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Biochemical Study and Molecular Approaching on Eight Wild Herbals Used in Folk Medicine in Al-Baha Region, KSA

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Abstract

Knowledge about the local treasure of natural medicinal resources is so important to define, evaluate and regulate the therapeutic use of herbs in a systematic and effective manner. Only recently, documented scientific studies describing the traditional therapeutic uses of natural medicinal and aromatic plants have been established in Al-Baha region, KSA. In this work, the antioxidant activity of eight herbals plant samples from Al-Baha region was studied using different solvents (ethanol, hexane and water) to evaluate their strong and total lipid-soluble antioxidant and water-soluble antioxidant activity (TLAC^{37,95} and TWAC^{37,95}). The eight herbal plants were also molecularly characterized using the PCR-based ISSR markers. The studied samples demonstrated valuable and variable antioxidant properties. The strong antioxidant capacity of Rumex (Rumex nervosus Vahl.) samples for ethanolic and aqueous extract samples was the highest followed by Fringed Rue (Ruta chalepensis L.) samples (47.68 and 42.72 & 81.74 and 74.18 µmoles/g respectively), while hexanic extracts of Euryops (Euryops arabicus Steud. ex Jaub. & Spach) samples revealed the highest strong antioxidant capacity followed by Fringed Rue samples (23.67 and 13.83 µmoles/g respectively). Besides that, ethanolic extracts of Fringed Rue samples demonstrated the highest total antioxidant capacity followed by olive leaves (Olea europaea subsp. cuspidata (Wall. & G.Don) Cif.) samples (686.95 and 462.62 µmoles/g respectively), while aqueous extracts of Rumex samples revealed superior total antioxidant capacity followed by olive leaves samples and Fringed Rue samples (869.10, 837.21 and 743.75 µmoles/g respectively. But the hexanic extracts of Fringed Rue samples revealed valuable total antioxidant capacity followed by Rumex samples (127.83 and 38.00 µmoles/g respectively. The herbal plants of Rumex, Fringed Rue and olive revealed huge both total and strong antioxidant activity. In the present study we began an initial approach to authenticate and identify different plant species in our study using inter-simple sequence repeats (ISSRs) molecular markers.

***Keywords:** Antioxidant activity, wild medicinal plants, traditional healers, molecular approaching, Al-Baha Region, Kingdom of Saudi Arabia.*

Introduction:

More than 400,000 flowering plants are figured out on the earth (**Govaerts, 2001**). About 50000 plant species of them are used in folk medicine (**Schippmann *et al.*, 2002; Mondal *et al.*, 2016; Hoekou *et al.*, 2016)**. Only 5% of those plant species have been already scientifically studied and described. In modern medicine system, higher plant-derived active ingredients form 25% of prescribed medications and nearly 90 of the 121 bioactive plant-derived compounds were identified through studies based on leads from folk medicine (**Rao *et al.*, 2004).**

In Saudi Arabia, over 50% of the total known flowering plants are expected to have medicinal value. Saudi Arabia folk medicine is ancient and still obtainable through the local tribal people and medicinal healers (**Abdel-Sattar *et al.*, 2015; Mossa *et al.*, 2000; Gazanfar Shahina, 1994; Chaudhary, 2001; Alqahtani *et al.*, 2013).** This human heritage could be lost through the urbanization of indigenous customs and dying of traditional healers without passing their precious knowledge. Wherefore, there is an urgent need for documenting those extensive stores of knowledge through different botanical studies. Al-Baha region is located in the Southwest of Saudi arabia (KSA), featuring with high biodiversity due to its different geographical regions (mountainous, plains and coastal).

Many plants have been identified as a potential source of antioxidants. The current focus is toward plant natural antioxidants, especially vitamins (E, C, A and etc.) and polyphenolics. It is interested to investigate the antioxidant capacities of some herbals especially those traditionally used in folk medicine, as: *Ruta chalepensis* L., *Anethum graveolents* L., *Pimpinella anisum* L., *Olea europaea var. Africana, Olea europaea* subsp. *cuspidata* (Wall. & G.Don) Cif) , *Ficus carica* L., *Rumex nervosus* Vahl, *Opuntia ficus-indica* (L.)Mill. and *Euryops arabicus* Steud. ex Jaub. & Spach. These herbals are used in folk medicine in different uses as: anti-inflammatory, antimicrobial, antispasmodic, anti-ulcer, anti-stomach ache, laxative, hypolipidemic and antipyretic (**Al-Daihan *et al.*, 2013; Alarif *et al.*, 2013; Al-Asmari *et al.*, 2015; Ammar *et al.*, 2015; Mansour *et al.*, 1990; and Ghazi *et al.*, 2012**). It is most repeatedly using different solvents for extraction of plant antioxidant agents. But, the nature of extracting solvent is so necessary for the extract yields and resulting antioxidant activities of the plant materials because of the presence of different antioxidant compounds of different chemical properties and polarities that could be soluble or not in a particular solvent (**Bushra Sultana *et al.*, 2009; Anabela Borges *et al.*, 2020**).

Analysis of genetic diversity and kinship between or within different species, populations and individual plant is essential requirement towards effective utilization and to protect the plant genetic resources (**Weising *et al.*, 1995**). It is prerequisite that plant genetic diversity changes in time and place. The range and distribution of genetic diversity in a plant species is surely depends on its evolution and breeding & ecological systems, geographical conditions and by many human interventions (**Ramanatha Rao and Hodgkin, 2002**). Molecular markers are valuable tools in the evaluation of genetic variation between and within species (**Powell *et al.*, 1996**). DNA-based markers are developed to be used for genotype fingerprinting, identification, genetic mapping and diversity evaluation (**Khazaei *et al.*, 2016**). Besides that, ISSRs as a DNA-based markers allow detection of polymorphisms in genome region between microsatellite loci **(Karaca and Izbirak, 2008).** ISSR DNA-based markers are widely used for studying genetic diversity of plant populations. It is more reliable and reproducible bands than some other molecular markers **(Nagaoka and Ogihara, 1997; Zhang and Dai, 2010).** It is used efficiently (**Etminan *et al.*, 2016**). In this current work, we plan to evaluate the total and strong antioxidant activity and the molecular genetic diversity in eight herbal plant species with different applications in folk medicine in Al-Baha region, KSA.

Materials and Methods:

Plant Material: The whole fruits or leaves of some herbals used in folk medicine (Table 1) were collected from Al-Baha Plateau, Kingdom of Saudi Arabia. Some plants grow naturally in the wild environment; others were purchased from the local market or private farms in the area (figure1).

Table 1: Plant material included in this study

Plant species	Abbreviations	Scientific name	Family	Used part
Fringed Rue	**Fri**	*Ruta chalepensis*	Rutaceae	leaf
Dill	**Dil**	*Anethum graveolens*	Apiaceae	Leaf
Anise	**Ani**	*Pimpinella anisum*		fruit
Wild Olive	**Oli**	*Olea europaea* subsp. *cuspidata*	Oleaceae	leaf
Fig	**Fig**	*Ficus carica*	Moraceae	leaf
Rumex	**Rum**	*Rumex nervosus*	Polygonaceae	Leaf
Cactus	**Cac**	*Opuntia ficus-indica*	Cactaceae	Fruit
Euryops	**Eur**	*Euryops arabicus*	Asteraceae	Leaf

Chemicals and reagents: Hexane, ethanol, analytical grade sodium phosphate, sulfuric acid and all other chemicals and reagents were of high analytical grade. Ammonium molybdate was obtained from Sigma-Aldrich Company, St Louis, Missouri.

Instrumentation: The molecular absorption spectra and absorbance at specific wavelengths were recorded with a UV-visible spectrophotometer.

- A TS-100 thermo shaker from BOECO Germany.
- 4K15 centrifuge from SIGMA.
- SHEL LAB shaking incubator.

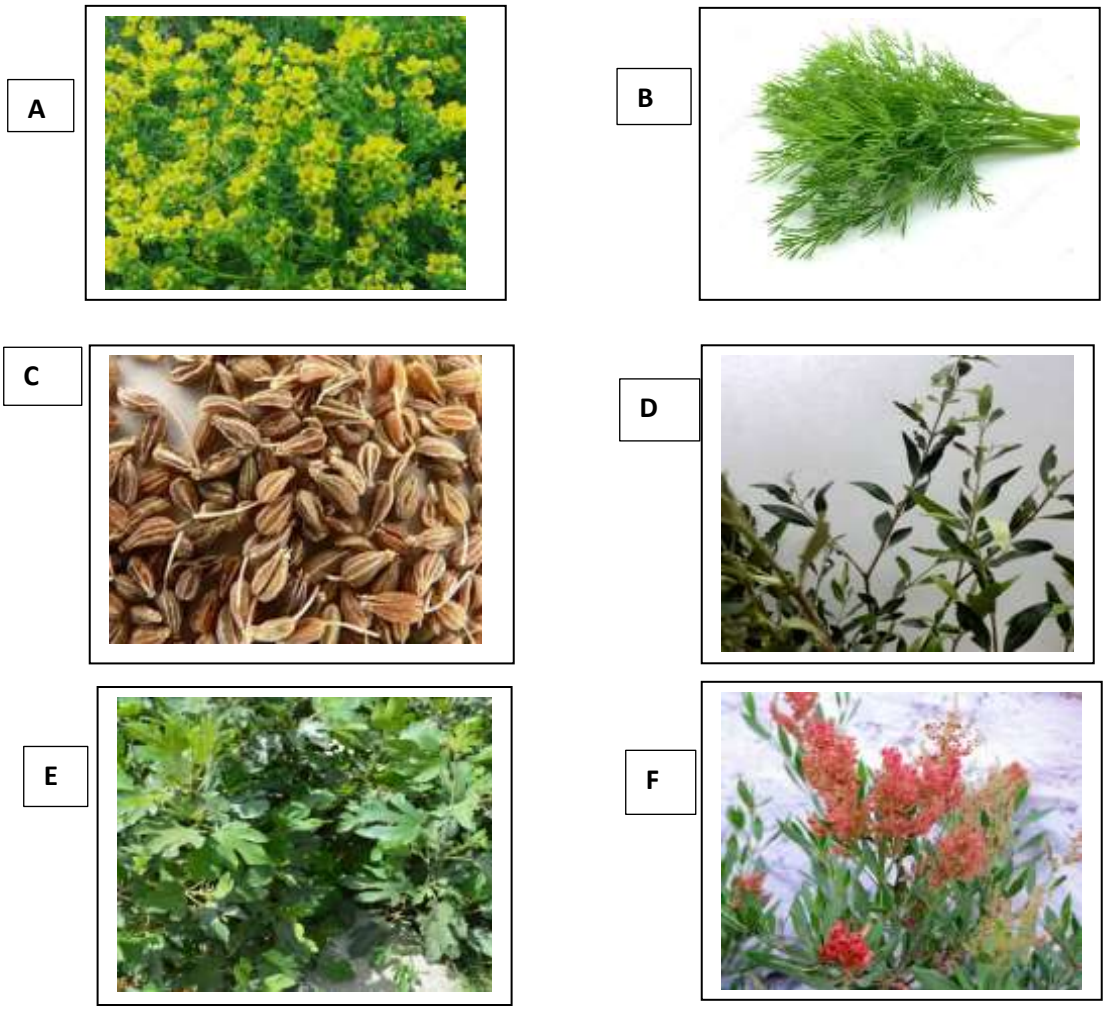


Figure (1): The eight wild herbs included in this study and its abbreviations.
A: *Ruta chalepensis* B: *Anethum graveolens* C: *Pimpinella anisum*
D: *Olea europaea* subsp. *cuspidata* E: *Ficus carica* F: *Rumex nervosus*
G: *Opuntia ficus-indica* H: *Euryops arabicus*

Determination of antioxidant activities

Sample preparation: Ethanolic, hexanic and Aqueous stock solutions of L-ascorbic acid and α-tocopherol were prepared. The exact concentrations at 294 nm were determined spectrophotometrically as described by **Windholz, 1976; Prieto *et al.*, 1999**. After freezing in liquid nitrogen, fresh plant samples (leaves and fruits) were prepared, extracted with ethanol, hexane ($TLAC^{E}$ and $TLAC^{H}$ respectively) and water and kept at 4ºC in the dark for immediate analysis as described by **Alshehri *et al.*, 2019**.

Determination of Total Lipid-soluble Antioxidant Capacity ($TLAC^{E\&H}$): TLAC was determined as described by a spectrophotometric method developed by **Prieto *et al.* 1999**. The method is based on the formation of a blue-green phosphomolybdenum complex. The preparation of samples and measurements was described by **Mohamed *et al.*, 2007**. TLAC per gram of plant material was obtained with the following formula:

TLAC (µmol α-tocoferol/g) = A695 × $Є^{-1}$ × RV × SV^{-1} × EV× m^{-1}

where A695 is the absorbance at 695 nm, $Є^{-1}$ is the inverse of the extinction coefficient (137 $µM^{-1}$), RV is the overall reaction volume, SV is the sample volume used in the reaction, EV is the volume of solvent used in the extraction of the plant material analyzed and m is the amount (in grams) of fresh plant material extracted. When the assay was performed at 37ºC ($TLAC^{37}$) instead of 95ºC, we got the total antioxidant capacity due to strong lipid-soluble antioxidant agents like vitamin E. All measurements were done in triplicate.

Determination of Total Water-soluble Antioxidant Capacity (TWAC): TWAC was determined also as described by **prieto *et al.*, 1999** and the preparations and measurements details were described by **Mohamed *et al.*, 2007.** Total water-soluble antioxidant capacity per gram of fresh plant material was obtained with the following formula:

TWAC (µmol L-ascorbic acid/g) = A695 × $Є^{-1}$ × RV × SV^{-1} × EV× m^{-1}

When the assays were performed at 37ºC ($TWAC^{37}$) instead of 95ºC, we got the total antioxidant capacity due to strong water-soluble antioxidant agents like vitamin C (L-Ascorbic acid). All determinations were done in triplicate.

Genomic DNA extraction

Genomic DNA was extracted from plant samples using Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit according to the manufacturer. Extracted DNA were stored at -20°C until further use.

Application of inter simple sequence repeat (ISSR) markers

Inter Simple Sequence Repeat (ISSR) analysis was applied according to **Williams *et al*. (1990)** using (ISSR) primers obtained from (Metabion AG Lena Christ Strasse, Martinsried, Deutschland) as shown in Table (2).

ISSR-PCR analysis:

ISSR-PCR reactions were conducted using anchored primers, which were synthesized by Bio-Neer South Korea. Their names and sequences are indicated in Table (2). Amplification was performed in a Reactions were carried out in a DNA thermocycler (Multigene Thermal Cycler Labnet USA) programmed using the temperature conditions as shown in Table (2). The PCR was performed with the cycling program of denaturation (one cycle) 94º C for 5 min, annealing 35 cycles for 1 min, 94º C, 1 min at (30-44) º C, 2 min at 72º C and with final extension of 10 min at 72ºC. Electrophoresis of DNA samples were performed on 1.2 % agarose gel and visualized with 0.5 mg/ml ethidium bromide. The amplified DNA bands were visualized under UV light and the sizes of the fragments were estimated based on a 1 Kb DNA ladder (Gene Direx) of 250 to 10,000 base pairs. The run was performed at 100 v in Bio-Rad submarine and photographed with Gel-Documentation UPV system.

Table 2: The list of primers and their nucleotide sequences used for ISSR markers in amplification of A. graecorum DNA (Bio-Neer South Korea).

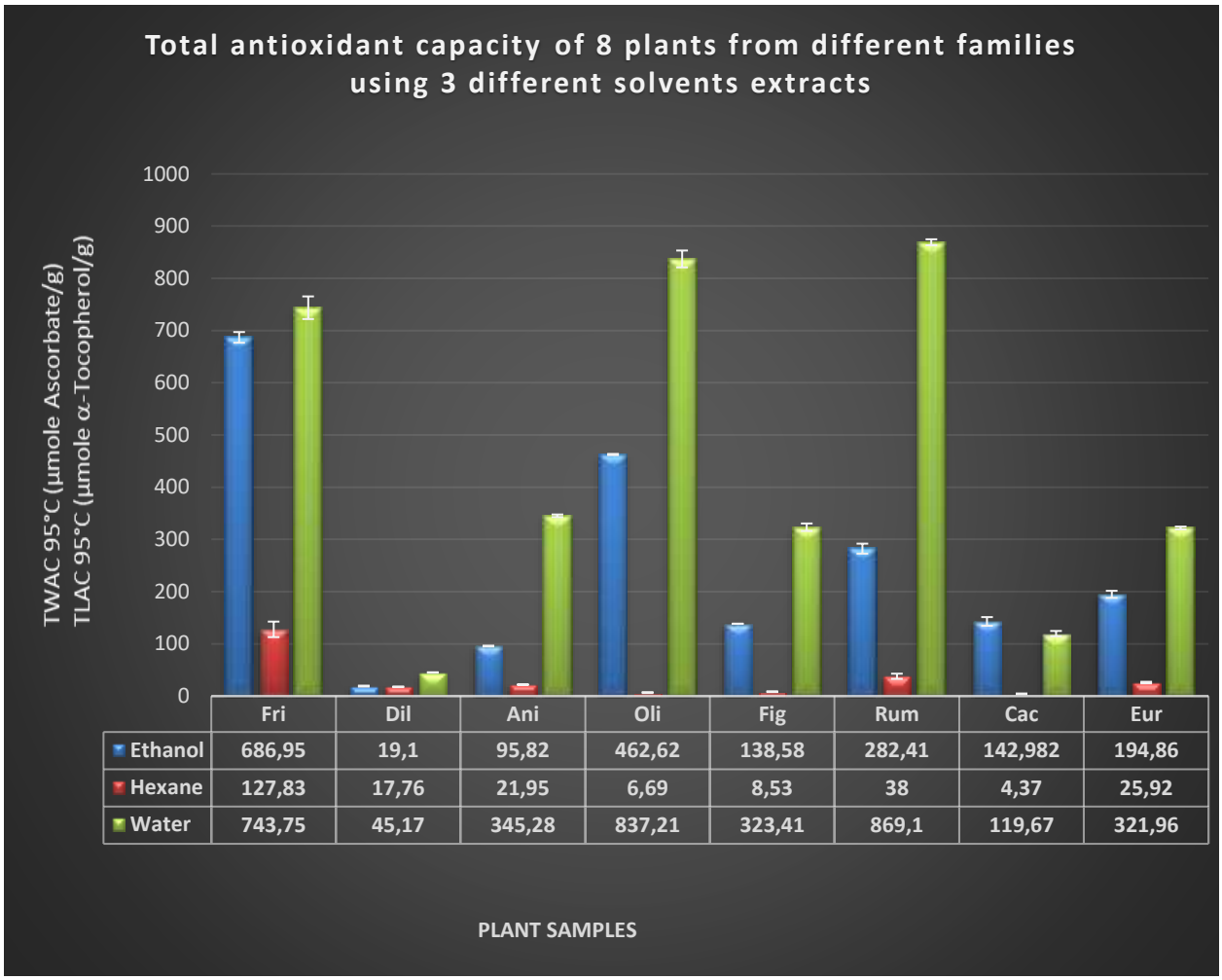
No	Primer name	Primer nucleotide sequence (5'→3')	Temperature
1	814	5'-CT CT CTCTCTCTCTCT TG-3'	35.7ºC
2	844A	5'-CT CT CTCTCTCTCTCT AC-3'	31.4ºC
3	844B	5'-CT CT CTCTCTCTCTCT GC-3'	38.8ºC
4	HB11	5'-GT GT GTGTGTGT CC-3'	44ºC
5	HB12	5'-CAC CAC CAC GC-3'	38ºC
6	HB13	5'-GAG GAG GAG GC-3'	38ºC

A: Adenine, **T**: Thymine, **G**: Guanine and **C**: Cytosine

Results and Discussion

Total antioxidant capacity (TWAC and TLAC) of the eight herbals included in this study: The present study comprises eight herbals (*Ruta chalepensis, Anethum graveolens, Pimpinella anisum, Olea europaea* subsp. *cuspidata* , *Ficus carica, Rumex nervosus, Opuntia ficus-indica* and *Euryops arabicus*), with the abbreviations: Fri, Dil, Ani, Oli, Fig, Rum, Cac and Eur respectively. These herbals are widely known for medicinal uses in traditional medicine in Al-Baha region, KSA as anti-inflammatory, antimicrobial, antispasmodic, anti-ulcer, anti-stomach ache, laxative, hypolipidemic and antipyretic and other medical applications for many years ago. The antioxidant capacity could be one of the most important biological properties of plants and is likely to make them of high pharmacological value. Figure 2 clarifies the total antioxidant capacity (TWAC and TLAC) for the eight studied plant samples using water, ethanol and hexane as organic solvents. Aqueous extract samples showed superior antioxidant capacity for the eight studied plant samples except for Cac aqueous extract sample where the ethanolic extract sample was the superior.

TWAC of Rum plant sample extract was the highest followed by Oli and Fri plant extract samples while the TWAC of Dil plant sample was the lowest. Ani, Fig and Eur plant extract samples respectively showed mild TWAC in comparison with the TWAC of Rum and Oli plant extract samples. On the other hand, ethanolic extracts of Fri plant sample extract revealed the highest $TLAC^{E}$ followed by Oli and Rum plant sample extracts respectively while Dil plant sample extract showed the lowest $TLAC^{E}$. Hexanic extracts of Fri plant sample extract showed the highest $TLAC^{H}$ while $TLAC^{H}$ of Cac plant sample extract was the lowest.



	Fri	Dil	Ani	Oli	Fig	Rum	Cac	Eur
Ethanol	686,95	19,1	95,82	462,62	138,58	282,41	142,982	194,86
Hexane	127,83	17,76	21,95	6,69	8,53	38	4,37	25,92
Water	743,75	45,17	345,28	837,21	323,41	869,1	119,67	321,96

Figure (2): Total antioxidant capacity of eight plant species samples (Fri, Dil, Ani, Oli, Fig, Rum, Cac and Eur respectively) belong to different plant families using three different solvents (ethanol, hexane and water). Data represent means of three independent determinations ± their respective standard deviations. Concentrations are relative to weight of fresh tissues. Total water-soluble antioxidant capacity (TWAC^{95}) is expressed as the equivalent of L-ascorbic acid in micromoles of L-ascorbic acid per gram of fresh tissue. Total lipid-soluble antioxidant capacity (TLAC^{95}) is expressed as the equivalent of α-tocopherol in micromoles of α-tocopherol per gram of fresh tissue.

Strong Antioxidant Capacity (TWAC^{37} and TLAC$^{37E, H}$) of the eight herbals included in this study:

Due to strong water-soluble and lipid-soluble antioxidant agents of our 8 plant sample extracts like vitamins C and E, we measured and got (TWAC37 and TLAC37 E, H) as shown in figure (3). TWAC37 of Rum sample extract was the highest value followed by Fri and Oli sample extracts respectively, while the lowest TWAC37 values were for Dil and Cac sample extract respectively. The relatively mild TWAC37 values were for Ani, Fig and Eur sample extracts respectively.

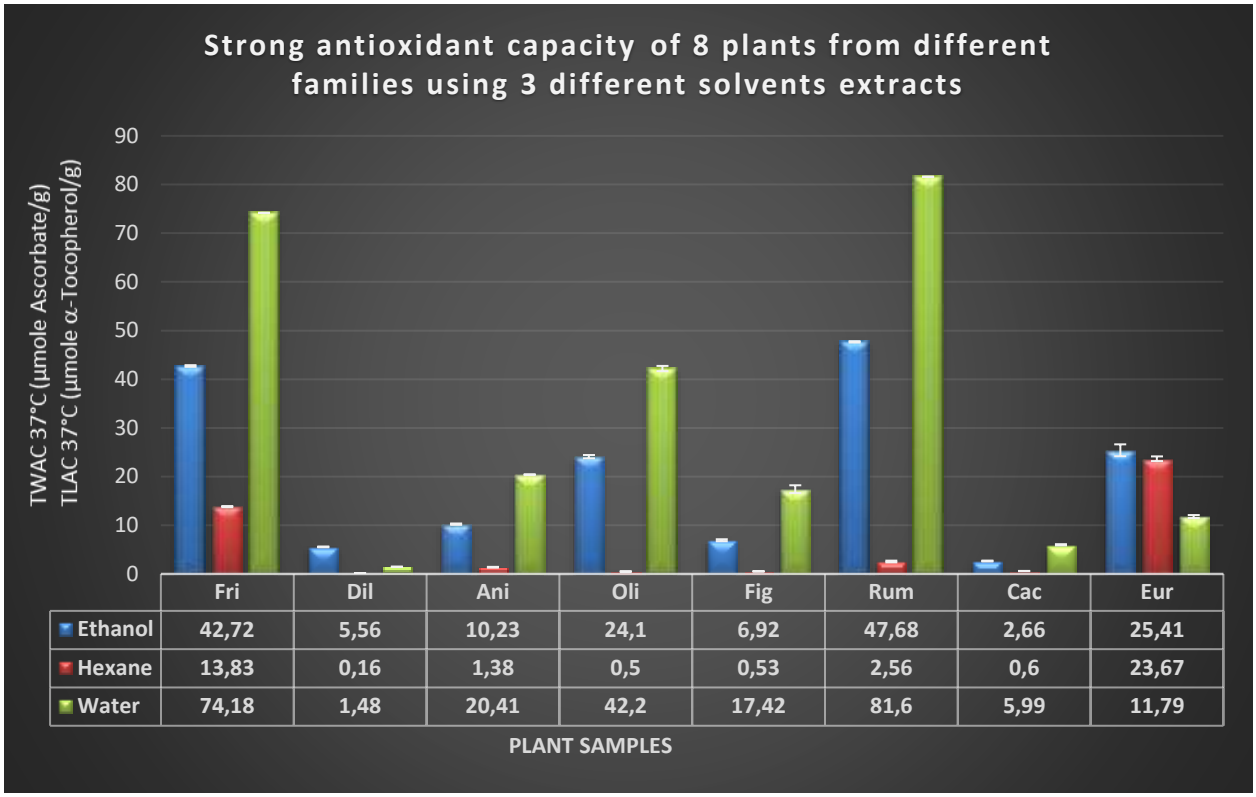


Figure (3): Strong antioxidant capacity of eight plant species samples (Fri, Dil, Ani, Oli, Fig, Rum, Cac and Eur respectively) belong to different plant families using three different solvents (ethanol, hexane and water). Data represent means of three independent determinations ± their respective standard deviations. Concentrations are relative to weight of fresh tissues. Total water-soluble antioxidant capacity ($TWAC^{37}$) is expressed as the equivalent of L-ascorbic acid in micromoles of L-ascorbic acid per gram of fresh tissue. Total lipid-soluble antioxidant capacity ($TLAC^{37}$) is expressed as the equivalent of α-tocopherol in micromoles of α-tocopherol per gram of fresh tissue.

Furthermore, figure (3) shows $TLAC^{37E}$ and $TLAC^{37H}$ of the Ethanolic and Hexanic extracts. Rum sample extract revealed the highest $TLAC^{37E}$ followed by Fri and Eur sample extracts respectively while the lowest $TLAC^{37E}$ values were for Cac and Dil sample extracts respectively. In the same time, $TLAC^{37H}$ of Eur sample extract values were the highest followed by Fri sample extract, the $TLAC^{37H}$ of the other 6 plant sample extracts were relatively low.

ISSR Profiling and Genetic Diversity Analysis of the studied eight herbals samples:

The current study attempts to indicate the genetic diversity among the eight studied herbs, which belong to different botanical families (table 1), with the exception of Dil and Ani, which belong to the family Apiaceae. Out of 6 ISSR primers tested as showed in Table 2, the six primers gave distinct polymorphic product. The results obtained with the ISSR primers are shown in Figure 4. The ISSR primers produced different polymorphic bands with a relatively high polymorphism level (**Vianna, *et al.*, 2019**). The highest number of polymorphic bands among the studied samples was obtained with the primer 844A, 844B and HB11 respectively, whereas, the lowest was scored for primer HB12.

The genetic distance values among different samples based on ISSR analysis are presented in figure 4. The highest value (0.25) was obtained between Oli and Fig samples followed by Dill and Ani (0.20) whereas, he lowest between Fri and Eur. The distance matrix based on ISSR data sets was used to construct a dendrogram, which is shown in Figure 4.

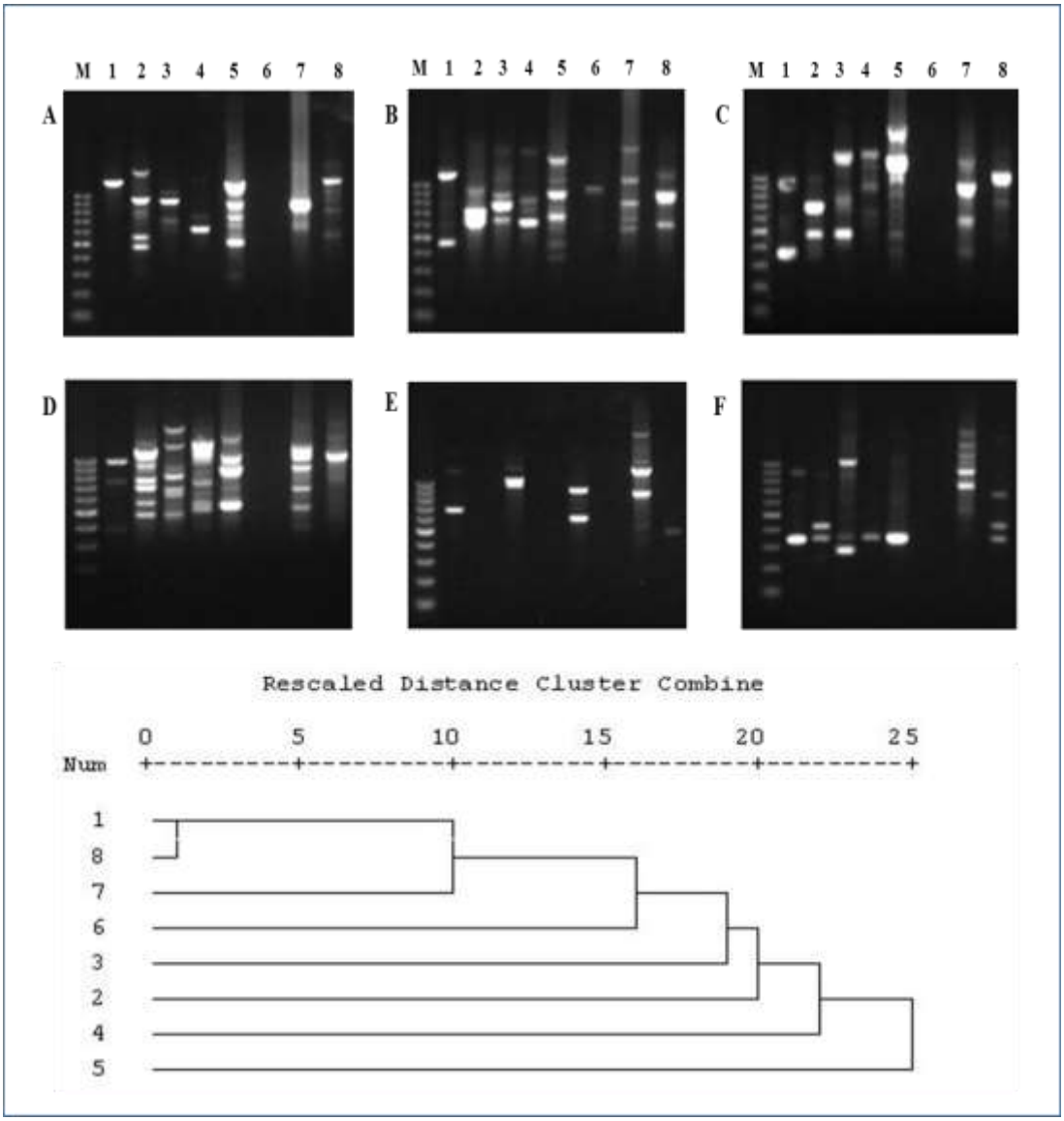


Figure (4): ISSR banding patterns obtained from 8 plant samples and its phylogeny dendogram. M refers to DNA standard (100-bp ladder, Bioron). Lanes 1, 2, 3, 4, 5, 6, 7 and 8 refers to plant samples (Fri, Dil, Ani, Oli, Fig, Rum, Cac and Eur respectively). A, B, C, D, E and F are the ISSR primers 814, 844A, 844B, HB11, HB12 and HB13 respectively.

Nowadays, the natural ingredients of wild herbs play a major role in the pharmaceutical, cosmetic and food preservation industries (**Parisa *et al.*, 2022**). from long ago until now, traditional healers have been used medicinal plants extracts to treat the bacterial, viral and other microbial diseases. A large body of scientific evidence is available demonstrating that natural ingredients have antioxidant, antimicrobial, anti-inflammatory, and anti-carcinogenic effects. The growing demand for herbal natural products requires further attention and particular focus to determinate their safety, effectiveness and their interactions with different drugs.

The flora of Al-Baha region as one of the richest biodiversity zones of Saudi Arabia, includes a large number of endemic plant species belong different plant families (**Ramu *et al.*, 2012; Vladimir-Knežević *et al.*, 2014; Sytar *et al.*, 2016; Alshehri *et al.*, 2019**).

*Ruta chalepensis (*Fri) belongs Rutaceae family is a famous plant species in Arabic countries with different pharmaceutical applications (**Boulos, 1983)**. Fri leaves ethanolic and aqueous extracts demonstrated high antioxidant capacity on both total and strong antioxidant capacity

levels. These results are in agreement with those of **Mohamed Barbouchi *et al.*,** (2020) and **Alexandra *et al.*,** (2020) on their study to provide a review about the different uses of *Ruta* species in folk medicine, as well as, on their multifactorial pharmacological and biological activities.

Anethum graveolens (Dil) and *Pimpinella anisum* (Ani) are from Apiaceae family consists of generally aromatic plants **(Mohamed *et al.*, 2007**; **Shah *et al.*, 2014)** in their study on the antioxidant capacity of some plant samples of family Apiaceae demonstrated that *Foeniculum vulgare* and *Cuminum cyminum* showed high antioxidant activity While, *Anethum graveolens* (Dil) and *Pimpinella anisum* (Ani) revealed minor antioxidant capacity between the studied Apiaceae family plant samples. Our results on Dil and Ani-antioxidant capacity are in agreement with these results.

Olea europaea subsp. *cuspidata* (Wall. & G.Don) Cif.) (Ole) belongs Oleaceae family. The main active constituents in olive leaves extracts act as anti-oxidant, anti-microbial and hypotensive activity (**Mossallam and Ba Zaid, 2000**). Our results on both total and strong antioxidant capacity of Ole leaves extracts revealed high antioxidant activity with aqueous and ethanolic extracts. These results are in agreement with **Mohamed *et al.*, (2007) and Yancheva *et al.*, (2016)** on their study of the olive leaves content of polyphenolics and vitamin E and other antioxidants agents.

Ficus carica L. (Fig) is one of famous plants belong Moraceae family. Fig plants are cultivated in Arabian Peninsula and north Africa for its edible and tasted good fruits (**Mossallam and Ba Zaid, 2000**). Fig leaves have been reported to have antidiabetic, stimulant, laxative and other therapeutic effects (**Ghazi *et al.*, 2012)**. Our study on aqueous and ethanolic extracts of Fig leaves revealed mild antioxidant capacity and these results are in agreement with **Raoufa *et al.*, (2021),** on their study on the therapeutic effects of Fig leaves extract as antioxidant and anticancer agent. **Ghazi *et al.*, (2012)** reported that, *Ficus carica* L. leaves contain strong antioxidant agents like ascorbic acid and vitamin E.

Rumex nervosus (Rum) belongs Polygonaceae family and locally known (Aathrab). It's widely spread in rocky lands in Asir and south western heights of Saudi Arabia. The edible leaves of Rum plant have many applications in traditional medicine in KSA. It is used to cure skin rashes, stomach ache also as anti-dysentery and effective treatment of warts **(Rahman *et al.*, 2004; Al-Asmari *et al.*, 2015)**. The present study demonstrated that, the antioxidant capacity of Rum leaf aqueous extracts was the highest between the eight studied plant samples in both total and strong antioxidant capacity levels. The ethanolic extract of Rum leaves revealed the highest strong antioxidant capacity. This likely reflects the richness of Rum leaf extracts in their content of various antioxidant agents. These results are in agreement with the investigation of **Tauchen *et al.*, 2015; AlMousa *et al.*, 2022** in their study on the antioxidant and anti-microbial activity of Rumex *nervosus*.

Opuntia ficus-indica L. Mill (Cactaceae) (Cac) is widely cultivated in arid and semi-arid areas of the world for its pleasant flavor fruit and with a high content of vitamins, minerals, dietary fiber and phytochemicals (**Lahsani *et al.*, 2003).** Cac. pear fruits are a rich source of nutrients due to their content of Vitamin C, amino acids and fibers (**Pretti *et al.*, 2014; Ammar *et al.*, 2015**). In comparation with the other plant samples of the present sudy, Cac fruit extracts revealed mild antioxidant activity. Aqueous extracts of Cac. fruit showed higher strong antioxidant activity than ethanolic and hexanic extracts. This result may explain what was mentioned in previous studies on the ascorbic acid content of Cac fruit **(Ghazi *et al.*, 2012; Rose and Norman, 2013)**.

Euryops arabicus (Asteraceae) (Eur), is grown in Arab Peninsula. Its leaves possess ethnomedicinal applications against several inflammatory conditions and wound healing **(Qashash, 2007; Ahmed *et al.*, 2020)**. In the present investigation, Eur. leaf hexanic extracts revealed the highest strong Fat-soluble antioxidant capacity in comparation with the other samples extracts included in this

study. These results may be demonstrating the Eur leaf richness of Fat-soluble antioxidant which is in agreement with the study of Hafez***, et al.,* (2015).**

Saudi Arabia contains a treasure of enormous numbers of wild and cultivated medicinal plants and comprises very important genetic resources biodiversity (**Rahman *et al.,* 2004**). Different types of DNA-based molecular approaches such as hybridization, polymerase chain reaction (PCR), and other techniques provide more objective and reliable methods to authenticate herbal medicines (**Joshi *et al.,* 2004; Shcher and Carles, 2008**). Among PCR-based methods, inter-simple sequence repeats (ISSRs) have been found to be an effective and reliable method established by **Zietkiewicz *et al.* (1994)** for the identification of species or varieties, population authentication and population genetic structure, etc. (**Shen *et al.*, 2006; Liu *et al.*, 2009; Ratnaparkhe *et al.*, 1998; Reddy *et al*., 2002)** showed that ISSR markers could be used as highly informative markers for genomic mapping and gene tagging because the evolutionary rate of change within microsatellites is considerably higher more than many other types of DNA markers. The inter-simple sequence repeats ISSr widely used in plants as a molecular marker and provide information about genomes analysis of of plant species **(Vijayan, 2005; Ratnaparkhe, *et al*., 1998).** The obtained results in our study using inter-simple sequence repeats (ISSRs) molecular markers indicated that wide genetic diversity and high genetic differentiation was found within studied plants in accordance with its differences in geographical areas **(Zietkiewicz *et al*., 1994).** The genetic diversity concluded that different polymorphic bands with a relatively high polymorphism level in studies plants this results in-agreement with **(Reddy *et al*., 2002; Charters and Wilkinson 2000)**. It is better to collection of large number of plants to understand the genetic diversity from wide range of geographical areas.

Conclusions:

The Middle East countries and Arabian Peninsula are recognized as a poor biodiversity region dominated by deserts. However, the Kingdom of Saudi Arabia has a wide range of flora containing a huge number of edible and medicinal plants thanks for its vast area of climates diverse and geographical landscapes. The traditional use of ethnomedicinal plants in KSA represents a strong interconnection among health, diet, familiar remedies, and traditional healing practices characterized by specific cultures. Plant natural extracts, essential oils and different natural sources are extremely demanded for various and valuable applications. Their antioxidant activity results from bioactive ingredients present in these natural sources. Antioxidant activity remains one of the most important biologically active properties that can be present in a plant species, as a result of its content of antioxidant agents. In comparing with the 8 studied samples, *Ruta chalepensis* and *Rumex nervosus* leaves show superior antioxidant capacity and deserve further investigations to be more useful in various medicinal applications.

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