International Journal of Anatomy and Physiology ISSN: 2326-7275 Vol. 7 (5), pp. 001-007, May, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antimicrobial activities of phenolic containing extracts of some tropical vegetables

S. O. Salawu[1,2]*, A. O. Ogundare[3], B. B. Ola-Salawu[4] and A. A. Akindahunsi[1]

[1]Department of Biochemistry, Federal University of Technology, P. M. B. 704, Akure, Ondo State, Nigeria.
[2]Department of Food Science, University of Pretoria, South Africa.
[3]Department of Microbiology, Federal University of Technology, P. M. B. 704, Akure, Ondo State, Nigeria.
[4]Department of Biology, Federal University of Technology, P. M. B. 704, Akure, Ondo State, Nigeria.

Accepted 19 October, 2017

The present study sought to investigate the antimicrobial properties of phenolic containing extracts of *Vernonia amygdalina (Va), Ocimum gratissimum (Og)* and *Manihot utilissima (Mu)*. Phenolic compound was characterized with the aid of a reversed phase HPLC/DAD/MS, and the antimicrobial activities of the extracts was assessed using agar-well diffusion method against some microorganisms, namely; *Bacillus cereus, Escherichia coli, Salmonella spp, Pseudomonas aeruginosa, Staphylococcus aereus, Shigella spp, Enterobacter, Clostridium sporogenes, Bacillus subtilis* and *Proteus vulgaris.* Ten, eight and four phenolic compounds were identified in *Va, Og,* and *Mu* respectively*.* The major phenolic compounds identified in *Va* were, Luteolin-7-O-glucoside, luteolin-7-O-glucuronide, luteolin and 1,5 dicaffeoyl quinic acid; nevadensin, vicenin-2, cichoric acid and rosmarinic acid in *Og*, while the major polyphenol in *Mu* were rutin and Kaempferol 3- O-rutinoside. The antimicrobial investigation showed that *M. utilissima* is active against only *B. cereus* of the entire tested organism. *V. amygdalina* is active against *B. cereus*, *S. aereus* and *Shigella* spp, while *O. gratissimum* is active against *B. cereus*, *S. aereus* and *Shigella* spp. The results obtained in the present investigation showed that the use of the vegetable materials as nutraceuticals may reduce the risk of microbial infections, which may partly be due to their phenolic composition.

Key words: Antimicrobial activity, phenolic compounds, *Ocimum gratissimum*, *Manihot utilissimam, Vernonia amygdalina.*

INTRODUCTION

Despite the wide availability of clinically useful antimicrobial drugs, several arguments (limited antimicrobial spectrum and emergence of previously uncommon infections) stimulate the development of new plant molecules with antibacterial activity. This has led to a rapidly growing interest in the investigation of the natural products from plant food for the discovery of new antimicrobial and antioxidant agents as well as an alternative route for the substitution of synthetic chemicals, side effects of which are always in question (Bisignano et al., 1999; Turkoglu et al., 2006).

The emergence of human pathogenic microorganisms that are resistant to major classes of antibiotics have increased in recent years, due to the indiscriminate use of antimicrobial drugs (Karaman et al., 2003; Paterson, 2006). This has caused many clinical problems in the treatment of infectious diseases and the antibiotics commonly used are sometimes associated with adverse effects on the host, which include hypersensitivity, allergic reaction and immuno-suppression (Mukherjee et al., 2002; Spellberg et al., 2004). Therefore, research for development of new antimicrobial agents is an urgent need.

Flavonoids and phenolic acids as dietary compounds are widely known as antioxidants that inhibit the oxidation of low-density lipoproteins and reduce thrombotic tendencies (Hertog et al., 1993; Tusda et al., 1999). Though

*Corresponding author. E-mail: sosalawu@yahoo.com. Tel: +27717523840. +2348137886171.

attention has been paid to their antimicrobial activity, no dramatic evidence of their effectiveness has been reported (Mori et al., 1978; Nishino et al., 1987; Barnabas and Nargarajan, 1988; Tsuchiya et al., 1996) *Ocimum gratissimum* (Labiatae) is widely distributed in tropical and warm temperate regions. The plant is com-monly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, ophthalmic, skin diseases, pneumonia, cough, fever and conjunctivitis (Onajob, 1986; Adebolu and Salau, 2005). In Nigeria, the plant is used as a spice in addition to its medicinal potential. However, as a green leafy vegetable it supplies to man essential nutrient such as minerals, vitamins and certain hormone precursors, in addition to protein and energy.

Vernonia amygdalina (Compositae) on the other hand is a small tree between 1 and 3 m in height, which grows throughout tropical Africa. Leaves of this plant are used in Nigeria as a green vegetable or as a spice in soup, especially in the popular bitter-leaf soup. The tops of this plant are also used in folk medicine as antimicrobial and antimalaria (Akinpelu, 1999; Abos and Raseroka, 2003; Okigbo and Emeka, 2008). Usually decoctions are prepared with local alcohol. In Tanzania, some wild chimpanzees were observed to use this plant for the treatment of parasite-related diseases.

There has not been any reported information on the antimicrobial properties of *Manihot utilissima*. Despite its non-popularity as a green vegetable in Nigeria, it is known as a staple food in some parts of Africa, Asia and Latin America (Joseph et al., 2001). The storage root crop is a good source of carbohydrate, and the leaves provide an inexpensive and rich source of protein (Rao and Hahn, 1984; Castelanos et al., 1993), albeit low in sulphur- containing amino acids (Cliff et al., 1985; Ngudi et al., 2003). The edible root and leaves also contain vitamins, minerals, dietary fibres and cyanogenic glucosides, mainly linamarin (Bradbury and Holloway, 1988; Balagopalan et al., 1988). Report had showed a prophylactic action of cassava as it relates to cancer; the incidence of bowel cancer is said to be very low in communities where cassava meal contributes fairly appreciably to its nutritional need (Balagopalan et al., 1988).

The aim of the present work is to evaluate the antimicrobial activities of the phenolic containing extracts of some vegetable (*O. gratissimum, V. amygdalina* and *M. utilissima*) against selected microorganism and to predict the possible relationship between the antibacterial activities and their phenolic profiles.

MATERIALS AND METHODS

Collection and preparation of materials

All the standards used to confirm the chemical structure of compounds were purchased from extrasynthese (Geney-France), with the exception of rutin from Sigma-Aldrich (St. Louis, MO-USA). The vegetables (*O. gratissimum*, *V. amygdalina* and *M. utilissima*) were harvested from local farms in Akure, South-Western Nigeria and voucher specimens were deposited at the Department of Biochemistry, Federal University of Technology, Akure, Nigeria and Department of Pharmaceutical Science, University of Florence, Italy. Fresh sample of about 4 kg was collected and dried in an oven at 30°C and powdered before storage.

Extraction methods for HPLC/MS/DAD analysis and antimicrobial assay

All solvents used were of analytical grade.

Hydro alcoholic extraction for HPLC/DAD/MS analysis

A dried sample (1 g each) was extracted with 40 ml (20 ml x 2) of ethanol/water 7:3 (v/v) acidified by formic acid (pH 2.5). The samples were filtered and the clear solution directly analysed by HPLC/DAD/MS.

Extraction method for antimicrobial assay

Powdered sample (100 g) was extracted with 350 ml each of ethanol-water (7/3) and filtered through Whatman No. 4 paper. The organic solvents were evaporated and subsequently freeze dried. The ethanol-water extract was re-dissolved in distilled water at a concentration of 50 mg/ml, and analyzed for their antimicrobial activity.

HPLC/DAD/MS analysis

Analyses were performed using an HP 1100 liquid chromatograph equipped with HP DAD and 1100 MS detectors. The interface was an HP 1100 MSD API-electro spray, the MS analyses were carried out in a negative mode with a fragmentor range between 80 to 150 V.

Method 1: A C12 column, 150 x 4 mm (4 µm) Synergi Max (Phenomenex-Torrance CA) maintained at 30°C and equipped with a 10 × 4 mm pre-column of the same phase was used; flow rate was 0.4 ml min^{-1}. The eluents were H_2O acidified to pH 3.2 with formic acid (A) and acetonitrile (B). The following linear solvent gradient was applied: from 95 to 85% A in 5 min, to 75% A in 8 min and a plateau of 10 min, to 55% A in 12 min and a plateau of 5 min, to 90% B in 3 min, and finally a plateau of 2 min to wash the column. The total time of analysis was 45 min.

Method 2: A different column was used for the analysis of the *O. gratissimum* samples. The column was a Polaris-ether (Varian) 250 x 4.6 mm, 5 µm; the eluents were H_2 O acidified to pH 3.2 with formic acid (A) and acetonitrile (B); the flow rate was 0.8 ml min^{-1}, oven temperature 30°C. The following linear solvent elution method was applied: from 92 to 80% A in 10 min, to 75% A in 18 min, to 55% A in 12 min, to 95% B in 3 min, and finally a plateau of 6 min to wash the column. The total time of analysis was 44 min.

Microorganisms and culture conditions

The bacterial strains used were *Bacillus cereus* NCIB 6349, *Bacillus subtilis* NCIB 3610, *Clostridium sporogens* NCIB 532, *Escherichia coli* NCIB 80, *Proteus vulgaris* NCIB 67, *Pseudomonas aeruginosa* NCIB 950, *Staphylococcus aureus* NCIB 8558, *Shigella sonnei*, *Salmonella typhi*, and *Enterobacter aerogenes*. All bacteria were obtained from Obafemi Awolowo University, Ile- Ife, Nigeria and were cultured aerobically at 37°C in nutrient agar medium. Before experimental use, cultures from solid medium were sub- cultivated

Table 1. Percentage yields of ethanol/water (7/3) extracts of some tropical vegetables.

Sample	Yield (% dry weight basis)
V. amygdalina	14.55
O. gratissimum	11.00
M. utilissima	13.45

Table 2. Identified phenolic compounds in the vegetable extracts (mg/g dried weight).

Peak no.	*V. amygdalina*	*O. gratissimum*	*M. utilissima*
1	Caffeoyl quinic acid	Vicenin-2	Rutin
2	Chlorogenic acid	Caffeic acid	Kaempferol 4'-rutinoside
3	Luteolin 7-O-glucoside	Rutin	Kaempferol 3-O-rutinoside
4	Luteolin 7-O-glucuronide	Kaempferol 4' O-rutinoside	Amentoflavone
5	1,5 dicaffeoyl quinic acid	Rosmarinic acid	-
6	Dicaffeoyl quinic acid	Cichoric acid	-
7	Dicaffeoyl quinic acid	Cirsimariti	-
8	Apigenin-O-glucuronide	Nevadensin	-
9	Luteolin	-	-
10-12	Unidentified flavonoids	-	-

in liquid media, incubated for 24 h and used as the source of inoculums for each experiment.

Antimicrobial activity

Antimicrobial activity was measured using agar-well diffusion method. Exactly 0.1 ml of each culture of bacteria was introduced into a sterile Petri dish. Sterile nutrient agar which has cooled to 45°C was poured on it and allowed to set. Three wells were borne on the set medium and suitably spaced apart. The wells were respectively filled with different concentrations (50, 25 and 12.5 mg/ml) of the extracts. The plates were incubated in an incubator at 37°C for 24 h. The results were recorded by measuring the zones of growth inhibition surrounding the wells. These clear inhibition zones around the wells indicate the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses. Sterile distilled water in the wells was used as control.

RESULTS AND DISCUSSION

The increasing resistance to antibiotic represents the main factor justifying the need to find and/or develop new antimicrobial agents. Thus, some studies have focused on the antimicrobial agents and on the antimicrobial properties of plant-derived active principles, which have been used for a long time in traditional medicine to overcome infections (Cowan, 1999). Thus, plant foods would be useful as medicine, in addition to the nutritional potentials.

The yield (percentage dry weight basis) of the vegetable extracts (*V. amygdalina, M. utilissima* and *O. gratissimum*) used for the present investigation was as presented on Table 1. The highest yield was obtained for *V. amygdalina w*hile the least was recorded for *O. gratissimum.* Identification of phenolic compounds in the extract (ethanol/water: 7/3) using HPLC/MS/DAD revealed the presence of eight, ten and four phenolic compounds respectively: *O. gratissimum*: vicenin-2, caffeic acid, rutin, kaempferol 4' O-rutinoside, rosmarinic acid, cichoric acid, cirsimaritin and nevadensin; *V. amygdalina*; Caffeoyl quinic acid, Chlorogenic acid, luteolin 7-O-glucoside, luteolin 7-O-glucuronide, 1,5 dicaffeoyl quinic acid, Dicaffeoyl quinic acid, Dicaffeoyl quinic acid, Apigenin-O-glucuronide, Luteolin and some unidentified flavonoids; *Manihot utilissima*: rutin, kaempferol 4'rutinoside, kaempferol 3-O-rutinoside and amentoflavone (Table 2). The result of the investigation shows nevadensin, cichoric acid and rosmarinic acid as the major phenolic compounds in *O. gratissimum*. On the contrary, results from a study on the flavonoid composition of *O. gratissimum* grown in UK, revealed rutin and cirsimaritin as the major flavonoids (Grayer et al*.,* 2000).

The main compounds detected in *V. amygdalina* were luteolin, luteolin 7-O-glucoside and luteolin 7-O-glucuronide, with luteolin-7-O-glucuronide been the most abundant flavonoid. The phenolic composition of *M. utilissima* showed rutin and kaempferol 3-O-rutinoside as the major phenolic compounds. The identified phenolic compounds in the three vegetable extract used for the antimicrobial investigation are almost in agreement with our previous report (Salawu et al., 2009). The result of our previous investigation revealed the presence of luteolin-7-O- rutinoside, luteolin-4-O- rutinoside in *V. amygdalina*; ferulic acid in *M. utilissima* and luteolin- 7-O-glucuronide, caffeoyl derivative and cirsiliol in *O. gratissimum* but were not identified in the present

Table 3. Antimicrobial activity of phenolic containing extracts of some tropical vegetables.

	Zones of inhibition (mm)								
	V. amygdalina			***O. gratissimum***			***M. utilissima***		
Microorganism	12.5	25	50	12.5	25	50	12.5	25	50
B. cereus	-	-	2.9	2.1	3.2	6.9	-	1.1	4.1
E. coli	-	-	-	-	-	-	-	-	-
Salmonella spp	-	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-	-	-
S. aereus	-	2.8	7.1	-	3.9	6.1	-	-	-
Shigella spp	-	1.1	2.2	-	1.1	2.3	-	-	-
Enterobacter	-	-	-	-	-	-	-	-	-
C. sporogenes	-	-	-	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-	-	-	-
P. vulgaris	-	-	-	-	-	-	-	-	-

Note: Each value is expressed as mean (n = 3).

investigation (Salawu et al., 2009). Report had shown that distribution of phenolic compounds in plants depends on several factors, including variation and the degree of light exposure. Also, the levels of individual and total phenolic compounds in food are influenced by genetic factors such as species, environmental conditions such as light, ripeness, and post-harvest treatments such as processing (Bravo, 1998; Duthie et al., 2000; Chu et al., 2000; Ksouri et al., 2008).

The crude extracts were used for the antimicrobial activity studies. The HPLC/DAD/MS analysis of the vegetable materials allowed us to identify the individual phenolic compounds present in the studied vegetable materials that could participate in the antimicrobial action of the extracts. However, we decided to use the crude extracts for the antimicrobial capacity of the phenolic compounds in the studied vegetables for several reasons. First, the antimicrobial capacity of phenolic compounds is well- known (Pereira et al., 2006; Prestos et al., 2005; Rauha et al., 2000; Zhu and Zhang, 2004; Puupponen-Pimiä et al., 2001; Pereira et al., 2007). They act by causing the leakage of cytoplasmic constituents such as protein, glutamate or potassium and phosphate from bacteria, which may be due to disruption of cell peptidoglycan or damage of the cell membrane. Also, extracts may be more beneficial than isolated constituents, since a bioactive individual component can change its properties in the presence of other compounds present in an extract (Barnabas and Nagarajan, 1988). Report had shown that additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent bioactive properties and the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods (Liu, 2003; Haidari et al., 2009).

The crude extracts were screened for their antimicrobial activities against *B. cereus, E. coli, Salmonella spp, Pseudomonas aeruginosa, S. aereus, Shigella spp, Enterobacter, C. sporogenes, B. subtilis, P. vulgaris.* The choice of these microorganisms was made due to the fact that some of them are causative agents of intestinal infection in human. The zone of inhibition (mm) of the extract and on the selected microorganism is as shown on Table 3, while Table 4 shows the zones of inhibition of some main polyphenols identified in the vegetal matter against the microorganism that are inhibited by the extracts. *M. utilissima* was active against only *B. cereus* out of the entire tested microorganism. *V. amygdalina* was active against the growth of *B. cereus*, *S. aereus* and *Shigella* spp while *O. gratissimum* was active against *B. cereus*, *S. aureus* and *Shigella* spp. The result of the zone of inhibition obtained in the three vegetable extracts indicates a higher inhibition at a concentration of 50 mg/ml. This is in agreement with previous report (Bradbury and Holloway, 1988; Furneri et al., 2002) that the mode of action of phenolic compounds is concentration dependent.

Rutin, being a minor phenolic compound in *O. gratissimum* and a major phenolic compound in *M. utilissima* might have contributed significantly to the antimicrobial properties of the two vegetable materials. The antimicrobial activity of quercetin was reported has been reported by Paolillo et al. (2011), also, report from Cowan (1999) showed that quercetin (a derivative of rutin) is one of the antimicrobial active principles of oak plant (*Quercus rubra*). Rutin (quercetin-3-beta-D-rutinoside) is also an important therapeutic substance that favorably influences the increase of blood vessel elasticity and the treatment of circulatory disorders and atherosclerosis, reduces blood pressure and stimulates vitamin C utilization. According to Quarenghi et al. (2000), kaempferol might have also contributed to the antimicrobial activity of *M. utilissima*. He concluded that the presence of quercetagetin, patuletin, kaempferol and

Table 4. Antimicrobial activity of the main phenolic compounds identified in the leafy vegetables.

		Zones of inhibition (mm)		
Phenolic compound	**(mg/ml)**	***S. aereus***	***B. cereus***	***Shigella spp***
	10	-	0.4	-
Luteolin-7-glucoside	25	0.69	0.81	0.52
	50	1.21	1.32	0.82
	10	-	0.71	-
Luteolin	25	0.92	1.1	0.67
	50	1.8	2.1	1.89
	10	0.52	0.63	-
Rutin	25	0.66	0.89	0.67
	50	1.23	1.41	1.22
	10	0.71	-	-
Caffeic acid	25	1.32	0.62	0.51
	50	1.33	0.71	0.67
	10	0.42	0.23	0.41
Rosmarinic acid	25	0.72	0.61	0.63
	50	1.52	1.31	1.23

Note: Each value is expressed as mean (n = 3).

quercetin glycosides might be related to the observed antimicrobial activities of *Anthemis cotula*. Report from Harsh et al. (2002), revealed the presence of rosmarinic acid in *O. basilicum* and also confirm the antimicrobial activity of rosmarinic acid. It has also been reported that rosmarinic acid also has antimicrobial properties - killing bacteria, fungi, viruses and parasites.

Bhanumathi et al. (1999) also reported the antibacterial and antiviral properties of Nevadensin. Caffeic acid in *Artemisia dracunculus* had been shown to exhibit some antimicrobial properties (Cowan, 1999). Therefore, cichoric acid, which is a caffeic acid ester would likely contribute to the antimicrobial properties in *O. gratissimum*. It is important to note that additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent bioactive properties (Liu, 2003; Haidari et al., 2009). This, by implication means that both the minor and the major phenolic compounds would have contributed to the antimicrobial activity exhibited by the studied vegetable materials. The high content of luteolin aglycone, its glycosides and the other phenolic compounds identified in *V. amygdalina* might have contributed to its antimicro-bial properties of *V*. *amygdalina*. In particular, luteolin has been described to possess antimicrobial activity against several bacterial species (Basile et al., 1999; Cottiglia et al., 2000; Sato et al., 2000; Tshikalange, 2005).

In general the antimicrobial properties of phenolic compounds have been reported. The mechanism of action involves the alteration of the permeability of the cell membrane that could result in the uncoupling of oxidative phosphorylation, inhibition of active transport, and loss of pool metabolites due to cytoplasmic membrane damage (Denyer and Hugo, 1991). Also, the presence of hydroxyl group in phenolic compound might influence their antimicrobial effectiveness by binding to the active site of enzymes, form hydrogen bonds with enzymes and alter their metabolism, and also the lipid solubility and the degree of steric hindrance of the phenolic compounds might determine their antimicrobial activity (Beuchat and Golden, 1989; Ceylan and Fung, 2004).

Conclusion

The results obtained in the present investigation reveals that the use of these vegetables as nutraceuticals may reduce the risk of microbial infections, which partly may be due to the protective action provided by its phenolic compounds.

ACKNOWLEDGEMENT

The authors wish to acknowledge the support of ICTP/IAEA for financing the stay of S.O. Salawu in some laboratories in Italy through a PhD Sandwich Training Educational fellowship award of the International Centre

for Theoretical Physics (ICTP), Trieste, Italy. We equally want to acknowledge the support of the research group of Professor Nadia Mulinacci of the department of Pharmaceutical Science, Florence, Italy for their technical support in the phenolic studies of the vegetable.

REFERENCES

Abos AO, Raseroka BH (2003). *In vivo* anti-malarial activity of *Vernonia amygdalina*.*British* J. Biomed. Sci., 60(2): 89-91.

Adebolu TT, Salau AO (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. Afr. J. Biotechnol., 4(7): 682-684.

Akinpelu DA (1999). Antimicrobial activity of *Vernonia amygdalina* leaves. Fitoterapia, 70(4): 432-434(3).

Balagopalan C, Padmaja G, Nanda SK, Moorthy SN (1988). Cassava in food, feed, and industry. *CRC* Press Inc., Florida.

Basile A, Giordano S, López-Sáez JA, Cobianchi RC (1999). Antibacterial activity of pure flavonoids isolated from mosses. Phytochemistry, 52: 1479-1482.

Barnabas CGG, Nagarajan S (1988). Antimicrobial activity of flavonoids of some medicinal plants. Fitoterapia, 59(6): 508–510.

Beuchat LR, Golden DA (1989). Antimicrobials occurring naturally in foods. Food Technol., 43(1): 134-142.

Bisignano G, Tomaino A, Lo Cascio R, Crisafi G, Uccella N, Saija A (1999). J. Pharm. Pharmacol., 51: 971-974.

Bradbury JH, Holloway WD (1988). Chemistry of tropical root crops: Significance for nutrition and agriculture in the Pacific. Australian Center for International Agriculture, pp. 5-22.

Bravo L (1988). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev., 56: 317–333.

Castellanos R, Altamirano SB, Moretti RH (1993). Nutritional characteristics of Cassava (*Manihot esculenta* Crantz) leaf protein concentrates obtained by ultrafiltration and acidic thermocoagulation. Plant Food Hum. Nutr., 45(4): 357-363.

Ceylan E, Fung DYC (2004). Antimicrobial activity of spices. J. Rapid Meth. Auto. Micro., 12(1): 1–55.

Cliff I, Lundquist P, Martensson I, Rosling H, Sorbo B (1985). Association of high cyanide and low sulphur intake in cassava induced spastic paraparesis. Lancet II, 1211-1213.

Chu YH, Chang CL, Hsu, HF (2000). Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric., 80: 561–566.

Cottiglia F, Loy G, Garau D, Floris C, Casu M, Pompei R, Bonsignore L (2000). Antimicrobial evaluation of coumarins and flavonoids from the stems of Daphne gnidium L. Phytomedicine, 8: 302-305.

Cowan MC (1999). Plant Products as Antimicrobial Agents. Clin Microbiol Rev., 12 (4): 564-582.

Denyer SP (1990). Mechanisms of action of biocides. Int. Biodeter., 26(2–4): 89-100.

Duthie GG, Duthie SJ, Kyle JAM (2000). Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. Nutr. Res. Rev., 13: 79–106.

Furneri PM, Marino A, Saija A, Uccella N, Bisignano G (2002). *In vitro* antimycoplasmal activity of oleuropein. Int. J. Antmicrob. Agents, 20: 293-296.

Grayer RJ, Kite GC, Abou-Zaid M, Archer LJ (2000). The Application of Atmospheric Pressure Chemical Ionization Liquid Chromatography-Mass Spectrometry in the Chemotaxonomic study of Flavonoids: Characterization of Flavonoids from *Ocimum gratissimum* var gratissimum. Phytochem. Anal., 11: 257-267.

Haidari M, Ali M, Casscells SW, Madjid M (2009). Pomegranate (Punicagranatum) Purified polyphenol extract inhibits influenza virus and has a synergistic effect with oseltamivir. Phytomedicine, 16: 1127–1136.

Harsh PB, Travis SW, Herbert PS, Jorge MV (2002). Plant Physiol. Biochem., 40(11): 983-995.

Hertog MGL, Feskens EJM, Hollman PC, Katan MB, Kromhout D (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet, 342: 1007-1011.

Joseph T, Yeoh H, Loh C (2001). Linamarin content and genetic stability of cassava plants derived by somatic embryogenesis. Euphytica, 120: 7-1.

Karaman I, Sahin F, Güllüce M, Ögütçü H, Sngül M, Adigüzel A (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol., 85: 231-235.

Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M, Smaoui A, Abdelly C (2008). Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. Pharmacol. Toxicol., 331: 865-873.

Liu RH (2003). Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals Am. J. Clin. Nutri., 78(3): 517S-520S.

Mori A, Nishino C, Enoki N, Tawata S (1987). Antibacterial activity and mode of action of plant flavonoids against *Proteus vulgaris* and *Staphylococcus aureus*. Phytochemistry, 26(8): 2231–2234.

Mukherjee PK, Saritha GS, Suresh B (2002). Antimicrobial potential of two different *Hypericum* species available in India. Phytother. Res., 16: 692-695.

Mulinacci N, Prucher D, Peruzzi M, Romani A, Pinelli P, Giaccherini C, Vincieri FF (2004). Commercial and laboratory extracts from artichoke leaves: estimation of caffeoyl esters and flavonoidic compound content. J. Pharm. Biomed. Anal., 34(2): 349-345.

Ngudi DD, Kou Y, Lambien F (2003). Cassava leaves, a non-negligible source of dietary exposure to cyanogens. Cassava Cyanide Diseases Network Issue, 2: 1-4.

Nishino C, Enoki N, Tawata S, Mori A, Kobayashi K, Fukushima M (1987). Antibacterial activity of flavonoids against *Staphylococcus epidermidis*, a skin bacterium. Agric. Biol. Chem., 51(1): 139–143.

Okigbo RN, Mmeka EC (2008). Antimicrobial Effects of Three Tropical Plant Extracts on *Staphylococcus Aureus, Escherichia Coli and Candida Albicans.* Afr. J Tradit Complement Altern. Med., 5(3): 226–229.

Onajobi FD (1986). Smooth muscle contracting lipidic soluble principle in chromatographic functions of *Ocimum gratissimum*. J. Ethnopharmacol., 18: 3-11.

Paolillo R, Carratelli CR, Rizzo A (2011). Effect of resveratrol and quercetin in experimental infection by Salmonella enteric serovar Typhimurium. Int. Immunopharmacol., 11: 149-156.

Paterson D.L. Resistance in Gram-negative bacteria (2006). *Enterobacteriaceae*. Am. J. Infect. Control, 119(5): 20-28.

Pereira JA, Pereira APG, Ferreira ICFR, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A (2006). Table olives from Portugal: phenolic compounds, antioxidant potential and antimicrobial activity. J. Agric. Food Chem., 54: 8425-8431.

Pereira AP, Ferreira IC, Marcelino F, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA (2007). Phenolic Compounds and Antimicrobial Activity of Olive (*Olea europaea* L.*Cv.* Cobrançosa) Leaves. Molecules, 12(5): 1153-1162.

Proestos C, Chorianopoulos N, Nychas GJE, Komaitis M (2005). RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. J. Agric. Food Chem., 53: 1190-1195.

Puupponen-Pimiä R, Nohynek L, Meier C, Kähkönen M, Heinonen M, Hopia A, Oksman-Caldentey KM (2001). Antimicrobial properties of phenolic compounds from berries. J. Appl Microbiol., 90: 494-507.

Quarenghi MV, Tereschuk ML, Baigori MD, Abdala LR (2000). Antimicrobial activity of flowers from *Anthemis cotula.* Fitoterapia, 71(6)6: 710-712.

Rao PV, Hahn SK (1984). An automated enzymatic assay for determining the cyanide content of cassava and cassava products. J. Sc. Food Agric., 35: 426-436.

Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int. J. Food Microbiol., 56: 3-12.

Salawu SO, Giaccherini C, Innocenti M, Vincieri FF, Akindahunsi, AA, Mulinacci N (2009). HPLC/DAD/MS characterization and analysis of flavonoids and cynnamoil derivatives in four Nigerian green-leafy vegetables. Food Chem., 115: 1568-1574.

Sato Y, Suzaki S, Nishikawa T, Kihara M, Shibata H, Higuti T (2000). Phytochemical flavones isolated from *Scutellaria barbata* and

antibacterial activity against methicillin-resistant *Staphylococcus aureus.* J. Ethnopharmacol., 72: 483-488.
Spellberg B, Powers JH, Brass EP, Miller LG, Edwards JE Jr (2004). Trends in antimicrobial drug development: Implications for the future. Clin. Infect. Dis., 38(9): 1279-1286.
Turkoglu A, Kivrak I, Mercan N, Duru ME, Gezer K, Turkoglu H (2006). Antioxidant and antimicrobial activities of *Morchella conica Pers*. Afr. J. Biotechnol., 5: 1146-1150.
Tusda T, Horio F, Osawa T (1999). Absorption and metabolism of cyanidin 3-*O*-_- D-glucoside in rats. FEBS Lett., 449: 179-182.
Tshikalange E, Meyer JJM, Hussein AA (2005). Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. J. Ethnopharmacol., 96: 515-519.
Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M (1996). Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*.J. Ethnopharmacol., 50: 27-34.
Zhu X, Zhang H, Lo R (2004). Phenolic compounds from the leaf extract of artichoke (Cynara scolymus L.) and their antimicrobial activities. J. Agric. Food Chem., 52: 7272-7278.