*Biomedical & Pharmacology Journal*, September 2023. Vol. **16**(3), p. 1451-1462

# Antioxidant, Antibacterial and Cytotoxic Activities of *Artemisia judaica, Ruta graveolens* and *Suaeda monoica* from Saudi Arabia

**Saida S. Ncibi[1]*, Aymen M. Madkhali[2,3], Magbool E. Oraiby[4], Jamilah A. Almalki[1], Hussein A. Khadashi[6], Abdullah A. Mobarki[5], Syam Mohan[6] and Hassan A. Hamali[2]**

[1]Department of Biology, College of Science, Jazan University, KSA.
[2]Department of Medical Laboratory Technology, Faculty of Applied Medical Science, Jazan University, Jazan, KSA.
[3]Medical Research Center, Jazan University, Jazan, Kingdom of Saudi Arabia.
[4]Poison control center Jazan, KSA.
[5]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Jazan University, Jazan, Kingdom of Saudi Arabia.
[6]Substance Abuse and Toxicology Research center, Jazan University Jazan, KSA.
*Corresponding Author E-mail:sncibi@jazanu.edu.sa

https://dx.doi.org/10.13005/bpj/2723

(Received: 12 August 2023; accepted: 21 September 2023)

**Artemisia judaica, Ruta graveolens, and Suaeda monoica, indigenous plants to Jazan, second smallest region of Saudi Arabia, have several uses in the local folk medicine. This research aims to study the chemical composition of their methanol extracts and to explore some related biological activities. The different extracts Gas Chromatography Mass Spectroscopy profiling revealed the occurrence of many compounds within these extracts. Besides, this study revealed varied and selective antibacterial activities of these extracts. Ruta graveolens methanol extract was effective in inhibiting the growth of all tested microorganisms. Furthermore, they exhibit an interesting cytotoxic effect on human breast cancer cell lines, especially Artemisia judaica methanol extract. These findings suggested that Artemisia judaica (Asteraceae), Ruta graveolens (Rutaceae), and Suaeda monoica (Chenopodiaceae) could be natural sources for the discovery of new drugs.**

**Keywords:** Antibacterial; Antioxidant; Artemisia Judaica; Cytotoxic; Suaeda Monoica; Ruta Graveolens.

During the last decades, several researchers reported the effectiveness of Plants' bioactive substances to fight communicable and non-communicable diseases[1], warranting their further exploitation as sources of new drugs[2].

Jazan, a coastal region on the Red Sea, southwestern area of Saudi Arabia; has a rich flora used in the local folk medicine, amongst *Artemisia judaica (*Asteraceae), *Ruta graveolens* (Rutaceae), and *Suaeda monoica* (Chenopodiaceae)[3].

According to previous studies, Artemisia species are widely spread and frequently used in traditional medicine to reduce phlegm, relieve cough, stop and minimize pain, induce sweat dieresis, and activate blood circulation[4–5]. *Ruta graveolens* has various properties; antioxidant,

antiepileptic, anti-pyretic, anti-cancer, anti-microbial (including fungal, bacterial and parasitic), as well as, diuretic, purgative, hepatoprotective, and hypotensive[6]. Besides, it is a toxin antidote and an insect repellent. *Suaeda monoica* treat sore throat, microbial infections, rheumatoid arthritis, asthma, snake bites, skin disease, ulceration, and toxic hepatitis[1].

This current study aims to analyze the chemical composition of *Artemisia judaica*, *Ruta graveolens,* and *Suaeda monoica* methanol extracts and investigate their antioxidant, antibacterial, and cytotoxic activities.

## MATERIALS AND METHODS

### Chemicals, bacteria, and cell lines

Chemicals, MCF-7 cell line, and bacteria strains were provided from Sigma Aldrich, Germany, American Type Culture Collection and the Centre of Biotechnology of Sfax, Tunisia, and, respectively.

### Plant material and extract preparation

Dried collected plants were grinded. Powdered matter (500 g) was extracted by continuous mixing in 1 L methanol (95%) for 72 h at room temperature with occasional shaking. Finally, methanol of the filtrate was evaporated and methanol extracts residues were stored at 4°C.

### Phytochemical screening

To carry out the phytochemical screening, aqueous solutions of different extracts were prepared as follows: In 10 ml distilled water, 100 mg of each extract was dissolved using a laboratory sonicator (30 min at 37°C).

### Total phenolic contents

The Folin–Ciocalteu method was used to measure the total phenolic contents of the different extracts[7]. Briefly, 2.5 ml of 10% Folin–Ciocalteu reagent (v/v) and 2.0 ml of 7.5% $Na_2CO_3$ was mixed with 0.5 ml of each plant aqueous solution. Gallic acid was used as a standard phenol in this assay. After 40 min incubation at 45°C period, the mixture absorbance was measured at 765 nm. Unless otherwise stated, all tests of the analysis were performed in triplicates. Total phenolic contents were expressed as gallic acid equivalents (eq GA mg/g dry weight of the different extracts).

### Total flavonoid contents

Total flavonoid contents of the extracts were determined using the formation of the flavonoid–aluminum complex procedure[8]. 1 ml aluminum chloride solution (2%) was added to 1 ml of each aqueous sample. Absorbance of the final mixture was measured at 430 nm after 15 min incubation at room temperature. Rutin was used to determine the standard curve. Results were presented as rutin equivalents (mg RE/g dry weight).

### Estimation of antioxidant property

### Free radical-scavenging activity assay

DPPH test was carried out to measure the free radical-scavenging activity[9]. 1 ml of a 0.1 mM methanolic solution of DPPH were added to 1 ml of the aqueous solutions (50–300 µg/ml). After 30 min incubation in darkness at 27°C, sample absorbance was determined at 517 nm. The standard of this assay was the Ascorbic acid.

### Ferric reducing power test

Chu *et al.* (2000) method was adopted to determine the Ferric reducing anti-oxidative power (FRAP) of the different extracts[10]. A mixture of 2.5 ml of potassium phosphate buffer (0.1 M, pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide was prepared. Then, 1.0 ml from varying concentrations (50–500 µg/ml) of plant extract solution was added to this mixture, followed by incubation 20 min at 50°C. Next, 2.5 ml of trichloroacetic acid (10% [w/v]). After that, 2.5 ml of water and 0.5 ml of FeCl3 (0.1% [w/v]) were added to 2.5 ml of the reaction mixture. Incubation of this solution 30 min at 28°C the color developed and its absorbance at 700 nm was determined. Standards for this experiment were ascorbic acid and ranitidine.

### Gas chromatography mass spectroscopy (GC-MS) analysis

A GC-MS analysis for the four methanol extracts were conducted to identify the bioactive compounds[11]. The model of the GC-MS used device was QP 2010 Plus, Shimadzu, Tokyo, Japan. The used column was VF-5ms fused silica capillary, its length 30 mm, 0.25 mm ID, and 0.25 5ØßmØ df. Electron impact mode at 70eV was adopted to ionize samples' components. In that experiment, the carrier gas used is helium (99.99%). The flow was 0.96 ml/min and the injection volume was 2.0 µI. Temperature was 250°C at the injector and 280°C at ion-source. Total GC running time is 36 min. Retention time, retention indices, and mass spectra were adopted to identify the compounds.

For the mass spectra, values were fixed at a scan interval of 0.5 seconds and fragments from 40 m/z–450 m/z.

The spectrum of the known compound stored in the database of National Institute of Standard and Technology (NIST) library were used as references to ascertain the name, molecular weight, and structure of the components of the extracts. Mass spectra analysis was done using Software version 2.71. To calculate the relative percentage amount of each compound, its average peak area was compared to the total area.

**Cell line and cell cultures**

To grow and maintain the cell line, RPMI-1640 medium (pH 7.4) supplemented with FBS (10%), penicillin (100 U/ml), and streptomycin (100 g/ml), was selected. Incubator temperature was fixed at 37°C with 90% humidity and 5% $CO_2$. The $CO_2$ incubator was from New Brunswick Scientific. DMSO was used to dissolve the extracts used in this study (DMSO < 0.05% in media). Control cell cultures received only DMSO.

**Cell viability assay**

*In vitro* cytotoxicity was determined using the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) microculture tetrazolium viability assay[12]. Unless otherwise stated, sample analyses were carried out in triplicates. Briefly, various samples with various concentrations (highest was 200 µg/ml) were incubated for 72 h at 37°C. Untreated cells and blank cell-free were used as controls. After the addition of 5mg/ml MTT to each well, a second incubation for further 4 h after was done. Finally, to solubilize the formazan crystals100 µl of DMSO were added to each well. The final mixture absorbance was read at 490 nm (BioTek microtiter plate reader, Winooski, VT, USA).

Cell proliferation inhibitory rate were calculated using the following formula:

$$Growth\ inhibition = \frac{OD\ control - OD\ treated}{OD\ control} \times 100$$

Extracts cytotoxicity on the cancer cell lines ($IC_{50}$) expressed the sample concentration that reduced the treated cells account by 50% with respect to control cells.

**Antibacterial activities**

In this study, DMSO was used to dissolve the different plant extracts, the initial concentration was 30 mg/ml. Agar-well diffusion method was adopted to carry out the antibacterial activities, as previously presented[13]

Onto the surface of agar plates, $10^6$ cfu/ml for bacteria were inoculated (200 ìl fresh cell suspension). Then, using a sterile Pasteur pipettes, 6 mm diameter wells were banged in the inoculated agar medium. After that, 100 ìl of each extract solution (30 mg/ml) were poured to these wells. To allow the diffusion of the extracts in the agar, plates were kept 4 h at 4°C. After 24 h incubation at 37°C, inhibition zone diameters (IZD) was performed to evaluate the antibacterial activity. In this assay, Gentamicin (150 ìg/ml) was used as positive control. The average values were taken from running the samples in triplicates.

Minimum inhibitory concentration (MIC) is defined as the lowest concentration that completely inhibits bacteria growth (microorganism). In this study, micro-well dilution method (125–2,5 mg/ml) was conducted to determine extracts MIC values. Bacterium suspensions (106 CFU/ml) were incubated, in plates, 24 h at 37°C. To determine extracts MIC values, 40 ìl of MTT (0.5 mg/ml dissolved in distilled water) were added to the wells, followed by an incubation 30 min at 37°C. In this experiment, red-purple color development indicated that the bacteria were biologically active.

**Statistical analysis**

Statistical Package for Social Sciences (SPSS) version 22.0 software (SPSS Inc., IBM, Chicago, Illinois, USA) was used to perform the statistical analysis study. Average value of the triplicates was calculated. Results are represented as means ± standard deviation (SD). Comparison between samples are carried out using ANOVA test followed by a post hoc. $p$ value < 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

Worldwide and throughout the human history, medicinal plants were a normative basis for the maintenance of good health[14]. The used herbal formulations (crude extract, tincture, teas, poultices, powders, and others) differ according to the intended aim[1]. In order to understand their healthiness mechanism and to rationalize plants use, several researches investigated plants'

secondary metabolites in depth during the last decades.

Amongst these compounds, phenolic compounds – phenolic acids and flavonoids – have been of great interest in research [15–17]. Scientific investigations proved that these natural phytochemicals have antioxidant, anti-microbial, and anticancer activities[2, 18, 19].

This study is dealing with *Artemisia judaica* (Asteraceae), *Ruta graveolens* (Rutaceae), and *Suaeda monoica* (Chenopodiaceae). Folk medicine in Saudi Arabia cited the use of these plants to treat different ailments[1].

Practitioners used *Artemisia judaica* to treat many disorders, such as gastro-intestinal disorders, poor eyesight, cardiovascular disease, risk of atherosclerosis, skin disorders, cancer and arthritis [20–21].

*Ruta graveolens* has anti-inflammatory and immune stimulant properties. It treats hypertension, cramps to hysteria, edema visual impairment as well as malaria and sclerosis [22-23].

**Table 1.** TPC and TFC are presented, respectively, as gallic acid equivalents (eq GA mg /g dry weight of the different extracts) and rutin equivalents (eq R mg /g dry weight of the different extracts) of *Artemisia judaica* methanol extract (AJME), *Ruta graveolens* methanol extract (RGME), *Suaeda monoica* methanol extract (SMME). Three independent runs data are displayed as mean ± standard deviation (SD)

| | TPC (µg G A/mg dry plant) | TFC (µg R/mg dry plant) |
|---|---|---|
| RGME | 89,03±3,6 | 3,34±0,9 |
| AJME | 51,39±4,2 | 2,41±0,78 |
| SMME | 34,54±2,75 | 3,056±0,61 |

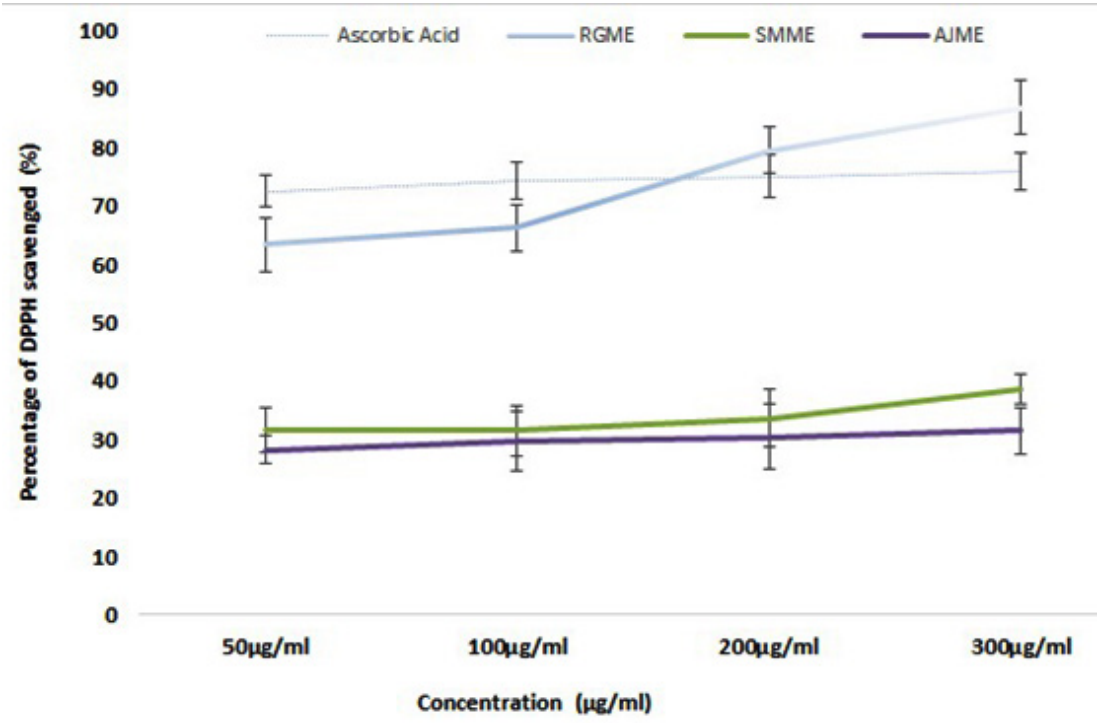


**Fig. 1.** DPPH free radical scavenging activities of AJME, RGME, SMME, and ascorbic acid. Three independent runs results are displayed as mean ± standard deviation (SD).

*Suaeda monoica* possesses a curative action for hepatitis and protects liver against paracetamol-induced injury[24]. Furthermore, this plant helps healing wounds[25].

Table 1 presents TPC and TFC of the different extracts. According to this study, RGME has the highest level of phenols and flavonoids compared to SMME and AJME. These amounts are different compared to the results of other studies. Asgharian (Asgharian *et al.,* 2020) reported different amounts of phenols and flavonoids within R. graveolens hydroalcholic extract, TPC were 14.1 ± 0.47 mg GAE/g and TFC were 15.8 ± 0.19 mg rutin equivalent/g[26]. Concerning *Artemisia Judaica,* Hashem (Hashem *et al.,* 2013) recorded TPC 726.8 mg GAE/g and TFC 705.73 mg rutin equivalent/ug fresh weight[27]. In addition, other studies proved that *Suaeda monoica* contains high amounts of phenols and flavonoids[28–29]. During experiment conduction, several factors such as extraction conditions and used solvent can lead to such variance. Allam (Allam *et al.,* 2019) suggested that Methanol extract contains the greatest amount of phenolics and flavonoids in *Artemisia Judaica*[30].

DPPH and FRAP tests were adopted to estimate the antioxidant activities of these extracts. The DPPH assay deals with the abilities in plants' methanol extracts to donate hydrogen to the DPPH radical, which leads to bleaching of the DPPH solution. Figure 1 presents changes in the free radical scavenging abilities of methanol extracts of the different plants based on their percent inhibition. Results show that RGME has an important scavenging activity tha is similar to ascorbic acid. However, *Artemisia judaica* methanol extract (AJME) and *Suaeda monoica* methanol extract (SMME) produced significantly lower scavenging activities than ascorbic acid.

One of the assays that has the ability to measure the total antioxidants levels in plant is the FRAP assay, where samples with antioxidant compounds had the ability to reduce Fe(III) in potassium ferricyanide to Fe(II) and thus to change the final solution color; yellow to light green.

Figure 2 represents the ferric reducing antioxidant activities of different extracts. Results show that activities of all extracts are significantly lower than the activity of ascorbic acid. However,

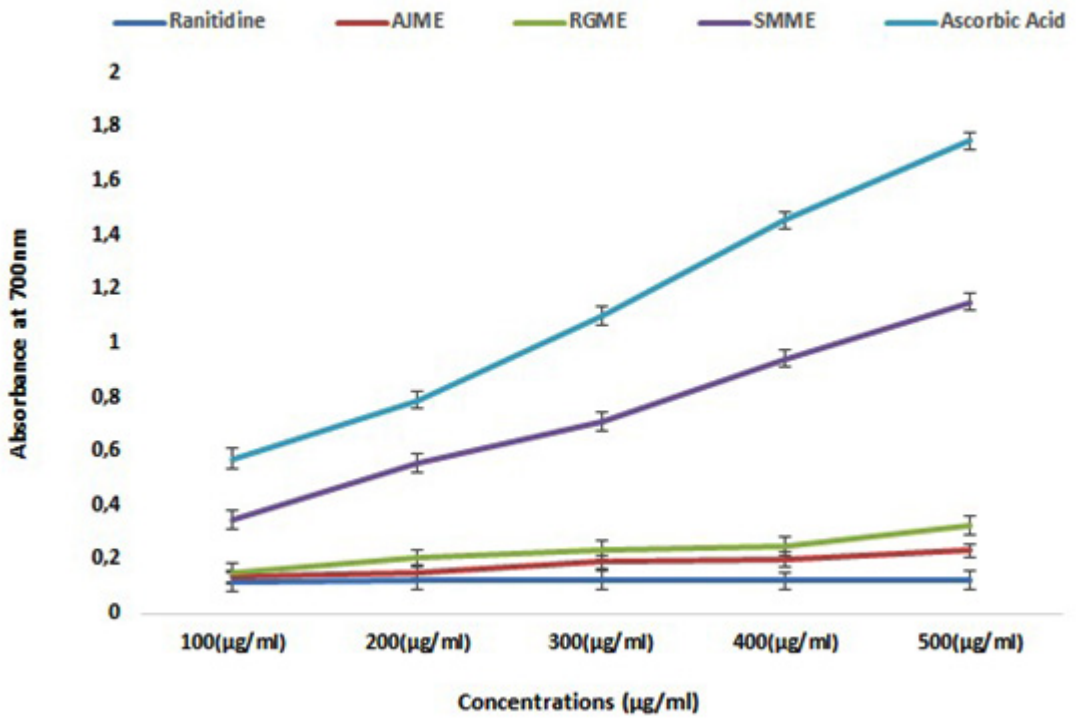


**Fig. 2.** Ferric Reducing Antioxidant power of AJME, RGME, SMME, ascorbic acid, and ranitidine. Three independent runs results are displayed as mean ± standard deviation (SD).

**Table 2.** Details of phytocompounds detected by GC-MS analysis of *Artemisia judaica* methanol extract (AJME)

| | Compound | Retention time | Percentage | Similarity | Biological activities |
|---|---|---|---|---|---|
| 1 | Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)- | 5.092 | 1.44 | 97 | curing dental composites[36] |
| 2 | 1,7-Octadiene-3,6-diol, 2,6-dimethyl- | 6.11 | 0.01 | 90 | Fatty alcohol |
| 3 | alpha-Fenchyl acetate | 6.26 | 0.22 | 97 | Fragrance Compounds |
| 4 | Eugenol | 6.903 | 0.03 | 90 | Immunomodulatory |
| 5 | DL-Proline, 5-oxo-, methyl ester | 7.154 | 0.06 | 93 | Antibacterial and antifungal |
| 6 | 2-Propenoic acid, 3-phenyl- | 7.536 | 0.08 | 91 | hepatoprotective agents |
| 7 | Davana ether | 8.133 | 0.76 | 94 | hepatoprotective agents |
| 8 | Dodecanoic acid (CAS) | 8.215 | 0.03 | 90 | *Lauric Acid* |
| 9 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- | 8.568 | 0.22 | 95 | Anti-microbial potential |
| 10 | Davanone | 8.761 | 0.34 | 92 | hepatoprotective agents |
| 11 | Isopropyl dodecanoate | 8.79 | 0.2 | 91 | |
| 12 | 1,2-Benzenedicarboxylic acid, diethyl ester | 8.878 | 0.17 | 95 | Antimicrobial[37] |
| 13 | 2-Naphthalenemethanol, decahydro-.alpha | 9.556 | 0.19 | 90 | |
| 14 | Mome Inositol | 10.143 | 1.28 | 90 | |
| 15 | *7–Ethyl–1,4*-dimethylazulene. | 10.172 | 1.09 | 94 | Chamazulene |
| 16 | Hexadecanoic acid, methyl ester | 11.2 | 0.46 | 94 | Insecticidal, |
| 17 | n-Hexadecanoic acid | 11.486 | 2.94 | 95 | Insecticidal |
| 18 | Heptadecanoic acid | 12.108 | 0.13 | 92 | Anti-cancer[38] |
| 19 | Cyclooctacosane | 12.289 | 0.11 | 95 | |
| 20 | Octadecanoic acid | 12.795 | 0.61 | 96 | |
| 21 | Matricarin | 14.395 | 1.15 | 90 | Sedative |
| 22 | Grossmisine | 14.436 | 0.34 | 92 | Insect repellent[39] |
| 23 | Dihydroartemisinin, 10-O-(t-butyloxy)- | 14.862 | 0.46 | 90 | |
| 24 | 1-Heptacosanol | 15.814 | 0.19 | 98 | |
| 25 | Heneicosane | 16.386 | 0.02 | 90 | |
| 26 | 13-Docosenamide, (Z)- | 16.518 | 0.09 | 94 | |
| 27 | 2,6,10,14,18,22-Tetracosahexaene | 16.58 | 0.09 | 95 | Antimicrobial[37] |
| 28 | alpha.-Tocopherol-. beta.-D-mannoside | 20.194 | 0.04 | 91 | Antimicrobial[37] |
| 29 | Stigmasterol | 22.68 | 0.83 | 93 | Antimicrobial[37] |
| 30 | Obtusifoliol | 23.476 | 0.15 | 94 | Anticancer : |
| 31 | Stigmast-5-en-3-ol, (3.beta.)- | 23.913 | 1.02 | 91 | |
| 32 | Artemetin | 24.357 | 1.47 | 93 | Anti-edematogenic |
| 33 | 9,19-Cyclolanost-23-ene-3, 25-diol, (3.beta.,23E)- | 25.312 | 0.06 | 91 | Pesticides[37] |
| 34 | Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)- | 5.092 | 1.44 | 97 | |
| 35 | 1,7-Octadiene-3,6-diol, 2,6-dimethyl- | 6.11 | 0.01 | 90 | |

SMME activity is significantly higher than ranitidine, RGME, and AJME activities.

In this study, the GC-MS analysis of the different extracts revealed the occurrence of a large number of compounds 196, 120, and 342 from AJME, RGME, and SMME, respectively. Compounds that have high similarity (e"90%) with the GC library are represented in tables 2, 3, and 4. The identified compounds are presented with their retention time (RT), similarity, percentage, and their reported biological activities.

GC-MS analyses revealed that these extracts contain several components that could be important in many fields: medicine, food industry, cosmetic industry, and others. This richness justifies their antioxidant, antibacterial, and cytotoxic activities.

These extracts are rich in phytosterols that have great interest in research due to their nutritional, biological, and medicinal activities, including anti-hypocholesterolemic, anti-inflammatory, anti-oxidative, and anti-tumor.

AJME contains 35 compounds with similarities higher than 90% with the GC library (Table 2). Some of them have anti-microbial activities. Others are antioxidant, hepatoprotective, and immunomodulatory agents[31–33]. Furthermore, several compounds have insecticidal activities or fragrance compounds[34]. Moreover, artemisinin, an essential compound of this plant extract, treats malaria according to many studies[35].

RGME contains 22 compounds that have similarity with GC library higher than 90% (Table 3). Some of them have neuroprotective,

**Table 3.** Details of phytocompounds detected by GC-MS analysis of *Ruta graveolens* methanol extract (RGME)

| | Compound | Retention time | Percentage | Similarity | Biological activities |
|---|---|---|---|---|---|
| 1 | 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl- | 5.012 | 1.43 | 96 | Antioxidant[41] |
| 2 | 1-Pyrrolidineethanamine | 5.535 | 0.51 | 95 | anti-anginal drug |
| 3 | 1,2,3-Propanetriol, monoacetate | 5.881 | 0.62 | 93 | Anti-cancer[42] |
| 4 | 2-Undecanone (CAS) | 6.242 | 0.45 | 93 | Anti-inflammatory |
| 5 | DL-Proline, 5-oxo-, methyl ester | 7.15 | 0.08 | 89 | Antimicrobial |
| 6 | 2-Acetoxytetradecane | 7.379 | 0.2 | 90 | Antibacterial[43] |
| 7 | Diethyl Phthalate | 8.886 | 0.68 | 98 | |
| 8 | 4-(3,4-Methylenedioxyphenyl)-2-butanone | 9.083 | 0.41 | 95 | Food flavor |
| 9 | 7H-Furo[3,2-g][1]benzopyran-7-one | 11.016 | 3.1 | 95 | |
| 10 | n-Hexadecanoic acid | 11.46 | 1.84 | 96 | Palmitic Acid |
| 11 | 2H-1-Benzopyran-2-one, 7-hydroxy-6-methoxy- (CAS) | 11.818 | 1 | 91 | Scopoletin |
| 12 | Xanthotoxin | 12.335 | 0.27 | 93 | Neuroprotective[44] |
| 13 | Phytol | 12.424 | 1.07 | 97 | Precursor of vitamin E |
| 14 | Methoxsalen | 12.514 | 3 | 92 | Xanthotoxin |
| 15 | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- | 12.651 | 4.53 | 97 | Linolenic acid |
| 16 | 7H-Furo[3,2-g][1]benzopyran-7-one, 2,3-dihydro-2-(1-hydroxy-1-methylethyl)-, (S)- (CAS) | 14.07 | 0.4 | 91 | —- |
| 17 | 1-Octadecanol (CAS) | 14.617 | 1.37 | 90 | Antimicrobial |
| 18 | Acetamiprid | 14.84 | 0.59 | 94 | Insecticide |
| 19 | dl-.alpha.-Tocopherol | 20.201 | 1.68 | 95 | Antimicrobial[37] |
| 20 | Ergost-5-en-3-ol, (3.beta.,24R)- (CAS) | 22.24 | 1.26 | 95 | Campesterol |
| 21 | Neophytadiene | 23.627 | 0.78 | 90 | Anti-inflammatory |
| 22 | Stigmast-5-en-3-ol, (3.beta.)- (CAS) | 23.887 | 1.13 | 91 | Phytodterol |

antioxidant, and anti-microbial effects[40]. Besides, Scopoletin is a pharmacologically active coumarin derivative compound found in various plant *Ruta* species.

SMME has 19 compounds that are similar to GC library, higher than 90%, including hydroxyamphetamine (Table 4). Hydroxyamphetamine is an indirect-acting adrenergic agonist. Its main effect is to induce the release of norepinephrine from the nerve ending, which indirectly initiates muscle contraction. Previous studies cited that *Suaeda monoica* flavonoids, saponins, alkaloids, polyphenols, resins, tannins, and coumarins are therapeutic phytoconstituents [45–47].

Stigmasterol, an essential biochemical present in the three extracts, protects against ketamine-induced psychotic symptoms[49]. Additionally, stigmast-5-en-3-ol increases glucose intake and overcomes insulin resistance[50].

On another hand, these extracts contain substances that could be toxic to animals

**Table 4.** Details of phytocompounds detected by GC-MS analysis of *Suaeda monoica* methanol extract (SMME)

| | Compound | Retention time | Percentage | Similarity | Biological activities |
|---|---|---|---|---|---|
| 1 | Benzeneacetic acid | 5,845 | 0,37 | 90 | antitumor |
| 2 | 1,3-BENZENEDIOL | 6,256 | 0,26 | 95 | catechol |
| 3 | 1H-Indole (CAS) | 6,494 | 0,06 | 89 | |
| 4 | 2-Methoxy-4-vinylphenol | 6,567 | 1,9 | 93 | Aromatic substance |
| 5 | Phenol, 2,6-dimethoxy- | 6,883 | 0,46 | 95 | Anti-fungalAnti-helminthic |
| 6 | 2,4-Imidazolidinedione (CAS) | 6,924 | 0,24 | 94 | Antioxidant[48] |
| 7 | DL-Proline, 5-oxo-, methyl ester | 7,152 | 0,33 | 90 | |
| 8 | p-Hydroxyamphetamine | 7,95 | 1 | 95 | |
| 9 | 1,2-Benzenedicarboxylic acid, diethyl ester (CAS) | 8,885 | 4,14 | 96 | |
| 10 | Neophytadiene | 10,56 | 0,51 | 94 | |
| 11 | Hexadecanoic acid, methyl ester (CAS) | 11,199 | 2,73 | 96 | |
| 12 | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) | 12,375 | 0,41 | 91 | |
| 13 | Phytol | 12,415 | 1,34 | 96 | |
| 14 | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- | 12,631 | 3,45 | 92 | |
| 15 | n-Tetracosanol-1 | 14,605 | 0,46 | 90 | Lignoceryl alcohol |
| 16 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | 14,862 | 0,69 | 94 | Anti-microbial |
| 17 | 1-Docosanol (CAS) | 19,568 | 0,25 | 90 | antiviral |
| 18 | Vitamin E | 20,189 | 4,85 | 95 | |
| 19 | Stigmasterol | 22,634 | 3,02 | 94 | |

**Table 5.** Anti-microbial activities of the different extracts (30mg/ml) and gentamycin (150 µg/ml). Inhibition zones are presented as mean ± SD. The extracts were *Artemisia judaica* methanol extract (AJME), *Ruta graveolens* methanol extract (RGME), *Suaeda monoica* methanol extract (SMME).

| | *E. coli* | *P. aeruginosa* | *S. typhimurium* | *M. luteus* | *S. aureus* | *B. cereus* |
|---|---|---|---|---|---|---|
| Gentamicin | 20±1,1 | 18±0,8 | 20±1 | 19±1,5 | 25±0,7 | 20±1 |
| RGME | 20±0,9 | 22±1 | 20±0,9 | 24±1,2 | 32±0,6 | 18±1,6 |
| AJME | 18±2 | 18±1.3 | ND | ND | ND | 14±2 |
| SMME | 24±0,5 | ND | ND | 18±2 | 10±1 | ND |

and humans. Diethyl phthalate, octadecanol, heptadecane, Tricosenoic acid are among these toxic compounds. They are interested in several industrial fields. Dihydrobenzofuran, known as coumaran, is an acetylcholinesterase inhibitor and can be used as a biofumigant[51].

Several gram-positive and gram-negative bacterial strains were used to test the potential antibacterial activities of the different extracts.

Tables 5 and 6 present the antibacterial activity results of this study. Table 5 shows the inhibition IZD, while Table 6 shows the MIC values. Gentamicin (150 ìg/ml), antibiotic that affects all used bacterial strain, is the positive control in this research.

Our current results showed the existence of varied and selective anti-microbial activities of the different extracts. RGME was effective in inhibiting the growth of all tested microorganisms. However, only *E. coli* appeared to be sensitive to SMME.

According to AlMousa (2021), the active fraction of the ethyl acetate *Artemisia judaica* extract as an anti-microbial effect is significantly observed using disc diffusion assays (at 30 µ/mL) against *P. aeruginosa*, *S. aureus*, *F. solani*, and *A. niger*[52].

A recent study by Helal *et al.* (2019) reported that *Ruta graveolens* extract showed total lack of anti-microbial activity against *E. coli* and *S. typhimurium*[40]. However, essential oils of this plant have high antibacterial activity on *S. aureus*, *K. pneumoniae*, *P. aeruginosa,* and *E. coli* with MIC values 3.5–3.9 ìg/ml, 4.5–5.2 ìg/ml, 5.8–6.3 ìg/

**Table 6.** Minimum inhibition concentrations of the different extracts. The extracts were *Artemisia judaica* methanol extract (AJME), *Ruta graveolens* methanol extract (RGME), *Suaeda monoica* methanol extract (SMME)

| | *E. coli* | *P. aeruginosa* | *S. typhimurium* | *M. luteus* | *S. aureus* | *B. cereus* |
|---|---|---|---|---|---|---|
| RGME | 6.25 | 12.5 | 12.5 | 3.12 | 1.55 | 12.5 |
| AJME | 12.5 | 12.5 | — | — | — | 25 |
| SMME | 12.5 | — | — | 12.5 | 25 | - |

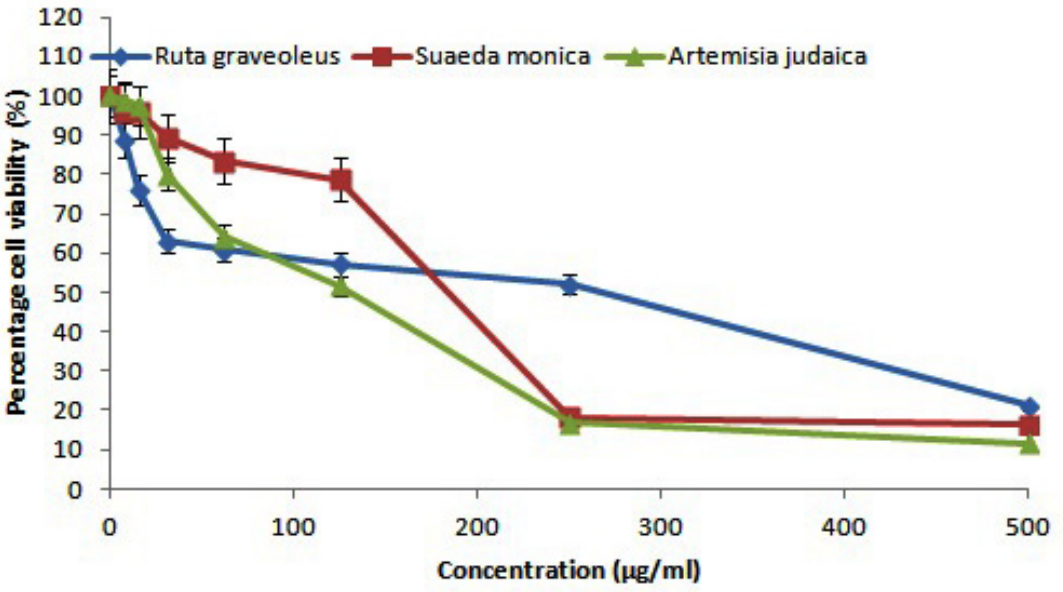


**Fig. 3.** Cytotoxic effect of different extract concentrations (AJME, RGME, SMME**)** on MCF-7 cell line. MTT assay was used to evaluate the percentage of cell viability. Three independent runs results are displayed as mean ± standard deviation (SD).

ml, and 7.5–7.94 ìg/ml, respectively[53]. In addition, according to previous research, *Suaeda monoica* leaves are a potential good source of antibacterial agents[29].

This research carried out MTT assay to investigate AJME, RGME, and SMME cytotoxic activities on the MCF-7 cell line. Current results showed that *Artemisia judaica (*Asteraceae), *Ruta graveolens (*Rutaceae), and *Suaeda monoica* (Chenopodiaceae) are natural sources of cytotoxic agents. AJME has the highest cytotoxic potential (IC50135.7 ± 17.51) against MCF-7 cell line compared to SMME and RGME (cytotoxic potential of SMME and RGME are 176.07 ± 14.39; 285.22 ± 24.1, respectively).

Several researchers confirmed these results, Nasr *et al.,* (2020); proved that *Artemisia judaica* methanol extract exhibited anti-proliferation activity against cell lines, including MCF-7, HepG2 Liver cells, and LoVo colon cells[53].

Additionally, Varamini *et al.,* (2009) study pointed the high cytotoxic activity of *R. graveolens* ethanol extract against different human tumor cell lines (such as; Burkitt's lymphoma cell lines RAJI, RAMOS, prostate adenocarcinoma cell line (LNCap-FGC-10), cell lung carcinoma (Mehr-80) with IC(50); 24.3 microg/ml, 35.2 microg/ml, 27.6 microg/ml, 46.2 microg/ml, respectivelly[54].

## CONCLUSION

In conclusion, this study demonstrated that the methanol extracts of Artemisia judaica (Asteraceae), Ruta graveolens (Rutaceae), and Suaeda monoica (Chenopodiaceae), indigenous plants in the Jazan Province of Saudi Arabia, contain various metabolites that have important antioxidant, antibacterial, and cytotoxic activities. Thus, further research are recommended to isolate and purify their active compounds.

## ACKNOWLEDGEMENT

Researchers would like to thank Jazan University membership and Scientific Research deanship for their support and efforts to succeed this project.

**Funding source**

This research was supported by a grant (000153/7/37) from Jazan University, Saudi Arabia.

**Conflict of interests**

None to declare.

## REFERENCES

1. Sofowora, A., Ogunbodede, E., & Onayade, A. The role and place of medicinal plants in the strategies for disease prevention. *Afr J Tradit Complement Altern Med*. 2013; 10.5:210-229.
2. Parvez, M. K., Al-Dosari, M. S., Rehman, M. T., Alajmi, M. F., Alqahtani, A. S., & AlSaid, M. S. New terpenic and phenolic compounds from *Suaeda monoica* reverse oxidative and apoptotic damages in human endothelial cells. *Saudi Pharmaceutical Journal,* 2021; 29:1102-1111.
3. Rahman, M.A., Mossa J.S., Al-Said, M.S., & Al-Yahya, M.A. Medicinal plant diversity in the flora of Saudi Arabia 1: a report on seven plant families. *Fitoterapia*. 2004; 75:149-61.
4. Al-Wahaibi, L.H.N., Mahmood, A., Khan M., & Alkhathlan, H.Z. Comparative study on the essential oils of Artemisia judaica and A. herba-alba from Saudi Arabia. *Arabian Journal of Chemistry*., 2020; 13: 2053-2065.
5. Abu-Darwish, M.S., Cabral, C., Gonçalves, M.J., Cruz, M.T., Zulfiqar, A., Khan, I.A., Efferth, T., & Salgueiro, L. *J. Ethnopharmacol*. 2016; 191:161-168.
6. Ghramh, H. A., Ibrahim, E. H., Kilnay, M., Ahmad, Z., Alhag, S. K., Khan, K. A., Taha, R., & Asiri, F. M. Silver Nanoparticle Production by *Ruta graveolens* and Testing Its Safety, Bioactivity, Immune Modulation, Anticancer, and Insecticidal Potentials. *Bioinorganic Chemistry and Applications*.2020; 2020:11.
7. Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. *Methods in Enzymology.* 1999; 299:152–178
8. Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. *Food Chemistry*, 2006; 97:654–660.
9. Grzegorczyk-Karolak, I., Matkowski, A., & Wysokinska H. *Food Chemistry.* 2007; 104:536–541.
10. Chu, Y.H., Chang, C.L., & Hsu, H.F. *J. Sci. Food Agric*. 2000; 80:561–566.
11. Fatima, N., Rizwan, M., Hobani, Y. H., Marwan, A. E., Kumar, B. V., Al Sunosi, R., Abdulwahab, S. I., Areeshi, M. Y., Alvi, A., & Oriaby, M. E. *Journal of Pharmacognosy and Phytochemistry*. 2017; 6:197-204.
12. Syam, S., Abdelwahab, S. I., Al-Mamary, M. A., & Mohan, S. Synthesis of Chalcones with Anticancer Activities. *Molecules* (Basel, Switzerland). 2012; 17:6179-6195.

13. Tagg, J. R., & McGiven, A. R. *Appl. Microbiol*. 1971; 21:943
14. Petrovska, B.B. Historical review of medicinal plants' usage. *Pharmacogn Rev*. 2012; 6(11):1-5. doi: 10.4103/0973-7847.95849.
15. Rahman, M. A., Mossa, J. S., Al-Said, M. S., & Al-Yahya, M. A. Medicinal plant diversity in the flora of Saudi Arabia 1: a report on seven plant families. *Fitoterapia*. 2004; 75:149-161
16. Tounekti, T., Mahdhi, M., & Khemira, H. Ethnobotanical study of indigenous medicinal plants of jazan region, saudi arabia. *Evidence - Based Complementary and Alternative Medicine*. 2019; 2019:45.
17. Alqethami, A., & Aldhebiani, A. Y. Medicinal plants used in Jeddah, Saudi Arabia: Phytochemical screening. *Saudi Journal of Biological Sciences*. 2021; 28:805-812
18. Almousa, A., Hassan, M., Abdallah, H., & Abo-Dahab, N. Anti-microbial and cytotoxic potential of an endophytic fungus Alternaria tenuissima AUMC14342 isolated from Artemisia judaica L. growing in Saudi Arabia. *Journal of King Saud University - Science*. 2021; 33(1). https://doi-org.sdl.idm.oclc.org/10.1016/j.jksus.2021.101462
19. Elansary, H. O., Szopa, A., Kubica, P., O. El-Ansary, D., Ekiert, H.; A. Al-Mana, F. Malus baccata var. gracilis and Malus toringoides Bark Polyphenol Studies and Antioxidant, Anti-microbial and Anticancer Activities. *Processes*, 2020; 8(3):283 https://doi-org.sdl.idm.oclc.org/10.3390/PR8050531
20. Janackovic, P., Novakovic, J., Sokoviæ, M., Vujisic, L., Giweli, A., Dajic, S.Z., & Marin, P. Composition and anti-microbial activity of essential oils of *Artemisia judaica*, *A. herba-alba* and *A. arborescens* from Libya. *Archives of Biological Sciences*. 2015; 67:455-466.
21. Abd-Elhady, H. Insecticidal Activity and Chemical Composition of Essential Oil from *Artemisia Judaica L*. Against *Callosobruchus Maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Plant Protection Research*. 2012; 52:347-352.
22. Reethi, K., Kuttan, G., & Kuttan, R. Anti-Tumour activity of *Ruta graveolens* extract. *Asian Pacific journal of cancer prevention*. 2006; 7:439-43.
23. Mahmoud, E. A., Elansary, H. O., & El-Ansary, D. O., Al-Mana, F. A. Elevated Bioactivity of *Ruta graveolens* against Cancer Cells and Microbes Using Seaweeds. *Processes*. 2020; 8:75
24. Elsharabasy, F. S., Metwally, N. S., Mahmoud, A. H., Soliman, M. S., Youness, E. R., Farrag, A. H., & Arafa, S. Phytoconstituents and Hepatoprotective Effect of *Suaeda monoica Forssk* and *Suaeda Pruinosa Lange*. *Biomed Pharmacol J*. 2019; 12(1):117-129
25. Padmakumar, K., & Ayyakkannu, K. Antiviral activity of marine plants. *Indian Journal of Virology.* 1997;13:33-6
26. Asgharian, S., Hojjati, M. R., Ahrari, M., Bijad, E., Deris, F., & Lorigooini, Z. *Ruta graveolens* and rutin, as its major compound: investigating their effect on spatial memory and passive avoidance memory in rats. *Pharm Biol*. 2020; 58(1):447-453
27. Hashem, H., Abdel Rahman, A. R., Kassem, H., & Aziz, N. Bio-Herbicidal potential of desert plants Artemisia judaica L., Asphodelus microcarpus Salzm. and Viv. and Solanum nigrum L. against Portulaca oleracea and Phalaris minor. *Egypt. J. Exp. Biol. (Bot.)*. 2019; 15(1): 99-109
28. Muthazhagan, K., Thirunavukkarasu, P., Ramanathan T., & Kannan, D. Studies on Phytochemical Screening, Antimicrobial and Anti Radical Scavenging Effect Coastal Salt Mash Plant of a Suaeda monoica. *Research Journal of Phytochemistry*, 2014; 8:102-111
29. Lincy, P., Paulpriya, K., & Mohan, V.R. Pharma science monitor an international journal of pharmaceutical sciences pharmacochemical characterization and antibacterial activity of *Suaeda monoica* leaf forssk ex gmel (Chenopodiaceae). *Pharma Science Monitor*. 2013; 4:3947-3963
30. Allam, H., Benamar, H., Ben Mansour, R., Ksouri, R. & Bennaceur M. Phenolic Composition, Antioxidant, and Antibacterial Activities of Artemisia Judaica Subsp. Sahariensis, *Journal of Herbs, Spices & Medicinal Plants,* 2019; 25(4):347-362
31. Abubakar, M. N., & Majinda, R. R. T. GC–MS analysis and preliminary anti-microbial activity of Albizia adianthifolia (Schumach) and Pterocarpus angolensis (DC). *Medicines*. 2016; 3(1):3p
32. Patra, J. K., Das, G., & Baek, K. H. Chemical composition and antioxidant and antibacterial activities of an essential oil extracted from an edible Seaweed, Laminaria japonica L. *Molecules*. 2015; 20:12093-12113
33. Ziaei, A., Ramezani, M., Wright, L., Paetz, C., & Schneider, A.Z. Identification of spathulenol in Salvia mirzayanii and the immunomodulatory effects. *Phytother. Res*. 2011; 25:557-562
34. Al-Wahaibi, L. H. N., Mahmood, A., Khan, M., & Alkhathlan, H. Z. Comparative study on the essential oils of Artemisia judaica and A. herba-alba from Saudi Arabia. *Arabian Journal of Chemistry*. 2020; 13(1):2053-2065
35. Kshirsagar, S., Rao, R. Antiviral and Immunomodulation Effects of Artemisia.

Medicina (Kaunas, Lithuania). 2021; 57. 10.3390/medicina57030217.

36. Dos Santos, D. C., da Silva Barboza, A., Schneider, L. R., Cuevas-Suárez, C. E., Ribeiro, J. S., & Damian, M. F., Campos, A. D., Lund, R. G. Anti-microbial and physical properties of experimental endodontic sealers containing vegetable extracts. *Sci Rep*. 2021; 11(1):6450
37. Ravi, R., Zulkrnin, N. S. H., Rozhan, N. N., Yusoff, N. R. N., Rasat, M. S. M., Ahmad, M. I., Hamzah, Z., Ishak, I. H., Mohd Amin, M. F. Evaluation of Two Different Solvents for Azolla pinnata Extracts on Chemical Compositions and Larvicidal Activity against Aedes albopictus (Diptera: Culicidae). *Journal of Chemistry,* 2018:8
38. Xu, C., Wu, P., Gao, J., Zhang, L., Ma, T., Ma, B., Yang, S., Shao, G., Yu, Y., Huang, X., Yang, X., Zhang, B. Heptadecanoic acid inhibits cell proliferation in PC 9 non small cell lung cancer cells with acquired gefitinib resistance. *Oncol Rep*. 2019; 41(6):3499-3507
39. Adekenov, S. M. & Mukhametzhanova, G. M. & Gayane A., & Juraj, H. Insect repellent and feeding deterrent activity of natural sesquiterpene lactones and their derivatives. *Czech Chemical Society Symposium Series*. 2015; 13:177-184
40. Helal, I. M., El-Bessoumy, A., Al-Bataineh, E., Joseph, M. R. P, Rajagopalan, P., Chandramoorthy, H.C., & Ben Hadj, A. S. Anti-microbial Efficiency of Essential Oils from Traditional Medicinal Plants of Asir Region, Saudi Arabia, over Drug Resistant Isolates. *Biomed Res Int.* 2019:9
41. Èechovská, L., Cejpek, K., Koneèný, M., & Velisek, J. On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one in antioxidant capacity of prunes. *European Food Research and Technology*. 2011; 233:367-376
42. Casuga, F. P., Castillo A. L., & Corpuz M. J. A. T. Bioactive Compounds and Cytotoxicity of Ethyl Acetate Extract From Broussonetia luzonica (Moraceae) Blanco Leaves against Hepatocellular Carcinoma (Hepg2) Cell Lines. *Pharmacognosy Journal*. 2016; 8(5):497-501
43. Chibani, S., Bouratoua, A., Kabouche, A., Laggoune, S., Semra, Z., Smati, F. & Kabouche, Z. Composition and antibacterial activity of the essential oil of Ruta chalepensis subsp. angustifolia from Algeria. *Der Pharmacia Lettre.* 2013; 5(5):252-255
44. Kurach, L., Kulczycka-Mamona, S., Kowalczyk, J., Skalicka-WoŸniak, K., Boguszewska-Czubara, A., El Sayed, N., Osmani, M., Iwaniak, K., & Budzyñska, B. Mechanisms of the Procognitive Effects of Xanthotoxin and Umbelliferone on LPS-Induced Amnesia in Mice. *Int J Mol Sci.* 2021; 22(4):1779
45. Kokpal, V., Miles, D. H., Payne, A. M., & Chittarwong, V. Chemical constituents and bioactive compounds from mangrove plants. *Stud. Nat. Prod. Chem*. 1990; 7:175–199
46. Lakshmi, K. P., & Narsimha, Rao G. M. Anti-microbial activity of *Suaeda monoica* (Forsst ex Geml) against Human and plant pathogens. *Res. J. Pharm. Biol. Chem. Sci*. 2013; 4:680–685
47. Muthazhagan, K., Thirunavukkarasu, P., Ramanathan, T., & Kannan, D. Studies on phytochemical screening, anti-microbial and antiradical scavenging effect of a coastal salt mash plant *Suaeda monoica*. *Res. J. Phytochem*. 2014; 8:102–111
48. Berczyñski, P., Duchnik, E., Kruk, I., Piechowska, T., Aboul-Enein, H.Y., Bozdað-Dündar, O., & Ceylan-Unlusoy, M. 6-Methyl 3-chromonyl 2,4-thiazolidinedione/2,4-imidazolidinedione/2-thioxo-imidazolidine-4-one compounds: novel scavengers of reactive oxygen species. *Luminescence*. 2014; 29(4):367-73
49. Yadav, M., Parle, M., Jindal, D. K., & Dhingr, S. *Pharmacological Reports*. 2018; 70:591–599
50. Sujatha, S., Anand, S., Sangeetha, K. N., Shilpa, K., Lakshmi, J., Balakrishnan, A., & Lakshmi, B. S. *International Journal of Diabetes Mellitus*, 2010; 2:101–109.
51. Rajashekar, Y., & Kumar, H. V., & Ravindra, K. & Nandagopal, B. Isolation and characterization of biofumigant from leaves of Lantana camara for control of stored grain pests. *Industrial Crops and Products*. 2013; 51:224-228.
52. Almousa, A., Hassan, M., Abdallah, H., & Abo-Dahab, N. Anti-microbial and cytotoxic potential of an endophytic fungus Alternaria tenuissima AUMC14342 isolated from Artemisia judaica L. growing in Saudi Arabia. *Journal of King Saud University - Science*. 2021; 33(1).
53. Nasr, F., Noman, O., Mothana, R., Alqahtani, A., & Al-Mishari, A. Cytotoxic, anti-microbial and antioxidant activities and phytochemical analysis of Artemisia judaica and A. sieberi in Saudi Arabia. *African journal of pharmacy and pharmacology*. 2020; 14:278-284
54. Varamini, P., Soltani, M., & Ghaderi, A. Cell cycle analysis and cytotoxic potential of Ruta graveolens against human tumor cell lines. *Neoplasma*. 2009; 56(6):490-3