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Antioxidant activity, total phenolic content and antimicrobial activity of two medicinal plants from Sulaimani City, Iraqi Kurdistan Region

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Abstract
Malabar spinach fruit with dark blue skin and deep red violet flesh is a potential source of natural colorant. Nasturtium officinal (watercress) of family Brassica ceae has been long used as a home remedy or a medical plant by the people Iraq- Sulaimaini City .The aim of this study was to investigate the antioxidant , antimicrobial activity of Malabar spinach &Nasturtium official (water cress) extracts using various in vitro assay systems including ferric reducing antioxidant power (FRAP),1,1-dipheny-2-picrylhydrazyl (DPPH), and 2,2-azinobis -3-ethylbenzothiozoline sulphonate (ABTS).Antibacterial activity were tested against five gram-positive ,three gram –negative bacteria and two fungi by disc diffusion method for different extract and determining their minimum inhibitory concentration (MIC)values .
Total phenolic content was determined by folin ciocalteu reagent for the extract (mg Gallic acid /g extract). From the result highest total phenol content was shown by Malabar spinach & Nastartium officinale in methanol extracts. Elemental analysis have been done element by inductive couple plasma (ICP) techniques for each this two medicinal plants high amount of Na ,K Ca, Mn ,Zn ,Mg, Fe, Cu ,and se elements have been detected in ppb.
Key words: Malabar spinach , Nasturtium officinal, antioxidant , antimicrobial ,phenolics

Introduction

Malabar spinach fig(2) it is also Knowing as Basella alba is a fast-growing ,soft stemmed vine ,reaching 10 meter (33ft) in length .Its thick ,Semi-Succulent ,heart-Shaped leaves have a mild flavor and mucilaginous texture. The stem of the cultivar basella alba ((Rubra)) is reddish-purple (**Jump,2012**).The fruit of Malabar Spinach is fleshy ,stalk less, avoid or spherical 5- 6mmlong and purple when mature it is also a good source of vitamins and minerals(**Elina,2007**) it is it is belongs to the Basell cease family , commonly found in the home gardens of many south Asian families **(Ali,1964).** Nasturtium officincle (watercress) fig(1) of the family Brassica ceae **(Ali,1964)** is a fast-growing, aquatic or semi-aquatic, perennial plant native to Europe and Asia, and one of the oldest knownleaf vegetables consumed by humans. It is a member of the family Brassicaceae, botanically related to garden cress, mustard and radish all noteworthy for a peppery, tangy flavour. The hollow stems of watercress are floating, and the leaves are pinnately compound. Small, white and green flowers are produced in clusters. If unharvested, watercress can grow to a height of 50–120cm (1.6–3.9 ft). Like many plants in this family, the foliage of watercress becomes bitter when the plants begin producing flowers.
Alocal name of Malabarspinach &Nasturtium officinale (watercress) in Kurdish are(spenakh), (kuzala). The free radicals produced the cell are responsible for oxidative stress, which is thought to contribute to the development of a wide range of disease including Alzheimers diseases, Parkinson's disease, pathologies caused by diabetes rheumatoid arthritis and neurodegradationin motor neuron disease. A class of molecules called antioxidants inhibits the formation of these free radicals by removing their intermediates**(Radimerk,2004),** while there are many foods contribute the overall health and gloving wellness, foods that contain antioxidants have a special ability to actually protect the body from the formation of free radicals. free radicals are the toxic off-shoots of many of the metabolic processes that go on the body on an every-day basis, studies show that antioxidant rich food can actually help to increase our life-span, as well as slow the signs of aging**(Halli,2007)** Natsturtium officinal(watercress)is used to cure abdominal pain and is eaten as a vegetable) and in salads in Iraq-Kurdistan region-Sulaimani City .This herbis used to treat diabetes, bronchitis and dieresis **(Miraldi,2001)**as anti-ulcerogenic **(lkofahi,1999),** in treatment of scurvy, tuberculosis ,influenza, asthma **(Duke,1992)** nutritional supplement and digestive aid**(Sezik,2001)**

The frequency of life -threatening infections caused by pathogenic microorganisms has increased world wide and is becoming an important cause of morbidity and mortality in immune compromised patients developed countries **(Al Bari,2006)** , There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross infection **(French L,2005))** in developing countries the world health

organization **(WHO,2002)** estimate that about there quarter of the population relies on plant based preparation used in their traditional medical system and as a basic need for human primary health care . Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of aliment of microbial origin **(Kameshwara,2000)** .

Fig(1) Nasturtium officinal plant

Fig(2)Garden Malabar spinach fruit

Experimental procedure

1-Sample collection and drying

Malabar spinach fruit from the house garden and Nasturtium officinal plant were collected in Sulaimani city in September 2012,these plants dehydrated by air-drying at ambient temperature of 23-25 ^{0}C in the dark in order to avoid the degradation of pigments and polyphenolic compounds. The plants were powdered to uniform particle size.

2-Preparation of the Extracts.

The dried and powdered aerial parts of the plant were extracted with methanol for 48 hours. The methanol extract was dissolved in water and fractionated with chloroform and ethyl acetate, respectively. The extracts were filtered using Whatman filter paper (no. 1) and then concentrated in vacuum at 40°C using a rotary evaporator. The residues obtained were stored in a freezer until further tests.

3-Determination of total phenols

The total phenolics content of the plant extract was determined according to the Folin and Ciocalteu procedure (Slinkard & Single-ton, 1977) and results were expressed as Gallic acid equivalents (mg Gallic acid equivalents/g dried extract).

4-Measurement of free radical-scavenging activity (DPPH^{o} assay)

Radical scavenging capacity was determined according to the technique reported by Blois (1958).An aliquot of 1.5 ml of 0.25 mM DPPH solution in ethanol and 1.5 ml of extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm with a Varian spectrophotometer. The DPPH radicals scavenging activity was calculated according to the following equation:

$$\text{Scavenging activity} = [(A0 - A1/A0) \times 100],$$

Where A0 is the absorbance of the control (blank, without extract) and A1 is the absorbance in the presence of the extract or standard sample.**5-**

5-Measurement of ferric reducing antioxidant power (FRAPassay) The reducing power was determined by using FRAP assay described by Benzie and Strains (1996) with some modifications. Briefly, 0.2 ml of sample compounds were mixed with 1.8 ml of the freshly prepared FRAP reagent, which consisted of 2.5 ml of 10 mM TPTZ solution in 40 mM HCl plus 2.5 ml of 20 mM FeCl3 and 25 ml of 0.3 M acetate buffer (pH 3.6). The absorption of the reaction mixture was measured at 595 nm. Ethanolic solutions of several Fe (II) concentrations were used to obtain the calibration curve. The FRAP values have been calculated by comparing the absorbance change at 593 nm in test samples with those containing ferrous ions in known concentration.

6-Measurement of Trolox equivalent antioxidant capacity (TEAassay)

TAEC assay is based on scavenging of ABTS^{o}+ (Madhujith et al., 2006).A solution of ABTS^{o}+ (7 mM) was prepared in 100 mM PBS (pH 7.4) and oxidized using potassium per sulfate (2.45 mM) for about 10 h in dark. The ABTS^{o}+ solution was diluted to an absorbance of 0.7 ± 0.05 at734 nm with PBS. For measuring antioxidant

capacity, 10 µL of the sample was mixed with 990 µL of $ABTS^{\circ+}$ solution. Absorbance of this mixture was measured at 734 nm after 6 min. The decrease in absorption was used for calculating TEAC values. A standard curve was prepared by measuring the reduction in absorbance of $ABTS^{\circ+}$ solution at different concentrations of Trolox. Appropriate blank measurements were carried out and the values recorded. TEAC values represent the Trolox equivalents of plants extracts.

7-Antimicrobial Activity:

The extracts were tested individually against a range of 11 microorganisms, including 9 bacteria and 2 fungi species.

a-Disk Diffusion Assay:

The antimicrobial activity of essential oil and extracts was determined by the disk diffusion method (NCCLS 1997). Briefly, 0.1 ml of a suspension of the test microorganism (10^8 cells ml^{-1}) was spread on Mueller–Hinton Agar plates for bacteria and Sabouraud Dextrose Agar for the fungi. Sterile 6 mm disks, each containing 10µl of samples were placed on the microbial lawns. The plates were incubated at 37 °C for 24 h for the bacteria and at 30 °C for 48 h for the fungi. The diameters of the zones of inhibition were measured and are reported in mm. Triplicate tests were performed in all experiments.

b-Determination of minimum inhibitory concentration(MIC):

The *MIC* values were determined by the broth micro dilution assay (NCCLS 1997). Serial two- fold dilutions of the samples were made in Mueller–Hinton Broth containing 0.5 % Tween 80 for the bacteria and Sabouraud Dextrose Broth with 0.5 % Tween 80 for the fungi in 96-well micro titer plates. Fresh microbial suspensions prepared from overnight-grown cultures in the same media were added to give a final concentration of 5×10^5 organisms ml^{-1}. Controls of medium with microorganisms or the samples alone were included. The micro plates were incubated at 37°C for 24 h for the bacteria and 30 °C for 48 h for the fungi. The first dilution with no microbial growth was recorded as the *MIC*.

8- Determination of elements

10gm of samples were weighted in to a porcelain crucibles, placed in a muffle furnace temperature was increased to 600 ^{0}C and held at the temperature for 6h., the samples were removed from the furnace and weighted, the determine percentage of ash for samples were (0.2gm), and (0.21gm)% respectively. The residual ash was dissolved in 1:1 nitric acid filtered and the volume was completed to (100ml) distilled water, the solution was injected to OES-ICP Perkin Elmer 2100 for determined

Results and discussion

1-**total phenolic content**

The stander Gallic acid was prepared and phenolic content of examined extracts was calculated based on this slandered and presented as Gallic acid equivalent(GAE)per gram of dry sample. Based on the results obtained in this experiment(Table 1),(Table 2)among all extract, methanol extract of each Malabar spinach fruit and Nasturtium officinal (watercress) showed the highest amount of phenolic compounds ,the evaluated phenolic content of methanol extract of Malabar spinach fruit (101.3mg/g)was higher than other extracts and the phenolic content of methanol extract of Nasturtium officinal (watercress)was(121.2mg/g) was higher than other extracts .Chloroform extract of each plant showed the lowest amount of phenolic compounds.Phenolic compounds exhibit a wide range of physiological properties, such as anti- inflammatory,anti-allergenic,anti-microbil,antioxidant,anti-trombotic,cardioprotective and vasodilator effects(19)

2-IC_{50} value in DPPH

The result of IC_{50} value of two plants in different extracts has been presented in Table -1 ,2 figure-3,4-.Among all the extracts a chloroform extract showed the IC_{50} value is significantly higher than other extracts which means the inhibition rate is lowest. It means that chloroform extract of each plants showed the high antioxidant activity against DPPH scavenging assay, reducing power assay.

3- ferric reducing antioxidant power (FRAPassay)

The FRAP assay measures the ability of antioxidant to reduce the 2,4,6-tripyridyl-S-triazine complex[Fe(III)-µmol /g $TpTz^2]^{2+}$ to intensely blue colored ferrous complex[Fe(II)-$TpTz^2]^{2+}$in acidic medium. FRAP values are calculated by measuring the absorbance increase of 593nm and relating it to a ferrous ion standard .The reduction capacity of plants extract may serve as a significant indicator of it potential antioxidant activity. The methanol extract exhibited higher antioxidant capacity (Table1),(Table 2) for Malabar spinach fruit and Nasturtium officinal{ 2460.3 µmol /g,1823.3 µmol /g} respectively

4-ABTS assay

ABTS is a method based on reduction of 2,2$^-$ - azinobis (3-ethylbenzothiozoline sulphonate)radical. Although ABTS have been widely used to measure the antioxidant capacity of natural extract based on their ability to

reduce radical cation ,the reaction of ABTS with free radical scavenges present in the test sample occur rapidly and can be accessed by following the decrease in the sample absorbance at 734nm.The highly significant correlation were observed for total antioxidant capacity in methanol extract(Table1),(Table2)for two plants was 723.1 µmol/g and 649.3 µmol/g and phenolic contens.

5- Antimicrobial Activity assay

The result of antimicrobial activity of methanol,water,ethylacetate,chloroformand BHT extracts of both plants were presented in table-3-showed that methanol extracts higher antimicrobial activity against all bacteria and fungi and chloroform extracts show no inhibition against most of microorganism

6-elemental analysis

The result of elemental analysis of Nasturtium officinal plant in ppb in table-5- showed that contained higher amount of calcium, one of calcium benefits is that it is essential for the proper growth and development of the human skeletal system. Therefore, right from babies to teenagers, all are advised to have calcium for proper growth. Uses of calcium also include its role in neuro-transmission. Calcium in the body helps in the release of neurotransmitters. Similarly, calcium is also required for maintenance of healthy teeth and gums. Calcium deficiency can lead to disorders of the teeth. The result of elemental analysis of garden Malabar spinach fruit in ppb in table-6- showed that contained higher amount of Se that play a role in the functioning of the thyroid gland and in every cell that uses thyroid hormone, by participating as a cofactor for the three of the four known types of thyroid hormone deiodinases, which activate and then deactivate various thyroid hormones and their metabolites: the iodothyronine deiodinases are the subfamily of deiodinase enzymes that use selenium as the otherwise rare amino acid selenocysteine. (Only the deiodinase iodotyrosine deiodinase,which works on the last break-down products of thyroid hormone, does not use selenium). Selenium may inhibit Hashimoto's disease, in which the body's own thyroid cells are attacked as alien. A reduction of 21% on TPO antibodies was reported with the dietary intake of 0.2 mg of selenium. Increased dietary selenium intakes reduce the effects of mercury toxicity[and it is now recognized that the molecular mechanism of mercury toxicity involves irreversible inhibition of selenoenzymes that are required to prevent and reverse oxidative damage in brain and endocrine tissues.(20)

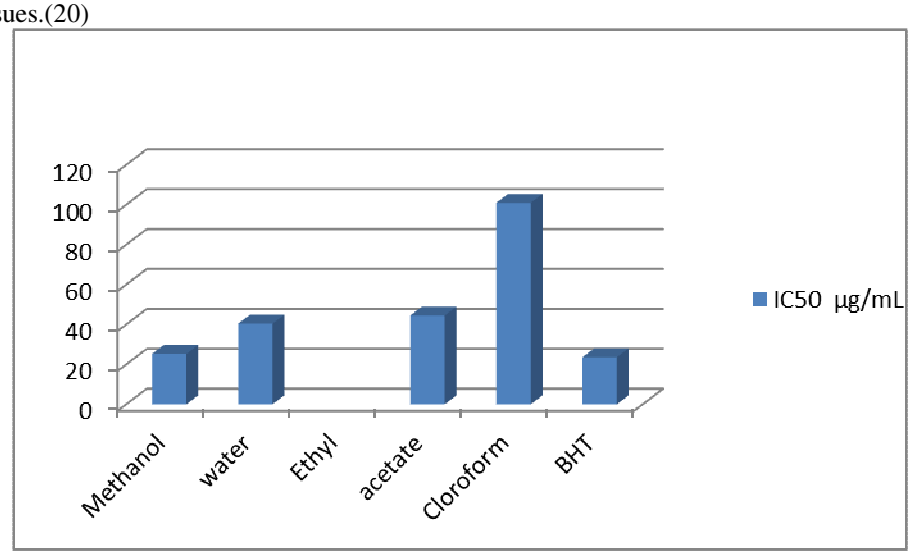

Figure-3-
Antioxidant activity of methanol,water,ethylacetate,chloroformand BHT extracts of gardene Malabar spinach fruit using DPPH free radical scavenging assay

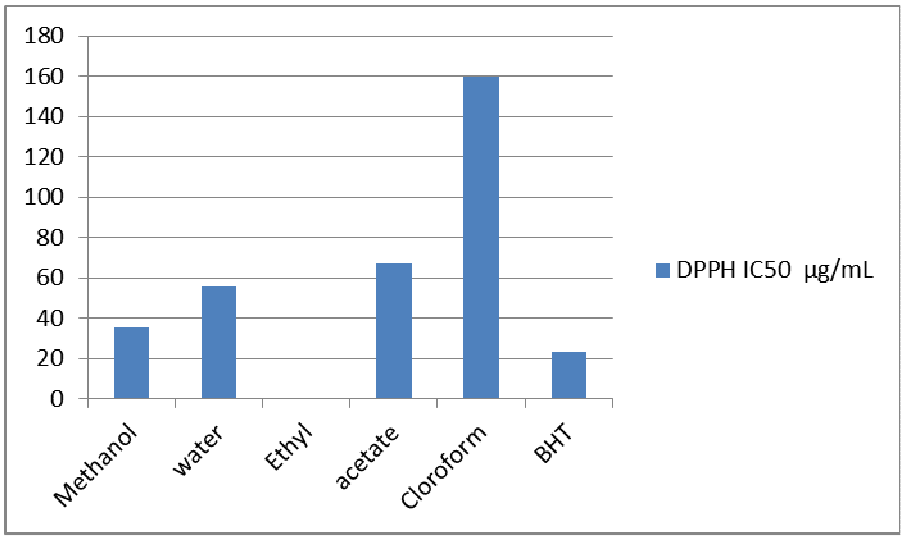

Figure-4-
Antioxidant activity of methanol,water,ethylacetate,chloroformand BHT extracts of Nasturtium officinale (watercress) using DPPH free radical scavenging assay

Table(1)

Total phenol and antioxidant activity of Garden *Malabar spinach fruit in sulaimani city*

Extracts	DPPH	FRAP	ABTS	Total phenol
	IC_{50} µg/mL	µmol Fe^{2+}/g extract	µmol Torolox /g extract	mg Gallic acid/g extract
Methanol	25.3±0.1	2460.3±3.4	723.1±2.3	101.3±1.9
water	40.4±0.3	1864.7±2.2	654.3±2.6	71.8±1.9
Ethyl acetate	44.5±0.5	1143.3±3.3	521.6±2.2	73.1±0.7
Chloroform	101.4±0.4	943.3±3.3	498.1±1.4	19.4±0.4
BHT	23.6±0.3	-	-	-

Table(2)

Total phenol and antioxidant activity of *Nastartium officinale in sulaimani city*

Extracts	DPPH	FRAP	ABTS	Total phenol
	IC_{50} µg/mL	µmol Fe^{2+}/g extract	µmol Torolox /g extract	mg Gallic acid/g extract
Methanol	35.2±0.2	1823.3±3.1	646.3±2.8	121.4±2.6
water	55.5±0.3	1534.5±3.2	604.1±2.1	99.2±1.8
Ethyl acetate	67.3±0.3	1245.2±2.5	518.4±2.3	83.3±o.8
Chloroform	160.4±0.6	544.5±1.1	509.6±1.3	34.5±0.8
BHT	23.6±0.3	-	-	-

Table (3)
Antimicrobial activity of Garden *Malabar spinach fruit* extracts

	Microorganism										
Extracts	B.PU	E.coli	S.au	B.Ce	KLb	E.NT	B.sub	St.Ep	PS	Can	Sac
Methanol	18[a] (7.5)[b]	18 (7.5)	20 (7.5)	18 (7.5)	12 (15)	11 (15)	12 (15)	11 (15)	11 (15)	11 (15)	12 (10)
water	14 (15)	20 (7.5)	14 (15)	12 (15)	12 (15)	11 (15)	0	11 (15)	0	12 (10)	11 (>10)
Ethyl acetate	14 (15)	12 (15)	14 (15)	12 (15)	11 (15)	12 (15)	0	-	0	12 (10)	10 (10)
Chloroform	-	12 (15)	0	11 (15)	-	0	-	0	0	-	-

a Diameter of inhibition zones (mm) including diameter of sterile disk (6 mm). **b**.Minimum Inhibitory Concentration, values as mg/ml
c -, not active; (7–14) moderately active; (>14) highly active.

Table(4)
Antimicrobial activity of *Nastartium officinale* extracts in sulamani city

	Microorganism										
Extracts	B.PU	E.coli	S.au	B.Ce	KLb	E.NT	B.sub	St.Ep	PS	Can	Sac
Methanol	15[a] (15)[b]	18 (7.5)	15 (15)	18 (7.5)	14 (15)	12 (10)	14 (15)	12 (15)	12 (10)	10 (10)	11 (10)
water	14 (15)	14 (15)	12 (>10)	12 (15)	14 (15)	14 (15)	11 (15)	12 (15)	-	10 (10)	11 (>10)
Ethyl acetate	14 (15)	12 (15)	14 (15)	11 (15)	11 (>10)	12 (10)	14 (15)	12 (10)	11 (>10)	0	10 (10)
Chloroform	11 (15)	-	0	11 (15)	11 (>10)	-	11 (>10)	-	-	0	0

a Diameter of inhibition zones (mm) including diameter of sterile disk (6 mm). b Minimum Inhibitory Concentration, values as mg/ml.
c -, not active; (7–14) moderately active; (>14) highly active.

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Table(5) Elemental analysis of Nasturtium officinal plant in ppb

elements	concentration
Cr	0.001
K	107.7
Na	5.451
Fe	0.219
Mn	1.835
Co	Nil.
Ca	127.3
Mg	55.23
Se	175.8

Table(6) Elemental analysis of Garden Malabar spinach Fruit in ppb

Elements	concentration
Cr	Nil.
Cu	18.14
Fe	11.7
Mn	3.532
Pb	Nil.
Zn	58.19
Se	149.4
Co	Nil.
Ni	3.088

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