

THE NUTRITIONAL VALUE AND ANTIOXIDANT ACTIVITY OF BAY LEAVES (*Laurus nobilis* L.)

Alaa G. AL-Hashimi ,Sawsan A. Mahmood

Department of food science, college of agriculture, university of Basrah

(Received 1 March 2016 ; Accepted 3 April 2016)

Key words Bay leaves (*L.nobilis*), Antioxidant, Chemical composition.

ABSTRACT

The present study is to identify the chemical composition of bay leaves (*Laurusnobilis* L.) protein, oil, ash and carbohydrate which were (7.62, 8.5, 3.63, 50.83)% respectively. This paper reports the concentration of many minerals calcium, Phosphorus, Potassium, Iron, Copper Magnesium, Manganese, and Zink. Calcium and Magnesium have the highest concentration (377, 550 mg /g) respectively, Vitamins concentration also determined riboflavin, ascorbic acid which were (45.33, 2, 0.90 mg/g) respectively. Bay oil. Was extracted from bay leaves (*L.nobilis*) with petroleum ether using Soxhlet apparatus. Bay oil showed high value in unsaturated fatty acids 55% include oleic, linoleic, linolenic, and saturated fatty acids 45% include lauric, myristic, palmitic, stearic. The present study describe the reducing power and antioxidant activity for alcoholic extracts of bay leaves. rates of antioxidant activity and reducing power increases as the concentration of bay leaves extract increased which were (173.81%) and (75.61%) respectively at 5% concentration.

INTRODUCTION

Laurusnobilis L. belongs to the family Lauraceae, which comprises numerous aromatic and medicinal plants (21).

Bay (*L.nobilis*) leaves, or Turkish laurel, is an industrial plant used in foods, drugs and cosmetics. The dried leaves and essential oils are used extensively in the food industry for seasoning of meat products, soups and fish (23). The plant is widely cultivated in Europe, America and in the Arabian countries, from Libya to Morocco (24). The dried leaves are

used extensively in cooking, and the essential oil is generally used in the flavorings industry (10). Leaves of this plant produce yellow oil known for many therapeutic indications, Leaves and their essential oil increase gastric fluid secretion and work against digestive disorders such as flatulent colic (28). The essential oil obtained from the leaves of this plant has been used for relieving hemorrhoid and rheumatic pains (51). It also has diuretic (6,51), antifungal (39) and antibacterial activities (46). In the study of (43) the main components of the essential oil were cineol (44.12%), eugenol (15.16%), sabinene (6.20%), 4-terpineol (3.60%), α -pinene (2.74%), methyleugenol (2.48%), α -terpineol (2.19%) and β -pinene (2.05%) (43). Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (12). Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils (31).

MATERIALS AND METHODS

Plant materials

Leaves of (Lauraceae) (*L.nobilis*) were purchased from Basrah local market and dried until constant weight.

Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade

Chemical Composition

Moisture content, crude protein, crude oil, ash content, crude fiber, total carbohydrates of (*L.nobilis*) leaves were determined according to (4). Moisture content of leaves of bay plant was determined by drying samples at 105 °C overnight (4). Total carbohydrates were obtained by subtraction of contents of the moisture, ash, oil, protein and crude fiber from 100.

Mineral analysis

The mineral contents (elements) of (*L.nobilis*) leaves: calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were determined using the atomic absorption spectrophotometer (AAS-Buck 205), as described the methods of the Association of Official Analytical Chemists

(4).Phosphorus was determined calorimetrically (4). Ascorbic acid determined by titration (4).Riboflavin was extracted according to the method described in (4). All the determinations were done in duplicates.

Oil extraction

Bay leaves extracted by Soxhlet according to the method of (4), the fatty acid detected according to (2).

Alcoholic extraction

Prepared according to (20), 5 g of powdered material along with 100 ml of alcohol was shaken well occasionally for the first 6 h and kept undisturbed for 18 h. The liquefied extract thus obtained was concentrated in a vacuum pump and the percentage was calculated with the weight of the bay leaves powder taken.

Determination of reducing power

The reducing power of extracts from bay leaves sample was determined according to the method of (35). Extracts solution in methanol and water at different amounts (0.2 to 1 mg) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After 2.5 ml of trichloroethanoic acidTCA (10%) was added, the mixture was centrifuged at 3000 rpm for 10 min. Supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of ferric chloride (0.1%) and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates greater reducing power.

Antioxidant activity assay

The antioxidant activity analysis using ferric thiocyanate was performed according to the method reported by (34). 0.6 g of bay leaves sample was dissolved in 0.12 ml of 98% ethanol, and 2.88 ml of a 2.51% linoleic acid solution in ethanol and 9 ml of a 40 mM phosphate buffer (pH 7.0) were added. The mixture was incubated at 40°C in a stoppered test tube in the dark for 3 days. During the incubation, 0.1 ml aliquot was taken from the mixture, and diluted with 9.7 ml of 75% ethanol, followed by the addition of 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after adding the 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid, the absorbance of the red color was measured

at 500 nm. The level of lipid peroxidation inhibition by each fraction was calculated from the absorbance ratio to that of a blank (without any sample).

RESULTS AND DISCUSSION

Nutritional Composition

The chemical composition of bay(*L.nobilis*) leaves showed in Table 1. The findings indicated that moisture content of leaves is 4.95% which is approach to those values obtained by (49),Protein content (7.62%) of leaves studied is coincided with those given by (49)Whereas, crude oil of leaves studied is 8.57%.and the ash content is 3.63%, the crude fiber content was 24.40% , while total carbohydrates of sample investigated is 50.83 %.

TABLE (1) Chemical composition of bay leaves.

Parameter Sample	Bay leaves studied
Moisture	4.95
Protein	7.62
Ash	3.63
Oil	8.57
Crude fiber	24.40
Carbohydrate	50.83

Table-2 showed that (*L.nobilis*) leaves contained essential minerals, Ca (377 mg/100g), P (112 mg/100g) , K(550 mg/100g) , Fe (45 mg/100g), Cu (0.63 mg/100g), Mg (112mg/100g), Mn (7.313 mg/100g), Zn (2.90 mg/100g)The differences in the composition may be due to the differences in the locality of its growth and the stage at maturity prior to harvesting,A number of heavy metals such as Co, Fe, Mn, Mo,Ni, Zn, Cu are essential micronutrients and required for normal plant growth and development since they are constituents of many enzymes and other proteins in plants, Heavy metals considered nonessential such as Pb,Cd, Cr, Hg are potentially toxic for plants (13,40,45).

In the adult human body, Ca content comprises approximately 1000 g in women and 1200g in men. Most of it (>99%) is located in the skeleton and the teeth as hydroxyapatite, The remaining Ca is found in blood, extracellular fluid, muscle and other tissues and cells (14). Calcium helps in regulation of muscle contraction required by children, pregnant and lactating women for bones and teeth development (26).

Phosphorus is an essential nutrient for the skeleton and deficiency causes rickets in children (38) and osteomalacia in adults (17). After oxygen, hydrogen, carbon, nitrogen and Ca, P is the 6th most abundant element in the human body, A 70-kg man has approximately 700 g of P in his body, Around 80-85% of the P is located in the skeleton as hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), The remaining (P.) is located in extracellular fluids and soft tissues, mainly as a component of proteins, phospholipids, nucleotides and nucleic acids (11).

High amount of potassium in the body was reported to increase iron utilization (1), and beneficial to people taking diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid (7).

Iron is said to be an important element in the diet of pregnant women, nursing mothers, infants convulsing patients and elderly to prevent anemia and other related diseases (33).

According to the literatures, the normal limits of Copper in plant tissues are in range of 4-15 mg/kg d. wt. and between 20-100 mg/kg d.wt. are accepted toxic levels (22).

Cu acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (27,42).

Magnesium plays fundamental roles in most reactions involving phosphate transfer; it is believed to be essential in the structural stability of nucleic acid and intestinal absorption while its deficiency in man is severe diarrhea and migraines (5) and plays a role in energy production and in supporting the immune system (32).

Manganese supports the immune system, regulates blood sugar levels and is involved in the production of energy and cell reproductions, It works with vitamin K to support blood clotting. Working with the B- complex vitamins, manganese helps to

control the effects of stress, Birth defects can possibly result when an expecting mother does not get enough of this important element (3).

Zinc is said to be an essential trace element for protein and nucleic acid synthesis and normal body development (30). Zinc is known for boosting the health of the hair, it is believed to play a role in the proper functioning of some sense organs such as ability to taste, sense and smell (36).

Riboflavin is essential for normal development, growth, reproduction, lactation, physical performance. It is participated in many essential biochemical oxidation – reduction reaction especially those yield energy (29).

Vitamin C is an electron donor therefore a reducing agent, all known physiological and biochemical actions of vitamin C are due to its action as an electron donor. Ascorbic acid donates two electrons from a double bond between the second and third carbons of the 6- carbon molecule. Vitamin C is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized, however by the very nature of this reaction, vitamin C itself is oxidized in the process (19).

Table (2) minerals and vitamins in bay leaves (mg/100g)

Element	Mg / 100g
Calcium	377
Phosphorus	112
Potassium	550
Iron	45
Copper	0.36
Magnesium	112
Manganese	7.313
Zinc	2.90
Riboflavin	0.90
Ascorbic acid	45.33

As shown in Table 3, many fatty acids were detected in the bay leaves oils. Lauric and oleic acids were considered as major fatty acids followed by linoleic and palmitic, while myristic, stearic and linolenic acids were low. Results illustrate Oleic acid, the major monounsaturated fatty acid (35%). The increase in oleic acid content is due to the triacylglycerols active biosynthesis which takes place throughout fruit ripening, involving a fall in the relative percentage of palmitic acid content. On the other hand, the increase in linoleic acid content (18.5) is due to the transformation of oleic acid into linoleic acid by the oleatedesaturase activity which is active during triacylglycerol biosynthesis (18, 44). The highest content of lauric acid (28.2) as important saturated fatty acid were found in bay leaves and the lowest value were found for myristics (0.6%). Variations in fatty acid composition observed in bay leaves oil might be related to both genetic factors and environmental conditions during the development and maturity of the fruit. These results are in accordance with the results of other researches (8, 25, 41).

TABLE (3) Fatty Acids content of bay leaves oil

Fatty Acid [%]	Bay leaves oil
Lauric (C12:0)	28.2
Myristic (C14:0)	0.6
Palmitic (C16:0)	15
Stearic (C18:0)	1.2
Oleic (C18:1)	35
Linoleic (C18:2)	18.5
Linolenic (C18:3)	1.5

Figure (1) shows the reducing power of bay (*L.nobilis*) leaves. The presence of reducers (the antioxidants) causes the conversion of the Fe^{3+} /ferricyanide complex to the ferrous form. The reducing power of all samples increased with increasing of concentration. The reducing power of ascorbic acid showed the highest reducing ability which was (218.22%) at the concentration 5 mg/ml, followed by The reducing power of citric acid which was (185.25%) at the same concentration reducing power of alcoholic extract of bay leave 173.81% at 5 mg/ml was found to be significantly higher than ButylatedHydroxyToluene (BHT) which was 92.11% at 5 mg/ml and α -tochopherol was (60.97%) at 5mg/ml. It was reported that, the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (47). Accordingly, might the bayleave contain higher amount of reduction, which could react with free radicals to stabilize and block radicalchain reactions.

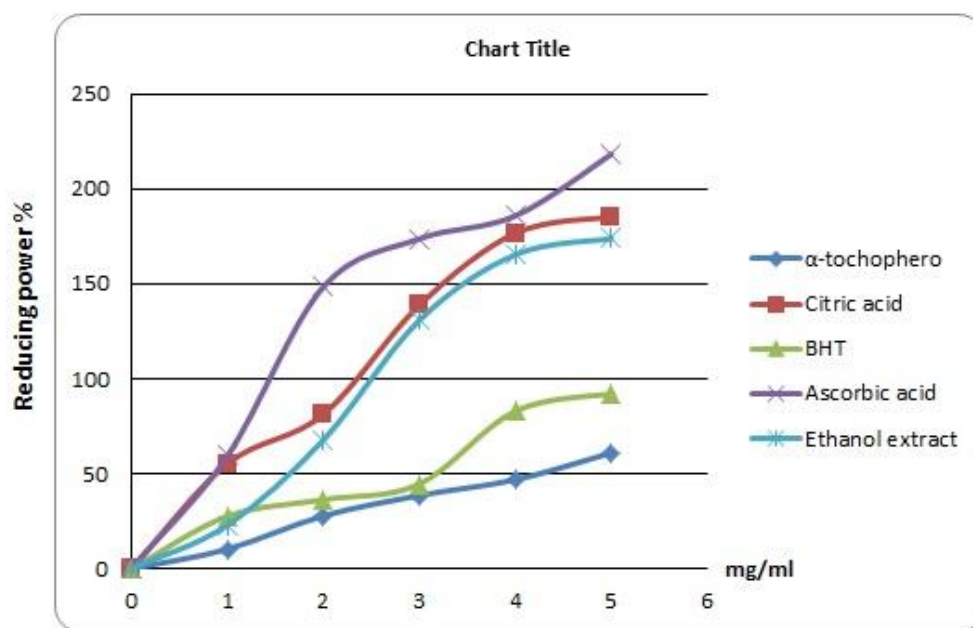
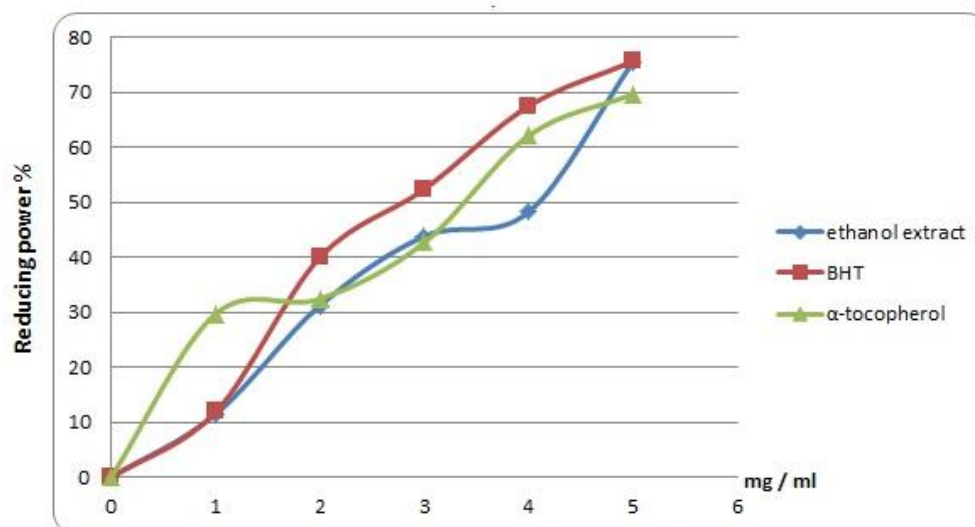


Figure (1) Reducing power of alcoholic bay leaves
(*L.nobilis*) extracts

Figure (2) represent The antioxidant activities of bay(*L.nobilis*) leave extracted by ethanol comparing with artificial antioxidant BHT, α -tocopherol. The data shows that the antioxidative activities of all samples increased when the concentration are increased. The antioxidative activities of ethanolic extraction and BHT was approximately equal in rates (75.61%) and (75.67%) followed by α - tocopherol (69.72%) at the concentrate 5 mg/ml.

In previous phytochemical investigations on *L. nobilis* leaves and fruits, different groups of chemicals were isolated: flavones (apigenin and luteolin) (48), flavonols (kaempferol, myricetin, and quercetin) (50), sesquiterpene lactones (16,37), glycosylated flavonoids (15). Amount of flavonoids in extracts plays a significant role in their antioxidative capacity.

Figure (2) Antioxidant activity alcoholic bay leaves (*L.nobilis*) extracts

القيمة الغذائية والفعالية المضادة للأكسدة لورق نبات الغار (*L.nobilis*)

آلاء غازي الهاشمي ، سوسن علي محمود

الخلاصة

درس التركيب الكيميائي لورق الغار (*L.nobilis*) إذ بلغت نسبة كل من البروتين والزيت والرماد والكربوهيدرات (7.62 و 8.5 و 3.63 و 50.83 %) على التوالي .

قدر تركيز العديد من المعادن الكالسيوم والفسفور والبوتاسيوم والحديد والنحاس والمغنيسيوم والمنغنيز والزنك وكان أعلى تركيز للكالسيوم والمغنيسيوم (377 و 550 ملغم /غم) على التوالي، كما درس تركيز فيتامينات الريبوفلافين والنياسين وحامض الاسكوربيك (0.90 و 2 و 45.33 ملغم /غم) على التوالي. أظهر الزيت المستخلص من ورق الغار ارتفاعاً في الاحماض الدهنية غير المشبعة 55% شملت الاوليك واللينوليك واللينولينيك، أما الاحماض الدهنية المشبعة 45% والتي شملت اللوريك والميرستيك والبالمتيك والستياريك. وشملت الدراسة الحالية تقدير القوة الاختزالية والفعالية المضادة للأكسدة للمستخلص الكحولي لورق الغار وزيادة كل منهما بزيادة التركيز إذ بلغت 173.81 و 75.61 % على التوالي عند تركيز 5%.

REFERENCES

- 1- Adeyeye, E. I. (2002). Determination of the Chemical Composition of the Nutritionally Valuable Parts of Male and Female Common West African Fresh Water Crab (*SudananoutesAfricanus*). International Journal of Food Sciences and Nutrition 53:189-196.

- 2- Al-Kaisey, M.T., (1992). Some chemical and nutritional proprieties of soy been seeds J. Agric. Sci., 5(1):21-28 .
- 3- Anhwange BA, Ajibola VO, Oniye SJ (2004). Chemical Studies of the seeds of *Moringaoleifera* (Lam) and *Deuterium microcarpum* (GuillandSperr). J. Biol. Sci. 4(6): 711-715.
- 4- AOAC (1990) Official methods of analysis of the Association of Official Analytical Chemists. 2 vols. 15th ed. Washington, DC.
- 5- Appel, L. J. (1999).NonpharmacologicTherapiesthat Reduce Blood Pressure.A fresh Perspective.Clin.Cadiol: 1111-1115.
- 6- AqiliKhorasani, M.S. 1992.Collection of drugs (Materiamedia), Enqelab-e-Eslami Publishing and EducationalOrganization, Tehran.624-630.
- 7- Arinanthan, V., Mohan, V. R. and Britto, A. J. (2003). Chemical Composition of Certain Tribal Pulses in South India. InternationalJournal of Food Sciences and Nutrition 3: 103-107.
- 8- Baccouri B, Temime SB, Campeol E, Lcioni P, Daoud D, Zarrouk M (2007) Application of solid-phasemicroextracation to the analysis of volatile compoundsin virgin olive oils from five new cultivars. Food Chem102: 850-856.
- 10- Bauer, K. and D. Garbe (1985) .Common fragrance and flavor materials, preparation, properties and uses.VCH.Weinheim, Germany.
- 11- Berner YN, ShikeM(1988). Consequences of phosphate imbalance.Annu Rev Nutr;8:121-148.
- 12- Burt, S., 2000. Essential oils: their antibacterial properties and potential applications in foods: a review. Inter J Food Microbial, 94: 223-253.
- 13- Devi, S.R. and M.N.V. Prasad. (1998). Copper toxicity in*Ceratophyllumdemeresum* L. (Coontail), a free floatingmacrophyte: Response of antioxidant enzymes andantioxidants. Plant Sci., 138: 157-165.
- 14- Favus MJ, Bushinsky DA, Lemann J Jr(2006). Regulation of calcium, magnesium, and phosphatemetabolism. In: Favus MJ (ed.) Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism.6th ed. Washington DC: American Society for Bone and MineralResearch; pp.76-83.
- 15- Fiorini, C.; David, B.; Fouraste, I.; Vercavteren, J. Acylatedkaempferol glycosides from *Laurusnobilis*leaves. *Phytochemistry*1998, 47, 821-824.

- 16- Fischer, H.; Oliver, J.; Fischer, D. *Progress in the Chemistry of Organic Natural Products*, 4th ed.; Herz, B., Grisebach, H., Kirby, G.W., Eds.; Springer-Verlag: New York, NY, USA, 1979;Chapter 2, pp. 47-390.
- 17- GenantHK(1993). Radiology of osteoporosis. In: Favus MJ (ed.) Primer on the metabolic bone diseasesand disorders of mineral metabolism. New York, US: Raven Press, Ltd;.p.229-240.
- 18- Gutiérrez F, Jiménez B, Ruiz A, Albi M A (1999) Effectof olive ripeness on the oxidative stability of virginolive oil extracted from the varieties Picual andHojiblanca and onthe different components involved. JAgric and Food Chem 47: 121–127.
- 19- Halliwell B,2000: Why and how should we measure oxidative DNA damage innutritional studies? How far have we come? Am J ClinNutr 72:1082–1087.
- 20- Harbone B (1973). Phytochemical methods, Chapman and Hall Limited, London. pp. 49-189.
- 21- Hogg, J.W., S.J. Terhune and B.M. Lawrenc(1974). Dehydro-1.8-cineole: A newmonoterpene oxide in Laurusnoblis oil. Phytochem., 13, 868-869.
- 22- Kabata-Pendias, A. and H. Pendias (2001). Trace Elements in Soils &Plants, CRC Press, LLC (Third Ed) Boca Raton,Florida.
- 23- Kilic, A., Hafizoglu, H., Kollmannsberger, H. and Nitz, S.(2004).Volatile constituents and key odorants inleaves, buds, flowers, and fruits of Laurusnobilis L. Journal of Agricultural and Food Chemistry 52, 1601–1606.
- 24- Kumar, S., Singh, J. and Sharma, A. (2001).Bay leaves. In: Peter, K.V(ed.) Handbook of Herbs and Spices.Woodhead Publishing Limited, Cambridge, UK, pp. 52–61.
- 25- Manai H, Haddada FM, Oueslati I, Daoud D, Zarrouk M (2008) Characterization of monvarietalViriginoliveoils from six crossing varieties. ScieHort 115: 252-260.
- 26- Margaret, L. and Vickery, B. (1997).Plant Productsof Tropical Africa.Macmillan in College ed. London.
- 27- Marschner H (1995) .Mineral nutrition of higher plants. Academic Press, London.
- 28- Matsuda, H.; Shimoda, H.; Ninomiya, K.; Yoshikawa, M (2002). Inhibitionmechanism of costunolide, a sesquiterpene lactone isolatedfromLaurusnobilis, on blood-ethanol elevation in rats: involvementof inhibition of gastric emptying and increase in gastric juicesecretion. Alcohol. Alcohol., , 37, 121-127.

- 29- McCormick DB(1999). Riboflavin. In: Shils M, Olson JA, Shike M, Ross AC, eds. Modern Nutrition in Health and Disease. 9th ed. Baltimore: Williams & Wilkins;:391–399.
- 30- Melaku, U., Clive, E. W. and Habtamon, F. (2005).Content of zinc, Iron, Calcium and TheirAbsorption Inhibitors in Ethiopia.Journal of FoodComposition Analysis18: 803-817.
- 31- Milhau, G., Valentin, A., Benoit, F., Mallie, M., Bastide, J., Pelissier, Y. and Bessiere, J. 1997. In vitro antimicrobial activity of eight essential oils. J. Ess. Oil Res. 9:329-333.
- 32- Muhammad, A, - Dangoggo, S.M, - Tsafe, A.I, - Itodo, A.U. and Atiku, F.A. 2011. Proximate, minerals and anti-nutritional factors of *Gardenia aqua lla* (*Gaudendutse*)fruit pulp. In *Pakistan Journal of Nutrition*, vol. 10, no. 6, p. 577-581.
- 33- Oluyemi EA, Akinlua AA, Adenuga AA, Adebayo MB (2006). MineralContents of some commonly consumed Nigerian foods. Sci. Focus.11(1): 153-157.
- 34- Osawa T, Namiki M (1981). A novel type of antioxidant isolated from leaf wax of eucalyptus leaves. Agric. Biol. Chem. 45:735-739.
- 35- Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307-315.
- 36- Payne, W. J. A. (1990). An Introduction to Animal Husbandry in the Tropics. Longman Publishers Singapore Pp 92-110.
- 37- Pech, B.; Bruneton, J. Alkaloids of *Laurusnobilis*. *J. Nat. Prod.* 1982, 45, 560-563.
- 38- PettiforJM(2008). What`s new in hypophosphataemic rickets? Eur J Pediatr;167:493-499.
- 39- Qamar, S. and Chaudhary, F.M. (1991).Antifungal activityof some essential oil from local plants. Pak. J. Sci. Indust.Res. 34: 30-31.
- 40- Rai, V., P. Vaypayee, S.N. Singh and S. Mehrotra.(2004). Effectof chromium accumulation on photosynthetic pigments,oxidative stress defense system, nitrate reduction, prolinelevel and eugenol content of *Ocimumtenuiflorum* L. Plant
- 41- Ramezani-Kharazi P (2008) Does amount of phenoliccompounds depend on olive varieties? J Food, AgricandEnvir 5: 125-129.
- 42- Raven JA, Evans MCW, Korb RE (1999). The role of tracemetals in photosynthetic electron transport in O2-evolvingorganisms.Photosynth. Res. 60:111-149.

- 43- Riaz, M., Ashraf, C.M. and Chaudhary, F.M. (1989).Studies of the essential oil of the Pakistani *Laurusnobilis*Linn in different seasons. Pak. J. Sci. Indust. Res. 32: 33-35.
- 44- Sanchez J, Harwood JL (2002). Biosynthesis of triacylglycerols and volatiles in olives. Euro J Lipid Scie and Techn 104: 564–573.Sci., 167: 1159-1169.
- 45- Sebastiani, L., F. Scebba and R. Tongetti.(2004). Heavy metal accumulation and growth responses in poplar clones Eridano (*Populus deltoides* x *maximowiczii*) and I-214 (*P.xeuramericana*) exposed to industrial waste. Env.Exp.Bot., 52: 79-88.
- 46- Seyed, M., Riaz, M. and Chaudhary, F.M. (1991).The antibacterial activity of the essential oil of the Pakistani *Acotusalmus*, *Callistemon lanceolatus* and *Laurusnobilis*. Pak. J. Sci. Indust. Res. 34: 456-458.
- 47- Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the autooxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40: 945-948.
- 48- Škerget, M.; Kotnik, P.; Hadolin, M.; Hraš, A.; Simonič, M.; Knez, Ž. Phenols. (2005) proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.*, 89, 191-198.
- 49- Tainter, D. R., & Grenis, A. T. (1993). Spices and seasonings. New York: VCH Publishers, Inc., p. 251
- 50- Vidal-Ollivier, E.; Elias, R.; Faure, F.; Babadjamian, A.; Crespin, F.; Balansard, G.; Boudon, G. Flavonal glycosides from *Calendula officinalis* flowers. *Planta Med.* 1989, 55, 73-74.
- 51- Zargari, A. 1990. Medicinal Plants, Tehran University press, Tehran, Vol. IV, pp.325-328.