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Chemical Characterization of *Reichardia tingitana* Methanolic Extract and Evaluation of Its Antioxidant and Anticancer Activity

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Abstract

The aim of this study was to employ GC-MS to assess the biological potency and chemical composition of the aerial parts of *Reichardia tingitana* (L.) Roth. Using this technique, 16 components were interpreted from the extracted plant, accounting for 100% of total volatile compounds. Commonly, dodecane (5.43%), tetradecane (9.11%), ethyl palmitate (8.56%), methyl (E)-octadec-11-enoate (11.50%), 2-hydroxypropane-1,3-diyl(9E,9'E)-bis(octadec-9-enoate) (7.04%), lup-20(29)-en-3-ol (Lupeol) (10.81%), and olean-12-en-3-one (23.99%) are positioned as the major components. Fatty acids and their esters are the major categories (38.06%), steroids (34.8%) and hydrocarbons (24.11%). The DPPH antioxidant activity of the *R. tingitana* extracted components revealed that the shoot extract is the most powerful, with an IC_{50} value of 34.91 mg L^{-1} and a radical scavenging activity percentage of 60.35%. According to the current result, the 50% methanol crude shoot extract of *R. tingitana* showed greater potential anticancer activity with high cytotoxicity for two tumor cells HepG-2 and PC3 cells (IC_{50} = 90.55 and 79.27 µg mL^{-1}, respectively), and non-cytotoxic activity for WI-38 normal cells (IC_{50} = >100 µg mL^{-1}). *R. tingitana* is a promising plant with biological activity. Further work will involve isolation of pure compounds and determination of the bioactivity of individual compounds.

Keywords*: Asteraceae, Reichardia tingitana, GC/MS; Antioxidant; Anticancer.*

1. Introduction

Since the beginning of time, people have sought for natural remedies for various illnesses. As with animals, the usage of medicinal herbs began as an instinctual behavior [1,2]. The old peoples used medicinal plants primarily as simple pharmaceutical forms, while in 16th and 18th centuries, the demand for compound drugs was increasing. The compound drugs comprised medicinal plants along with drugs of animal and plant origin [3,4]. Now, plants are essential to healthcare and represent the finest source for secure future drug supply.

Asteraceae family (Compositae) is among the largest plant families in the world, accounting for around 10% of the total flora on all continents with the exception of Antarctica, with more than 23,000 and 1,620 genera [5,6]. Also, Asteraceae is one of the biggest family of the Egyptian flora [7]. The majority of plant members representing this family are herbaceous in nature, but shrubs and trees, as well as creepers and climbers, are also reported [8]. Plants under this family have been widely utilized in the past and are still used today for their medicinal properties. Members of Asteraceae have been reported to possess many biological activities including antibacterial, antifungal, antidiabetic, antihelminthic, immunostimulatory, anticancer and cytotoxic activities [9-15].

Reichardia tingitana (L.) Roth, is a glabrous annual plant that grows wildly in Egypt's inland and coastal desert. The plant has a taproot that extends up to 40 cm, which first produces a rosette of big radical leaves, then the stem branches out from the base. Its flowering season lasts from March to May. Aboveground biomass has undergone preliminary phytochemical analysis, which reveals the presence of phenolics, tannins, flavonoids, coumarins, volatile oils, glycosides, flavonoids, lactones, esters, significant levels of sterols, and/or triterpenes [15-17].

Understanding plant toxicity and defending people and animals against natural toxins are two benefits of studying therapeutic plants. The synthesis of secondary metabolites by plants is what gives them their therapeutic properties [18,19]. With this in mind, the field of study in natural product chemistry has seen a surge in attention. Therapeutic needs, the remarkable diversity of chemical structure and biological activities of naturally occurring secondary metabolites, the use of novel bioactive natural compounds as biochemical probes, improved methods to isolate, purify, and structurally characterize these active constituents, and

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advanced techniques to characterize these active constituents are some of the reasons for this interest [20,21].

Despite its significance, little study has been done on *R. tingitana* extract. Therefore, in this study we investigated the chemical composition of air-dried aerial parts of this plant used GC-MS analysis. The study looked at the antioxidant activity of the extracted plant using the DPPH free radical scavenging assay, as well as in vitro cytotoxicity against Hepatocellular carcinoma (HePG-2), Mammary gland carcinoma (MCF-7) and Human prostate cancer (PC3) cell lines.

2. Materials and methods

2.1. Plant material and extraction process

The aerial parts of *Reichardia tingitana* were collected in April 2022 during the blooming season in Wadi Araba, Eastern Desert, Egypt (28°58'50.54"N 32° 7'37.41"E). In accordance with Tackholm [22] and Boulos [23], the plant was identified. After being washed, the samples were given time to air dry. 30 gram of dried plant material were put into a conical flask (500 mL) and could hold 250 mL of methanol. The mixture was then placed in a water bath shaker (Memmert WB14, Schwabach, Germany), where it was continually stirred for two hours at room temperature. Whatman filter sheets were used to filter the mixture (no. 1, 125 mm, Cat. No. 1001 125, Germany). The obtained alcoholic extracts were evaporated to dryness using rotary-evaporator at 40 °C and the viscous residue were kept at 4 °C for storage [24].

2.2. Gas chromatography-mass spectrometry analysis (GC-MS)

By using the plant extract on a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 m film thickness), the chemical composition of the extracted *R. tingitana* plant was characterized [25]. The column oven's temperature was initially held constant at 50 °C, then increased by 5 °C per minute to reach 250 °C and hold for 2 minutes, and then increased by 30 °C per minute to achieve the final temperature of 300 °C and hold for 2 minutes. Temperatures of 260 and 270 °C, respectively, were maintained for the MS injector and transfer line. Helium (He) was used as a carrier inert gas at a constant flow rate of 1 mL/min. After the solvent had been removed after 4 minutes, 1 l of diluted samples were fed into the GC in split mode with an Autosampler AS1300 right away. At an ionization voltage of 70 EV, EI mass spectroscopy data were collected in packed scan mode over the m/z range of 50–500. The temperature of the ion source was fixed at 200 °C. It was feasible to determine the chemical components of each unique plant material by comparing the mass spectrum data of the numerous extracted plant components to those of the mass spectrometry databases WILEY 09 and NIST 14. The GC-MS analysis found five possible components for each observed peak. The chosen structure in the case of the many recommended components was determined by the likelihood factors and the primary structure's fragmentation patterns.

2.3. Antioxidant DPPH Assay

The desired concentrations of *R. tingitana* were obtained by diluting a stock solution of the extracted plant in methanol (5, 10, 20, 30, 40, and 50 mg L^{-1}). The antioxidant activity of the extracted *R. tingitana* was performed using DPPH assay following the previously reported protocol [15,26].

2.4. Cytotoxicity assay

Three specific human tumor cell lines, including hepatocellular carcinoma (HePG-2), mammary gland carcinoma (MCF-7), and human prostate cancer (PC3), were purchased from the ATCC holding organization for biological goods and vaccines (VACSERA), Cairo, Egypt. Doxorubicin was a typical chemotherapeutic anticancer drug. Fetal bovine serum, MTT, RPMI-1640 medium, and Sigma Co.'s DMSO were the chemical reagents employed (FBS; Gibco Life Technologies, Paisley, UK). The cytotoxicity of the extracted *R. tingitana* against cell lines HePG-2, MCF-7 and PC3 was performed using MTT based assay following the previously reported protocol [27,28].

2.5. Statistical Analysis

Using the Costat software (CoHort Software, Monterey, CA, USA), the antioxidant, and cytotoxic activity experiments were conducted three times with three replications. The outcomes were then subjected to a one-way ANOVA to assess the significance of the differences between samples.

3. Results & Discussion

3.1. Gas-Chromatography Mass Spectroscopy "GC-MS"

The *R. tingitana* extract's chemical components were determined by GC-MS mass spectroscopic analysis. The chart in Figure 1 showed the relationship between the relative abundance and the retention time at which a specific constituent was recorded in a chromatogram from a GC-MS mass spectrometer. The results in Table 1 indicated that sixteen chemical components were interpreted at different retention times with diverse composition percentages. The major components with the highest percentages of composition of the total constitutes of the extract were identified as dodecane (5.43%), tetradecane (9.11%),

ethyl palmitate (8.56%), methyl (E)-octadec-11-enoate (11.50%), 2-hydroxypropane-1,3-diyl(9E,9'E)-bis(octadec-9-enoate) (7.04%), lup-20(29)-en-3-ol (Lupeol) (10.81%), and olean-12-en-3-one (23.99%). It was found that hydrocarbons expressed by five components constitute the major composition percentage for tetradecane (9.11%), while 4a-Hydroxy-4,8a-dimethyl-6-(prop-1-en-2-yl)octahydro naphthalen-1(2H)-one is the only characterized component for terpenes with composition percentage at 3.05%. In addition, fatty acids, and esters of fatty acids represented by eight constitutes, in which ethyl palmitate (8.56%), methyl (E)-octadec-11-enoate (11.50%), and 2-hydroxypropane-1,3-diyl(9E,9'E)-bis(octadec-9-enoate) (7.04%) are the major components by highest composition percentages. The steroids were represented by two components with the most abundant ratios from the total composition percentages that were identified as lup-20(29)-en-3-ol (Lupeol) (10.81%), and olean-12-en-3-one (23.99%).

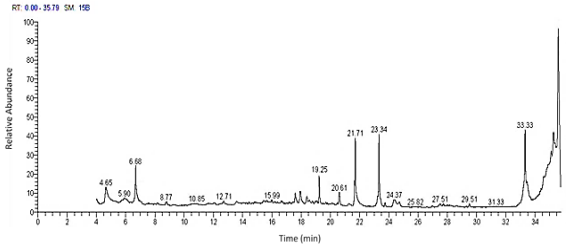

Figure 1. Chromatogram of the methanol extract of *R. tingitana* aerial parts by GC-MS.

Table 1. The interpreted components of the MeOH extract of *R. tingitana* aerial parts by GC-MS.

No.	Chemical name	Classification	RT (min)	Molecular Weight	Molecular formula	Composition %
	Hydrocarbons					
1	Dodecane	Hydrocarbon	4.65	170.34	$C_{12}H_{26}$	5.43
2	Tetradecane	Hydrocarbon	6.67	198.39	$C_{14}H_{30}$	9.11
3	Heptadec-13-yn-1-ol	Oxygenated hydrocarbon	17.94	252.44	$C_{17}H_{32}O$	3.24
4	6,10,14-Trimethylpentadecan-2-one	Oxygenated hydrocarbon	19.25	268.49	$C_{18}H_{36}O$	4.05
5	psi.,.psi.-Carotene, 1,2-dihydro-1-hydroxy-(Rhodopin)	Oxygenated hydrocarbon	34.72	554.90	$C_{40}H_{58}O$	2.28
	Terpenes					
6	4a-Hydroxy-4,8a-dimethyl-6-(prop-1-en-2-yl)octahydronaphthalen-1(2*H*)-one	Sesquiterpene	17.60	236.36	$C_{15}H_{24}O_2$	3.05
	Fatty acids, and esters of fatty acids					
7	Oleic acid	Fatty acid	18.39	282.47	$C_{18}H_{34}O_2$	2.14
8	Methyl 14-methylpentadecanoate	Fatty acid ester	20.61	270.46	$C_{17}H_{34}O_2$	3.24
9	Ethyl palmitate	Fatty acid ester	21.71	284.48	$C_{18}H_{36}O_2$	8.56
10	Methyl (7*E*,10*E*)-octadeca-7,10-dienoate	Fatty acid ester	23.24	294.48	$C_{19}H_{34}O_2$	1.71
11	Methyl (*E*)-octadec-11-enoate	Fatty acid ester	23.33	296.50	$C_{19}H_{36}O_2$	11.50
12	3-(Octadecyloxy)propyl oleate	Fatty acid ester	35.24	593.03	$C_{39}H_{76}O_3$	2.26
13	2-Hydroxypropane-1,3-diyl (9*E*,9'*E*)-*bis*(octadec-9-enoate)	Fatty acid ester	35.40	621.00	$C_{39}H_{72}O_5$	7.04
14	(2-Phenyl-1,3-dioxolan-4-yl)methyl oleate	Fatty acid ester	35.50	444.66	$C_{28}H_{44}O_4$	1.61
	Steroids					
15	Lup-20(29)-en-3-ol (Lupeol)	Steroid	33.33	426.73	$C_{30}H_{50}O$	10.81
16	Olean-12-en-3-one	Steroid	35.72	424.71	$C_{30}H_{48}O$	23.99
	Total					Σ= 100.0

RT: Retention time (min)

There are four classes of the investigated chemical constitutes interpreted from the GC-MS mass spectroscopic analysis of the R. tingitana extract involving hydrocarbons, terpenes, fatty acids, esters of fatty acids, and steroids. The class of fatty acids and their esters are the major categories with a percentage of composition at 38.06%, while steroids and hydrocarbons are located in the second, and third orders with percentages of the composition of 34.8, and 24.11%, respectively, from the total composition of the extracted components (Figure 2).

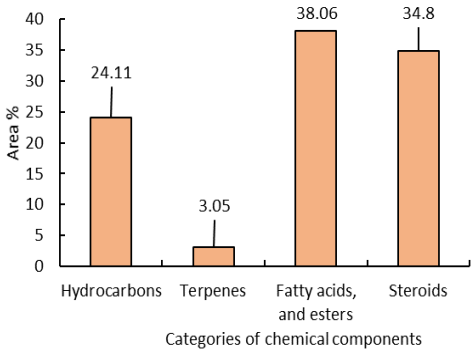

Figure 2. The classified chemical constitutes deduced from the extracted *R. tingitana* by GC-MS.

3.2. Biological characteristics of the plant extracts

3.2.1. Antioxidant activity-DPPH assay

The capacity of *R. tingitana* methanolic extract to scavenge DPPH free radicals in comparison to ascorbic acid was used to determine its antioxidant activity. Half maximum inhibitory concentration (IC_{50}) values were used to express the scavenging effects of plant extracts and the standard on the DPPH radical; the findings are shown in Table 2. A lower IC_{50} value indicates a greater capacity to scavenge DPPH radicals. Subsequently, the results as presented in Table 2, confirmed that the extract from the shoot has the most antioxidant scavenging activity, with an IC_{50} of 34.91 mg/L. The main element regulating the mechanism of the reactions engaged in the assessment of the antioxidant ability of the examined extract is the predominance of fatty acids and their derivatives (38.06%) and hydrocarbons (24.11%) of total separated components. The present results of *R. tingitana* were in agreement with the results of Cornara et al. [29], Abd-ElGawad et al. [15 & 30], and Salama et al. [27].

On the other hand, fatty acids and lipids, which were extracted from *Sisymbrium irio, Aesculus indica*, *Abies pindrow*, and *Rumex vesicarius* shown strong antioxidant capabilities for scavenging the free radicals in the solution [31, 28]. The major components with the highest percentages of composition of the total constitutes of the extract were identified as olean-12-en-3-one (23.99%); methyl (E)-octadec-11-enoate (11.50%), lup-20(29)-en-3-ol (Lupeol) (10.81%), tetradecane (9.11%) and ethyl palmitate (8.56%). The ability of reactive oxygen species, such as phenolics, fatty acids, terpenes, oxygenated hy-drocarbons, or carbohydrates, to scavenge or stabilize free radicals generally determines the antioxidant capacity of bioactive chemicals [32-34].

Table 2. Radical scavenging activity percent (%), and IC_{50} values (mg/L) at various concentrations of the methanol extracted of *R. tingitana* and the standard ascorbic acid by DPPH assay.

Treatment	Conc. (mg /L)	Scavenging activity percentage
		Shoot
R. tingitana	5	30.50±0.92
	10	32.24±0.98
	20	40.40±1.09
	30	46.02±1.24
	40	53.66±1.45
	50	60.35±1.63
	IC_{50} (mg /L)	**34.91**
	$LSD_{0.05}$	0.0001***
Ascorbic acid	1	2.73±0.09
	2.5	10.99±0.35
	5	38.20±0.75
	10	45.19±0.82
	15	56.23±1.23
	20	64.97±1.52
	IC_{50} (mg /L)	**13.30**
	$LSD_{0.05}$	0.0001***

In this investigation, the antioxidant activity of *R. tingitana* shoot extract was superior to that of other wild plant extracts in many countries. Different studies have demonstrated that the quantity of bioactive chemicals, particularly phenolic compounds such as flavonoid, phenolic acids, ascorbic acid and carotenoids, directly affects the antioxidant capabilities of plants [35]. From our study this plant contains nonvolatile compounds such as tannins, flavonoids and phenolics [27].

2.2.3. Anticancer Activity

Cancer is one of the important causes of mortality and morbidity around the world and the variety of instances are continuously growing estimated to be 21 million via 2030 [36,37]. Nearly, 80% of the world's population rely upon traditional drug remedies and extra than 60% of clinically permitted anticancer capsules are derivatives of those medicinal plant [38]. Medicinal plants serve as nature's gift to humans to help them pursue better health. Plants and their bioactive compounds are in medicinal practices since ancient times [39]. In this study, the cytotoxic activity of the prepared plant sample extract was evaluated using an MTT assay. The sample was tested in vitro against two tumor cells, i.e., HepG-2, and PC3 cell lines (Table 3). A decision was made on doxorubicin as the reference drug, by comparing the results of the tested sample against the different tumor cells. The control sample is a benefit for calculating the cell viability percent, as it produces 100% viability of healthy cells. The experiments were run using five concentrations of each plant extract (1.56, 3.125, 6.25, 12.50, 25, 50 and 100 µg mL^{-1})

prepared in a serial dilution. According to the results, the methanolic extracts of plant exhibited cytotoxic activity in a dose-dependent manner, which was comparable with doxorubicin as a reference standard. At 100 µg mL^{-1}, the extract of *R. tingitana* showed inhibition activities of 51.88%, 56.11%, and 11.60% for HepG-2 and PC3 human tumor cells and normal cell (WI-38), respectively. However, the lowest concentration (1.56 µg mL^{-1}) shows the lowest cytotoxic activity in all samples (Table 3).

The curves generated by plotting the percentages of cell survival vs drug concentration (µM) were used to calculate the IC_{50} values, which expressed the concentration that represented 50% of the inhibition of cell growth. When a result, as the extract concentration and IC_{50} values decline, the potency of cytotoxicity will increase. For HepG-2 and PC3 cells as well as WI-38 normal cells, the IC_{50} values of the MeOH extract from the *R. tingitana* sample were 90.55, 79.27, and >100 µg mL^{-1}, respectively. To compare the outcomes of the tested compounds against the various cancer cells, which achieved IC_{50} values of 5.24, 8.80, and >100 µg mL^{-1} for HepG-2, PC3, and WI-38, respectively, doxorubicin was used as a reference drug (Table 3 and Figure 3).

According to the IC_{50} data, the extracted shoot of this plant shows non-cytotoxic activity for normal cells (WI-38) and weak cytotoxic activity for two human tumor cells (HepG-2 and PC3). This result is inconsistent with the results obtained by Salama et al. [27] who pronounced that the cytotoxic activity of *R. tingitana* methanolic extract collected from Mediterranean coast gives moderate activities towards WI-38, HepG-2 and PC3 cells. But, this result is agree with result obtained by Csupor-Löffler et al. [40] who studied, the ant proliferative activities of aqueous and organic extracts prepared from 26 Hungarian species of the family Asteraceae were tested in vitro against HeLa (cervix epithelial adenocarcinoma), A431 (skin epidermoid carcinoma) and MCF7 (breast epithelial adenocarcinoma) cells by using the MTT assay.

It is important to note that the structural nature of each extract's components and the nature of the cancer cell line are frequently correlated with the cytotoxicity of extracted samples as cytotoxic agents on HepG2 and PC3 tumor cell lines. Furthermore, the characteristics of the extracted plant particles, such as their size, aggregation, and surface shape, may influence their cytotoxicity [41]. Many polyphenols, such as isoflavones, are phytoestrogens that may bind to estrogen receptors and have an estrogenic impact on the tissues or organs they are intended to affect. Additionally, newly identified substances like polyphenol and flavonoids could be to blame for the anti-inflammatory and cytotoxic effects of plant extracts [42,28]. *R. tingitana* contains non-volatile substances such flavonides and phenolics in the current investigation [15,27].

Table 3. Cytotoxic activity and the IC_{50} values of the prepared plant samples against the tumor and normal cells at different concentrations, and doxorubicin as standard. Hepatocellular carcinoma (HePG-2), Human prostate cancer (PC3) and normal cell (WI-38).

Samples	Conc. (µg/mL)	In Vitro Cytotoxicity		
		HePG-2	PC3	WI-38
Doxorubicin	100	93.34	91.62	10.99
	50	88.55	81.82	9.77
	25	85.28	75.52	7.02
	12.5	68.72	58.42	5.11
	6.25	50.80	41.92	2.92
	3.125	39.20	25.59	1.51
	1.56	25.82	23.14	0.00
	[(a)]IC_{50}	**5.24**	**8.80**	**>100**
R. tingitana-Inland sample	100	51.88	56.11	11.60
	50	44.76	44.75	10.52
	25	31.72	32.04	5.91
	12.5	20.81	24.89	4.31
	6.25	10.20	19.33	2.06
	3.125	4.21	14.03	0.00
	1.56	0.00	0.00	0.00
	IC_{50}	**90.55**	**79.27**	**>100**

[(a)]IC_{50}: inhibitory concentration (µg): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), and above 100 (non-cytotoxic).

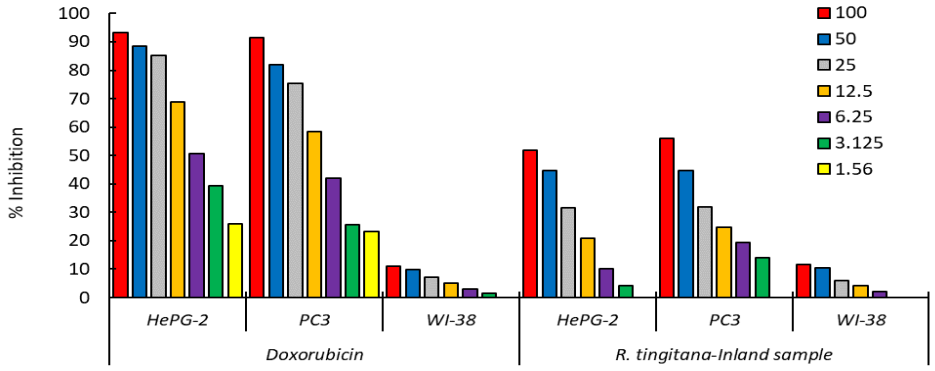

Figure 3. The inhibition percentage of the prepared plant samples against the tumor and normal cells at different concentrations, and doxorubicin as standard. Hepatocellular carcinoma (HePG-2), Human prostate cancer (PC3) and normal cell (WI-38)

4. Conclusions

In conclusion, by using GC-MS analysis seventeen components were interpreted from the MeOH extract of *R. tingitana* aerial parts. The major components with the highest percentages of composition of the total constitutes of the extract were identified as olean-12-en-3-one (23.99%), methyl (E)-octadec-11-enoate (11.50%), lup-20(29)-en-3-ol (Lupeol) (10.81%), tetradecane (9.11%), ethyl palmitate (8.56%),2-hydroxypropane-1,3-diyl(9E,9'E)-bis(octadec-9-enoate) (7.04%) and dodecane (5.43%). Fatty acids and their esters are the major categories with a percentage of composition at 38.06%, while steroids and hydrocarbons are located in the second, and third orders with percentages of the composition of 34.8, and 24.11%, respectively, from the total composition of the extracted components. Shoot extract exhibited the highest antioxidant activity, displaying a higher potential to trap free radicals in the DPPH solution with an IC_{50} value of 34.91 mg/L. Moreover, in comparison to control, the methanol extract of *R. tingitana* shown increased potential anticancer activity with significant cytotoxicity for the two tumor cells HepG-2 and PC3 cells. The significant biological findings and the remarkable percentage of phenolic, flavonoid, and tannin contents found in the *R. tingitana* extract, in addition to the terpenes, fatty acids, and their esters, supported the possibility of further study on this plant for the creation of drugs from natural sources.

Conflicts of interest

There are no conflicts to declare.

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