International Journal of Scientific Research and Engineering Studies (IJSRES)
Volume 3 Issue 3, March 2016
ISSN: 2349-8862

Evaluation Of Antimicrobial Activity Of Oil Extracted From Three Different Citrus Seeds (Citrus Limon, Citrus Aurantifolia And Citrus Aurantium)

Aladekoyi G.

Omosuli S.V.

Orungbemi O.O.

Food Science And Technology Dept, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria

Adesuyi A. T.

Science Laboratory Technology Dept, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria

Abstract: ***As pathogenic organisms are becoming resistant to present day antibiotics, this study focuses on antimicrobial activity and future prophylactic potential of oil extracted from lemon seed (citrus limon), lime seed (citrus aurantifolia) and bitter orange seed (citrus aurantium). Antimicrobial analysis was done by using agar well diffusion method against bacterial pathogens. Oil from Lime, Bitter Orange and Lemon seeds have 0.15mm, 0.10mm and 0.20mm against Proteus spp , and 0.20mm, 0.25 and 0,10mm against Pseudomonas respectively. The value against Staphylococcus is 0.10mm for all the three samples. Lemon seed oil has 0.20mm against Klebsiella while there was no action against Lime and Bitter orange seed oil respectively. Lime and Lemon seed oil has 0.10mm and 0.15 against E. colli while there is no zone against Bitter orange seed. Bitter orange has a maximum zone of inhibition of 0.25mm against Pseudomonas, followed by zone of inhibition of 0.20mm against Proteus and Klebsiella in Lemon seed Oil and 0.20mm against Pseudomonas in Lime seed oil respectively.***

Keywords: Pathogenic, organisms, Antimicrobial, Citrus Seeds, Oil

I. INTRODUCTION

The Citrus species, belonging to the family *Rutaceae*, is an annual plant that is widely distributed in the Mediterranean countries of the Middle East and Southern Europe but also grows abundantly in other warm climates worldwide. The plants of most species of Citrus are large evergreen shrubs or small trees, and their fruits are among the most important tree fruit crops in the world. Citrus fruits are of great economic importance because of their varied uses (Saidani *et al*., 2004). Citrus species are of prime economic importance in both fresh and processed fruit markets. Consequently, large amounts of Citrus seeds are discharged at processing plants. At present Citrus fruits are processed to produce juice, jam or marmalade and the wastes of this industry such as peels, seeds and pulps represent about 50% of the raw processed fruit (Ben, 1967). This not only wastes a potentially valuable resource, but also aggravates already serious disposal problems. So, expansion in the Citrus industry in recent years attracts attention to the further use of the Citrus seeds as a potential source for other nutrients. Obviously, such an objective would improve the utilization of the Chemical evaluation of citrus seeds, an agro-industrial waste, as a new potential source of vegetable oils.

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacterial, fungi, protozoa or viruses. Antibiotics are those substances which are produced by micro-organisms that kill or prevent the growth of other micro-organisms. Antibiotics are generally used against bacteria, antiviral are specifically for treating viral infections. Antifungal are used for treating fungal infections, some of those side effects can be life threatening if the drug is not used properly. Several micro-organism derived antibiotics are currently in use to treat a variety of human diseases, therefore the action must be taken to control the use of antibiotics, develop new drugs either synthetic or natural for a long period of time, plant have a valuable source of a natural products for maintaining human health. India has a rich tradition in use of medicinal plants to develop drugs. According to World Health Organization (WHO), any plant which contain substances that can be used for therapeutic purpose or which are precursor of chemo-pharmaceuticals semi-synthetic new drugs is referred as medical plant (Salih and Abass, 2003). Medical plant would

be the best source to obtain a variety of drugs as the phytochemical are more specific phytochemical offer unique platform for structural diversity and biological functionality which is indispensable for drug discovery.

Citrus lemon of *Rutaceae* family which common name is lemon is originated from South East Asia, probably in India or Southern China. Lemon is a pale yellow, elliptically shaped berry fruit. Citrus fruit in general contain sugar, polyssacharide, organic acid, lipids, carolenoids, and vitamins.Limon or lime juices have been reported to exhibit antimicrobial activity against Vibrio cholera (Hiroyuki *et al*., 2006).

The antimicrobial potential of some Indian herbal oils with a view to exploring their potential to application in food industries as botanical preservation (Gupta *et al*., 2008). Peel waste are highly perishable and seasonal is a problem to the processing industries and pollution maintaining agencies. Suitable methods have to be adopted to utilise them for the conversion into value added products (Kumal *et al.,* 2011). Citrus peels are employed for a variety of uses, as fodder at fisheries, activated carbon, raw materials for traditional paper (Javed *et al.,* 2011). Citrus fruit products are known to protect antimicrobial agent's juice bacterial, fungus (Mathur *et al.*, 2011). The antimicrobial activity of plants had been received attention many years ago as one of the most effective mechanism for the control of micro-organisms (Saadi *et al*., 2003). Pharmacologically, lemon is primarily important for its vitamin C and potassium content. German studies in the late 1980s related to effect of peel lemon oil also posses antioxidant activity clinical trails are lacking. Lemon juice may increase iron absorption.

Rutaceae is a large fruit family comprising of 140 genera and 1300 species. According to the "Flora of Pakistan", 27 species of *Rutaceae* family grow in Pakistan, out of these 27; species 10 belong to genus *Citrus*. Genus *Citrus* is one of the most important genera because of its fruit, which are esteemed primarily as articles of diet. Apart from the international value of Citrus fruit, it has very important medicinal, as well as commercial values. The oil yielded from the fruits, rinds, flowers and seeds of various Citrus species are used in confectionary, toiletry and perfumery industry (Hua and Yahong, 1986).

The international fresh orange lime and lemon producing sectors evolved substantially in recent years, driven by increased competition, and significant shifts in production, consumption, trade, and marketing. Over the last two decades, developing countries less growth in production and consumption, while output and consumption among traditional developed-country suppliers was stable or declined. Technological advancements in storage and shipping, and trade liberalization, resulted in expanded trade in the subject products. The rise in market power of global retailers also profoundly affected the sectors.

Sweet orange (*Citrus sinensis* (L.) is the world's most commonly cultivated fruit tree. It belongs to the Rutaceae family which comprises mandarins, limes, lemons, grapefruits, sour and sweet oranges (Karoui and Marzouk, 2013). Citrus fruits are of immense economic value; occupying the top position in fruit production. Orange trees are widely cultivated in tropical and subtropical climates for the sweet fruit, which is peeled or cut (to avoid the bitter rind) and eaten whole, or processed to extract orange juice (Pandharipande and Makode, 2009; Kamal *et al*., 2011).

Citrus fruits are known to contain substantial quantities of vitamin C, a potent water-soluble vitamin essential for healthy living. They are also known to contain other bioactive components such as carotenoids and a wide array of phenolic compounds. The consumption of citrus fruits is also believed to confer some protection against diseases such as cardiovascular disease and cancer (Baghurst, 2003; Guimaraes *et al*., 2010; Atolani *et al*., 2012). The orange fruit is composed of an external layer (peel) formed by flavedo (epicarp or exocarp) and albedo (mesocarp), and an inner material called endocarp that contains vesicles with juice (Liu *et al*., 2007). The seeds are usually embedded at the centre of the fruit, in direct contact with the juice sacs. The peels obtained from citrus fruits constitute between 50 and 65% of the total weight of the fruits. When not processed further, this by-product becomes a very worrisome waste capable of causing serious environmental pollution (Mandalari *et al*., 2006; Hegazy and Ibrahim, 2012). In Nigeria, citrus fruit wastes are discarded carelessly in the environment. This leads to the release of odors, serving as fertile ground for insect proliferation and generally, the production of an unsightly environment with reduced aesthetic outlook.

CONSTITUENTS AND COMPOSITION: Lime exocarp contains an essential oil (7%) or typical volatile, whose main components are citral, limonene, B-pinene and fenchone (up to 15%). Further aroma compounds are terpineol, bisabolene and other terpenoids. Limonene was regarded as the major volatile component. Major fractions of peel oil include: monoterpenes (76%), sesquiterpenes (3.8%) and oxygen containing compounds (18.1%) such as aldehydes, esters and alcohols. Citrus essential Oils are made up mainly of hydrocarbons designated as terpenes and of a smaller amount of sesquiterpenes; these two components serve as carrier for more important class of oxygenated compounds which are usually the bearers of the characteristic odor of the oil in which they are contained (Nagy et al., 1977).

The composition of lime juice varies considerably with the variety of fruit and with the location where the fruit is grown which is based on analyses of juice from different varieties and from different production areas (Braverman, 1949). The fruit of citrus structure is called a hesperidium. The exocarp colored yellow, orange, or green and is called flavedo. Located in the exocarp are spherical oil glands containing. e.g., lemon oils. The white, spongy portion of the fruit wall is the mesocarp and is called albedo. The locule is an air-filled chamber in which the future seeds, the ovules, are found. In the hesperidium, the layer of endocarp lining the outside of the locule grows as hairs that project into the locule. Cells of these hairs store water, organic acids and sugars, and each hair therefore becomes juice sacs. These juice sacs eventually fill the locule. The locule, when mature, becomes the section of a tangerine containing the juice sacs (hairs) within (Nagy *et al*., 1977).

BENEFITS: Citrus contain unique flavonoid compounds that have antioxidant and anti-cancer properties. Within these

flavonoids, d-limonene has been shown to inhibit mammary tumours in rats. The interesting property of flavonoids in lime juice is perhaps their antibiotic effects, for example against Vibrio cholero (Mata *et al.,* 1994). Limes are an excellent source of vitamin C, one of the most important antioxidants in nature (Wood, 1988).

CITRUS LIMONOIDS: Citrus fruits are one of the most popular foods in the world with worldwide agricultural production over 100 million metric tons per year. While a significant quantity of citrus is consumed as fresh fruit, more and more of the crop is being consumed in the form of processed products such as juices, concentrates, flavored, citrus beverages and other food products. However, one of the long-standing problems in processed citrus products has been bitter. The level of bitterness varies among different cultivars. Bitterness in citrus juices is caused by two groups of chemicals: the flavanone neohesperidosides, such as maringin and limonin, found in species relates to the pummelo and lemon, respectively, and the limonoids (Maier *et at.,* 1980 and Nagy *et al.,* 1977).

LIMONIN AND OTHER LIMONOIDS: A group of chemically related triterpene derivatives found in the Ruiaceae and Meliaceae families has been named limonoids, after the first known compound of this type, limonin. The latter has received considerable attention due to the bitter taste of most limonoids: Ruiaceae includes the citrus species of commerce (Nagy *et al.,* 1977). Limonoids have recently attracted attention because compounds belonging to this group have exhibited a range of biological activities like insecticidal, insect antifeedant and growth regulating activity on insects as well as antibacterial, antifungal, anticancer, antiviral and a number of other pharmacological activities on humans. Interest in limonoids research has become greater than before also because some of them are responsible for producing bitterness in citrus fruits, which has negative impact on citrus fruit and juice industry worldwide. The major limonoid in this family is limonin, accountable for the bitterness of poor quality juices (Mark *et al.,* 2000).

STRUCTURE OF LIMONIN: Characteristic structural features of limonoids are seen in the formula of limonin (Figure 2.3). All naturally occurring citrus limonoids contain a furan ring attached to the D-ring. at C-17, as well as oxygen containing functional groups at C-1. C-3, C-7, C-16 and C-17 (Somrutai et al., 2005). The structural variations of limonoids found in Ruiaceae are less than in Meliaceaie and are generally limited to the modification of A and B rings, the limonoids of Meliaceae are more complex with very high degree of oxidation and rearrangement exhibited in the parent limonoid structure (Suarez *et al.,* 2002).

PROPERTIES OF LIMONIN: Limonin's chemical composition is $C_{26}H_{30}O_8$ with a molecular weight of 470 which contains two lactones groups. Limonoids are heavily oxygenated, modified terpenoids dominant in the plant family Meliaceae, and to a lesser extent in Ruiaceae. They may be the most extreme examples of oxidation of triterpenes in nature. They have moderate polarity and are insoluble in water and hexane but soluble in hydrocarbons, alcohol and ketone. The limonoids are mostly bitter in taste and account for the scent of fresh peels of citrus fruits. Limonoids are present in neutral (noncarboxylated/aglycon) as well as acidic (carboxylated/ glucoside) forms, the former are insoluble and bitter while the latter are soluble and tasteless (Mark *et al.,* 2000; Kale and Adsule, 1995 and Nagy *et al.,* 1977).

II. MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL: Seeds of citrus plants (*C. aurantifolia, C limolin and C.limon*), were obtained from the whole fruits bought from "Oja Oba"market in Owo town, Owo local government area, in Ondo state.

PREPARATION OF THE SAMPLE: The epicarp of dried seeds of Limes (*C. aurantifolia, C limolin and C.limon*), samples were manually dehulled and the endosperm were chopped into pieces. The three samples were reduced to fine powder with the aid of a mechanical grinder to pass through 40 mesh sieve to increase the surface area for proper analysis. The milled powder samples were collected and stored in glass jars, tightly covered and kept for analysis.

PROCEDURE FOR OIL EXTRACTION: The extraction method used for this research is soxhlet extraction. 10 g by weight of the milled sample was filled in a thimble was weighed and recorded as W1 and then filled with the milled sample, reweighed and recorded as W2. The round bottom flask was weighed and recorded as W3 and then filled with the solvent (normal hexane) up to two-third of the flask. The reflux condenser was fitted to the top of the extractor and the water flow was turned on. The round bottom flask was placed in the heating mantle and the temperature of the mantle adjusted to 650C so the solvent is brought to the vaporization point. Each extraction occurs over a period of 3 to 4 hours. When the solvent has just siphoned over the barrel, the condenser is detached and the thimble removed. The filtrate is exposed to the atmosphere and the residual solvent is allowed to evaporate.

CHARACTERIZATION OF THE EXTRACTED OIL: In evaluating the quality of the extracted oil, the physico-chemical such as specific gravity, refractive index, viscosity, acid value, iodine value, peroxide value, saponification value and the Antimicrobial activity of the oil were analysed using AOAC, 2000 and agar – well technique (Pelczer and Black, 1993) respectively.

STERILIZATION OF GLASS WARE: All the glassware used the for this study such as Petri dishes, Agar bottle, test tube, conical flask, beakers, pipette and forcepts were soaked with detergent and rinsed with water. They were sterilized using hot air oven at a temperature of 120^{o}C for 2 hours. The wire loop was sterilized by heating it in the blue flame of the bursen burner until red hot and allowed to cool before using. 95% alcohol was used to swab the work bench area to prevent contamination. The process was carried out aseptically.

MEDIA AND REAGENT: Nutrient Agar (N.A) and Nutrient broth used were prepared according to manufacturer's instructions and autoclaved at 121^{o}C for 15mins.

ANTIMICROBIAL SCREENING TEST AND PHYSICAL CHARACTERISTICS OF African Bassil (*O.basilicum) and Sweet Bassil* (*O.gratissimum)* Essential oils

The extracts were tested for their antibacteria properties using the agar – well technique (Pelczer and Black, 1993). The assay for antibacteria activities was carried out with *E. coli, Staphylococcus aureus, Bacillus cereus, Salmonella typhii, and Klebsiella pneumonia*. Triplicate plates of media for each organism were inoculated with the appropriate suspension of bacteria. Agar well were aseptically made in the media with a sterile 6.0mm diameter cork borer. The different concentrations of the test solutions of extracts were dispensed (0.5ml) aseptically into the wells. The plates were kept in sterilized inoculation chambers for two hours to facilitate diffusion of solutions. The plates were then inoculated at 37^{o}C for 24hours for the bacteria. The diameters of the zones of inhibitions of bacteria growth were measured in the plates and the mean value and standard error for each organism was recorded.

III. RESULTS AND DISCUSION

Pathogens	Zone of inhibition (mm)		
	A	B	C
Proteus	0.15±0.02	0.10±0.01	0.20±0.01
Pseudomonas aeruginosa	0.20±0.03	0.25±0.02	0.10±0.02
Staphylococcus aereus	0.10±0.01	0.10±0.02	0.10±0.02
Klebsiella	No zone	No zone	0.20±0.02
Escherichia. coli	0.10	No zone	0.15±0.01

± DSV of triplicate result
A= lime seeds oil, B= bitter orange seeds oil, C= lemon seeds oil

Table 1

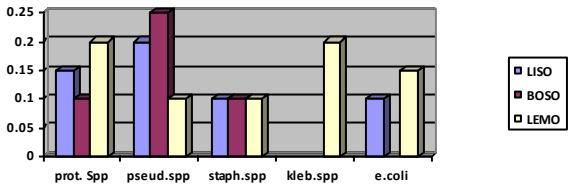

Chart 1: Bar chart representing susceptibility of each pathogenic organisms from different citrus seed oil LISO= lime seeds oil, BOSO= bitter orange seeds oil, LEMO= lemon seeds oil

DISCUSSION: Herbal medicines are a valuable and readily available resources for primary health care and complementary health care system, undoubtedly the plant kingdom still holds many species of plants containing substances of medicinal value that are yet to be discovered, though large number of plants are constantly being screened for their antimicrobial effects, these plants may prove to be a rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary.

Agar well diffusion method was used to get the antimicrobial activity of oil extracted from lemon, lime and bitter orange seeds against five pathogenic organisms. lime oil shows greater antimicrobial effect on *Pseudomonas aeruginosa*, having an inhibition zone of 0.20mm; it also has an inhibition zone of 0.15mm against *Proteus*, inhibition zone of 0.10mm against *Staphylococcus aureus*, and an inhibition zone of 0.10mm against *Escherichia coli*. But it has no zone of inhibition against *Klebsiella*.. bitter orange oil has a zone of inhibition of 0.10mm against *Proteus*, 0.25mm against Pseudomonas *aeruginosa* and 0.10mm against *Staphylococcus aureus* respectively, but it has no zone of inhibition against *Klebsiella* and *Eschirichia*. lemon has a zone of inhibition of 0.20mm against *Proteus*, 0.10mm against *Pseudomonas aeuruginosa*, 0.10mm against *Staphylococcus aureous*, 0.20mm against *Klebsiella* and 0.15mm against *Escherichia coli* respectively. These showed that oil with higher zone of inhibition has higher tendency of killing the target micro-organism when applied at the appropriate proportion, especially against Pseudomonas *aeruginosa* in bitter orange seed oil . This study shows that the oil extracted from the seeds of lemon, lime and bitter orange seeds are not only an astringent but also a good antimicrobial agent.

IV. CONCLUSION AND RECOMMENDATION

CONCLUSION: The result of antimicrobial susceptibility assay showed promising evidence for the antimicrobial effects of oil from lemon, lime and bitter orange seeds against bacterial (*Staphylococcus, Proteus* and *Pseudomonas*) pathogens. Oil from bitter lemon showed maximum zone of inhibition (0.25mm) against *Pseudomonas*, while oil from lemon seeds showed maximum zone of inhibition (0.20mm) against *Proteus*, (0.20mm) against *Klebsiella* and maximum zone of inhibition (0.15mm) against *E.coli* while *Staphylococcus* has the same zone of inhibition (0.10) against all tested oil samples

RECOMMENDATION: Pseudomonas and Staphylococcus can grow in presence of sebum, especially when it is secreted in excess (in certain person), and cause Purulent skin infections. Sometimes, it can serve as a predisposing factor for other types of infections like acne. Simple use of oil extracted from seeds of citrus species of lemon, lime and bitter orange can prevent such type of infections and could help in keeping a good and healthy skin. Moreover, oil from citrus species of lemon, bitter orange and lime are essential as an antimicrobial preservative of foods such as cream filled cakes and pastries, thereby reducing the microbial population of food that can lead to the risk of food poisoning.

REFERENCES

[1] Atolani,O., Omere, J., Otueehere, C.A and Adewuyi, A. (2012). Antioxodant and cytotoxicity effects of seed oils from edible fruits. Journal of Acute Disease: 130-134 (doi:10.1016/S2221-61899(13) 60073-6).
[2] Baghurst,K. (2003). The health benefits of citrus fruits. CSIRO Health Sciences and Nutrition full report. Project

No: CT02057. Horticulture Australia Ltd., Sydney, Australia.

[3] Ben Gem J.B. (1967). Citrus products: chemical composition and chemical technology. Inter-science publishers.

[4] Braverman, J.B (1949). Citrus products: chemical composition and chemical technology. Inter-science, New York.

[5] Guimaraes, R., Barros, L., Barreira, J.C., Sousa, M.J., Carvalha, A.M and Ferreira, I.C.F.R (2010). Targeting excessive free radicals with peels and juice of citrus fruits grapefruits, lemon, lime and orange. Food and Chemical Toxicology, 48(1):99-106.

[6] Gupta, C., Garey, A.P., Uniyal, R.C and Kumari, A. (2008). Antimicrobial activity of some herbal oils against common food borne pathogens. African Journal of Microbiology Research, 5(2):258-261

[7] Hegazy, A.E. and Ibrahim, M.I. (2012). Antioxidant Activities of Orange Peel extracts. World Applied Science Journal, 18(5): 684-688.

[8] Hiroyuki, T., Tetsuro, K., Masaguki, A.K., Fusao, O. (2006). Antimicrobial Activity of Citrus Fruits juice against vibro species, 52(2): 157-160.

[9] http://www.botgard.ucla.edu/html/botanytextbooks/economicbotany/Citrus/b1241tx.html)

[10] Hua and Yahong (1986). Fatty acid composition of neutral lipid: classes of citrus seed oil. Sepu, volume 4, issue 1-2, pp. 74-80.

[11] Javed, S., Jarid, A., Mahmood, Z., Nasim, F. (2011). Biocidal activity of citrus peels essential oils against some food spoilage bacterial. Journal of medical plants research 5(16): 3697-3701.

[12] Kale, P.N and Adsule, P.G. (1995). Citrus: Handbook of fruit science and technology-production, composition storage and processing. Van Nostrand Reinhod, New York.

[13] Kamal, G.M., Anwar, F.J., Hussain, A.L., Sarri, N and Ashrf, N.Y. (2011). Yield and chemical composition of citrus essential oils as affected by drying pre-treatment of peels. International Food Research Journal, 18(4): 1275-1282.

[14] Karoui, I.J and Marzouk, B. (2013). Characterization of bioactive compounds in Tunisian bitter orange *(Citrus aurantium L.).* Peel and juice determination of their antioxidant activities. Bio-medical Research International (http://dx.doi.org/10.1155/2013/345415).

[15] Kumar, K.A., Narayani, M., Subanthini, A., Jayakumar, M. (2011). Antimicrobial activity and phytachemical analysis of citrus fruit peels-utilization of fruit waste. IJEST, 3:0975-5462.

[16] Liu, Q., Xu, J., Liu, Y., Zhao, X., Deng, X., Guo, L and Gu, J. (2007). A novel bud mutation that confers abnormal lycopene accumulation in sweet orange fruit (*Citrus sinensis* L. Os beck). Journal of Experimental Botany, 58 (15-16):4161-4171.

[17] Maier, V.P, Hasegawa, S., Bennet, R.D and Echols, L.C (1980). Limonin and Liomonoids: Chemistry, bio-chemistry and juice bitterness. Journal of the American Chemical Society, 43:63-82.

[18] Mandalari, G., Bennett, R.N., Bisignao, G., Saija, A., Dugo, G., Faulds, C.B and Waldron, K.W. (2006). Characterization of flavonoids and pectin from bergamot (*Citrus bergamia Risso*) peel, a major by product of essential oil extraction. Journal of Agriculture and Food Chemistry, 54:197-203.

[19] Mark, A.B., Hasegawa, S. and Gary, D.M. (2000). Citrus Limonoids: functional chemicals in agriculture and food. American Chemical Society, Washington, D.C.

[20] Mata, L. Vargas, C., Saboria, D. and Vives, M. (1994). Extinction of *vibrio choleral* in acidic subtrata: contaminated cabbage and lettuce treated with lime juice. Revista de bidogia tropical. 42(3):487-492.

[21] Nagy, S., Shaw, P.E. and Veldhuis, M.K. (1977): Citrus Science and technology: Nutrition, anatomy, chemical composition and bio-regulation. Vol. 1, AV, Publishing, United State of America.

[22] Pandharipande, S. and Makode, H. (2009). Separation of oil and pectin from orange peel and study of effect of pH of extracting medium on the yield of pectin. Journal of Engineering Research and Studies, 3(2):06-09.

[23] Pelczer O.S. and Black P (1993). Internet Journal of Food Safety. Vol. 13.107-114.

[24] Saadi, M.A.K., Salih, H.A.M., Abbas, A.M. (2003). Study of the fruit peels of *Citrus sinesis*and *punica granatum*. Journal of Babylon University, 3(9):243-342.

[25] Saidani, N., Krucken, J., Sachroesel, R.M., Muller, I.U., Narinovski, P. (2004). Comparative analysis of the human gimap gene cluster encoding a novel Gr Pase family, Gene 341:291-304.

[26] Salih, H.AL.M., Abbas, A,N. (2003). Study of the fruit peels of *Citrus sinensis* and *Punica granatum*. Journal of Babylon University, (9)3:243-342.

[27] Suarez, L.E.C., Menichini, F. and Monache, F.D. (2002). Tetranortri-terpenoids and dinydrocinnamic acid derivatives from *Hortia Colombiana*. Journal of Babylon Chemical Society 13:339-344.

[28] Wood, S.A. (1988). The whole foods encyclopaedia. Prentice-Hall press, New York.