ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Polyphenolic constituents and antimicrobial activity of *Rhapis excels* (*Arecaceae, Coryphoideae*).

Hassanein HD[1]*, Elsayed WM[1], Abreu AC[2], Simões M[2], and Abdelmohsen MM[1].

[1]Phytochemistry Department, National Research Centre, 12311 Dokki, Cairo Egypt.
[2]LEPAE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal.

ABSTRACT

The chromatographic fractionation of *Rhapis excelsa* f.,Arecaceae, leaves extract, a plant known as lady palm, resulted in the isolation of four flavonoids: Apigenin-8-*C*-glucoside (vitexin), Apigenin-6,8-Di-*C*-*β*-glucopyranoside (vicenin-2), Luteolin-6-*C*-glucoside (isoorientin) and Luteolin-8-*C*-glucoside (orientin). The structural elucidations of these compounds were performed by means of the comparison of their spectral data (UV systematic identification and ^{1}NMR) with those ones of the literature. Ethyl acetate and butanol fractions showed remarkable antioxidant activity (86.2 and 75.6 respectively), when investigated for their DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The major polyphenols were identified, as benzoic acid, ferulic acid with others by means of RP-HPLC, they were quantified in methanolic crude extract. Also, the antibacterial activity of the extract was assessed against *Staphylococcus aureus*strains, including methicillin-resistant *S. aureus* (MRSA). The extracts had no antimicrobial activity alone but they revealed ability to potentiate the antibacterial activity of ciprofloxacin, tetracycline and oxacillin.

Keywords: Rhapis excels, Arecaceae, Flavonoids, Antibacterial activity

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Plants have been an important source of medicine for thousands of years. Most valuable phytochemicals are products of plant secondary metabolites, which may be used as purified compounds or complex mixtures and serve as medicines, pesticides, flavoring, herbicides, food preservatives, etc. The historic use of herbal remedies to treat and prevent infectious diseases has been replaced with the emergence of specific synthetic drugs and antimicrobial agents. However, in the last decade the use of plant remedies, known to possess natural antioxidant, antimicrobial, cytotoxic and other activities, has increased in human medicine, as it serves as a natural approach to treat diseases (Rochfort and Panozzo, 2007).

The Arecaceae is a very large family, of great commercial importance as source of nuts, edible fruits, starchy foods, oils, fibre and woods. Palm trees include many important agricultural plants including Coconut (*Cocosnucifera*), Date Palm (*Phoenix dactylifera*), Oil Palm (*Elaeisguineensis*), True Sago Palm (*Metroxylonsagus*) and Rattan (*Calamus* sp.). The genus *Rhapis* (*Arecaceae, Coryphoideae*) consists of clustering unarmed palms, commonly known as lady palms. The species of *Rhapis* are distributed from southern China southwards through Indochina to peninsular Thailand and northern Sumatra (Dransfield and Uhl, 1998). *Rhapis* is represented by small under-growth palms of tropical evergreen, lowland forest (Averyanove*t al.*, 2006; Hastings 2003; Uhl and Dransfield 1987**)**. In fact, palm fruits have already been found to contain a number of components with established biological function, such as, carotenes, tocopherols, polyphenols, phenolic acids and flavonoids (Tan *et al.,* 2007**)**. Recent research has shown that palm kernel cake protein inhibits the growth of spore-forming bacteria (Tan *et al.,* 2011).

Rhapis excelsa is a palmate species and an outdoor plant, which is very undemanding and grows well in a variety of soils, so long as poor drainage and salt are avoided. It may be considered as ideal subject for research. Few studies have been carried out to date regarding its active constituents as well as its medical uses.

In this study, we describe the chromatographic fractionation, the isolation and the structural elucidation of four flavonoids that occur in the methanolic crude extract of *R. excelsa* leaves. Investigation of the antioxidant activity of this extract which may be responsible for the antimicrobial activity was performed. Analysis of polyphenolic compounds was carried out by a RP-HPLC method. Also, the antibacterial activity of the extract was assessed against *Staphylococcus aureus*strains, including methicillin-resistant *S. aureus* (MRSA). The ability of this extract to potentiate the antibacterial activity of five antibiotics,ampicillin (AMP), oxacillin (OXA), ciprofloxacin (CIP), tetracycline (TET) and erythromycin (ERY), was evaluated as well. The disc diffusion method was used for this assay.

EXPERIMENTAL

Materials and apparatus

Plant material

Rhapisexcelsa fresh leaves were collected during 2013 from El-Orman Botanical Garden. The Authors would like to thank Dr. Mohammed El-Gibali Senior botanist for his help in plant identification. Leaves of the plant were air dried at room temperature and coarsely powdered, packed in dark coloured tightly closed containers and kept for extraction. A voucher specimen are deposited at the herbarium of the NRC.

Chemicals and reagents

Column chromatography (CC) was performed using polyamide and sephadex LH-20 (Pharmacia, Merck, Darmstadt, Germany). Whatmann 3 was used for paper chromatography (PC), developed with *n*-butanol: acetic acid: water 4:1:5 (BAW) and 15 % acetic acid (AC). Spots were detected using Neu's reagent (1% 2-aminoethyl diphenylborinate in methanol) Aldrich. DMSO, DPPH and Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid were purchased from Aldrich chem. Co. All chemicals and reagents used were of analytical or HPLC grade. AMP, OXA, CIP, TET and ERY were purchased from Sigma (Portugal) and prepared according to manufacturer recommendations.

Apparatus

UV-spectrophotometer (Shimadzu, Kyoto, Japan), NMR on Delta 2 spectrometer operating at 500 MHz, samples were dissolved in DMSO. HPLC determination of phenolic compounds was conducted on Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. Column: Eclipse XDB-C_{18} (4.6 X 250 mm; 5 µm) with a C_{18} guard column (Phenomenex, Torrance, CA). Column temperature: ambient temperature; flow rate: 0.8 ml/min; Detector at 280, 320 nm; injection volume: 20 µl; Mobile phase: acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B) with gradient programmed as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. Run time: 70 min. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection as previously mentioned in Kim *et al.* (2006).

Methods

Extraction, fractionation and isolation

Two kg of the air dried powdered leaves of *R. excelsa* were extracted by maceration in methanol – water (4:1, v/v) at room temperature for three successive times. The combined extracts were then filtered and concentrated under reduced pressure at 45ºCto obtain the methanolic crude extract (150 g). 140 g were suspended in methanol –water (4:1, v/v), left overnight, and then filtered. The supernatant was extracted with solvents of increased polarity to afford hexane soluble layer (Hex, 5 g), a dichloromethane soluble layer (DCM, 7 g), an ethyl acetate soluble layer (EtOAc, 8 g), and finally *n*- butanol soluble layer (But, 15 g). An aliquot of butanol fraction (12 g) was chromatographed using polyamide column (80 ×7cm) eluted by water, with the gradient increase of methanol (10 % each). Six main fractions were pooled after PC analysis. Spots were visualized under UV light at 254 and 356 nm before and after spraying. Factions with main compounds were further rechomatographed on sephadex column (80 × 4cm) using gradient concentrations of methanol- water. Compounds **1**, **2** were obtained as yellowish powder (30 and 50 mg respectively). Compounds **3** and **4** were purified by preparative PC to give 35 and 40 mg respectively. The isolated compounds were developed on PC, spots were detected under UV light, colored by spraying with Neu's reagent, and the R_f values were taken. Structures were established by correlating spectral data (UV, MS, ^{1}H-NMR) with those of literature (Mabry *et al.*, 1970, 1976; El-Desoky and Sharaf, 2004; Koolen*et al.,* 2012).

DPPH Assay

The determination of DPPH radical scavenging capability was estimated as described (Clarke *et al.,* 2013). 20 µL of extract diluted appropriately in dimethyl sulfoxide (DMSO) was mixed with 180 µL of DPPH in methanol (4 mg/mL) in wells of a 96-well plate. The plate was kept in the dark for 15 min, after which the absorbance of the solution was measured at 540 nm in a Multiskan automatic kinetic microplate reader ((LabsystemsMultiskan RC reader). Appropriate blanks (DMSO) and standard (trolox solutions in DMSO) were run simultaneously. Extracts were first tested at a single concentration of 4 mg/mL, and those showing good evidence of antioxidant activity (high percentage reduction of the initial DPPH absorption) were tested over a range of concentrations to establish the EC_{50} (extract concentration providing 50% inhibition) (Hassanein*et al.,* 2014). Tests were carried out in triplicate.

Preparation of the sample

Leaves powder was extracted according to Kim *et al*. (2006). Briefly, 10 mg was submitted to an alkaline hydrolysis by shaking with 200 mL of 2 M NaOH, then PH was adjusted to 2 with 6 M HCL, then filtered. The supernatant was collected, then extracted twice with ethyl ether and ethyl acetate. The organic phase was separated and evaporated. The residue was extracted by sonication with methanol 80% at room temperature, the extract was filtered, clarified by centrifugation. Pure standards were used as external standards to identify the compounds (All standards were purchased from Sigma). Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

ISSN: 0975-8585

Antimicrobial Activity

Bacterial strains

Seven *S. aureus* strains were used. SA1199B is a fluoroquinolone-resistant NorAoverexpresser derived from a methicillin-susceptible *S. aureus* bloodstream isolate from a patient with endocarditis(Kaatz*et al.*, 1991), RN4220 contains plasmid pU5054 (that carries the gene encoding the MsrA macrolide efflux protein), and XU212 possesses the TetK efflux pump and is also a MRSA strain.These strainswere provided by S. Gibbons (University College London, UK)(Smith *et al.* 2007, Oluwatuyi*et al.* 2004, Gibbons*et al.* 2003, Gibbons &Udo 2000).Three clinical MRSA (MJMC001, MJMC002, MJMC004) were isolatedfrom the Hospital Centre of Trás-os-Montes and Alto Douro, EPE, Vila Real (Portugal). *S. aureus* CECT 976 was already used in other studies with phytochemical compounds (Abreu *et al.* 2014 & 2012, Saavedra *et al.* 2010,Simões*et al.* 2008). Bacteria were grown overnight at 37 ºC and underagitation (150 rpm) in Mueller-Hinton (MH) broth (Merck, Germany).

Antibacterial susceptibility testing

The Minimum Inhibitory Concentration (MIC) of each extract was determined by broth microdilution testing according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010). MIC was defined as the lowest concentration of the antimicrobial compound that inhibited bacterial growth. Three independent experiments were performed for each compound. The highest concentration of DMSO remaining after dilution (10%, v/v of the well) caused no growth inhibition (data not shown).

Disc Diffusion Method

Antibiotic-extracts antibacterial effects were determined according to Abreu *et al.* (2014). Each extract was added to MH agar (after autoclaved and cooled) yielding the final concentration desired, which was chosen according to the checkerboard results. Then, the medium was poured into 90 mm Petri dishes to give a uniform depth of approximately 4 mm (~20 mL). The bacterial suspensions were adjusted to 0.5 McFarlandstandards and seeded over hardened MH agar Petri dishes using a sterilized cotton swab and allowed to set (for 10 to 15 min).Sterile blank discs (6 mm diameter; Oxoid, Portugal) were placed on the agar plate seeded with the respective bacteria. A volume of 15 µL of each antibiotic prepared according to the CLSI guidelines (AMP – 10 µg/disc; CIP – 5 µg/disc; ERY – 15 µg/disc; TET – 30 µg/disc; and OXA – 1 µg/disc) was added to the blank discs[26].After incubation at 37 °C for 24 h, each inhibition zone diameter (IZD) was recorded and analyzed according to CLSI guidelines (CLSI, 20013).Concerning the disc diffusion method, according to the scheme proposed in a previous study (Abreu *et al.* 2014) for non-antibacterial compounds, it was characterized the combination between two bioactive agents as additive if 4 ≤ (IZD combination – IZD most active agent) < 6 mm, as potentiation if (IZD combination – IZD most active agent) ≥ 6 mm, indifferent if -6 <(IZD combination – IZD most active agent) < 4 and asnegative if (IZD most active agent – IZD combination) ≥ 6 mm.

RESULTS AND DISCUSSION

Isolated Compounds

Apigenin-8-*C*-glucoside, vitexin**(1)**, yellowish powder, $C_{21}H_{20}O_{10}$, purple spot turns yellowish green after spraying, R_f = 0.43 (BAW), 0.65 (AC). UV (λ_{max}, nm) MeOH: 271, 333; NaOMe: 279, 329, 397; $AlCl_3$: 279, 303, 348; $AlCl_3$/HCl: 279, 346; NaOAc: 278, 378; NaOAc/H_3Bo_3: 272, 341. ^{1}H-NMR (300 MHz, DMSO) δ: 7.79 (2H, d, *J*=8.8 Hz, H-2′, H-6′), 6.93 (2H, d, *J*=8.8 Hz, H-3′, H-5′), 6.62 (1 H, s, H-3), 6.21 (1 H, d, *J*=2.5 Hz, H-6), 4.61 (1H, d, H-1″), 3.93 (1H, m, H-2″), 3.65 (2H, m, H-3″, H-5″), 3.33 (1H, m, H-4″), 3.93 (1H, m, H-6b″), 3.85 (1H, m, H-6a″).

Apigenin-6,8-Di-*C*-*β*-glucopyranoside, vicenin-2 **(2)**, yellowish powder, $C_{27}H_{30}O_{15}$,purple spot turns yellowish green after spraying, R_f = 0.36 (BAW), 0.65 (AC). UV (λ_{max}, nm) MeOH: 271, 327; NaOMe: 275, 394; $AlCl_3$: 277, 344, 378; $AlCl_3$/HCl: 278, 343, 378; NaOAc: 270, 390; NaOAc/H_3Bo_3: 271, 330. ^{1}H-NMR (300 MHz, DMSO) δ: 7.92(2H, d, *J*=8.8 Hz, H-2′, H-6′), 6.94 (2H, d, *J*=8.8 Hz, H-3′, H-5′), 6.82 (1 H, s, H-3), 4.64 (1H, d, H-1″), 4.02 (1H, m, H-2″), 3.19 (H, m, H-3″), 3.22 (1H, m, H-4″), 3.16 (1H, m, H-5″), 3.39, 3.45 (2H, m, H-6b″, H-6a″), 4.97

(1H, d, H-1‴), 3.81(1H, m, H-2‴), 3.16(H, m, H-3‴), 3.32 (1H, m, H-4‴), 3.29(1H, m, H-5‴), 3.66, 3.71(2H, m, H-6b‴, H-6a‴).

Luteolin-6-*C*-glucoside, isoorientin**(3),** yellow powder, $C_{21}H_{20}O_{11,}$ purple spot turns yellowish green after spraying, R_f = 0.38 (BAW), 0.57 (AC). UV (λ_{max}, nm)MeOH: 270, 344; NaOMe: 277, 401; $AlCl_3$: 276, 404,$AlCl_3$/HCl: 273, 387; NaOAc: 272, 397; NaOAc/H_3Bo_3: 270, 353.^{1}H-NMR (300 MHz, DMSO) δ: 6.47 (1H, s, H-8), 6.53 (1H, s, H-3), 7.34 (1H, m, H-2'), 7.33 (1H, dd, *J*=8.5, 2.1 Hz, H-6'), 6.89 (1H, d, *J*=8.5 Hz, H-5'), 4.98 (1H, d, *J*=9.0 Hz, H-1"), 4.13 (1H, d, *J*=9.0 Hz, H-2"), 3.86 (1H, dd, *J*=12.0, 2.0 Hz, H-6b"), 3.77 (1H, d, *J*=12.0, 5.0 Hz, H-6a"), 3.46 (2H, m, H-4", H-5"), 3.40 (1H, m, H-3").

Luteolin-8-*C*-glucoside, orientin **(4),** yellow powder, $C_{21}H_{20}O_{11,}$ purple spot turns yellowish green after spraying, R_f = 0.28 (BAW), 0.37 (AC). UV (λ_{max}, nm)MeOH: 269, 342;NaOMe: 277, 403; $AlCl_3$: 274, 348, 415,$AlCl_3$/HCl: 275, 354; NaOAc: 271, 381; NaOAc/H_3Bo_3: 265, 359.^{1}H-NMR (300 MHz, DMSO) δ: 6.33 (1H, s, H-6), 6.57 (1H, s, H-3), 7.10 (1H, m, H-2'), 7.70 (1H, dd, *J*=8.5, 2.1 Hz, H-6'), 6.87 (1H, d, *J*=8.5 Hz, H-5'), 4.89 (1H, d, *J*=9.0 Hz, H-1"), 4.12 (1H, m, H-2"), 4.03 (1H, dd, *J*=12.0, 2.0 Hz, H-6b"), 3.82 (1H, d, *J*=12.0, 5.0 Hz, H-6a"), 3.42 (2H, m, H-4", H-5"), 3.41 (1H, m, H-3").

DPPH Assay

The results showed thatboth the ethyl acetate and butanol fractions exerted remarkable activity (86.2 and 75.6 % respectively), when compared with the oxidative potentialof the standard compound (Trolox, 98.2%). The EC_{50} values of both were 30±1.06 and 32±1.26 µg/mL respectively (Fig. 1).

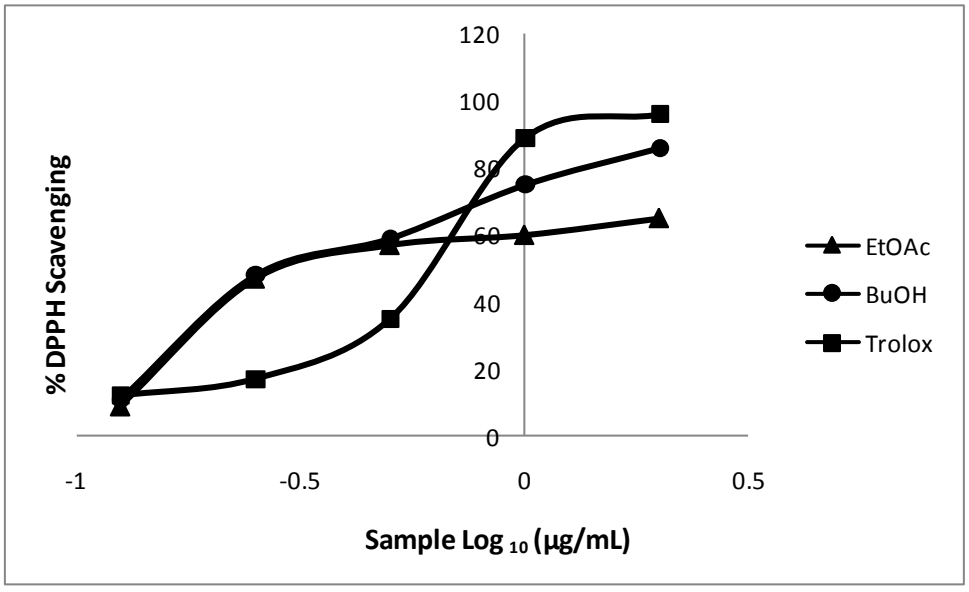

Figure 1: Concentration-response curves for the DPPH radical scavenging activity of trolox (positive control), butanol fraction and ethyl acetate fraction of *R. excelsa.*

Determination of the phenolic compounds

The HPLC result of the leaf methanolic extract of *Raphis* showed that the main constituents were benzoic acid (1882.5 µg/g of the dry plant material) and ferulic acid (1254.4 µg/g). Caffeic, syrngic, vanillic and sinapic acids luteolin and rutin were also identified (Table 1, Fig. 2).

ISSN: 0975-8585

Table 1: HPLC data for the phenolic acids and flavonoids detected in *R. excels* leaves extract.

Compound	Rt	ug/20ul	ug/g	Peak no. on chart
Protocatechuic	9.62	1.474056	715.1443	1
Benzoic Acid	16.76	3.880286	1882.537	2
Catachine	18.29	0.455183	220.8338	3
Caffeic	20.38	0.348048	168.8568	4
Syrngic	21.89	0.207959	100.8924	5
Vanillic	23.41	0.185202	89.85172	6
Ferulic	31.13	2.585604	1254.417	7
Sinapic	32.65	0.836012	405.595	8
Ruten	35.55	0.616564	299.1289	9
Coumarin	36.54	0.059158	28.7006	10
Cinnamic	42.19	0.017294	8.390186	11
Luteolin	42.87	0.033762	16.37978	12

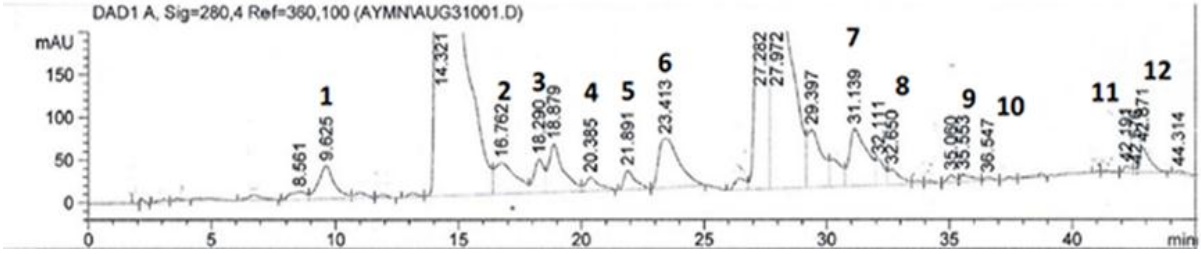

Figure 2: HPLC–DAD chromatograms at 280 nm of the EtOH extracts of Rhapis leafs; Peak numbers refer to Table 1.

Antimicrobial activity

Table 2 presents the disc diffusion method results evaluated for the antibiotics alone and combined with LP extract (at 1mg/mL). For *S. aureus* SA1199B, RN4220 and XU212, only CIP, ERY and TET were tested, respectively. According to the analysis of IZDs by the susceptibility breakpoints of the NCCLS (NCCLS/ CLSI, 2003)**,** *S.aureus* CECT976 was considered susceptible to all antibiotics, *S. aureus* SA1199B, RN4220 and XU212 were resistant to CIP, ERY and TET, respectively, the MRSA strains were classified as resistant to AMP, OXA, ERY and CIP and only susceptible to TET; MSSA strains were classified as susceptible to all antibiotics, with exception of AMP. The extract had no antibacterial activity at the highest concentration tested, 1mg/mL (data not shown).

The LP extract was able to potentiate the activity of CIP against *S. aureus* SA1199B and of TET against XU212. Interestingly, this extract was also able to potentiate OXA against MRSA strains MJMC002 and 4, but not MRSA-1. However, with AMP (also a β-lactam antibiotic) no interaction was observed. Additive interactions were obtained in combination with TET against *S. aureus* CECT976, MRSA-1 and MRSA-2. Since the strains possess different resistance profiles, it may be difficult to explain how the extract can potentiate different antibiotics against some strains, but not against others, and what mechanisms may be behind these potentiating activities. This result is probably related to the fact that strains vary in the extent of the expression of resistance mechanisms. Also, the extract possesses different compounds that may act in distinct ways against the *S. aureus* strains.

Becker *et al.,* (2005) reported that *Lythrumsalicaria* extracts showed activity against some bacteria, including *S.aureus.* Theflavon-*C*-glucosidesvitexin, isovitexin, orientin and isoorientin were isolated. However, these compounds were not considered responsible for the antibacterial activities. Cottigli *et al.* (2001) evaluated the antimicrobial activity of stems methanol extract from *Daphne gnidium* L. collected from Italy, against strains of *S.aureus*, amongst others. Luteolin, orientin, isoorientin and apigenin-7-*O*-glucoside were

detected in the plant extract but were not considered active compounds. To our knowledge, there are no studies about the activity of these compounds in potentiating antibiotics. The activities detected in the extract and fractions suggest a synergistic action of some compounds (well known for their antioxidant properties), as well as the presence of other bioactive compounds that already had been isolated from the leaves and recognized as antimicrobial agents against several micro-organisms (Pretto *et al.* 2004). More tests are necessary in order to conclude if any of these compounds alone or in combination are responsible for the potentiating activities of the extract.

Table 2: IZDs obtained by disc-diffusion method for the antibiotics alone and in combination with *R.excelsa*ethanolic extract (at 1mg/mL) against the *S.aureus* strains tested. Classifications of the combinations as potentiating (P), additive (A) or indifferent (I) are given in parentheses.

IZD disc diffusion method			
Isolates	**Antib.**	**Control**	**LP**
CECT 976	**CIP**	38.9 ± 2.1	38.8 ± 2.4 (I)
	TET	29.7 ± 2.1	35.0 ± 4.7 (A)
	ERY	32.0 ± 1.4	34.8 ± 3.0 (I)
	AMP	22.2 ± 2.0	22.0 ± 1.4 (I)
	OXA	27.2 ± 2.9	27.5 ± 2.8 (I)
SA1199B	**CIP**	18.1 ± 1.5	**29.9 ± 2.3 (P)**
XU212	**TET**	0.0 ± 0.0	**10.3 ± 0.6 (P)**
RN4220	**ERY**	9.9 ± 1.9	12.7 ± 1.7 (I)
MRSA MJMC001	**CIP**	NG	NG (I)
	TET	24.5 ± 0.0	30.0 ± 1.4 (A)
	ERY	13.0 ± 0.0	13.5 ± 0.7 (I)
	AMP	NG	NG (I)
	OXA	NG	NG (I)
MRSA MJMC002	**CIP**	NG	NG (I)
	TET	20.0 ± 0.0	25.5 ± 0.7 (A)
	ERY	12.5 ± 0.7	15.0 ± 0.0 (I)
	AMP	NG	NG (I)
	OXA	NG	**20.0 ± 0.0 (P)**
MRSA MJMC004	**CIP**	NG	NG (I)
	TET	27.0 ± 1.0	25.0 ± 0.0 (I)
	ERY	12.0 ± 0.0	15.0 ± 0.0 (I)
	AMP	NG	NG (I)
	OXA	NG	**31.5 ± 0.6 (P)**

NG: no growth. Data are means and SD from at least three independent experiments.

CONCLUSION

This study displays some biological properties of interest related to the polyphenols (including phenolic acids and flavonoids) and their antioxidant efficacy which are the key in the beneficial properties of *R.excelsa.* The use of phenolic compounds as antimicrobial agents can provide synergistic benefits with their natural biological properties. Their increasing prevalence is one of the major challenges for the health systems worldwide as antibiotic-resistant infections imposes enormous health expenditure, which in need for the development of new generations of antibiotic drugs, mostly in developing countries. We suggest that leaves of *R. excelsa* could afford an interesting raw material for the production of health-benefit products as food preservatives and provide a solution to an environmental problem.

REFERENCES

[1] Abreu AC, Borges A, Saavedra MJ, Simões M (2012). Antibacterial activity of phenyl isothiocyanate on *Escherichia coli* and *Staphylococcus aureus*. Medicinal Chemistry,12:756-761.

[2] Abreu AC, Serra S, Borges A, Saavedra MJ, Salgado A, Simões(2014).M. Evaluation of the best method to assess antibiotic potentiation by phytochemicals against *Staphylococcus aureus*. Diagnostic Microbiology and Infectious Diseases, 79: 125-134.

[3] Averyanov LV, Nguyen,TH,Phan,KL (2006). *Rhapisvidalii*anewpapyrus-likepalmfromVietnam. Palms, 50: 11–22.
[4] Becker H, Scher JM, Speakman JB, Zapp J (2005). Bioactivity guided isolation of antimicrobial compounds from Lythrum salicaria. Fitoterapia,76:580-584.
[5] Clarke G, Ting T, Wiart C, Fry J (2013). High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Ferricreducing activity potential and total phenolicscontentindicates redundancy in use of all three assays to screen forantioxidant activity of extracts of plants from the Malaysianrainforest. Antioxidants, 2: 1-10.
[6] Clinical and Laboratory Standards Institute (CLSI) (2013). Performance standards forantimicrobial susceptibility testing; approved standard - sixth Edition, M7-A6.Document M100-S17. Wayne, PA, USA.
[7] Cody RB, Laramée JA, Durst HD (2005). Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions.Analytical Chemistry,77: 2297-2302.
[8] Cottigli F, Loy G,Garau D, Floris C, Caus M,Pompei R, Bonsignore L (2001).. Antimicrobial evaluation of coumarins and flavonoids from the stems of *Daphne gnidium* L. Phytomedicine,8:302-305.
[9] Dransfield J, Uhl, NW(1998). Palmae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants. IV. Flowering Plants, Monocotyledons: Alismatanae and Commelinanae, Except Gramineae. Springer, Berlin, pp. 306–389.
[10] El-Desoky S, Sharaf M (2004). A new prenylatedbiflavone from *Origanumsyriacum*. Revista Latino Americana de Quimica, 32: 21- 24.
[11] Gibbons S, Oluwatuyi M, Kaatz GW (2003). A novel inhibitor of multidrug efflux pumps in *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy,51:13-17.
[12] Gibbons S, Udo EE (2000). The effect of reserpine, a modulator of multidrug efflux pumps, on the *in vitro* activity of tetracycline against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) possessing the Tet(K) determinant. Phytotherapy Research,14:139-140.
[13] Hassanein HD, Said-Al Ahl HAH, Abdelmohsen MM (2014). Antioxidant Polyphenolic Constituents of *Satureja Montana* L. Growing in Egypt. International Journal of Pharmacy and Pharmaceutical Sciences,6:578-581.
[14] Hastings LH (2003).A revision of *Rhapis*, the Lady Palms. Palms, 47: 62–78.
[15] Kaatz GW, Seo SM, Ruble CA (1991). Mechanisms of Fluoroquinolone Resistance in *Staphylococcus aureus*.Journal of Infectious Diseases,163:1080-1086.
[16] Kim, k.H., Tsao, R., Yang, R., Cui, S.W. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. FoodChemistry, 95: 466-473.
[17] Koolen HHF, Soares ER, Silva FMA, Souza AQL, Filho ER, Souza ADL (2012). Triterpenes and flavonoids from the roots of *Mauritiaflexuosa*. RevistaBrasileira deFarmacognosia, 22: 189- 192.
[18] Mabry TJ, Markham KR, Thomas MB (1970). The systematic identification of flavonoids, Springer-Verlag, New York.
[19] Mabry TJ, Markham KR, Thomas MB (1976). The systematic identification of flavonoids. New York:Springer-Verlag, 354.
[20] National Committee for Clinical Laboratory Standards (NCCLS/ CLSI)(2003). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. NCCLS/ CLSI, Wayne, Pa, USA..
[21] Oluwatuyi M, Kaatz GW, Gibbons S(2004). Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. Phytochemistry, 65:3249-3254.
[22] Pretto JB, Cechinel-Filho V, Noldin VF, Sartori MRK, Isaias DEB, Cruz AB (2004). Antimicrobial Activity of Fractions and Compounds from *Calophyllum brasiliense* (Clusiaceae/Guttiferae). *Z. Naturforsch. 59c, 657D662*.
[23] Rochfort S, Panozzo J (2007). Phytochemicals for health, the role of pulses. Journal of Agricultural and Food Chemistry,55: 7981-7994.
[24] Saavedra MJ, Borges A, Dias C, Aires A, Bennett RN, Rosa ES, Simões M(2010).Antimicrobial activity of phenolics and glucosinolate hydrolysis products and their synergy with streptomycin against pathogenic bacteria. Medicinal Chemistry, 6:174-183.
[25] Simões M, Rocha R, Coimbra MA, Vieira M (2008). Enhancement of *Escherichia coli* and *Staphylococcus aureus* antibiotic susceptibility using sesquiterpenoids. Medicinal Chemistry,. 4:616-623.
[26] Smith E, Williamson M, Wareham N, Kaatz G, Gibbons S(2007). Antibacterials and modulators of bacterial resistance from the immature cones of *Chamaecyparis lawsoniana*. Phytochemistry,68:210-217.

[27] Tan, Y. A., Sambanthamurthi, R., Sundram, K., & Wahid, M. B. (2007). Valorisation ofpalm by-products as functional components. European Journal of Lipid Scienceand Technology, 109: 380–393.

[28] Tan, Y. N., Ayob, M. K., Osman, M. A., & Matthews, K. R. (2011). Antibacterial activityof different degree of hydrolysis of palm kernel expeller peptides against spore-formingand non-spore-forming bacteria. Letters in Applied Microbiology, 53: 509–517.

[29] Uhl NW, Dransfield J (1987). Genera Palmarum: A Classification of Palms Based on the Work of Harold E. Moore Jr. Allen Press, Lawrence, Kansas.

[30] Wolverton BC, Johnson A, Bounds K (1989). Interior landscape plants for indoor air pollution abatement, final report. This work was jointly supported by the NASA Office of Commercial Programs-Technology Utilization Division, and the Associated Landscape Contractors of America (ALCA). National Aeronautics and Space Administration John C. Stennis Space Center Science and Technology Laboratory Stennis Space Center, MS 39529-6000)

[31] Wolverton BC, Wolverton,DV (1996). Interior Plants: Their Influence on Airborne Microbes inside Energy- efficient Buildings. Journal of Mississipi Academy of Science; 41(2): 99-105.

http://www.richarlyonsnursery.com