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Comparative Study of the Alkaloidal and Tannin Contents of Some *Reseda* species

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Abstract: The total alkaloids of leaves, stems and roots of *Reseda ducrsiva*, *Reseda muricata* and *Reseda Pruinosa* were determined by two different methods. The percentages of total alkaloids of roots were found to be the highest values for the three plants. The alkaloid constituents of each plant were fractionated on silica gel column, purified by PTLC and identified through Mass Spectrum, H^1NMR and IR Spectrum. Three alkaloids were isolated from *R.ducrsiva*, β-hydroxyphenyl-N-methyl amine, hordenin and 5-phenyl-2-oxazolidone, while the alkaloids, β-hydroxyphenyl-N-methyl amine, hordenin and 2-amilino 1,4-naphthoquinone were isolated from *R.muricata*. The alkaloid, β-hydroxyphenyl-N-methyl amine, was isolated from *R.Pruinosa*. The percentages of total tannins of the leaves were found to be the highest values for the three plants. Quantitative determination of gallotannins using HPLC revealed that *R.ducrsiva* plant contained the highest concentration of gallotannins. Fractionation of the tannin constituents of each plant on sephadex LH-20 column and purification by TLC revealed that *R.ducrsiva* contained, 1,2,3,4,6-pentagalloyl-o-D-glucose and 6-m-digalloyl-1,2,3,4-tetragalloy-o-D-glucose, while *R.muricata* contained 1,2,3,4,6-pentagalloyl-1-o-D-glucose and 3,4,5-tri-o-galloyl quinic acid. *R.Pruinosa* contained only 3,4,5-tri-galloyl quinic acid. Chloroform extract of *R.ducrsiva*, *R.muricata* and *R.Pruinosa* showed cytotoxic activity at concentration 50µg/ml, while ethyl alcohol 96% extracts archived less potent at the same conc. against colon carcinoma (HCT 116).

Key words: *Reseda*, alkaloids, tannins, gallotannins.

INTRODUCTION

Numerous investigations have proved that medicinal plants contained diverse class of bioactive compounds such as tannins, alkaloids and flavonoids, which exhibit various pharmacological properties such as anticarcinogenic, anti-inflammatory and antiallergic[1]. *Reseda* species are rich in biologically active constituents with anti-inflammatory and analgestic activities. Some of these species were used in traditional medicine in external hemorrhodal treatment[2].

Sener *et al*.,[3] also reported that the extract of *Reseda Lutea* possesse pharmacological activities against insects, brine shrimp, nematodes and microorganisms. Lutfullin *et al*.,[4] isolated four alkaloids, resedine, resdinine, phenyl-β-naphthylamine and β-hydroxyphenylethylamine from the epigeal part of *R.luteola* L. Three alkaloids identified as kokusaginine, maculine and kolbisine have anti-microbial activity against Gram-positive and negative bacteria, fungi and *Mycobacterium smegmatis*[5]. The alkaloids vincaleukoblastine and vincristine are anticancerous while raubasine is used to check fragility of capillaries[6]. Anon[7] found that toxic alkaloids such as hermol alkaloids have a hypotensive action on human peripheral blood.

Some alkaloids are effective in the treatment of chemotherapy for patients suffering from leukemia, lymph node and spleen cancer[8].

Other alkaloids affect the central nervous system, including nerve cells of the brain and spinal cord which control many body systems, heart beat, circulation and breathing[9].

Vinson *et al*.,[10] found that tannins have some beneficial effects on human and animal food stuff, but they are known as antinutrients as they can complex proteins by interaction between hydroxyl and carbonyl groups. Tannins are among the most abundant secondary metabolites polyphenolic compounds found in plants and thus they are frequently found in animal feeds human foods[11]. Tannin sometimes act as a toxin rather than a digestion inhibitor[12]. Tannins are able to form soluble and insoluble complexes with proteins, carbohydrates, nucleic acids and alkaloids[13]. Most tannin molecules contain adjacent phenolic hydroxyl moieties which stable chelates with from many metal ions, e.g. iron, copper and zinc or reduce their solubility[14]. Tannins can disturb the absorption of minerals through the gastrointestinal tract and increase

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the endogenous losses of minerals such as calcium, magnesium and phosphorus[15]. Tannins have shown potential antiviral[16], antibacterial[17] and antiparasitic effects[18].

Studies have shown that a diet containing phenolics associated with a reduced risk of cancer[19,20].

MATERIALS AND METHODS

2.1. Plant Materials: *R.ducrsiva* Forssk, *R. muricata* presl and *R. pruinosa* were collected from North Sinai, during growth season.

2.2. Extraction of Total Alkaloids[21]: Air dried plant powdered of *R pruinosa*, *R.muricata* and *R.ducrsiva* were separately extracted with rectified spirit for 7 days, filter and the filterate was evaporated under reduced pressure at 40°C to obtain brownish residues. Each crude extract was suspended into water and extracted with petroleum ether, in a separating funnel to remove pigments and fatty substances. The aqueous layer was separated and extracted with chloroform and the solvent was evaporated under reduced pressure to obtain neutral chloroform extract. The residual aqueous layer was made acidic (pH 3) by adding HCl and extracted with chloroform and the solvent was evaporated under reduced pressure to afford acidic chloroform extracts. Then the acidic aqueous layer was made alkaline (pH 9) by adding NH_4OH solution and again extracted with chloroform and the solvent was evaporated under reduced pressure to obtain a basic chloroform extracts. All chloroform extracts were tested for alkaloids using Dragendorff s.

2.3. Estimation of Total Alkaloids: The total alkaloids of the leaves, stems and roots of *R.ducrsiva*, *R.muricata* and *R.pruinosa* were determined by two methods:

1- Spectrophotometric Method[22]: Total alkaloids of the three organs of each plant were estimated by spectrophotometric method using ion-pair complexation of methyl orange dye with alkaloids, the complex was extracted with chloroform and by treatment with hydrochloric acid,the dye was liberated from the complex. The pink colour of the liberated dye is proportional to the amount of alkaloids which was measured at 530 nm. Beer s law was obyed between 5-40 μg/ml.

2- Gravimetric Method: Was done on 90% extract of 100 gm of each organ. The percent w/w calculated on the bases of weight sample taken.

2.4. Investigation of Chloroformic Soluble Alkaloids: The chloroformic residue of each plant was dissolved in chloroform and subjected to thin layer chromatography (TLC) using the solvent system chloroform:methanol (9: 1 v/v). The developed chromatograms were air-dried, examined under UV light and sprayed with Dragendorff s reagent,where orange colour indicate the presence of alkaloids.

Detection of Alkaloids:

1. Alkaloids are detected under UV light[21]
2. Reagents for alkaloids[23]

a- Dragendorff s reagent : potassium bismuth iodide
b- Wagner s reagent : potassium tri-iodide.
c- Mayer s reagent : potassium –mercuric iodide.

2.5. Isolation of the Alkaloid Constituents: The chloroformic extract of each plant was mixed with silica gel for column and applied on the top of a silica gel column. Elution was started with benzene, then the polarity was gradually increased by addition of chloroform. The obtained fractions were concentrated under reduced pressure, then chromatographically screened on silica gel G plates using Dragendorff s reagent, where similar fractions were pooled together and concentrated.

Preparative TLC: Preparative TLC silica gel G plates were used for isolation and purification of alkaloid compounds using the solvent system chloroform : methanol (9:1). The chromatograms were air dried and the bands were scraped off separately and eluted with methanol, where the elute was freed from solvent under vacuum.

The Chloroform Insoluble Alkaloids (Quaternary Bases):

Isolation: The alkaline aqueous layer, for each plant was tested for alkaloids with Dragendorff s reagent, acidified with dil. HCl to pH 1-2 and filtered. An equal volume of hot saturated aqueous solution of ammonium reineckate was added with stirring to the acidic aqueous phase, cooled and kept in refrigerator over night. The precipitated reineckate were filtered, washed with water then with ether and dried. They were dissolved in 100 ml acetone and filtered. Ten ml silver sulphate solution (0.02 M) were added to the filterate, where silver reineckate was precipitated and filtered. Barium chloride solution (1M) was added to the filtrate, to precipitate sulphate ions and filter again. The filterates, which contained quaternary bases as chloride salts were evaporated under vaccum and dried.

TLC Investigation: Each quaternary base residue was dissolved in (50%) ethanol and subjected to TLC

investigation alongside with authentic choline chloride and betain chloride, using the solvent system ethyl acetate : formic acid (75: 25 v/v). The chromatograms were air-dried and sprayed with Dragendorff s reagent.

Preparative TLC: Preparative TLC technique was adapted for the separation of quaternary bases using the solvent system:ethyl acetate:formic acid (75:25 v/v). Chromatograms were air dried and the major bands were scraped off and eluted with 50% methanol, where the eluate was freed from solvent under vacuum and purified by the same manner.

Estimation of Total Tannins:

1- Hide Powdered Method[24]: Accurate 0.75 g of each plant organs, were boiled, separately, with 150 ml distilled water and kept on a boiling water bath for 30 minutes. The mixtures were, separately, cooled, where the volume was completed to 250 ml with distilled water and filtered. 25 ml of each filtrate was evaporated and the weight of the obtained residue (G1) resembled the total water-soluble extractives containing tannins. 50 ml of each filtrate was mixed with 0.5 gm hide powder for one hour with shaking, filter, 25 ml of the filtrate was evaporated and the weight of the residue (G_2) represent the non adsorped substances by hide powder. Blank determination was carried out using distilled water, and the weight of the residue (G_0=0). The tannin percentages were calculated from the equation:

$$\text{Tannin \%} = G_1-(G_2-G_0)\text{x } 1000 \text{ x } 100 \text{ x } \frac{10}{W}$$

2- Copper Acetate Method[25]: Two gm of each plant organs were separately extracted with 100 ml of acetone :water (1 :1), filtered and the filterates were separately completed to 250 ml with distilled water. Each extract was boiled, where 30 ml of aqueous solution was boiled and 30 ml of aqueous solution of copper acetate was added with stirring. The preciptitate of copper tannate was collected on ashless filter paper and ignited to a constant weight. Drops of nitric acid were added to the residue and reignited. The weight of copper oxide was determined and the percentage of tannin was calculated according to the following correlation

Each 1 g of CuO =1.305 g tannins.

Quantitative Determination of Gallotannins Using HPLC[26]:

Determination of Free Gallic Acid: one ml of each tannin extract of the three plants was evaporated, where 750 µl of distilled water were added to the residue and filter the contents before loading to the HPLC.

Determination of Gallic Acid Present in Free and in Gallotannin Forms: To 2 ml of each hydrolysed tannin extract, 2 ml buffer solution were added, adjust pH between (6.3-6.8), using 8M KOH solution, freeze overnight and filter before loading to the HPLC using nucleosil 120-5 C18 column with a flow rate 1.2 ml/min and column temperature 22ºC.

Isolation and Identification of Tannins:

Extraction: The defatted powder of *R.pruinosa*, *R.muricata* and *R.ducrsiva* were separately extracted with 70% acetone, filtered and evaporated under reduced pressure at 40 °C.

Isolation: The obtained residues were chromatographed on Sephadex LH-20 column and elution was started with ethanol followed by gradual increase of H_2O. Fractions obtained were investigated by TLC using solvent systems S_1 and S_2 (acetic acid : H_2O, 2:98 v/v) and (butanol: acetic acid : water 60 : 15 : 25 v/v/v). Combined fractions which gave positive responses with UV and ferric chloride reagent were rechromatographed on silica gel column using chloroform followed by chloroform/methanol system, and were further purified on Sephadex LH-20 column.

Determination of Cytotoxic Activity: Potential cytotoxicity of the chloroform and ethyl alcohol 96% extracts of *R.ducrsiva*, *R.muricata* and *R.Pruinosa* were tested using the method of Skehen *et al*.,[27].

Tumor cell lines:

1. HCT116 (Colon carcinoma cell line).
2. MCF7 (Breast carcinoma cell line).

Procedure:

1. Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 hour before treatment with the extracts to allow attachment of cell to the wall of the plate.
2. Different concentration of the extracts under test (0,1,2.5,5 and 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose.
3. Monolayer cells were incubated with the extracts for 48 hour at 37ºC and atmosphere of 5% CO2.
4. After 48 hour, cells were fixed, washed and stand with sulforhodamine B straine (SRB).
5. Excess stain was washed acetic acid and attached stain was recovered Tris-EDTA buffer.
6. Color intensity was measured in an ELISA reader.
7. The relation between surviving fraction and extract concentration is plotted to get the survival curve of each tumor cell line after the specified extract under investigation was added.

Determination of Free Radical Scavenging Activity: Free radical scavenging activity of chloroform and ethyl alcohol extracts of *R.pruinosa*, *R.muricata* and *R.ducrsiva* using picrylhydrazyl radical (DPPH) were carried according to Yildirin *et al*.,[28]. One ml of various concentration of each extract was mixed 1 ml of DPPH solution (0.1 mM) and equal amount of methanol and DPPH used as control. After incubation in the dark for 20 min, absorbance was recorded at 517 nm, the percentages scavenging were calculated as follows:

$$\% \text{ Scavenging} = \frac{Ac-As}{Ac} \times 100$$

Ac= absorbance of control.
As= absorbance of sample

RESULTS AND DISCUSSION

3.1. Estimation of Total Alkaloids: The total alkaloids of the leaves, stems and roots of *R.ducrsiva*, *R.muricata* and *R.pruinosa* were determined by spectrophotometric and gravemetric methods. Data illustrated at table (1) showed that the highest values of the alkaloid were detected in the roots of *R.ducrsiva*, *R.muricata* and *R.pruinosa* where the result obtained from the spectrophotometric method were 0.180, 0.135 and 0.091while the obtained results from gravimetric method were 0.190, 0.145 and 0.098, for *R.ducrsiva*, *R.muricata* and *R.pruinosa*, respectively. The percentages of total alkaloids of leaves were found to be the lowest values for the three plants 0.123, 0.112 and 0.053 % (spectrophotometric method) and 0.135, 0.123 and 0.064% (gravimetric method) for *R.ducrsiva*, *R.muricata* and *R.pruinosa*, respectively These results were in agreement with those of Lutfullin *et al*.,[29] on studying the alkaloid content in different organs of *R. luteola* during its vegetation periods, where its root contained the highest amount of alkaloid. On the other hand *R. ducrsiva* contained the highest amount of alkaloid in its all organ

3.2. Investigation of Chloroformic Soluble Alkaloids: TLC investigation of the chloroformic extracts of the three plants, revealed that both *R.ducrsiva* and *R.muricata* contained three major alkaloid constituents and traces of others while *R. pruinosa* contained one major alkaloid compound beside traces of others, which were detected under UV light and gave orange colour with Dragendorff s reagent.

3.3. Isolation of the Alkaloid Constituents: Fractionation of the alkaloid constituents of each plant on silica gel column revealed the presence of three main fractions for both *R.ducrsiva* and *R.muricata* while *R.pruinosa* gave only one main fraction when subjected to TLC investigation.

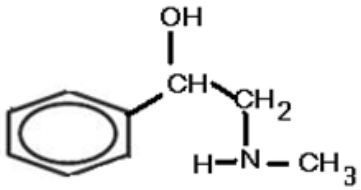
3.4. Preparative TLC: Preparative TLC silica gel G plates were used for purification of alkaloid compounds using the solvent system chloroform:methanol (9:1). The eluate of each band was concentrated under vacuum and subjected to TLC screening which revealed that both *R.ducrsiva* and *R.muricata* contained three alkaloid compounds and *R.pruinosa* contained one alkaloid compound. *Reseda ducrsiva* contained the compounds A_1, A_2 and A_3, while *R.muricata* contained the compounds A_1, A_2 and A_4 .On the other hand *R. pruinosa* contained the compound A_1.

Identification of Compound A_1:

Mass Spectroscopy: The mass spectrum of compound A_1 revealed a molecular ion peak (M^+) at m/e 151 (10%) and other ions at, m/e 107 (20%), 105 (40%), 79 (90%) and 44 (100%).

^{1}H-NMR (DMSO): ^{1}H-NMR spectrum of compound A_1 showed signals at δ 7.13(5H,S,monosubstituted benzene ring), 4.6 (1H, triplet Ar-CH-CH_2-), 4.37 (2H, S, NH, OH), 2.4 (2H,d,CH-CH_2) and 2.18 (3H, S, N-CH_3).

IR Spectrum: IR spectrum of compound A_1 showed absorption bands at 710 and 770 cm^{-1} (monosubstituted benzene ring), 3500 cm^{-1}(broad) (OH),300cm^{-1}(NH), 1600, 1510, 1470 (C=C aromatic). Analysis of the spectra of A_1 indicated that the compound A_2 was identified as β-hydroxyphenyl-N-methylethyl amine.



β-hydroxyphenyl-N-methylethyl amine

Identification of Compound A_2:

IR Spectrum: IR spectrum of the compound A_2 showed peaks at 3500 cm^{-1} (broad) (OH), 1600, 1510, 1470 cm^{-1} (C=C aromatic).

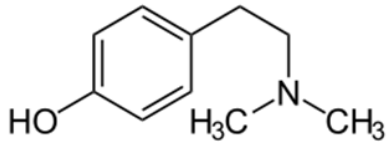
^{1}H-NMR Spectrum: ^{1}H-NMR spectrum of the compound A_2 showed signals at δ 6.98 (2H, d, J=7.6Hz, H=3, H-5), 6.66 (2H,d,J=7.6Hz,H-2,H-6),4.4 (1H,S,OH),2,27(6H,S,N- $(CH_3)_2$), 2.59 (4H,S,H-7and H-8).

Table 1: Percentages of total alkaloids of different organs of *R.ducrsiva*, *R.muricata* and *R. pruinosa*

Methods	Percentages								
	Reseda ducrsiva			*Reseda muricata*			*Reseda pruinosa*		
	Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots
Spectrophotometric	0.123	0.145	0.180	0.112	0.114	0.135	0.053	0.072	0.091
Gravimetric	0.135	0.156	0.190	0.123	0.126	0.145	0.064	0.079	0.098

^{13}C-NMR Spectrum: ^{13}C-NMR Spectrum showed signals at 155.41 (C-1), 114.97 (C-2,C-6), 129.28 (C-3, C-5), 130.2 (C-4), 34.69 (C-7), 53.58 (C-8) and 35.93 N-$(CH_3)_2$

Mass Spectrum: Mass spectrum of compound A_2 revealed a molecular ion peak (M^+) at m/e 165 and other important fragments. By comparing the above data with those reported in the literature, compound A_2 was identified as hordenin.



Hordenin.

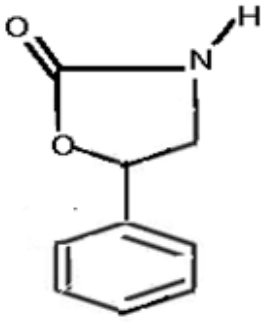
Identification of Compound A_3:

I.R. Spectrum: I.R. spectrum of compound A_3 showed peaks at 710, 770 cm^{-1} (monosubstituted benzene ring), 1680 cm^{-1} (C=0), 3300 cm^{-1} (NH) (broad) and 1600, 1510, 1470 (C=C aromatic).

Mass Spectrum: Mass spectrum of compound A_3 revealed the presence of a molecular ion peak (M^+) at m/e 163 and other important ions, at m/e 119, 104, 91 and 77.

^{1}H-NMR Spectrum: ^{1}H-NMR spectrum of compound A_3 showed signals at δ 8.5ppm (1H,S,N-H), 7.34 (5H, S, monosubstituted benzene reing),5.8,4.1,3.65 (1H, triplet) due to the protons of vicinal methylene and methine groups.

From the previous data, compound A_3 was identified as 5-phenyl-2-oxazolidone.



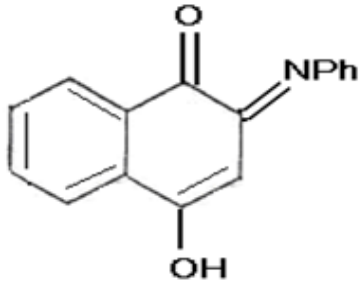
5-phenyl-2-oxazolidone.

Identification of Compound A_4:

I.R. Spectrum: I.R. spectrum of compound A_4 showed absorption bands at 3320 cm^{-1} (OH), 1670 cm^{-1} (C=0), 710 and 750 cm^{-1} (mono substituted benzene ring) and 750 cm^{-1}. The mass spectrum of compound A_4 showed a molecular ion peak (M^+) at m/e 249 (100%) and other important fragments.

UV spectral data showed absorption maxima at 216, 222 and 274 nm. ^{1}H-NMR spectrum of compound $_4$A showed signals at δ 8.12 (dJ=3.9Hz,H-5), 7.78 (d,J=3.0Hz,H-7,H-8), 7.43 (t,H-6) and 5.98 (1H,δ,H-2), and 4.37 (1H,δ,OH). The ^{1}H-NMR spectra of (A_4) showed the presence of four adjacent aromatic proton signals and olefinic proton singlet.

A study of the IR, Mass, UV and ^{1}H-NMR spectra, showed that compound A_4 was identified as 2-amilino-1,4-naphthoquinone.



2-amilino-1,4-naphthoquinone

Harborne[30] reported that alkaloids are often toxic to man and many of them have dramatic physiological activities, hence they have wide use in medicine e.g., quinine used for the treatment of malaria, and morphin alkaloids are narcotics.

Many alkaloids have physiological effects, they are used as muscle relaxant, for relief of pain and as local anesthetic[31].

3.5. the Chloroform Insoluble Alkaloids (Quaternary Bases): The alkaline aqueous layer of each plant gave positive test with Dragendorff s reagent. TLC investigation of the residue (from each plant) containing quaternary bases alongside with authentic choline chloride and betain chloride after drying and spraying with Dragendorff s reagent, revealed the presence of choline (B_1) in the three plants, while betain (B_2) was found in *R. ducrsiva* and *R. muricata* only.

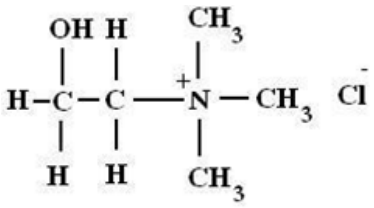
Identification:

Compound B_1: Compound B1 was obtained as white crystal, soluble in water and methanol, m.p. (303-305°C). It showed one spot chromatographically identical with that of choline chloride.

IR Spectrum: IR spectrum of compound B_1 gave peak at 3000 cm^{-1} broad peak indicating the presence of OH group and another peak at 1400 cm^{-1} indicating the presence of C-H. IR spectrum of the compound B_1 was superimposed with that of authentic choline chloride.

^{1}H-NMR Spectrum: ^{1}H-NMR spectrum of compound B_1 showed signals at δ 4.6, broad (1H,OH), 3.5 (9H,S,N $(CH_3)_3$) and δ 2.5 (4H,S,CH_2-CH_2).

Thus from the previous data compound B_1 was identified as choline chloride.

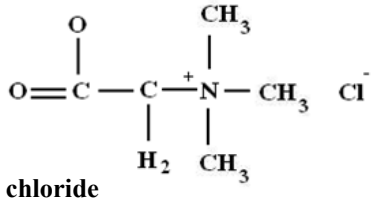


Choline chloride

Compound B_2: Compound B_2 obtained as white crystals; it is soluble in methanol and water, m.p. (290-293°C). It showed one spot chromatographically identical with that of betaine chloride.

IR Spectrum: Spectrum of this compound gave peak at 1710 cm^{-1} for C=O and 1400 cm^{-1} for CH. IR spectrum of this compound was superimposed with that of betaine chloride.

^{1}H-NMR Spectrum: ^{1}H-NMR spectrum of compound B_2 showed signals at δ 3.5, broad (1H,OH), 3.25 (9H,S,N-$(CH_3)_3$) and 2.5 (2H,S,CH_2).



Betaine chloride

Estimation of Total Tannins: The total tannins of the leaves, stems and roots of *R.ducriva*, *R.muricata* and *R.pruinosa* were determined by hide powder and copper acetate methods (Table 2). The obtained results showed that the percentages of total tannins of leaves were found to be more accurate with the copper acetate meted., where the highest values (8.65,7.64 and 5.93) were detected in leaves of *R.ducriva*, *R.muricata* and *R.pruinosa*, respectively. Meanwhile the lowest values (2.82, 2.51and 1.92) were detected in roots of *R.ducriva*, *R.muricata* and *R.pruinosa*, respectively. The obtained results from hide powdered method of the leaves were (8.47, 7.45 and 5.75) and those for the roots were (2.78, 2.43 and 1.81) for *R.ducriva*, *R.muricata* and *R.pruinosa*, respectively. The association of tannins with protein molecules produced molecules which are large enough to link adjacent collagen chains and which have sufficient phenolic groups to permit-cross linking at several sides. If the molecule was too large it failed to penetrate the hide powder.

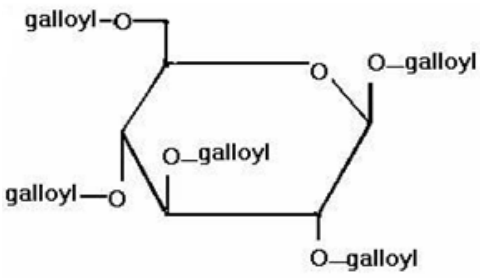
Quantitative Determination of Gallotannins Using HPLC: Quantitative determination of free gallic acid in *R.ducriva*, *R.muricata* and *R.pruinosa* using HPLC showed that *R.ducriva* contained the highest concentration of free gallic acid (3.8%) while *R.pruinosa* contained the lowest concentration of free gallic acid (2.7%) and *R.muricata* contained (3.1%) free gallic acid. Total gallic acid present in free and in gallotannins forms in the three plant were 18.6%, 15.2% and 10.8% for *R.ducriva*, *R.muricata* and *R.pruinosa*, respectively. Thus the concentration of gallotannins as gallic acid in the three plants were (15, 12.1 and 8.1%, respectively).

Isolation of the Tannins Constituents: Fractionation of the tannin constituents of each plant on sephadex LH-20 column revealed the presence of 2 main fractions for both *R.ducriva*, *R. muricata* while *R.pruinosa* gave one main fraction when subjected to TLC investigation. Tannin compounds were purified through preparative TLC and subcolumn sephadex LH-20. TLC for the isolated compounds showed that *R. ducriva* contained the compounds T_1, T_2, while *R.muricata* contained the compounds T_1, T_3 and *R.pruinosa* contained the compound T_3 only.

Identification of the Compound T_1: UV spectral data of compound T_1 in ethanol revealed the presence of a peak at λmax= 280 nm. ^{13}C-NMR spectrum (in d6 acetone) revealed signals due to the glucose carbons at 93.3(C-1), 74(C-5), 73.3(C-3), 71.7(C-2), 69.3(C-4) and 62.7ppm (C-6). ^{13}C-NMR also showed signals characteristic for galloyl moieties. ^{1}H-NMR spectrum of compound T_1 showed signals for the glucose protons at δ 6.5 (d,J=5.9Hz,H-1), 5.40 (d,J=5.9Hz,H-2), 4.84 (s,H-4), 4.56 (t,H-6), 4.14 (dd,J=10.6,8.5Hz,H-6') also ^{1}H-NMR showed signals characteristic for galloyl moieties. From the previous data and by comparison these spectral data with those of authentic samples, compound $T_1$was identified as 1, 2, 3, 4, 6-penta galloyl-o-D-glucose.

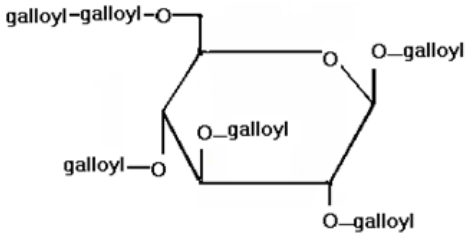
Table 2: Percentages of total tannins of different organs of *R.ducrsiva, R. muricata* and *R. pruinosa*.

Methods	Percentages								
	Reseda ducrsiva			*Reseda muricata*			*Reseda pruinosa*		
	Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots
Hide powder	8.47	6.59	2.78	7.45	5.58	2.43	5.75	3.68	1.81
Copper acetate	8.65	6.73	2.82	7.64	5.77	2.51	5.93	3.78	1.92



1, 2, 3, 4, 6-penta galloyl-o-D-glucose

Identification of the Compound T_2: UV spectrum of compound T_2 in methanol showed a peak at λmax=280nm, sh at 300nm. ^{13}C-NMR spectrum of T_2 showed the signals of the glucose carbons at 93.3 (C-1), 73.8 (C-5), 73.3 (C-3), 71.7 (C-2), 69.4 (C-4) and 63.1 (C-6). In comparison with the spectrum of T_1, C-6 was shifted to down field, because m-hydroxyl group was galloylated, ^{13}C-NMR also showed signals characteristic for galloyl moieties. From the previous data and by comparison with those of authentic samples compound T_2 was identified as 6-m-digalloyl-1,2,3,4-tetra galloyl-o-D-glucose



6-m-digalloyl-1,2,3,4-tetra galloyl-o-D-glucose

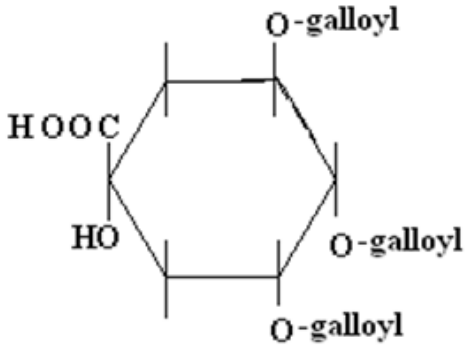
Identification of Compound T_3 :

^{1}H-NMR Spectrum: ^{1}H-NMR spectrum of compound T_3 showed three singlets characteristic for the orthoprotons of the three galloyl groups, at δ 7.12 (S,H-2a and H-6a), δ 6.93 (S,H-2b,H-6b) and δ 6.77 (S,H-2C and H-6C) and the raimaining proton signals were attributed to the methine and methylene protons of the quinic acid moiety, at δ 2.47 (2ax dd), 2.25 (2eq,dd), 4.9 (H-3,m), 4.3 (H-4,dd,J=3.2,9.2) 5.62 (H-5,m) and 2.35 (6ax.eq, m)

^{13}C-NMR Spectrum: ^{13}C-NMR spectrum showed signal at δ177.86 ppm attributed to carboxyl (C-7) of quinic acid, at 74.57 (C-1), 35.30 (C-2), 68.82 (C-3), 73.21 (C-4), 70 (C-5), 37.2 (C-6), 177.87 (C-7), 120.5 (C1a), 116.41 (C2a,C6a), 138.59 (C4a, C4b), 144.68 (C3a, C5a), 138.55 (C4a, C4b, C4c), 166.95 (C7a, C7c), 199.82 (C1b), 110.13 (C2b, C6b, C2c, C6c), 144.45 (C3b, C5b, C3c, C5c), 167 (C7b), 120.07 (C1c).

Mass Spectral Analysis: Mass spectral analysis showed molecular ion peak at m/e 648.

From the obtained data and comparison with literature values, compound T3 was identified as 3, 4, 5 tri-o-galloylquinic acid.



3, 4, 5 tri-o-galloylquinic acid

Tannins can influence digestibility, absorption of nutrients such as proteins, amino acids, carbohydrates and lipids and also the activity of digestive enzymes in monogastrics[32].

Several of polyphenolic compounds regulate the genes that are critical for the control of proliferation, cell cycle and apoptosis pathway in cancer cells[33].

Determination of Cytotoxic Activity: Chloroform extract of *R.ducrsiva, R.muricata and R.pruinosa* showed cytotoxic activity (9.12%, 8.2% and 8.23%, respectively) at 50 µg /ml while ethyl alcohol 96% extracts achived less potent 4.78%, 3.43% and 3.15% at the same conc. against colon carcinoma (HCT116). Chloroform extract of *R. ducrsiva, R.muricata and R.Pruinosa* showed remarkable cytotoxic activity (2.56, 2.31 and 2.23) respectively. Meanwhile ethyl alcohol 96% showed cytotoxic activity (1.78, 1.43 and 1.05) against breast carcinoma (MCF7). The obtained results may be due to the presence of phenolic acids and

Table 3: Percentages of inhibition of tumor cells MCF7 and HCT116 in the chloroform and ethyl alcohol of *R. ducrsiva*, *R. muricata* and *R. Pruinosa*

Extract	Cell lines											
	MCF7						HCT_{116}					
	IC_{50}			IC_{10}			IC_{50}			IC_{10}		
	R. ducrsiva	*R. muricata*	*R. Pruinosa*	*R. ducrsiva*	*R. muricata*	*R. Pruinosa*	*R. ducrsiva*	*R. muricata*	*R. Pruinosa*	*R ducrsiva*	*R. muricata*	*R. Pruinosa*
Chl.	2.56	2.31	2.23	-	-	-	9.12	8.82	8.23 l	-	-	-
Ethyl Alc.	1.78	1.43	1.05	-	-	-	4.78	3.43 l	3.15 l	-	-	-

alkaloids in the chloroform extract, hydrolysable tannins and flavonoids in the ethyl 96% extract[34].

Phenolic compounds have antioxidant, antimutagenic and free radicals scavenging activities[35].

Determination of Free Radical Scavenging Activity: For both chloroform and ethyl alcohol 96% extracts of *R.ducrsiva*, *R. muricata* and *R. Pruinosa* the percentages of scavenged DPPH increased with concentration increment. Maximum scavenging activity was found at concentration 50 μg ml^{-1} while the minimum scavenging activity was found at 5 μg ml^{-1}. For chloroform extract *R.ducrsiva* showed lower effects (65.1%) than that of *R. muricata* Meanwhile *R.pruinosa* showed the lowest effect (51.6%).

For ethyl alcohol extracts *R.ducrsiva* showed higher effect (67%), *R. muricata* showed lower effect (50%) meanwhile *R. pruinosa* showed the lowest effect (45%).

Plant phenolics have multiple biological activities in addition to their antioxidants or free radical terminators activity.[36,37]. The alkaloid extracts exhibited strong total antioxidant activity using DPPH free radical scavenging[38].

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