**In-vitro** evaluation of antioxidant and antiradical potential of successive extracts, semi-purified fractions and biosynthesized silver nanoparticles of *Rumex vesicarius*

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Abstract

The aim of the present study was to assess in vitro the antiradical and antioxidant activities of successive extracts and semi-purified fractions from *Rumex vesicarius* L. In the present work, three extracts (n-Hexane, ethyl acetate and methanol) and 22 column fractions of methanolic extract (as promising extract) were evaluated against 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging methods as antiradical and antioxidant activities compared with Butylated hydroxytoluene (BHT) as synthetic standard and silver nanoparticles of methanolic extract (Ag-NPs-Me), in addition to analysis of chemical constituents of extract and fraction using Gas chromatography–mass spectrometry (GC-MS). The obtained results revealed that, both methods go parallel showing that the concentration of extract and incubation time are dependent and proportional with phenolic compounds concentration. Absolute methanol extract recorded the highest antioxidant activity when compared with the other crude extracts with 79.3 and 78.8% against DPPH and ABTS respectively when compared with BHT as synthetic standard (89.4 and 89.9%) against DPPH and ABTS respectively. Calculation of the antiradical activity units showed the highest values of methanolic extract and its promising fraction (No. 12) after 300 seconds (5 minutes) comparing with antioxidant activity (30 min). Also, the antioxidant activity increased with synthetic Ag-NPs-Me when compared with methanolic extract by (IC50= 53.9 and 74.6 µg/ml respectively). Thus, the GC-MS analysis of successive extracts of *R. vesicarius* L showed a highly complex profile, containing approximately 24 different components. One pure compound was identified from fraction No. 12. The identified compound was l-(+)- ascorbic acid 2, 6-dihexadecanoate. The data also revealed presence of closely similar antioxidant activities in methanolic extract or its pure compounds with BHT when mixed at different proportions. From the obtained results it could be concluded that *R. vesicarius* methanolic extracts and...
fractions can be extensively used in the production of potential antioxidant, antiradical and AgNPs-Me for biomedical application on the consumer’s health.

**Keywords:** biological activities; chemical constituents; *Rumex vesicarius*; silver nanoparticles; successive extracts

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**Introduction**

*Rumex vesicarius* L. is a wild edible plant, known in Arabic as Humeidh and in English as Bladder dock, possible eaten fresh or in cooked form and can be used in the daily diet. It is distributed in many parts of Middle East regions especially Kingdom of Saudi Arabia and semi-desert areas of North Africa. In Hail region, KSA, *R. vesicarius* L. is antioxidant source and widely used as food and as a medicinal herb (Farooq *et al*., 2020).

Various highly active free radicals are responsible for human disease (e.g: ageing, cancer, inflammation, etc) and food deterioration could be delayed by the use of antioxidants compounds present in different *Rumex* species. The phytochemicals of crude organic extracts of *R. vesicarius* L. was analysed and identified using GC-MS by Farooq *et al*. (2020) who found that thirty-five active compounds were identified in the stem extract of *Rumex* sp. From the major compounds present are propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester, butane, 1,2,3-tris(trimethylsiloxy), and butanedioic acid, bis (trimethylsilyl) ester. The biological activities of *R. vesicarius* L. crude extracts were evaluated by (Al-Abd *et al*., 2017; Shalaby and Hameed, 2020; Farooq *et al*., 2020) they reported that the crude extracts possess antioxidant activity against DPPH and ABTS radical assay in addition to anticancer activity against human breast cancer (MCF7), human colon carcinoma (Lovo, and Caco-2), human hepatocellular carcinoma (HepG2) cell lines. Most of the crude extracts did not show any significant toxicity.

The radical scavenging (antioxidant) activity of *R. vesicarius* was studied by Al Aboody (2015) who revealed that the maximum percentage of DPPH inhibition was exhibited by ethyl acetate followed by distilled water extract and the lowest activity was recorded by hexane extract with concentration 1000 ppm. This investigation supports the folkloric uses of the Rumex species with different biological activities such as antioxidant, anticancer and anti-inflammatory.

There is a great variation between two expression “antiradical” and “antioxidant” activity as recorded by Shalaby and Shanab (2013) and according to Tirzitis and Bartosz (2010) the antiradical activity characterizes the ability of active ingredients or chemical compounds to react with different types of free radicals. However, antioxidant activity reflects the ability of active ingredients or chemical compounds to inhibit the steps or the process of oxidation reaction. Moreover, all test methods using a stable free radical (such as, ABTS or DPPH) give information on the antioxidant or antiradical activity (El-Beltagi *et al*., 2018; 2019a, b; Gaber *et al*., 2021).

The whole plant in addition to its extracts are currently used in silver nanoparticles preparation, because of their contents from bioactive compounds especially reducing agent compounds such as phenolic compounds, amines, sugars, vitamins ---etc. Several biological activities such as cytotoxicity, antimicrobial, antioxidant and anticancer of the biosynthesized nanoparticles have been reported (Mohamed *et al*., 2009; Huo *et al*., 2018; Jin *et al*., 2018; Lakshmanan *et al*., 2018; Chahardoli *et al*., 2018; Khattak *et al*., 2019; El-Beltagi *et al*., 2020a, b; Dawi *et al*., 2021). Moreover, Adewale *et al*. (2020) revealed the green plants contain a wide variety of natural products that could serve as reducing and capping/stabilizing agent in biosynthesis of silver nanoparticles (SNPs). These active compounds also enhance the antioxidant activity of the synthesized nanoparticles based on the results of *in vitro* antioxidant methods performed.

The synergism effect of methanolic plant extract as antioxidant was determined by Aboul-Enein *et al*. (2014) who reported the synergistic action of a wide spectrum of antioxidants may be more effective than the activity a single antioxidant.
The present work was designed to investigate the phytochemical contents of *R. vesicarius* plant and evaluate the activity of its successive extracts as an antioxidant and antiradical and to compare the results with silver nanoparticles prepared from its methanolic extract.

**Materials and Methods**

**Chemicals and reagents**

Pure hexane, chloroform, ethanol, ethyl acetate, ethanol and methanol were purchased from E. Merck Co. (Darmstadt, Germany). Sulfarhodamine, 2, 2 diphenyl-1-picrylhydrazyl (DPPH), 2, 2’ azino-bis (ethylbenzthiazoline-6-sulfonic acid (ABTS+)) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid and butylated hydroxyl toluene (BHT), purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Collection of plant materials**

*R. vesicarius* L. plant was collected from the desert of Hail region, KSA during February month 2020. The collected plant samples were kindly identified by the Botany Department, Faculty of Science, Cairo University, Giza, Egypt. The following is the taxonomy or classification of the plant under study:

- **Class**: Dicotyledons
- **Order**: Polygonales
- **Family**: Polygonaceae
- **Genus**: *Rumex*
- **Species**: *vesicarius* L.

**Plant extraction**

The collected plant was air-dried and then grinded to fine powder. The dried powder (100 g) was subjected to extraction with successive selective solvents according to Rosenthaler (1930). Hexane, ethyl acetate, and methanol were used. The polarity was increased from non-polar to highly polar, the extraction process was repeated three times. The organic solvent extract was combined and concentrated under vacuum at 40 °C to obtain a dry crude extract for each solvent used.

**Fractionation of methanolic plant extract as promising extract**

The chromatographic column (40 cm length, 2.5 cm diameter) was packed with 150 g silica gel (60-120 mesh for column chromatography) using hexane as solvent. 5.0 g of methanol crude extract of *R. vesicarius* L. (as promising crud extract) were grounded very well with silica gel powder and then placed on the top of the packed column. The column was then sequentially eluted with 100% hexane and increased the polarity with chloroform followed by ethyl acetate and ethanol solvent (Table 1), the polarity increased by 15% between each mobile phase mixtures (total 21 fractions were obtained) as the following:

| Solvent/ Sample no. | Fractions No. |
|---------------------|---------------|
|                     | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
| Hexane              | 100| 85 | 70 | 55 | 40 | 25 | 10 | 0  |
| Chloroform          | 0  | 15 | 30 | 45 | 60 | 75 | 90 | 100|
| Sample no.          | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Chloroform          | 85 | 70 | 55 | 40 | 25 | 10 | 0  |    |
| Ethyl acetate       | 15 | 30 | 45 | 60 | 75 | 90 | 100|
| Sample no.          | 17 | 18 | 19 | 20 | 21 | 22 |    |    |
| Ethyl acetate       | 85 | 70 | 55 | 40 | 25 | 10 | 0  |    |
| Ethanol             | 15 | 30 | 45 | 60 | 75 | 90 | 100|    |
Qualitative phytochemical screening

Qualitative phytochemical analysis of *R. vesicarius* L. extracts were done by following the method described by Harborne (1973) and Trease and Evans (1983).

**DPPH radical scavenging activity**

The scavenging effects of *R. vesicarius* L. extracts and fractions were determined by the method of Yen and Chen (1995), where 2.0 ml of 0.16 mM DPPH solution (in methanol) was added to a test tube containing 1.0 mL aliquot of sample (extracts, fractions and Ag-Nps-Me) at 100 and 200 µg ml\(^{-1}\). The mixture was vortexed for 1 min and kept at room temperature for 30 min. in the dark. The absorbance of all the sample solutions and BHT as synthetic standard were measured at 517 nm. The percentage (%) of scavenging activity was calculated as the following:

\[
\% \text{Antioxidant activity} = \frac{(\text{Control} - \text{Sample} \times 100)}{\text{Control}}
\]

Where: control in DPPH solution (0.16 mM).

**ABTS radical cation scavenging assay**

This assay was based on the ability of different substances to scavenge [2, 2'- azino-bis ethylbenzthiazoline-6-sulfonic acid (ABTS\(^+\))] radical cation in comparison to a standard (BHT). The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4-16 hrs. until the reaction was completed and the absorbance was stable. The (ABTS\(^+\)) solution was diluted with ethanol until it gives an absorbance of 0.700 \pm 0.05 at 734 nm for measurements according to Re *et al.* (1999). The photometric Assay was conducted on 0.9 mL of (ABTS\(^+\)), and 0.1 mL of tested samples at 100 and 200 µg ml\(^{-1}\), mixed for 45 s, and the measurements were taken at 734 nm after 1 min. The antioxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

\[
E = \left(\frac{\text{Ac} - \text{At}}{\text{Ac}}\right) \times 100, \text{where: } \text{At and Ac are the respective absorbance of tested samples and ABTS}\(^+\)
\]

**Determination of total phenolic content**

The total phenolic contents of methanolic extract as a promising antioxidant and RV-SNPs was determined by the Folin-Ciocalteu method (Wen *et al.*, 2010). Briefly, 0.25 mL of each extract was mixed with 1.25 mL of 1 N Folin-Ciocalteu reagent. After 5 min, 1 mL of sodium carbonate aqueous solution (7.5 %, w/v) was added to the mixture and completed the reaction for 120 min at room temperature. The absorbance was measured at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). The results were expressed in equivalent milligrams of gallic acid per gram of dry weight of plant extract (mg GAE g\(^{-1}\) DW).

**Measurement of antiradical activity of promising extract and fraction**

DPPH\(^•\) and ABTS radicals in its radical form have characteristic absorbance at 517 and 734 nm respectively, which disappears after its reduction by an antiradical compound. The reduction of DPPH and ABTS can thus be monitored by measuring the decrease in its absorbance at 517 and 734 nm when react with plant extracts or its fractions during the reaction time (30-300 sec) at 100 µgml\(^{-1}\). All details related to the method are described by Shalaby and Shanab (2013). The antiradical activity (AU515) was calculated according to the equation:

\[
\text{AU515} = (A0 - A1) - (A0K - A1K)
\]

where AU515 is the antiradical activity of the extract, A0K the absorbance of the control sample at the beginning of the reaction, and A1K the absorbance of the control sample after incubation times (30-300 sec) of the reaction. Because A0K–A1K was always equal to 0, the above equation was simplified to: AU515 = A0– A1.
Gas-chromatographic analysis
GC-MS analysis was performed to identify and quantify active ingredients extracted from Rumex vesicarius L. extracts. The analysis was carried out using Trace GC1300-TSQ mass spectrometer from Thermo Scientific, Austin, TX, USA using He at 1ml/min as carrier gas. An TG–5MS capillary column (30 m x 0.25 mm x 0.25 µm film thickness) was used. The oven temperature was programmed as follow: initial temperature of 60 °C was kept for 1 min. and temperature was then increased, at rate of 5.0 °C /min to 200 °C and maintain for 2 min at this temperature. Injector temperature was 260 °C, and injections were made in the split mode with a split flow 1:25. Mass spectrometer was operating as follow: ion source temperature 250 °C, ionization energy 70 eV (electron impact ionization), m/z scanning range 50-650 Da. The acquisition of chromatographic data was performed by means of WILEY 09 and NIST 11 mass spectral database.

Thin layer chromatography (TLC)
The separation of active compounds from the promising fraction of Rumex vesicarius L. (Fraction No. 12) was performed using Precoated silica gel plates (TLC F254) with using Benzene: acetone (9:1 v/v) as a mobile phase and the separated spot was scratched. Two dimensions TLC that was done for this spot confirmed presence of a pure compound. GC-MS analysis with the same previous conditions was performed to identify and quantify the separated active compound (El-fayoumy et al. , 2021).

Bio-autography for antioxidant activity
A rapid TLC screening method for antioxidant activity was done using the 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) as a spray reagent. TLC was performed for all R. vesicarius L extracts (hexane, ethyl acetate and methanol) in addition to semi-purified fraction (No. 12) as described earlier (Nair et al., 2005). The plates were dried and antioxidant activity was detected by spraying 0.2% 2,2-diphenyl-2-picrylhydrazyl (DPPH) in methanol onto TLC plates. The development of yellow or white spots against a purple background indicated the presence of antioxidant compound.

Preparation of silver nanoparticles (Ag-NPs)
Ten mg of R. vesicarius L. methanolic extract was directly dissolved in 100 mL of 1 mM AgNO3 aqueous solution with stirring at room temperature. The pH of the obtained solutions was adjusted to 10 by KOH. After that, the reaction mixture was kept on a magnetic stirrer for 30 min under constant heating (70 °C). The reduction of Ag+ ions to silver nanoparticles was monitored by visual inspection of the colour change in solution and was apparent immediately after the beginning of the reaction. Particles synthesized with methanolic extract were designated Ag-NPs-Me. The nanoparticles were repeatedly centrifuged at 20,000xg for 30 min and washed with sterile bi-distilled water before further analysis (Khattak et al., 2019).

Characterization of Ag-NPs-Me
UV–vis spectrophotometric analysis
The colour change of the reaction medium was monitored initially by periodic sampling of reaction solutions and then by measuring its UV–VIS absorption. The aliquots of reaction mixture were analysed by Uv-visible spectrophotometer in the range of 200–800 nm as described by Khattak et al. (2019).

Fourier Transform Infrared (FTIR) spectroscopy
Fourier transform infrared spectroscopy (FTIR) analysis was done for methanolic extract of Rumex vesicarius L and silver NPs-Me with Shimadzu FTIR spectrometer at room temperature over the range of 4000-400 cm⁻¹ at a resolution of 3 cm⁻¹ in KBr pellets.
**Blending of plant extract and fractions with BHT as synthetic standard**

The methanolic extract of *R. vesicarius* L as a promising extract and the pure compound separated from promising fraction (No. 12) were used for the determination of its antioxidant activity using DPPH method (as mentioned before) after blending with synthetic antioxidant standard (BHT) at 100 ug/ml as described by Shalaby and Shanab (2013).

**Statistical analysis**

Values are analysed as means ± SE or SD. Statistical analysis was done utilizing “costat” statistic computer program. Statistical analysis was established on One-way analysis of variance ANOVA followed by student-Newman Keuls test, and least significant difference (LSD) at P < 0.05.

**Results and Discussion**

**Phytochemical screening**

A lot of medicinal plants are considered to be a biochemical factory as it contains multitude of active ingredients or secondary metabolites such as phenolic compounds, flavonoids, alkaloids, plant acids and glycosides.

The preliminary qualitative screening for phytochemicals of *R. vesicarius* L. successive extracts revealed that the secondary metabolites such as flavonoids, phenols, terpenoids and tannins were detected in the examined three organic extracts (Table 2). However, only methanolic extract contains all secondary metabolites tested (terpenoids, saponin, phenolic, flavonoids, carbohydrates, tannins and alkaloids). Many phytochemicals and other microelements like ascorbic acid, tocopherol, carotenoids, flavonoids, anthocyanins, have antioxidant properties (Abdel-Rahim et al., 2010; Shallan et al., 2010; Afify et al., 2011, 2012; El-Beltagi et al., 2017; Abd El-Maksoud et al., 2018; Mohamed et al., 2018a, b).

**Table 2. Phytochemical screening of successive extracts from *R. vesicarius* L.**

| Phytochemical com | n-Hexane | Ethyl acetate | Methanol |
|------------------|---------|--------------|---------|
| Terpenoids       | +       | +            | +       |
| Phenolic         | +       | +            | +       |
| Carbohydrates    | -       | -            | +       |
| Tannins          | +       | +            | +       |
| Saponin          | -       | -            | +       |
| Alkaloids        | -       | +            | +       |
| Flavonoids       | +       | +            | +       |

+: present; -: absent

These results were in agreement with previous data obtained by Panduraju et al. (2009), Amira et al. (2011), Hariprasad and Ramakrishnan (2011), Husain Khan et al. (2014), Al Aboody (2015), Shalaby and Hameed (2020), as they reported the presence of different natural products in various extracts of *R. vesicarius* L. such as tannins, plant acids, phenolic, alkaloids, steroids, amino acids derivatives and glycosides. These active compounds are considered to be antifungal, antibacterial, antioxidant and antitumor agents as recorded by (Rao, 2003; Alberto et al., 2006; Stevic et al., 2010; El-Beltagi, 2011; Imran et al., 2011; Khan et al., 2014).

**Antioxidant activity of *R. vesicarius* L. successive extracts**

DPPH and ABTS methods are widely applied to determine the free radical scavenging effect of different antioxidant agents. The DPPH and ABTS possess scavenging abilities due to presence of the hydrogen or
electron donating activities of antioxidant agents. When DPPH and ABTS results are investigated, it was observed that antioxidant activity has increased in a dose-dependent manner (Ahmeda et al., 2020).

The antioxidant activity of successive extracts from *R. vesicarius* L. was evaluated using DPPH and ABTS radical scavenging method. The obtained results that are recorded in Table 3, revealed that, both methods go parallel and were shown to be dependent on both concentration of extract and incubation time.

The obtained results reported that methanolic extract recorded significantly highest antioxidant activity against both methods (DPPH and ABTS radical) by 79.3±2.4 and 78.8±2.9% at 100 µg mL$^{-1}$ in addition to 88.6±3.1 and 89.5±1.8% at 200 µg mL$^{-1}$ respectively during 30 min of incubation as shown in Table (2) followed by ethyl acetate extract by 71.8±4.1 and 76.1±1.8% at 100 µg mL$^{-1}$ in addition 82.0±0.9 and 84.3±1.1% at 200 µg mL$^{-1}$ respectively followed by n-hexane extract and compared with BHT as synthetic standard which recorded the highest percentage as antioxidant against both radical methods by 92.8±3.2 and 93.0±2.1% at 200 µg mL$^{-1}$ against DPPH and ABTS respectively. These results were in agreement with the results obtained by Al Aboody (2015) who mentioned that the maximum percentage of DPPH activity by testing different extracts of *R. vesicarius* L. extracts was recorded in ethyl acetate extract followed by distilled water and the lowest activity percentage was exhibited by n-hexane extract.

| Extract      | DPPH     | ABTS     |
|--------------|----------|----------|
|              | 100 µg mL$^{-1}$ | 200 µg mL$^{-1}$ | 100 µg mL$^{-1}$ | 200 µg mL$^{-1}$ |
| n-Hexane     | 54.3±3.2 | 68.5±1.5 | 56.5±4.3 | 72.4±1.9 |
| Ethyl acetate| 71.8±4.1 | 82.0±0.9 | 76.1±1.8 | 84.3±1.1 |
| Methanol     | 79.3±2.4 | 88.6±3.1 | 78.8±2.9 | 89.5±1.8 |
| BHT          | 89.4±1.4 | 92.8±3.2 | 89.9±0.8 | 93.0±2.1 |

Data are given as mean ± SE (n = 3). a,b and c Means within the same column with different letters are significantly differed (p < 0.05).

Determination of phenolic compounds in the three extracts revealed that, absolute methanol extract has recorded the highest percentage (83.7 as mg GAE/g) followed by ethyl acetate (71.5 mg GAE/g) and finally n-hexane with 35.70 mg GAE/g as shown in Figure 1.

In this regard, methanolic extract of *R. vesicarius* L has recorded the highest antioxidant activity which may be mainly due to its contents from natural products as shown in Table 1 and highest amount of phenolic compounds as shown in Figure 1. These results are in agreements with the results obtained by Shanab et al. (2012); Aly et al. (2013); Aboul-Enein et al. (2014); Akladious and Mohamed, 2017; El-Fayoumy et al. (2021) they reported that there is strong correlation between the antioxidant activity (determined by DPPH and ABTS) and phenolic compounds concentration in algae and plant species.
Figure 1. Phenolic compounds content (as mg GAE/g) of successive extracts from *R. vesicarius* L.

Antioxidant activity of semi-purified fractions

Twenty-two fractions were separated and identified from methanolic extract (as promising antioxidant extract) of *R. vesicarius* L. DPPH and ABTS radical methods were used for determination the antioxidant activity of each fraction at 100 µg mL⁻¹. The obtained results in Table 4 indicated that fraction No. 12 show the highest biological activities as antioxidant against both DPPH and ABTS methods by 75.8 and 77.4% respectively, followed by fraction No. No. 9 by 68.4 and 68.9% respectively then fraction 11 by 65.3 and 67.4% respectively.

Table 4. Antioxidant activity (%) of semi-purified fractions of methanolic extract from *R. vesicarius* against DPPH and ABTS at 100 µg mL⁻¹

| Fraction no. | Against DPPH   | Against ABTS   | Fraction no. | Against DPPH   | Against ABTS   |
|--------------|----------------|----------------|--------------|----------------|----------------|
| 1            | 5.1±0.3        | 6.0±0.7        | 12           | 75.8±1.1       | 77.4±1.8       |
| 2            | 4.6±0.0        | 5.2±0.7        | 13           | 3.5±0.1        | 5.9±0.3        |
| 3            | 6.4±0.2        | 6.2±0.0        | 14           | 33.3±2.0       | 32.5±0.6       |
| 4            | 6.7±1.0        | 6.7±0.4        | 15           | 49.4±1.2       | 51.1±3.2       |
| 5            | 8.3±0.3        | 9.2±1.0        | 16           | 49.4±1.2       | 50.8±1.7       |
| 6            | 5.1±0.0        | 5.0±0.3        | 17           | 41.2±0.4       | 39.6±2.8       |
| 7            | 3.5±0.0        | 3.9±0.4        | 18           | 36.7±1.4       | 37.5±1.9       |
| 8            | 56.4±3.1       | 55.0±0.3       | 19           | 30.2±0.0       | 31.2±0.7       |
| 9            | 68.4±0.9       | 68.9±4.1       | 20           | 30.2±0.3       | 30.4±0.5       |
| 10           | 16.7±1.1       | 20.5±1.2       | 21           | 13.4±0.5       | 12.7±1.0       |
| 11           | 65.3±1.5       | 67.4±0.7       | 22           | 12.0±0.1       | 14.6±0.5       |

Data are given as mean ± SE (n = 3). a, b and c Means within the same column with different letters are significantly differed (p < 0.05).

GC-MS analysis of successive extracts

The GC-MS analysis of successive extracts of *R. vesicarius* showed the presence of various phytocomponents. The phytocomponents of each extract are presented separately in Table 5 and the GC-MS chromatogram with peak area of each extract is also shown in Figure 2. Totally 35 constituents were identified in *R. vesicarius* from all the three successive extracts. Methanol extract has recorded the highest number of (19) phytocomponents, while lower number of (6) phytocomponents was observed in hexane extract including both major and minor constituents. Three constituents were commonly present in all the three extracts as the...
following 9,12,15-Octadecatrienoic acid; 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl) and gamma-tocopherol.

Figure 2. GC-MS chromatogram of *R. vesicarius*

a) hexane extract (above chromatogram); b) ethyl acetate extract (center); c) methanol extract (down).
The methanol extract of *R. vesicarius* showed 19 constituents, the major constituents were 9,12,15-octadecatrienoic acid (30.8%); gamma-sitosterol (12.7%) and ascorbic acid 2, 6-dihexadecanoate (9.68%). All of these major compounds represent antioxidant activity as reported by Sayik *et al.* (2017); Baskar *et al.* (2012) and Begum *et al.* (2017). In addition to 11 compounds from the total of 19 compounds of methanol extract reported antioxidant activity as shown in Table 5. These findings were going parallel with the results obtained in Table 3 and Figure 3.

The ethyl acetate extract of *R. vesicarius* revealed presence of 10 constituents (Table 5). The major constituents were 9,12,15-octadecatrienoic acid (45.34%), n-hexadecanoic acid (14.1%) and pentatriacontane (12.62%). Along with major constituents, minor constituents were also reported. Moreover, the hexane extract of the plant showed the lowest constituent's number (only 6 constituents) as shown in (Table 5). The major constituents were 9,12,15-octadecatrienoic acid (41.11%), pentatriacontane (24.02%) and ascorbic acid 2, 6-dihexadecanoate (18.91%). Along with major constituents, minor constituents were also recorded. The GC-MS chromatogram with peak area was given in Figure 2. The obtained results were in agreement with the results obtained by Hariprasad and Ramakrishnan (2011); Ammar *et al.* (2015).

**Antiradical activity of methanolic extract and its fraction**

Antiradical activity of methanolic extract and fraction No. 12 (chloroform: ethyl acetate 40:60 v/v) were determined using both DPPH and ABTS radical scavenging methods at 100 µg mL\(^{-1}\) during the incubation times (30-300 sec).

The obtained results recorded in Figures (3 a and b), revealed that, the antiradical activity was shown to be incubation time dependent. Methanolic extract showed the highest antiradical activity represented as AU or antiradical unit (0.056 and 0.061) against DPPH and ABTS respectively when compared with fraction No. 12 by AU (0.026 and 0.036) at 300 seconds of incubation.

From the obtained results of antioxidant and antiradical activity of crude extracts and obtained fractions shown in Tables 3 and 4 and Figure 3, it could be concluded that the activity was decreased upon separation of the fractions and it was lower than that of the crude extract. The potent antioxidant activity manifested by the crude extract in comparison with those of the separated fractions may be due to the synergistic action of the collective biologically active compounds of one or more of the twenty-two fractions in the crude extract. In addition, the crude extract may have secondary metabolites in very low concentration which enhance the active principles and increase the antiradical and antioxidant activity. This suggestion was previously confirmed by Chu *et al.* (2010) and Aboul Enein *et al.* (2014) they reported that crude methanolic extract of *Arthrospira* sp gave higher antioxidant activity than pure chemical compounds. They mentioned that the extract might contain other constituents (e.g. flavonoids, phenolic compounds) which recorded a higher combined antioxidant activity than pure compound. The synergistic effect of a wide spectrum of antioxidants may be more efficiency than the activity a single antioxidant.

The obtained results were found to be in agreement with those recorded by Nivas *et al.* (2010) and Tiryitis and Bartosz (2010). In the same context Kaviarasan *et al.* (2007) studied the antiradical and antioxidant activities (by ABTS and DPPH) using aqueous methanolic extract of fenugreek seeds and they found that the activities could be correlated with the phenolic concentration in the extract. The same results were reported by Huyut *et al.* (2017) who mentioned that there was a very significant relationship between antiradical, antioxidant activities and total content of phenolic compounds.
Table 5. List of phytochemical constituents (as Relative percentage) in different successive extracts of *Rumex vesicarius* L.

| S.No. | Rt   | Chemical name                                      | Extracts | Biological activities as antioxidant | References          |
|-------|------|----------------------------------------------------|----------|--------------------------------------|---------------------|
|       |      |                                                    | Hexane   | Ethyl acetate | Methanol              |                     |
| 1     | 17.298 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl |           |              | 3.68                  | Antioxidant activity Sayik *et al.* (2017) |
| 2     | 20.028 | Coumaran                                           |           |              | 3.72                  | Antioxidant activity Ilya *et al.* (2018)  |
| 3     | 20.159 | 1-Propanone, 2-methyl-1-(4-(methylthio)phenyl)-2-(4-morpholino) | | | 1.82                  |                     |
| 4     | 23.266 | N,N,N-Trimethyl-1,4-phenylenediamine               |           |              | 4.85                  |                     |
| 5     | 25.408 | Methyl 5-oxo-2-pyridinedicarboxylate              |           |              | 1.52                  | Antioxidant activity Wondrak *et al.* (2008) |
| 6     | 27.372 | 2-hydroxy-4-methylbenzaldehyde                    |           |              | 4.61                  | Antioxidant activity Wang *et al.* (2010)  |
| 7     | 29.758 | Heptose                                            |           |              | 0.65                  |                     |
| 8     | 30.146 | Folic acid                                         |           |              | 0.4                   | Antioxidant activity Atteia *et al.* (2009) |
| 9     | 30.538 | 2-cyclohexylpiridine                              |           |              | 4.83                  |                     |
| 10    | 31.305 | Formy glutamine                                    |           |              | 0.3                   | Antioxidant activity Zabot *et al.* (2017) |
| 11    | 41.868 | n-Hexadecanoic acid                               |           |              | 14.1                  | Antioxidant activity Sheela and Uthayakumaria (2013) |
| 12    | 42.026 | Ascorbic acid 2, 6-dihexadecanoate                | 18.91     |              | 9.68                  | Antioxidant activity Begum *et al.* (2017) |
| 13    | 45.305 | Oleic acid                                         |           |              | 3.68                  |                     |
| 14    | 46.136 | 9,12,15-Octadecatrienoic acid                     | 41.11     | 45.34       | 30.8                  | Antioxidant activity Sayik *et al.* (2017) |
| 15    | 46.537 | Paromomycin                                        |           |              | 1.96                  |                     |
| 16    | 55.302 | beta-Sitosterol                                     |           | 7.59        | 12.7                  | Antioxidant activity Baskar *et al.* (2012) |
| 17    | 55.726 | gamma-Sitosterol                                    |           | 2.48        | 12.0                  |                     |
| 18    | 57.229 | 11-Octadecenal                                     |           | 5.77        | 0.4                   |                     |
| 19    | 57.682 | 2-Myrutnynol pantetheine                           |           | 0.64        | 4.0                   |                     |
| 20    | 60.941 | Pentatriacontane                                   | 24.02     | 12.62       | 7.62                  |                     |
| 21    | 61.485 | 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyloctyl) | 5.71     | 4.23        | 7.62                  |                     |
| 22    | 61.629 | 2-Morpholino ethane sulfonic acid                  | 0.49      |             | 4.9                   | Antioxidant activity Baker *et al.* (2007) |
| 23    | 61.79  | Dihydroxanthin                                     |           | 0.75        | 0.4                   | Antioxidant activity Khan *et al.* (2019)  |
| 24    | 62.996 | gamma-Tocopherol                                   | 9.76      | 6.47        | 2.79                  | Antioxidant activity Abdulla *et al.* (2018) |

Meanwhile, Sroka (2006) determined the antiradical and antioxidant efficiency in 100% and 50% methanol extracts of tea samples (green and black). He mentioned that 100% methanol extracted the tea tannins. Higher antiradical activity unit TAU/g was those of green and black tea leaves in ethyl acetate fraction of aqueous methanol extract. Moreover, Melichacova *et al.* (2010) revealed that the antioxidant activity of 50% methanol extract of both sweet cherry and tart cherry fruit were due to and correlated with the soluble phenolics in tested solvent.
Figure 3. Antiradical Unit (AU) against DPPH (above) and ABTS (down) of methanolic extract and pure fraction (No.12) from *R. vesicarius* at 100 µg/ml

Bio-autography for antioxidant activity

Antioxidant potential compounds on TLC plates were identified in situ through using of DPPH reagent (Figure 4). The fractions produced yellowish or white bands on the purple background were considered as strong antioxidants. All of *Rumex vesicarius* L extracts showed white or yellow band (antioxidant compounds) being formed at the region at Rf 0.287, 0.81 and 0.90 on exposure to DPPH, in addition to single antioxidant compound separated from fraction No. 12 with white colour after sprayed by DPPH reagent with Rf 0.081 as shown In Figure 4. DPPH reagent with method measures electron-donating activity (free radical scavenging activity) of compounds and provides an evaluation of antioxidant activity (Prema *et al.*, 2012).

Semi-purified antioxidant constituent (from fraction No. 12) was scrapped and collected after performing silica gel preparative TLC separation of methanolic extract. The sample obtained from preparative TLC was subjected to GC-MS analysis to identify the antioxidant compound (Figure 5).

GC-MS of pure compound

One main compound was identified from fraction No. 12 of methanolic extract using GC-MS (Figure 5) after scratching from preparative TLC. The identified compound was 1-(+)- Ascorbic acid 2, 6-dihexadecanoate (with molecular weight 652.9 Da and molecular formula C₃₈H₆₈O₈) which been found in different saudi medicinal plants (Ara *et al.*, 2012) and also reported to have antioxidant activity by Begum *et al.* (2017). The efficiency of ascorbates derivatives as antioxidants is dependent upon the substrate and the compounds to be protected. Because the 2- and 3-positions of ascorbic acid must be unsubstituted, the two free
radicals formed at these positions may be intermediates in scavenging oxygen and inhibiting radical formation at double bonds.

Upon mixing the promising absolute methanol extract (recorded 79.3% antioxidant activity) with the standard synthetic antioxidant BHT in gradually proportion (from 100% BHT to 100% of methanol extract or pure compound), the results in Table 6, revealed presence of closely similar activities (with very few differences) in all extracts or pure compounds and BHT proportions. This means that the antioxidant activity of standard BHT was increased by each proportion of extract with an obvious synergism between them and these results were in agreement with the results obtained by Shalaby and Shanab (2013). Also, the obtained results revealed that the effect of the crude extract was more effective when mixing with BHT when compared with pure compound separated from methanolic extract as shown in Table 6.
Figure 5. GC-MS chromatogram of pure compound isolated from fraction No. 12 of crude methanolic extract of *R. vesicarius*

Table 6. Antioxidant activity (%) of synthetic antioxidant (BHT, 100 µg ml\(^{-1}\)) blending with methanolic extract and pure compound (scratched from fraction No. 12 at 100 µg/ml) of *R. vesicarius* at different ratio against DPPH assay

| Sample                  | Antioxidant % | Sample                  | Antioxidant % |
|-------------------------|---------------|-------------------------|---------------|
| 100% BHT                | 89.4±1.4      | 100% BHT                | 89.4±1.4      |
| 90% BHT: 10% Me extract | 86.0±2.6      | 90% BHT: 10% pure compound | 83.1±0.8      |
| 80% BHT: 20% Me extract | 85.9±1.1      | 80% BHT: 20% pure compound | 81.4±2.7      |
| 70% BHT: 30% Me extract | 83.2±0.9      | 70% BHT: 30% pure compound | 80.7±3.2      |
| 60% BHT: 40% Me extract | 84.7±2.5      | 60% BHT: 40% pure compound | 80.0±2.0      |
| 50% BHT: 50% Me extract | 84.1±1.4      | 50% BHT: 50% pure compound | 79.6±3.2      |
| 40% BHT: 60% Me extract | 82.0±3.0      | 40% BHT: 60% pure compound | 78.9±1.5      |
| 30% BHT: 70% Me extract | 82.7±0.9      | 30% BHT: 70% pure compound | 76.8±0.7      |
| 20% BHT: 80% Me extract | 82.5±1.9      | 20% BHT: 80% pure compound | 75.0±2.8      |
| 10% BHT: 90% Me extract | 81.0±1.8      | 10% BHT: 90% pure compound | 75.6±1.0      |
| 100% Me extract         | 79.3±2.4      | 100% pure compound       | 73.0±3.5      |

Data are given as mean ± SE (n = 3). a, b and c Means within the same column with different letters are significantly differed (p < 0.05).

Synthesis of silver nanoparticles (Ag-NPs-Me)

UV-visible of NPs

When the methanolic extract of *R. vesicarius* L was added to silver nitrate solution, pH was adjusted and the solution was heated. The colour of the reaction was formed immediately and started to be converted gradually from colourless to brown. The intensity of the brown colour increased rapidly by time, from seconds to minutes and remained stable within one hour. It is well known that Ag-NPs-Me have brown colour due to
their characteristic excitation of surface plasmons in the range of 400–490 nm (Panja et al., 2020). Therefore, a transition of the solution from colourless to brown colour indicates the synthesis of Ag-NPs (Vanaja et al., 2013).

This result means that the methanolic extract of _R. vesicarius_ L. have high reduction potential for reduced silver ions and formation of silver nanoparticles. The UV–VIS spectra of synthesized Ag-NPs-Me demonstrated the maximum peak at 390 nm as shown in Figure 6, which was consistent with the spectra of spherical AgNPs within the wavelength range of 380-450 nm. Similar surface plasmon resonance (SPR) peaks were observed in many studies of green synthesis for silver nanoparticles as reported by several studies (Desai et al., 2012; Ndikau et al., 2017; Yugay et al., 2020).

![Figure 6. UV-VIS spectra of _R. vesicarius_ methanolic extract with SNPs (Ag-NPs-Me); above picture illustrate bio reduction of silver nitrate colorless solution (1 mM) by methanolic _R. vesicarius_ extract and formation of plant silver nanoparticles (AgNPs) with brown color source](image)

**Figure 6.** UV-VIS spectra of _R. vesicarius_ methanolic extract with SNPs (Ag-NPs-Me); above picture illustrate bio reduction of silver nitrate colorless solution (1 mM) by methanolic _R. vesicarius_ extract and formation of plant silver nanoparticles (AgNPs) with brown color source

**FTIR of NPs**

FTIR measurements were carried out to identify the promising biomolecules in the _Rumex vesicarius_ L. methanolic extract accountable for the silver ion reduction and also the capping agent liable for the reduced AgNPs stability.

As shown in Figures 7 a and b and Table 7, the FTIR spectra of methanolic extract and Ag-NPs-Me, respectively, were recorded in the frequency range between 4400 and 350 cm$^{-1}$ in the mode of % transmittance (%T). It was shown that there were slight shifts in the FTIR peaks of _R. vesicarius_ L extract (3383, 2948, 2834, 2526, 2052, 1654, 1453, 1412, 1112, 1031 and 663 cm$^{-1}$) and the synthesized AgNPs (3455, 2067, 1638 and 443 cm$^{-1}$). The absence of some peaks (2948, 2834, 2526, 1453, 1412, 1112 and 1031 cm$^{-1}$) in the synthesized AgNPs compared to the methanolic extract, and the slight shifts noted in the peaks suggests the involvement of some functional groups in the reduction process. The bands from 3455 up to 3383 cm$^{-1}$ in the FTIR spectra corresponds to O-H stretching vibration, which indicates the presence of alcohol and phenol. It was reported that hydroxyl groups (O-H) have stronger binding ability with silver ions. It was noted that there were shifts in the FTIR peaks of _R. vesicarius_ L synthesized AgNPs (3383, 2052, and 1654 cm$^{-1}$) when compared to _R. vesicarius_ L extract. This suggests the presence of various functional groups responsible for the reduction of silver ion to the nanoparticles form. The FT-IR analysis suggested that the reasonable mechanism of AgNPs formation may be due to the reduction of Ag$^+$ ions that takes place together with oxidation of phenolic components of polyols or other reducing components in plant extract (Gandhi et al., 2020).
Figure 7. FTIR spectra of *Rumex* sp methanolic extract (a) and silver nanoparticles synthesized solution (b)

Table 7. Wavenumbers range of characteristic bands and corresponding assignments for *R. vesicarius* and Ag-NPs-Me

| Wavenumber range (cm⁻¹) | Function groups assigned | Sample | Methanolic extract | Ag-NPs-Me |
|-------------------------|--------------------------|--------|-------------------|-----------|
| 3300-4000               | Polymeric hydroxyl compound O-H stretching |       | 3383              | 3455      |
| 3100-2723               | C-H stretching vibrations specific to CH3 and CH2 |       | 2948              | ND        |
| 1700-1630               | C=O stretching vibration, C-N stretching, Lipids, Ester carbonyl – COOR and carboxylate ion stretching (-COO-) |       | 1654              | 1638      |
| 1600–1400               | C-O stretching vibration (amide) and C-C stretching from phenyl groups, |       | 1453              | ND        |

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Antioxidant activity of Ag-NPs-Me of R. vesicarius L

Regarding the biological activity of synthetic silver nanoparticles from *R. vesicarius* L methanolic extract, the antioxidant activity of Ag-NPs-Me against DPPH radical was evaluated. The obtained results revealed that green synthesis of SNPs using methanolic extract led to increase of antioxidant activity (with IC50=53.9 µg/ml) when compared with crude methanol extract (IC50=74.6 µg/ml) but lower than BHT as synthetic standard (IC50=13.64 µg/ml) as shown in Figure 8. These results may be due to that the nanoparticles synthesized using methanolic extract of *R. vesicarius* showed antioxidant activity because of capped phenolic compounds.

![Figure 8. IC50 (µg ml⁻¹) of plant methanolic extract and Ag-NPs-Me against DPPH radical compared with BHT as synthetic standard](image)

Phenolic group facilitates the conversion of silver nitrate to AgNPs due to its electron donating ability. These results were in agreement with the results obtained by Yousaf *et al.* (2020), who mentioned that the methanol-SNPs exhibit greater inhibition of DPPH radicals with IC50 7.03 ± 0.31 µg/mL. Also, Sudha *et al.* (2017) who reported that the maximum inhibition of superoxide radical scavenging activity was about 70% by biosynthesized AgNPs as compared to the activity of butylated hydroxy toluene as synthetic standard (84%). Moreover, Salari *et al.* (2019) revealed that the phenols and flavonoids in AgNPs-containing plant extract were 462.69 mg GAE/g extract and 386.94 mg QE/g extract respectively, which were significantly greater than native extract. Biosynthesized silver nanoparticles showed a higher antioxidant compared to native extract alone.
Conclusions

From the results obtained in the present study, it can be concluded that the crude extracts of *R. vesicarius* L contains a wide variety of secondary metabolites that could serve as antioxidant, antiradical and reducing or capping agents in the synthesis of nanoparticles. Antioxidant and antiradical activity of plant extracts was dependent on concentration of extract and incubation timed. Also, the obtained results conclude that methanolic extract of *R. vesicarius* L recorded the highest antioxidant and antiradical activity when compared with other crude extracts and BHT as synthetic standard. As, the antiradical activity being defined as the ability of a compound to react with free radicals in a single free radical reaction, the calculated antiradical activity units showed the highest values after 300 seconds (5 minutes) comparing with antioxidant activity (30 min) which is important in time saving and considered more sensitive especially with the electron reacting ABTS radicals. Moreover, the antioxidant activity increased with synthetic Ag-NPs-Me when compare with methanolic extract of *R. vesicarius* L. The data also revealed presence of closely similar antioxidant activities in methanolic extract or its pure compounds with BHT when mixed at different proportions.

Authors’ Contributions

Conceptualization: E.A.S., K. M. Y. and H. S. E; Data curation; E.A.S. and K. M. Y. Formal analysis; Funding acquisition; R. M. R. and E. A. S. Investigation; E. A. S., K. M. Y., H. S. E. and H. E. Methodology; E.A.S., K. M. Y., S. R and H. E Project administration; R. M. R., E. A. S and H. S. E. Resources; E. A. S and K. M. Y. Software; E. A. S. R and H. E. Supervision; E. A. S., H. S. E. and R. M. R. Validation; E. A. S. and K. M. Y. Writing - original draft; E. A. S., H. S. E and K. M. Y. Writing - review and editing E. A. S., H. A. E., R. M. R., S. R and H. E.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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