Evaluation of Antibacterial Activity and Phenolic Contents of Four Nigerian Medicinal Plants

Arzu Cagri-Mehmetoglu[1,*], Abimbola Sowemimo[2] and Maryna van de Venter[3]

[1]*Sakarya University, Dept. of Food Engineering, Sakarya, Turkey*

[2]*University of Lagos, Faculty of Pharmacy, Department of Pharmacognosy, Lagos, Nigeria*

[3]*Nelson Mandela Metropolitan University, Dept. of Biochemistry and Microbiology, Port Elizabeth, South Africa*

Abstract: In this study, phenolic compounds and antimicrobial properties of four medicinal plants from Nigeria was investigated. The antibacterial properties and minimum inhibition concentration of *Microdesmis puberula*, *Hypoestis verticillaris*, *Icacina tricantha*, and *Enterolobium cyclocarpum* against 21 different bacteria was carried out using the disc diffusion assay. These plant extract were subjected to phytochemical screening by reverse phase HPLC (high pressure liquid chromatography) coupled with diode array detection and GC-MS (gas chromatography- mass spectrum). *H. verticillaris* had the most significant activity and showed inhibitory activity against most of the Gram-positive bacteria. However, *M. puberula* was only effective against the growth of *Staphylococcus aureus* and *Enterobacter sakkai* with inhibition zone of 8 mm. Furthermore, both *I. tricantha* and *E. cyclocarpum* only had antimicrobial effect on *S. warneri* with the inhibition zone of 12 and 13 mm, respectively. The results showed that *M. puberula* mostly contained 0.46 mg100 g^{-1} dry sample quarcetin and *H. verticillaris* contained approximately 0.7 mg 100 g^{-1} dry sample mangiferin and quarcetin. Moreover, both *I. tricantha* and *E. cyclocarpum* contain mostly quarcetin and rutin. According to GC-MS results, *M. puberula* contained thymol and methyl cinnamic acid and *H. verticillaris* contained methyl cinnamic acid and gallic acid. These plant extracts can be considered to be used in the cosmetic and food industries or even as a safe alternative to synthetic antimicrobial drugs.

Keywords: Plant extracts, Antibacterial effect, Phenols, HPLC, GC-MS.

INTRODUCTION

Medicinal plants containing components of healing value have been used as medications for human illnesses for a long time. Recently, scientific research into the antimicrobial activity of medicinal plants has been increasing because of the approval of those plants as an alternative remedy [1-4]. Moreover, increasing consumer concern over chemically synthesized food preservatives has also resulted in growing pressure on the food industry to use natural food ingredients as alternatives [5]. Most of the plants were suggested to be fine sources of phenolic compounds that could be used for food preservation and also for contribution to a healthy diet [6, 7].

The plant (poly)phenols are a diverse group of higher secondary metabolites, possessing an aromatic ring bearing one or more hydroxy substituents, derived from the shikimate pathway and phenylpropanoid metabolism [8]. They include mainly simple phenols, phenolic acids, coumarins, tannins and flavonoids. These compounds usually occur in the form of glycosides or esters in plants [9].

Phenolic compounds show an important free radical scavenging activity. They have reactivity as electron donating agents and metal chelation properties. They can provide the stability of radicals derived by antioxidation and reactivate with other antioxidants [6]. The consumption of natural antioxidants reduces risk of cancer, chronic inflammation and cardiovascular disease [7, 10-12].

Plant polyphenols were also recognised to be antimicrobial agents, and they were recommended as possible food natural preservatives [13]. Mechanisms such as hydrogen peroxide production, bacterial protein/enzyme inhibition and disinfectant activity of phenolic acids were all well documented [14].

Thousands of plants in the nature need to be investigated in order to find safe active compounds which can be beneficial antioxidants and antimicrobials in the food, cosmetic and pharmaceutical industries. Objectives of this study were to investigate the antimicrobial activity and the phenolic compounds of the extracts from four Nigerian medicinal plants (*Microdesmis puberula*, *Hypoestis verticillaris*, *Icacina tricantha*, and *Enterolobium cyclocarpum*) by using HPLC and GC-MS.

*Address correspondence to this author at the Sakarya Universitesi, Muhendislik Fakultesi, Gıda Muhendisligi Bolumu, Esentepe, Sakarya, Turkey; Tel: 90 264 295 5920; Fax: 90 264 295 5601; E-mail: acagri@sakarya.edu.tr

E-ISSN: 2408-9826/17

MATERIALS AND METHODS

Plant Collection and Extraction

The leaves of *Microdesmis puberula* Hook. f. ex Planch., *Hypoestis verticillaris* (Linn f.) Soland, *Icacina tricantha* Oliv., and *Enterolobium cyclocarpum* (Jacq.) Griseb were collected in Ibadan, Oyo State, Nigeria. They were identified by Mr. Daramola and authenticated at the Herbarium of the Department of Botany and Microbiology, University of Lagos, Akoka, Lagos, Nigeria where voucher specimens (LUH 233, LUH 3374, LUH 3513 and FH1 2941 respectively) were deposited. The plants were dried in a hot air oven at 40 ºC. The dried plant materials (250 g) were extracted with 1L ethanol. The extracts were filtered and concentrated *in vacuo* at 30 ºC using the rotary evaporator (Buchi, Switzerland).

Organisms and Media

The test organisms used in this study were as follows: Bacillus cereus, Bacillus subtilis (ATCC 6633), Bacillus pantothenticus, Bifidobacter infantis (clinical isolate), Citrobacter freundii, Enterococcus sakkai, Escherichia coli O157:H7 (NCTC 12900), Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 13883), Listeria monocytogenes (ATCC 9111), Micrococcus flavus, Proteus vulgaris (ATCC 13315), Pseudomonas aeruginosa (ATCC 9027), Salmonella enteritidis (ATCC 13076), Salmonella Ttyphimurium (ATCC 14028), Serratia liquefaciens (ATCC 19980), Shigella sonnei, Staphylococcus aureus (ATCC 25923), Stapylococcus epidermidis, Staphylococcus warneri, Xantomonas fragariae,. The strains were maintained and tested on Tryptone Soya Agar (Merck). For the antimicrobial tests, the cells were grown overnight in Tryptone Soya Broth (Merck) at 37°C.

Disc Diffusion Test

For antimicrobial testing, a 15% (w/v) stock solution of each dry extract was prepared in pure dimethylsulfoxide (DMSO). Overnight broth cultures, adjusted to yield approximately 10^8 cfu ml^{-1} were streaked with a calibrated loop on plates containing appropriate solid medium. Filter paper discs of 6 mm diameter were placed on the inoculated agar surfaces and impregnated with 10 µl of stock solutions. Pure DMSO (10 µl) was used as a negative control while vancomycin discs (30 mg), amoxicillin discs (10 mg; Oxoid) and amphotericin B discs (100 mg; Pasteur, Milan, Italy) were used as positive controls. The plates were observed after 18 h at 37 °C. All tests were performed in duplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts.

Minimum İnhibitory Concentration

The extracts giving an inhibition zone of 8 mm in diameter were chosen to assay the minimum inhibitory concentration (MIC) with the agar dilution method. A stock solution of each extract was serially diluted two fold in pure DMSO from 0.0025 to 2% and 10 µl of each dilution were pipetted on the filter discs on the inoculated agar medium with each overnight bacteria cultured. The agar plates were incubated at 37°C for 24 h. DMSO (98%) was used as control. The MIC was defined as the lowest concentration of the extract inhibiting the growth of each micro-organism with clear inhibition zones around the discs.

Standards

Gallic acid, mangiferin, (+)-catechin, quercetin, rutin, caffeic acid, thymol, epicatechin, hesperidin, phlorid, alantoin, polyditin, and chlorogenic acid were purchased from Sigma–Aldrich (Steinheim, Germany). All standards were prepared as stock solutions in methanol. Working standards were made by diluting stock solutions in 62.5% aqueous methanol containing butylated hydroxytoluene 1 g l^{-1}, and 6 M HCl to yield concentrations ranging from 0.5–25 mg l^{-1}. Stock working solutions of the standards were stored in darkness at - 18 °C. All solvents and reagents from various suppliers were of the highest purity needed for each application.

HPLC Analysis

The analytical HPLC system employed consisted of a high performance liquid chromatograph coupled with a diode array detector (Agilent 1200 Infinity Series, U.S.A). The separation was achieved on an Agilent proshell 120 EC-C18 270 mm 30 x 50 mm column at 30°C. The mobile phase consisted of water with 2.5% glacial acetic acid (solvent A) and acetonitrile (solvent B). The gradient used was similar to that used for the determination of phenolics in wine [15] with some modifications: 97% A 0–1 min, 91% A 1-4 min, 89% A 4-6 min, 82% A 6-10 min, 70% A 10-15 min, 68% A 15-20 min, 55% A 20-25 min, 50% A 25-30 min, 45% A 30-40 min, 40% A 40-50 min, 100% B 50-60 min; post-time 10 min before next injection. The flow rate was 0.5 ml min^{-1} and the injection volume was 20 µl.

GC-MS Analysis

The samples were analysed on GC-MS system based on a modified method by Yu *et al*. [16]. GC-MS system (Agilent 5975C Series) with triple-axis detector was used in this study. The detector has electron impact mode set at 70 eV and the mass range at m/z 25–700. A capillary column (HP-5ms Ultra Inert, 30 m · 0.32 mm, i.d.) with 0.25 m film coated material was used. Temperature of injector and detector were set at 280 °C and 290 °C, respectively. The spitless mode with 1 min was applied. The temperature programme was determined as follows: from 70 to 135 °C with 2 °C min^{-1}, hold for 10 min, from 135 to 220 °C with 4°C min^{-1}, hold for 10 min, from 220 to 270 °C with 3.5 °C $min^{-1}$and then hold for 20 min. A post-run of 10 min at 70 °C was sufficient for the next injection. The flow rate of carrier gas (helium) was continued at 1.9 ml min^{-1}. The compounds were identified by matching the retention times with those of authentic compounds and the spectral data obtained from the Wiley and NIST libraries. Each determination was carried out in duplicate.

RESULTS

Disc Diffusion Test

Antimicrobial activity of 4 plant extracts used for medicinal purposes in Nigeria was measured against selected Gram-positive and Gram-negative bacteria. The results obtained indicated that *H. verticillaris* had the most significant activity and showed inhibitory activity against *B. subtilis*, *B. pantothenticus, S. aureus, S. warneri, K. pneumonia*, *S. liquefaciens*, and *E. sakkai* with inhibition zones recorded in the range of 8 mm to 16 mm (Table **1**). The other plant extracts were only effective on some of the Gram-positive bacteria.

Table 1: Antimicrobial Activity and Minimum Inhibition Concentration (MIC) of Four Medicinal Plant Extracts

	Microdesmis Puberula **Hook. f. ex Planch**		***Hypoestis Verticillaris*** **(Linn f.) Soland**		***Icacina Tricantha*** **Oliv.**		***Enterolobium Cyclocarpum*** **(Jacq.) Griseb.**	
Microorganisms	**IZ (mm)**	**MIC (%, w/v)**	**IZ (mm)**	**MIC (%, w/v)**	**IZ (mm)**	**MIC (%,w/v)**	**IZ (mm)**	**MIC (%, w/v)**
Bacillus cereus	-	-*	-	-	-	-	-	-
Bacillus pantothenticus	-	-	12	2	-	-	-	-
B. subtilis	-	-	8	2	-	-	-	-
Bifidobacter infantis	-	-	-	-	-	-	-	-
Citrobacter freundii	-	-	-	-	-	-	-	-
Enterococcus sakkai	8	0.5	10	0.5	-	-	-	-
Escherichia coli O157:H7	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	16	0.1	-	-	-	-
Listeria monocytogenes	-	-	-	-	-	-	-	-
Micrococcus flavus	-	-	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-
Salmonella enteritidis	-	-	-	-	-	-	-	-
S. Thyphimurium	-	-	-	-	-	-	-	-
Serratia liquefaciens	-	-	8	2	-	-	8	2
Shigella sonnei	-	-	-	-	-	-	-	-
Staphylococcus aureus	8	2	8	2	-	-	-	-
S. epidermidis	-	-	-	-	-	-	-	-
S. warneri	-	-	9	2	12	0.1	13	2
Xantomonas fragariae	-	-	-	-	-	-	-	-

*Minimum inhibitory concentration not determined because inhibition zone (IZ) diameters by disc diffusion test were <8 mm.

For example, *M. puberula* was only effective against the growth of *S. aureus* and *E. sakkai* with inhibition zone of 8 mm. Furthermore, both *I. tricantha* and *E. cyclocarpum* had antimicrobial effect on *S. warneri* with inhibition zones of 12 and 13 mm, respectively.

The MIC values of ethanol extracts are also given in Table **1**. The DMSO control showed no toxic effect at 98% (v/v). The higher sensitivity of Gram-positive bacteria was confirmed by the agar dilution method; the MIC values ranged from 0.1 to 2% (w/v), with *I. tricantha* showing the maximal activity on *S. warneri*. The MIC value for *H. verticilaris* against *K. pneumoniae* and *E. sakkai* was 0.5%.

HPLC Analysis

Phenolic compounds in the four plant extracts were identified by HPLC on the basis of their retention time and absorbance spectrum pattern and, where possible, by chromatography with an authentic standard (Table **2**). It should be noted that a number of the phenolics found in the plants were not available in a purified form. The most abundant phenolic compounds detected were caffeic acid (0.06–0.31 mg 100 g^{-1} dry sample), quercetin (0.45–1.08 mg 100 g^{-1} dry sample), and rutin (0.03-1.08 mg 100 g^{-1} dry sample) (Table **2**). The results showed that *M. puberula* mostly contained 0.46 mg 100 g^{-1} dry sample quercetin and *H. verticillaris* contained approximately 0.7 mg 100 g^{-1} dry sample mangiferin and quercetin. Moreover, both *I. tricantha* and *E. cyclocarpum* contained mostly quercetin and rutin. None of the plant extracts contained allontoin and chlorogenic acid. *M. puberula* and *I. tricantha* had the lowest (0.76 mg 100 g^{-1}) and the highest (3.15 mg 100 g^{-1}) total phenolic compounds, respectively. Unfortunately, no phytochemical data for the plants examined except *I. tricantha* in this study were found in the literature for comparison.

GC-MS Analysis

Phenolic compounds of the plant extracts analysed by GC-MS are shown at the Table **3**. According to the results, *M. puberula* contained only two phenolic compounds including thymol (0.23% of volatile compounds) and ρ-methyl cinnamic acid (0.06% of volatile compounds). However, more phenolic compounds were analysed in the extract of *H. verticillaris* such as mangiferin, piperonol, methylcinnamic acid, and gallic acid, with 0.12, 0.12, 0.19, and 0.23% of volatile compounds, respectively. Methylcinnamic acid is the common phenolic compound detected in the tested four plant extracts by GC-MS. Thymol in *M. puberula*, mangiferin in *H. vertillaris*, quercetin in *I. tricantha*, and rutin in *E. cyclocarpum* were detected by both HPLC and GC-MS. Total percentage of phenolic compounds in the volatile

Table 2: Contents of Phenolic Compounds in Four Medicinal Plant Extracts Detected by HPLC

Phenolic Compounds	*Microdesmis Puberula* Hook. f. ex Planch (mg/100 g dry sample)	*Hypoestis Verticillaris* (Linn f.) Soland (mg/100 g dry sample)	*Icacina Tricantha* Oliv. (mg/100 g dry sample)	*Enterolobium Cyclocarpum* (Jacq.) Griseb. (mg/100 g dry sample)
Allantoin	ND	ND	ND	ND
Caffeic acid	0.064 ± 0,003	0.316± 0,053	0.090± 0,008	0.082± 0,009
Catechin	ND	0.073± 0,022	0.228± 0,012	ND
Chlorogenic acid	ND	ND	ND	ND
Epicatechin	0.011± 0,005	0.068± 0,005	0.110± 0,024	0.182± 0,033
Gallic acid	ND	0.067± 0,007	0.478± 0,027	ND
Hesperidin	0.036± 0,009	0.048± 0,006	0.036± 0,007	0.018± 0,006
Mangiferin	0.234± 0,014	0.637± 0,045	ND	ND
Quercetin	0.458± 0,031	0.75± 0,054	1.083± 0,005	0.533± 0,056
Polyditin	ND	ND	0.039± 0,003	0.057± 0,004
Phlorid	0.005± 0,001	0.003± 0,001	0.003± 0,001	ND
Rutin	0.033± 0,001	0.136± 0,083	1.080± 0,092	0.692± 0,073
Thymol	0.077± 0,009	ND	ND	0.034± 0,005
Total Phenols	0.761	2.099	3.148	1.598

ND: Not detected

Table 3: The Phenolic Compounds from Four Examined Plant Extracts Detected by GC-MS

***Microdesmis Puberula* Hook. f. ex Planch**			***Hypoestis Verticillaris* (Linn f.) Soland**			***Icacina Tricantha* Oliv.**			***Enterolobium Cyclocarpum* (Jacq.) Griseb.**		
Compounds	**RT * (min)**	**Area (%)**	**Compounds**	**RT (min)**	**Area (%)**	**Compounds**	**RT (min)**	**Area (%)**	**Compounds**	**RT (min)**	**Area (%)**
Adenosine, 4-de (hydroxymethyl)-4`- [N-ethylaminoformyl]	21.39	0.12	Piperonal	19.78	0.12	Phenol, 2, 4-bis (1,1-dimethylethyl)	30.08	0.18	Rutin	18.34	0.02
Thymol	17.67	0.23	Benzo thiophene, 2-ethyl	31.38	0.56	ρ-Methylcinnamic acid	31.33	0.34	ρ-Methylcinnamic acid	31.34	0.03
ρ-Methylhydrocinnamic acid	25.55	0.06	Mangiferin	32.17	0.12	Quarcetin	33.13	0.09	1, 4-Eicosadie	51.50	0.15
			ρ-Methylcinnamic acid	32.34	0.19	1, 4-Eicosadiene	62.65	0.98	Retinoic acid, methyl ester	63.96	0.56
			Gallic acid	60.34	0.23	3-Eicosene	62.67	0.56	Eicosane	84.77	0.07
			Pterin-6-carboxylic acid	63.68	0.12	4-Benzenesulfonyl-5-methyl-2-phenyl-2H-pyrazol-3-ol	63.95	0.56	β-amyrin	95.16	0.45
									α-amyrin	95.23	0.35
Total area (%)		**0.41**			**1.24**			**2.71**			**1.63**

*RT: Retention time (min).

compounds detected by GC-MS were found as 0.41, 1.24, 2.71 or 1.63% for *M. puberula, H. verticillaris, I. tricantha* or *E. cyclocarpum,* respectively.

TIC chromatograms of phenolics from *M. puberula, H. verticillaris, I. tricantha and E. cyclocarpum* are presented in Figure **1a**, **b**, and Figure **2a**, **2b**, respectively. Data obtained showed excellent resolution between all compounds of interest. Retention times of volatile compounds in the examined plant extracts are presented in Table **3**. Peaks related to complex volatile compounds with high molecular masses were also identified by the electronic libraries. Other phenolic compounds, such as piperonol, methylcinnamic acid, and gallic acid were identified by the present method as TMS derivatives, based upon the Wiley and NIST libraries.

DISCUSSION

The results showed that different bacterial species exhibited different sensitivities towards the plant extracts. In general, the extracts inhibited Gram-positive but not Gram-negative bacteria. These variations may reflect differences in cell surface structures between Gram-negative and Gram-positive bacteria. In particular, the outer membrane of Gram-negative bacteria functions as a preventive barrier against hydrophobic compounds [17]. The Gram-positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier [18]. Other studies also reported that plant extracts rich in phenolic compounds were more active against Gram-positive than Gram-negative bacteria [16, 19].

M. puberula, one of the medicinal plants tested in the current study, is widely used in Africa continent. For example, it is traditionally used as aphrodisiac and a medicine against spleen attacks for babies, diarrhoea, abscesses, gonorrhoea, gastrointestinal disorders, colics, ovarian troubles, otitis, ulcers and feverish stiffness [20]. It is also chewed for sore gum and teeth cleaning especially for elders in Nigeria [21]. In the current study, although *M. puberula* showed slight antimicrobial effect against *S. aureus* and *E. sakkai*, Atindehou *et al*. [22] could not observe any antimicrobial trace of the same plant against the same bacteria.

E. cyclocarpum is a species of flowering tree in the pea family, Fabaceae, that is native in the tropical regions of the Americas**.** Extracted leaves of this tree only inhibited the growth of *S*. warneri and *S. liquefaciens* in the current study. No inhibition was observed against *E. coli, B. subtilis, S. aureus* or *P. aureginosa* and this was supported by the work of Lendz *et al*. [23] who also reported the non-inhibition of

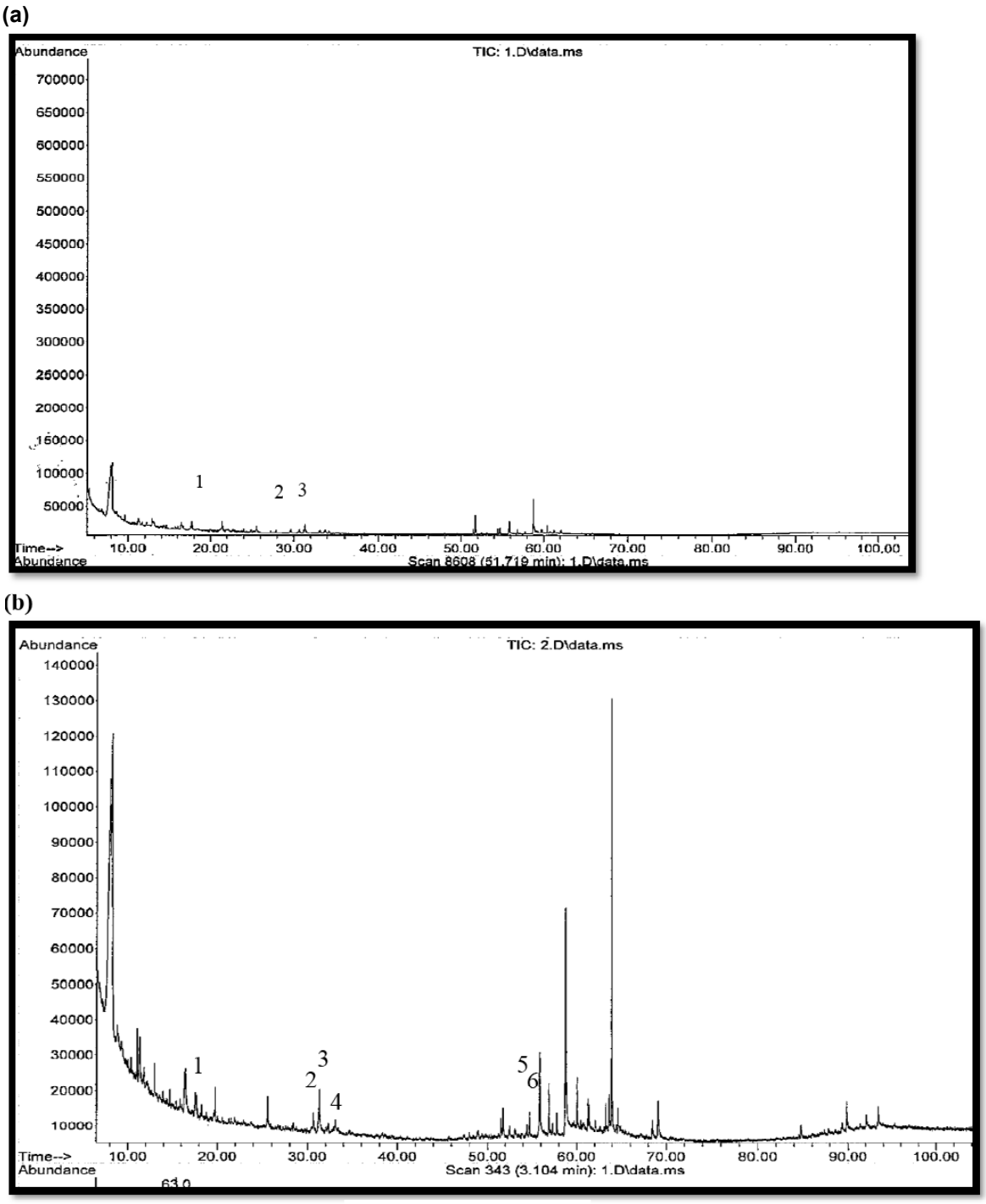

Figure 1(a): TIC chromatogram of phenolics from *Microdesmispuberula*: (1) Adenosine, 4-de (hydroxymethyl)-4`- [N-ethylaminoformyl], (2) thymol, (3) ρ -Methylcinnamic acid.
(b): TIC chromatogram of phenolics from *H.verticillaris*: (1) Piperonal, (2) benzothiophene, 2-ethyl (3) mangiferin(4) p-methylcinnamic acid,(5) gallic acid, (6) pterin-6-carboxylic acid.

the bacteria by the plant extract. Supplementation of the leaves of *E. cyclocarpum* was reported to decrease the number of protozoa but increased the cellulolytic bacterial counts in rumen of sheep because of its saponin content [24, 25].

The flavonoids detected in the present study have been studied by Rauha *et al*. [26] for determining antimicrobial effect in pure form. They claimed that pure flavonoids generally showed more effectiveness than the plant extracts. They explained that plant

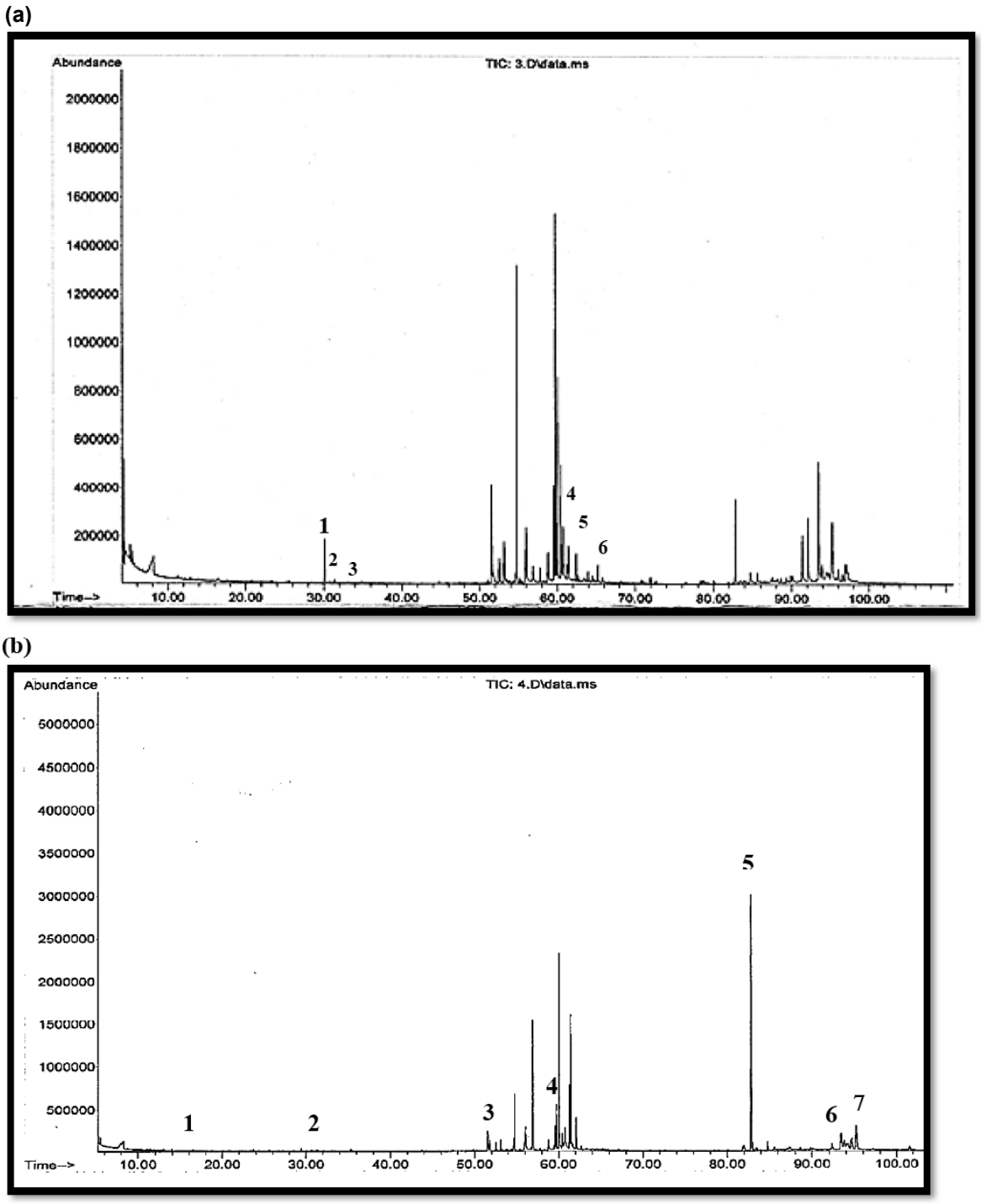

Figure 2(a): TIC chromatogram of phenolics from *Icacinatricantha*: (1) Phenol, 2, 4-bis (1,1-dimethylethyl), (2) p-methyl cinnamic acid, (3) quarcetin, (4) 1, 4-eicosadiene (5) 3-eicosene (6) 4-benzenesulfonyl-5-methyl-2-phenyl-2H-pyrazol-3-ol, **(b):** TIC chromatogram of phenolics from *Enterolobiumcyclocarpum*: (1) Rutin, (2) p-methylcinnamic acid , (3) 1, 4-eicosadie (4) retinoic acid, methyl ester, (5) eicosane (6) β-amyrin, (7) α-amyrin.

extracts generally contained flavonoids in glycosidic form and this may be the reason why the plant extracts did not produce as marked inhibition as many of the pure compounds.

In the current study, *S. warneri* was found as the most sensitive pathogen to three tested plant extracts including *H. verticillaris*, *I. tricantha* and *E. cyclocar pum*. This pathogen was found as part of the skin flora on humans and animals and has been suggested as a

cause of abortion in cattle and humans [27]. Furthermore, it has been also associated with urinary tract infection [28], meningitis [29], orthopedic infections [27], and endocarditis [30]. On the other hand, *K. pneumonia,* inhibited by ethanol extract of *H. verticillaris* in the present study*,* a pathogen*,* can cause inflammation and hemorrhagic in human lungs.

I. tricantha, traditionally used in the treatment of diabetes, is a shrub that arises from a stout hairy underground tube with a stem straggling with soft brown hairs [31]. It is usually grown in West Africa and Southern Nigeria [32]. Moderate antioxidant property and free radical scavenging activity for this plant was reported by Oke and Hamburger [33] and Sofidiya *et al.* [34]. The amount of total phenolic compounds was also reported as 5 mg g^{-1} gallic acid equivalent which was considerably higher than total phenolic compounds detected in the present study.

Quercetin was the most abundant phenol detected in the plant extracts in this study. This phenol, one of the most studied flavonoids, has been reported to have antioxidant, antimicrobial, and antiviral activities. At high concentrations (500 µg/ml), it appeared active against different microorganisms including *B. subtilis*, *M. luteus*, *S. aureus* and *S. epidermidis*; *A. flavus* and *A. parasiticus* [35, 36]. However, quercetin at low concentrations (100 µg/ml) or its esters did not have any antimicrobial effects on *S. aureus*, *B. subtilis, L. ivanovi*, *L. monocytogenes*, *L. serligeri*, *E. coli*, *S. flexneri*, *S. sonnei*, *S. enteritidis* and *S. tiphymurium* [37]. Similarly, in the present study, the plant extracts containing 0.45-1.08 mg 100 g^{-1} quercetin could inhibit only 7 bacteria types including *B. subtilis*, *S. aureus, S. warneri*, *K. pneumonia, E. sakkai, S. liquefaciens* and *B. pantothenticus* out of 21 different bacteria tested.

A variety of naturally occurring plant components has been demonstrated to be antimicrobial [19, 26, 38]. For example, several hydroxycinnamic acid derivatives, aldehydes and ketones have been shown to be antibacterial, antifungal and in some instances to be potent anticarcinogenic agents. In the present study, piperonal and p-methylcinnamic known antimicrobial agents were detected from *H. verticillaris*. Piperonal, a pepper-flavored GRAS aromatic aldehyde, had antimicrobial effects against *Y. enterocolitica, L. monocytogenes, S. typhimurium* and *B. cereus* at 3.13 mM, and *S. flexneri, A. hydrophila* and *E. coli* 0157:H7 at 6.25 mM [39]. Furthermore, cinnamic acid has inhibition effect against *Enterobacter aerogenes*, *E. coli* and *S. aureus* [40]. However, in this study, *H. verticillaris* did not show the same antibacterial effect against the same bacteria type most probably due to its insufficient piperonal (0.12% of total volatile compounds) and p-methylcinnamic acid (0.19% of total volatile compounds) content.

In conclusion, ethanolic extracts of the plants investigated in this study, especially *H. verticillaris,* showed inhibition against some of the selected Gram-positive and few Gram-negative bacteria. Some active phytochemicals such as quercetin, rutin, mangiferin, thymol or gallic acid in the plant samples were detected by HPLC and confirmed by GC-MS. The applications of these plant extracts could be considered as either natural preservative in the cosmetic and food industries or a safe alternative to synthetic antimicrobial drugs. For future research, the authors recommend that different extraction methods should be evaluated for the same plants for comparison.

ABBREVIATIONS

- HPLC: High pressure liquid chromatography.
- GC-MS: Gas chromatography-mass spectrum.
- DMSO: dimethylsulfoxide.
- MIC: Minimum inhibitory concentration.
- TMS: Transcranial magnetic stimulation.

REFERENCES

[1] Bisignano G, Germano MP, Nostro A, Sanogo R. Drugs used in Africa as dyes: antimicrobial activies. Phyto Res 1996; 9: 346-350.

[2] Lis-Balchin M, Deans SG. Antimicrobial effects of hydrophilic extracts of Pelargonium species (Geraniacee). Lett App Micro 1996; 23: 205-207. https://doi.org/10.1111/j.1472-765X.1996.tb00066.x

[3] Maoz M, Neeman I. Antimicrobial effects of aqueous plant extracts on the fungi Microsporum canis and Trichophyton rubrum and on three bacterial species. Lett App Micro 1998; 26: 61-63. https://doi.org/10.1046/j.1472-765X.1998.00277.x

[4] Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J App Microbiol 1999; 86: 985-990. https://doi.org/10.1046/j.1365-2672.1999.00780.x

[5] Roller S. The quest for natural antimicrobials as novel means of food preservation: status report on a European research project. Int Biodet Biodeg 1995; 36: 333-345. https://doi.org/10.1016/0964-8305(95)00074-7

[6] Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci 1997; 2: 152-159. https://doi.org/10.1016/S1360-1385(97)01018-2

[7] Justesen U, Knuthsen P. Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in

traditional Danish dishes. Food Chem 2001; 73: 245-250. https://doi.org/10.1016/S0308-8146(01)00114-5

[8] Ryan D, Robards K, Prenzler P, Antolovich M. Applications of mass spectrometry to plant phenols. Trends Anal Chem 1999; 18: 362-372. https://doi.org/10.1016/S0165-9936(98)00118-6

[9] Harborne JB. Phenolic compounds. In: Harborne JB, editor. Phytochemical methods; a guide to modern techniques of plant analysis. London: Chapman and Hall; 1998; p 40-106.

[10] Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, editor. The Flavonoids. London: Chapman and Hall; 1994; p 619-652.

[11] Hollman PCH, Katan MB. Dietary flavonoids: intake, hlth effects and bioavailability. Food Chem Toxic 1999; 37: 937-942. https://doi.org/10.1016/S0278-6915(99)00079-4

[12] Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. J Nutri Biochem 2002; 13: 572-584. https://doi.org/10.1016/S0955-2863(02)00208-5

[13] Tranter HS, Tassou SC, Nychas GJ. The effect of the olive phenoliccompound, oleuropein, on growth and enterotoxin B production by Staphylococcus aureus. J App Bac 1993; 74: 253-259. https://doi.org/10.1111/j.1365-2672.1993.tb03023.x

[14] Nychas, GJE. Natural antimicrobials from plants. Springer US, 1995. https://doi.org/10.1007/978-1-4615-2105-1_4

[15] Parrilla MCG, Heredia JF, Troncoso MA. Sherry wine vinegars: phenolic composition changes during aging. Food Res Int 1999; 32: 433-440. https://doi.org/10.1016/S0963-9969(99)00105-2

[16] Yu J, Lei J, Yu H, Cai X, Zou G. Chemical composition and antimicrobial activity of the essential oil of Scutellaria barbata. Phytochem 2004; 65: 881-884. https://doi.org/10.1016/j.phytochem.2004.02.005

[17] Helander LM, Alakomi HL, Latva-Kala K. Characterization of the action of selected essential oil components on Gram-negative bacteria. J Agri Food Chem 1998; 46: 3590-3595. https://doi.org/10.1021/jf980154m

[18] Malanovic N, Lohner K. Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides. BBA-Biomemb 2016; 1858: 936-946. https://doi.org/10.1016/j.bbamem.2015.11.004

[19] Nostro A, Germanoa MP, D'Angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett App Micro 2000; 30: 379-384. https://doi.org/10.1046/j.1472-765x.2000.00731.x

[20] Idu M, Onyibe HI. Medicinal plants of Edo State, Nigeria. Res J Med Plant 2008; 1: 32-41.

[21] Idu M, Umweni AA, Odaro T, Ojelede L. Ethnobotanical plants used for oral healthcare among the Esan Tribe of Edo State. Nigeria Ethnobot Leaf 2009; 13:548-563.

[22] Atindehou KK, Kone M, Terreaux C, Traore D, Hostettmann K, Dosso M. Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast Pho Res 2002; 16: 497-502.

[23] Lentz D L, Clark AM, Hufford CD, Meurer-Grimes B, Passreiter CM, Cordero J, Ibrahimi O, Okunade AL. Antimicrobial properties of Honduran medicinal plants. J Ethnopharm 1998; 63: 253-263. https://doi.org/10.1016/S0378-8741(98)00100-7

[24] Diaz A, Avendan OM, Escobar A. Evaluation of Sapindus saponaria as a defaunating agent and its effects on different ruminal digestion parameters. Liv Res Rural Dev 1993; (epublication: http://www.cipav.org.co/lrrd/lrrd5/cefe.htm).

[25] Ivan M, Koenig KM, Teferedegne B. Effect of the dietary Enterolobium cyclocarpum foliage on the population dynamics of rumen ciliate protozoa in sheep. Small Rum Res 2004; 52: 81-91. https://doi.org/10.1016/S0921-4488(03)00230-X

[26] Rauha JP, Remes S, Heinonen M, Hopia A, Kahkonen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Micro 2000; 56: 3-12 https://doi.org/10.1016/S0168-1605(00)00218-X

[27] Barigye R, Schaan L, Gibbs PS, Schamber E, Dyer NW. Diagnostic evidence of Staphylococcus warneri as a possible cause of bovine abortion. J Vet Dia Inv 2007; 19: 694-696. https://doi.org/10.1177/104063870701900613

[28] Dimitriadi D, Charitidou C, Charvalos E. Urinary tract infection due to beta-lactams-resistant Staphylococcus warneri: A case report. Int Arabic J Antimic Agen 2015; 4: 1-4. https://doi.org/10.3823/760

[29] Incani RN, Hernández M, Cortez J, González ME, Salazar YD. Staphylococcus warneri meningitis in a patient with Strongyloides stercoralis hyperinfection and lymphoma: first report of a case. J São Paulo Ins Trop Med 2010; 52: 169-170. https://doi.org/10.1590/S0036-46652010000300011

[30] Campoccia D, Montanaro L, Visai L, Corazzari T, Poggio C, Pegreffi F, Maso A, Pirini V, Ravaioli S, Cangini I, others. Characterization of 26 Staphylococcus warneri isolates from orthopedic infections. Int J Artif Org 2010; 33: 575-581.

[31] Fatokun FK. Investigation of the possible anti-diabetic activity of Icacina trichantha, Ananas cosmos and Uraria picta in a rat model. A Dissertation for Master of Science. Department of Pharmacy and Pharmacology, University of the Witwatersr and, Johannesburg, South Africa. 2010

[32] Gbile ZO, Adesina SK, Odukoya OA, Akinwusi DD, Illoh HC, Jayeola A. The United Nations University Programme on Natural resources in Africa. Survey on indigenous useful plants of West Africa with Special emphasis on medicinal plants and issues associated with their management. 1993; p 131-132.

[33] Oke JM, Hamburger MO. Screening of some Nigerian medicinal plants for antioxidant activity using 2,2, diphenyl-picryl-hydrazyl radical. Afr J Biomed Res 2002; 5: 77-79.

[34] Sofidiya MO, Odukoya OA, Familoni OB, Inya-Agha SI. Free radical scavenging activity of some Nigerian medicinal plant extracts. Pak J Bio Sci 2006; 9: 1438-1441. https://doi.org/10.3923/pjbs.2006.1438.1441

[35] Aziz NH, Farag SE, Mousa LA, Abo-Zaid MA. Comparative antibacterial and antifungal effects of some phenolic compounds. Microbiol 1998; 93: 43-51.

[36] Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, Vuorela P. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Mic 2000; 56: 3-12. https://doi.org/10.1016/S0168-1605(00)00218-X

[37] Gatto MT, Falcocchio S, Grippa E, Mazzanti G, Battinelli L, Nicolosi G, Lambusta D, Saso L. Antimicrobial and anti-lipase activity of quercetin and its C2-C16 3-O-Acyl-Esters. Bio Med Chem 2002; 10: 269-272. https://doi.org/10.1016/S0968-0896(01)00275-9

[38] Puupponen-Pimia R, Nohynek L, Meier C, Kahkonen M, Heinonen M, Hopia A, Oksman-Caldentey KM. Antimicrobial properties of phenolic compounds from Berries. J App Micro 2001; 90: 494-507 https://doi.org/10.1046/j.1365-2672.2001.01271.x

[39] Bowles BL, and Juneja V K. Inhibition of foodborne bacterial pathogens by naturally occurring food additives. J Food Safety 1998; 18: 101-112. https://doi.org/10.1111/j.1745-4565.1998.tb00206.x

[40] Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Bra J Micro 2000; 31: 247-256. https://doi.org/10.1590/s1517-83822000000400003

Received on 02-02-2017 Accepted on 20-02-2017 Published on 28-02-2017

http://dx.doi.org/10.15379/2408-9826.2017.04.01.03