

Anti-Candida and antioxidant activities of hydroalcoholic extract of *Rumex obtusifolius* leaves

Farahnaz Bineshian¹, Neda Bakhshandeh²,
Mohammad reza Freidounian³ and Hossein Nazari^{2*}

¹Department of Mycology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran

²Department of Biochemistry, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran

³Semnan Technical College, Semnan, Iran

Abstract: Due to increasing the use of antifungal drugs, the development of resistance in some *Candida* species and the consumption of the side effects of chemical drugs, use of new resources, especially medicinal plants are very important. The aim of this study was to investigate of anti-Candida and antioxidant activities of hydroalcoholic extract from leaves of *Rumex obtusifolius*. The *Rumex obtusifolius* Leaves were extracted using Ethyl acetate; methanol and distilled water (6:3:1) by Soxhlet system. The hydroalcoholic extraction of *Rumex obtusifolius* was evaluated for their antioxidant capacities using in vitro methods; including 1-diphenyl-2-picrylhydrazyl radical scavenging, β -Carotene bleaching test and reducing power assay. Total free phenolics, total flavonoids content and as well as the antifungal activity were also examined. The components of extract were analyzed via GC-Mass instrument. The extract was screened against 40 isolated pathogenic *Candida* species such as *C. albicans* and *C. glabrata* through agar diffusion method. The hydroalcoholic extract can strongly scavenge DPPH radical and its antioxidant capacities which are high correlated with the total free Phenolics and total flavonoids. Also, the extract had high capability inhibition of linoleic acid oxidation and the reducing ability. This study revealed a higher antioxidant capacity in the leaves of *Rumex obtusifolius* compared with control groups. The minimum inhibitory concentration values within 24 and 48 hours were 200-250 μ g/ μ L for *C. albicans* and 250 μ g/ μ L for *C. glabrata*. The extract includes high amounts of phenolic compounds and antioxidant activity showing is significant. Also, the results confirmed that leaves extract had a potential in anti-Candida activity and suggesting that it could be utilized as a potential source of herbal medicine drugs and natural antioxidants to prevent diseases associated with free radical, anti-fungal disease and food preservation.

Keywords: *Rumex obtusifolius*, *Candida*, antioxidant activity, hydro alcoholic extract.

INTRODUCTION

Rumex includes approximately 200 species widely distributed all over the world. *Rumex* species belong to family Polygonaceae used as a traditional herbal medicine, such as treating the sores, burns, blisters, and is also used as a drug for treating astringent, diarrheal, laxative, stomachic, tonic diseases and an antidote for nettle, cancer, tumors and anti-inflammation. It is used as antioxidant, antimicrobial, cytotoxic substance (Harshaw *et al.*, 2010, Rao *et al.*, 2011) as well. The plant can also be used to reduce the biliary disorders and control cholesterol levels (El-Bakry *et al.*, 2012). Many researchers have shown the existence of anthracene derivatives, flavonoids, procyanidins and oxalic acid in plant may play key role in treatment of some diseases (Spencer *et al.*, 2007).

Dietary consumption of antioxidant-containing plants such as flavonoids will lead to the protection against non-communicable diseases in humans; cancer, cardiovascular diseases and cataract (Matkowski, 2008). *R. obtusifolius*, a member of Polygonaceae family, is a perennial plant

widely distributed in North America, Europe and Iran. It is native to these regions. *R. obtusifolius* is commonly known as "Torshak" in Iran and the local name is "toopa" in Mahdishahr (Sangsar). The leaves of this plant added in food and soups are also eaten as vegetables. The leaves and roots also have medicinal properties (Vasas *et al.*, 2015).

Due to increasing the number of Acquired Immune Deficiency Syndrome (AIDS) patients, organ transplantations, other immune-compromised patients, neoplastic diseases, using the broad-spectrum antibiotics and immunosuppressive drugs has led to increased Candidiasis disease especially those caused by *Candida albicans*.

In recent years, enough attention has been paid to find natural antioxidants within plants because using the natural products with therapeutic properties are both cost-effective and beneficial and also available. The present study was carried out to evaluate the anti-Candida and antioxidant activities of hydroalcoholic extract from leaves of *R. obtusifolius*.

*Corresponding author: e-mail:hossen253@yahoo.co.uk "mailto:hossen253@yahoo.co.uk"@yahoo.co.uk

MATERIALS AND METHODS

Materials

L-ascorbic acid, 2, 2-Diphenyl-1-picryl hydrazyl (DPPH), Gallic acid, Quercetin, Butylated Hydroxy Toluene (BHT), Folin-Ciocalteu reagent, trichloroacetic acid (TCA) were purchased from Sigma-Aldrich. Also reagents and chemicals had analytical grades. Spectrophotometric measurements were performed using a Spectronic. Spectronic Spekol, 2000 spectrophotometer (Analytik Jena, Germany).

Plant collection

R. obtusifolius were collected from Jashloobar garden located in the North West of Semnan, Iran. Plants were identified by experienced botanists from University of Applied Sciences and Technology (UAST) at Education Center in Semnan branch. A voucher specimen *R. obtusifolius* were deposited in the herbarium of Medicinal Plants Research Center at UAST.

Plant Leaves were washed with running tap water. Leaves were dried in the shade for a week and then crushed into small pieces and finally were powdered using an electric blender. Plant leaves powder was then stored in plastic bags for further use.

Procurement of the plant extract

Plant extracts were obtained by procedures as follows: Ethanolic extract was obtained by incubating 50grams of powdered leaves with 500ml of Ethylacetate; methanol; distilled water (6:3:1) for overnight at room temperature (RT) before adjusting 40°C for 8hours in a Soxhlet system. In all cases, the extracts were centrifuged (3000 rpm) for 15min. and the supernatants were harvested and filtered using Whatman paper No. 1. The solvents were evaporated by incubation at RT. The extraction yields were calculated as the percentage of the used powder.

Determination of total phenolic contents

Total phenolic content of the *R. Obtusifolius* Hydroalcoholic extracts was estimated using the Folin-Ciocalteu colorimetric method described previously (Zheng and Wang, 2001, Liu *et al.*, 2002) with a little modification. Briefly, the reaction mixture contained: 200 µl of diluted *R. Obtusifolius* leaves extracts, 800µl of freshly prepared diluted Folin Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The final mixture was diluted to 7ml with deionized water. The appropriate dilutions of the filtered extracts were oxidized with 0.2 N Folin-Ciocalteu reagents and then the reaction was neutralized with saturated sodium carbonate (75g/L). The optical density (OD) of the resulting blue color was measured at 765nm by Spectronic Spekol, 2000 spectrophotometer (Analytik jena, Germany) after incubation for 2hours in darkness at the ambient conditions to complete the reaction at RT. Quantification was done on the basis of the standard curve of Gallic acid. Results were expressed

as gram of Gallic acid equivalent (GAE) per 100grams of dry weight (Wolfe *et al.*, 2003).

Determination of total flavonoid contents

1 ml of extract in methanol and different dilution of a standard solution of Quercetin was added to 10ml volumetric flask containing 4ml of distilled water. To the above mixture, 0.3ml of 5% sodium nitrite was added via 5 minutes incubation time. 0.3ml of 10% aluminum Chloride before 6 minutes incubation time and then 2ml of 1M Sodium Hydroxide was added and the total volume was made up to 10ml with distilled water. The OD of the mixture with pink in color was measured against a freshly prepared reagent blank at 510nm. Total flavonoid content of the extracts was expressed as mg quercetin equivalents (QE) per gram of sample (mg/g) (Tesoriere *et al.*, 2007, Tepe *et al.*, 2006, Tlili *et al.*, 2011).

Concentration values of extracts were obtained from Quercetin standard curve by interpolating to the X- axis. Total Flavonoid Contents was calculated by using the following equation:

$$TFC = R \times D.F \times V \times 100/w$$

Where,

R = Result which has been obtained from the standard curve,

D.F= Dilution factor,

V= Volume of stock Solution;

100= for 100 grams dried plant,

W= Weight of plant which was used in experiment.

Antioxidant assays

Samples were dissolved in 95% methanol at a concentration of 1mg/ml and then diluted to prepare the series of concentrations for antioxidant assays. Reference chemicals were used to be compared in all assays.

DPPH radical scavenging activity

DPPH radical scavenging activity was assessed according to Burits and Bucar, 2000; Cuendet *et al.*, 1997; Kirby and Schmidt, 1997. In this assay a 50µl of various dilutions of the *R. Obtusifolius* Hydroalcoholic extract solution (stock 1mg/ml concentration) was added with 5ml of 0.004% Methanolic DPPH. The mixture was shaken strongly using vortex and left for 30 minutes at RT in a dark room. The scavenging effect on the DPPH radical was read using a spectrophotometer at 517nm. Ascorbic acid and Butylated Hydroxy Toluene (BHT) were used as positive control. The radical scavenging activity was expressed as the radical scavenging percentage using the following equation:

$$\% \text{ percentage DPPH radical scavenging: } I\% = (1 - A_s/A_c) \times 100$$

Where,

Ac= absorbance of control

As= absorbance of sample solution

The DPPH solution without sample solution was used as a control. IC₅₀ value is the concentration of sample required to scavenge 50% of DPPH free radical and was calculated

from plotted graph of radical scavenging activity across from the concentration of extracts.

Reducing power assay

Different extracts were measured according to the method used by Hinneburg (Hinneburg *et al.*, 2006) in order to determination of reducing power. Briefly, one milliliter of extracts with different concentrations was added to 2.5 ml of phosphate buffer (200mm; pH6.6) and 2.5ml of potassium ferricyanide 1% and incubated at 50°C for 20 min after gently mixed. Then, 2.5ml of 10% TCA was added to the mixture and centrifuged at 3000rpm for 10 min. The absorbance was measured through spectrophotometer at 700nm after mixing 2.5ml of supernatant with 2.5ml of distilled water and 0.5ml of FeCl₃ (0.1%). Increase in absorbance of the reaction mixture was interpreted as an increase in reducing activity of the extract and the results were compared with ascorbic acid which was used as a positive control.

β -Carotene bleaching test (BCBT)

Solution of β -carotene-linoleic acid mixture stock was prepared as follows: 0.5mg of β -carotene dissolved in 1ml of chloroform; 25 μ l of linoleic acid and 200mg of Tween 40 were added as emulsifier, because β -carotene is not water soluble. Chloroform was completely evaporated using a vacuum evaporator. Then, 100ml of distilled water saturated with oxygen was added with vigorous shaking at a rate of 100ml/min for 30min; 2500 μ l of this reaction mixture was dispersed to test tubes, and 350 μ l portions of extracts, prepared in 2g/l concentrations, were added. The emulsion system was incubated for up to 48 hours at RT in a dark room. OD of the mixture was measured at 490nm after incubation time. The same procedure was repeated with a positive control BHT and a blank which antioxidant capacities of extracts were compared with those at the BHT and the blank. Tests were carried out in triplicate. The relative antioxidant activity (RAAs) of the samples was calculated using the following equation: RAA=A sample/A BHT (Tomaino *et al.*, 2005).

Candida strain and inoculum preparation

Preparation serial dilution of hydroalcoholic extract

Two grams of the dried Hydroalcoholic Extract of *R. Obtusifolius* was dissolved in 5mL Dimethyl Sulphoxide (DMSO, 100%) to a final concentration of 400 μ g/ μ L as a stock (Nejad *et al.*, 2014) and serial dilutions were prepared using sterile distilled water from 400 , 350 , 300, 250 , 200 , 150, 100, 50, 25, 12/5 μ g/ μ L.

In current study, the reference strain used *Candida albicans* (ATCC10231). A total of 40 *Candida* spp isolates from women with vulvovaginal candidiasis that thirty-four *C. albicans* isolates and six *C. glabrata* isolates were from vulvovaginal candidiasis (Bineshian *et al.*, 2015). *Candida* spp isolates were inoculated into Sabouraud dextrose broth (SDB, Merk, Germany) and

were incubated for 24hours at 35°C. Then yeasts were washed three times with sterile distilled water and the suspension was adjusted to make a conidial concentration of 1×10^6 CFU/mL (0.5 Mac-Farland standard). The commercial antifungal drugs "fluconazole disc" (10 μ g/disc Hi-Media, Mumbai, India) were used as positive control and DMSO was used as negative control.

Broth Microdilution Method for Determination of MIC

One hundred microliter of yeast inoculums (10^6 cells/mL) was spread onto Sabouraud dextrose agar medium plates (SDA, Merck, Germany) using a bent glass rod. Then six wells of 7 mm diameter were punched by a borer into the SDA medium and filled with 100 μ L of two-fold serial dilutions of plant extracts as well as sterile DMSO 100% as negative control. Anti- *Candida* activity was determined by measuring the zone of inhibition after incubated for 24hours at 35°C. Experiments were repeated three times.

Gas chromatography-Mass spectrometry analysis

The Gas chromatography-Mass spectrometry (GC-MS) analysis of the extracts was performed using a GC-MS (Agilent) equipped with a VF-5ms fused silica capillary column, DB-35ms, 30m $\times$ 250 μ m $\times$ 0.15 μ m. Electron ionization system with ionization energy of 70eV was used for GC-MS detection. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1ml/min. Injector and mass transfer line temperature were set at 200 and 250°C respectively. The oven temperature was programmed from 60 to 280°C at 10°C/min, held isothermal for 1min and finally raised to 280°C at 10°C/min. 1 μ L ml of respective diluted samples was manually injected in the split less mode, with split ratio of 1: 10 and with mass scan of 50-600.

Total running time of GC-MS is 57min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization. The identity of the components in the extracts was assigned by the comparison of their retention times and mass spectra fragmentation patterns with those stored on the computer library and also with published articles. Willey library sources were used for matching the identified components from the plant material.

STATISTICAL ANALYSIS

Statistical analysis was performed based on one way Analysis of variance (ANOVA) at confidence level 95% using SPSS version 16 software. Linear regression to correlate between total phenolic as well as total flavonoid with antioxidant activity was carried using Excel 2003.

RESULTS

The hydro alcoholic extraction of *R. obtusifolius* was evaluated for their antioxidant capacities using in vitro

methods, including DPPH radical scavenging, β -Carotene bleaching test and reducing power assay. Total free phenols and total flavonoids contents as well as antifungal activity were also examined. The components of extract which were analyzed by GC-Mass instrument were investigated. The extract was screened against 40 isolated pathogenic *Candida* species such as *C. albicans* and *C. glabrata* by agar well diffusion method. *R. Obtusifolius* extraction of leaves was found to be the most antioxidant effect which total antioxidant activity and DPPH scavenging activity was determined by using spectrophotometry.

Extraction yield, total polyphenols and flavonoid contents

The phenolics and flavonoids contents as well as anti-radical activity vary and depend on the extraction methods, genetic factors and climatic/growing conditions. However, we prefer to use a different solvent system that is more effective than one's solvent according to (Aliyazicioglu *et al.*, 2013) reported, the Ethyl acetate and Methanol; distilled water (60:30:10) system is well-known to extract the antioxidant compounds. Table 1 showed w/w% extraction yield.

Table 1: % yield of *R. obtusifolius* hydroalcoholic extracts

Extract	Yield(%w/w)
Leaves	4.33

Antioxidant activity

The anti-radical activity of the hydro alcoholic extracts which are prepared from *R. obtusifolius* aerial organ collected from Semnan city located in Iran was examined. The extract showed high antioxidant activities in the three assays tested: DPPH method, Reducing power assay and β -Carotene bleaching test (BCBT).

DPPH radical scavenging activity

This assay is known as a primary test since quenching odd electron of DPPH by the extract to decrease the absorbance at 517nm. *R. obtusifolius* leaves extract showed a high level anti-radical activity in scavenging DPPH radical (comparable to the standard, BHT) with a maximum inhibition of about 18.10 at a concentration of 1000 μ g/ml (table 2).

Table 2: Effect of the test compounds in the DPPH assay

	Test compound	IC ₅₀ ^a	Inhibition%
1	<i>Rumex obtusifolius</i>	25.50	4.34
2	Butylhydroxytoluene	15.02	18.10

Reducing power activity

The capacity of reducing a compound serves as a remarkable indicator of its potential antioxidant activity. The reducing power (RP) of the extracts was determined

by direct electron donation in the reduction of ferri cyanide to ferro cyanide and the change in the optical density of the final mixture which is measured at 700nm. Increase in the optical density indicates the higher reductive ability. The reducing capacity of the hydro alcoholic extract of *R. obtusifolius* was found not to be in dose- dependent manner when compared with Vit. C. The percent of reducing power of the extract has been shown in table 3. The *R. obtusifolius* leave extract has the highest reducing power which accorded with the total phenolic and total flavonoid content. The correlation between reducing power activity of extract and its phenolic contents revealed R²=0.89, while with the flavonoid content showing R²=0.88. Taking into account of R² values, it suggested the phenolic compounds had more probably contribution to its reducing activity than dose of flavonoid compounds.

Table 3: The reducing power of ethyl acetate: methanol; distilled water (60:30:10) *R. obtusifolius* extract with respect to standard Vit.c at 700 nm.

	Extract	Reducing power %
1	<i>Rumex obtusifolius</i>	91.39 $\pm$ 3.09

β -carotene bleaching assay

Due to reaction of β -carotene with radicals formed by the oxidation of linoleic acid, its yellow color is decreased. However, the rate of β -carotene bleaching slows down at the presence of antioxidants. The relative antioxidant activity (RAAs) of the *R. obtusifolius* extraction was calculated using the following equation:

$$RAA = A_{\text{sample}} / A_{\text{BHT}}$$

A_{BHT} is the absorbance of the control (BHT) and A_{sample} is the absorbance of the *R. obtusifolius* extract. The calculated RAAs for the *R. obtusifolius* leave extract and standard antioxidant compound (BHT) are given in table 4.

Table 4: The relative antioxidant activity (RAAs) of the *R. obtusifolius* with respect to standard BHT.

	Extract	β -carotene bleaching(RAA%)
1	<i>Rumex obtusifolius</i>	48.92 $\pm$ 0.81
2	BHT	100

Bioactive contents

Total free phenols

The most common secondary metabolites were found in plants are phenolic compounds including flavonoids, tannins, and phenolic acids. The Total Phenolic Content (TPC) of the *R. obtusifolius* was determined spectrophotometry according to the method of Folin-Ciocalteu and the results were expressed as Gallic acid equivalent. The standard curve equation which was used as follows: y (absorbance) = 0.038 $\times$ (μ g Gallic acid) -

Table 5: Antioxidant constituents of *R. obtusifolius*

	Extract	Total phenolics (GAE) ^a (mg/g)	Total flavonoids (QU) ^b (mg/g)
1	<i>Rumex obtusifolius</i> (leaf)	97.49±5.36*	332 ±5.66

*aValues represent averages ± standard deviations for triplicate experiments.

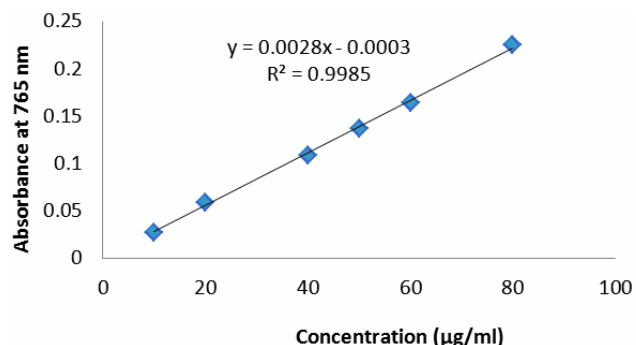
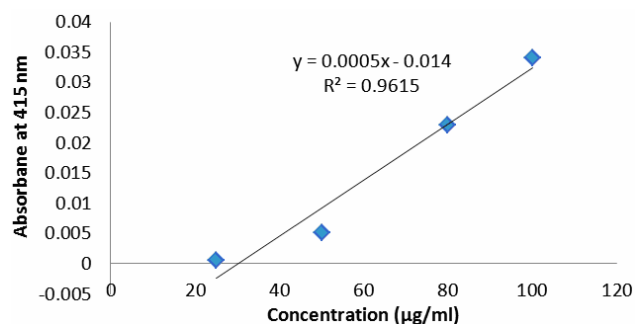
^a Expressed as Gallic acid equivalent (GAE).

^b Expressed as Quercetin equivalent (QU).

Table 6: Anti-Candida activity of hydroalcoholic extract of *R. obtusifolius*

	400 µg/µL	250 µg/µL	200 µg/µL	Fluconazole
<i>Candida albicans</i>	13	12	11	20
<i>Candida glabrata</i>	11	10	-	-

0.0132, $R^2=0.9859$ (fig. 1). The absorbance value was inserted in the above equation and the total amount of phenolic compound was calculated. As shown in table 5, the Total Phenolic content of the *R. obtusifolius* leaves was 97.49 ± 5.36 .

**Fig. 1:** Calibration curve of standard Gallic acid to determine the total Phenol content**Fig. 2:** Calibration curve of standard Quercetin to determine the total Flavonoid content in *R. obtusifolius*

Determination of total flavonoid content

Flavonoids are polyphenolic molecules that protect the plants against stress effects. The flavonoids are divided into six major subtypes including chalcones, flavones, isoflavonoids, flavanones, anthoxanthins and anthocyanins. The total flavonoids content of the *R. obtusifolius* was determined spectrophotometric according to the method of Folin-Ciocalteu and the results were expressed as Quercetin equivalent. The standard curve equation which was used as follow; y (absorbance) =

$0.0005 \times (\mu\text{g Quercetin}) - 0.014$, $R^2=0.9615$ (fig. 2). The absorbance value was inserted in the above equation and the total amount of flavonoid compound was calculated. The overall mean± SD of total flavonoids was 332 ± 5.66 mg/g (table 5).

Anti-candida

To investigate the anti-*Candida* activity of hydro-alcoholic extract of *R. obtusifolius* against *Candida Albicans*, *Candida Glabrata*. The results are shown in table 6. Hydro-alcoholic extract of *R. obtusifolius* has shown anti-*Candida* activities against *C. albicans* ($n=34$) and *C. glabrata* ($n=6$). The highest inhibition zone was observed against *C. albicans* with a range of 13 ± 2.0 mm and a minimum inhibition zone of 11 ± 2.0 mm and 200-250 µg/µL MIC values.

Gas chromatography, mass spectrometry analysis

The GC-MS analysis of the extracts showed the presence of photo-components, Totally Twenty-nine constituents was identified in the *R. obtusifolius* leave extract (table 7). Major components of leave extract were Linolenic acid, Palmitic acid and 2-Methoxy-4-vinylphenol, -D-glucopyranose, Benzofuran.

DISCUSSION

Today the approach to the natural resources of medicinal plants as the effective compounds for treatment is paid special attention. Since, the flavonoids have antioxidant effects and can be found in abundance in edible plants in the family Polygonaceae. Medical importance of the plant is due to its chemical composition, because it contains many active substances such as flavonoids (vitexin, isovitexin, orientin and isorientin), anthraquinones (emodin and chrysophanol), quinones, carotenoids, vitamins, proteins, lipids, carbohydrates, reducing sugars, phenols, tannins, saponins, triterpenoids, organic acids and minerals. One of the most important groups of phenolic is flavonoids found in nature. Flavonoids are the major chemical compounds of the *Rumex* (Zhang *et al.*, 2012, Prasad and Ramakrishnan, 2012b, Mostafa *et al.*,

Table 7: Chemical analyses constituents of Hydro alcoholic extract of leave *R. obtusifolius* based on GC peak areas

No.	Components	% (Peak area)	Retention Time (RT)
1	Valeraldehyde	0.261	3.270
2	1-Pentyl-3,3-D2 Acetate	0.049	5.556
3	4-Amino-1-hexanol	0.235	8.840
4	3-Butoxypropylamine	0.049	8.969
5	2-Furancarboxaldehyde,	0.139	10.528
6	2-Pyrrolidinone	1.601	17.206
7	4H-Pyran-4-one,	0.177	18.623
8	2-Methoxy-4-vinylphenol	15.59	22.243
9	Benzofuran	4.854	22.691
10	Acetamide	0.263	25.45
11	Pyrrolidin-2-one	2.157	26.040
12	benzoquinone	3.537	28.929
13	1,7-Dimethylxanthine	4.224	29.229
14	D-glucopyranose (levoglucosan)	7.123	32.505
15	Myristic acid	0.594	34.274
16	4-Methoxy-3-(methylthio)phenol	1.089	34.702
17	Glucopyranoside	0.082	34.962
18	galactopyranoside	0.693	35.263
19	Hexadecanoic acid (Palmitic acid)	16.727	35.901
20	Octadecatrien-1-ol (Linolenic acid)	29.100	39.791
21	L Ketone	3.018	40.761
22	dodecadien-1-ol	0.355	41.570
23	Pyrido[3,4-b]indole1- soquinolinecarbonitrile	0.82	42.405
24	2-Pentadecanone,	0.834	42.731
25	9-Octadecenoic acid (Oleic acid)	3.888	43.367
26	Nonylphenol	0.837	44.419
27	Pyridinecarboxaldehyde,	1.357	44.469
28	Octadec-9-enoic acid	0.214	46.434
29	1,2'-Binaphthalene	0.131	46.475
Total		99.99%	

2011, Hariprasad and Ramakrishnan, 2012, Prasad and Ramakrishnan, 2012a). The present study showed that the leaves of *R. obtusifolius* have antioxidant activity. Antioxidant activity can be linked to the presence of phenolic and the flavonoid contents.

The nature of polyphenolic flavonoids is to collect the free radicals such as superoxide and hydroxyl radicals. Harshaw showed that the methanol extracts of the leaves of *R. obtusifolius* had the highest levels of free radical scavenging property are accorded with our study (Harshaw *et al.*, 2010).

However, the leaves of *R. obtusifolius* contain the high amounts of phenolic compounds which have exhibited significant antioxidant activities and they could be utilized as a natural source of antioxidant in food industry. In our research, Twenty-nine chemical constituents have been identified from leaves hydroalcoholic extract of *R. obtusifolius*. The major chemical constituents are Linolenic acid (Octadecatrien-1-ol) (29.1%), Palmitic acid (Hexadecanoic acid) (16.73%), 2-Methoxy-4-vinylphenol (15.6 %), D-glucopyranose (7.12%),

Benzofuran (4.85%), Oleic acid (Octadecenoic acid) (3.9%), aldehyde compounds (.86%), 4H-Pyran (.177%) and 2-Furancarboxaldehyde (.14%).

Many studies have reported that Linolenic acid has important properties such as antioxidant, antitumor, antifungal and antimicrobial and Palmitic acid with properties of antioxidant, hypocholesterolemic, nematocide, pesticide, flavor, Lubricant, antiandrogenic and hemolytic 5-alpha reductase inhibitor and 2-Methoxy-4-vinylphenol which is main polyphenol compound of *R. obtusifolius* extract contains the properties of antimicrobial, antioxidant, anti-inflammatory and analgesic and also D-glucopyranose with preservative properties. Benzofuran which is included in extract, has wide range of therapeutic usage with antibacterial, antifungal, anti-inflammatory, analgesic, antidepressant, anticonvulsant, antitumor, imaging, anti-HIV, antidiabetic, antitubercular, antioxidant and miscellaneous properties and Oleic acid with properties of anti-inflammatory, anti-androgenic cancer, dermatogenic, hypo-cholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge properties and flavor. 4H-Pyran

is a flavonoid compound has properties of antimicrobial, anti-inflammatory and 2-Furancarboxaldehyde with properties antimicrobial, preservative with content 0.177%, 0.14%; respectively (Agoramoorthy *et al.*, 2007, Ashraf *et al.*, 2015, Vadivel and Gopalakrishnan, 2011). Also, the paper indicates that synthetic compounds have Benzofuran nucleus possess with broad range of biological activities, such Griseofulvin as antifungal and also exhibits antifungal activity against *C. albicans* in vitro (Kamal *et al.*, 2011, Masubuchi *et al.*, 2003).

Our results showed that the leaves of *R. obtusifolius* have anti-candidial activity. This seems to be related to the presence of high levels of polyphenols (2-Methoxy-4-vinylphenol), flavonoids (4H-Pyran), Benzofuran and fatty acids such as Linolenic acid (highest amount), Palmitic acid, Oleic acid which is in concordance with other investigations (Agoramoorthy *et al.*, 2007, Chandrasekaran *et al.*, 2011, Abdelillah *et al.*, 2013, Walters *et al.*, 2004).

CONCLUSION

Therefore, the extract includes high amounts of phenolic compounds and antioxidant activity as well as fatty acids indicating the significance. Also, the results confirmed that leave extract had a potential anti-Candida activity and suggesting that it could be utilized as the potential sources of herbal medicinal drugs and natural antioxidants to prevent the diseases associated with free radical, anti-fungal disease and food preservation.

REFERENCES

- Abdelillah A, Houcine B, Halima D, Sari Meriel C, Imane Z, Eddine SD, Abdallah M and Sari Daoudi C (2013). Evaluation of antifungal activity of free fatty acids methyl esters fraction isolated from *Algerian Linum usitatissimum* L. seeds against toxigenic *Aspergillus*. *Asian Pac. J. Trop. Biomed.*, **3**: 443-448.
- Agoramoorthy G, Chandrasekaran M, Venkatesalu V and Hsu M (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. *Braz. J. Microbiol.*, **38**: 739-742.
- Aliyazicioglu R, Eyupoglu OE, Sahin H, Yildiz O and Baltas N (2013). Phenolic components, antioxidant activity, and mineral analysis of *Capparis spinosa* L. *Afr. J. Biotechnol.*, **12**: 6643-6649.
- Ashraf A, Sarfraz RA, Anwar F, Shahid SA and Alkharfy KM (2015). Chemical composition and biological activities of leaves of *Ziziphus mauritiana* L. native to Pakistan. *Pak. J. Bot.*, **47**: 367-376.
- Bineshian F, Yadegari MH, Sharifi Z, Eidgahi MA and Nasr R (2015). Identification of *Candida* species using MP65 gene and evaluation of the *Candida albicans* MP65 gene expression in BALB/C mice. *Jundishapur J. Microbiol.*, **8**.
- Chandrasekaran M, Senthilkumar A and Venkatesalu V (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. *Eur. Rev. Med. Pharmacol. Sci.*, **15**: 775-780.
- El-Bakry A, Mostafa H and Alam E (2012). Antioxidant activity of *Rumex vesicarius* L. at the vegetative stage of growth. *Asian J. Pharm. Clin. Res.*, **5**: 111-117.
- Hariprasad P and Ramakrishnan N (2012). Chromatographic finger print analysis of *Rumex vesicarius* L. by HPTLC Technique. *Asian Pac. J. Trop. Biomed.*, **2**, S57-S63.
- Harshaw D, Nahar L, Vadla B, Saif-e-naser GM and Sarker SD (2010). Bioactivity of *Rumex obtusifolius* (Polygonaceae). *Arch. Biol. Sci. Belgrade*, **62**, 387-392.
- Kamal M, Shakya AK and Jawaidd T (2011). Benzofurans: a new profile of biological activities. *International Journal of Medical and Pharmaceutical Sciences*, **1**: 1-15.
- Liu M, Li XQ, Weber C, Lee CY, Brown J and Liu RH (2002). Antioxidant and antiproliferative activities of raspberries. *J. Agric. Food Chem.*, **50**: 2926-2930.
- Masubuchi M, Ebike H, Kawasaki KI, Sogabe S, Morikami K, Shiratori Y, Tsujii S, Fujii T, Sakata K and Hayase M (2003). Synthesis and biological activities of benzofuran antifungal agents targeting fungal N-myristoyltransferase. *Bioorg. Med. Chem.*, **11**: 4463-4478.
- Matkowski, A. (2008). Plant *in vitro* culture for the production of antioxidants: A review. *Biotechnol. Adv.*, **26**: 548-560.
- Mostafa H, Elbakry A and Eman AA. (2011). Evaluation of antibacterial and antioxidant activities of different plant parts of *Rumex vesicarius* L. (Polygonaceae). *Int. J. Pharm. Pharm. Sci.*, **3**: 109-18.
- Nejad BS, Rajabi M, Mamoudabadi AZ and Zarrin M (2014). In vitro anti-candida activity of the hydroalcoholic extracts of heracleum persicum fruit against pathogenic candida species. *Jundishapur J. Microbiol.*, **7**.
- Prasad P and Ramakrishnan N (2012a). Antioxidant assay of *Rumex vesicarius* L. *Int. J. Curr. Res.*, **3**: 074-6.
- Prasad P and Ramakrishnan N (2012b). In vitro lipid peroxidation assay of *Rumex vesicarius* L. *Int. J. Pharm. Pharm. Sci.*, **4**: S368-S370.
- Rao K, Ch S and Banji D (2011). A study on the nutraceuticals from the genus *Rumex*.
- Spencer, P., Sivakumaran, S., Fraser, K., Foo, L. Y., Lane GA, Edwards PJ and Meagher LP (2007). Isolation and characterisation of procyanidins from *Rumex obtusifolius*. *Phytochem. Anal.*, **18**: 193-203.
- Tepe B, Sokmen M, Akpulat HA and Sokmen A (2006). Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem.*, **95**: 200-204.
- Tesoriere L, Butera D, Gentile C and Livrea MA (2007). Bioactive components of caper (*Capparis spinosa* L.) from Sicily and antioxidant effects in a red meat

- simulated gastric digestion. *J. Agric. Food Chem.*, **55**: 8465-8471.
- Tlili N, Nasri N, Khaldi A, Triki S and Munné-Bosch S (2011). Phenolic compounds, tocopherols, carotenoids and vitamin C of commercial caper. *J. Food Biochem.*, **35**: 472-483.
- Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A and Saija A (2005). Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food Chem.*, **89**: 549-554.
- Vadivel E and gopalakrishnan S (2011). Gc-MS Analysis of some bioactive constituents of *Mussaenda frondosa* Linn. *Inter. J. Pharm. Bio. Sci.*, **2**: 313-320.
- Vasas A, Orban-Gyapai O and Hohmann J (2015). The Genus *Rumex*: Review of traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacol.*, **175**: 198-228.
- Walters D, Raynor L, Mitchell A, Walker R and Walker K (2004). Antifungal activities of four fatty acids against plant pathogenic fungi. *Mycopathologia*, **157**, 87-90.
- Wolfe K, Wu X and Liu RH. (2003). Antioxidant activity of apple peels. *J. Agric. Food Chem.*, **51**: 609-614.
- Zhang H, Guo Z, Wu N, Xu W, Han L, Li N and Han Y (2012). Two novel naphthalene glucosides and an anthraquinone isolated from *Rumex dentatus* and their antiproliferation activities in four cell lines. *Molecules*, **17**: 843-850.
- Zheng W and Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.*, **49**: 5165-5170.