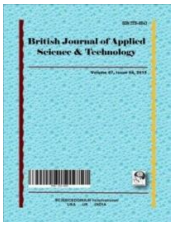

*British Journal of Applied Science & Technology*
*11(1): 1-8, 2015, Article no.BJAST.18249*
*ISSN: 2231-0843*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Determination of Antioxidants and Total Phenolic Content in Some Wild Vegetables Used Widely in Lesotho

**Mosotho J. George[1*], Relebohile Lets'aba[1], Makhotso J. Khotso[1], Lineo E. Bese[1], Keneuoe Taole[1] and Mamotlotlisi Maroba[1]**

*[1]Department of Chemistry and Chemical Technology, National University of Lesotho, P.O. Roma 180, Lesotho.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author MJG designed and supervised the study, wrote the manuscript and updated the literature. Authors RL, MJK, LEB, KT and MM performed the experiment and prepared the first draft. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/BJAST/2015/18249
Editor(s):
(1) Sumit Goswami, Senior Scientist, Pfizer Inc., Chesterfield, MO, USA.
Reviewers:
(1) Anonymous, University of Carthage, Tunisia.
(2) Egharevba, Henry Omoregie, National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.
(3) Anonymous, University of Ghana, Ghana.
Complete Peer review History: http://sciencedomain.org/review-history/10335

***Short Research Article***

***Received 11th April 2015***
***Accepted 26th May 2015***
***Published 26th July 2015***

## ABSTRACT

Antioxidants, important chemicals that combat uncontrollable oxidation processes in the living cells, occur naturally in plants in the form of phenolic compounds such as phenolic acids and flavanoids. The total antioxidant capacity and total phenolic content of different wild edible plants consumed widely in Lesotho as vegetables, namely Moetse-oa-pere -Tragopogon dubius (Scopoli), Papasane - Rorippa nudiuscula, Leharasoana - Sonchus oleraceus (Linnaeus), Bobatsi - Urtica urens (Linnaeus) and Seruoe - Chenopodium album (Linnaeus) were determined from the ethanol extracts resulting in the linoleic acid peroxidation inhibition of 73.7%, 64.6%, 76.3%, 69.5% and 60.3% respectively using the iron (II) thiocyanate spectrophotometric method. The results also demonstrated high levels of total phenolic content (39.2–54.4 mg GAE per gram of sample) with a significant correlation ($R^2$=0.9277) between antioxidant capacity and total phenolic content. The

**Corresponding author: E-mail: maluti2005@gmail.com, jm.george@nul.ls;*

extraction efficiency demonstrated temperature dependence as showed by the levelling off at around 80°C with some slight drop at boiling temperature of water (96°C). The observed abundance of the antioxidant capacity in these plants is gratifying considering how widely these vegetables are consumed in the rural areas of Lesotho – a small mountainous country in the Southern Africa.

*Keywords: Antioxidants; phenolic content; cooking; wild vegetables; Lesotho.*

## 1. INTRODUCTION

Oxidation reactions are important to produce energy required to support all other processes that take place in living cells. Oxygen is readily activated by ultraviolet radiation and heat from sunlight to produce reactive oxygen species most of which are dangerous to the cells and as such the process requires strict control [1]. The peroxidation of unsaturated lipids, one type of the oxidation processes with deleterious effects in living organisms, has been linked the onset of a wide range of diseases such as cancer, diabetes, cardiovascular diseases as well as many degenerative diseases linked to aging such as rheumatoid arthritis, cirrhosis and arteriosclerosis to name but a few [2,3]. Various potent compounds against this process called antioxidants, are classified as either free radical or active oxygen scavengers [4]. They mostly occur naturally in plants to counteract these reactive oxygen species and enhance cell survival; thus making plants to be a potent source of natural antioxidants. This, therefore, makes antioxidants not only to be important in defence mechanisms of living cells but also in food protection against oxidative stress [5]. Consequently, natural antioxidants have received extensive attention for their capacity to protect organisms and cells from damage brought on by oxidative stress [6]; as well as searching for naturally occurring antioxidants as alternatives to synthetic counterparts [7]. Plant-derived natural antioxidants are generally classified as vitamins, phenolic compounds including flavonoids and phenolic acids, as well as volatile compounds in herbs and spices [8]. These natural antioxidants are becoming increasingly important, not only in food but also in preventive medicine against diseases mentioned earlier that are associated with oxidative stress [9]. This is more important considering that excess supplementation with certain putative antioxidants is reportedly suspected to be harmful [10,11].

There has been increase in the use of organic foods owing to the belief that these reduce the exposure to chemically tainted foods that are feared to be exposing humans to potential carcinogens. In light of these, consumption of wild vegetables especially from rural areas has also been recommended, as rural areas are far less prone to chemical exposure. Wild vegetables are also reportedly rich in natural antioxidants [12].

Lesotho, as a low-income nation with a population of just over 2 million people [13] has been plagued with food insecurity for some years [14]. Typical of developing countries, there is an outflow of males and younger people to the urban areas in search of jobs to earn a living to the detriment of the families that are often headed by elderly women with relatively poor capacity to support the families [15]. This outflow also promotes a phenomenon of co-residence where most of the children reside with one elderly woman per household [16]. Due to poverty and general poor access to most agro-crops, these communities rely mostly on wild vegetables to take with starch for their daily meals.

In this study the levels of total antioxidant activity and total phenolic content were determined from some wild vegetables that are commonly consumed in Lesotho on daily bases as vegetables using the thiocyanate reduction and the Folin–Ciocalteu methods respectively. Five different wild vegetables namely Moetse-oa-pere (*Tragopogon dubius),* Papasane (*Rorripa nudiuscula*), Leharasoana (*Sonchus oleraceus*), Bobatsi *(Urtica urens) and* Seruoe *(Chenopodium album)* were studied. The effect of temperature, mimicking traditional deep boiling cooking, was also investigated using water as an extracting solvent in place of ethanol.

## 2. EXPERIMENTAL

### 2.1 Chemicals

Ethanol, potassium phosphate, iron (II) chloride, potassium thiocyanate were all obtained from Associated Chemical Enterprises (Pty) Ltd (Johannesburg, South Africa); linoleic acid and Folin-Ciocalteu reagents were obtained from Sigma Aldrich (Johannesburg, South Africa).

### 2.2 Collection, Storage and Treatment of the Samples

Fresh leaves of the wild vegetables were collected from the fields around the main University campus in Roma (about 35 km South-East of Maseru, the capital of Lesotho). The samples were washed with water to remove dust and soil particles; thereafter they were dried in the oven at 40°C to a constant mass (dry weight). The dried vegetables were ground into a fine powder using a mortar and pestle and kept in a refrigerator at 5°C till further use.

Individual portions of known mass (recorded in Table 1) of the dried and ground samples were extracted with portions of ethanol at room temperature repeatedly until the extracting solvent became colourless. The obtained extracts were combined and filtered. The filtrates were collected and the ethanol was evaporated using a rotary evaporator at 40°C to obtain dry extracts. The dried extracts were placed in the refrigerator at 5°C before the subsequent analyses.

### 2.3 Determination of Antioxidant Capacity

The total antioxidant activities were determined according to the thiocyanate method used originally by Elmastas and his colleagues [3]. Pre-weighed portions of the dried extracts of each vegetable were dissolved in 10 mL ethanol. 2.5 mL aliquots were drawn and added to 2.5 mL of potassium phosphate buffer (0.04M, pH 7.0). This solution was incubated at 37°C overnight. After the incubation the solutions were mixed with equal volumes of iron (II) chloride and potassium thiocyanate solutions and allowed to stand for 3 minutes before the absorbance at 500 nm using a 1201 Shimadzu UV-Vis Spectrophotometer (Kyoto, Japan). The absorbance of the control was taken as 100% and the absorbances of the other solutions were appropriately compared with it.

For the control analysis, the 2.5 mL solutions of the vegetables were replaced by the same volume of pre-filtered saturated solution of linoleic acid. The antioxidant capacity was calculated on the basis of the inhibition of linoleic acid oxidation using the formula as follows:

$$\% \text{ inhibition} = 100 - [(A_s/A_c) \times 100\%]$$

where $A_c$ and $A_s$ are the absorbances of the control and sample solutions respectively. All the analyses were performed in triplicates to enable evaluation of the precision of the results.

### 2.4 Determination of Total Phenolic Content

Total phenolic content of the vegetables was determined by the Folin–Ciocalteu colorimetric method reported in 1965 by Singleton and Rossi [17]. A series of gallic acid solutions were prepared in the range of 1-100 mg and their absorbances at 750 nm were measured using the spectrophotometer. The absorbances were plotted on a calibration curve from which the estimation of the total phenolic contents of the extracts were determined per unit gram of the sample. The absorbances of the analytes were measured in a similar fashion and the estimated phenolic content calculated from the calibration curve. All the sample analyses were carried out in replicates (n=3). Total phenolic content was expressed as gallic acid milli-equivalents (mg GAE) per gram of dry weight.

### 2.5 Correlation of Antioxidant Capacity to Total Phenolic Content

The values obtained for antioxidant were plotted as a function of total phenolic content and the linearity of the plot was determined, from which a relationship could be deduced.

### 2.6 Effect of Temperature on Antioxidant Capacity

The samples were extracted using distilled water at different temperatures and analysed for the total antioxidants as in section 2.3. The water bath was used for this purpose. The highest temperature, 96°C, was used as the boiling temperature. To prevent water loss during boiling, this extraction was carried out in a reflux apparatus for the 15 minutes duration.

## 3. RESULTS AND DISCUSSION

### 3.1 Determination of Total Phenolic Content

The total phenolic content of the vegetable samples were determined from the regression analysis using Windows Excel Software, yielding the calibration equation y=0.0144x+0.4421 with the correlation coefficient ($R^2$) of 0.9975 which demonstrated sufficient linearity of the method. The method demonstrated the statistical estimated detection limit in the range 3.6–6.4 mg

GAE using the standard error of the intercept and that of the regression respectively.

Table 1 shows the calculated total phenolic content reported as milli-gram gallic acid equivalent (mg GAE) per gram of dry mass of each sample. As can be seen these vegetables contain considerable amounts of phenolics with the darker leaved vegetables (*U. Urens and R. nudiuscula*) yielding higher values and the lighter *C. album* showing lowest value. This is in agreement with the report that darker-leaved vegetables contain higher levels of total phenolic content than lighter counterparts. However, *T. dubius* which is considerably lighter compared to the other vegetables showed higher phenolics content and hence higher antioxidants than the other plants. This plant produces milky sticky extrusion when cut, that possibly contains this chemicals. The values obtained in this study are higher than those reported elsewhere for *C. album, R. nudiuscula* and *U. urens* [18]. These differences in the total phenolic contents could be attributed to the developmental and physiological state of the plants at the time of collection.

## 3.2 Determination of Antioxidant Capacity

Table 2 shows the averaged amounts of the each of the vegetables used and the average absorbance for n=3 analyses. It further depicts the percentage inhibition of these extracts per unit mass of sample used as a percentage inhibition of linoleic acid by iron (II) thiocyanate solution. The results show that the contents of antioxidants degrease in the order *S. oleraceus* > *T. dubius* > *U. urens* > *R. nudiuscula* > *C. album* with values of 76.3, 73.6, 69.5, 64.6 and 60.3% inhibitions respectively.

The values from the table show that *C. album* has lower inhibition than the rest of the samples. However, this observation is debatable since taking the confidence interval at p=0.05 the percentage inhibition of *C. album* of 60.3±6.6% ranges between 53.7% and 66.9%. The next lowest level of 64.6 would then range from 58.5 to 70.1 taking the confidence interval of 5.8, thus rendering the degree of overlap to be too significant to be ignored. Fig. 1 depicts the same results in Table 2 pictorially and it shows considerable overlaps between most of the values obtained which is not as explicit with the tabular presentation in Table 2.

## 3.3 Correlation of Antioxidant Capacity with Total Phenolic Content for Ethanol Extracts

Fig. 2 shows the correlation of the antioxidant capacity to the total phenolic content for the samples. The error bars in the figure demonstrate the respective standard deviations for n=3 on either axes.

**Table 1. Total phenolic content of the wild vegetables reported as gallic acid equivalent**

| Sample | Mass of sample (g) | Absorbance | Concentration[a] |
|---|---|---|---|
| *T. dubius* | 1.0153 | 1.2032 | 53.1 (1.2)[b] |
| *R. nudiuscula* | 1.0421 | 1.0880 | 45.3 (2.8) |
| *S. oleraceus* | 1.0212 | 1.2176 | 54.4 (1.5) |
| *U. urens* | 1.0087 | 1.1456 | 48.9 (2.7) |
| *C. album* | 1.0214 | 1.0016 | 39.2 (1.7) |

[a] *Concentration given in mg GAE per gram of sample*

[b] *The values in parentheses are the standard deviations obtained with n = 3*

**Table 2. The amount of sample used and % inhibition measured as absorbance at 500 nm**

| Sample | Amount (g) | Absorbance | % Inhibition (/g sample) |
|---|---|---|---|
| Control (linoleic acid solution) | | 1.920 | 0 (0)[a] |
| *T. dubius* | 1.1035 | 0.506 | 73.6 (5.5) |
| *R. nudiuscula* | 1.0961 | 0.585 | 64.6 (5.3) |
| *S. oleraceus* | 1.3553 | 0.455 | 76.3 (6.3) |
| *U. urens* | 1.2784 | 0.677 | 69.5 (4.7) |
| *C. album* | 1.0282 | 0.762 | 60.3 (6.5) |

[a] *The values in parentheses indicate the % RSD for n=3*

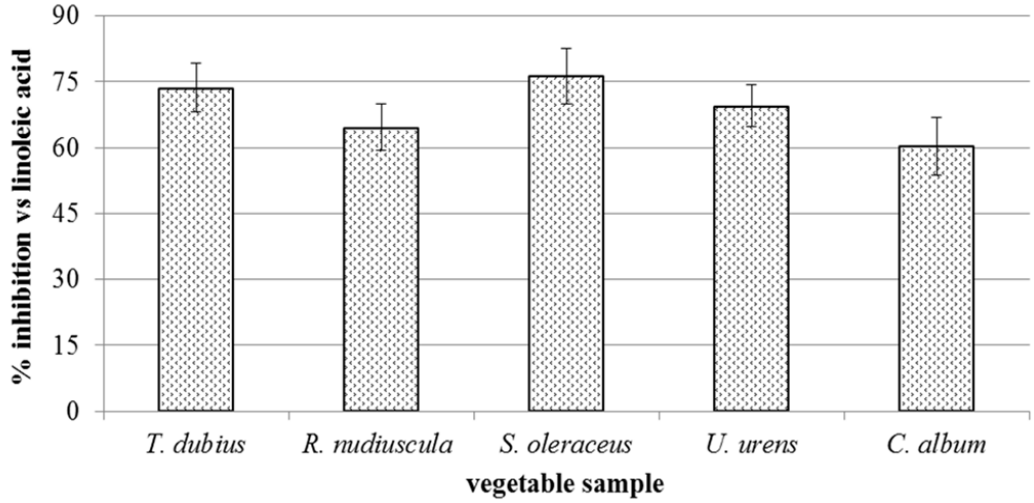


**Fig. 1. Inhibition of peroxidase activity by difference vegetables extracts**

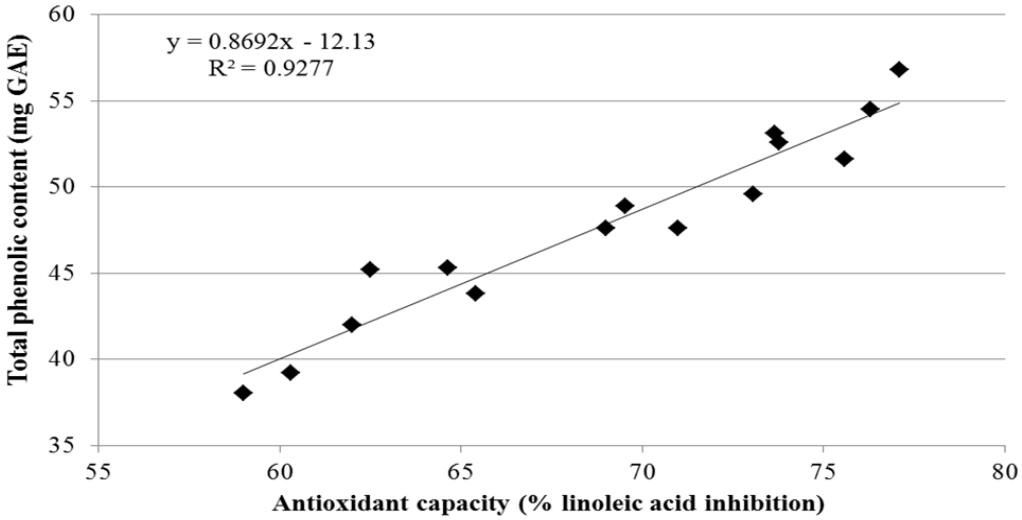


**Fig. 2. Correlation of total phenolic content with antioxidant capacity**

As can be seen there is considerable correlation ($R^2$=0.9277) indicating that the antioxidant capacity is considerably attributable to the phenolic compounds. These results are consistent with a number of reports on determination on antioxidants and total phenolic compounds in plant and food samples [19,20]. The comparison of the obtained values with those reported for other food stuffs indicate that these vegetable have significantly higher total phenolic content than most agro-crop vegetables such as cabbage, spinach, peas, cauliflower and broccoli ranging between 10 and 20 mg/g Pakade *et al.* [21]. The comparison of different parts of the *U. pilulifera* plant for the antioxidant capacity, Özen *et al.* [1] found the following order: the seeds > the flowers > the leaves > the roots. The difference in the values obtained in that report (86.76% inhibition) compared to the present study (69% inhibition) maybe due to the developmental stage of the plant as well as the fact that these are different species of the same genus.

### 3.4 Effect of Temperature on Antioxidant Capacity

Fig. 3 shows the effect of temperature on the antioxidant behaviour. This, by implication, further demonstrates the effect of temperature on extraction efficiency of the phenolic compounds from the plant materials.

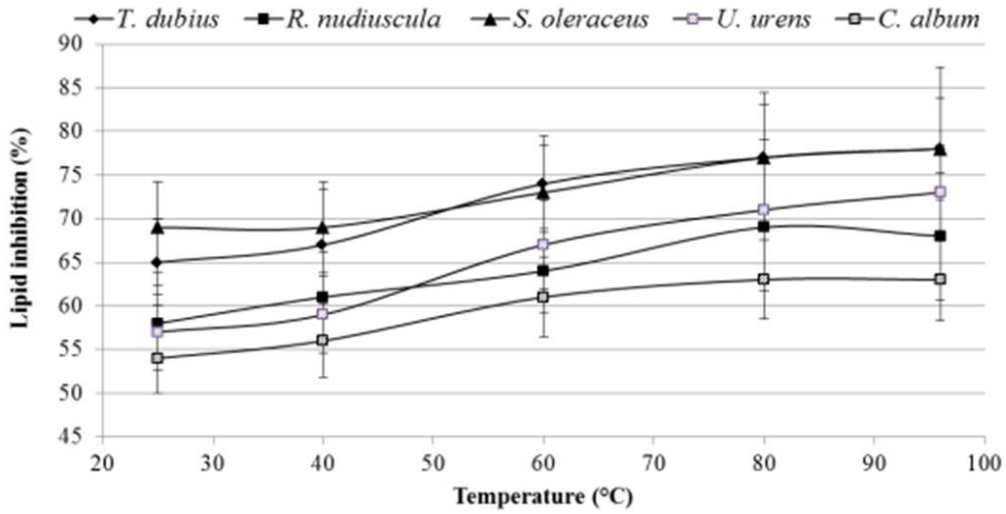


**Fig. 3. Effect of temperature on antioxidant capacity for different vegetables**

According to Fig. 3, it can be seen that the inhibition increases with temperature and peaks around the boiling point of water since the trends lines level between 80°C and 96°C except for *U. urens* that seem to increase. This behaviour is significant since it has a bearing on food processing. It confirms the assertion that boiling food and discarding the extracted juice afterwards leads to loss of nutrients with that juice. The drop in the extraction efficiency at the boiling does not indicate less extractability but rather could be attributable to the decomposition of some of the components responsible for antioxidant ability such as ascorbic acid which is known to decrease with increase in temperature. Some glycosylated compounds could lose the glycoside thereby become less water soluble; or get more volatile and hence evaporate during the boiling/cooking process, thus reducing the antioxidant capacity.

It is also worth noting that the extraction efficiency of water is lower than ethanol at the same temperature. For example, *T. dubius* showed inhibition of 73% for ethanol and gave 65% for distilled water at 25°C (room temperature); and the extraction efficiency at boiling water is higher than that of ethanol at room temperature. The comparison with hot ethanol was not made, but rather with water to mimic cooking. However, other studies using variation of temperature revealed that a maximum of about 65°C would be suitable for effective extraction of the antioxidants using 40% ethanol-water mixture [22]. However this 65°C could be attributable to the boiling point of ethanol hence this temperature could be used to avoid excess loss of solvent at near boiling temperatures. This solvent dependency of the extraction efficiency has been attributable to the organic solvent's ability to interact with the cell walls of the plants and dissolve more sample as opposed to water with interacts less with the cell wall [23]. The same study revealed that variation of the solvent did not show similar variation in extraction efficiency. Only time showed clear variation with efficiency improving with the longer extraction processes.

## 4. GENERAL DISCUSSION AND CONCLUSION

This study has demonstrated that the studied wild vegetables contain significant levels of antioxidant capacity and phenolic compounds which are essential therapeutically ranking as follows *S. oleraceus* (76.3%) > *T. dubius* (73.7%) > *U. urens* (69.5%) > *R. nudiuscula* (64.6%) > *C. album* (60.3%). The total phenolic content correlated significantly ($R^2$=0.9277) with antioxidants indicating that the observed antioxidant capacity is attributable to the phenolic content. The effect of temperature also indicated that although high temperatures improve extraction, this could also be detrimental to the nutritional value of these vegetables if cooked by excessive boiling.

These results are gratifying since these vegetables are consumed routinely in rural areas of Lesotho. Moreover, these vegetables can be

used as an easily accessible source of natural antioxidants and as a possible food supplement in pharmaceuticals. They should be recommended to rural people with limited financial resources to afford food supplements, not merely as vegetables for daily meals but for their nutritious and therapeutic value as well. The practice of uprooting these vegetables during weeding for other agricultural crops should be controlled so that they are not completely eliminated.

## ACKNOWLEDGEMENTS

Authors would like to acknowledge Mr J.M. Polaki for his assistance in identifying the plants and providing their scientific names.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Özen T, Çöllü Z, Korkmaz H. Antioxidant Properties of *Urtica pilulifera* Root, seed, flower, and leaf extract. Journal of Medical Foods. 2010;13(5):1224-1231.
2. Yagi K. Lipid peroxides and human disease. Chemistry and Physics of Lipids. 1997;45:337-341.
3. Elmastas M, Isildak O, Turkekul I, Temur N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. Journal of Food Composition and Analysis. 2007;20:337-345.
4. Duh P-D. Antioxidant activity of burdock (*Arctium lapa Linné*): its scavenging effect on free radical and active oxygen. Journal of the American Oil Chemists' Society. 1998;75:455-465.
5. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. American Journal of Medicine. 1991;91(3C):14-22.
6. Cazzi R, Ricardy R, Aglitti T, Gatta V, Petricone P, de Salvia R. Ascorbic acid and b-carotene as modulators of oxidative damage. Carcinogenesis. 1997;18:223-228.
7. Frankel EN. Antioxidants in lipid foods and their impact on food quality. Food Chemistry. 1996;57:51-55.
8. Namiki M. Antioxidants/antimutagens in food. Critical Review in Food Science and Nutrition. 1990;29:273-300.
9. Halliwell B. Gutteridge JMC, Cross CE. Free radicals, antioxidants, and human disease - where are we now? Journal of Laboratory and Clinical Medicine. 1992; 119:598-620.
10. German JB. Food processing and lipid oxidation. Advances in Experimental and Medical Biology. 1999;459:23-50.
11. Jacob RA. Three eras of vitamin C discovery. Subcellular Biochemistry. 1996;25:1-16.
12. Boari F, Cefola M, Di Gioia F, Pace B, Serio F, Cantore V. Effect of cooking methods on antioxidant activity and nitrate content of selected wild Mediterranean plants. International Journal of Food Science and Nutrition. 2013;64(7):870-876.
13. World Bank. Gross national income per capita Atlas method and PPP. World Bank, Geneva, Switzerland; 2009.
14. World Food Programme. Lesotho food security monitoring system quarterly bulletin, World Food Programme. 2011; Available:http://documents.wfp.org/stellent/groups/public/documents/ena/wfp240315.pdf
(Accessed: 17/09/2013)
15. Cobbe J. Lesotho: From Labor Reserve to Depopulating Periphery? Migration Information Source, N.D.
Available:http://www.migrationinformation.org
(Accessed: 22/11/2013)
16. Mba CJ. Determinants of Living Arrangements of Lesotho's Elderly Female Population. International Journal of Women's Studies. 2002;3(2):1-22.
17. Baydar NG, Baydar H. Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (*Rosa damascena* Mill.) extracts. Industrial Crops and Products. 2013;41:375-380.
18. Mavi A, Lawrence GD, Kordali SA, Yildrim A. Inhibition of iron-fructose-phosphate-induced lipid peroxidation in lecithin liposome and linoleic acid emulsion systems by some edible plants. Journal of Food Biochemistry. 2011;35:833-844.
19. Anesini C, Ferraro GE, Filip R. Total Polyphenol Content and Antioxidant Capacity of Commercially Available Tea (*Camellia sinensis*) in Argentina. Journal of Agriculture and Food Chemistry. 2008; 56:9225-9229.
20. Paixão N, Perestrelo R, Marques JC, Câmara JS. Relationship between antioxidant capacity and total phenolic

content of red, rose´ and white wines. Food Chemistry. 2007;105:204-214.

21. Pakade V, Cukrowska E, Chimuka L. Comparison of antioxidant activity of *Moringa oleifera* and selected vegetables in South Africa. South African Journal of Science. 2013. 109 (3/4), Art. #1154. Available:http://dx.doi.org/10.1590/sajs.2013/1154 (Accessed: 01/05/2014)
22. Thoo YY, Ho SK, Liang JY, Ho, CW, Tan CP. Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). Food Chemistry. 2010;120:290-295.
23. Lapornik B, Prošek M, Wondra AG. Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering. 2005;71:214-222.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://sciencedomain.org/review-history/10335*