

## Research Article

# GC-MS Analysis, Heavy Metals, Biological, and Toxicological Evaluation of *Reseda muricata* and *Marrubium vulgare* Methanol Extracts

Riaz Ullah  and Ali S. Alqahtani

Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Correspondence should be addressed to Riaz Ullah; rullah@ksu.edu.sa

Received 8 February 2022; Accepted 8 March 2022; Published 21 March 2022

Academic Editor: Ruchika Garg

Copyright © 2022 Riaz Ullah and Ali S. Alqahtani. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The usage of herbal remedy is growing vividly all around the world. Though, ecological contamination particularly with heavy metals carries a thoughtful problem on quality of medicinal plants and their foodstuffs. In the world, 80% of the population depend on traditional medicine, while information on the levels of heavy metal such as Zn, Mn, Cu, Cr, Pb, As, Cd, and Cr in plants utilized for making of herbal remedies is unavailable. Therefore, the purpose of this study was to assess phytochemicals, biological activities, and heavy metal analysis of *Reseda muricata* and *Marrubium vulgare* grown in different parts of Saudi Arabia. Qualitative phytochemical analysis of *R. muricata* and *M. vulgare* confirmed the presence of alkaloids, flavonoids, tannins, phenol, and saponins. Methanol extracts of both *Reseda muricata* and *Marrubium vulgare* were characterized with the help of GC-MS. Antioxidants, antimicrobial, and brine shrimp lethal toxicity of the both species were also evaluated.

## 1. Introduction

The medicinal potential of plants species is because of the occurrence of secondary phytoconstituents which have numerous functions such as antioxidant, antimicrobial, cytotoxic, anticancer, and antiviral. Plants have been the main root of traditional medicines since ancient times [1–3]. The plants are used as primary healthcare all over the world, but South American countries in particular [4]. About 300–315 thousand species of plants are present on this planet, and few of these provide food to humans, aquatic, and terrestrial animals [5]. Plants are used as antimicrobial agents that are used to kill microorganisms such as bacteria and fungi or inhibit their growth [6, 7]. They also have antioxidant, anticancer, antiviral, and cytotoxic and much more effects. Antioxidants inhibit the oxidation of substrate such as free radicals [8, 9]. Cytotoxicity is the quality of being toxic to cells. Cytotoxic potential of crude extract is ascertained against various cells using methods such as MTT and brine shrimp lethality assays [10, 11]. *Reseda muricata* is an

herb belonging to family and spread in southeast Egypt. Family Resedaceae consists of 6 genera and 75 species. While, very small data about *R. muricata* appeared till date. Previously, chemical screening of *R. muricata* has reported a flavonoid trioxide, and its coumaryl ester together with some flavonoids and phenolic acids were identified from the leaves of *Reseda muricata*. Pharmacological studies of extracts of various *Reseda* species presented antifungal, antibacterial, and anti-inflammatory activities. *R. muricata* is traditionally used for the treatment of hemorrhoids, stomach aches, and diarrhea [12].

*Marrubium vulgare* is a plant of family Lamiaceae, whose genus comprises of 97 species. It is spread far and wide along the Mediterranean Sea and growing in the temperate areas of the Eurasian region. The plant has been utilized as a substitute for hops in beer breweries and is presently used to make herbal teas. In the past times, it was augmented to boiled vegetables, sauces, and salads. It is also utilized as traditional medicine. *M. vulgare* is habitually a significant foundation for the pharmaceutical and food industries. For

instance, just in India, there are 33 registered herbal formulations comprising of white horehound. *M. vulgare* showed diaphoretic, expectorant, stimulant, tonic, diuretic, and aromatic potential. Its phytochemical constituents are diterpenic lactones, phenolic compounds, and phenylpropanoids [13]. Folk usage of *Reseda muricata* fruit is reported as menstruation tonic [14]. Similarly, in Morocco, the leaf infusion utilization of *M. vulgare* is used for the treatment of metabolic disorder [15]. Furthermore, in Serbian language, *M. vulgare* is called očajnica, which means desperate woman; because its tea is considered good remedy for women who were unable to conceive and regulate menstrual cycle, it is also traditionally utilized for the treatment of respiratory and gastrointestinal disorders [16]. Medicinal uses of the plants are due to the presence of phytochemicals. The most generally responsible phytoconstituents as antidiabetic are benzoic acid derivatives, phenol and its derivatives, flavonoid, amino acid and its derivatives, vitamins, saponin, alkaloid, and carbohydrate [17]. Phytochemicals such as flavonoids, polyphenols, steroids, terpenoids, and alkaloids have balanced usages and are existed in different amounts in different plants species. The presence of these classes of phytochemicals in plants species and dietary food plays a substantial part to defend against ailment [18]. Keeping in mind the importance of these plants species, we have designed the current study to evaluate its biological potential.

## 2. Experimental

**2.1. Plant Collection.** The plants *R. muricata* and *M. vulgare* were collected from the Hawdaf Sudayer Dam on 18-3-2017 and identified by Dr. Rifayatullah, a plant taxonomist at our college. "The specimen vouchers were deposited in the Herbarium of the Medicinal Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, with voucher numbers SAID 326 and MV-2019, respectively."

**2.2. Extraction Procedure.** The plants *R. muricata* and *M. vulgare* parts were ground to fine powder by a mechanical grinder. The powder plant material (100 g, each) was soaked in 3 liters of methanol. The greenish methanol was filtered and evaporated with the help of a rotary evaporator. The greenish residue of methanol was further assessed for phytochemicals screening, antimicrobial, antioxidant, and cytotoxic potential using reported protocols.

**2.3. Phytochemicals Screening.** Phytochemicals such as alkaloids, flavonoids, tannins, phenols, and saponins were determined using different reagents such as Mayer's reagent and ferric chloride reagent [19–24].

**2.4. GC-MS Analysis.** Agilent GC 7890A combined with a triple axis detector 5975 C single quadrupole mass spectrometer were used for GC-MS analysis. The chromatographic column was an Agilent HP 5MS column

(30 m × 0.25 mm × 0.25 μm film thickness), with high-purity helium as the gas carrier, at a flow rate of 1 mL/min. The injector temperature was 250°C, and it was equipped with a splitless injector at 20:1. The source temperature of MS was set at 230°C, and the quad temperature was set at 150°C. The oven temperature was initially at 40°C (held for 1 min), then was increased to 150°C at 10°C min<sup>-1</sup> (held for 1 min), and then increased further to 300°C at 10°C min<sup>-1</sup> for 1 min. The injection volume was 1 μL, and the scan range was set at 50–800 mass ranges at 70 eV electron energy and the solvent delay of 3 minutes. Finally, unknown compounds were identified by comparing the spectra with that of the NIST 2008 (National Institute of Standard and Technology library). The total time required for analyzing a single sample was 29 minutes.

### 2.5. Antimicrobial Activity

**2.5.1. Agar Well Diffusion Assay (Antibacterial Activity).** Methanol extracts of the plants *R. muricata* and *M. vulgare* were evaluated for antibacterial potential using the well diffusion assay. Nutrient agar media plates were inoculated and were placed in the incubator at 37°C for 18–24 h. Wells were designed in Petri dish having 6 mm diameter with the help of sterile cork borers. Using a sterile swab, the inocula of the respective bacterial strains were spread on nutrient agar plates and then dried at 37°C for 15 min. Stock solutions of n-hexane, chloroform, ethyl acetate, and butanol extracts were prepared using DMSO as solvent. Concentration of each extract solution was kept as 2 mg/mL and 3 mg/mL. Then, 100 μL of each extract was administered in each well containing *E. coli*, *K. pneumonia*, *Xanthomonas*, and *S. aureus*. The Petri plates were placed for incubation at temperature 37°C for 24 h. The zone of inhibition in (mm) for antibacterial activity was determined after incubation. The positive control used showed antibacterial activity [25, 26].

**2.5.2. Agar Well Diffusion Assay (Antifungal Activity).** Agar well diffusion assay was used to find out the antifungal activity of *R. muricata* and *M. vulgare*. The fungal strains were first grown on Petri plates. Wells of 6 mm in diameter were made in Petri plates containing nutrient agar medium using sterile cork borers. Using a sterile swab, the inocula of the respective bacterial strains were spread on nutrient agar plates and then dried at 37°C for 15 min. Stock solutions of n-hexane, chloroform, ethyl acetate, and butanol extracts were prepared using DMSO as solvent. Concentration of each extract solution was kept as 2 mg/mL and 3 mg/mL. Then, 100 μL of each extract was administered in each well containing *Aspergillus niger*, clinical *Candida*, *Acremonium*, *Rhizopus*, and *Trichoderma*. The Petri plates were placed for incubation at temperature 37°C for 24 h. The zones of inhibition in mm for antibacterial activity were determined after incubation. The positive control used showed antibacterial activity [27, 28].

**2.6. Antioxidant Activity.** The antioxidant activity of *R. muricata* and *M. vulgare* was measured by using 2,2-diphenyl picrylhydrazyl radical (DPPH). The molecule responsible for antioxidant activity reacts with DPPH and converts it into diphenyl-picryl hydrazine having yellow color. This change in color is measured with the help of a spectrophotometer. Using the following equation, the DPPH radical inhibiting activity or antioxidant activity of the plant extract with different solvents was calculated.

$$\text{DPPH scavenged (\%)} = \frac{\text{Abs (control)} - \text{Abs (test)}}{\text{Abs (control)}} \times 100, \quad (1)$$

where Abs (control) and Abs (test) are the absorbance of the control and absorbance of the tested sample, respectively [29].

**2.7. Brine Shrimp Lethality Protocol.** The cytotoxic activity of the plant was assessed using the brine shrimp lethality bioassay method where totally 6 graded doses (3 graded doses for each plant) (1000  $\mu\text{g/mL}$ , 100  $\mu\text{g/mL}$ , and 10  $\mu\text{g/mL}$ ) were used. Brine shrimps (*Artemia salina* Leach) nauplii (Ocean 90, USA) were used as test organisms. For hatching, eggs were kept in brine with a constant oxygen supply for 48 h. The mature nauplii were then used in the experiment. DMSO was used as a solvent and also as a negative control. Vincristine sulfate was used as a reference standard in this case. To gage the percentage mortality (% M), the number of dead shrimps is divided by the total number of shrimps and is then multiplied by 100%. The death of the shrimps confirmed the presence of bioactive compounds in the plant extracts [21, 28, 30].

**2.8. Determination of Heavy Metals.** The protocol described by W. Khan et al. [31] was used. Analytical grade concentrated per chloric acid ( $\text{HClO}_4$ ) and nitric acid ( $\text{HNO}_3$ ) were used for the digestion. Samples (0.2 g) of dry grounded whole plant (each) were weighed into 100 mL beakers. Predigestion of the samples was performed with  $\text{HNO}_3$  (5 mL), followed by cooling and digestion again to  $\text{HClO}_4$  fumes. Heavy metals analyses for zinc (Zn), copper (Cu), manganese (Mn), chromium (Cr), lead (Pb), arsenic (As), cadmium (Cd), and chromium (Cr) were performed in triplicates for both medicinal plants using the atomic absorption spectrophotometer (Analyst 700, Perkin Elmer).

### 3. Results and Discussion

**3.1. GC-MS Analysis of *Reseda muricata* (RMM) and *Marrubium vulgare* (MVM) Methanol Extracts.** GC-MS analysis of *Reseda muricata* and *Marrubium vulgare* methanol extracts is shown in Figure 1, Table 1 and Figure 2, Table 2, respectively. GC-MS analysis of RMM showed 17 constituents in methanol extracts. The highest abundance of 2-propenenitrile, 3-phenyl-, (E)- followed by 1H-indole-3-acetic acid-methyl ester has been observed as shown in Figure 1. Similarly, 42 phytoconstituents were noted in the

methanol extract of MVM. 5-Methoxy-2-nitrobenzoic acid was the highest in abundance followed by hexadecanoic acid and methyl ester in MVM. Phytochemicals as collective in the extracts are responsible for biological activities of plant extracts. Therefore, detail of phytochemicals is important.

**3.2. Qualitative Phytochemicals Analysis of *R. muricata* and *M. vulgare*.** Qualitative investigation of methanol extract of *R. muricata* and *M. vulgare* confirmed the presence of different kinds of phytochemicals such flavonoids, phenols, saponins, alkaloids, and tannins, as given in Table 3.

**3.3. Antibacterial Activity.** The bacterial strains used are *E. coli*, *Klebsiella pneumonia*, *Xanthomonas*, and *Staphylococcus aureus*. The methanol extract of *R. muricata* and *M. vulgare* showed antibacterial activity (Table 4). Both the extracts showed a significant zone of inhibition against the test bacterial strain, which confirmed that the methanol extract of *R. muricata* and *M. vulgare* can be used for further investigation.

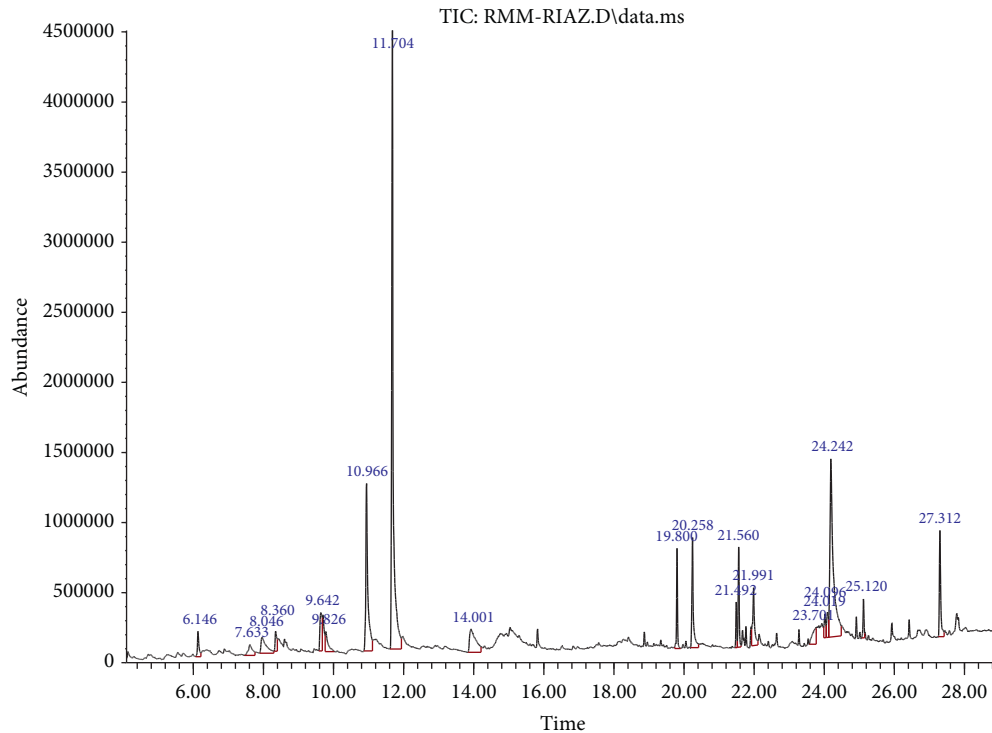
**3.4. Antifungal Activity.** *A. niger*, *Candida*, *Rhizopus*, *Acremonium*, and *Trichoderma* fungal stains were used. The results obtained are given in Table 5. The methanol extract of *R. muricata* and *M. vulgare* showed significant antifungal activities.

**3.5. Antioxidant Activity.** The DPPH free radical scavenging assay was used to evaluate the antioxidant potential of the methanol extract of *R. muricata* and *M. vulgare* which showed that both extracts are active (Table 6). The results were compared with ascorbic acid which was used as a standard antioxidant having  $\text{LC}_{50}$  31.59  $\mu\text{g/mL}$ . The  $\text{LC}_{50}$  of both extracts was above 100  $\mu\text{g/mL}$  (Table 6).

**3.6. Cytotoxicity.** Brine shrimp lethality assay (BSLA) was applied to evaluate the cytotoxicity of the extracts of *Reseda muricata*. It can be seen that with increased in concentration, the mortality rate was increased. The extracts were found to have high mortality above 1000  $\mu\text{g/mL}$  mortality rate and  $\text{LD}_{50}$ , as given in Table 7.

**3.7. Determination of Heavy Metals.** The results given in Table 8 provide the levels of heavy metal in the whole part of *R. muricata* and *M. vulgare*. These results show that the concentration of bioessential elements is within the permissible limit as well as the toxic heavy metal not detected in it.

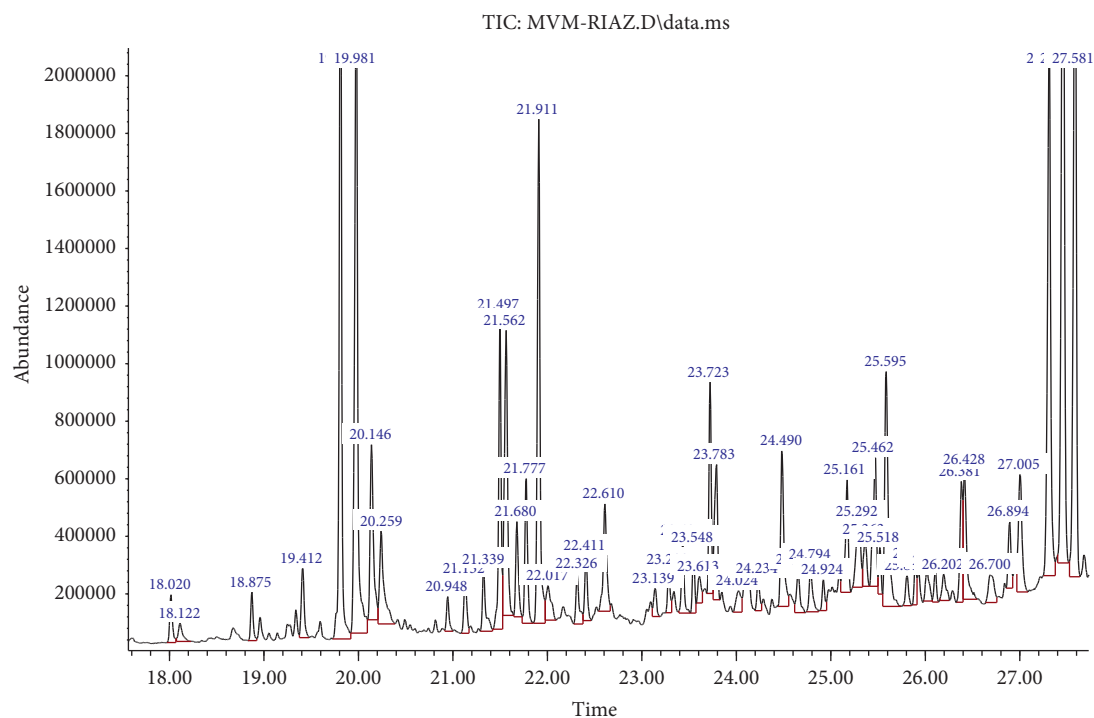
According to the FAO/WHO [31], the acceptable limit for Zn, Cu, Cr, and Mn is 27.4, 3.00, 0.02, and 2 ppm, respectively. From the given findings, it is concluded that the levels of these elements are within the standard parameters. Our study completely agreed with the results which confirmed that concentration of heavy metals in both species *R. muricata* and *M. vulgare* were within the toxic limits. Though, its concentration varies based on different

FIGURE 1: GC-MS analysis of *Reseda muricata* (RMM).TABLE 1: GC-MS analysis of *Reseda muricata* (RMM).

| S. no. | Rt (min) | Area (Ab*s) | Absolute height (ab) | Name of compounds                                      | Mol. weight (amu) |
|--------|----------|-------------|----------------------|--|-------------------|
| 1      | 6.145    | 557970      | 224607               | 5-Isothiazolemethanol                                  | 115.009           |
| 2      | 7.634    | 643368      | 129714               | Cyclopentanecarboxylic acid, 1-amino-                  | 129.079           |
| 3      | 8.047    | 1190894     | 184042               | 2-Butanone, 4-hydroxy-3-methyl-                        | 102.068           |
| 4      | 8.359    | 493253      | 226255               | Piperazine, 1,2,4-trimethyl-                           | 128.131           |
| 5      | 9.642    | 1203054     | 357987               | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-    | 144.042           |
| 6      | 9.823    | 719452      | 222876               | 2,5-Difluoroanisole                                    | 144.039           |
| 7      | 11.706   | 13822257    | 4511047              | 2-Propenenitrile, 3-phenyl-, (E)-                      | 129.058           |
| 8      | 14.002   | 2029488     | 241307               | Benzeneacetic acid, alpha-hydroxy-, methyl ester, (S)- | 166.063           |
| 9      | 19.8     | 1288777     | 817118               | Pentadecanoic acid, 14-methyl-, methyl ester           | 270.256           |
| 10     | 20.257   | 2262071     | 893465               | n-Hexadecanoic acid                                    | 256.24            |
| 11     | 21.489   | 561457      | 434086               | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester         | 294.256           |
| 12     | 21.558   | 1440001     | 827838               | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-  | 292.24            |
| 13     | 21.99    | 1513742     | 536962               | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-                | 278.225           |
| 14     | 23.704   | 891444      | 252326               | 2,5-Dimethylbenzonitrile                               | 131.073           |
| 15     | 24.016   | 482012      | 348804               | Octadecanoic acid, 3-oxo-, methyl ester                | 312.266           |
| 16     | 24.098   | 614148      | 361747               | Heptadecanoic acid, 3-oxo-, methyl ester               | 298.251           |
| 17     | 24.242   | 8477297     | 1450949              | 1H-indole-3-acetic acid-methyl ester                   | 204.09            |

environmental conditions [32]. These metals are very necessary for the development of plants, and its excess or shortage can cause serious problems in plants and humans. Zn is an essential trace nutrient for plant growth due to its role in several cell functions. It is also vital for brain growth, normal growth, bone formation, wound-healing, and behavioral response with a dietary limit in humans of 100 ppm [33]. Its deficiency causes diabetes and loss of smell and touch. Cu is important for normal plant growth, but its extreme levels (>100 ppm) can cause phytotoxicity. The concentration of Cu in seed and flower parts exceeded the

prescribed WHO limits, but its concentration was lower in leaf, stem, and root. Meanwhile, with respect to bodyweight, the acceptable lower limit of Cu is 20  $\mu\text{g}/\text{mg}$  bodyweight per day [31, 34]. In both *R. muricata* and *M. vulgare* samples, Pb, As, Cd, and Cr were not detected. Industries, sewage, air, water pollution, and the fly ash are the main sources of heavy metal contamination. These plants were collected from the Hawdaf Sudayeer Dam of Saudi Arabia, the dam constructed only to store rain water. It is away from cities and industries or industries sewage. This may be the reason why these plants are safe. A similar study reports that three other

FIGURE 2: GC-MS analysis of *Marrubium vulgare* (MVM).TABLE 2: GC-MS analysis of *Marrubium vulgare* (MVM).

| S. no. | Rt (min) | Area (Ab*s) | Absolute height (ab) | Name of compounds   | Mol. weight (amu) |
|--------|----------|-------------|----------------------|---|-------------------|
| 1      | 15.609   | 917256      | 441083               | Bicyclo[2.2.2]octane, 2-methyl-   | 124.125           |
| 2      | 18.124   | 192669      | 98391                | 2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (E)-(+/-)-                                       | 224.214           |
| 3      | 18.875   | 305308      | 206206               | Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)-                     | 138.141           |
| 4      | 19.412   | 526019      | 288389               | 4-(1,3,3-Trimethyl-bicyclo[4.1.0]hept-2-yl)-but-3-en-2-one                                | 206.167           |
| 5      | 19.813   | 4914762     | 2914174              | Hexadecanoic acid, methyl ester   | 270.256           |
| 6      | 20.144   | 1490568     | 718319               | Phenol, o-amino-  | 109.053           |
| 7      | 20.257   | 1050891     | 418235               | n-Hexadecanoic acid   | 256.24            |
| 8      | 20.945   | 228251      | 190697               | Benzenesulfinothioic acid, 4-methoxy-, S-phenyl ester                                     | 264.028           |
| 9      | 21.495   | 2040641     | 1171386              | 9,12-Octadecadienoic acid, methyl ester   | 294.256           |
| 10     | 21.564   | 2275132     | 1115652              | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-                                     | 292.24            |
| 11     | 21.683   | 650401      | 450996               | Phytol  | 296.308           |
| 12     | 21.777   | 1018339     | 600656               | Octadecanoic acid, methyl ester   | 298.287           |
| 13     | 21.908   | 3522375     | 1849605              | 1H-Indene, 2-butyl-5-hexyloctahydro-  | 264.282           |
| 14     | 22.015   | 424602      | 228408               | 1H-Indene, 5-butyl-6-hexyloctahydro-  | 264.282           |
| 15     | 22.327   | 421674      | 271332               | Carane, 4,5-epoxy-, trans   | 152.12            |
| 16     | 22.409   | 447050      | 331946               | 1,2-Dioctylcyclopropene   | 264.282           |
| 17     | 22.609   | 965818      | 512697               | 7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]octane     | 234.162           |
| 18     | 23.141   | 211430      | 218876               | [1,1'-Biphenyl]-3-amine   | 169.089           |
| 19     | 23.291   | 283469      | 285892               | Tricosane   | 324.376           |
| 20     | 23.435   | 495501      | 383643               | Methyl 2-octylcyclopropene-1-octanoate  | 308.272           |
| 21     | 23.547   | 383518      | 362077               | Methyl 18-methylnonadecanoate   | 326.318           |
| 22     | 23.61    | 231395      | 260954               | 2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4.alpha.,7.beta.,8.alpha.)- | 208.183           |
| 23     | 23.722   | 1294950     | 935884               | cis,cis-2,9-Dimethylspiro[5.5]undecane  | 180.188           |
| 24     | 23.785   | 1072281     | 649412               | 2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane                         | 222.198           |
| 25     | 24.023   | 278775      | 211072               | 3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-  | 152.12            |
| 26     | 24.235   | 235821      | 253211               | Butyramide, 4,N-bis(4-methoxyphenyl)-2,4-dioxo-   | 327.111           |
| 27     | 24.492   | 1286972     | 696867               | 3-Buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl)                                   | 208.146           |



TABLE 2: Continued.

| S. no. | Rt (min) | Area (Ab*s) | Absolute height (ab) | Name of compounds  | Mol. weight (amu) |
|--------|----------|-------------|----------------------|--|-------------------|
| 28     | 24.654   | 361709      | 283653               | 1,1'-Bicyclohexyl, 2-propyl-, trans-   | 208.219           |
| 29     | 24.792   | 393996      | 301014               | Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-                                   | 207.162           |
| 30     | 25.161   | 935181      | 595906               | Docosanoic acid, methyl ester  | 354.35            |
| 31     | 25.292   | 794788      | 450542               | (7,7-Dimethyl-2-oxobicyclo[2.2.1]hept-1-yl)methanesulfonic acid, methyl ester                              | 246.093           |
| 32     | 25.461   | 1111821     | 672632               | 2-Heptanone, 6-(3-acetyl-1-cyclopropen-1-yl)-3-hydroxy-6-methyl-, (R*,R*)-                                 | 224.141           |
| 33     | 25.593   | 2166410     | 972489               | Longipinane, (E)-  | 206.203           |
| 34     | 25.805   | 232096      | 262661               | Phenol, 4-methyl-2-nitro-  | 153.043           |
| 35     | 25.893   | 263570      | 304601               | 3,5-Dihydroxybenzamide   | 153.043           |
| 36     | 26.106   | 195485      | 282498               | Ursodeoxycholic acid   | 392.293           |
| 37     | 26.425   | 1019036     | 631125               | Trifluoroacetyl-.alpha.-fenchol  | 250.118           |
| 38     | 26.7     | 412469      | 265361               | 2,4,5,5,8a-Pentamethyl-4a,5,6,7,8,8a-hexahydro-2h-chromene   | 208.183           |
| 39     | 27.006   | 1106824     | 614740               | 1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.,9R*)]- | 222.198           |
| 40     | 27.313   | 4354006     | 2167785              | Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (+/-)-   | 152.12            |
| 41     | 27.457   | 6087633     | 2947769              | 5-Methoxy-2-nitrobenzoic acid  | 197.032           |
| 42     | 28.282   | 703407      | 401338               | Cyclohexane-1-methanol, 3,3-dimethyl-2-(3-methyl-1,3-butadienyl)-  | 208.183           |

TABLE 3: Qualitative phytochemical analysis of *R. muricata* and *M. vulgare*.

| S. no. | Plant species                         | Alkaloids | Flavonoids | Tannins | Phenol | Saponins |
|--------|---------------------------------------|-----------|------------|---------|--------|----------|
| 1      | <i>R. muricata</i> (methanol extract) | +         | +          | +       | +      | +        |
| 2      | <i>M. vulgare</i> (methanol extract)  | +         | +          | +       | +      | +        |

TABLE 4: Antibacterial activity of methanol extract of *R. muricata* and *M. vulgare*.

| Samples                               | Gram negative           |                              |                    | Gram positive                |  |
|---------------------------------------|-------------------------|------------------------------|--------------------|------------------------------|--|
|                                       | Zone of inhibition (mm) |                              |                    |                              |  |
|                                       | <i>E. coli</i>          | <i>Klebsiella pneumoniae</i> | <i>Xanthomonas</i> | <i>Staphylococcus aureus</i> |  |
| Negative control (DMSO)               | —                       | —                            | —                  | —                            |  |
| <i>R. muricata</i> (methanol extract) | 8.03 ± 0.35             | 7.13 ± 0.12                  | 5.13 ± 0.12        | 4.13 ± 0.22                  |  |
| <i>M. vulgare</i> (methanol extract)  | 5.17 ± 0.35             | 6.12 ± 0.39                  | 8.11 ± 0.31        | 6.11 ± 0.31                  |  |
| Streptomycin                          | 17.00 ± 1.02            | 16.20 ± 0.14                 | 15.91 ± 0.81       | 18.10 ± 0.07                 |  |

TABLE 5: Antifungal activity of methanol extract of *R. muricata* and *M. vulgare*.

| Samples                               | Zone of inhibition (mm) |                |                 |                   |                    |
|---------------------------------------|-------------------------|----------------|-----------------|-------------------|--------------------|
|                                       | <i>A. niger</i>         | <i>Candida</i> | <i>Rhizopus</i> | <i>Acremonium</i> | <i>Trichoderma</i> |
| Negative control (DMSO)               | —                       | —              | —               | —                 | —                  |
| <i>R. muricata</i> (methanol extract) | 3.09 ± 0.31             | 4.12 ± 0.10    | 1.19 ± 0.91     | 5.11 ± 0.21       | 1.08 ± 0.11        |
| <i>M. vulgare</i> (methanol extract)  | 2.91 ± 0.16             | 4.01 ± 0.29    | 2.10 ± 0.01     | 7.12 ± 0.31       | 0.11 ± 0.12        |
| Flumetazole                           | 9.89 ± 2.08             | 10.25 ± 1.01   | 4.29 ± 0.01     | 13.21 ± 1.22      | 2.22 ± 1.29        |

TABLE 6: LC<sub>50</sub> values of the methanol extract of *R. muricata* and *M. vulgare*.

| Samples                               | LC <sub>50</sub> (µg/mL)    |
|---------------------------------------|-----------------------------|
| <i>R. muricata</i> (methanol extract) | 154.80 ± 12.10 <sup>a</sup> |
| <i>M. vulgare</i> (methanol extract)  | 127.20 ± 10.10 <sup>b</sup> |
| Ascorbic acid                         | 31.59 ± 6.01 <sup>c</sup>   |

Values are presented as means ± SD ( $n = 3$ ). Means with different superscript (<sup>a-c</sup>) letters in the rows are significantly ( $p < 0.01$ ) different from one another.

TABLE 7: Cytotoxic activity of *R. muricata* and *M. vulgare* (methanol extract).

| Samples                               | % mortality at different concentrations |           |           | LD <sub>50</sub> (μg/mL) |
|---------------------------------------|---|-----------|-----------|--------------------------|
|                                       | 1000 μg/mL                              | 100 μg/mL | 10 μg/mL  |                          |
| <i>R. muricata</i> (methanol extract) | 45 ± 1.92                               | 22 ± 2.21 | 10 ± 0.11 | >1000                    |
| <i>M. vulgare</i> (methanol extract)  | 60 ± 1.87                               | 35 ± 1.39 | 12 ± 1.18 | >1000                    |

TABLE 8: Heavy metal concentrations (mg/kg) of *R. muricata* and *M. vulgare* (methanol extract).

| Samples            | Zn          | Cu          | Cr          | Mn          | Pb | As | Cd | Cr |
|--------------------|-------------|-------------|-------------|-------------|----|----|----|----|
| <i>R. muricata</i> | 8.91 ± 0.02 | 1.98 ± 0.90 | 1.02 ± 0.02 | 0.91 ± 0.01 | ND | ND | ND | ND |
| <i>M. vulgare</i>  | 5.42 ± 0.03 | 2.37 ± 0.12 | 1.91 ± 0.03 | 0.52 ± 0.03 | ND | ND | ND | ND |

species, namely, *Juniperus communis*, *Ocimum basilicum*, and *Commiphora opobalsamum* were collected from Saudi Arabia where Pb and Cd were not detected [35], which is an agreement with our findings. Concentration of Cr between 5 and 30 mg kg<sup>-1</sup> is measured serious for the plants and can affect the plant by curbing its yield. It is vital to show that lead is very hazardous both for plants and animals, especially humans. The maximum acceptable limit for food stuff is 1 mg kg<sup>-1</sup> [36]. The poisonous effects of these heavy metal are because of their hindrance with the regular body biochemistry in normal metabolic processes. Usually, Cr and arsenic are the heavy metals most often concerned in morbidity and death [37]. Likewise, the plant metabolites are fundamental phytoconstituents that display a few observable impacts in the human body. Flavonoids and alkaloids possess strong antioxidant, anticancer, and antimalarial activities. Steroids are very vital class of alcohols with variable implication. The structures are so appropriate to be efficiently transformed by the microbial activities to commercially valued constituents; otherwise, it is hard to synthesize [38]. The presence of alkaloid and tannin contents in *R. muricata* was also quantitatively and qualitatively confirmed by another study [39]. Though, till date, very fewer biological investigations have been made with *R. muricata*, *Reseda* species have been described to hold numerous biological potential such as antimicrobial, antioxidant, and anti-inflammatory, which agree with our findings. The presence of steroids in all extracts modeled a limitless anticipation. The saponins are also identified as the soap forming constituents and are commercially significant [40]. The extracts displayed a good variety of dose-reliant antimicrobial actions against the tested pathogenic fungi and bacteria. So, this plant may be useful for the preparation of broad-spectrum antibacterial drugs. To summarize, this study is a decent help for diverse folklore usages of plants, but still needed a broad study and care because different geographical conditioned may affect the heavy metal contents as well as phytochemical parameters.

#### 4. Conclusion

Traditionally, both plant species have many biological potentials. Numerous phytoconstituents, counting tannins, steroids, alkaloids, phenol, saponin, and flavonoid, are

present in methanol extract of *R. muricata* and *M. vulgare*. The presence of heavy metals such as Zn, Cu, Cr, and Mn are in permissible limits. The paper disc diffusion test displayed some significant antimicrobial effectiveness compared to the standard antibiotics. Antioxidant effectiveness possessed by a plant is certain to keep the free radicals in the body and scavenge them if they are high and a very excellent protector to DNA. Hence, this study recommended further investigation to isolate new bioactive compounds from *R. muricata* and *M. vulgare*.

#### Data Availability

The data used to support this study are included within the article.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

#### Acknowledgments

The authors wish to thank researcher supporting project number (RSP-2021/110) at King Saud University, Riyadh, Saudi Arabia, for financial support.

#### References

- [1] R. Islam, A. S. M. A. Reza, A. A. K. Chawdhury, J. Uddin, and K. Farhana, "Evaluation of in vitro antioxidant activity and cytotoxicity of mButanolic extract of *Sidacordataleaves*," *International Journal of Biological & Pharmaceutical Research*, vol. 5, no. 2, pp. 196–200, 2014.
- [2] Y. Hara-Kudo, A. Kobayashi, Y. Sugita-Konishi, and K. Kondo, "Antibacterial activity of plants used in cooking for aroma and taste," *Journal of Food Protection*, vol. 12, no. 5, pp. 2820–2824, 2004.
- [3] D. Liu, Z. Hu, Z. Liu, B. Yang, W. Tu, and L. Li, "Chemical composition and antimicrobial activity of essential oil isolated from the cultured mycelia of *Ganoderma japonicum*," *Journal of Nanjing Medical University*, vol. 23, no. 3, pp. 168–172, 2009.
- [4] M. A. M. Maciel, A. C. Pinto, V. F. Veiga, N. F. Grynberg, and A. Echevarria, "Plantas medicinais: a necessidade de estudos multidisciplinares," *Química Nova*, vol. 25, no. 3, pp. 429–438, 2002.

- [5] M. M. Cowan, "Plant products as antimicrobial agents," *Clinical Microbiology Reviews*, vol. 12, no. 4, pp. 564–582, 1999.
- [6] R. Dabur, A. Gupta, T. K. Mandal et al., "Antimicrobial activity of some Indian medicinal plants," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 4, no. 3, pp. 313–318, 2007.
- [7] A. Zulqarnain, A. Rahim, K. Ahmad, F. Ullah, H. Ullah, and U. Nishan, "In vitro Antibacterial activity of selected medicinal plants from lower Himalayas," *Pakistan Journal of Pharmaceutical Sciences*, vol. 28, no. 2, pp. 581–587, 2015.
- [8] E. A. Brisibe, U. E. Umoren, F. Brisibe et al., "Nutritional characterisation and antioxidant capacity of different tissues of *Artemisia annua* L.," *Food Chemistry*, vol. 115, no. 4, pp. 1240–1246, 2009.
- [9] A. Karadeniz, I. Cinbilgel, S. Gun, and A. Cetin, "Antioxidant activity of some Turkish medicinal plants," *Natural Product Research*, vol. 29, pp. 1–5, 2015.
- [10] S. Pisutthanan, P. Plianbangchang, N. Pisutthanan, S. Ruanruay, and O. Muanrit, "Brine shrimp lethality activity of Thai medicinal plants in the family Meliaceae," *Naresuan University Journal*, vol. 12, no. 2, pp. 13–18, 2004.
- [11] H. Bhattacharya, Q. Xiao, and L. Lun, "Toxicity studies of nonylphenol on rosy barb (*Puntius conchonius*): a biochemical and histopathological evaluation," *Tissue and Cell*, vol. 40, no. 4, pp. 243–249, 2008.
- [12] N. H. El-Sayed, N. M. Omara, A. K. Yousef, A. M. Farag, and T. J. Mabry, "Kaempferol triosides from *Reseda muricata*," *Phytochemistry*, vol. 57, no. 4, pp. 575–578, 2001.
- [13] M. Rezgui, M. Basma, N. Neng, J. M. Nogueira, L. Bettaieb Ben-Kaab, and M. E. Machado Araújo, "Evaluation of *marrubium vulgare* growing wild in Tunisia for its potential as a dietary supplement," *Foods*, vol. 10, no. 11, Article ID 2864, 2021.
- [14] R. Ullah, A. S. Alqahtani, O. M. A. Noman, A. M. Alqahtani, S. Ibenmoussa, and M. Bourhia, "A review on ethno-medicinal plants used in traditional medicine in the Kingdom of Saudi Arabia," *Saudi Journal of Biological Sciences*, vol. 27, no. 10, pp. 2706–2718, 2020.
- [15] N. Chaachouay, O. Benkhniq, M. Fadli, H. El Ibaoui, and L. Zidane, "Ethnobotanical and ethnopharmacological studies of medicinal and aromatic plants used in the treatment of metabolic diseases in the Moroccan Rif," *Heliyon*, vol. 5, no. 10, Article ID e02191, 2019.
- [16] M. Aćimović, K. Jeremić, N. Salaj et al., "*Marrubium vulgare* L.: a phytochemical and pharmacological overview," *Molecules*, vol. 25, no. 12, Article ID 2898, 2020.
- [17] A. S. Alqahtani, R. Ullah, and A. A. Shahat, "Bioactive constituents and toxicological evaluation of selected antidiabetic medicinal plants of Saudi Arabia," *Evidence-based Complementary and Alternative Medicine*, vol. 2022, Article ID 7123521, 23 pages, 2022.
- [18] A. Shad, S. Ahmad, R. Ullah et al., "Phytochemical and biological activities of four wild medicinal plants," *The Scientific World Journal*, vol. 2014, Article ID 857363, 7 pages, 2014.
- [19] B. S. Abhijit and R. M. Yogini, "Phytochemical analysis and antibacterial properties of some Indian medicinal plants," *International Journal of current Microbiology and Applied sciences*, vol. 4, no. 3, pp. 228–235, 2015.
- [20] H. Iqbal, K. MoneeburRahman, U. Riaz et al., "Phytochemical screening and antimicrobial activities of selected medicinal plants of Khayberpaktunkhwa Pakistan," *African Journal of Pharmacy and Pharmacology*, vol. 5, no. 6, pp. 746–750, 2011.
- [21] R. Umbreen, K. R. Muhammad, J. Shumila, B. Jasia, and S. A. Naseer, "Assessment of phytochemicals, antimicrobial and cytotoxic activities of extract and fractions from *Fagoniaolivieri* (Zygophyllaceae)," *BioMed Central complementary and alternative medicine*, vol. 13, p. 167, 2013.
- [22] M. S. El-Abyad, N. M. Morsi, D. A. Zaki, and M. T. Shaaban, "Preliminary screening of some Egyptian weeds for antimicrobial activity," *Microbios*, vol. 62, pp. 47–57, 1990.
- [23] A. J. Aldesanmi, A. Sofowora, and J. D. Leary, "Preliminary biological and phytochemical investigation of two Nigerian medicinal plants," *International Journal of crude drugs Res*, vol. 24, pp. 147–153, 1986.
- [24] G. F. S. Al-Saleh, A. Y. Gamal El-Din, J. A. Abbas, and N. A. Saeed, "Phytochemical and biological studies of medicinal plants in Bahrain: the family chenopodiaceae-Part 2," *International Journal of Pharmacognosy*, vol. 35, no. 1, pp. 38–42, 1997.
- [25] B. Mahesh and S. Satish, "Antimicrobial activity of some important medicinal plant against plant and human pathogens," *World Journal of Agricultural Sciences*, vol. 4, pp. 839–843, 2008.
- [26] C. F. Bagamboula, M. Uyttendaele, and J. Debevere, "Antimicrobial effect of spices and herbs on *Shigella sonnei* and *Shigella flexneri*," *Journal of Food Protection*, vol. 66, no. 4, pp. 668–673, 2003.
- [27] R. Dall'Agnol, A. Ferraz, and A. P. Bernardi, "Antimicrobial activity of some *Hypericum* species," *Phytomedicine*, vol. 10, pp. 511–516, 2003.
- [28] M. Al-Fatima, M. Wurster, G. Schroder, and U. Lindequist, "Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen," *Journal of Ethnopharmacology*, vol. 11, pp. 657–666, 2007.
- [29] R. Ullah, M. S. Alsaid, A. A. Shahat et al., "Antioxidant and hepatoprotective effects of methanolic extracts of zilla spinosa and hammada elegans against carbon tetrachlorideinduced hepatotoxicity in rats," *Open Chemistry*, vol. 16, no. 1, pp. 133–140, 2018.
- [30] M. O. Ullah, M. Haque, K. F. Urmi et al., "Anti-bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh," *Asian Pacific journal of tropical biomedicine*, vol. 3, no. 1, pp. 1–7, 2013.
- [31] W. Khan, S. Subhan, D. Farhan Shams et al., "Antioxidant potential, phytochemicals composition, and metal contents of *datura alba*," *BioMed Research International*, vol. 2019, Article ID 2403718, 8 pages, 2019.
- [32] A. Chehregani, F. Mohsenzade, and F. Vaezi, "Introducing a new metal accumulator plant and the evaluation of its ability in removing heavy metals," *Toxicological and Environmental Chemistry*, vol. 91, no. 6, pp. 1105–1114, 2009.
- [33] J. P. Dzoyem and J. N. Eloff, "Anti-inflammatory, anticholinesterase and antioxidant activity of leaf extracts of twelve plants used traditionally to alleviate pain and inflammation in South Africa," *Journal of Ethnopharmacology*, vol. 160, no. 3, pp. 194–201, 2015.
- [34] J. Mohmand, S. A. M. A. S. Eqani, M. Fasola et al., "Human exposure to toxic metals via contaminated dust: bio-accumulation trends and their potential risk estimation," *Chemosphere*, vol. 132, pp. 142–151, 2015.
- [35] I. A. Maghrabi, "Determination of some mineral and heavy metals in Saudi Arabia popular herbal drugs using modern techniques," *African journal of Pharmacy and Pharmacology*, vol. 8, no. 36, pp. 893–898, 2014.



- [36] N. M. Abd Ei-Salam, I. Hussain, R. Ullah, S. Ahmad, and S. Khan, "Essential oil and heavy metals analysis of *Boerhaavia procumbens*," *Life Science Journal*, vol. 10, pp. 955–958, 2013.
- [37] H. N. Bhatti and R. A. Khera, "Biological transformations of steroidal compounds: a review," *Steroids*, vol. 77, no. 12, pp. 1267–1290, 2012.
- [38] R. A. Street, "Heavy metals in medicinal plant products - an African perspective," *South African Journal of Botany*, vol. 82, pp. 67–74, 2012.
- [39] S. S. Emam, "Comparative study of the alkaloidal and tannin contents of some *Reseda* species," *Journal of Applied Sciences Research*, vol. 6, pp. 888–896, 2010.
- [40] A. Phuyal, P. K. Ojha, B. Guragain, and N. K. Chaudhary, "Phytochemical screening, metal concentration determination, antioxidant activity, and antibacterial evaluation of *Drymaria diandra* plant," *Beni-Suef University Journal of Basic and Applied Sciences*, vol. 8, no. 1, p. 16, 2019.