



Asian Journal of **Biochemistry**

ISSN 1815-9923



Academic
Journals Inc.

www.academicjournals.com

Antimicrobial and Antioxidant Activities of *Artemisia abyssinica* Extracts and DNA Degradation Effects

^{1,2}Magdy M. Youssef, ^{1,3}Ahmed A. Mahmoud and ¹Yasair S.S. Al-Faiyz

¹Department of Chemistry, College of Science, King Faisal University, Saudi Arabia

²Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt

³Department of Chemistry, Faculty of Science, El Minia University, El Minia, Egypt

Corresponding Author: Magdy M. Youssef, Department of Chemistry, College of Science, King Faisal University, Saudi Arabia

ABSTRACT

Artemisia abyssinica, belongs to the family Asteraceae, is an herb commonly used in folk medicine. Antibacterial and antioxidant activities of six organic solvent extracts (*n*-hexane, dichloromethane [CH₂Cl₂, 100%], dichloromethane:methanol [CH₂Cl₂:MeOH, 1:1], methanol [MeOH, 100%], methanol:water [MeOH:H₂O, 8.5:1.5] and water [H₂O, 100%]) of the aerial parts of *A. abyssinica* extracts were investigated. Antibacterial activity was evaluated using the agar diffusion assay against Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus* and *Bacillus megaterium* bacterial strains. The minimum inhibitory concentration of the *Artemisia abyssinica* extracts was tested using a two-fold dilution method at concentrations ranging from 32-512 µg mL⁻¹. Antioxidant activities were measured using the 1,1-diphenyl-2-picrylhydrazyl and superoxide dismutase-like activity methods. Among the tested extracts, *n*-hexane and water extracts exhibited strong antibacterial activity. These extracts also exhibited good antioxidant activity with a 50% inhibition concentration. The extracts were also screened for their degradation effects of high molecular weight biomolecules, such as DNA and bovine serum protein.

Key words: Herbs, *Artemisia abyssinica*, antibacterial, antioxidant, DNA, protein

INTRODUCTION

Medicinal plants are valuable sources of natural products for maintaining human health. Many studies have investigated the potential uses of these plants for health purposes because of the widespread interest in the use of traditional plant-derived products (drug and supplements). Among the numerous herbs used in oriental medicine, growing interest has focused on *Artemisia* spp., which may have great possibilities based on its use in home remedies (Choi *et al.*, 2013). The genus *Artemisia* L. (family Asteraceae, tribe Anthemideae) is large with approximately 500 species widely distributed in Europe, North America, Asia and South Africa (Marco and Barbera, 1990; Bora and Sharma, 2011). Species of this genus are important medicinal plants used by many cultures in folk and modern medicine due to their therapeutic effects (Tan *et al.*, 1998; Abad *et al.*, 2012; Choi *et al.*, 2013). Herbal teas from these species are used as analgesic, antispasmodic, antihelminthic, antidiarrheal and diuretic agents (Darias *et al.*, 1986; Tan *et al.*, 1998; Benjumea *et al.*, 2005) while several extracts and essential oils also have a number of important biological effects, such as antihyperglycemic (Ribnický *et al.*, 2006), antimicrobial (Setzer *et al.*, 2004), antioxidant (Kim *et al.*, 2003; Kordali *et al.*, 2005; Gouveia and Castilho, 2011;

Akrout *et al.*, 2011) and anti-inflammatory (Mino *et al.*, 2004) properties. Furthermore, some species of genus *Artemisia* are frequently utilized for the treatment of diseases such as malaria (Dhingra *et al.*, 2000; Mueller *et al.*, 2000; Willcox and Bodeker, 2004; Willcox, 2009), hepatitis, cancer and infection by fungi, bacteria and viruses (Lee and Lin, 1988; Wilairatana and Looareesuwan, 2002; Lee *et al.*, 2002, 2003; Seo *et al.*, 2003). Chemically, *Artemisia* has been a productive genus in the search for new chemical constituents and biologically active compounds; artemisinin has not only antimalarial activity but also profound cytotoxicity against tumor cells (Efferth, 2007) and arglabin was used to treat certain types of cancer in the former USSR (Wong and Brown, 2002). Therefore, many *Artemisia* species of the world have been extensively investigated. This genus is rich in sesquiterpenoids, monoterpenoids, flavonoids and coumarins (Marco and Barbera, 1990; Tang *et al.*, 2000; Wong and Brown, 2002; Mahmoud and Ahmed, 2006; Bora and Sharma, 2011). *Artemisia abyssinica* is distributed in regions of North Africa and the Middle East. It is quite commonly used in folk medicine as a remedy for heart troubles, cough, rabies, tonsillitis, gonorrhoea, syphilis and leprosy (Geyid *et al.*, 2005; Nibret and Wink, 2010). This plant grows abundantly in various parts of Saudi Arabia and is locally known as "Aathir", where decoction of the fresh whole plant is traditionally used to treat diabetes mellitus (Mossa, 1985). The essential oil composition, diabetic activity and preliminary toxicity of the methanol extract of this species have been previously investigated (Qureshi *et al.*, 1990).

Antibiotic resistance by bacteria causing infectious diseases has become an international concern. The worldwide development of multidrug-resistant *Escherichia coli*, *Pseudomonas aeruginosa* and many other β -lactamase originators has become a major therapeutic problem. Plants are appreciated sources of natural products. The use of compounds from *Artemisia* spp., for pharmaceutical leads has gradually increased (Ribnicky *et al.*, 2009; Fiamegos *et al.*, 2011). The antibacterial activity of *Artemisia* spp., against multidrug-resistant strains of *Staphylococcus aureus*, *E. coli* and *P. aeruginosa*, opportunistic pathogens widely distributed in hospitals as well as increasingly isolated from community acquired infections, has been reported (Zheng *et al.*, 1996). Information regarding the chemical constituents of plants is necessary, not only for the discovery of beneficial agents but also because such knowledge may facilitate the identification of new sources. Conventionally obtainable synthetic antibacterial and antioxidant medications are often associated with unwanted side effects and antibacterial drug resistance. The application of phytochemicals with known antibacterial properties can be a highly beneficial treatment against resistant bacterial strains. *Artemisia* species have a high phenolic and flavonoid content which provides strong antioxidant properties and radical scavenging activities (Ramezani *et al.*, 2004; Shi *et al.*, 2010). The aim of the present study was to investigate the antimicrobial activity of various extracts of *Artemisia abyssinica* against Gram-negative and Gram-positive bacterial strains using the agar diffusion technique. The antioxidant activities of the *Artemisia abyssinica* extracts were also tested using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide dismutase (SOD)-like methods.

MATERIAL AND METHODS

Plant material: The aerial parts of *Artemisia abyssinica* were collected from the desert near Riyadh, Saudi Arabia, in April 2010 and identified by a specialized taxonomist. A voucher specimen (Art-1-10) was deposited in the Natural Products Laboratory, Department of Chemistry, College of Science, King Faisal University, Saudi Arabia. The collected plant materials were stored in a dry and dark place at room temperature with passive ventilation for 2 weeks. The dried plant materials were ground to powder using a plant grinder. The plant powder was stored in airtight containers and maintained at 4°C until use.

Table 1: Extraction scheme of *Artemisia abyssinica*

| Sample code | Weight of dry plant material (g) | Extraction solvent | Weight of total extract (g) |
|----------------------|----------------------------------|---|-----------------------------|
| <i>Artemisia</i> I | 30.0 | <i>n</i> -Hexane | 1.23 |
| <i>Artemisia</i> II | 30.0 | CH ₂ Cl ₂ (100 %) | 1.66 |
| <i>Artemisia</i> III | 30.0 | CH ₂ Cl ₂ +MeOH (1:1) | 3.30 |
| <i>Artemisia</i> IV | 30.0 | MeOH (100 %) | 3.83 |
| <i>Artemisia</i> V | 30.0 | MeOH +H ₂ O (8.5:1.5) | 3.93 |
| <i>Artemisia</i> VI | 30.0 | H ₂ O (100%) boiling | 3.89 |

Extraction procedure: Extraction of the chemical constituent of the dry powdered aerial parts of *Artemisia abyssinica* was performed using the following organic solvent systems according to the polarity: *n*-hexane, dichloromethane (CH₂Cl₂, 100%), dichloromethane:methanol (CH₂Cl₂:MeOH, 1:1), methanol (MeOH, 100%), methanol:water (MeOH:H₂O, 8.5:1.5) and water (H₂O, 100%). Soxhlet and flask extraction procedures were adapted for extraction. Thirty grams of the powdered samples was packed in muslin cloth and used for extraction by a Soxhlet apparatus at a temperature below the boiling temperature of each solvent. A portion of the powdered plant samples was soaked in a conical flask containing solvent, wrapped with aluminum foil and placed in a shaker for 48 h. After 48 h, the extracts were filtered using Whatman filter paper No. 1 and concentrated using a vacuum rotary evaporator. The extracts were stored at 4°C for measurement of antioxidant and antimicrobial activities. The extraction scheme is given in Table 1.

Bacterial strains and antibacterial activities: Bacterial strains used in the present study were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Antibacterial analyses were performed against standard strains of Gram-negative *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 and Gram-positive *S. aureus* ATCC 25923 and *B. megaterium* ATCC 14591. All procedures were approved by the Committee of Scientific Research Ethics of King Faisal University.

The antibacterial activity of *A. abyssinica* extracts (I-IV) was examined individually by the agar diffusion technique (Bauer *et al.*, 1966), using cotton swabs for each bacterial suspension (10⁶ mL⁻¹) and inoculated plates on which the bacteria were spread uniformly on the agar surface. The agar surface was perforated with 6 mm diameter holes, aseptically cut and filled with the various *A. abyssinica* extracts. The antibacterial test was performed against the Gram-negative strains *E. coli* and *P. aeruginosa* and Gram-positive *S. aureus* and *B. megaterium*. The tested extracts were used at a concentration of 10 mg extract/mL of dimethylsulfoxide (DMSO) 10% because DMSO at this concentration does not inhibit microorganism growth. Luria-Bertani (LB) agar medium (10 g bacto-tryptone, 5 g yeast extract, 20 g agar and 10 g NaCl in 1 L de-ionized water) was made for inoculation and bacterial growth. An aliquot of the solution of the verified *A. abyssinica* extracts (I-IV) equivalent to 100 µg was placed separately in agar, cut in the agar. The LB agar plates were incubated for 24 h at 37°C and the resulting inhibition zones were measured. Based on the inhibition zone diameter data analysis, the antibacterial activities were determined against Gram-negative and Gram-positive bacteria.

Minimum Inhibitory Concentrations (MIC): Minimum Inhibitory Concentration (MIC) values of *A. abyssinica* extracts (I-IV) were determined for the bacterial strains (Gram-negative *E. coli*, *P. aeruginosa* and Gram-positive *S. aureus* and *B. megaterium*). All bacterial strains were grown in LB broth separately with 50 µL containing approximately 5×10⁴ CFU of 18 h grown cultures of each organism to be tested. The extracts were first dissolved in 10% DMSO and then diluted to the highest concentration (20 mg mL⁻¹) for testing; serial two-fold dilutions were

made to obtain a final concentration of the extracts in nutrient broth ranging of 512-16 $\mu\text{g mL}^{-1}$. MIC values of the extracts against bacterial strains were determined based on a microwell dilution method (NCCLS., 2001). In brief, 96-well plates were prepared by dispensing into each well 95 μL nutrient broth and 5 μL inocula. A 100 μL aliquot from the stock solutions of the *A. abyssinica* extracts (1-4) in DMSO initially prepared at a concentration of 20 mg mL^{-1} was added to the first wells. Then, 100 μL from their serial dilutions was transferred into six consecutive wells. The last well, containing 195 μL nutrient broth without compound and 5 μL inocula on each strip, was used as negative control. The final volume in each well was 200 μL . The plate was covered with a sterile plate sealer and incubated for 18 h at 37°C. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms after incubation. The values were the means from triplicate experiments.

Estimation of antioxidant activities of *Artemisia abyssinica* extracts using DPPH:

Radical scavenging activity of *A. abyssinica* extracts (I-IV) against stable DPPH was determined spectrophotometrically. When DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. Changes in the color (from purple to yellow) were measured at 520 nm on a UV/Visible light spectrophotometer. Radical scavenging activity of *A. abyssinica* extracts (I-IV) was tested according to a previously reported method (Gulcin, 2010), as described below. Extract solutions were prepared by dissolving 200 μg of *A. abyssinica* extracts (I-IV) in 400 μL DMSO and mixed vigorously. The solution of DPPH in ethanol (6×10^{-5} M) was prepared daily, before UV measurements. Three milliliters of this solution was mixed with 77 μL *A. abyssinica* extract solution in 1 cm path length microcuvettes. A negative control was prepared by adding 77 μL DMSO and 3 mL DPPH ethanolic solution. The samples were kept in the dark for 15 min at room temperature and the decrease in absorption was measured. Absorption of blank sample containing the same amount of ethanol and DPPH solution was prepared and measured daily. Ascorbic acid was used as an antioxidant standard. Determination of antioxidant activities of each *A. abyssinica* extract (I-IV) using DPPH was performed in triplicate. The percentage of antioxidant activity was determined according to the following equation:

$$\text{Antioxidant activity (\%)} = 100 - [(\text{Absorbance of sample} - \text{Absorbance of blank}) \times 100]$$

Determination of SOD-like activity: The *Artemisia abyssinica* extracts were assayed for SOD enzyme-like activity (Bridges and Salin, 1981). The SOD-like activity of *A. abyssinica* extracts (I-IV) was assayed by using phenazine methosulfate to generate superoxide anion radicals at pH = 8.3 (phosphate buffer). Reduction of nitroblue tetrazolium to form blue formazan was used as an indicator of superoxide production and measured spectrophotometrically at 560 nm. The addition of phenazine methosulfate (9.3×10^{-5} M) to a solution of nitroblue tetrazolium (3×10^{-5} M), NADH (4.7×10^{-4} M) and phosphate buffer (final volume of 1 mL) led to a change in the OD ($\Delta 1$) 560 nm per 4 min. The reactions in blank samples and in the presence of *A. abyssinica* extracts (I-IV) were measured. For comparative purposes, the activity of native horseradish SOD was also determined. The superoxide radical scavenging ratio (%) was calculated according to the following equation:

$$\text{Superoxide radical scavenging ratio (\%)} = \frac{\Delta A - \Delta A_1}{\Delta A} \times 100$$

where, A is the absorbance of positive control and A_1 is the absorbance of the test samples.

Agarose gel electrophoresis: The *Artemisia abyssinica* extracts (I-IV) (20 μg) were added individually to 1 μg of the DNA isolated from *E. coli* (Youssef and Al-Omair, 2008). The samples were incubated for 1 h at 37°C. The DNA was analyzed using horizontal agarose gel electrophoresis. The electrophoresis was performed using 0.7% (w/v) agarose gels in TAE buffer (5 mM sodium acetate, 1 mM EDTA and 0.04 M Tris-HCl) at pH 7.9. The agarose gels were stained with ethidium bromide (0.5 $\mu\text{g mL}^{-1}$) and the DNA was visualized on a UV transilluminator (Sambrook *et al.*, 1989).

Polyacrylamide gel electrophoresis: Bovine serum albumin (BSA; 1 mg) was treated with *A. abyssinica* extracts (I-IV; 20 μg) individually. The reaction mixtures were incubated for 1 h at 37°C. The protein samples were analyzed by using vertical one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Laemmli, 1970).

RESULTS AND DISCUSSION

Antibacterial activity: The *Artemisia abyssinica* extracts were tested for antibacterial activity against Gram-negative and Gram-positive bacterial strains using the agar diffusion method. All the *A. abyssinica* extracts exhibited broad inhibitory activity against the tested bacteria (Table 2). The *n*-hexane, 100% H₂O, 100% MeOH and 8.5:1.5 MeOH-H₂O extracts showed good activity compared to the 100% CH₂Cl₂ and 1:1 CH₂Cl₂-MeOH extracts. Growth of *E. coli* was inhibited by *n*-hexane (20 mm), 100% H₂O (19 mm), 100% MeOH (17 mm), 8.5:1.5 MeOH-H₂O (14 mm), 100% CH₂Cl₂ (12 mm) and 1:1CH₂Cl₂-MeOH (11 mm). The MIC for *n*-hexane, 100% H₂O, 100% MeOH, 8.5:1.5 MeOH-H₂O, 100% CH₂Cl₂ and 1:1 CH₂Cl₂-MeOH extracts of *E. coli* ranged from 32-256 $\mu\text{g mL}^{-1}$ (Table 3). A larger inhibition zone was observed with 100% H₂O (20 mm),

Table 2: Effect of *Artemisia abyssinica* extracts on some microorganisms

| <i>Artemisia abyssinica</i> solvent | <i>E. coli</i> ATCC 25922 | <i>P. aeruginosa</i> ATCC 27853 | <i>S. aureus</i> ATCC 25923 | <i>B. megaterium</i> ATCC 14591 |
|---|------------------------------|------------------------------------|--------------------------------|------------------------------------|
| <i>n</i> -Hexane | 20 | 18 | 15 | 16 |
| CH ₂ Cl ₂ (100%) | 12 | 13 | 14 | 12 |
| CH ₂ Cl ₂ +MeOH (1:1) | 11 | 10 | 12 | 10 |
| MeOH (100%) | 14 | 15 | 16 | 14 |
| MeOH+H ₂ O (8.5:1.5) | 17 | 18 | 16 | 18 |
| H ₂ O (100%) boiling | 19 | 20 | 18 | 18 |
| Ampicillin | 22 | 23 | 21 | 20 |

Results are expressed as zone inhibition in millimeters diameter

Table 3: Minimum inhibitory concentration of *Artemisia abyssinica* extracts ($\mu\text{g mL}^{-1}$) against some microorganisms

| <i>Artemisia abyssinica</i> solvent | <i>E. coli</i> ATCC 25922 | <i>P. aeruginosa</i> ATCC 27853 | <i>S. aureus</i> ATCC 25923 | <i>B. megaterium</i> ATCC 14591 |
|---|------------------------------|------------------------------------|--------------------------------|------------------------------------|
| <i>n</i> -Hexane | 32 | 16 | 32 | 32 |
| CH ₂ Cl ₂ (100%) | 256 | 256 | 64 | 128 |
| CH ₂ Cl ₂ +MeOH (1:1) | 256 | 256 | 516 | 256 |
| MeOH (100%) | 128 | 256 | 256 | 128 |
| MeOH+H ₂ O (8.5:1.5) | 32 | 64 | 64 | 32 |
| H ₂ O (100%) boiling | 32 | 32 | 32 | 32 |
| Ampicillin | 8 | 4 | 16 | 8 |

n-hexane, 8.5:1.5 MeOH-H₂O (18 mm) for *P. aeruginosa* while other extracts, such as 100% CH₂Cl₂ (13 mm) and 1:1 CH₂Cl₂-MeOH (10 mm) had comparatively smaller inhibition zones. *Artemisia abyssinica* extracts of *n*-hexane, 100% H₂O, 100% MeOH and 8.5:1.5 MeOH-H₂O, 100% CH₂Cl₂ and 1:1 CH₂Cl₂-MeOH showed a MIC ranging from 32-256 µg mL⁻¹ for *P. aeruginosa*. Gram-positive bacterial strain (*S. aureus*) was inhibited by the *A. abyssinica* extracts. The MIC of *A. abyssinica* extracts for *S. aureus* ranged from 32-516 µg mL⁻¹. Inhibition of *B. megaterium* bacterial strain by *A. abyssinica* extracts was observed with 100% H₂O (16 mm) and 8.5:1.5 MeOH-H₂O (18 mm), *n*-hexane (16 mm), 100% MeOH (14 mm), 100% CH₂Cl₂ (12 mm) and 1:1 CH₂Cl₂-MeOH (10 mm). The MIC of *A. abyssinica* extracts for *B. megaterium* ranged from 32 to 265 µg mL⁻¹.

Antioxidant activity

DPPH radical scavenging and SOD-like activities: The capacity of *Artemisia abyssinica* extracts to scavenge DPPH free radical activity is shown in Fig. 1. The DPPH free radical is stable at room temperature which produces a violet solution in ethanol. In the presence of antioxidant compounds DPPH reduces, producing an uncolored ethanolic solution. The 100% H₂O, 8.5:1.5 MeOH-H₂O, *n*-hexane and 100% MeOH *A. abyssinica* extracts inhibited DPPH free radicals by 61.7±1.18, 57.1±0.69, 54.8±0.98 and 50.6±0.87%, respectively. These extracts showed a high scavenging capacity compared to 1:1CH₂Cl₂-MeOH and 100% CH₂Cl₂ extracts which inhibited the DPPH free radicals by 47.8±1.12% and 43.6±1.03%, respectively. This finding indicates that *A. abyssinica* extracts have inhibitory activities against the DPPH radicals. In the present study, a DPPH assay was used to evaluate the antioxidant activity of extracts of *A. abyssinica*, demonstrating that polar extracts had good antiradical activity. Differences in polarity (and thus extract ability) of antioxidative components may explain why the antioxidant activity of the extracts differed (Galvez *et al.*, 2005).

Free radicals are injurious byproducts generated during normal cellular metabolism which cause oxidative damage to cells (Abdi and Ali, 1999) antioxidants are thought to have a significant role in cellular defense against free radicals. Many recent reports have described the presence of

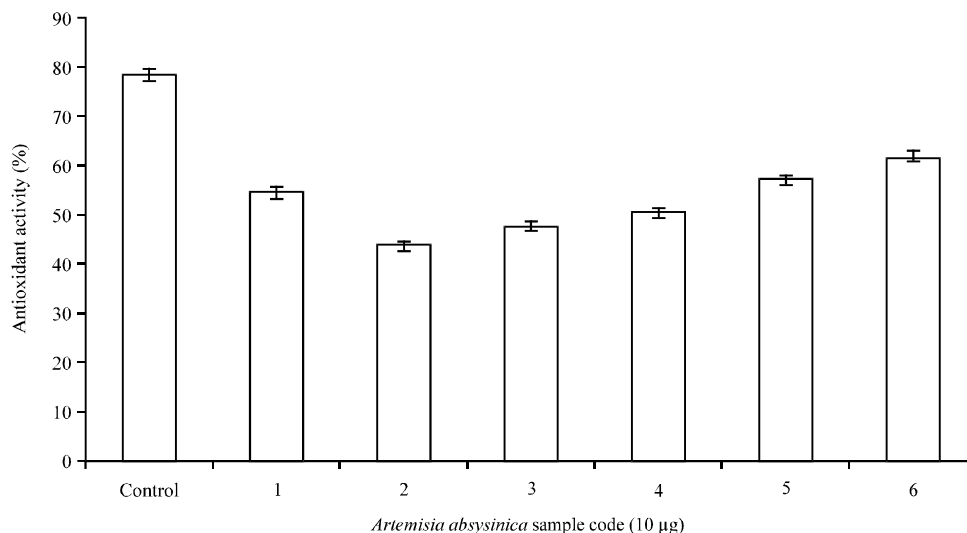


Fig. 1: Antioxidant activities of *Artemisia abyssinica* extracts using DPPH

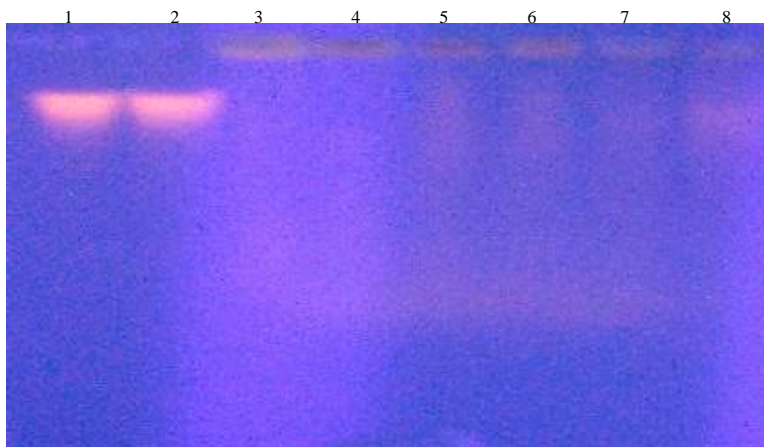


Fig. 2: Degradation effect of *Artemisia abyssinica* extracts on DNA isolated from *E. coli* 7, Lane 1: DNA, Lane 2: DNA-DMSO, Lane 3: *n*-Hexane, Lane 4: CH₂Cl₂ (100%), Lane 5: CH₂Cl₂-MeOH (1: 1), Lane 6: MeOH (100%), Lane 7: MeOH-H₂O (8.5: 1.5) and Lane 8: H₂O (100%) boiling

Table 4: Superoxide dismutase (SOD) like activity of *Artemisia abyssinica* extracts as antioxidant enzyme

| Parameters | SOD like activity | |
|---|-------------------|----------------|
| | Δ through 4 min | Inhibition (%) |
| Control | 0.698 | - |
| HR SOD | 0.157 | 77.5 |
| <i>n</i> -Hexane | 0.339 | 51.4 |
| CH ₂ Cl ₂ (100%) | 0.392 | 43.8 |
| CH ₂ Cl ₂ +MeOH (1:1) | 0.375 | 46.3 |
| MeOH (100%) | 0.354 | 49.3 |
| MeOH+H ₂ O (8.5:1.5) | 0.327 | 53.2 |
| H ₂ O (100%) boiling | 0.308 | 55.9 |

antioxidants and compounds with radical-scavenging activity in fruits, vegetable, herbs and cereals (Hou *et al.*, 2005). DPPH radicals have been extensively used to estimate the free radical scavenging capability of antioxidants (Mensor *et al.*, 2001).

Determination of the antioxidant activity of different *A. abyssinica* extracts based on SOD-like activity is presented in Table 4. The 100% H₂O, 8.5:1.5 MeOH-H₂O and *n*-hexane extracts were more active than the 100% MeOH, 100% CH₂Cl₂ and 1 CH₂Cl₂-MeOH extracts. For *A. abyssinica*, the water, methanol/water and *n*-hexane extracts exhibited strong antioxidant activity based on the DPPH and SOD-like activity methods. These findings, especially for *Artemisia campestris*, were comparable to these reported by Akrouit *et al.* (2010).

Effect of *Artemisia abyssinica* extracts on DNA *in vitro*: The DNA degradation effects of 10 µg of the *A. abyssinica* extracts *in vitro* is illustrated in Fig. 2. The negative control (only DNA) and the positive control (DNA in DMSO) exhibited no DNA degradation through the incubation period, as illustrated in Fig. 2, lanes 1 and 2, respectively. The *A. abyssinica* extracts of *n*-hexane,



Fig. 3: Degradation effect of *Artemisia abyssinica* extracts on Bovine Serum Albumin (BSA), Lane 1: BSA, Lane 2: BSA-DMSO, Lane 3: *n*-Hexane, Lane 4: CH₂Cl₂ (100%), Lane 5: CH₂Cl₂+MeOH (1:1), Lane 6: MeOH (100%), Lane 7: MeOH+H₂O (8.5:1.5) and Lane 8: H₂O (100%) boiling

100% CH₂Cl₂, 1:1 CH₂Cl₂-MeOH, 100% MeOH and 8.5:1.5 MeOH-H₂O completely degraded the DNA, as shown in Fig. 2, lanes 3-7, respectively. The *A. abyssinica* extracts of 100% H₂O had strong DNA degradation effects, as shown in Fig. 2, lane 8. Therefore, the polar and nonpolar *A. abyssinica* extracts are promising as antitumor agents *in vivo* to inhibit the DNA replication in cancer cells and to prevent tumor growth. More work *in vivo* must be performed to elucidate the therapeutic roles of these extracts and to determine the exact pathway of *A. abyssinica* extracts *in vivo*.

Additional biochemical studies to reveal the effects of *A. abyssinica* extracts on BSA as a high molecular-weight biologic compound were performed. The effects of the *A. abyssinica* extracts on BSA are illustrated in Fig. 3, BSA and BSA in DMSO were used as controls, as shown Fig. 3, lanes 1 and 2, respectively. The *A. abyssinica* extracts of *n*-hexane and 100% CH₂Cl₂ completely degraded the BSA, as shown in Fig. 3, lanes 3 and 4, respectively. The *A. abyssinica* extracts of 1:1 CH₂Cl₂- MeOH, 100% MeOH and 8.5:1.5 MeOH-H₂O, also exhibited strong degradation effects on BSA as compared to the control as shown in Fig. 3, lanes 5, 6 and 7, respectively. Moreover, the degradation effects of the *A. abyssinica* extracts of 100% H₂O exhibited significant degradation effects on BSA, as shown in Fig. 3, lane 8.

Polar and nonpolar extracts of *A. abyssinica* interact with nucleophilic molecules, including DNA and proteins. The findings of the present study demonstrated that *A. abyssinica* extracts degraded DNA and protein *in vitro*. The *A. abyssinica* extracts may interact with DNA forming inter and intra-strand adducts, hindering DNA replication, thus leading to cell cycle arrest and apoptosis.

CONCLUSION

All extracts exhibited antibacterial activity against the tested strains. Of all the extracts, the water, *n*-hexane and methanol-water extracts had the highest inhibitory effects against the tested Gram-negative and Gram-positive bacterial strains. The results also revealed that *Artemisia abyssinica* extracts have strong DNA degradation effects. Hence, potentially therapeutic compounds isolated and purified from *Artemisia abyssinica* could be used as an effective source of antioxidants to degrade the DNA in cancer cells and treat bacterial diseases in humans and plants.

ACKNOWLEDGMENT

Financial support from the Deanship of Scientific Research (Project No. 130268) King Faisal University, Saudi Arabia is gratefully acknowledged.

REFERENCES

- Abad, M.J., L.M. Bedoya, L. Apaza and P. Bermejo, 2012. The *Artemisia* L. Genus: A review of bioactive essential oils. *Molecules*, 17: 2542-2566.
- Abdi, S. and A. Ali, 1999. Role of ROS modified human DNA in the pathogenesis and etiology of cancer. *Cancer Lett.*, 142: 1-9.
- Akrout, A., H. El-Jani, S. Amouri and M. Neffati, 2010. Screening of antiradical and antibacterial activities of essential oils of *Artemisia campestris*, *Artemisia herba alba* asso and *Thymus capitatus* hoff. et link. growing wild in the Southern of Tunisia. *Recent Res. Sci. Technol.*, 2: 29-39.
- Akrout, A., L.A. Gonzalez, H. El Jani and P.C. Madrid, 2011. Antioxidant and antitumor activities of *Artemisia campestris* and *Thymelaea hirsuta* from Southern Tunisia. *Food Chem. Toxicol.*, 49: 342-347.
- Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45: 493-496.
- Benjumea, D., S. Abdala F. Hernandez-Luis, P. Perez-Paz and D. Martin-Herrera, 2005. Diuretic activity of *Artemisia thuscula* an endemic canary species. *J. Ethnopharmacol.*, 100: 205-209.
- Bora, K.S. and A. Sharma, 2011. The genus *Artemisia*: A comprehensive review. *Pharm. Biol.*, 49: 101-109.
- Bridges, S.M. and M.L. Salin, 1981. Distribution of iron-containing superoxide dismutase in vascular plants. *Plant Physiol.*, 68: 275-278.
- Choi, E., H. Park, J. Lee and G. Kim, 2013. Anticancer, antiobesity, and anti-inflammatory activity of *Artemisia* species *in vitro*. *J. Tradit. Chin. Med.*, 33: 92-97.
- Darias, V., L. Bravo, E. Barquin, D. Martin-Herrera and C. Fraile, 1986. Contribution to the ethnopharmacological study of the Canary Island. *J. Ethnopharmacol.*, 15: 169-193.
- Dhingra, V., K.V. Rao and M.L. Narasu, 2000. Current status of artemisinin and its derivatives as antimalarial drugs. *Life Sci.*, 66: 279-300.
- Efferth, T., 2007. Antiplasmodial and antitumor activity of artemisinin-from bench to bedside. *Planta Med.*, 73: 299-309.
- Fiamegos, Y.C., P.L. Kastiris, V. Exarchou, H. Han and A.M.J.J. Bonvin *et al.*, 2011. Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from *Artemisia absinthium* against gram positive pathogenic bacteria. *PLoS One*, Vol. 6. 10.1371/journal.pone.0018127
- Galvez, M., C. Martin-Cordero, P.J. Houghton and M.J. Ayuso, 2005. Antioxidant activity of methanol extracts obtained from *Plantago* species. *J. Agric. Food Chem.*, 53: 1927-1933.
- Geyid, A., D. Abebe, A. Debella, Z. Makonnen and F. Aberra *et al.*, 2005. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. *J. Ethnopharmacol.*, 97: 421-427.
- Gouveia, S. and P.C. Castilho, 2011. Antioxidant potential of *Artemisia argentea* L'Her alcoholic extract and its relation with the phenolic composition. *Food Res. Int.*, 44: 1620-1631.
- Gulcin, I., 2010. Antioxidant properties of resveratrol: A structure-activity insight. *Innovative Food Sci. Emerg. Technol.*, 11: 210-218.

- Hou, W.C., R.D. Lui, T.H. Lee, Y.H. Huang, F.L. Hsu and M.H. Lee, 2005. The phenolic constituents and free radical scavenging activities of *Gynura formosana*. *J. Sci. Food Agric.*, 85: 615-621.
- Kim, K.S., S. Lee, Y.S. Lee, S.H. Yung, Y. Park, K.H. Shin and B.K. Kim, 2003. Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*. *J. Ethnopharmacol.*, 85: 69-72.
- Kordali, S., R. Kotan, A. Mavi, A. Cakir, A. Ala and A. Yildirim, 2005. Determination of the chemical composition and antioxidant activity of the essential of *Artemisi dracuncululus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *Artemisia dracuncululus*, *Artemisia santonicum* and *Artemisia spicigera* essential oils. *J. Agric. Food Chem.*, 53: 9452-9458.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.
- Lee, H. and J.Y. Lin, 1988. Antimutagenic activity of extracts from anticancer drugs in Chinese medicine. *Mutat. Res./Genet. Toxicol.*, 204: 229-234.
- Lee, S.H., H.K. Kim, H.M. Kang, J.M. Seo, K.H. Son, H.S. Lee and B.H. Kwon, 2002. Arteminolides B, C and D, new inhibitors of farnesyl protein transferase from *Artemisia argyi*. *J. Org. Chem.*, 67: 7670-7675.
- Lee, S.H., M.Y. Lee, H.M. Kang, D.C. Han and K.H. Son et al., 2003. Anti-tumor activity of the farnesyl-protein transferase inhibitors arteminolides, isolated from *Artemisa*. *Bioorgan. Med. Chem.*, 11: 4545-4549.
- Mahmoud, A.A. and A.A. Ahmed, 2006. a-Pinene-type monoterpenes and other constituents from *Artemisia suksdorfii*. *Phytochemistry*, 67: 2103-2109.
- Marco, J. and O. Barbera, 1990. Natural Products from the Genus *Artemisia*. In: *Studies in Natural Products*, Atta-ur-Rehman, X. (Ed.). Vol. 7. Elsevier, Amsterdam, pp: 201-264.
- Mensor, L.L., F.S. Menezes, G.G. Leitao, A.S. Reis, T.C. dos Santos, C.S. Coube and S.G. Leitao, 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.*, 15: 127-130.
- Mino, J., V. Moscatelli, O. Hnatyszyn, S. Gorzalczany, C. Acevedo and G. Ferraro, 2004. Antinociceptive and antiinflammatory activities of *Artemisia copa* extracts. *Pharmacol. Res.*, 50: 59-63.
- Mossa, J.S., 1985. Phytochemical and biological studies on *Artemisia abyssinica* and antidiabetic herb used in Arabian folk medicine. *Fitoterapia*, 56: 311-314.
- Mueller, M.S., I.B. Karhagomba, H.M. Hirt and E. Wemakor, 2000. The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: Agricultural, chemical and clinical aspects. *J. Ethnopharmacol.*, 73: 487-493.
- NCCLS., 2001. Performance standard for anti-microbial susceptibility testing: Eleventh informational supplement. Document M100-S11, National Committee for Clinical Laboratory Standard, Wayne, PA., USA.
- Nibret, E. and M. Wink, 2010. Volatile components of four Ethiopian *Artemisia* species extracts and their *In vitro* antitrypanosomal and cytotoxic activities. *Phytomedicine*, 17: 369-374.
- Qureshi, S., A.M. Ageel, M.A. Al-Yahya, M. Tariq, J.S. Mossa and A.H. Shah, 1990. Preliminary toxicity studies on ethanol extracts of the aerial parts of *Artemisia abyssinica* and *A. inculta* in mice. *J. Ethnopharmacol.*, 28: 157-162.
- Ramezani, M., F.B.S. Bazzaz, F. Saghafi-Khadem and A. Dabaghian, 2004. Antimicrobial activity of four *Artemisia* species of Iran. *Fitoterapia*, 75: 201-203.

- Ribnicky, D.M., A. Poulev, M. Watford, W.T. Cefalu and I. Raskin, 2006. Antihyperglycemic activity of Tarralin™, an ethanolic extract of *Artemisia dracuncululus* L. *Phytomedicine*, 13: 550-557.
- Ribnicky, D.M., P. Kuhn, A. Poulev, S. Logendra, A. Zuberi, W.T. Cefalu and I. Raskin, 2009. Improved absorption and bioactivity of active compounds from an anti-diabetic extract of *Artemisia dracuncululus* L. *Int. J. Pharmaceut.*, 370: 87-92.
- Sambrook, J., E.F. Fritsch and T.A. Maniatis, 1989. *Molecular Cloning: A Laboratory Manual*. 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY., USA., ISBN-13: 9780879695774, Pages: 397.
- Seo, J.M., H.M. Kang, K.H. Son, J.H. Kim and C.W. Lee *et al.*, 2003. Antitumor activity of flavones isolated from *Artemisia argyi*. *Planta Med.*, 69: 218-222.
- Setzer, W.N., B. Vogler, J.M. Schmidt, J.G. Leahy and R. Rives, 2004. Antimicrobial activity of *Artemisia douglasiana* leaf essential oil. *Fitoterapia*, 75: 192-200.
- Shi, F., X. Jia, C. Zhao and Y. Chen, 2010. Antioxidant activities of various extracts from *Artemisia selengensis* Turcz (LuHao). *Molecules*, 15: 4934-4946.
- Tan, R.X., W.F. Zheng and H.Q. Tang, 1998. Biologically active substances from the genus *Artemisia*. *Planta Med.*, 64: 295-302.
- Tang, H.Q., J. Hu, L. Yang and R.X. Tan, 2000. Terpenoids and flavonoids from *Artemisia* species. *Planta Med.*, 66: 391-393.
- Wilairatana, P. and S. Looareesuwan, 2002. The Clinical Use of Artemisinin and its Derivatives in the Treatment of Malaria. In: *Artemisia, Medicinal and Aromatic Plants: Industrial Profiles*, Wright, C.W. (Ed.). Vol. 18, Taylor and Francis, London, UK., pp: 289-307.
- Willcox, M., 2009. *Artemisia* species: From traditional medicines to modern antimalarials-and back again. *J. Altern. Complement. Med.*, 15: 101-109.
- Willcox, M.L. and G. Bodeker, 2004. Traditional herbal medicines for malaria. *Br. Med. J.*, 329: 1156-1159.
- Wong, H.F. and G.D. Brown, 2002. Germacranolides from *Artemisia myriantha* and their conformation. *Phytochemistry*, 59: 529-536.
- Youssef, M.M. and M.A. Al-Omair, 2008. Cloning, purification, characterization and immobilization of L-asparaginase II from *E. coli* W3110. *Asian J. Biochem.*, 3: 337-350.
- Zheng, W.F., R.X. Tan, L. Yang and Z.L. Liu, 1996. Two flavones from *Artemisia giraldii* and their antimicrobial activity. *Planta Med.*, 62: 160-162.