Identification of non-polar compounds of aerial organs of *Malva sylvestris L.* and its antioxidant effects

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Abstract: Herbal medicines are an important part of the culture and traditions of African people. Antioxidants are substances that may protect your cells against the effects of free radicals. The aim of present study was to identification of non-polar compounds of aerial organs of Malva sylvestris L. and its antioxidative effects. Present research was carried out during 2012 in the laboratory of Islamic Azad University, Ahar branch. In order to extraction, dried grounded powder from total aerial parts of the plants and were Soxhlet-extracted with 250 ml of Petroleum benzine. All of the extracts were filtered (through 0.45 mm filters under sterile conditions), dried using a rotary evaporator at 35 °C to achieve the stock concentration 2 mg/ml. The GC-MS analysis was performed on an Agilent 7890A GC system equipped with 5975C VL MSD. A HP-5 MS capillary column (0.25 mm i.d., 30 m length, and 0.25 µm film thicknesses) was used. The antioxidant activity was discovered by running a TLC plate with the non-polar extract, with concentration 50 mg. The non-polar extract of aerial parts of Malva sylvestris L. was analyzed by GC-MS. The results were identification of 10 compounds which were comprised 99.78% of total non-polar extract. The most part of the non-polar extract was a compound with the name Bis (2-ethylhexyl) phthalate (86.03%). Also, it has been shown that this plant has an antioxidant activity. In conclusion it can be said that 86.03% of detected compounds in the non-polar fraction was due to Bis(2-ethylhexyl) phthalate. Because this element has much application in many industries, so, it can be introduced as a good replacement. On the other hands, this plant has antioxidant activity as shown.

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Introduction:

Herbal medicines are an important part of the culture and traditions of African people. Today, most of the population in urban South Africa, as well as smaller rural communities, is reliant on herbal medicines for their health care needs. Apart from their cultural significance, this is because herbal medicines are generally more accessible and affordable (Mander, 1998). As a consequence, there is an increasing trend, worldwide, to integrate traditional medicine with primary health care.

Renewed interest in traditional pharmacopoeias has meant that researchers are concerned not only with determining the scientific rationale for the plant's usage, but also with the discovery of novel compounds of pharmaceutical value. Instead of relying on trial and error, as in random screening procedures, traditional knowledge helps scientists to target plants that may be medicinally useful (Cox and Balick, 1994). Already an estimated 122 drugs from 94 plant species have been discovered through ethnobotanical leads (Fabricant and Farnsworth, 2001). Various assays can be used to test for biological activity, firstly in vitro and later, for promising natural products, in vivo. Crude or fractionated extracts and sometimes individual compounds reported here were screened for antibacterial, anti-inflammatory, antioxidant, anthelmintic, anti-amoebic, antischistosomal and antimalarial activity, as well as psychotropic and neurotropic properties.

Malva sylvestris L. (Malvaceae), usually known as common mallow, is native to Europe, North Africa and Asia, and its traditional use has been documented since a long-time ago, although little clinical evidence is available. The Greeks and Romans claimed for its emollient and laxative properties and several ethnobotanical surveys conducted in Europe, highlight the potential of such neglected local resource, which is use today at the brink of disappearance. Roots, shoots, leaves, flowers, fruits, and seeds are applied in infusions, decoctions, poultices, liniments, lotions, baths, and gargles (Barros et al., 2010). Valuable gains have been documented on the bactericidal, bacteriostatic, antifungal, immune-modulatory and antioxidative properties of M. sylvestris extracts (Grierson and Afolayan, 1999).

Antioxidants are substances that may protect your cells against the effects of free radicals. Free radicals are molecules produced when your body breaks down food, or by environmental exposures like tobacco smoke and radiation. Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases (Sies, 1997). The aim of present study was to identification of non-polar compounds of aerial organs of Malva sylvestris L. and its antioxidative effects.

Materials and methods:

Present research was carried out during 2012 in the laboratory of Islamic Azad University, Ahar branch. All chemicals used in this study were prepared from Merk Company. The plant was gathered from the gardens of Ahar suburb during summer. Then, plants were dried in out of sunlight and room temperature.

Extraction of non-polar extract

Dried grounded powder from total aerial parts of the plants and were Soxhlet-extracted sequentially with 250 ml of Petroleum benzine. All of the extracts were filtered (through 0.45 mm filters under sterile conditions), dried using a rotary evaporator at 35 °C to achieve the stock concentration 2 mg/ml (Barros et al., 2010).

Preparation of non-polar extract in order to inject into GC/MS apparatus

Dechlorophyllation:

In order to remove the pigments, we used of thin layer chromatography. So, 40g silica gel GF_{254}, 50mg extract and 50 ml a mixture of chloroform and ethyl acetate solvents (ratio 70:30) and methanol were applied.

Esterification:

The dechlorophyllated contents were solved in 5ml n-hexane and were added into the centrifuge tube 20 ml. 0.2 ml potassium hydroxide (2M) was added then was put into Vortex Shaker for 30 seconds. Finally, 2 ml saturated sodium chloride was added in order to separate the organic phase. Organic phase was obtained and a little amount of sodium sulfate added.

Identification and determination of non-polar extract constituents:

The GC-MS analysis was performed on an Agilent 7890A GC system equipped with 5975C VL MSD. A HP-5 MS capillary column (0.25 mm i.d., 30 m length, and 0.25 µm film thicknesses) was used. Sample was injected in split mode (50:1). The injection volume was 1 µl and the injector temperature was 270°C. The carrier gas (He) flow rate was 1 ml/min (constant pressure mode). The column temperature was 50°C (3 min) then programmed linearly 50-270°C (10°C/min) with a final 5-min hold. Mass spectrometer was an electron impact (EI) type (70 eV) and the ion source temperature was set at 230°C.

Antioxidant TLC Assay

The antioxidant activity was discovered by running a TLC plate with the non-polar extract, with concentration 50 mg. The plates were then dried, sprayed with a DPPH solution (2 mg/mL in methanol), and left for an hour.

Results:

Proximate composition analysis

The non-polar extract of aerial parts of Malva sylvestris L. was analyzed by GC-MS. The results were identification of 10 compounds which were comprised 99.78% of total non-polar extract. A list of constituents of plant is given in table 1. The most part of the non-polar extract was a compound with the name Bis (2-ethylhexyl) phthalate (86.03%).

Table 1: Proximate compositions of non-polar extract of Malva sylvestis L

#	Compound	RT(min)	%
1	Bis(2-ethylhexyl) phthalate	25/350	86/028
2	Naphthalene, decahydro-1,5-dimethy	11/030	5/868
3	2(1H)-Naphthalenone, octahydro-4a-methyl-, trans-	10/928	1/950
4	Naphthalene, decahydro-2,3-dimethy	10/656	1/311
5	Naphthalene, decahydro-2,6-dimethy	10/530	1/267
6	trans,trans-1,10-Dimethylspiro[4.5] decane	10/440	0/837
7	Dodecane	10/488	0/810
8	Margaric acid methyl ester	19/841	0/663
9	Eicosane	13/737	0/543
10	2,6-Dimethylbicyclo[3.2.1]octane	10/371	0/502

Antioxidant activity

The antioxidant properties of natural products are investigateblt by using 2,2-diphenyl-picrylhydrazyl (DPPH) radical. This compound is a stable radical, and in the presence of radical scavengers (antioxidants) it is converted from a purple to a yellow color. This contrasting difference in color is distinct and enables recognition of antioxidant substances. In our investigation we could see antioxidant compounds appeared as yellow spots against a purple background.

Discussion:

Plants that are eaten as foods were shown to also provide important health benefits in the form of

antioxidant activity (Lindsey et al., 2002). Plants were evaluated for antioxidant activity by testing for inhibition of lipid peroxidation. This in vitro assay is well recognized and allows for the screening of a large number of plant extracts at a time (Lindsey et al., 2002). Extracts with inhibition values greater than 70%, and thus with high antioxidant activity, were found in the potherbs Amaranthus sp. (Amaranthaceae), Sisymbrium thellungii O.E. Schulz (Brassicaceae) and Urtica dioica L. (Urticaceae). Traditionally, these plants are used in the preparation of ‘imfino’ which forms an important part of the diet. High activity (greater than 70%) was also shown by the tuberous Colocasia esculanta Schott (Araceae) and the teas Galium aparine L. (Rubiaceae) and Aspalathus linearis (Burm.f.) R. Dahlgren (Fabaceae) (rooibos) and Malva sylvestris. Plants commonly used in traditional medicine are assumed to be safe. This safety is based on their long usage in the treatment of diseases according to knowledge accumulated over centuries. However, recent scientific research has shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic (Schimmer et al., 1988, 1994; Higashimoto et al., 1993; Kassie et al., 1996; De Sã Ferrira and Ferrão Vargas, 1999).

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling.

In one study by Barros et al., 2012 it has been shown that Kaempferol-3-O-rutinoside (0.84 mg/gdw) and quercetin-3-O-rutinoside (14.9 mg/gdw) were the main flavonols present in M. sylvestris and S. nigra, respectively. Due to the well-established antioxidant activity of phenolic compounds, the studied wild medicinal flowers could be selected for processing extracts with health-promoting properties or to be incorporate into functional beverages or products with bioactive properties related to oxidative stress.

A complete review involving the ethnobotanical and scientific aspects of M. sylvestris has been made by Gasparetto et al., 2012. Their research has provided evidence that M. sylvestris has potential use as a medicinal plant and has highlighted a need for more studies involving clinical and toxicological aspects of its use.

Marouane et al., 2011 showed that of 80 rats exposed to ammonium metavanadate (0.24 mmol/kg body weight in drinking water) for 90 days, lipid peroxidation levels and superoxide dismutase, catalase and glutathione peroxidase activities were measured in kidney. A significant increase in the formation of free radicals and antioxidant enzyme activities was noticed. In addition, a histological examination of kidney revealed a structural deterioration of the renal cortical capsules and a shrinking of the Bowman space. In animals intoxicated by metavanadate but also given a Malva sylvestris decoction (0.2 g dry mallow/kg body weight), no such pathologic features were observed: lipid peroxidation levels, antioxidant enzyme activities and histological features appeared normal as compared to control rats.

Analysis of the extract, carried out by different chromatographic techniques, led to the isolation of eleven compounds: 4-hydroxybenzoic acid, 4-methoxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 4-hydroxycinnamic acid, ferulic acid, methyl 2-hydroxydihydrocinnamate, scopoletin, N-trans-feruloyl tyramine, a sesquiterpene, (3R,7E)-3-hydroxy-5,7-megastigmadien-9-one, and (10E,15Z)-9,12,13-trihydroxyoctadeca-10,15-dienoic acid. The antioxidant activities of all these compounds are reported (DellaGreca et al., 2009).

Redzić et al., 2005 showed that species Malva moschata L. grows on ecologically clear soils as opposed to well-known medicinal species Malva sylvestris L., and considering the production of phytomass, phytochemical structure and pharmacological influence it can be considered very medical and be given advantage over this wider spread category.

In conclusion it can be said that 86.03% of detected compounds in the non-polar fraction was due to Bis(2-ethylhexyl) phthalate. Because this element has much application in many industries, so, it can be introduced as a good replacement. On the other hands, this plant has antioxidant activity as shown.

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