Universidad Autónoma del Estado de México

Facultad de Ciencias

Anti-staphylococcal properties of four plant extracts against sensitive and multi-resistant bacterial strains isolated from cattle and rabbits

Artículo publicado en revista arbitrada

QUE PARA OBTENER EL TÍTULO DE:

LICENCIATURA EN BIOTECNOLOGÍA

P R E S E N T A:

Mayra Fernanda Echeverría-Medina

ASESORES:

Dr. Abdelfattah Zeidan Mohamed Salem (Asesor)

Dr. Valente Velázquez Ordoñez (Coasesor)

TOLUCA, MÉXICO, ABRIL 2018

Microbial Pathogenesis 113 (2017) 286–294

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Anti-staphylococcal properties of four plant extracts against sensitive and multi-resistant bacterial strains isolated from cattle and rabbits

Mayra Fernanda Echeverría Medina[a], Peter Adeniyi Alaba[b], María Elena Estrada-Zuñiga[a], Valente Velázquez-Ordoñez[c], Alberto Barbabosa-Pliego[c], Mohmaed Z.M. Salem[d], María Uxúa Alonso-Fresán[c], Luis Miguel Camacho-Díaz[e,**], Abdelfattah Z.M. Salem[c,*]

[a] Facultad de Ciencias, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico
[b] Department of Chemical Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia
[c] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico
[d] Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt
[e] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Guerrero, Km. 3.5 Carretera Cd. Altamirano-Iguala, CP 40660 Cd. Altamirano, Guerrero, Mexico

ARTICLE INFO

Keywords:
Antimicrobial agents
Cattle
Plant extracts
Staphylococcus aureus
Rabbits

ABSTRACT

The aim of this study is to investigate the biopotency of methanolic extracts of Vitex mollis, Psidium guajava, Dalbergia retusa, and Crescential alata leaves against various staphylococcal strains isolated from cattle and rabbits. Methicillin-resistant S. aureus strains were isolated from cattle, while other strains were isolated from rabbits using standard methodology. The total phytochemical phenolic and saponins contents were obtained being the main groups of the antinutritional factors. The antimicrobial activity of the extracts against the standard culture of S. aureus (control) and S. aureus isolated from cattle and rabbits were investigated comparatively relative to that of oxacillin. It was found that both the control S. aureus and the isolated S. aureus are susceptible to all the four plant extracts, and sensitive to oxacillin. Of all the S. aureus including the control, MRSA2 is the most susceptible to all the extracts at 1000 μg/mL, except that of V. mollis where it is the least susceptible. Among all the plant extracts, P. guajava is the most active against MRSA2 and SOSA2. Therefore, the isolates from cattle (MRSA1 and MRSA2) are more susceptible to all the plant extracts than the isolates from rabbits. Among all the rabbit isolates, CoNS3 is the least susceptible to the extracts. Since all the plant extracts exhibit remarkable inhibitory activities against all the S. aureus strains, they are promising towards the production of therapeutic drugs.

1. Introduction

Staphylococcus aureus is one of the most challenging of all bacterial pathogens owing largely to the dogged occurrence of antibiotic-resistant strains. This is obvious in the recent emergence of oxacillin sensitive S. aureus (SOSA1 and SOSA2), and methicillin-resistant S. aureus (MRSA1 and MRSA2), which was isolated in Denmark and United Kingdom [1,2]. The recalcitrance of many S. aureus infections to antimicrobials is yet another evidence.

These infections represent a vital cause of mortality and morbidity among animals [3]. Therefore, pharmaceutical companies have been saddled recently with the responsibility of developing new antimicrobial agents, particularly due to the perpetual development of microorganisms resilient to conventional antibiotics. Some bacterial species genetically exhibit capability to develop and transmit resistance against existing antibiotics owing to the regular information on the isolation of bacteria, which are sensitive to habitually used antibiotics and develop diverse resistances to other existing conventional antibiotics [4,5]. Therefore, the common tactics approved by pharmaceutical companies to design new antibiotics is by altering the molecular structure of the prevailing drugs, making them more efficient or develop the ability to recover loss of activity due to bacterial resistance ability [6]. Consequently, this has resulted in an urgent requirement for novel antimicrobial agents. Prominent among the drugs are Rifampicin, Chloramphenicol, Cefepime, Ciprofloxacin, Sulfazotrin, Tetracycline, Gentamicin, and Cephalothin. These drugs are mostly toxic and unhealthy for human consumption [6]. However, the use of those drugs are discouraged due to their side effect, hence, the need for an

* Corresponding author.
** Corresponding author.
E-mail addresses: caamacho@hotmail.com (L.M. Camacho-Díaz), asalem70@yahoo.com (A.Z.M. Salem).

http://dx.doi.org/10.1016/j.micpath.2017.10.053
Received 7 October 2017; Received in revised form 26 October 2017; Accepted 27 October 2017
Available online 31 October 2017
0882-4010/

alternative antibacterial drugs [7].

There are numerous medicinal plants like Moringa oleifera leaves [8], Salix babylonica [9], P. guajava, and Cymbopogon citratus (lemongrass) [6], V. mollis [10], and Zingiber officinale (ginger) [6], with a long history of curative properties against various ailments and diseases. However, it is essential to urgent screen these plants for their activity to determine their biological activity. This screening could be achieved either by their ethnobotanical understanding of a particular disease or their chemotaxonomic analysis. It is quite challenging to identify a particular compound against a specific disease due to the long process involved. Plant chemicals could be classified into two, primary metabolites such as chlorophylls, amino acids, proteins, sugars, etc. and other category is known as the secondary metabolites, which are saponins, terpenoids, alkaloids and phenolic compounds. These chemicals are potential antioxidants and/or hypocholesterolemic agents, which play a vital physiological effect on the mammalian system [11,12]. Several secondary metabolites of antimicrobial importance have been isolated from about 12,000 plants [12–14]. These vast potentials of plants as sources of therapeutic drugs with reference to antibacterial agents have placed an urgent demand for the development of new anti-staphylococci drugs from natural sources.

Several authors have reported the use of plant extracts as antibiotics against bacterial strains isolated from difference animal species [13,15,16]. There are many factors responsible for the activity of the extracts on bacterial strains. These include the chemical form and bioavailability of the plant extract, and the level of K, Na and proton in the Bacterial isolates. The category of the bacterial strain (Gram-positive and Gram-negative bacteria) is also a vital factor. Gram-negative bacteria are said to be more susceptible to antibiotics than Gram-positive bacteria [16].

This in vitro systemic study was undertaken to investigate the bioactive potential of the methanioc extract of V. mollis, P. guajaya, D. retusa, and C. alata leave against some selected standard cultured S. aureus and isolated S. aureus from rabbits and cattle.

2. Materials and methods

2.1. Collection and identification of plant samples

V. mollis, P. guajava, D. retusa, and C. alata were collected in the State of Guerrero, municipality of Acapulco de Juárez (20 m above sea level) during the winter period of 2016, taking care that they did not show signs of stress such as discoloration, chlorosis, and leaf color senescence. The fresh and disease free plants were sorted, cleaned, and air-dried at room temperature for 8–10 days. The leaves were cut from the petiole and allowed to dry further at room temperature. Leaves were separated from the branches in order to obtain homogeneous samples and ground in a mill (Pulvex model 2000, mesh 20, Mexico City). The fine powder was stored at 20 °C in dark and moisture-free place until required for further experimental purposes.

2.2. Preparation of plant extracts

Two grams of the powdered leaves of each plant were mixed successively into 400 mL of methanol, and obtained using an ultrasound device (Shanghai Xiwen Biotech Co., model XW-650Y, China, Shanghai) in 30 min cycles concentrating in a rotary evaporator (BUCHI model R-3000, Brazil, São Paulo) at 40°C until reaching a final volume of 20 mL. The vacuum filtration technique was used to separate the biomass from the extract. The extracts were stored in amber flasks at room temperature for further experimental analysis [36].

2.3. Phenotypic identification of Staphylococcus aureus

Of the typical S. aureus colonies that were identified in the medium selective agar Baird Parker, a single colony was selected from the medium and seeded on agar and mannitol salt agar (BD Bioxon, Mexico). The colonies used as positive salt and mannitol were seeded in 13 × 100 mm glass tubes on blood-based agar for storage cooled to 4 °C. Subsequently, they were tested for coagulase, catalase, anaerobic fermentation of mannitol, fermentation of carbohydrates (Trehalosa and Malthosa), Voges Proskauer, Gram stain and hemolysis β and α, and triple sugar iron agar. S. aureus strain ATCC 43300 was used as the positive control, while S. epidermidis ATCC 12228 was used as the negative strain. Phenotypic identification of Staphylococcus spp Staphylococcus samples that were negative for the coagulase test were processed using a commercial analytical profile index Staphy kit following the manufacturer's recommendations. For the interpretation of the positive and negative reactions, the color gallery of the analytical profile index staphy color was used.

Identification of the S. aureus antibiotic for the detection of the S. aureus antibiotic from oxacillin resistant S. aureus, the oxacillin agar screen was tested from each isolation. A direct suspension of colonies was made in Mueller-Hinton broth, with a bacterial suspension at a density of 0.5 McFarland.

Each culture was inoculated in duplicate into Mueller-Hinton agar plates added with 4% NaCl and 6 μg/mL oxacillin and incubated for 24 h at 35° C. For this test, isolates that resisted this concentration were considered as MRSA. Strain ATCC 29213 (sensitive) and strain ATCC 43300 (resistant) were used as positive controls. In vitro susceptibility to β-lactam antibiotics of S aureus isolation was assessed by the Mueller-Hinton agar diffusion method, with amoxycillin/clavulanic acid (10/20 mg) units incubated at 37 °C and Mueller-Hinton agar plates (4% NaCl) and oxacillin-methicillin units (1 μg and 6 μg) incubated at 35 and 42° C (López et al., 2013).

2.4. Antimicrobial susceptibility testing

The indicator bacteria viz. Staphylococcus aureus (S. aureus) ATCC 25923, S. aureus ATCC 29213, S. aureus ATCC 43300, methicillin-resistant S. aureus (MRSA1 and MRSA2), oxacillin sensitive S. aureus (SOSA1 and SOSA2), and coagulase negative Staphylococcus epidermidis (CoNS1, CoNS2, and CoNS3) were used for the antibacterial assay. Control strains viz. ATCC 25923, ATCC 29213, and ATCC 43300 were obtained from the Center for Research and Advanced Studies in Animal Health (CIESA), Autonomous University of the State of Mexico (UAEMex). Methicillin-resistant S. aureus was isolated from cattle, while other strains were isolated from rabbits using standard methodology. Brain-heart infusion broth (BIOXON, DF, Mexico) medium was used for sub-culturing bacterial strains.

2.5. Disc diffusion assay

Bacterial cultures were prepared in 5 mL of Brain-heart infusion broth, adjusted to a 0.5 McFarland scale (1×10^6 CFU/mL), and incubated at 37 °C for 24 h in a rotatory shaker. Bacterial cultures were swabbed on sterilized Mueller-Hinton agar plates. Subsequently, 25 μL of methanolic extracts of leaves at various concentrations (62.5–1000 μg/mL) were transferred to sterile discs (6 mm) and allowed to soak for 30 min. The discs were transferred aseptically to the plates seeded with the respective staphylococci pathogens and incubated at 37 °C for 24 h. After the required period of incubation, the zone of inhibition (mm) formed by plant extracts against Staphylococcus sp. were measured. Oxacillin (1μg/disc) was used as positive control, while the negative control was dimethyl sulfoxide, with no inhibition zones found. All the experiments were carried out in triplicate.

2.6. Determination of relative percentage inhibition

The relative percentage inhibition (RPI) of the leaf extracts based on positive control was computed as described below.

$$RPI = \frac{IHD_{EXT} - IHD_{NC}}{IHD_{PC} - IHD_{NC}} \times 100$$

Where, IHD = Inhibition halo diameter; EXT = Extract; NC = Negative control; PC = Positive control.

2.7. Determination of total phenolic and saponins content

Total phenolic content in the leaf extracts of the respective plant was calculated based on the methodology of [17] with slight modifications. One millilitre of solvent extract (1 mg/mL), 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% Na_2CO_3 were used as the reaction mixture. The reaction mixture was incubated at 45 °C for 15 min and the absorbance was recorded at 765 nm. Ethanol was used as Blank while the calibration curve was prepared using gallic acid as standard at the concentrations of 20–100 μg/mL. The total phenolic content was calculated as milligrams of gallic acid equivalent per gram of dry weight (mg gallic acid equivalent/g) of extract.

The total saponins content in the leaf extracts of the respective plant was determined according to the modified methodology of [18]. Approximately 50 μL of leaf extract was added after the addition of 250 μL of distilled water. Subsequently, about 250 μL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol) and 2.5 mL of 72% sulphuric acid was added. The solution was further incubated at 60 °C for 10 min. After that, it was chilled in ice-cold condition and the absorbance was observed at 544 nm. The total saponins was calculated as diosgenin equivalents (mg diosgenin equivalents/g extract).

2.8. Statistical analyses

Experiments were performed in triplicate and results were expressed as mean ± standard deviation. Statistical analyses were performed using Microsoft Excel 2007.

3. Results

3.1. Total phytochemical composition

Table 1 presents the preliminary phytochemical composition, which shows the presence of phenolics (activity expressed as mg gallic acid equivalent/g of extract) and saponins (activity expressed as mg diosgenin equivalents/g of extract) in the leaf extracts, indicating that all the plants are potentials, antimicrobial agents. The total phenolics concentration were in the order of V. mollis > C. calata > D. retusa > G. guajava, while the saponins concentration follows C. alata > D. retusa=V. molis > P. guajava.

3.2. Oxacillin free radical scavenging activity in the presence of various S. aureus

In Table 2, presents the zone of inhibition determined free radical scavenging activity of oxacillin against standard cultured S. aureus and S. aureus isolated from cattle and rabbits using disc diffusion assay.

Table 1
Total phenolics and saponins content in leaf extracts.

Plants	Total phenolics (mg GAE/g extract)	Saponins (mg DE/g extract)
Dalber giaretusa	31	26
Crescentia alata	35	43
Psidium guajava	12.5	5.6
Vitex mollis	68	26

mg GAE/g: milligrams of gallic acid equivalent per gram of dry weight; mg DE/g extract: milligrams of diosgenin equivalent per gram of dry weight.

Table 2
Zone of inhibition determined anti – microbial activity of oxacillin against standard cultured S. aureus and S. aureus isolated from cattle and rabbits.

Extracts	ATCC 25923	ATCC 29213	ATCC 43300	MRSA1	MRSA2	SOSA1	SOSA2	CoNS1	CoNS2	CoNS3
V. mollis	14.50 ± 0.30	14.63 ± 0.51	12.37 ± 0.40	12.43 ± 0.45	18.53 ± 0.40	14.34 ± 0.30	18.60 ± 0.23	18.60 ± 0.23	14.25 ± 0.30	18.53 ± 0.50
P. guajava	18.37 ± 0.40	18.33 ± 0.42	12.43 ± 0.17	12.30 ± 0.32	12.43 ± 0.23	14.24 ± 0.41	10.45 ± 0.35	14.60 ± 0.30	12.65 ± 0.51	18.60 ± 0.36
D. retusa	14.78 ± 0.51	14.32 ± 0.60	18.10 ± 0.45	12.60 ± 0.53	12.32 ± 0.37	18.42 ± 0.52	14.62 ± 0.47	14.63 ± 0.15	14.16 ± 0.16	18.53 ± 0.31
C. alata	14.78 ± 0.51	14.32 ± 0.60	18.10 ± 0.45	12.60 ± 0.53	12.32 ± 0.37	18.42 ± 0.52	14.62 ± 0.47	14.63 ± 0.15	14.16 ± 0.16	18.53 ± 0.31

MRSA1 and MRSA2: methicillin-resistant S. aureus isolated from cattle; SOSA1 and SOSA2: oxacillin sensitive S. aureus isolated from the rabbit; CoNS1, CoNS2, and CoNS3: coagulase negative Staphylococcus epidermidis isolated from the rabbit.

However, ATCC 25923 and ATCC 29213, P. guajava exhibited the highest activity due to low phenolic content. The oxacillin scavenging activity of the studied plant extracts was in the order of P. guajava > D. retusa=C. alata > V. mollis for ATCC 25923, and P. guajava > V. mollis > D. retusa=C. alata for ATCC 29213. For ATCC 43300, D. retusa and C. alata exhibited the highest activity due to moderate phenolic content. The oxacillin scavenging activity of the studied plant extracts was in the order of D. retusa=C. alata > P. guajava > V. mollis. For MRSA1, D. retusa and C. alata exhibited the highest activity due to moderate phenolic content. The oxacillin scavenging activity of the tested plant extracts was in the order of D. retusa=C. alata > V. mollis > P. guajava. For MRSA2, V. mollis exhibited the highest activity due to high phenolic content. The oxacillin scavenging activity of the studied plant extracts was in the order of V. mollis > P. guajava > D. retusa=C. alata. For SOSA1, D. retusa and C. alata exhibited the highest activity due to moderate phenolic content. The oxacillin scavenging activity of the tested plant extracts was in the order of D. retusa=C. alata > V. mollis > P. guajava. For SOSA2, V. mollis exhibited the highest activity due to high phenolic content. The oxacillin scavenging activity of the studied plant extracts was in the order of V. mollis > D. retusa=C. alata > P. guajava. For CoSN1 and CoSN1, V. mollis exhibited the highest activity due to high phenolic content. The oxacillin scavenging activity of the tested plant extracts was in the order of V. mollis > D. retusa=C. alata > P. guajava. For CoSN3, The oxacillin scavenging activity of the studied plant extracts was in the order of P. guajava > D. retusa=C. alata > V. mollis for ATCC 25923, and P. guajava > V. mollis=D. retusa=C. alata. D. retusa and C. alata exhibited similar activity due to the closeness of their phenolic contents.

3.3. Susceptibility of S. aureus against the plant extracts

Table 3 shows the zones of inhibition determined anti–microbial activity of the plant extracts against standard cultured S. aureus and S. aureus isolated from cattle and rabbits, while Fig. 1 presents the bioactivity based on relative percentage inhibition (RPI). The activity was tested by varying the extract concentration from 62.5 to 1000 μg/mL in bacteria inoculum of 1×10^6 CFU/mL. The oxacillin antibacterial generated zones of inhibition ranging from 10.45 to 18.60 mm, and CoNS3 exhibits the highest (10.45 mm), while SOSA2 the lowest (18.60 mm). For all the plant extracts, the value of RPI increases with increase with an increase in the concentration of the extract in the bacteria inoculum (Fig. 2). P. guajava gives the best RPI value, and MRSA1 and MRSA2 exhibit the highest susceptibility to all the plant extracts almost throughout the concentrations particularly at 1000 μg/mL. An exception to this claim was observed with V. mollis, which shows the least RPI to MRSA2.

It is obvious that the all the plants extracts are more potent on the S. aureus isolated from cattle than those isolated from rabbits, except V. mollis that exhibits the least activity all through the concentration variation. C. alata and D. retusa are more potent towards the cattle isolates, except at 62.5 and 1000 μg/mL extract concentration that P. guajava became the most potent. Among the oxacillin sensitive S. aureus (SOSA1 and SOSA2), rabbit isolates, P. guajava exhibits the best activity. It is also worthy to note that CoNS3 exhibits the least vulnerability to all the plant extracts among the coagulase negative Staphylococcus epidermidis (CoNS1, CoNS2, and CoNS3), as well as among all the isolates (Fig. 3).

4. Discussion

The free radical scavenging activities of antibiotic have been reported as a function of the phenolic content [19,20]. This is because phenolic compounds are potential antioxidants, which function by the oxidative free radicals scavenging because of the presence of hydroxyl groups and conjugated ring structures [21]. Oxygen is very vital towards aerobic respiration, nevertheless, it can cause a serious health to the living organism by formation of free radicals (reactive oxygen species) under certain conditions. This could possibly lead to some hazardous diseases such as ulcer, diabetes, atherosclerosis, cancer, neurodegenerative disorders (AD & Dementia), aging, immune-suppression and coronary heart disease [22,23]. Although, almost all living organisms are immune to free radicals attack via defence system like the protective antioxidant system, which weaken the rate of formation of free radicals alongside with additional system that generates antioxidants (chain-breaker) to alleviate free radicals scavenging. Nevertheless, when the rate of formation of free radical surpasses the defence mechanisms capability, it leads to an extensive tissue injury [24]. Therefore, therapeutic drugs that exhibit abilities to scavenge free radicals are essential towards the therapy and prevention of these diseases in living organisms [25]. Antioxidant compounds function biochemically through a number of mechanisms, which include radical scavenging, metal ions chelation, sustained hydrogen abstraction, breakdown of peroxides, reductive ability, and prevention of chain initiation. Therefore, several plants are proposed as a source of antioxidant.

V. mollis [26], P. guajava [27], D. retusa [28] and C. alata [29] leaf extract have been extensively used as herbal medicine to treat diverse diseases like diabetes, pain, inflammation and hypertension due to their antibiotic properties. Their extracts consist of phenolics ranging between 12.5 and 68 mg gallic acid equivalent/g, and saponins ranging between 5.6 and 43 mg diosgenin equivalents/g extract. Phenolics are a group of the second metabolite used as antioxidant agents due to their abilities to scavenge free radical [19]. Saponins are a group of the second metabolite, which consist of isoprenoidal, a derivative of aglycone, popularly known as sapogenin or genin. They are covalently linked to one or more moieties of sugar [30]. Saponins are promising antioxidant [31] due to their anti-cancer properties in spite of their hemolytic side effects [32,33].

The extracts are used against standard cultured S. aureus (ATCC25923, ATCC29213, and ATCC43300) and S. aureus isolated (MRSA1, MRSA2, SOSA1, SOSA2, CoNS1, CoNS2 and CoNS2) from rabbits and cattle. We observed that all the investigated plant extracts are potential antibiotic with antioxidant abilities to inhibit all the examined S. aureus.

For all the plant extracts, the inhibitory activity increases with increase in the concentration of the extract in the bacteria inoculum. The inhibitory activity of the plant extracts on each S. aureus solely depend on the concentration applied. For instance, at 62.5 μg/mL, it was observed that the best antimicrobial agent for MRSA1 and MRSA2 is D. retusa and C. alata. V. mollis is the best for CoNS1, CoNS2, CoNS3, and ATCC43300, while P. guajava is the best for SOSA2, MRSA2, and CoNS1. The best antimicrobial agent for ATCC29213 is C. alata, while that of ATCC25923 is D. retusa. The observation at 125 μg/mL is almost the same as that at 62.5 μg/mL, it was observed that the best antimicrobial agent for MRSA1 and MRSA2 is D. retusa and C. alata. V. mollis is the best for CoNS2, CoNS3, and ATCC43300, while P. guajava is the best for SOSA1, SOSA2, and CoNS1. The best antimicrobial agent for ATCC29213 is C. alata, while that of ATCC25923 is D. retusa. At 250 μg/mL, it was observed that the best antimicrobial agent for MRSA1 and CoNS1 is D. retusa and C. alata. V. mollis is the best for ATCC25923 and ATCC43300, while P. guajava is the best for SOSA1 and SOSA2. The best antimicrobial agent for CoNS3, ATCC29213, and MRSA2 is C. alata, while that of CoNS2 is D. retusa.

At 500 μg/mL, it was observed that the best antimicrobial agent for MRSA1 and MRSA2 is C. alata. V. mollis is the best for ATCC29213 and ATCC43300, while P. guajava is the best for SOSA1 and SOSA2. The best antimicrobial agent for and ATCC25923 is C. alata, while that of CoNS3 and CoNS1 is D. retusa. Furthermore, at 1000 μg/mL extracts concentration, the best antimicrobial agent for MRSA is D. retusa and C. alata. V. mollis is the best for only MRSA1 and ATCC43300, while P. guajava is the best for only MRSA2. The best antimicrobial agent for SOSA and CoNS3 is P. guajava, while for CoNS1 and CoNS2 is D. retusa

Table 3
Zone of inhibition determined anti – microbial activity of the plant extracts against standard cultured S. aureus and S. aureus isolated from cattle and rabbits.

	Conc. (μg/mL)	ATCC 25923	ATCC 29213	ATCC 43300	MRSA1	MRSA2	SOSA1	SOSA2	CoNS1	CoNS2	CoNS3
Control	0	14.50 ± 0.30	14.63 ± 0.51	12.37 ± 0.40	12.43 ± 0.45	18.53 ± 0.40	14.34 ± 0.30	18.60 ± 0.23	18.60 ± 0.23	14.25 ± 0.30	18.53 ± 0.50
V. mollis	62.5	5.37 ± 0.15	4.65 ± 0.14	6.31 ± 0.25	5.47 ± 0.38	5.73 ± 0.25	6.10 ± 0.17	6.80 ± 0.20	5.99 ± 0.62	6.68 ± 0.28	6.92 ± 0.10
	125	7.15 ± 0.27	6.71 ± 0.25	6.84 ± 0.15	6.64 ± 0.41	6.64 ± 0.15	7.18 ± 0.28	8.44 ± 0.21	6.79 ± 0.23	7.58 ± 0.10	9.42 ± 0.09
	250	9.15 ± 0.30	8.32 ± 0.65	8.63 ± 0.50	8.60 ± 0.28	8.57 ± 0.28	9.23 ± 0.21	10.37 ± 0.27	9.00 ± 0.26	8.63 ± 0.32	9.58 ± 0.13
	500	10.23 ± 0.52	10.54 ± 0.50	9.50 ± 0.30	9.32 ± 0.16	9.37 ± 0.24	10.40 ± 0.20	11.50 ± 0.21	10.23 ± 0.11	9.25 ± 0.51	10.33 ± 0.30
	1000	11.20 ± 0.25	11.79 ± 0.26	10.37 ± 0.35	10.10 ± 0.26	10.40 ± 0.33	11.52 ± 0.21	12.60 ± 0.25	11.63 ± 0.16	10.43 ± 0.16	11.30 ± 0.50
P. guajaba	62.5	6.54 ± 0.32	5.30 ± 0.20	5.65 ± 0.22	5.54 ± 0.23	6.40 ± 0.26	6.12 ± 0.16	6.23 ± 0.61	7.23 ± 0.65	5.67 ± 0.21	5.32 ± 0.13
	125	8.30 ± 0.20	6.65 ± 0.65	6.23 ± 0.58	6.78 ± 0.40	6.98 ± 0.30	8.47 ± 0.25	6.66 ± 0.30	8.60 ± 0.20	7.32 ± 0.40	8.13 ± 0.61
	250	9.43 ± 0.25	8.63 ± 0.10	8.50 ± 0.42	8.45 ± 0.12	8.52 ± 0.13	10.34 ± 0.09	7.68 ± 0.15	9.16 ± 0.82	7.83 ± 0.50	9.73 ± 0.60
	500	10.40 ± 0.21	9.23 ± 0.23	9.46 ± 0.65	9.73 ± 0.61	9.30 ± 0.19	11.43 ± 0.26	9.38 ± 0.57	10.30 ± 0.25	9.61 ± 0.60	10.53 ± 0.26
	1000	10.63 ± 0.15	10.51 ± 0.58	10.57 ± 0.31	10.33 ± 0.40	12.10 ± 0.46	12.63 ± 0.76	10.12 ± 0.28	11.30 ± 0.17	10.88 ± 0.67	11.78 ± 0.30
D. retusa	62.5	6.80 ± 0.20	5.45 ± 0.12	6.61 ± 0.20	6.42 ± 0.45	6.33 ± 0.14	6.29 ± 0.20	6.42 ± 0.24	6.23 ± 0.32	5.85 ± 0.23	6.09 ± 0.14
	125	8.48 ± 0.09	6.54 ± 0.21	8.30 ± 0.11	8.50 ± 0.22	8.22 ± 0.17	8.16 ± 0.16	8.30 ± 0.16	8.76 ± 0.35	6.28 ± 0.43	8.39 ± 0.13
	250	9.12 ± 0.30	7.62 ± 0.21	9.35 ± 0.27	9.83 ± 0.30	8.56 ± 0.23	10.34 ± 0.21	9.39 ± 0.51	10.55 ± 0.24	9.25 ± 0.34	10.15 ± 0.16
	500	11.55 ± 0.15	8.32 ± 0.16	10.42 ± 0.14	10.67 ± 0.21	9.30 ± 0.14	12.57 ± 0.24	11.22 ± 0.27	12.43 ± 0.11	10.65 ± 0.24	10.92 ± 0.32
	1000	12.66 ± 0.23	10.41 ± 0.13	12.26 ± 0.34	11.28 ± 0.34	11.61 ± 0.18	13.30 ± 0.14	12.53 ± 0.31	12.75 ± 0.15	12.46 ± 0.15	11.10 ± 0.18
C. alata	62.5	5.80 ± 0.20	6.45 ± 0.12	5.61 ± 0.02	6.42 ± 0.45	6.22 ± 0.14	6.39 ± 0.20	6.12 ± 0.24	5.23 ± 0.32	5.45 ± 0.23	6.29 ± 0.14
	125	6.48 ± 0.09	7.54 ± 0.21	6.30 ± 0.11	8.40 ± 0.22	8.12 ± 0.17	8.56 ± 0.16	8.20 ± 0.16	7.76 ± 0.35	6.18 ± 0.43	8.19 ± 0.13
	250	8.12 ± 0.30	8.65 ± 0.21	7.35 ± 0.27	9.89 ± 0.30	9.56 ± 0.23	10.24 ± 0.21	9.29 ± 0.51	9.55 ± 0.24	8.25 ± 0.34	10.25 ± 0.16
	500	10.51 ± 0.15	9.32 ± 0.16	9.42 ± 0.14	10.62 ± 0.21	10.30 ± 0.14	11.57 ± 0.24	10.22 ± 0.27	10.43 ± 0.11	10.25 ± 0.24	10.92 ± 0.32
	1000	12.56 ± 0.23	10.61 ± 0.13	10.26 ± 0.34	11.38 ± 0.34	11.71 ± 0.18	12.30 ± 0.14	12.33 ± 0.31	12.65 ± 0.15	12.26 ± 0.15	11.50 ± 0.18

MRSA1 and MRSA2: methicillin-resistant S. aureus isolated from cattle; SOSA1 and SOSA2: oxacillin sensitive S. aureus isolated from the rabbit; CoNS1, CoNS2, and CoNS3: coagulase negative Staphylococcus epidermidis isolated from the rabbit.

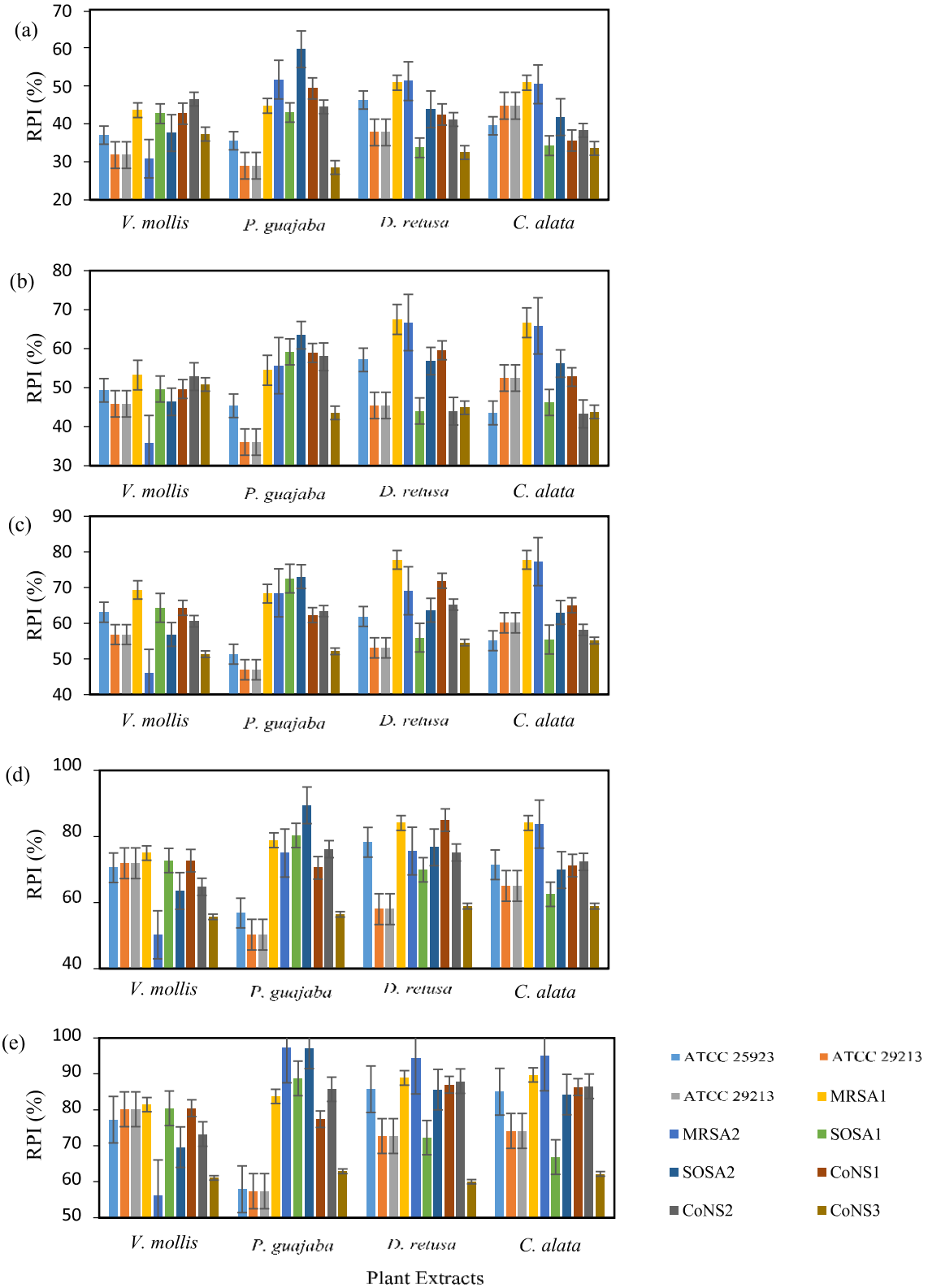

Fig. 1. Bioactivity of the plant extracts based on RPI (%) at various extract concentrations (µg/mL); (a) 62.5, (b) 125, (c) 250, (d) 500, and (e) 1000 µg/mL.

and C. alata. The best antimicrobial extract agent for ATCC25923 is C. alata, while that of ATCC29213 is V. mollis.

All the plant extracts observed a higher bioactivity against the methicillin-resistant S. aureus (MRSA1 and MRSA2) isolates from cattle than those of the isolates from the rabbits (oxacillin sensitive S. aureus (SOSA1 and SOSA2), and coagulase negative Staphylococcus epidermidis (CoNS1, CoNS2, and CoNS3). These differences in bioactivity could be ascribed to variable effects of the antibiotics on the proton and metal movement direction across the cell membrane of the S. aureus, which is eventually a function of the magnitude of ion gradients through the cell membrane [34]. This shows that the resistance of the cattle isolates to V. mollis is attributable to translocation of metal ions throughout the cell membrane of the cattle, which limits the therapeutic utilization of V. mollis. Furthermore, CoNS3 exhibits a higher resistance capacity to

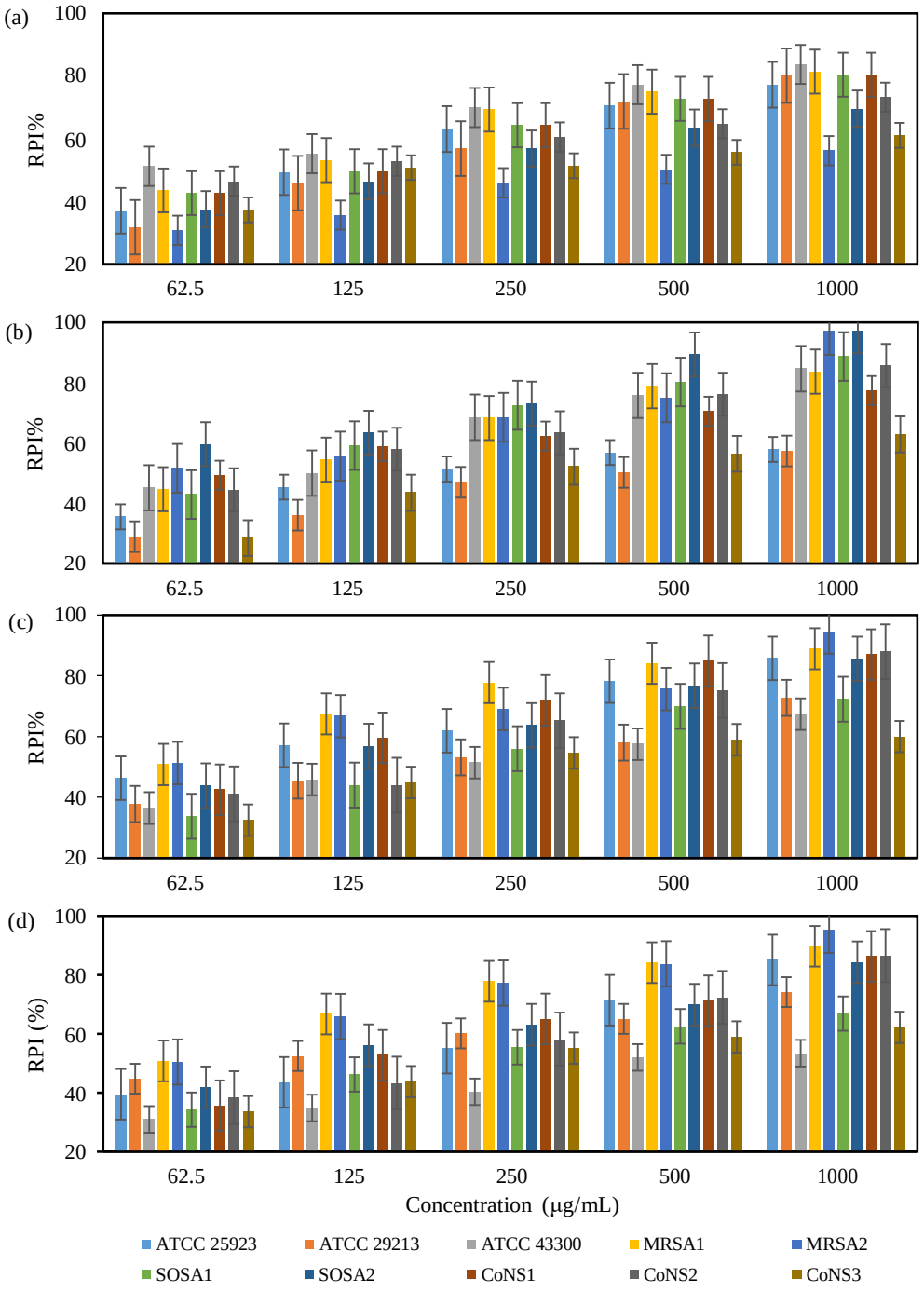

Fig. 2. Susceptibility of standard cultured S. aureus and S. aureus isolated from cattle and rabbits on (a) V. mollis, (b) P. guajaba, (c) C. retusa, (d) C. alata, and (e) 1000; based on RPI (%) at various extract concentrations (µg/mL).

all the plant extracts. This is probably because CoSN3 maintains a higher K concentration in its cells, and oust protons and Na [16], thereby limiting the biopotency of the plant extracts. This shows that the toxicity of antibiotic does not only depend on the bioavailability and chemical form of the antibiotic but also the bacterial species [35]. Despite the stubbornness of S. aureus, being gram positive bacterial strain, yet the plant extracts exhibit a remarkable activity against the isolated Staphloccocus strains, making them a promising therapeutic drug.

5. Conclusion

The antimicrobial activity of extracts of Vitex mollis, Psidium guajava, Dalbergia retusa, and Crescential alata leaves against various staphylococcal strains (standard culture and isolated from cattle and rabbits) was found to be remarkable. All the plant extracts demonstrated significant antimicrobial activity mainly by their antioxidant ability, making them suitable broad-spectrum antibiotics for restriction of common pathogens growth. Both control S. aureus and isolated S. aureus are susceptible to all the four plant extracts. Of all the S. aureus including the control, MRSA2 is the most susceptible to all the extracts except that of V. mollis where it is the least susceptible. Among all the plant extracts, P. guajava is the most active against MRSA2 and SOSA2. The control S. aureus (ATCC 25923, ATCC 29213 and ATCC 43300) are the least susceptible S. aureus strain to P. guajava, while CoNS3 is the least susceptible S. aureus strain to both D. retusa and C. alata extracts. Therefore, the methicillin-resistant S. aureus (MRSA1 and MRSA2) isolates from cattle are more susceptible to all the plant extracts than

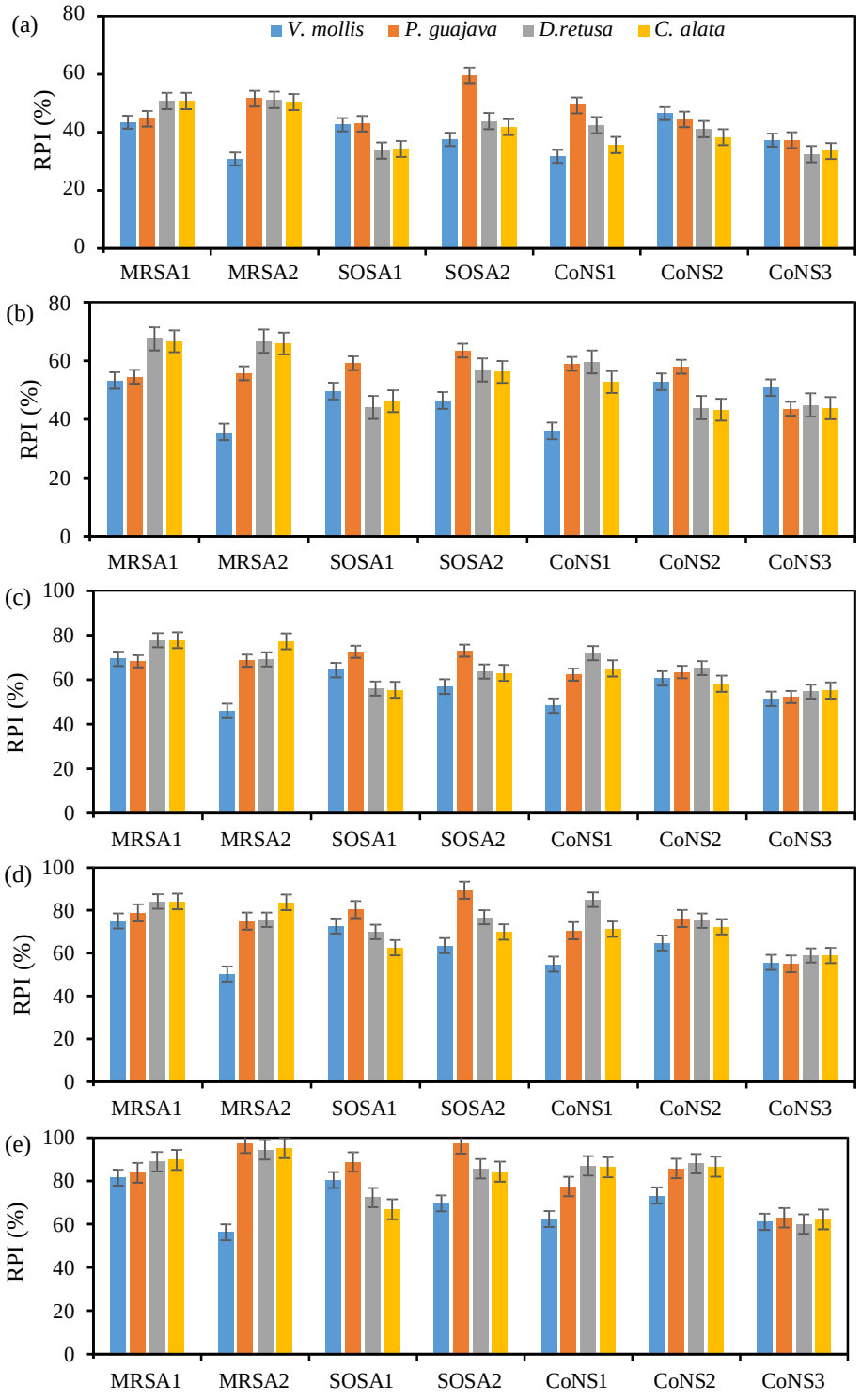

Fig. 3. Susceptibility of isolated S. aureus from cattle and rabbits to plant extracts based on RPI (%) at various extract concentrations (μg/mL); (a) 62.5, (b) 125, (c) 250, (d) 500, and (e) 1000 μg/mL.
MRSA1 and MRSA2: methicillin-resistant S. aureus isolated from cattle; SOSA1 and SOSA2: oxacillin sensitive S. aureus isolated from the rabbit; CoNS1, CoNS2, and CoNS3: coagulase negative Staphylococcus epidermidis isolated from the rabbit.

the isolates from rabbits. Among all the rabbit isolates, CoNS3 (coagulase negative Staphylococcus epidermidis) is the least susceptible to the extracts. Since all the plant extracts exhibit remarkable inhibitory activities against all the S. aureus strains, they are promising towards production of therapeutic drugs.

References

[1] T. Yamamoto, A. Nishiyama, T. Takano, S. Yabe, W. Higuchi, O. Razvina, D. Shi, Community-acquired methicillin-resistant Staphylococcus aureus: community transmission, pathogenesis, and drug resistance, J. Infect. Chemother. 16 (2010) 225–254.

[2] A.I. Hidron, C.E. Low, E.G. Honig, H.M. Blumberg, Emergence of community-acquired meticillin-resistant Staphylococcus aureus strain USA300 as a cause of necrotising community-onset pneumonia, Lancet Infect. Dis. 9 (2009) 384–392.

[3] N. Saeed, M.R. Khan, M. Shabbir, Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L, BMC Compl. Altern. Med. 12 (2012) 221.

[4] G.G. Nascimento, J. Locatelli, P.C. Freitas, G.L. Silva, Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria, Braz. J. Microbiol. 31 (2000) 247–256.

[5] Y. Sakagami, K. Kajimura, Bactericidal activities of disinfectants against vancomycin-resistant enterococci, J. Hosp. Infect. 50 (2002) 140–144.

[6] N. Silva, A. Fernandes Júnior, Biological properties of medicinal plants: a review of their antimicrobial activity, J. Venom. Animals Toxins Incl. Trop. Dis. 16 (2010)

402–413.
[7] A. Salem, M. Ronquillo, L. Camacho, S. Cerrillo, I. Domínguez, J. Borquez, Beneficial effects of plant extracts in ruminant nutrition: a review, Indian J. Animal Sci. 82 (2012) 1117–1121.
[8] A. Leone, A. Spada, A. Battezzati, A. Schiraldi, J. Aristil, S. Bertoli, Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: An overview, Int. J. Mol. Sci. 16 (2015) 12791–12835.
[9] A. Salem, Oral administration of leaf extracts to rumen liquid donor lambs modifies in vitro gas production of other tree leaves, Animal Feed Sci. Technol. 176 (2012) 94–101.
[10] F. Delgado-Vargas, F. Félix-Favela, J. Pío-León, G. López-Angulo, J. López-Valenzuela, S. Díaz-Camacho, M. Uribe-Beltran, Antibacterial activity and qualitative phytochemical analysis of Vitex mollis fruit, Int. J. Green Pharm. 4 (2010) 288.
[11] T. Johns, R. Mahunnah, P. Sanaya, L. Chapman, T. Ticktin, Saponins and phenolic content in plant dietary additives of a traditional subsistence community, the Batemi of Ngorongoro District, Tanzania, J. Ethnopharmacol. 66 (1999) 1–10.
[12] S.Z. Shaheen, K. Bolla, K. Vasu, M.S. Charya, Antimicrobial activity of the fruit extracts of Coccinia indica, Afr. J. Biotechnol. 8 (2009).
[13] M.Z. Salem, Y.M. Gohar, L. Camacho, N.A. El-Shanhorey, A. Salem, Antioxidant and antibacterial activities of leaves and branches extracts of Tecoma stans (L.) Juss. ex Kunth against nine species of pathogenic bacteria, Afr. J. Microbiol. Res. 7 (2013) 418–426.
[14] I. Zampini, S. Cuello, M. Alberto, R. Ordonez, R. D'almeida, E. Solorzano, M. Isla, Antimicrobial activity of selected plant species from "the Argentine Puna" against sensitive and multi-resistant bacteria, J. Ethnopharmacol. 124 (2009) 499–505.
[15] A. Salem, P. Robinson, S. Lopez, Y. Gohar, R. Rojo, J. Tinoco, Sensitivity of sheep intestinal lactic acid bacteria to secondary compounds extracted from Acacia saligna leaves, Animal Feed Sci. Technol. 161 (2010) 85–93.
[16] A.Z.M. Salem, Y.M. Gohar, S. Lopez, M. Gonzalez-Ronquillo, Sensitivity of ruminal bacterial isolates of sheep, cattle and buffalo to 13 therapeutic antibiotics, Afr. J. Microbiol. Res. 6 (2012) 4727–4733.
[17] V. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.
[18] H. Makkar, K. Becker, Nutrients and antiquality factors in different morphological parts of the Moringa oleifera tree, J. Agric. Sci. 128 (1997) 311–322.
[19] M. Ayaz, M. Junaid, J. Ahmed, F. Ullah, A. Sadiq, S. Ahmad, M. Imran, Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from Polygonum hydropiper L, BMC Compl. Altern. Med. 14 (2014) 145.
[20] V.B. Maisuria, Z. Hosseinidoust, N. Tufenkji, Polyphenolic extract from maple syrup potentiates antibiotic susceptibility and reduces biofilm formation of pathogenic bacteria, Appl. Environ. Microbiol. 81 (2015) 3782–3792.
[21] F. Shahidi, P. Janitha, P. Wanasundara, Phenolic antioxidants, Crit. Rev. Food Sci. Nutr. 32 (1992) 67–103.
[22] O.I. Aruoma, Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods, Mutat. Research/ Fundamental Mol. Mech. Mutagen. 523 (2003) 9–20.
[23] H.R. Jadhav, K. Bhutani, Antioxidant properties of Indian medicinal plants, Phytother. Res. 16 (2002) 771–773.
[24] S.R. Hasan, M.M. Hossain, R. Akter, M. Jamila, M.E.H. Mazumder, S. Rahman, DPPH free radical scavenging activity of some Bangladeshi medicinal plants, J. Med. Plants Res. 3 (2009) 875–879.
[25] S.A. Saeed, M.Z.S. Urfy, T.M. Ali, F. Khimani, A. Gilani, Antioxidants: their role in health and disease, Int. J. Pharmacol. 1 (2005) 210–217.
[26] E. Cuevas-Juárez, K.Y. Yuriar-Arredondo, J.F. Pío-León, J. Montes-Avila, G. López-Angulo, S.P. Díaz-Camacho, F. Delgado-Vargas, Antioxidant and α-glucosidase inhibitory properties of soluble melanins from the fruits of Vitex mollis Kunth, Randia echinocarpa Sessé et Mociño and Crescentia alata Kunth, J. Funct. Foods 9 (2014) 78–88.
[27] E. Díaz-De-Cerio, A. Rodríguez-Nogales, F. Algieri, M. Romero, V. Verardo, A. Segura-Carretero, J. Duarte, J. Galvez, The hypoglycemic effects of guava leaf (Psidium guajava L.) extract are associated with improving endothelial dysfunction in mice with diet-induced obesity, Food Res. Int. 96 (2017) 64–71.
[28] M. Gregson, W.D. Ollis, B.T. Redman, I.O. Sutherland, H. Dietrichs, O.R. Gottlieb, Obtusastyrene and obtustyrene, cinnamylphenols from Dalbergia retusa, Phytochemistry 17 (1978) 1395–1400.
[29] G. Rojas, J. Lévaro, J. Tortoriello, V. Navarro, Antimicrobial evaluation of certain plants used in Mexican traditional medicine for the treatment of respiratory diseases, J. Ethnopharmacol. 74 (2001) 97–101.
[30] J.M. Augustin, V. Kuzina, S.B. Andersen, S. Bak, Molecular activities, biosynthesis and evolution of triterpenoid saponins, Phytochemistry 72 (2011) 435–457.
[31] J. Hu, S.-O. Lee, S. Hendrich, P.A. Murphy, Quantification of the group B soyasaponins by high-performance liquid chromatography, J. Agric. Food Chem. 50 (2002) 2587–2594.
[32] X. Tian, H. Tang, H. Lin, G. Cheng, S. Wang, X. Zhang, Saponins: the potential chemotherapeutic agents in pursuing new anti-glioblastoma drugs, Mini Rev. Med. Chem. 13 (2013) 1709–1724.
[33] S. Man, W. Gao, Y. Zhang, L. Huang, C. Liu, Chemical study and medical application of saponins as anti-cancer agents, Fitoterapia 81 (2010) 703–714.
[34] J.B. Russell, H. Strobel, Effect of ionophores on ruminal fermentation, Appl. Environ. Microbiol. 55 (1989) 1.
[35] A.Z.M. Salem, H. Ammar, S. Lopez, Y. Gohar, J. González, Sensitivity of ruminal bacteria isolates of sheep, cattle and buffalo to some heavy metals, Animal Feed Sci. Technol. 163 (2011) 143–149.
[36] A.Z.M. Salem, M.Z.M. Salem, M.M. El-Adawy, P.H. Robinson, Nutritive evaluations of some browse tree foliages during the dry season: secondary compounds, feed intake and in vivo digestibility in sheep and goats, Animal Feed Sci. Technol. 127 (2006) 251–267.