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Phytochemical and Cytotoxic Studies of *Rumex pictus* Forssk. and *Rumex vesicarius* L. (Family Polygonaceae), Growing in Egypt

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Authors' contributions

All authors have equally contributed to the manuscript and read and approved the final manuscript.

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ABSTRACT

Aims: *Rumex pictus* Forssk. and *Rumex vesicarius* L., Family Polygonaceae, wildly growing in Egypt possess many biological activities. The current study was performed to study the phytochemical constituents and the *in vitro* cytotoxic activity of the successive extracts of the aerial parts of *Rumex pictus* and *Rumex vesicarius.*
Place and Duration of Study: Department of Pharmacognosy, National Research Centre, Giza, Egypt, between March 2014 and January 2015.
Methodology: The preliminary phytochemical screening of the aerial parts of *Rumex pictus* and *Rumex vesicarius*, revealed the presence of volatiles, phenolic compounds, sterols, terpenes and carbohydrates in both species. The volatile constituents were prepared from the fresh aerial parts of each of the plants by hydro-distillation and their chemical composition was identified using GC and GC/MS. The results revealed the presence of hexahydrofarnesyl acetone (7.29%) and *o*-xylene (5.63%) as the major components in *R. pictus* and *R. vesicarius*, respectively. The lipoidal content of the aerial parts of each species was prepared and analyzed after saponification by GC/MS. The results revealed the presence of unsaturated fatty acids as oleic (6.54%) in *R. pictus*

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and ricinoleic (13.7%) in *R. vesicarius.* Sterols including campesterol, stigmasterol, β-sitosterol, α- and β-amyrin were present in both species while taraxasterol was detected only in *R. pictus.* The amino acid content of each of the species was analyzed and the results revealed the presence of phenylalanine, threonine, valine, methionine, isoleucine, leucine, histidine and lysine as essential amino acids in both species at different proportions. The study of carbohydrates revealed the presence of the free sugars glucose, fructose, sucrose and mannitol in both species with different percentages, while the polysaccharide content was prepared and analyzed qualitatively and quantitatively after hydrolysis by GC and GC/MS. Glucose, rhamnose, arabinose, galactose and ribose were detected in both species, while sorbitol was present in *R. pictus* only. The different successive extracts of the aerial parts of the two species were tested for their cytotoxic activity on breast cancer (MCF-7), lung carcinoma (A549) and hepatocellular carcinoma (HepG2) cell lines. The results revealed that the chloroformic extract of the aerial parts of *R. pictus* showed significant cytotoxic activity against hepatocellular carcinoma (HepG2) cell line (IC_{50}= 1.33 µg/mL) and lung carcinoma (A549) (IC_{50}= 3.35 µg/mL) cell line when compared to Doxorubicin, while, the 70% methanolic extract of the aerial parts of *R. pictus* showed significant cytotoxic activity towards breast cancer (MCF7) cell line (IC_{50}= 15.5 µg/mL). This is the first report of the cytotoxic activity of *R. pictus.*

Conclusion: In conclusion, the preliminary phytochemical screening of the aerial parts of *R. pictus* and *R. vesicarius* (Family Polygonaceae) revealed the presence of a variety of constituents including volatile compounds, carbohydrates and /or glycosides, flavonoids, anthraquinones, sterols and/or terpenes, tannins and amino acids, as well as, significant cytotoxic activity of the different successive extracts with variable proportions.

Keywords: Rumex pictus; rumex vesicarius; volatile constituents; lipoidal matter; amino acids; carbohydrates; cytotoxic activity.

1. INTRODUCTION

The genus *Rumex*, belonging to the Polygonaceae includes more than 250 species, which are distributed worldwide [1]. The *Rumex* includes many edible plants, which attracted the attention of many investigators because of their medicinal importance [2]. For centuries, *Rumex* spp. have been used in folk medicine for treating a wide range of ailments including; colds, sore throat, indigestion, scurvy, as well as a cooling drink for fevers. Also, they have been used to treat cancer, rheumatism, liver disorders, foul ulcers and skin conditions. Moreover, roots have been made into poultices for treating nettle and bee stings and other inflammations [3]. *Rumex pictus* Forssk. "Veined dock" and *Rumex vesicarius* L. "Bladder dock" are two species that grow wild in Egypt. Both plants are edible, collected in Spring and eaten fresh or cooked [4]. No information has been found reporting the biological activity of *R. pictus*. And very little information has been reported on its chemical composition. However, *R. vesicarius* is reported to have various biological activities, as an antioxidant, antibacterial, hepatoprotective, antiemetic and cytotoxic [4-7]. The medicinal importance of *R. vesicarius* is a reflection to its chemical composition, since it contains many bioactive substances such as flavonoids (vitexin, isovitexin, orientin and isorientin), anthraquinones particularly in the roots (emodin and chrysophanol), quinines, carotenoids, vitamins (especially vitamin C), proteins, lipids, carbohydrates, reducing sugars, phenols, tannins, saponins, triterepenoids and organic acids. This plant is also a good source of minerals, such as; K, Na, Ca, Mg, Fe, Mn and Cu [8]. Therefore, this study was performed to assay the *in vitro* cytotoxic activity of different extracts of the aerial parts of *R. pictus* and *R. vesicarius* and to investigate these extracts chemically by performing preliminary phytochemical screening to obtain an idea of the bioactive agents that may be responsible for this activity. Also, to evaluate the nutritional importance of the edible parts of the two species under investigation by analyzing their carbohydrate and amino acid contents.

2. MATERIALS, METHODS AND APPARATUS

2.1 Materials

2.1.1 Plant material

Aerial parts of *R. pictus* Forssk. and *Rumex vesicarius* L. (Polygonaceae) were collected from Damietta-Port Said road, North of Al-Manzala Lake and from Wadi Hagol, Cairo-Suez road,

Egypt, respectively. The plants were authenticated by Dr. Abd ElHaleem Abd ElMotagaly, Department of flora, the Agricultural Museum, Giza, Egypt. Voucher specimens of the authenticated plants (RP201 and RV202) were deposited in the National Research Centre Herbarium. The collected plants under investigation were air dried, powdered and reduced to mesh no. 36 and kept in tightly sealed containers.

2.1.2 Reference material

Reference standards of carbohydrates, sterols, hydrocarbons, fatty acids and amino acids were obtained from Merck, Germany.

2.1.3 Material for *in vitro* cytotoxic assay

2.1.3.1 Chemicals and kits

3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT), (Sigma Aldrich, MO), DMSO (Lab Scan, Ireland), Doxorubicin,(Merck, Germany), used as a reference anticancer agent.

2.1.3.2 Cancer cell lines

The following cell lines are available at the Department of Pharmacology, Faculty of Pharmacy, AlAzhar University MCF7 (breast carcinoma cell line), A549 (lung carcinoma cell line) and HEPG2 (hepatocellular carcinoma cell line). These were obtained from Karolinska Institutet, Stockholm, Sweden.

2.2 Methods

2.2.1 Preparation of successive extracts with selective organic solvents

Amounts of 500 grams of air-dried powdered aerial parts of each of *R. pictus* and *R. vesicarius* were separately extracted in continuous extraction apparatus (Soxhlet) successively and exhaustively using solvents of analytical grade with increasing polarity in the following order: petroleum ether (60-80℃), diethyl ether, chloroform, 70% methanol. For each solvent the extraction was continued till no residue was obtained when a small aliquot of the colorless extract was evaporated to dryness in a small watch glass. Before applying the next solvent, the powder was taken out of the extractor and carefully dried on paper sheets. In each case, the solvent was stripped off by distillation under reduced pressure at a temperature below 40℃ and dried to constant weight in a vacuum dessicator over anhydrous calcium chloride.

2.2.2 Phytochemical screening tests

The air-dried powdered aerial parts of *R. pictus* and *R. vesicarius* were, separately subjected to preliminary phytochemical screening study [9] for testing carbohydrates and /or glycosides, tannins, saponins, sterols and /or triterpenes, alkaloids and /or nitrogenous compounds, flavonoids, anthraquinones, coumarins.

2.2.3 Investigation of volatile constituents

The volatile constituents of each of the two species under investigation were prepared according to MacLeod [10] using a modified Likens and Nickerson apparatus. Analysis of the volatile constituents was carried out on a Finnigan SSQ 7000 gas chromatograph directly coupled to mass spectrophotometer. Capillary column: DB-5 fused silica (5% phenyl methyl polysiloxane), 30 m length, 0.25 mm id, 0.25 µm thickness. Carrier gas: Helium at 1 ml/min and pressure 13 psi. Oven temperature: was programmed at 60℃ isothermal for 3min., then heating to 260℃ at a rate of 4℃/min. then isothermal at 260℃ for 5 min. Ion source temperature: 180℃. Ionization voltage: 70 eV. Injector temperature: 220℃. Injection volume: 1µl. Identification of the constituents was performed by comparison of their spectral fragmentation patterns with those of the available database libraries Wiley 9 (Wiley Int., USA) and NIST 11 (National Institute of Standards and Technology, USA) and /or published data (Adams, 1995). Quantitative determination was carried out based on peak area integration.

2.2.4 Investigation of lipoidal matter and preparation of unsaponifiable matter (USM) and fatty acids (FA)

The solvent-free petroleum ether residue (1 g) of each of the two species, was saponified according to reported methods [11]. Both the USM and fatty acid methyl esters (FAME) were analyzed using a Finnigan SSQ 7000 gas chromatograph directly coupled to mass spectrophotometer under the following conditions:

2.2.4.1 Unsaponifiable matter (USM)

Capillary column: DB-5 fused silica (5% phenyl methyl polysiloxane), 30 m length, 0.25 mm id,

0.25 µm thickness. Carrier gas: Helium at 1ml/min and pressure 13 psi. Oven temperature: was programmed at 70-290℃ at a rate of 4℃/min. Ion source temperature: 180℃. Ionization voltage: 70 eV. Injector temperature: 220℃. Injection volume: 1 µl.

2.2.4.2 Fatty acid methyl esters (FAME)

Capillary column: DB-WAX fused silica, 30 m length, 0.25 mm id, 0.25 µm thickness. Carrier gas: Helium at 1ml/min and pressure13 psi. Oven temperature: was programmed at 50-260℃ at a rate of 4℃/min. Ion source temperature: 180℃. Ionization voltage: 70 eV. Injector temperature: 220℃. Injection volume: 1µl.

Identification of the constituents was performed by comparison of their spectral fragmentation patterns with those of the available database libraries Wiley 9 (Wiley Int., USA) and NIST 11 (National Institute of Standards and Technology, USA) and /or published data (Adams, 1995). Quantitative determination was carried out based on peak area integration.

2.2.5 Investigation of amino acid content

Determination of the amino acid contents of the aerial parts of each species was carried out as described by [12] using amino acid analyzer (LC 300 amino acid analyzer, Eppendorf, Germany) under the following conditions: Flow rate: 0.2 ml/min. Pressure of buffer: 0- 50 bar. Pressure of reagent: 0- 150 bar. Reaction temperature: 123℃.

2.2.6 Investigation of carbohydrate content

2.2.6.1 Free sugars

Extraction and analysis of free sugars of each species were, separately performed according to Gertz [13] using a model HP1050 HPLC equipped with Refractive index (RI) detector. Column: APS (4.6 mm × 200 mm). Mobile phase: acetonitrile: water (76:24, v/v). RI detector was used at flow rate 2 ml/min.

2.2.6.2 Polysaccharides

Polysaccharides of the aerial parts of each of the two species under investigation were, separately prepared according to [14]. The isolated polysaccharide was subjected to acid hydrolysis according to [15]. Silylation of polysaccahride hydrolysate was performed according to Kirk and Sawer [16]. Analysis of the polysaccharide hydrolysate was performed using HP 6890 GLC under the following conditions: Column: ZB-1701 (14% cyanopropyl phenyl methyl), 30 m length, 0.25 mm id, 0.25 µm thickness. Carrier gas: Helium at 1.2 ml/min 10.6 psi. Injector chamber temperature: 250℃. Temperature programming: 150-120℃ at a rate of 7℃ /min. Flame ionization detector temperature: 270℃ and air flow: 45 ml/min.

2.2.7 Determination of *in vitro* cytotoxic activity (MTT assay)

Successive extracts of the aerial parts of *R. pictus* and *R. vesicarius,* using solvents of analytical grade with increasing polarity (petroleum ether, diethyl ether, chloroform, and methanol 70%) were chromatographed on silica gel plates. The yields of the successive extracts were as follows: 0.2%, 0.15%, 0.19% and 20.5% for *R. pictus* and 0.4%, 0.1%, 0.42% and 16.25% for *R. vesicarius*. Similar extracts were combined together resulting in three major extracts. The first extract (total ether extract) consists of petroleum ether and diethyl ether extracts, while the second one is the chloroform extract and the third one is the 70% methanolic extract. The three extracts were assessed for their cytotoxic activity according to Mosmann [17]. Exponentially growing cells were trypsinized, counted and seeded at the appropriate densities (5000 cells/0.33 cm2 well) into 96-well microtiter plates. Cells were incubated in a humidified atmosphere at 37℃ for 24 hours. Then, cells were exposed to successive extracts of the aerial parts of *R. pictus* and *R. vesicarius* at the desired concentrations, (0.1, 1, 10, 100 and 1000 µg/µl) for 72 hours. At the end of the treatment period, media were removed; cells were incubated with 200 µl of 5% MTT solution/well and allowed to metabolize the dye into a colored-insoluble formazan complex for 2 hours. Medium was discarded from the wells and the formazan crystals were dissolved in 200 µl/well acidified isopropanol for 30 min, covered with aluminum foil and with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc, MI) at room temperature. Absorbance was measured at 570 nm using a SpectraMax plus Microplate Reader (Molecular Devices, CA). The cell viability was expressed relative to the untreated control cells.

2.3 Apparatus

Modified Likens and Nikerson apparatus was used for preparation of the volatile constituents. GC/MS: Gas chromatograph coupled with a mass spectrometer GC/MS Finnigan Mat SSQ 7000 (Thermo Fischer Scientific, Bremen, Germany) for analysis of volatile oils and lipoidal matter. HP 1050 HPLC (Agilent Technologies, CA, USA) equipped with refractive index detector for free sugars analysis. HP 6890 GLC (Agilent Technologies, CA, USA) for polysaccharide hydrolysate analysis. Amino acid analyzer (LC 300 amino acid analyzer, Eppendorf, Germany). MaxQ 2000 plate shaker (Thermo Fisher Scientific, Bremen, Germany) and SpectraMax plus Microplate Reader (Molecular Devices, CA, USA) for cytotoxic assay.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening Results

Preliminary phytochemical screening of the aerial parts of the two plants under investigation revealed that both *R. pictus* and *R. vesicarius* contained; volatiles, carbohydrates and /or glycosides, flavonoids, anthraquinones, sterols and/or terpenes and tannins. While, alkaloids, saponins and coumarins were all absent according to the methods used.

3.2 Identification of Volatile Constituents

The results of the GC/MS analysis of the volatile constituents (Table 1) of the aerial parts of *R. pictus* revealed the identification of forty-two compounds representing about 90.94% of the total volatile constituents. The major components were found to be hexahydrofarnesyl acetone (7.29%), *n*-nonanal (6.18%), safranal (4.52%) and *n*-heptacosane (4.10%). The volatile constituents were dominated by the presence of aliphatic saturated hydrocarbons (from *n*-C_{11} to *n*-C_{30}) constituting 39.25% of the total volatile constituents composition, followed by aliphatic saturated and unsaturated aldehydes (17.26%), sesquiterpenes (14.78%), monoterpenes (11.42%), miscellaneous compounds (5.07%), an aromatic aldehyde (3.01%) and aliphatic alcohols (2.72%). This is the first report of the volatile constituents of *R. pictus* aerial parts. While, the volatile constituents of the aerial parts of *R. vesicarius* was found to contain a total of thirty-two compounds representing 74.23% of the total volatile constituents. The principal components of *R. vesicarius* were found to be *n*-hexadecane (5.39%), *n*-octadecane (4.33%), 2E-decenal (3.41%) and *n*-heptadecane (3.35%). The volatile constituents were dominated by the presence of aliphatic saturated hydrocarbons (from *n*-C_9 to *n*-C_{30}) constituting 52.72% of the total volatiles composition, followed by aliphatic saturated and unsaturated aldehydes (10.23%), miscellaneous compounds (5.02%), aromatic hydrocarbons (3.7%), a sesquiterpene (2.71%), a phenyl propene (1.79%) and an aliphatic acid (1.16%). The volatile constituents of *R. vesicarius* determined in this study showed a relatively different pattern to those already reported in literature for other geographical regions. The major constituents of the oil of *R. vesicarius* growing in six different parts of Saudi Arabia were: nonacosane (27.7%), pentatriacontane (16.7%), palmitic acid (12.9%), 3,8-dimethylundecane (6.4%), and 2-ethyl-2-methyl-l-decanol (5.8%) from Sudier; 9,12-octadecadienoic acid (41.4%), palmitic acid (39.4%) and phytol (7.7%) from Dawadmi; palmitic acid (30.7%) and phytol (17.4%) from Kharj, 9,12,15-octadecatrienoic acid (46.7%) and palmitic acid (26.0%) from Riyadh Airport; palmitic acid (37.2%) and 9,12,15-octadecatrienoic acid (29.5%) from Aljenadriah and 9,12-octadecadienoic acid (29.1%) and palmitic acid (20.3%) from Riyadh city. Palmitic acid was the major constituent (ranging from 12.9–39.4%) in the essential oil of *R. vesicarius* growing in different parts of Saudi Arabia [18].This was different from the results obtained in this study as palmitic acid was absent in the volatile constituents of the aerial parts of *R. vesicarius* grown in Egypt. Thus, there would appear to be considerable seasonal and geographic variations in the chemical composition of the volatiles of *R. vesicarius.* Total ion chromatograms of the volatile constituents of the aerial parts of *R. pictus* and *R. vesicarius* are presented in Figs. (1) and (2), respectively.

3.3 Identification of Lipoidal Matter

3.3.1 Unsaponifiable matter (USM)

GC/MS analysis of the USM (Table 2) of the petroleum ether extract of the aerial parts of *R. pictus* revealed the identification of twenty-eight compounds constituting 90.84% of the total USM content, of which butylated hydroxytoluene (19.08%), cycloeicosane (10.97%), 1-hexadecene (9.71%) and phytol (8.53%) were the main components. In case of *R. vesicarius,* GC-MS analysis of the USM

revealed the identification of twenty compounds constituting 80.8% of the total USM content, of which *n*-nonacosane (18.53%), 6,10,14-trimethyl-2-pentadecanone (17.87%), *n*-hentriacontane (12.49%) and butylated hydroxytoluene (6.25%) were the main components. Unoxygenated compounds predominated in both species, representing 58.94% and 43.37% of the total USM content of *R. pictus* and *R. vesicarius,* respectively. On the other hand, oxygenated compounds represented 31.9% and 37.33% of the total USM content of *R. pictus* and *R. vesicarius,* respectively. Five triterpenoidal steroidal compounds were identified in both species, including campesterol, stigmasterol, β-sitosterol, α- and β-amyrin, whereas taraxasterol was identified in *R. pictus,* only. Seventeen chemical constituents were common in the USM of both species. This is the first report on the USM matter of *R. pictus* aerial parts. The results of analysis of the USM of the aerial parts of *R. vesicarius* agreed with Al-Easa et al. findings [19]. Butylated hydroxytoluene (BHT), a major component in the USM of both species, is a lipid-soluble antioxidant commonly used in food processing and preservation. BHT has been shown to inhibit the induction of cancer by a wide variety of chemical carcinogens [20]. Moreover, it has been shown to inhibit the growth of *Staph. aureus*, to the extent that enterotoxin A could not be detected after 24h of incubation [21]. Besides, being a potent inactivator of lipid-enveloped viruses [22]. β –Sitosterol, the major sterol in both species, has several medicinal activities, being an antidiabetic, an anticancer and an antimicrobial against *E. coli, P. aeruginosa, Staph. aureus* and *Klebsiella pneumoniae* [23]. In addition to, its use as a pharmacologic treatment option for men with mild to moderate BPH particularly men who are at increased risk for adverse effects from alpha-blockers or surgical intervention. However, the long term effectiveness and safety of β -sitosterols and their ability to prevent complications from BPH are not known [24]. Total ion chromatograms of the USM of the aerial parts of *R. pictus* and *R. vesicarius* are illustrated in Figs. (3) and (4), respectively.

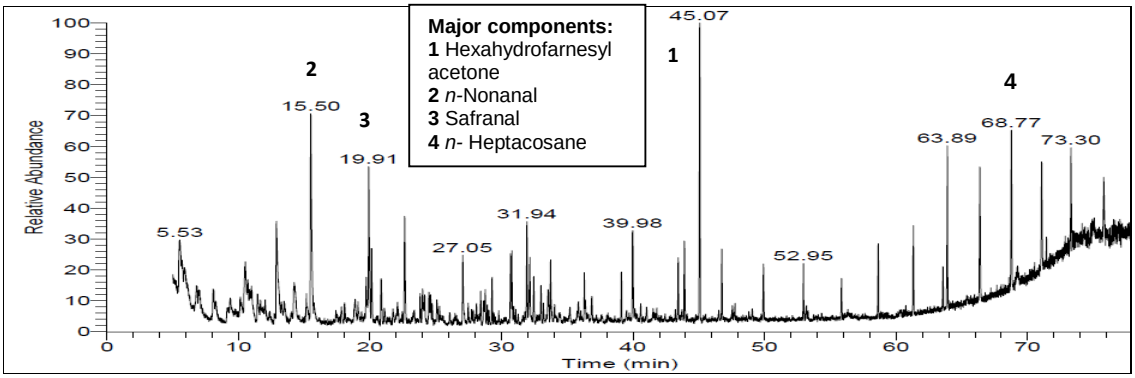

Fig. 1. Total ion chromatogram of the volatile constituents from the aerial parts of *R. pictus*

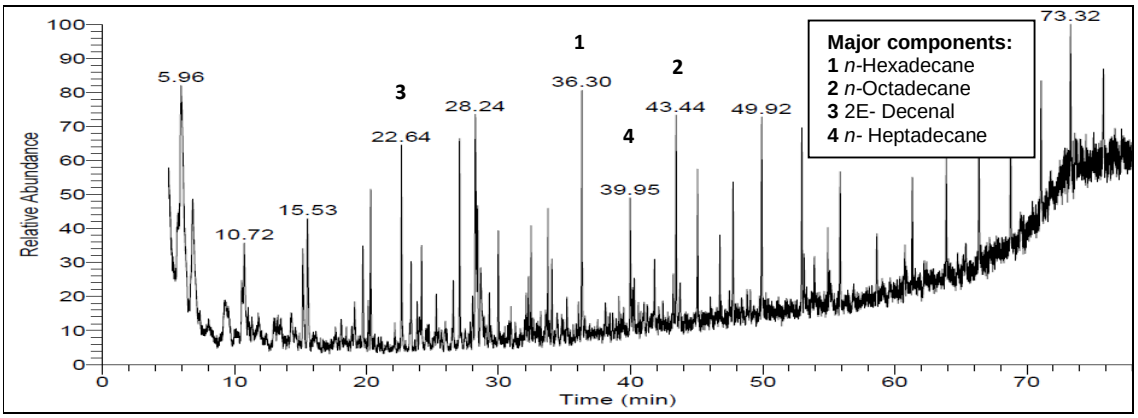

Fig. 2. Total ion chromatogram of the volatile constituents from the aerial parts of R. vesicarius

Table 1. GC/MS analysis of the volatile constituents from the aerial parts of *R. pictus* and *R. vesicarius*

No.	Compounds	Rt (min.)	Relative area (%)	
			R. pictus	*R. vesicarius*
1.	2E- Hexenal	5.50	2.15	-
2.	*n*-Nonane	6.82	-	2.44
3.	α-Pinene	8.08	0.97	-
4.	Pentanoic acid	9.24	-	1.16
5.	1-Octen-3-ol	10.14	0.93	-
6.	2-Pentyl furan	10.47	0.97	-
7.	Mesitylene	10.54	-	0.78
8.	*n*-Decane	10.73	-	1.37
9.	2E,4E-Heptadienal	11.44	0.95	-
10.	Phenylacetaldehyde	12.88	3.01	-
11.	1-Octanol	14.23	1.79	-
12.	*p*-Cymene	14.31	-	0.90
13.	*n*-Undecane	15.16	1.22	2.68
14.	*n*-Nonanal	15.52	6.18	2.76
15.	Terpinen-4-ol	18.87	0.91	-
16.	Naphthalene	19.10	-	0.85
17.	*n*-Dodecane	19.71	1.00	1.83
18.	Safranal	19.91	4.52	-
19.	*n*-Decanal	20.12	2.11	0.84
20.	Cyclocitral	20.84	1.65	-
21.	2E-Decenal	22.63	3.03	3.79
22.	*n*-Tridecane	24.16	-	1.57
23.	Thymol	24.48	0.95	-
24.	(E)1- (2,3,6-Trimethylphenyl) buta-1,3-diene(TPB, 1)	26.57	-	1.17
25.	2E- Undecenal	27.05	1.94	3.22
26.	*n*-Tetradecane	28.41	0.82	1.50
27.	3-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-propenal	28.63	-	2.06
28.	Hexahydro pseudoionone	28.77	0.97	-
29.	Benzoin	29.98	-	1.79
30.	Humulene	30.69	1.81	-
31.	E- Geranyl acetone	30.79	1.53	-
32.	Curcumene	31.39	2.40	-
33.	Ionone	32.15	1.93	-
34.	*n*-Pentadecane	32.46	1.12	2.71
35.	Tridecanal	33.01	0.89	-
36.	Cadinene	33.55	1.11	-
37.	*n*-Hexadecane	36.30	1.48	5.39
38.	*n*-Heptadecane	39.98	3.05	3.35
39.	*n*-Octadecane	43.44	1.57	4.33
40.	Nootkatone	43.91	2.17	-
41.	Hexahydrofarnesyl acetone	45.07	7.29	2.71
42.	*n*-Nonadecane	46.76	1.67	1.94
43.	*n*-Eicosane	49.92	1.35	3.21
44.	*n*-Heneicosane	52.95	1.46	-
45.	*n*-Docosane	55.86	1.07	3.24
46.	*n*-Tricosane	58.64	1.78	1.15
47.	*n*-Tetracosane	61.32	2.43	1.88
48.	*n*-Pentacosane	63.90	3.97	2.05
49.	*n*-Hexacosane	66.37	3.41	2.39
50.	*n*- Heptacosane	68.77	4.10	2.31
51.	*n*-Octacosane	71.07	2.89	2.40
52.	*n*-Nonacosane	73.32	2.72	2.88

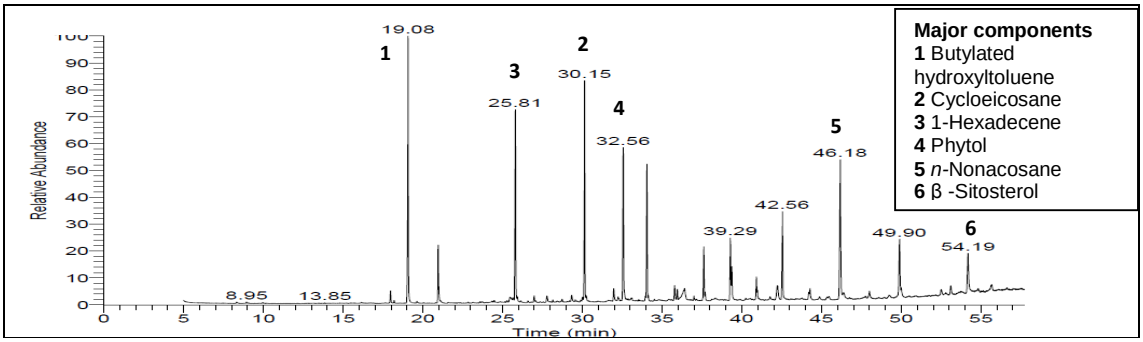

Fig. 3. Total ion chromatogram of USM from the petroleum ether extract of the aerial parts of *R. pictus*

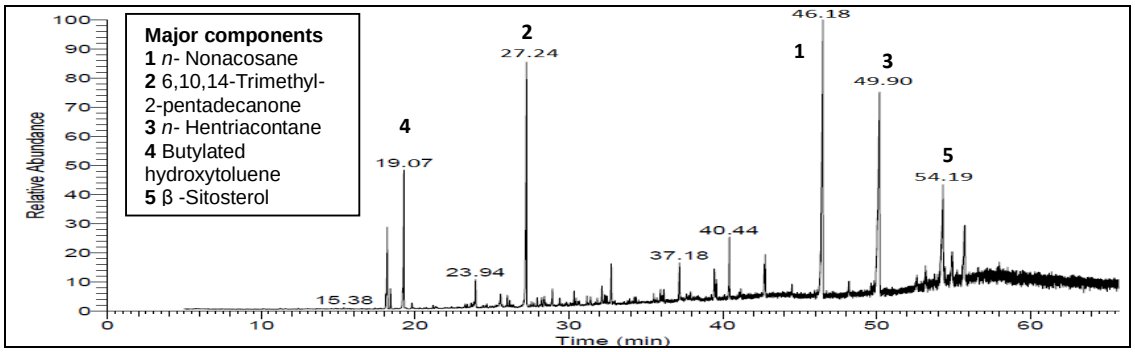

Fig. 4. Total ion chromatogram of USM from the petroleum ether extract of the aerial parts of *R. vesicarius*

3.3.2 Fatty acid methyl esters (FAME)

GC/MS analysis of the FAME (Table 3) of the aerial parts of *R. pictus* revealed the identification of sixteen FAME derivatives; representing 69.14% of the total composition. While that of *R. vesicarius* revealed the identification of ten FAME derivatives, representing 44.15% of the total composition. The major fatty acids identified in *R. pictus* were palmitic (28.16%), stearic (25.71%) and oleic (4.66%), while in *R. vesicarius* the major fatty acids were ricinoleic (13.7%), palmitic (10.3%) and oleic (6.54%). Saturated fatty acids predominated in *R. pictus*, constituting 63.63% of the total identified fatty acids, whereas mono- and di-unsaturated fatty acids constituted 4.99% and 0.52%, respectively. However, unsaturated fatty acids predominated in *R. vesicarius* constituting 26.32% of the total identified fatty acids, while saturated fatty acids constituted 17.83%. Nine fatty acids were common in both species. This is the first report of the GC/MS analysis of the fatty acid content of *R. pictus.* The results reported here for the GC/MS analysis of the FAME of the aerial parts of *R. vesicarius* corresponded to Al-Easa et al. findings [19]. It is worthy to mention that free fatty acids (FFA) have diverse and potent biological activities. Amongst these activities is their ability to inhibit the growth of bacteria. Whilst the antibacterial activity of FFA is under-studies as a subject, it is believed that the prime target of FFA is the cell membrane. Thus, FFA are considered attractive antibacterial agents for various applications in medicine, agriculture and food preservation [25]. Palmitic acid (C16:0), the major fatty acid in *R. pictus,* has been thought for many years to raise cholesterol levels. However, cholesterol elevating effects are controversial. Clanidin et al. assessed the effect of high vs. low palmitic acid intakes on plasma lipoprotein cholesterol levels and on rates for endogenous synthesis of cholesterol in healthy and hyperlipidemic subjects and concluded that palmitic acid has no effect on serum lipoprotein profiles in the presence of recommended intakes for linoleic acid [26]. However, Joshi- Barve et al. reported that palmitic acid induced production of proinflammatory cytokine interleukin-8 from hepatocytes, thereby potentially contributing to hepatic inflammation and consequent liver injury [27]. On the other hand, palmitic acid has shown

selective cytotoxic activity to human leukemic cells, but no cytotoxicity to normal human dermal fibroblast (HDF) cells *in vitro* [28]. Ricinoleic acid (12-hydroxy-9-*cis*-octadecenoic acid), the major fatty acid identified in *R. vesicarius* is an unsaturated omega-9 fatty acid, which has potent antioxidant [29], antimicrobial [30] and cytotoxic [31] activities. Total ion chromatograms of the FAME of *R. pictus* and *R. vesicarius* are illustrated in Figs. (5) and (6), respectively.

Table 2. GC/MS analysis of the USM from the petroleum ether extract of the aerial parts of *R. pictus* and *R. vesicarius*

No.	Compounds	Rt (min.)	Relative area (%)	
			R. pictus	***R. vesicarius***
1.	Butylated hydroxytoluene (BHT)	19.07	13.89	6.25
2.	(2E,4E)- Dodecadienol	20.97	3.18	-
3.	2-Pentadecanol	23.94	-	1.28
4.	1-Hexadecene	25.81	9.71	-
5.	6,10,14-Trimethyl-2-pentadecanone	27.24	0.36	17.78
6.	11-Octdecenal	27.78	0.45	-
7.	Methyl palmitate	28.65	-	0.84
8.	Cycloeicosane	30.15	10.97	-
9.	Phytol	32.57	8.53	1.66
10.	1-Docosene	34.05	6.85	-
11.	*n*-Docosane	34.14	0.21	-
12.	*n*-Tricosane	35.96	0.47	0.44
13.	1-Tetracosene	37.62	6.32	-
14.	*n*-Tetracosane	37.70	1.61	1.69
15.	*n*-Pentacosane	39.38	1.49	0.76
16.	1-Hexacosene	40.92	1.44	-
17.	*n*-Hexacosane	42.24	1.20	1.17
18.	*n*-Heptacosane	42.56	4.52	1.30
19.	1-Octacosene	44.20	0.32	-
20.	*n*-Octacosane	44.28	0.63	0.68
21.	*n*-Nonacosane	46.18	9.86	18.53
22.	*n*-Triacontane	46.51	0.31	1.03
23.	*n*-Hentriacontane	49.90	3.93	12.49
24.	17-Pentatricontene	50.00	0.45	-
25.	Campesterol	52.50	0.47	0.83
26.	Stigmasterol	53.11	0.69	1.25
27.	β -Sitosterol	54.19	3.06	6.30
28.	Taraxasterol	54.83	0.39	-
29.	α -Amyrin	54.92	0.63	2.07
30.	β-Amyrin	55.67	0.25	4.36

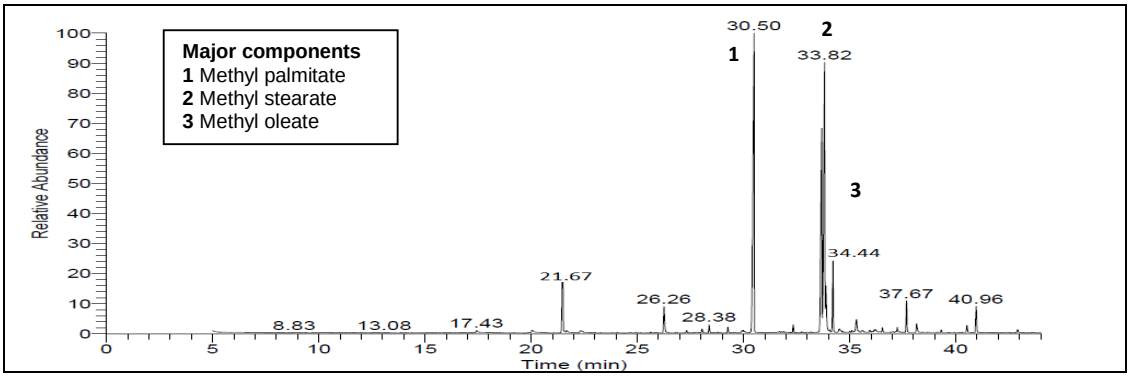

Fig. 5. Total ion chromatogram of the FAME of the aerial parts of *R. pictus*

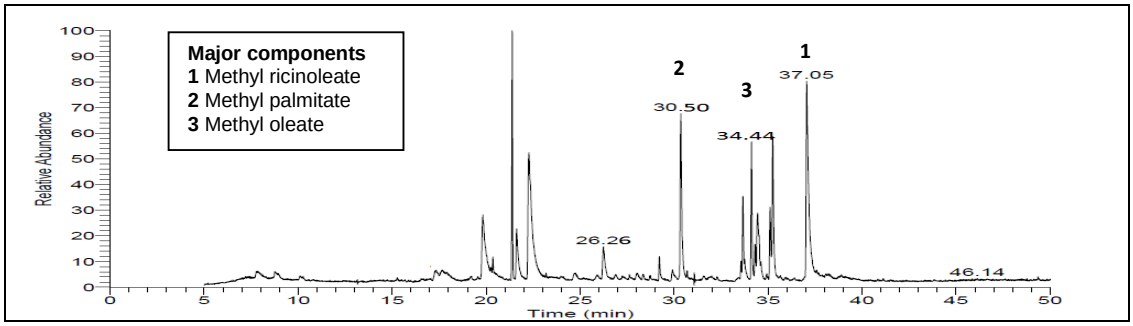

Fig. 6. Total ion chromatogram of the FAME of the aerial parts of *R. vesicarius*

Table 3. GC/MS analysis of the FAME from the petroleum ether extract of the aerial parts of *R. pictus* and *R. vesicarius*

No.	Compounds	Rt (min.)	Relative area (%)	
			R. pictus	*R. vesicarius*
1.	Methyl laurate (12:0)	21.67	2.01	3.61
2.	Methyl myristate	26.26	2.06	2.34
3.	Methyl pentadecanoate (15:0)	28.38	0.6	0.44
4.	Methyl palmitate (16:0)	30.35	28.16	10.30
5.	Methyl palmitoleate (9-16:1)	31.89	0.33	0.9
6.	Methyl heptdecanoate (17:0)	32.34	0.50	-
7.	Methyl 2-hydroxyhexadecanoate	32.75	0.10	-
8.	Methyl stearate (18:0)	34.21	25.71	1.14
9.	Methyl oleate (9-18:1)	34.44	4.66	6.54
10.	Methyl 6,9-octadecadienoate (6,9-18:2)	34.52	0.45	4.83
11.	Methyl 5,12-octadecadienoate (5,12- 18:2)	34.99	0.07	0.35
12.	Methyl phytanate	35.10	0.11	-
13.	Methyl ricinoleate	37.05	-	13.7
14.	Methyl arachidate	37.67	2.19	-
15	Methyl heneicosanoate	39.31	0.2	-
16.	Methyl docosanoate	40.95	2.02	-
17.	Methyl tricosanoate	42.91	0.3	-

3.4 Identification of Amino Acid Content

Results of the analysis of the amino acid content of the aerial parts of both species (Table 4) revealed the presence of phenylalanine, threonine, valine, methionine, isoleucine, leucine, histidine and lysine as essential amino acids in the two species with different proportions. The major essential amino acids in *R. pictus* and *R. vesicarius* were leucine (11.23%) and phenyl alanine (12.35%), respectively. While, the major non essential amino acid in both species was glutamic acid (17.46% and 16.7%) in *R. pictus* and *R. vesicarius*, respectively. Tyrosine (4.66%), a non-essential amino acid was only present in *R. pictus.* It's noteworthy that an optimal balance in nutritional diet amino acids is crucial for the whole body homeostasis and regulating key metabolic pathways. Both species are rich in functional amino acids including leucine, glutamic acid, proline and arginine, among others, which are of particular importance in ameliorating health problems at various stages of the life cycle (obesity, diabetes, cardiovascular disease); and optimizing efficiency of metabolic transformations to enhance muscle growth, milk production and preventing excess fat deposition thus reducing adiposity [32].

3.5 Identification of Carbohydrate Content

3.5.1 Free sugars

HPLC analysis of the prepared free sugars of the aerial parts of *R. pictus* and *R. vesicarius* (Table 5) revealed the presence of glucose, fructose, sucrose and mannitol in both species with different proportions. The major sugar in *R. pictus* and *R. vesicarius* was sucrose (19.62% and 20.64%), respectively.

Table 4. Amino acid content of the aerial parts of *R. pictus* and *R. vesicarius*

Amino acids		*R. pictus*	*R. vesicarius*
		Relative area%	Relative area%
Essential amino acids			
1	Threonine	4.76%	4.36%
2	Valine	8.66%	6.73%
3	Methionine	0.74%	1.99%
4	Isoleucine	2.5%	2.79%
5	Leucine	11.23%	8.69%
6	Phenyl alanine	7.59%	12.35%
7	Histidine	2.41%	2.03%
8	Lysine	4.13%	4.19%
Non essential amino acids			
1	Aspartic acid	8.5%	8.5%
2	Serine	8.75%	6.57%
3	Glutamic acid	17.46%	16.7%
4	Proline	0.98%	0.77%
5	Glycine	7.31%	6.72%
6	Alanine	16.87%	14.21%
7	Tyrosine	4.66%	-
8	Arginine	5.47%	3.31%

Table 5. Results of HPLC analysis of free sugars of the aerial parts of *R. pictus* and *R. vesicarius*

Authentic sugars	Retention time	*R. pictus*	*R. vesicarius*
		Relative area %	Relative area %
Sucrose	6.73	19.62	20.64
Glucose	7.77	16.5	17.83
Fructose	9.57	14.03	10.12
Mannitol	12.2	4.51	3.10
Total identified sugars		54.66%	51.69%

3.5.2 Polysaccharides

The yield of the isolated polysaccharide was 2.28% w/w as compared to the dry weight of the dry aerial parts of *R. pictus* and 6.73% w/w as compared to the dry aerial parts of *R. vesicarius.* GLC analysis of the polysaccharide hydrolysate of *R. pictus* (Table 6) revealed the presence of arabinose (5.74%), ribose (0.20%), rhamnose (3.40%), sorbitol (2.10%), galactose (5.56%) and glucose (12.27%). While that of *R. vesicarius* revealed the presence of arabinose (8.28%), ribose (2.30%), rhamnose (11.31%), galactose (10.90%) and glucose (7.25%).

3.6 Determination of *in vitro* Cytotoxic Activity

Total ether, chloroformic and 70% methanolic extracts of both species were tested for their cytotoxic activities. The results (Table 7) revealed that the potential response of cytotoxic activity is a dose-response manner, which corresponds to Gomaa and Saleh [33] findings. The chloroformic extract of *R. pictus* aerial parts showed strong cytotoxic activity against hepatocellular carcinoma (HepG2) cell line (IC_{50}= 1.33 µg/mL) and lung carcinoma (A549) cell line (IC_{50}= 3.35 µg/mL) when compared to doxorubicin. While, the 70% methanolic extract of *R. pictus* aerial parts showed cytotoxic activity towards breast cancer (MCF7) cell line (IC_{50}= 15.5 µg/mL) when compared to doxorubicin.

Table 6. Results of GLC analysis of polysacharide hydrolysate of the aerial parts of *R. pictus* and *R. vesicarius*

Authentic sugars	Retention time	*R. pictus*	*R. vesicarius*
		Relative area %	Relative area %
Arabinose	8.03	5.74	8.28
Ribose	8.46	0.20	2.30
Rhamnose	9.14	3.40	11.31
Sorbitol	10.98	2.10	-
Galactose	11.98	5.56	10.90
Glucose	12.12	12.27	7.25
Total identified sugars		29.27%	40.04%

Table 7. IC_{50} of different extracts of the aerial parts of *R. pictus* and *R. vesicarius* (in comparison with Doxorubicin)

Tumor cell line	IC_{50} (µg/mL)						
	R. pictus			*R. vesicarius*			DOX
	1	2	3	4	5	6	
HepG-2	955	1.33	97.7	>500	151	257	0.772
A549	0.29	3.55	75.8	>500	426.6	>500	0.29
MCF-7	602.5	107	15.5	676	275.4	>500	0.0912

1-3: successive extracts of R. pictus aerial parts [total ether (1), chloroform (2), 70% methanol (3)];
4-6: successive extracts of R. vesicarius aerial parts [total ether (4), chloroform (5), 70% methanol (6)];
DOX: Doxorubicin reference drug

These results can possibly be attributed to the presence of phenolic compounds, including anthraquinones and flavonoids, as well as to their antioxidant activities [34,35]. This is the first report of the cytotoxic activity of R. pictus.

4. CONCLUSION

In conclusion, the preliminary phytochemical screening of the aerial parts of *R. pictus* and *R. vesicarius* (Family Polygonaceae) revealed the presence of a variety of constituents including volatiles, carbohydrates and /or glycosides, flavonoids, anthraquinones, sterols and/or terpenes, amino acids and tannins. These constituents may have contributed to the cytotoxic activities revealed in this study. Bioassay directed fractionation of the potent active extract is in progress to confirm the phytochemical components attributing to the biological activities of these species.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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