Evaluation of the phytochemical composition and antibacterial activities of methanolic and aqueous leaf extract of *psidium guajava* (l.)

Ugoh, S. C. And Nneji, L. M.

Department of Biological Sciences, University of Abuja, P.M.B. 117, Abuja, Nigeria
Sylvaugoh@hotmail.com, lotannanneji@gmail.com

Abstract: Phytochemical composition and antibacterial properties of methanolic and aqueous leaf extract of *Psidium guajava* (L) was carried out. Fresh leaves of the plant were collected, washed, dried at room temperature and were grounded to fine powder. It was then extracted with 95% methanol and distilled water and further screened for the presence of chemically active compounds by standard methods. The antibacterial activity was carried out using agar well diffusion method. Phytochemical analysis showed that the methanolic leaf extracts of *P. guajava* contain reducing sugar, saponins tannins, steroids, flavonoids, alkaloids, terpenoids and phlobatanins while the aqueous leaf extract contain saponins, tannins, steroids and flavonoids. The zone of inhibition increased with increase in concentrations of the extract. However, the highest concentration (500mg/ml) of the methanolic extract revealed a higher significant activity ($P > 0.05$) compared to Gentamicin (control). The extracts were more active against gram positive microorganisms than gram negative microorganisms. Furthermore, this study has revealed that the leaf extract can be harnessed for the production of antibiotics which can enhance our health care delivery system.
[Ugoh, S. C. And Nneji, L. M. **Evaluation of the phytochemical composition and antibacterial activities of methanolic and aqueous leaf.** *Rep Opinion* 2013;5(09):14-20]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 4

Keywords: Phytochemical, antibacterial, agar well diffusion, *Psidium guajava*

1.0 introduction

Herbal medicine was practiced by the ancient people, and at present, it is estimated that about 80% of the world population relies on herbs as medicines to meet their health needs (Wargovish *et al*., 2001). The use of herbal products for medicinal benefits has an important role in many cultures on earth. Plant extracts can be of great significance in therapeutic treatments. In the past few years, a number of studies have been conducted which proved efficiency of plant extracts in the treatment of human diseases (Newall *et al*., l996). However, the rapid development of organism's resistance to available antibiotics led to the search for plants with antibacterial activities (Nastro *et al*., 2000).

Psidium guajava (L.) is a medicinal plant belonging to the myrtaceae family. It is an evergreen shrub or small tree growing from 2 to 8m in height and up to 4cm in diameter at breast height. It is an important herbal medicinal plant which is planted and grown all over tropics including Nigeria and is commonly known as guava (Iwu, 1993). It is a well known edible fruit and is common in the backyards. Native to tropical America, *P. guajava* is now cultivated in many tropical and sub-tropical countries for its edible fruit (Perez *et al*., 2008).

P. guajava has been known to have antimicrobial (Arima and Danno, 2002, Chah *et al*., 2006), anti-inflammatory (Ojewole, 2006), antimalarial (Tona *et al*, 1999) and antiglycemic (Ojewole, 2005., Mukhtar *et al.,* 2006) activities. It has been used to treat wounds (Chah et *al.,* 2006), acne lesions (Qadan *et al*., 2005), cough (Jairaj *et al*., 1999) and dental diseases (Razak *et al*., 2006). Belemtougri *et al*.(2006) reported that the strong bactericidal activity exhibited by the leaf extracts of *Psidium guajava* was possibly due to the protein degrading activity of the extracts.

The leaves of *Psidium guajava* have been shown to inhibit both gram-positive and gram-negative bacteria such as *Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeruginosa, Salmonella enteridis*, *Bacillus cereus*, *Proteus* species, *Shigella* species and *Escherichia coli* (Perez *et al*., 2008).The leaves and bark also act as an antidiarrhoeic (Lutterodt, 1999., Tona *et al.,* 1999).

The aim of this research is to identify the phytochemical compositions as well as evaluate the invitro antibacterial properties of the aqueous and methanolic leaf extracts of *Psidium guajava* against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus pyogenes.*

Plate 1: *Psidium guajava* (Guava) Tree

2.0 materials and method

2.1 Sample Collection

Fresh leaves of *Psidium guajava* (L) were collected from a residential home in Gwagwalada, Abuja, Nigeria. A branch of the plant was identified by trained plant taxonomist in the Biotechnology Garden of Department of Biological sciences, University of Abuja, Nigeria.

2.2 Processing of Plant Samples

The fresh leaves were properly rinsed with tap water. Thereafter, the leaves were dried under room temperature and then blended to fine powder using electric blender.

2.3 Sterilization of Apparatus

All glass wares such as conical flask, beakers, and test tubes were washed and rinsed properly. Glass wares were sterilized in an autoclave at 121°C for 20 minutes before use. The work bench was disinfected with cotton wool soaked in 98% alcohol.

2.4 Extraction of plant material

Hundred grammes (100g) each of the powdered leaf material of *P. guajava* (L) were soaked in 500 ml each of distilled water and 95% methanol separately in 100 ml sterile conical flasks at room temperature for 72 hours, to obtain the aqueous and methanolic extracts. The content was filtered through a Whatman filter paper lined funnel into a conical flask. The filtrates were gently evaporated to dryness on a water bath and then stored in separate clean dry bottles and kept at room temperature until required. After preparation of the crude extract as described, the methanolic extract and aqueous extract were reconstituted using distilled H_2O to obtain concentrations of 500, 250, 100 and 50 mg/ml.

2.5 Phytochemical screening of plant extracts.

The methanolic and aqueous extracts were subjected to phytochemical screening. Reducing sugars, saponins, tannins, steroids, flavonoids, alkaloids, antracenosides, glycosides, terpenoids and phlobatanins were determined using the standard methods described by Brain and Turner (1975).

Reducing Sugars

1.0cm³ of the methanolic and the aqueous extracts was taken into separate test tubes. These were diluted with 2.0cm³ of distilled water followed by addition of Fehling's solutions (A+B) and the mixture was warmed. Brick-red precipitates at the bottom of the test tubes would denote the presence of reducing sugars.

Saponins

0.5g of the extracts was taken into separate test tubes.5.0cm³ of distilled water was added and vigorously shaken. A persistant frost that last for at least 15 minutes indicates the presence of saponin.

Tannins

2.0 cm³ of each extract was diluted with 4.0 cm³ of distilled water in a test tube. 2-3 drops of 5% ferric chloride solution was added. A green-black or blue-black colouration would indicate the presence of tannins/

Steroids

2.0 cm³ of the extracts were taken into test tubes and evaporated to dryness. The residue was dissolved in acetic anhydride and chloroform was then added. By means of a pipette, concentrated sulphuric acid was added by the side of the test tube. A brownish ring at the interface of the two liquids and the appearance of violet colour in the supernatant layer would indicate the presence of steroids.

Flavonoids

2.0 g of the extracts were dissolved separately in 20 cm³ of 50% (v/v) methanol by heating on a water-bath. Magnesium metal and 5-6 drops of concentrated hydrochloric acid were added. Appearance of a red colour would indicate the presence of flavonoids.

Alkaloids

To 1.0 cm³ of each extract in 2 separate test tubes,2-3 drops of Dragendoff's and Mayer's reagents

were separately added. An orange red precipitate/ turbidity with Dragendoff's or white precipitates with Mayer's denote the presence of alkaloids.

Artracenosides

4.0 cm^3 of the hydrolysed and partitioned extracts was concentrated to about 2.0 cm^3, and then 1.0 cm^3 of 35% ammonia solution was added. A cherish red colour indicates the presence of antracenosides.

Glycosides

5.0 g of each extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1 ml of concentrated Tetraoxosulphate (VI) acid to give a brown ring formation at the interface.

Terpenoids

About 5.0 g of each extract was mixed with 2 ml of chloroform. Concentrated Tetraoxosulphate (VI) acid was carefully added to form a layer. A reddish brown colouration of interface indicates the presence of terpenoids.

Phlobatanins

About 0.5 g of each plant extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

2.6 Microbial Cultures

Fresh plates of the test bacteria which include *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa* were made from the isolate cultures obtained from Microbiology Laboratory of the University of Abuja Teaching Hospital, Gwagwalada, Abuja. Colonies of fresh cultures of the different bacterial isolates were picked and suspended in 5 ml nutrient broth in well labeled sterile bijoux bottles, and were incubated for 24 hours at 37°C. For standard inocula preparation, a loopful of the 24 hours culture was transferred to Nutrient broth and incubated for 3 hours. This was to ensure that the microorganisms were at the lag phase at the onset of the experiment.

2.7 Agar Well Diffusion Technique

Semi solid Mueller Hinton agar plates were seeded with 0.5ml of the standard inocula dilution of the test bacterial isolates. The plates were swirled allowing the inocula to spread on the surface of the agar and the excess was discarded in a disinfectant jar. The plates were allowed on the bench for 20 minutes to set. With the aid of a sterile 6 mm diameter cork borer, five wells were bored at equal distances around the plates. The bottoms of the wells were sealed with one drop of Mueller Hinton agar to prevent diffusion of the extracts under the agar. The 5^{th} wells served as controls.

0.2ml of each of the prepared concentration of the extracts was aseptically introduced into wells with the 5th well filled with Gentamycin as control antibiotics. The plates were allowed on the bench for 30 minutes for pre diffusion and then incubated at 37°c for 24 hours. The resulting zones of inhibition were measured using a ruler calibrated in millimeters. The average of the three readings was taken to be the zone diameter of inhibition of the bacterial isolate in question at that particular concentration.

2.8 Minimal Inhibitory Concentration (MIC)

The MIC of the methanolic and aqueous extract was determined according to the macro broth dilution method. Standardized suspensions of the test organisms were inoculated into five sterile tubes of containing nutrient broth containing 0.2 ml of two fold dilutions of the extracts beginning with 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml. These were then incubated for 24 hours at 37°C. The MICs were read as the least concentration at which the growths of the tested organisms were inhibited.

2.9 Minimum Lethal Concentration (MLC)

MLC of the extracts were determined by first selecting tubes that showed no growth during MIC determination. A loopful from each tube was inoculated on Mueller Hinton agar plate and incubated for another 24 hours at 37°C. The result of the minimum lethal concentration was determined using the minimum concentration at which no growth was observed.

Statistical Analysis

The results obtained in the zone diameter of inhibition (ZDI) were expressed as Mean ± Standard Deviation. The results were further subjected to t-test at P=0.05 to test the significance differences between the mean values.

3.0 RESULTS

Table 1 shows the phytochemical compositions of the methanolic and aqueous leaf extract of *Psidium guajava* L. The result obtained showed that the methanolic leaf extracts of *P. guajava* contains reducing sugar, saponins tannins, steroids, flavonoids, alkaloids, terpenoids and phlobatanins. However, the aqueous leaf extract contains saponins, tannins, steroids and flavonoids.

Table 1: Phytochemical Composition of the Methanolic and Aqueous Leaf Extracts of *Psidium guajava* L.

Chemical component	Methanolic extract	Aqueous extract
Reducing sugar	+	_
Saponins	+	+
Tannins	+	+
Steroids	+	+
Flavonoids	+	+
Alkaloids	+	_
Artracenoides	_	_
Glycosides	_	_
Terpenoids	+	_
Phlobatanins	+	_

Note: + = present; - = Absent

Table 2 shows that the zone diameter of inhibition of methanolic and aqueous leaf extracts of *P. gaujava* on the test organisms. The mean zone of inhibition of 500mg/ml and 250mg/ml of methanolic leaf extract of *P. guajava* against *E. coli* was 7.0±1.2mm and 4.0±0.5mm respectively. No antimicrobial activity was recorded for 100mg/ml and 50mg/ml of methanolic leaf extract of *P. guajava* against *E. coli.* However, the mean zone of inhibition of 500mg/ml, 250mg/ml, 100mg/ml and 50mg/ml of aqueous leaf extract of *P. guajava* against *E. coli* was 8.0±1.1mm, 7.0±1.3mm, 5.0±1.2mm and 2.0±0.2mm respectively.

The mean zone of inhibition of 500mg/ml, 250mg/ml and 100mg/ml of methanolic leaf extract of *P. guajava* against *S. pyogenes* was 9.0±1.2mm, 5.0±1.2mm and 4.0±0.5mm respectively. No antimicrobial activity was recorded for 50mg/ml of methanolic leaf extract of *P. guajava* against *S. pyogenes.* The mean zone of inhibition of 500mg/ml and 250mg/ml of aqueous leaf extract of *P. guajava* against *S. pyogenes* was 6.0±1.0mm and 4.0±1.3mm respectively. No activity was recorded for 100mg/ml and 50mg/ml of the aqueous leaf extract of *P. guajava* against *S. pyogenes*.

The mean zone of inhibition of 500mg/ml and 250mg/ml of methanolic leaf extract of *P. guajava* against *P. aeruginosa* was 5.0±1.0mm and 3.0±0.2mm respectively. No antimicrobial activity was recorded for 100mg/ml and 50mg/ml of methanolic leaf extract of *P. guajava* against *P. aeruginosa.* However, the mean zone of inhibition of 500mg/ml, 250mg/ml, 100mg/ml and 50mg/ml of aqueous leaf extract of *P. guajava* against *E. coli* was 9.0±1.1mm, 6.0±1.2mm, 5.0±1.1mm and 3.0±0.1mm respectively.

Table 2: Zone diameter of inhibition of the Methanolic and Aqueous Leaf Extract of *P. guajava* on Test Organisms

Mean Zone of Inhibition (mm)						
Organism	Solvent	500mg/ml	250mg/ml	100mg/ml	50mg/ml	Control
E. coli	Meth	7.0±1.2	4.0±0.5	0.0±0.0	0.0±0.0	13.0±2.1
	Aq.	8.0±1.1	7.0±1.3	5.0±1.2	2.0±0.2	13.0±2.2
S. pyogenes	Meth	9.0±1.2	5.0±1.2	3.0±0.4	0.0±0.0	15.0±2.3
	Aq.	6.0±1.0	4.0±1.3	0.0±0.0	0.0±0.0	15.0±2.3
P. aeruginosa	Meth	5.0±1.0	3.0±0.2	0.0±0.0	0.0±0.0	12.0±2.0
	Aq.	9.0±1.1	6.0±1.2	5.0±1.1	3.0±0.1	12.0±2.0
S. aureus	Meth	12.0±1.1	7.0±1.2	5.0±1.2	3.0±0.3	15.0±3.1
	Aq.	6.0±1.1	3.0±0.1	2.0±0.8	0.0±0.0	15.0±3.1

Results are expressed as Mean of the Triplicates ± Standard Deviation.
Note: Meth. = Methanolic, Aq. = Aqueous

Table 3 shows the Minimum inhibitory concentration (MIC) of the methanolic and aqueous leaf extract of *P. gaujava* on the test organisms. The MIC of the methanolic and aqueous leaf extract against *E. coli* was 125mg/ml and 62.5mg/ml respectively. However, the MIC of the methanolic and aqueous leaf extract against *S. pyogenes* was 125mg/ml and 250mg/ml respectively. The MIC of the methanolic and aqueous leaf extract against *P. aeruginosa* was 250mg/ml and 62.5mg/ml respectively. Furthermore, the MIC of the methanolic and aqueous leaf extract against *S. aureus* was 125mg/ml respectively.

Table 3: Minimum inhibitory concentration of the Methanolic and Aqueous Leaf Extract of *P. gaujava* on the test organisms

Concentration of the Extract (mg/ml)							
Test organism	**Solvent**	**500**	**250**	**125**	**62.5**	**31.25**	**Inference**
E. coli	Meth	-	-	-	+	+	MIC is 125mg/ml
	Aq.	-	-	-	-	+	MIC is 62.5mg/ml
S. pyogenes	Meth	-	-	-	+	+	MIC is 125mg/ml
	Aq.	-	-	+	+	+	MIC is 250mg/ml
P. aeruginosa	Meth	-	-	+	+	+	MIC is 250mg/ml
	Aq.	-	-	-	-	+	MIC is 62.5mg/ml
S. aureus	Meth	-	-	-	+	+	MIC is 125mg/ml
	Aq.	-	-	-	+	+	MIC is 125mg/ml

Note: Meth. = Methanolic, Aq. = Aqueous, + = turbid, - = clear

Table 4 shows the Minimum lethal concentration (MLC) of the methanolic and aqueous leaf extract of *P. gaujava* on the test organisms. The MLC of the methanolic and aqueous leaf extract against *E. coli* was 250mg/ml and 125mg/ml respectively. It was also observed that the MLC of the methanolic and aqueous leaf extract against *S. pyogenes* was 250mg/ml and 500mg/ml respectively. However, the MLC of the methanolic and aqueous leaf extract against *P. aeruginosa* was 500mg/ml and 125mg/ml respectively. Furthermore, the MIC of the methanolic and aqueous leaf extract against *S. aureus* was 250mg/ml respectively.

Table 4: Minimum Lethal Concentration of the Methanolic and Aqueous Leaf Extract of *P. gaujava* on the test organisms

Concentration of the Extract (mg/ml)						
Test organism	**Solvent**	**500**	**250**	**125**	**62.5**	**Inference**
E. coli	Meth	-	-	+	NT	MLC is 250mg/ml
	Aq.	-	-	-	+	MLC is 125mg/ml
S. pyogenes	Meth	-	-	+	NT	MLC is 250mg/ml
	Aq.	-	+	NT	NT	MLC is 500mg/ml
P. aeruginosa	Meth	-	+	NT	NT	MLC is 500mg/ml
	Aq.	-	-	-	+	MLC is 125mg/ml
S. aureus	Meth	-	-	+	NT	MLC is 250mg/ml
	Aq.	-	-	+	NT	MLC is 250mg/ml

Note: Meth. = Methanolic, Aq. = Aqueous, NT= Not Tested
+ = Growth, - = No growth

4.0 Discussion

In the present investigation, the active phytochemical components inherent in the aqueous and methanolic leaf extract of *P. guajava* was studied and the antimicrobial activity of the plant extracts was further tested against four potentially pathogenic micro-organisms viz *E. coli, S. pyogenes*, *S. aureus* and *P. aeruginosa* at different concentrations of the extract.

The results of the phytochemical tests showed that the methanolic leaf extract of *P. guajava* contains reducing sugar, saponins tannins, steroids, flavonoids, alkaloids, terpenoids and phlobatanins. However, the aqueous leaf extract contains saponins, tannins, steroids and flavonoids. It has been documented that different solvents have diverse solubility capacities for different phytochemical constituents (Marjorie, 1999). So also, it has been recorded that these bioactive compounds inherent in the methanolic and aqueous leaf extract of *P. guajava* possess antibacterial properties, thus suggesting the potential of the use of plant for the treatment and prevention of bacterial infection.

However, the test organisms- *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa* were found to be sensitive to the different concentrations of the methanolic and aqueous leaf extracts of *Psidium guajava* as evidenced by the mean zones of inhibition. The effectiveness varied with the different concentrations used, with *Staphylococcus aureus* showing significantly higher susceptibility ($P \geq 0.05$) to the methanolic extract and *Pseudomonas aeruginosa* showing the highest susceptibility to the aqueous extract ($P \geq 0.05$) than other test organisms. The test organisms were increasingly inhibited with increase in the concentration of the extracts. On the other hand, among the extracts tested, aqueous extract showed broader spectrum of activity, being active to both Gram-positive and Gram-negative organisms compared to methanolic extract. Activities of the various extracts were comparable to those of standard antibacterial agent Gentamicin as control. Of all the bacteria tested the Gram-positive were slightly more susceptible to the extracts than the Gram-negative bacteria. The differences in the observed activities of the various extracts may be due to varying degree of solubility of the active constituents in the two solvents used.

The pharmacological activities of the drug may be attributed to the presence of the secondary metabolites. Hence, the presence of some metabolites suggests the antibacterial activities of aqueous and methanolic leaf extract of *P. guajava* against test organisms.

4.1 Conclusions

The results obtained from this research show that the plant used (*Psidium guajava* L.) contain bioactive chemical compounds and also possess antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa.* The methanolic and aqueous leaf extract of *P. guajava* is more active against the gram positive bacteria than gram negative bacteria. Furthermore, advanced researches should be encouraged to find out more about the medicinal properties of *Psidium guajava.*

References

1. Arima, H. and Danno, G. (2002). Isolation of antimicrobial compounds from guava (*Psidium guajava* L.). *Biological science, Biotechnology and Biochemistry*, *66*: 1727-1730.
2. Belemtougri, R.G., Constantin, B., Cognard, C., Raymond, G.,Sawadogo, L., (2006). Effects of *Sclerocarya birrea* (A.rich) hochst (anacardiaceae) leaf extracts on calcium signalling in cultured rat skeletal muscle cells. *Journal of Ethnopharmacology*, *76:*247-252.
3. Brain, K.R. and Turner, T.D.(1975). *The Practical Evaluation of Phytochemicals.* Wright Scientechica, Bristol; Pp 57-58.
4. Chah, K.F., Eze, C.A., Emuelosi, C.E., Esimone, C.O. (2006). Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. *Journal of Ethnopharmacology, 140*: 164-167.
5. Iwu, M. (1993). *Handbook of African Medicinal Plants*. CRC Press.
6. Jairaj, P., Khoohaswan, P., Wongkrajang, Y., Peungvicha, P., Suriyawong, P., Saraya, M.L., Ruangsomboon O (1999). Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. *Journal of Ethnopharmacology, 67:* 203-212.
7. Lutterodt, G.D., Ismail, E., Basheer, R.H., Baharudin, H. (1999) Antimicrobial effects of *Psidium guajava* extract as one mechanism of its anti-diarrhoeal action. *Malayan Journal of Medical Science, 6* (2): 17-20.
8. Marjorie M.C. 1999. Plant products as antimicriobial agents. *Clin. Microbiol. Rev.* 12(4): 564-582.
9. Mukhtar, H.M., Ansari, S.H., Bhat, Z.A., Naved, T., Singh, P.(2006). Antidiabetic activity of an ethanol extract obtained from the stem bark of *Psidium guajava* (Myrtaceae). *Pharmazie*, *61*: 725-727.
10. Nastro, A., Germano, M.P., D'Angelo, V., Marino, A., Cannatell, M.A. (2000). Extraction

methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Journal of Applied Microbiology, 30*: 379-382.

11. Newall, C.A., Anderson, L.A., Phillipson, J.D. (1996). *Herbal Medicines. A guide for health-care professionals*. Royal Pharmaceutical Society of Great Britain, London. Pp 296.
12. Olajide, O. A., Awe, S. O., Makinde, J. M. (1999) Pharmacological studies on the leaf of *Psidium guajava*. *Fitoterapia, 70* (1):25-31
13. Ojewole, J.A. (2006). Antiinflamatory and analgesic effects of *Psidium guajava* Linn (Myrtaceae) leaf aqueous extract in rat and mice. *Methods Findings Experimental Clinical Pharmacology, 28*: 441-446.
14. Ojewole, J.A. (2005). Hypoglycemic and hypotensive effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract. *Methods Findings Experimental Clinical Pharmacology, 27*: 689-695.
15. Perez, R.M., Mitchell, S., Solis, R.V. (2008). *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*. *117*: 1-27.
16. Razak, F.A., Othman, R.Y., Rahim, Z.H. (2006). The effect of *Piper beetle* and *Psidium guajava* extracts on the cell-surface hydrophobicity of selected early settlers of dental plaque. *Journal of Oral Science, 48*: 71-75.
17. Tona, L., Kambu, K., Mesia, K., Cimanga, K., Apers, S., de Bruyne, T., Pieters, L., Totte, J., Vlietinck, A.J.and T.(1999). Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. *Phytomedicine*, *6(1)*: 59-66.
18. Wargovish, M.J.,Woods,C., Holis, D.M., Zander, N.E.(2001). Harbalist, Cancer Prevention and Health. *Journal of Nutrition, 131*:30345-30365.

9/12/2013