

IN VITRO ANTIBACTERIAL ACTIVITY AND THE EFFECT OF EXPLANT TYPE AND EXTRACTION SOLUTION ON SOME BIOCHEMICAL PROPERTIES OF *RUMEX TUBEROSUS*

YOUSEF GHASEMI*¹, MOHAMMAD ALI EBRAHIMZADEH², SAREH REZAEI³,
MAHDIEH TAHERI⁴, SEYYEDEH FATEMEH HASHEMIAN⁵ AND ABBAS ALI
DEHPOUR⁵

1. Genetic and Agricultural Biotechnology Institute of Tabarestan. University of
Agricultural Sciences and Natural resources, Sari, Iran. PO Box 578, Sari, Iran.

2. Pharmaceutical Sciences Research Center, School of Pharmacy,
Mazandaran University of Medical Sciences, 48189, Sari, Iran.

3. Department of biology, Sari University of payame noor, Iran

4. MSc student of Health care Service Management of Sari branch Islamic Azad university.

5. Department of Biology, Qaemshahr branch Islamic Azad University of Qaemshahr, Iran.

ABSTRACT

Medicinal plants contain wide range of secondary metabolites which have being used in many aspects of pharmaceutical science. This paper was conducted to determine the effect of extraction solution and plant part on the antioxidant activities, flavonoid and phenol contents and assay of antimicrobial activity of *Rumex tuberosus*. High antioxidant activity with 0.65 mg/ml was observed in flower when it extract by methanol solvent. Also methanol increase the total phenol content in all parts of the plant in comparison with chloroform but flavonoid result is completely different and the highest flavonoid was obtained by chloroform extraction. Antimicrobial test approved that Gram-positive bacteria have been more impressed than gram-negative bacteria by methanol extraction of *R. tuberosus*. Two strains (*P. mirabilis*, *K. pneumonia*) could not control by *R. tuberosus* ethanol extract.

KEY WORDS: Antioxidant activity; Antibacterial activity; Phenols; Flavonoids; *Rumextuberosus*.

INTRODUCTION

The role of free radicals in many diseases has been demonstrated. Several biochemical pathways in our body produce reactive oxygen species (ROS) which damage crucial biomolecules. If they are not effectively scavenged by cellular constituents, they cause different diseases in our body (Halliwell B et al. 1992). Antioxidants are chemical substances that donate an electron to the free radicals and convert them to the harmless molecule. They may decline the energy of the free radical or suppress radical formation or break chain propagation or repair damage. Nature has been a source of medicinal materials for thousands of years that numerous numbers of modern drugs isolated from natural sources (Cragg GM and Newman DJ, 2001). Medicinal plants used for traditional

remedies, contain different phytochemical compound which can be utilized to treat many diseases. The genus *Rumex*, (Polygonaceae) contains about 150 species widely distributed around the World. The main chemical constituents of *Rumex* are anthraquinones and flavonoids (Zhang H et al. 2012). There are many reports on *Rumex* medicinal properties in literature. El-Bakry et al., (2002) demonstrated existence of some phytochemicals compounds such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid with high antioxidant activity in *Rumex vesicarius* L. They investigated the alteration of biologically active constituents in different vegetative stages of plant. The effect of fractionation (n-hexane, ethyl acetate, chloroform, butanol and aqueous) in *Rumex hastatus* leaf has been studied for its antioxidant activity (Sahreen S

et al. 2011). This genus is used as laxative, alterative, tonic, used in rheumatism (Shinwari zk and Gilani SS, 2003) skin diseases, bilious complaints, piles and bleeding of lungs (Gorsi MS and Miraj S, 2002). The strong antibacterial activity has been reported for *Rumex ecklonianus* L. and *Rumex japonicas* Hoult extraction against both Gram-positive and Gram-negative bacteria (Jimoh FO et al. 2008; Elzaawely AA et al. 2005). There is just one preliminary report on antioxidant activity of *rumex tuberosus* in turkmen sahara region of Iran (Mohammadi S and Naghibi F, 2010). The objective of this study was assessment of the effect of different extraction solvents (chloroform and methanol) and different parts of plant (stem, flower, root) on total phenol, flavonoid and antioxidant activity in *Rumex tuberosus*. Also in vitro antibacterial activity of ethanol extract of this plant was studied.

MATERIALS AND METHODS

1. PLANT MATERIALS AND PREPARATION OF FREEZE-DRIED EXTRACT

Rumex tuberosus stem, flower and root were collected in Sari, Iran in June 2012 and identified by Dr Bahman Eslami (Department of Biology, Islamic Azad University of Qaemshahr, Iran). A voucher specimen (No 1257) has been deposited at the Sari School of Pharmacy Herbarium. Plant material was dried under dark conditions at room temperature and coarsely grounded (2-3 mm). 50 g of dried powdered sample were macerated for 24h with 250 ml of chloroform or methanol, separately. Extraction was repeated three times and the resulting extracts were concentrated over a rotary vacuum evaporator until a solid extract sample was obtained. The resulting crude extract was freeze-dried for complete removal of solvent. The yields are represented in Table 1.

2. TOTAL PHENOLIC COMPOUNDS AND FLAVONOID CONTENT

Total phenolic compound contents were determined by the Folin-Ciocalteu method (Ebrahimzadeh MA et al. 2010). The extraction samples (0.5 ml) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagents for 5 min and 2.0 ml of 75 gL⁻¹ sodium carbonate. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. The total flavonoid content was measured by a colorimetric aluminum chloride method (Ebrahimzadeh MA et al. 2010a). 0.5 ml solution of each extracts in

methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). The total flavonoid contents are expressed in terms of quercetin equivalent (QE), mg/g of extract.

3. DPPH RADICAL-SCAVENGING ACTIVITY

The stable DPPH radical was used for determination of free radical scavenging activity of the extracts (Ebrahimzadeh MA et al. 2010a). Different concentrations of each extracts were added, at an equal volume, to methanol solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm.. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

4. REDUCING POWER

The Fe³⁺ reducing power of the extract was determined by the methods of Yen and Chen with a slight modification. Different concentrations (50 - 800 µg/ml) of the extract (1 ml) were mixed with 1 ml phosphate buffer (0.2 M, pH 6.6) and 1 ml potassium hexacyanoferrate (1%), followed by incubation at 50°C in a water bath for 20 min.. All tests were performed triplicate (Ebrahimzadeh MA et al. 2010b). Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance (p < 0.05) and the means separated by Duncan's multiple range tests. The IC50 values were calculated from linear regression analysis.

5. ANTIBACTERIAL ACTIVITY

5.1. Preparation of extract

The whole plant was dried at room temperature before extraction. Powdered sample (2 g) was extracted with 50 mL 80% methanol and then freeze-dried by a Heto FD3 freeze-dryer (Heto-Holten A/S, Allerød, Denmark). The samples were stored at 4 °C until water at room temperature (□23 °C) for 24 h in a shaking water bath (Shaking Bath 5B-16, Techne, Cambridge, UK). The extract was filtered by a Millipore filter with a 0.45-µm nylon membrane under vacuum at 23 °C. The filtrates were concentrated by Rotavapor (R-114) (Buchi, Flawil, Switzerland) and then freeze-dried by a freeze-dryer (Heto-Holten A/S, Allerød, Denmark).

6. ASSAY FOR ANTIBACTERIAL ACTIVITY OF PLANT EXTRACT

Antibacterial activity of plant extracts was determined by disc diffusion method as described by (Bauer AW. 1966). Three Gram negative bacteria (*Proteus mirabilis* PTCC (1076); *Enterobacter cloacae* PTCC (1003), and *Klebsiella pneumonia* PTCC (1290) and two Gram positive bacteria (*Staphylococcus aureus* PTCC (1112) and *Bacillus subtilis* PTCC (1023) were used for the present study. All the test bacteria were collected from Pastor Institute of Iran. Dried filter paper discs (4mm in diameter) impregnated in known amount of test substances (400 µg/discs) were placed on Mueller-Hinton agar medium uniformly seeded with the test organisms. Ampicillin, Cephalothin and Chloramphenicol discs (30µg/disc) soaked in respective solvent were used as positive control. The diffusion occurred according to the physical law that controls the diffusion of molecules through agar gel the plates were then incubated at 37°C for 24 hours to allow maximum growth of the microorganisms. The antibacterial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

RESULTS

According to Table 1, yield of extraction is varied in different parts of the plant and change by extraction solvents. The yield of extraction was higher by methanol. Among different plant parts, root exhibited good extraction percentage by usage of methanol.

Total phenol and flavonoids content

The results in Table 1 showed that plant parts and the extraction solvent influenced all measured parameters in *R. tuberosus*. Significantly differences were observed among different treatments. Total phenol compounds are reported as gallic acid equivalents by reference to standard curve ($y = 0.0063x$, $r^2 = 0.987$) and flavonoids content, by reference to standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$). In this paper the amount of total phenols in stem part was higher (193 mg GAE/g of extract) when it extracted with methanol, while the lowest content was shown in stem part with chloroform extraction (85 mg GAE/g of extract).

Table 1
The yields, total phenols, total flavonoids, total antioxidant and reducing power of *R. tuberosus* different parts.

Solvent	Plant parts	Yield of extraction (%)	Total phenols (mg GAE/g of extract)	Total flavonoids (mg QE/g of extract)	DPPH free radical scavenging, IC ₅₀ (mg/ml) ^a
Methanol	stem	9.7	193.4 ± 4.8	60.56 ± 3.2	0.69 ± 0.02
	flower	15	147.8 ± 11.3	140.40 ± 7.7	0.65 ± 0.01
	root	19.2	119.1 ± 8.9	40.41 ± 3.1	0.74 ± 0.03
Chloroform	stem	4.5	85.1 ± 2.1	191.5 ± 4.2	2.96 ± 0.12
	flower	2.1	137.1 ± 6.7	221.1 ± 5.5	2.61 ± 0.14
	root	1.5	106.2 ± 7.6	206.1 ± 8.9	3.01 ± 0.27

1. ^a IC₅₀ of BHA was 53.96 ± 3.1, Vitamin C, 5.05 ± 0.1 and Quercetin 5.28 ± 0.2, respectively. The extraction of flower portion with chloroform in *R. tuberosus* was enriched in flavonoids content but root segment showed the weak flavonoids content with application of methanol as extraction solvent (40.41 mg QE/g of extract). Chloroform extracts more total flavonoid contents than Methanol generally. Extraction with methanol as a polar solvent cause high phenol contents while more flavonoid contents were measured in chloroform extract. It concluded that other phenol materials such as plant pigments, anthocyanins,

carotenoids and some vitamins specially vitamin C and E compensate low quantity of flavonoid in stem parts.

DPPH radical-scavenging activity

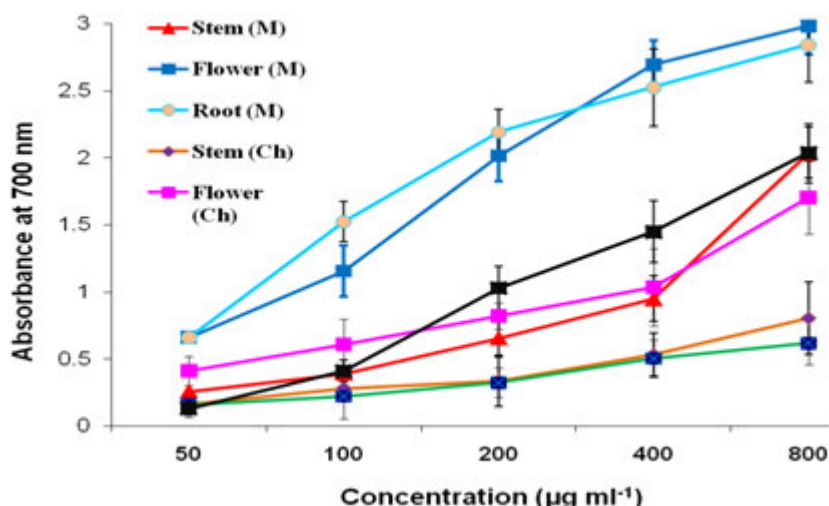
Clearly, as it shown in Table 1, extraction with chloroform in comparison with methanol reduced the scavenging free radical in this plant. The maximum content of antioxidant activity in *R. tuberosus* was recorded in flower when extract with methanol (IC₅₀=0.65 mg/ml). Contrary, the weak antioxidant activity obtained with root by usage of chloroform extraction (IC₅₀=3.01 mg/ml).

Reducing power

According to Figure 1 reducing power of all extracts increase by increasing their concentration. Good reducing power was observed in some extraction at 50 and 800 $\mu\text{g/ml}$ but some was less than that of vitamin C ($p < 0.001$). Among the plant parts, root and flower exhibited better reducing power than vitamin C which is used as a

positive control. Also methanol fraction has significantly differences than chloroform to reducing the Fe^{3+} to Fe^{2+} as an antioxidant activity indicator. It reveals that in addition of plant portion, the solvent influence reductive potential as well, and could serve as strong electron donors.

Figure 1
Reducing power of extracts. Vitamin C used as control.



Assessment of antibacterial activity

The *in vitro* antimicrobial activity of crude extract of *Rumex tuberosus* collected from Firozkoh region (north of Iran) were studied on Gram-positive and Gram-negative bacteria. The results of antibacterial activity of crude extract are shown in table 2 by measuring the minimum inhibitory concentrations (MICs). The maximum activity was observed on *Proteus mirabilis* PTCC (1076) (10 mm) and non activity on *Enterobacter cloacae* PTCC(1003), *Klebsiella pneumonia* PTCC(1290), *Staphylococcus aureus* PTCC (1112) and *Bacillus subtilis* PTCC(1023). Two types of Gram-positive bacteria (*Staphylococcus aureus* PTCC (1112) and *Bacillus subtilis* PTCC (1023) and Gram-negative bacteria (*Proteus mirabilis* PTCC (1076); *Enterobacter cloacae* PTCC (1003), and *Klebsiella pneumonia* PTCC (1290) were selected to measure the antibacterial activity of *Rumex tuberosus*. Methanol for negative control did not have any growth inhibition effect on all bacteria. According to table 2, methanol extraction of *Rumex tuberosus* exhibited moderate inhibitory activity against PTCC (1112) and PTCC (1023) with 12 and 11.7 mm diameter. There was no any antimicrobial activity in PTCC (1076) and PTCC

(1290) strains and weak antibacterial activity obtained in PTCC (1003).

DISCUSSION

Polyphenolic compounds act as an antioxidant activity and can play reducing agent to stable the single oxygen. It is impressed by plant materials, methods of extraction, solvent and environmental conditions. Jamshidi M et al. (2014) concluded that total phenol content of *Lythrum salicaria* was changed by plant portion, and flower part indicated rich source of this compound more than leaf. Methanol extracts contained more total phenolic contents than chloroform extract. Our result is supported by the report of Sharipah SA et al. (2009). They indicated that methanol extracts contained more total phenol than chloroform extract in *Ficus deltoidea*. Phenols and Polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Ebrahimzadeh MA et al. 2010b). Usually, there is a direct relation between total phenol and flavonoid content, but as it derived with Figure1 there is no

correlation between them. Ghasemi Y et al. (2012) revealed that no correlation was found between total phenol and flavonoid content of fruit ($r^2 = 0.71$). High amount of phenolic and flavonoid compounds could directly relate to antioxidant activity of fruit (Ghasemi Y et al. 2012). Like this study, phenol compound indicated good antioxidant activity. The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables (Klimczak I et al. 2007; Kiselova Y et al. 2006). As reported, antioxidant activity of fruits and vegetables significantly increases with the presence of high concentration of total polyphenol content. These compounds can be impressed by the solvent extraction too. DPPH free radical scavenging activity and phenol content were higher in methanol rather than chloroform. Sharipah SA et al. (2009) reported that the content of total phenol and antioxidant activity in *Ficus detoidea* var. *angustifolia* were the highest in methanol by comparison with chloroform. Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action (Yildirim A et al. 2001). In the reducing power method, the presence of some substances in the sample cause the reduction of Fe^{3+} to Fe^{2+} by donating electrons. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Nabavi SF et al. 2010). Increasing absorbance at 700 nm indicates an increase in reductive ability. Flower and root in methanol exhibited more reducing power activity in this study. Both reducing power and DPPH free radical scavenging activity method proved that methanol is better solution than chloroform in antioxidant activity. It may be due to the high extraction of material that plays more antioxidant activity in polar solvent such as methanol.

Antimicrobial activity The antimicrobial activity of medicinal plants against various pathogenic bacteria has been approved (Tadhani M, and Subhash R, 2006; Lino A, and Deogracious O, 2006). It seems that antimicrobial properties of alcoholic extract, generally derived from presence of secondary metabolite especially phenols, terpenoids and saponins (Stojanovic G et al. 2005). Mostafa HAM et al. (2011) reported that *Rumex vesicarius* show good antibacterial potential on Gram-positive and Gram-negative bacteria and they found that leave is the best portion for antibacterial activity. Gram-positive bacteria were more susceptible than Gram-negative bacteria to the methanol extraction in this research. Shan B et al. (2007) reported that Gram-positive bacteria indicated more sensitivity to the extract rather than the gram-negative bacteria in this test and good correlation was observed between total phenol content and antibacterial activity ($R^2=0.73-0.93$). Antibiotics which selected as positive control showed more antibacterial activity against both Gram-positive and Gram-negative bacteria than crude extract. According to Cimanga k et al. (2002), 15 or more diameter of prevented growth area indicate excellent antibacterial activity, between 10-15 diameter moderate activity and less than 10 diameters is sign of weak antimicrobial activity of the extract. Resistance of Gram-negative bacteria to plant extract probably depends on lipo polysaccharide composition of cell wall. It plays the obstacle to reach the antimicrobial compound from plant to cytoplasmic membrane of Gram-negative bacteria (Duraipandiyar V et al. 2006). Mothana R et al. (2009) revealed that most of the plant extraction has preventing effects on Gram-positive bacteria and weak effects on Gram-negative strains.

Table 2
Antibacterial activities of R. tuberosus methanol extract as minimum inhibitory concentrations (MICS) in g/ml

	Extract (700µg /disc)	Cephalothin (15µl/ disc)	Ampicilin (15µl /disc)	Choramphenicol (15µl /disc)
<i>Proteus mirabilis</i> PTCC(1076)	0	23	9	12
<i>Enterobacter cloacae</i> PTCC(1003)	11.3	24	7	12
<i>Klebsiella pneumonia</i> PTCC(1290)	0	24	0	13
<i>Staphylococcus aureus</i> PTCC (1112)	12	22	21	31
<i>Bacillus subtilis</i> PTCC(1023)	11.7	30	24	40

Data are in mm diameter

CONCLUSION

In recent decades researchers have an especial regard on medicinal plant to improve the human health. In these studies some plants have equal or more effect than chemical drugs with fewer side effects. *Rumex* genus contains many species with different capacity in total phenol, flavonoid, antioxidant and antibacterial activity. There has not been report on *R. tuberosus* antimicrobial activity and there is just less information about medicinal properties of this genus. Numerous factors such as plant parts, solvent and the methods of extraction influence the antioxidant and antibacterial activity. In our research it is cleared that methanol is better against chloroform solvent and antioxidant activity and phenol content. Methanol is polar solvent and could extract more compound of sample in comparison

with chloroform as non-polar solvent. It cause that methanol induce high phenol content and antioxidant activity of *R. tuberosus*. Of course flower portion of this plant indicated high capacity in total phenol and flavonoid content rather than other parts. Up to now no paper has been published about antibacterial activities of *R. tuberosus*. Gram-positive and Gram-negative bacteria were selected to test antimicrobial activity of *R. tuberosus*. The result showed that ethanol extract have more inhibitory effect on Gram-positive bacteria. It is may be due to the construction of cell wall of the bacteria in witch lipopolysaccharide influence the resistance of bacteria to active materials in medicinal plant. Totally we can suggest that *R. tuberosus* could select as suitable representative for antibacterial against infection disease.

REFERENCES

1. Bauer AW, Kirby WM, Sherris JC and Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966; 45 (4): 493-496.
2. Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totté J, Pieters L, Vlietinck AJ. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. Journal of ethnopharmacology. 2002 Feb 28;79(2):213-20.
3. Cragg GM, Newman DJ. Medicinals for the millennia. Annals of the New York Academy of Sciences. 2001 Dec 1;953(1):3-25.
4. Duraipandiyar V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC complementary and alternative medicine. 2006 Oct 17;6(1):1.
5. Ebrahimzadeh MA, Nabavi SF, Nabavi SM and Eslami B. Antihemolytic and antioxidant activities of *Allium paradoxum*. Cent. Eur. J. Biol. 2010a; 5(3): 338-345.
6. Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslami B. Antihemolytic and antioxidant activities of *Allium paradoxum*. Cent.Eur. J. Biol. 2010b; 5(3): 338-345.
7. El-bakry AA, Mostafa HAM and Eman A. Antioxidant activity of *Rumex vesicarius* L. at the vegetative stage of growth. Asian Journal of Pharmaceutical and Clinical Research. 2012; 5(4): 111-117.
8. Elzaawely AA, Xuan TD and Tawata Sh. Antioxidant and antibacterial activity of *Rumex japonicas* Houtt. Aerial parts. Biological and Pharmaceutical Bulletin. 2008; 28(12): 2225-2230.
9. Ghasemi Y, Nematzadeh Gh, A, Ebrahimzadeh MA, and Dehpour AA. Influence of harvesting date on some physicochemical properties of nectarine leaf and fruit. Journal of Medicinal Plant Research. 2012; 6(43): 5552-5556.
10. Gorski MS, Miraj S. Ethnomedicinal survey of plants of Khanabad village and its allied areas, district Gilgit. Asian J Plant Sci. 2002;1(5):604-15.
11. Halliwell B, Gutteridge JMC and Cross CE. Free radicals, antioxidants and human disease: where are we now? J. Lab. Clin. Med. 1992; 119:598-620.
12. Jamshidi M, Shabani E., Hashemi Z. and Ebrahimzadeh MA.. Evaluation of three methods for the extraction of antioxidants from leaf and aerial parts of *Lythrum salicaria* L. (Lythraceae). International Food Research Journal. 2014; 21(2): 783-788.
13. Jimoh FO, Adedapo AA, Aliro AA, and Afolayan AJ. Polyphenolic Contents and biological Activities of *Rumex ecklonianus*.

- Pharmaceutical Biology. 2008; 46(5):333-340.
14. Kiselova Y, Ivanova D, Chervenkov T, Geroval D, Galunska B and Yankova T. Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytother Res.* 2006; 20(11): 961- 965.
 15. Klimczak I, Małeczka M, Szlachta M, Gliszczyńska-Świąło A. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis.* 2007 May 31;20(3):313-22.
 16. Lino A, Deogracious O. The in-vitro antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steganotaenia araliacea*-Ugandan medicinal plants. *African health sciences.* 2006 Mar 1;6(1):31-5.
 17. Mohammadi Motamed S and Naghibi F. Antioxidant activity of some edible plants of the Turkmen sahara region in north of Iran. *Food Chemistry.* 2010; 119(4):1637-1642.
 18. Mostafa HAM, El-Bakry, AA and Eman AA. Evaluation of antibacterial and antioxidant activities of different plant parts of *Rumex vesicarius* L. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2011; 3(2):109-118.
 19. Mothana RA, Lindequist U, Gruenert R, Bednarski PJ. Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqatra. *BMC Complementary and Alternative Medicine.* 2009 Mar 25;9(1):1.
 20. Nabavi SF, Ebrahimzadeh MA, Nabavi SM and Eslami B. Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss. *Grasas y Aceites.* 2010; 61(3): 244-250.
 21. Sahreen S, Rashid khan M and Ali khan R. Phenolic compounds and antioxidant activities of *Rumex hastatus* D. Don. Leaves. *Journal of Medicinal Plants Research.* 2011 5(13): 2755-2765.
 22. Shan B, Cai YZ, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *International Journal of food microbiology.* 2007 Jun 10;117(1):112-9.
 23. Sharipah Ruzaina SA, Sunalti M, Norizan A and Faridahanim MJ. Phenolic content and antioxidant activity of fruits of *Ficus Deltoidae* var *angustifolia* SP. *The Malaysian Journal of Analytical Sciences.* 2009; 13(2): 146 – 150.
 24. Shinwari ZK and Gilani SS. Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Northern Pakistan). *J. Ethnopharmacol.* 2003; 84: 289-298.
 25. Stojanovic G, Radulovic N, Hashimoo T and Palic R. In Vitro Antimicrobial Activity of Extracts of four *Achillea* Species: The Composition of *Achillea Clavennae* L. (Asteraceae) Extract. *J Etnopharmacol.* 2005; 101(1-3):185-190.
 26. Tadhani MB, Subhash R. In vitro antimicrobial activity of *Stevia rebaudiana* Bertoni leaves. *Tropical Journal of Pharmaceutical Research.* 2006 Jun;5(1):557-60.
 27. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *Journal of agricultural and food chemistry.* 2001 Aug 20;49(8):4083-9.
 28. Zhang H, Guo Z, Wu N, Xu W, Han L, Li N, Han Y. Two novel naphthalene glucosides and an anthraquinone isolated from *Rumex dentatus* and their antiproliferation activities in four cell lines. *Molecules.* 2012 Jan 17;17(1):843-50.