal house of National Research Center. A pilot test was carried out to determine the range of doses, which were estimated to cause 0-100% deaths in the final test. Rats were divided into groups, each composed of 8 animals. The test was carried out during November under a laboratory temperature of about 19-22°C. The extract of the plant was suspended in dist. water of tween 80 (polyoxyethylene sorbitan) (3:1) V/V for 1 g of alcohol extract. After oral and intra peritoneal administration of the extract, the animals were kept under observation during the following 24 h. Deaths occurring during this period were recorded in 24, 48 and 72 h for each group. Dose mortality curves were constructed covering responses between 0-100% lethalities. Toxicity data were analyzed in accordance with Behrens and Karber (1935) and

statistically by Litchfield and Wilcoxon (1949) procedure. The latter method also allowed comparison of the oral and intra peritoneal dose mortality curves with respect to their slope function, parallelism and relative toxicity ratios.

RESULTS AND DISCUSSION

The results obtained from the preliminary phytochemical screening of *Zygophyllum album* L.f. revealed the presence of alkaloids, flavonoids and saponins as major components. Also, the presence of carbohydrates and/or glycosides, coumarins, sterols and/or triterpenes, tannins and cardiac glycosides. The average percentages of the constants of the plant were calculated as shown in Table 1.

The results obtained from the GLC chromatogram of hydrocarbon fraction, eluted with petroleum ether-benzene (90:10), revealed the presence of a series of 16 n-alkanes (n-C_{12} to n-C_{32}) with $C_{26}H_{54}$ as the major constituent of the mixture (19.36%) and the percent of the other constituents ranging from 0.39-11.13% as shown Table 2 and Fig. 1. Odd-numbered n-alkanes were not the major constituents in *Zygophyllum album* L.f. Therefore, selection of morphologically uniform samples for chemotaxonomic comparison of n-alkane content is essential for search. As similar to the hydrocarbon content of *Cassia obtusifolia* L. which give even-number n-alkanes as major constituents (Wilkinson, 1970). This phenomenon is unusual comparing with the distribution of n-alkanes in angiosperm in which, the predominant n-alkanes have odd-carbon numbers (Chikaraishi and Naraoka, 1982; Ohkouchi *et al*., 2000; Disnar and Harouna, 1994).

The residue obtained from fractions eluted with petroleum ether-benzene (75:25) gave white crystalline needles in MeOH. It melted at 197-199°C both alone and upon admixture with authentic β-amyrin. It gives positive Liebermann-Burchardt reaction, indicating that it is a triterpenoid in nature. TLC on Silica gel using different solvent systems showed to be a single spot possessing the same R_f as authentic β-amyrin. The IR-spectrum of the isolated substance showed the same absorption bands characteristic for authentic β-amyrin (Barreiros *et al*., 2002).

Table 1: Constants of *Zygophyllum album* L

			[3]Extractive (%)				
Plant Material	[1]Moisture (%)	[2]Ash (%)	Pet. ether (60-80°C)	Diethyl ether (%)	$CHCl_3$ (%)	MeOH (%)	Hot water (%)
Whole plant	8.28	25.2	5.5	3.2	0.95	21.5	25.2

[1]Average percentages (means of duplicate analysis), [2]Calculated on the air-dried plant material, [3]Calculated on the oven-dried plant material

Table 2: Gas liquid chromatographic analysis of the hydrocarbon fraction

n-alkane	Retention time (min)	Relative percentage
n-C_{12}	5.38	1.92
n-C_{14}	8.47	10.96
n-C_{15}	9.29	11.13
n-C_{16}	10.47	9.68
n-C_{18}	12.65	2.59
n-C_{20}	14.80	11.12
n-C_{21}	15.73	9.08
n-C_{22}	16.55	1.82
n-C_{23}	18.55	7.05
n-C_{24}	18.28	5.45
n-C_{25}	19.30	5.32
n-C_{26}	19.79	19.36
n-C_{28}	20.90	1.39
n-C_{30}	22.00	1.88
n-C_{31}	23.54	0.86
n-C_{32}	25.21	0.39

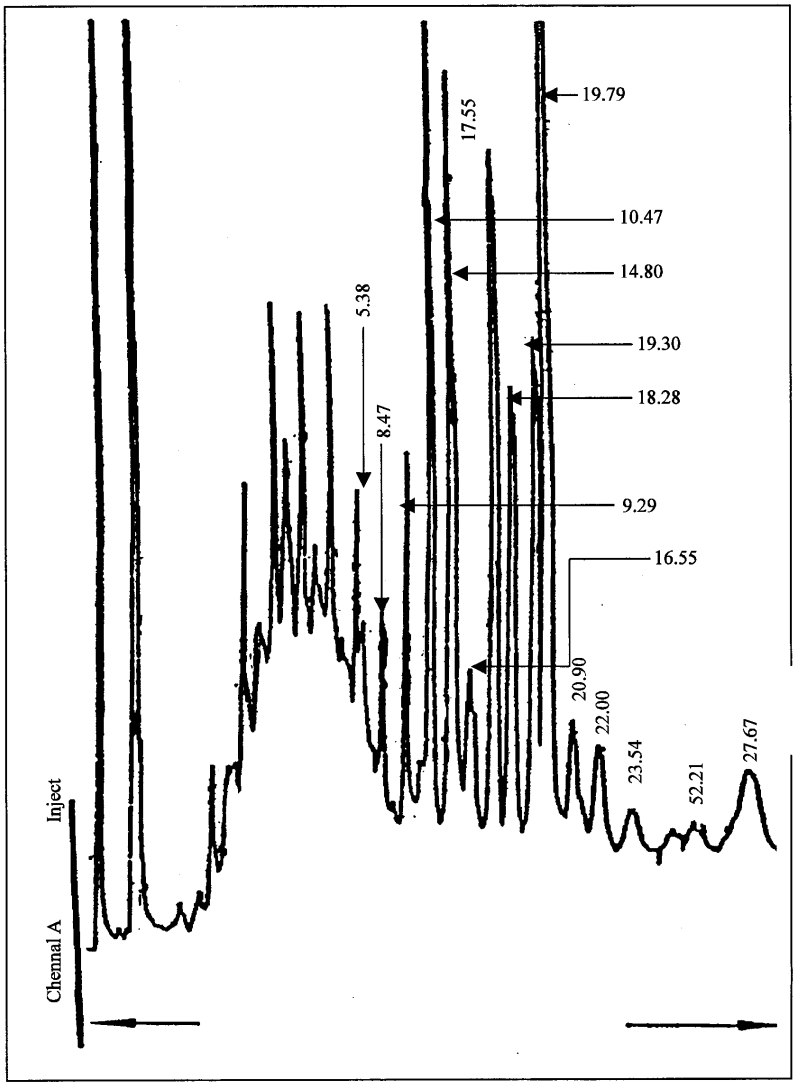

Fig. 1: GLC chromatogram of hydrocarbon fraction

The combined fractions eluted with petroleum ether-benzene (50:50) gave white crystalline needles in ethanol, m.p. 133-135°C. The substance showed a single spot on TLC, using toluene-acetone system (90:10), possessing R_f; 0.44. It was identical with authentic β-sitosterol. However, several authors (Ghaleb *et al*., 1972; Bennett and Erich Heftmann, 1966; Marsili and Morelli, 1968; Singh *et al*., 1970; Nagasampagi *et al*., 1971) reported that the sterols isolated from plants, showed single substances on TLC and their physical properties confirmed this. Yet on subjecting to GLC they were proved to be mixtures. So the sterol fraction was subjected to GLC. The obtained chromatogram revealed that, the isolated sterol fraction is a mixture of two compounds possessing the same retention times (18.21 and 20.02, respectively) as authentic Stigmasterol and β-sitosterol (Tian-Jye *et al*., 1999). The percentages of them were 74.24 and 25.76%, respectively from the total sterol fraction.

GLC chromatogram of the methyl ester of fatty acids revealed the presence of nine fatty acids according to their retention times in comparison with authentic. The saturated fatty acids represent 92.68% while the unsaturated fatty acids represented by 7.32% of the total fatty acids. The major fatty acids were decanoic acid 33.46% and palmitic acid 31.18% as shown in Table 3 and Fig. 2.

PC of the $CHCl_3$ extract was found to give better separation revealing the presence of one major flavonoid Z-I R_f; (0.76, BAW 4:1:5), beside others as minor constituents. The ethyl acetate (EtOAc) extract was found to contain two flavonoidal spots Z-II, Z-III R_f; (0.64, 0.56, BAW 4:1:5), beside others as minors. Compound (Z-I), UV-spectrum of Z-I in MeOH, (Table 4 and Fig. 3) exhibited λ_{max} at 367.8 nm (band-I) and band-II at 269.8 nm. This indicates that it is a flavonol type (Mabry *et al.*, 1970). The NaOMe spectrum showed a bathochromic shift of 54 nm in band-I with a marked decrease in its intensity and with rapid degeneration indicating to the presence of 3-OH groups in 3, 3', 4'-positions. Also, showed a shoulder peak at 320 nm, which is an indicative to the presence of 7-hydroxy group. $AlCl_3$ spectrum showed a bathochromic shift (54.6 nm) indicating the existence of *ortho*-dihydroxy system and/or 3, 5-di-hydroxy groups. However, $AlCl_3$/HCl spectrum exhibited slightly hypsochromic shift (3.9 nm) in band-I relative to $AlCl_3$ spectrum, which is an indication of the presence of 3, 5-OH groups. NaOAc spectrum showed a bathochromic shift (5.4 nm) in band-II,

Table 3: Gas liquid chromatographic analysis of the methyl esters of fatty acids fraction

Methyl esters of fatty acids	Retention time (min)	Relative percentage
Octanoic acid	6.62	0.69
Decanoic acid	9.35	33.46
Dodecanoic acid	12.34	5.65
Myristic acid	15.11	6.47
Palmitic acid	18.21	31.18
Heptadecanoic acid	20.81	2.43
Stearic acid	22.77	4.51
Linoleic acid	25.73	7.32
Arachidic acid	31.49	8.29

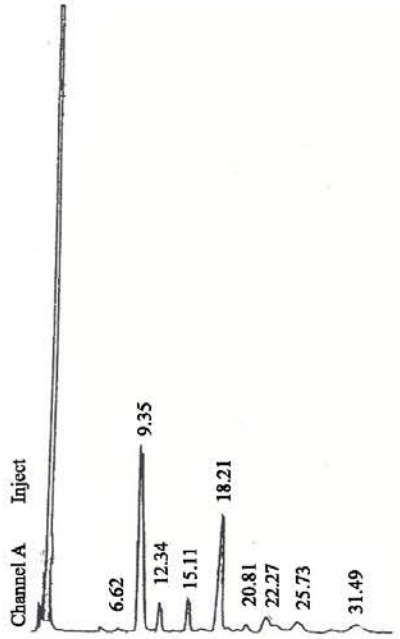

Fig. 2: GLC chromatogram of saponifiable fraction

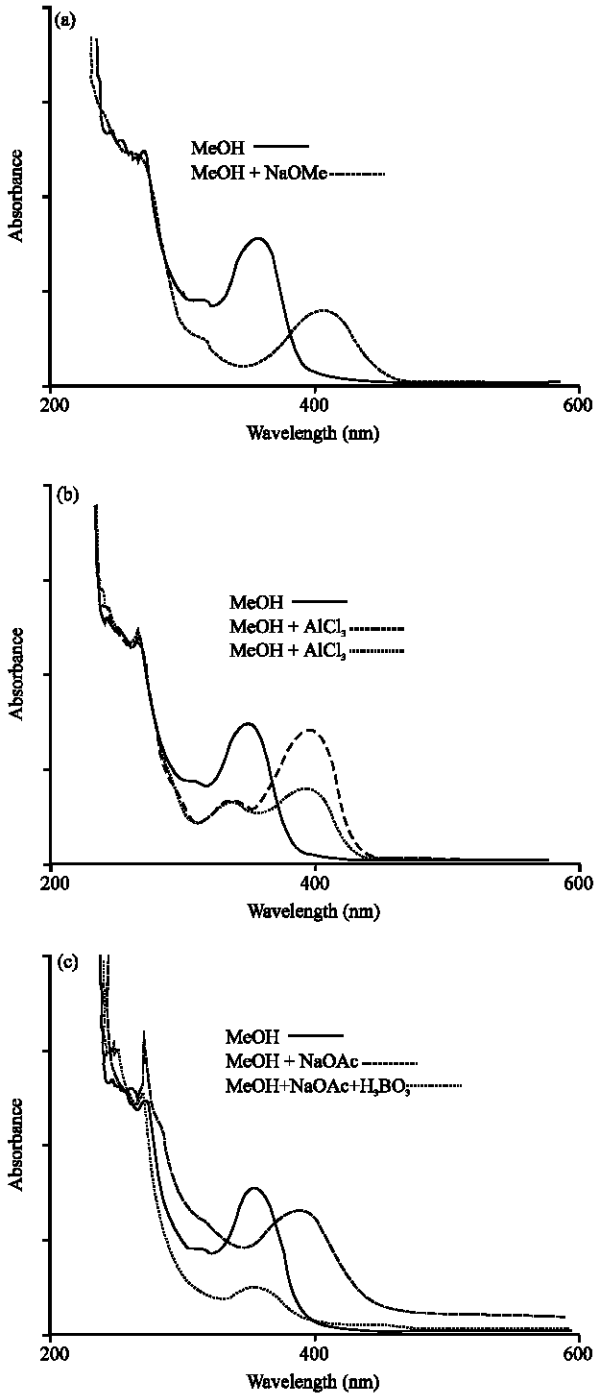

Fig. 3: UV/Vis absorption spectra of compound Z-I in (a) MeOH and MeOH/NaOMe, (b) MeOH, MeOH/$AlCl_3$ and MeOH/$AlCl_3$/HCl and (c) MeOH, MeOH/NaOAc and MeOH/NaOAc/H_3BO_3

indicates the presence of free 7-OH group, also a bathochromic shift (27.5 nm) in band-I with slow degeneration indicating the presence of 3, 3', 4'-trihydroxy groups. IR-spectrum of Z-I showed a strong band at 3494 cm^{-1} corresponding to -OH group, band at 1665 cm^{-1} corresponding to an α, β-unsaturated ketone (C = O group of γ-pyrone), absorption bands at 1605, 1590 and 1540 cm^{-1} (an aromatic system); in addition to the absorption bands at 1464, 1377, 1082 and 1029 cm^{-1}. MS of Z-I showed a molecular ion (M^+) at m/z 286 (100%), which corresponds to the molecular formula $C_{15}H_{10}O_6$ of four hydroxy substitution patterns. Hence, the fragmentation pathway undergoes the Retero-Diels Alder reaction-giving rise to ring-A fragment at m/z 153 (4.97) and m/z 152 (0.60). However, the hydrogen transfer ion at m/z 153 is much intense than that of the normal fragment ion at m/z 152, indicating that it has 5,7-di-hydroxy groups. Furthermore, loss of CO directly from the molecular ion (M^+-CO) was also shown, leading to the phenylbenzofuran fragment ion at m/z 258 (5.57) which further fragments giving rise to the benzoyl ion at m/z 121 (14.97) and lose CO directly giving m/z 93 (4.59). Moreover, the molecular ion (M^+) m/z 286 loses hydrogen to give the molecular ion (M^+-1) at m/z 285 (33.56). The previous data are in agreement with those reported for kaempferol as shown in Fig. 4 (Hamzah and Lajis, 1998; Coelhoa *et al*., 2003; Hadizadeh *et al*., 2003).

Compound (Z-II), UV-spectrum in MeOH, (Table 4 and Fig. 5) exhibited a λ_{max} at 372 nm (band-I) and band-II at 276.6 nm which indicates that it is a flavonol type (Mabry *et al*., 1970). The results obtained from UV-spectrum, IR-spectrum, MS and ^1H NMR are in agreement with those reported for Isorhamnetin as shown in Fig. 4 (Shahat *et al*., 2002).

Compound (Z-III), UV-spectrum in MeOH, as shown in Table 4 and Fig. 6 exhibited a band of max. absorbance at 356 nm (band-I) and a shoulder band at 300 nm indicating that it is a flavonol in nature. The R_f-values as well as the color under UV-light showed that, it is probably a flavonoidal glycoside in nature. PC analysis of Z-III residue after hydrolysis revealed the presence of glucose as a sugar moiety while the aglycone was identified as quercetin by comparison with authentic reference. The previous data are in agreement with those reported for quercetin 3-O-glucoside (isoquercetrin) (Assaf, 1980; Aderogba *et al*., 2005).

Table 4: Ultra-violet absorption spectra of the isolated flavonoidal constituents

Compound	λ_{max}nm MeOH	λ_{max}nm NaOMe	λ_{max}nm $AlCl_3$	λ_{max}nm $AlCl_3$/HCl	λ_{max}nm NaOAc	λ_{max}nm NaOAc/ H_3BO_3
Z-I	269.8 319sh 367.8	268.3, 320, 422.4 (dec.)	261.3sh, 269, 351.6, 422.4	256sh 268.2, 351, 418.5	275.2 285.6, 395.3	268, 299.9, 366.5
Z-II	276.6, 372	284.1, 330, 436.2 (dec.)	277.5, 363.9, 428.4	277.5 358.5, 425.7	281.1, 410.1 (dec.)	255.9, 282.6
Z-III	255, 258, 300sh, 347 356	278, 320sh, 378, 381, 413.1	271.2, 304sh, 361, 423	270, 300sh, 350.1, 394	270.4, 319, 347, 397.2	263, 299sh, 369

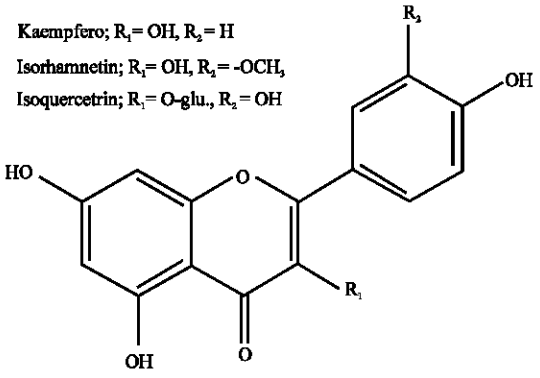

Fig. 4: Structures of isolated flavonoids

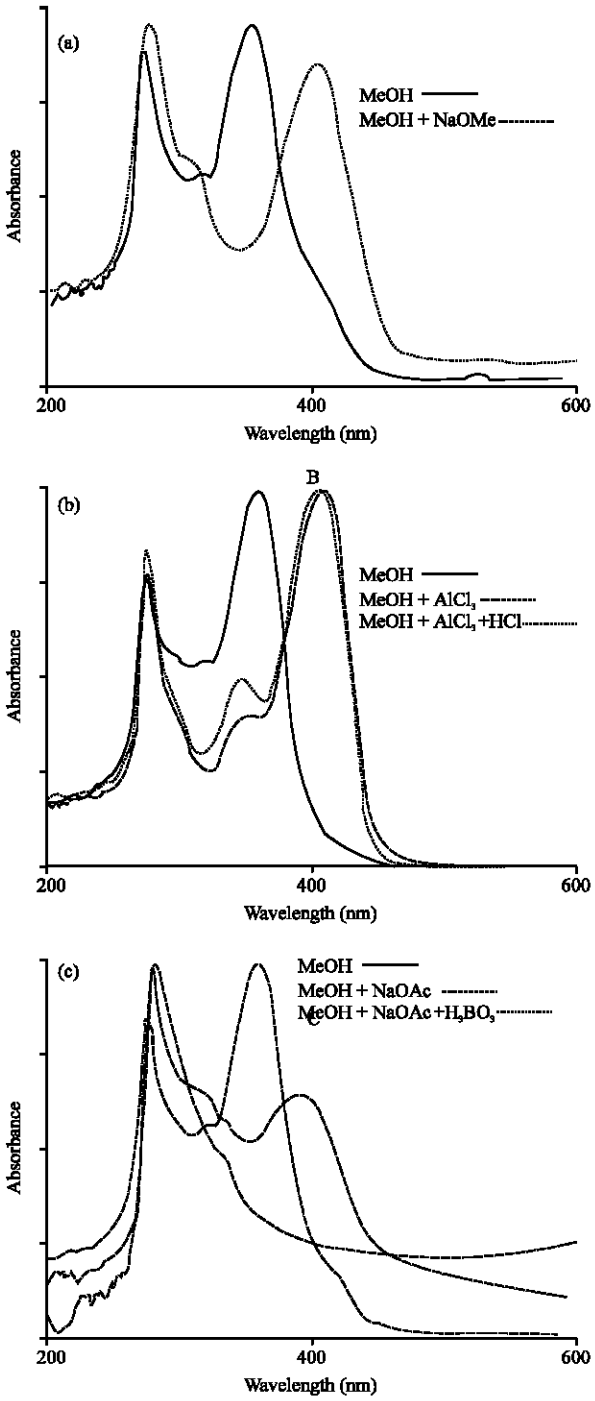

Fig. 5: UV/V is absorption spectra of compound Z-II in (a) MeOH and MeOH/NaOMe, (b) MeOH, MeOH/$AlCl_3$ and MeOH/$AlCl_3$/HCl and (c) MeOH, MeOH/NaOAc and MeOH/NaOAc/H_3BO_3

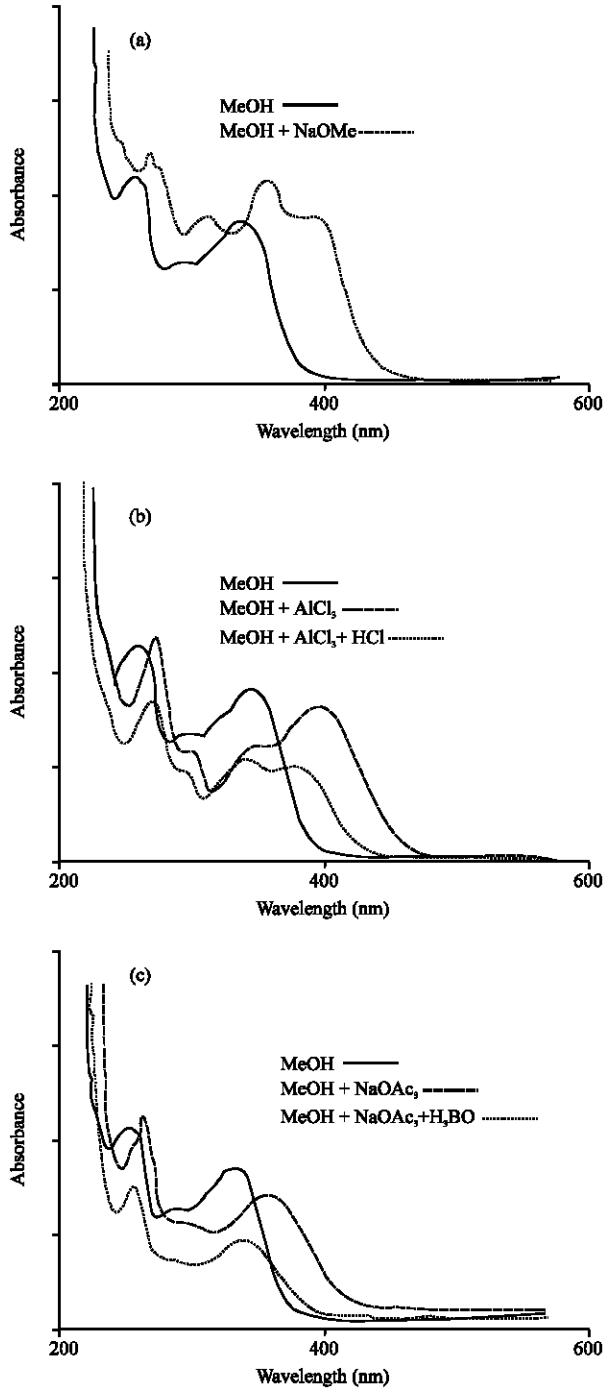

Fig. 6: UV/V is absorption spectra of compound Z-III in (a) MeOH and MeOH/NaOMe, (b) MeOH, MeOH/$AlCl_3$ and MeOH/$AlCl_3$/HCl and (c) MeOH, MeOH /NaOAc and MeOH/NaOAc/H_3BO_3

The major alkaloid spot Z-IV (R_f; 0.63, $CHCl_3$-MeOH (85:15)) appeared as fluorescent blue under UV-light before spraying the reagent and colored bright orange red after spaying. The other alkaloidal spots appeared as minor orange spots on a yellow background with R_f; 0.83, 0.57, 0.47, 0.35, 0.28, 0.25 and 0.17. *Compound (Z-IV)* (30 mg), the data obtained from MS and ^{1}H NMR are in agreement with those reported for harmine (7-methoxy-1-methyl-β-carboline) as shown in Fig. 7 (Olga *et al*., 2005; Baisa, 2002).

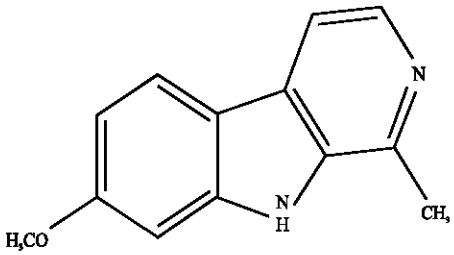

Fig. 7: Structure of isolated Harmine

The qualitative features recorded during the 24 h period following oral and i.p. administration with steadily progressive increases in test doses of total alcoholic extract of the plant resulted in: Behavioral; gradual cessation of spontaneous motility, ataxia and partial tail elevation, skeletal muscle hypotonia, loss of rigidity reflex and motor incapacitation, progressive sedation, eventually passing into hypnosis verified by closure of eyelids, analgesia, lack of response to painful stimuli and tail compression. Respiration; tachyponea, alternating with phases of apnea, dyspnea with irregular breathing and terminal gasping respiration. Heart; feeble cardiac contractions, associated with cyanosis, asphysine, convulsive fits ending by cardiorespiratory failure. Itching; presumably reflecting histamine release from H_1-receptors in cutaneous mast cells. Diarrhoea and loose stools excessive urination evidenced by wetting of perineal region. Well-marked swelling and engorgement of testicles with diffuse hyperaemia and congestion of the skin of the scrotum. Analgesia arises from antagonistic interactions. This type of interactions is responsible for the blocking of endogenous hypothalamic opioid receptors. Likewise, tail elevation (strobes phenomenon) constituting could be considered as a cardinal sign of agonist/antagonist interaction with opioid receptors in alimentary tract. According to Zbindin (1963), tests inhibition of spermatogenesis is enhanced particularly in the stage of transformation from spermatid to immature spermatozoa. It is apparent from the obtained toxic manifestations and comparing them with the standard manifestation of acute toxicity by Häyes (1982), *Zygophyllum album* L.f. extract has mast cell H_1 histamine releasing property from skin, autonomic, CNS and neuromuscular effects. These findings are in agreement with those recorded by Steyn and Liebig (Steyn, 1929 ; Liebig *et al*., 1974), who studied the toxic activity of some species of the genus *Zygophyllum*.

On the other hand, the LD_{50} of the alcoholic extract of the plant was achieved by two methods of analysis for 24 h, to allow a comparison between them. By using the method of Behrens, LD_{50} of the plant extract for oral and i.p administration were 5.975 and 2.575 g kg^{-1} body weight, respectively, as shown in Table 5 and 6. It is remarked that, some rats which did not die in the first 24 h might survive and escape the fatal outcome, but others might die in the second or third day, which shown in Table 7 and 8. Whereas, oral LD_{50}±standard error and their 95% fiducial limits of the extract calculated by Lichfield procedure were 5.9±0.25 and (5.5924-6.2245) g kg^{-1} bw., respectively. While, the intra peritoneal LD_{50}±standard error and their 95% fiducial limits of the extract were 2.60±0.15

Table 5: The effect of oral route administration of different doses of the alcoholic extract of *Zygophyllum album* L.f. on the mortality rate in rats

Dose of alcoholic extract in g kg^{-1} b.wt	No. of group of animals	No. of dead animals	a	b	a x b
5.0	8	0	0.20	0.50	0.10
5.2	8	1	0.20	1.00	0.20
5.4	8	1	0.20	1.50	0.30
5.6	8	2	0.20	2.50	0.50
5.8	8	3	0.20	3.50	0.70
6.0	8	4	0.20	4.50	0.90
6.2	8	5	0.20	5.50	1.10
6.4	8	6	0.20	6.50	1.30
6.6	8	7	0.20	7.50	1.50
6.8	8	8	-	-	-
		Total			6.60

Table 6: The effect of intra peritoneal injection of different doses of the alcoholic extract of *Zygophyllum album* L.f. on the mortality rate in rats

Dose of alcoholic extract in g kg^{-1} b.wt	No. of group of animals	No. of dead animals	a	b	a x b
2.0	8	0	0.20	0.50	0.10
2.2	8	1	0.20	1.50	0.30
2.4	8	2	0.20	3.00	0.60
2.6	8	4	0.20	5.00	1.00
2.8	8	6	0.20	7.00	1.40
3.0	8	8	-	-	-
		Total			3.40

Table 7: Oral toxicity of the total alcoholic extract of *Zygophyllum album* L.f. in adult normal male *albino* rats showing the number of animals that died during 72 h after oral administration of the extract. Each group consists of 8 rats

		The mortality rate							
		Acute		Delay					
		24 h		48 h		72 h			
Oral dose levels									Total
(g kg^{-1} body weight)	No. of tested rats	No.	(%)	No.	(%)	No.	(%)	No.	(%)
5.0	8	-	0	-	0	-	0	-	0
5.2	8	1	12.5	-	0	-	0	1	12.5
5.4	8	1	12.5	-	0	-	0	1	12.5
5.6	8	2	25.0	-	0	1	12.5	3	37.5
5.8	8	3	37.5	-	0	1	12.5	4	50.0
6.0	8	4	50.0	-	0	1	12.5	5	62.5
6.2	8	5	62.5	-	0	1	12.5	6	75.0
6.4	8	6	75.0	1	12.5	1	12.5	8	100.0
6.6	8	7	87.5	1	12.5	-	0	8	100.0
6.8	8	8	100.0	-	0	-	0	8	100.0
Total	80	37	46.25	2	2.5	5	6.25	44	55.0

Table 8: Intra-peritoneal (i.p.) toxicity of the total alcoholic extract of *Zygophyllum album* L.f. in adult normal male *albino* rats showing the number of animals that died during 72 h after (i.p.) administration of the extract. Each group consists of 8 rats

		The mortality rate							
		Acute		Delay					
		24 h		48 h		72 h			
I.P. dose levels								Total	
(g kg^{-1} body weight)	No. of tested rats	No.	(%)	No.	(%)	No.	(%)	No.	(%)
2.0	8	-	0	-	0	-	0	-	0
2.2	8	1	12.5	-	0	-	0	1	12.5
2.4	8	2	25.0	-	0	1	12.5	3	37.5
2.6	8	4	50.0	1	12.5	1	12.5	6	75.0
2.8	8	6	75.0	1	12.5	1	12.5	8	100.0
3.0	8	8	100.0	-	0	-	0	8	100.0
Total	48	21	43.75	2	4.17	3	6.25	26	54.17

Table 9: Acute oral toxicity of total alcoholic extract of *Zygophyllum album* L.f. in adult normal male *albino* rats. Body weight range of rats 190-210 g; average 200 g. Graded test doses of *Zygophyllum album* L.f. administered by intra gastric intubation of 100 g % w/v. aqueous solution (incorporating tween 80 in proportion of 3:1) in volumes not exceeding than 1.6 mL per rat. Animals observed for mortality over 24 h following oral administration. Data obtained by Lichfield and Wilcoxon (1949) procedure of calculation

	A1 observed 24 h effects				
A1 oral dose levels (g kg^{-1} body weight)	Dead/ tested	Observed Percentage mortality	B1 Expressed Percentage mortality	C1 Observed minus expected	C2 Contribution to $(Chi)^2$ Nomograph No.1
5.0	2/8	Zero (1.3)	4.0	2.7	0.019
5.3	1/8	12.5	12.5	-	-
5.6	0/8	25.0	25.0	-	-
5.9	N 4/8	50.0	50.0 D3	-	-
6.2	5/8	62.5	62.5	-	-
6.5	7/8	87.5	87.5	-	-
6.8	8/8	100 (98.2)	94.5	3.7	0.0275
Tween 80	0/8	Zero	–	–	–
Solubilizing agent 0.4 mL kg^{-1} B.W			Total		0.0465

Total animals = 56

C3 Number of doses, k =7 $(Chi)^2 = 0.0465 \times 8 = 0.372$

Animals/ Dose = 56/7 = 8 Degrees of freedom, n = k-2 = 5

$(Chi)^2$ from Table[28] (Mathematical Tables, 1941) for n of 5 = 11.1

C4 0.372 is less than 11.1. Therefore, the data are not significantly heterogeneous

Oral LD_{16} = 5.4 g kg^{-1} body weight **D2** S = 1.0887

D1 Oral LD_{50} = 5.9 g kg^{-1} body weight **D3** N` = 24

Oral LD_{84} = 6.4 g kg^{-1} body weight **D4** f_{LD50} = 1.055 (from Nomograph No.2)

Standard error of acute oral LD_{50} (SE) = 0.250

D5 Acute oral $LD_{50} \pm SE = 5.90 \pm 0.250$

LD_{50} and 95% confidence limits of LD_{50}. 5.9 (5.5924-6.2245) g kg^{-1} body weight

E1 R = 1.2264

E2 A = 1.19 (from Nomograph No.3)

E3 f_s = 1.35 (from Nomograph No.2)

E4 S and 95% confidence limits of S 1.0887 (0.8064 - 1.4697)

Conclusion

-Percentage content of the total alcoholic extract in powdered *Zygophyllum album* L.f. was 34g% (w/w)

-Oral LD_{16} in crude powdered plant = 15.8824 g kg^{-1} b.w.

-Oral LD_{50} in crude powdered plant = 17.3529 g kg^{-1} b.w.

-Oral LD_{84} in crude powdered plant = 18.8235 g kg^{-1} b.w.

-Oral median lethal dose LD_{50} and 95% fiducial limits expressed per g. weight of crude powdered plants were 17.3529 (16.4482-18.3073)

and (2.4413-2.7690) g kg^{-1} bw., respectively. Details of calculations of LD_{50} and its 95% fiducial limits are shown in Table 9 and 10. The oral and intra peritoneal LD_{50} values of the plant obtained by Behrens are enclosed within the 95% confidence limits of acute LD_{50} determined by Lichfielde procedure indicating the practical equality of acute lethal toxicity of the total alcoholic extract obtained by the two methods. The obtained results point out that, the dose mortality lines after oral and i.p. administration are parallel and the toxicity ratio of the oral to i.p. LD_{50} is about 2.2692 as shown in Table 11.

In other words, the total extract of the plant is about 2.2692 times toxic when injected i.p. than in oral administration of such an extract. The oral and i.p. LD_{50} of the total alcoholic extract of the plant, in rats was extrapolated to human and a variety of other mammalian species (e.g., guinea-pigs, monkeys, ...etc.) (Paget and Barnes, 1964), Table 12. Moreover, the three conventual oral lethal doses and 95% confidence limits of acute oral LD_{50} in rats expressed per gram weight of the crude powdered plant were extrapolated to four species of farm animals (goats, sheep, cows and buffaloes). The data

Table 10: Acute Intra peritoneal (i.p.) toxicity of total alcoholic extract of *Zygophyllum album* L.f. in adult normal male *albino* rats. Body weight range of rats 190-210 g; average 200 g. Graded test doses of *Zygophyllum album* L.f. administered by intra gastric intubation of 100 g% w/v. aqueous solution (incorporating tween 80 in proportion of 3:1) in volumes not exceeding than 1.6 mL per rat. Animals observed for mortality over 24 h following i.p. administration. Data obtained by Lichfield and Wilcoxon (1949) procedure of calculation

	A1 observed 24 h effects				
A1 Oral dose levels ($g\ kg^{-1}$ body weight)	Dead/ tested	Observed Percentage mortality	B1 Expressed Percentage mortality	C1 Observed minus expected	C2 Contribution to $(Chi)^2$ Nomograph No.1
2.0	0/8	Zero (0.85)	2.50	1.65	0.015
2.2	1/8	12.5	13.0	0.50	-
2.4	2/8	25.0	25.0	-	-
2.6	N 4/8	50.0	50.0 D3	-	-
2.8	6/8	75.0	75.0	-	-
3.0	8/8	100 (96.8)	90.0	6.8	0.055
Tween 80	0/8	Zero	–	–	–
Solubilizing agent 0.4 mL kg^{-1} b.w.			Total		0.070

Total animals = 48

C3 Number of doses, k = 6 — $(Chi)^2 = 0.070 \times 8 = 0.56$

Animals/Dose = 48/6 = 8 — Degrees of freedom, n = k-2 = 6-2 = 4

$(Chi)^2$ from Table[28] (Hodgman, 1941) for n of 4 = 9.49

C4 0.56 is less than 9.49. Therefore, the data are not significantly heterogeneous

I.P. LD_{16} = 2.3 g kg^{-1} body weight

D1 I.P. LD_{50} = 2.6 g kg^{-1} body weight

I.P. LD_{84} = 2.9 g kg^{-1} body weight

D2 S = 1.1229

D3 N` = 24

D4 f_{LD50} = 1.065 (from Nomograph No.2)

Standard error of I.P. acute LD_{50} (S.E.) = 0.15

D5 Acute I.P. $LD_{50} \pm S.E = 2.60 \pm 0.15$

LD_{50} and 95% confidence limits of LD_{50}. 2.60 (2.4413-2.7690) g kg^{-1} body weight

E1 R = 1.2727

E2 A = 1.14 (from Nomograph No.3)

E3 f_s = 1.325 (from Nomograph No.2)

E4 S and 95% confidence limits of S 1.1229 (0.8475 - 1.4878)

Conclusion

-Percentage content of the total alcoholic extract in powdered *Zygophyllum album* was 34 g% (w/w)

-I.P. LD_{16} in crude powdered plant = 6.7647 g kg^{-1} b.w.

-I.P. LD_{50} in crude powdered plant = 7.6471 g kg^{-1} b.w.

-I.P. LD_{84} in crude powdered plant = 8.5294 g kg^{-1} b.w.

-I.P. median lethal dose LD_{50} and 95% fiducial limits expressed per g. weight of crude powdered plants were 7.6470 (7.1803-8.1441)

Table 11: Compiled data showing test of parallelism of best-fitting log. dose probit mortality lines of acute oral and intra peritoneal i.p. lethal toxicities of the total alcoholic extract of *Zygophyllum album* L.f. in adult ormal male *albino* rats and the statistical significance of lethal toxicities between two routes of systemic administration. Data obtained by Litchfield and Wilcoxon (1949) procedure of calculation

	Calculated values of specified toxicity parameters				Test of parallelism			Relative acute toxicity	
Route of administration	Median lethal dose LD_{50} $g\ kg^{-1}$ body weight	f_{LD50}	Slope function S	f_s	Slope ratio S.R.	$f_{S.R}$	Slope ratio S.R.and 95% fiducial limits	$f_{T.R}$	Toxicity ratio,T.R and 95% fiducial limits
Oral	5.9	1.055	1.0887	1.35	1.0314	1.50*	1.0314 (0.6876 -1.5471)	1.009*	2.2692 2.2489-2.2896)#
Intra peritoneal i.p.	2.6	1.065	1.1229	1.325	No significant deviation from positive parallelism between two lines. (The curves may consider parallel within experimental error)			Significantly higher acute toxicity of extract by confirmed i.p. injection relative to oral administration	

*From Nomograph No. 4, #Significant

Table 12: Hypothetical values for the intra peritoneal and oral median lethal doses (LD_{50}) and 95% fiducial limits of the total alcoholic extract of *Zygophyllum album* L.f. in a variety of mammalian species as obtained by Paget and Barnes method (1964)

	Oral			Intra peritoneal		
		95% fiducial limits			95% fiducial limits	
	LD_{50}	Lower	Upper	LD_{50}	Lower	Upper
Rats	5.900	5.5924	6.2245	2.600	2.4413	2.7690
Mice	8.260	7.8295	8.7143	3.640	3.4181	3.8766
Guinea-pigs	5.133	4.8655	5.4153	2.262	2.1241	2.4090
Rabbits	3.068	2.9081	3.2367	1.352	1.2696	1.4399
Cats	2.832	2.6844	2.9878	1.248	1.1719	1.3291
Monkey	2.714	2.5726	2.8633	1.196	1.1231	1.2737
Dogs	1.750	1.6591	1.8466	0.771	0.7243	0.8215
Humans	0.944	0.8948	0.9959	0.416	0.3906	0.4430

Table 13: Extrapolated values for three conventual oral lethal toxic doses and 95% confidence limits of acute oral LD_{50} expressed per gram weight of the crude powdered plant in four species of farm animals (interspecies dosage conversion, according to revised Mellet, 1969

				95% confidence limits		
Mammalian species		LD_{16}	LD_{50}	Lower	Upper	LD_{84}
Rodent	Rats	15.8824	17.3529	16.4482	18.3073	18.8235
Farm animals	Goats	7.0200	7.6699	7.2701	8.0918	8.3199
	Sheep	7.2424	7.9129	7.5004	8.3481	8.5835
	Cows	6.1941	6.7676	6.4148	7.1398	7.3412
	Buffaloes	6.7500	7.3749	6.9905	7.7806	7.9999

obtained according to Mellet (1969) and Krupp (1982), revised were tabulated in Table 13. From the public health point of view, it is important to extrapolate the oral LD_{50} in rats to man using the method of Paget and Barnes (1964). The results registered in Table 12 showed that, the oral LD_{50} and their 95% confidence limits in man was found to be 0.944 (0.8948-0.9959) g kg^{-1} b.w. The obtained results revealed that, the total alcoholic extract of the plant could be considered as slightly toxic to man. About 7.5-8.4 g kg^{-1} b.w. of crude powdered plant was found to be toxic to the sheep and caused high mortality. These results are in agreement with Steyn, (1929, 1933 and 1934) and Watt and Breyer-Brandwijk (1962) who recorded that, *Zygophyllum microcarpum* Cham. and Schlect. was toxic and caused higher mortality in sheep.

CONCLUSIONS

Investigation of *Zygophyllum album* L.f. resulted in the isolation and identification of flavonoids, β-carboline alkaloids and lipid constituents with percentages 1.27, 0.12 and 2.33% relative to the total powder plant. The flavonoid constituents include Kaempferol, Isorhamnetin and Quercetin-3-O-glucoside as the major constituents. Harmine was isolated and identified as the major constituent of β-carboline alkaloids. The isolation and identification of lipid fraction resulted in 16 n-alkanes (C12-C32) with $C_{26}H_{54}$ as the major constituent and β-amyrin, stigmasterol, β-sitosterol and nine fatty acids. The saturated fatty acids represent 92.68% while the unsaturated fatty acids represented by 7.32% of the total fatty acids. The major fatty acids were decanoic acid 33.46% and palmitic acid 31.18%.

It is apparent from the obtained toxic data and comparing them with the standard manifestation of acute toxicity that the total alcoholic extract of the plant could be highly toxic for rats. The oral LD_{50}±standard error and their 95% fiducial limits of the total alcoholic extract for rats were 5.9±0.25 and 5.59-6.22 g kg^{-1} b.w., respectively. The intra peritoneal LD_{50}±standard error and their 95% fiducial limits of the total alcoholic extract were 2.60±0.15 and 2.44-2.77 g kg^{-1} b.w., respectively. The

calculated percentage of crude powdered plant was toxic to sheep (7.5-8.4 g kg $^{-1}$ b.w.) and caused high mortality. The extrapolated calculation to human, revealed that, this plant could be considered as slightly toxic for man.

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