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Anti-staphylococcal properties of *Eichhornia crassipes, Pistacia vera*, and *Ziziphus amole* leaf extracts: Isolates from cattle and rabbits

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ABSTRACT

The desideratum aim of the present context was to assess the biopotency of methanolic extracts of *Eichhornia crassipes* (*E. crassipes*), *Pistacia vera* (*P. vera*), and *Ziziphus amole* (*Z. amole*) leaves against various staphylococcal strains, and to quantify the phenolics as well as saponin content in them. The antibacterial activity of various concentrations (62.5–1000 μg/mL) of plant extracts was tested against control clinical strains (*Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, and *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) using disc diffusion assay. Leaf extracts of the three plants exhibited pronounced growth inhibitory characteristics against staphylococci in a dose dependent manner. *E. crassipes* extract depicted the highest relative percentage inhibition values against control clinical strains (68.6 ± 0.5%), while *P. vera* (68.6 ± 0.3%) and *Z. amole* (74.79 ± 0.3%) extracts showed pronounced relative inhibition values against staphylococcal strains isolated from cattle. Total phenols and saponin content of leaf extracts were investigated by standard *in vitro* methods. The methanolic extracts of these plants were found to comprise substantial content of phenolics and saponin at varying levels. The highest value of phenolics was estimated in *P. vera* extract (60.0 ± 1.3 mg gallic acid/g extract), followed by *Z. amole* (33.6 ± 1.4 mg gallic acid/g extract), and *E. crassipes* (23.0 ± 1.3 mg gallic acid/g extract). Saponin content for *P. vera*, *Z. amole*, and *E. crassipes* extracts were estimated as 41.0 ± 1.3, 35.8 ± 1.3, and 25.0 ± 1.2 mg diosgenin/g extract, respectively. The outcome of this study suggested the exploitation of methanolic extract of *P. vera*, *Z. amole*, and *E. crassipes* leaves for their possible application in ethnomedicine, particularly as drugs preparation against staphylococcal infections. In conclusion, the study indicates the biopotency of these plants against pathogenic MRSA present in cattle, and SOSA as well as CoNS bacteria present in rabbits, which could be a serious issue for livestock.

1. Introduction

The emergence and development of drug resistant bacterial pathogens have substantially threatened the existing antibacterial therapy. In general, bacteria have the genetic potentiality to transmit and acquire resistance to therapeutic drugs, and thus, incidences of epidemics due to drug resistant bacteria are now a common global problem posing enormous public health concerns [1].

Staphylococcus sp. is one of the commensal bacteria that constitute a major component of the normal skin and mucosal microflora of humans [2]. In recent years, these bacteria have emerged as an opportunistic pathogen, causing bacteremia as well as nosocomial infections [3]. Some *Staphylococcus* sp. are involved in the pathogenesis of respiratory and skin infections [4], and also form biofilm on the surfaces of the medical devices. Staphylococci strains have acquired resistance to several other antibiotics and most antibiotic resistance genes are plasmid-encoded and are more often found in methicillin-resistant strains [5].

The high cost and non-availability of new generation antibiotics have resulted in increase in morbidity and mortality [1]. Consequently, this has led to the search for more effective agents of plant origin, with the aim of developing active ingredients that can serve as source and

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template for the synthesis of new antibacterial drugs [1].

Medicinal plants have been known to exhibit myriad benefits to mankind from ancient periods due to their ample pharmacological aspect. Traditional applications of the medicinal plants have fewer side effects that lead the development of varied phytomedicines globally [6]. Medicinal plants derived secondary metabolites are vital sources of distinct phytochemicals that could be used for the production of pharmaceutical products. At present, approximately 80% of the world's populace still relies on the plants associated traditional medicine for health care needs. Therefore, in the current scenario, the demand for herbal medicines has surged in comparison to the synthetic drugs.

Eichhornia crassipes (Water hyacinth), belonging to the family Pontideriaceae is one of the most productive aquatic perennial herbs on earth, and it has been known for its unique medicinal importance. The phytoconstituents of this plant have vast biological properties including antiviral, antifungal, antitumor, and antibacterial activities [7]. Additionally, its secondary metabolites have been considered to be involved in the chemical defense of plants against plant pathogens [8].

Pistacia vera, a member of Anacardiaceae family, is one of the most economically important aromatic plants and widely distributed in the Mediterranean region as well as USA. *P. vera* plants are remarkably rich in linoleic and linolenic acids, the fatty acids vital for human health [9]. In addition to this, *Pistacia* sp. were previously reported to depict various biological activities such as anti-atherogenic, hypoglycemic, antioxidant, anti-inflammatory, antifungal, and insecticidal [10–12].

Ziziphus sp. (Rhamnaceae) comprises about 40 species distributed in warm-temperate and sub-tropical regions. *Ziziphus* plants possess bioactive components that are traditionally used as for the treatment of various diseases such as digestive disorders, urinary troubles, diabetes, skin infections, diarrhea, fever, bronchitis, liver complaints, anaemia, etc. [13]. Antimicrobial activity of some members of genus *Ziziphus* had already been reported in the previous literature [14,15].

Considering the vast potentiality of plants as sources for therapeutic drugs with reference to antibacterial agents and the urgent demand of the current scenario for developing new anti-staphylococci drugs from natural sources, the present *in vitro* systemic study was undertaken to investigate the bioactive potential of methanolic extract of *E. crassipes, P. vera*, and *Z. amole* leaves against ten different strains of staphylococci.

2. Materials and methods

2.1. Plants collection

E. crassipes, *P. vera*, and *Z. amole* 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 curling senescence. The fresh and disease free plants were separated from the branches, 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. After drying, the leaves were ground in a mill (Pulvex model 2000, mesh 20, Mexico City). The resulting fine powder was stored in plastic and kraft paper bags at 20 °C in a dark and moisture-free place until required for extraction process.

2.2. Extract preparation

The powdered leaves (2 g) 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 rota evaporator (BUCHI model R-3000, Brazil, São Paulo) at 40 °C until reaching a final volume of 20 mL. The biomass was separated from the extract by vacuum filtration using filter paper and vacuum pump. The resulting extracts were stored in amber flasks at room temperature. At the same time, 5 mL of each sample was stored at 4 °C in capped tubes for further *in vitro* experimental analysis.

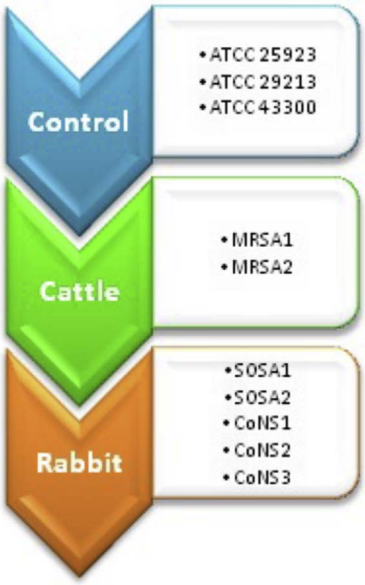

Fig. 1. Different sources of staphylococci viz. *S. aureus* (Control), methicillin-resistant *S. aureus* (Cattle), oxacillin sensitive *S. aureus* (Rabbit), and coagulase-negative *S. epidermidis* (Rabbit).

2.3. In vitro antibacterial evaluation

2.3.1. Bacteria of interest

The indicator bacteria used for the antibacterial test include *Staphylococcus 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). Fig. 1 depicts the various control staphylococci strains, as well as isolates from cattle and rabbits. Excluding control bacteria (*S. aureus*), methicillin-resistant *S. aureus* strains were isolated from cattle, while, oxacillin sensitive *S. aureus* and coagulase-negative *S. epidermidis* were isolated from rabbits. 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 using selective MRSA agar medium. The plates were incubated at 35 °C for 48 h and plates were examined for *Staphylococcus* sp. Isolates were screened for methicillin resistance using disc diffusion assay. Oxacillin sensitive and coagulase-negative strains were isolated from rabbits on MRSA and MRS (de Man, Rogosa and Sharpe) medium respectively using standard protocol. All bacterial cultures were sub-cultured into Brain-heart infusion (BHI) broth (BIOXON, DF, Mexico) medium for further experimental purpose.

2.3.2. Disc diffusion assay

Each bacterial inoculum was prepared in 5 mL of BHI 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. After the required period of incubation, bacterial cultures were swabbed on selective agar medium plates. Subsequently, methanolic extracts (25 µL) of leaves at the concentrations of 62.5, 125, 250, 500, and 1000 µg/mL were transferred to sterile discs (6 mm) and allowed to soak for 10-15 min. The discs were transferred aseptically to the plates seeded with the respective staphylococci pathogens with the help of ethanol dipped and flamed forceps, and incubated at 37 °C for 24 h. After 24 h, zone of inhibition (mm) formed by different plant extracts against the indicator

pathogenic bacteria were measured. Oxacillin (1μg/disc) was used as positive control and the experiments were carried out in triplicate.

2.3.3. Determination of relative percentage inhibition

The relative percentage inhibition (RPI) of the leaf extracts with respect to positive control was calculated as described below.

$$\text{Relative percentage inhibition} = \frac{IHD_{EXT} - IHD_{NC}}{IHD_{PC} - IHD_{NC}} 100$$

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

2.4. Estimation of total phenolics and saponin content

Total phenolics content in the leaf extracts of the plant was estimated according to the methods of Singleton et al. [16] with some modifications. The reaction mixture contains 1 mL 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. The samples were incubated at 45 °C for 15 min and the absorbance was read at 765 nm. Blank includes ethanol, instead of extract solution. The calibration curve was prepared using Gallic acid as standard at the concentrations of 20–100 μg/mL. The total phenolics content was calculated as milligrams of gallic acid equivalent per gram of dry weight of extract (mg gallic acid/g extract).

The total saponin content in the leaf extracts of plant was estimated according to the method described by Makkar et al. [17] based on vanillin-sulphuric acid colorimetric reaction with slight modifications. Approximately 50 μL of plant extract was added with 250 μL of distilled water. To this, about 250 μL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol) as well as 2.5 mL of 72% sulphuric acid was added and it was mixed well. The solution was incubated in a water bath at 60 °C for 10 min. After that, it was cooled in ice cold water and the absorbance was read at 544 nm. The total saponin content was calculated as diosgenin equivalents (mg diosgenin/g extract).

2.5. Statistical analyses

All experiments were carried out in triplicate and results were expressed as mean ± SD. Statistical analyses were performed in factorial design with three factors (extract of tree species, extract concentrations, and bacterial strains) using the GLM Procedure.

3. Results

3.1. In vitro antibacterial assessment

The methanolic extracts of plant leaves showed broad-spectrum antibacterial activity against various staphylococci strains in a dose dependent manner. *E. crassipes* extract showed potent bactericidal activity against CoNS1 with maximum zone of inhibition of 14.63 ± 0.16 mm at 1000 μg/mL of concentration. A minimum zone of inhibition of 10.17 ± 0.35 mm was observed against ATCC 43300 at higher concentration of *E. crassipes* extract. The methanolic extract of *P. vera* was found to be the most active against ATCC 25923 with maximum zone of inhibition of 14.63 ± 0.15 mm at 1000 μg/mL of concentration. The extract was found to be less effective against SOSA2 with minimum zone of inhibition of 10.32 ± 0.28 mm. In like manner, *Z. amole* exhibited potent growth inhibitory property against ATCC 25923 with maximum zone of 14.76 ± 0.23 mm at higher concentration. In accordance to the bactericidal zone of plant extracts, the relative percentage inhibition (i.e., RPI) values were found to be affected (Table 1).

3.2. Relative percentage inhibition (RPI) of extracts against staphylococci strains

Fig. 2a depicts the RPI values for *E. crassipes* against control staphylococcal strains as well as *Staphylococcus* sp. isolated from cattle and rabbit. The methanolic extract of this plant was found to be the most active against control strains (RPI% - 68.6 ± 0.5), followed by cattle (RPI% - 64.6 ± 0.6) and rabbits (RPI% - 61.7 ± 0.3) strains. On the other hand, *P. vera* revealed maximum RPI values against cattle isolates (68.6 ± 0.3%), followed by rabbits (65.7 ± 0.5%) and control (59.9 ± 0.4%) strains (Fig. 2b). Similar to *P. vera* extract, the methanolic extract of *Z. amole* showed promising RPI values against staphylococcal strains in the order of 74.79 ± 0.3% (cattle) > 67.3 ± 0.4% (rabbits) > 66.5 ± 0.5% (control) (Fig. 2c).

3.3. Quantification of total phenolics and total saponins

The present findings showed that the content of total phenolics and total saponins differed significantly among the methanolic extract of plants. *P. vera* extract showed substantial amount of total phenolics content with the highest value of 60.0 ± 1.3 mg gallic acid/g extract, followed by *Z. amole* (33.6 ± 1.4 mg gallic acid/g extract) and *E. crassipes* (23.0 ± 1.3 mg gallic acid/g extract) (Fig. 3a). The total saponin content for *Z. amole*, *E. crassipes*, and *P. vera* extracts were estimated as 35.8 ± 1.3, 25.0 ± 1.2 and 41.0 ± 1.3 mg diosgenin (DE)/g extract, respectively (Fig. 3b).

4. Discussion

Humankind has been relied on the traditional uses of plants as therapeutics from ancient periods. Secondary metabolites obtained from the plants are found to be an important source of diversified phytoconstituents that could be used for the production of several pharmaceuticals. At present, in the developing as well as developed countries, human populace still rely on the plants derived traditional medicine for health care needs. Thus, the demand for herbal medicines as potent therapeutic agents is continuously increasing day by day in comparison to the synthetic drugs. *Staphylococcus* sp. is predominant among the microorganisms responsible for infective complications following surgical vascular grafts or the implantation of prosthetic devices. *Staphylococcus* sp. is the chief organism accountable for infections of prosthetic heart valves, artificial joints, urinary tract, and cerebrospinal fluid shunts.

Researchers mainly focus on the medicinal plants rather than on the common weeds which are also the source of many phytochemicals. In the present study, *E. crassipes* was tested for its antibacterial activity against staphylococci strains, and depicted pronounced inhibition on the growth of *Staphylococcus* sp. tested. Furthermore, the RPI value for the plant extract was observed to be the maximum against control staphylococcal strains. The cattle and rabbits strains were found to be less susceptible to the extract. Similar observation was reported by Shehnaz and Vijayalakshmi [18] who demonstrated the bioactivity of methanolic extract of *E. crassipes* flowers against *Staphylococcus* sp. In another report, Zhou et al. [19] observed pH, concentration, and time dependent antibacterial activity of *E. crassipes* extract against *Staphylococcus* sp.

Although the biological activities of some species of the genus *Pistacia* has been investigated, studies on the antibacterial properties of methanolic extract of *P. vera* are currently very limited, probably not available. The present context evaluated the strong antibacterial activity of the methanolic extract of *P. vera* against few staphylococci strains in a dose dependent manner. *Staphylococcus* sp. isolated from cattle, in a comparison with control and rabbit strains were observed to be highly susceptible to the methanolic extract of *P. vera* in terms of RPI determination. According to the report of Smeriglio et al. [20], essential oil of *P. vera* was found to be markedly effective against clinical strains

Table 1
Antibacterial activity and relative percentage inhibition of plant extracts against *Staphylococcus* sp.

Plants	Strains	Strains origin	Extract activity		Oxacillin activity	RPI (%)*
			Concentrations	IZ (mm)*	IZ (mm)	
			(μg/mL)			
E. crassipes	ATCC 25923	Control	62.5	7.57 ± 0.15	14.50 ± 0.30	52.2
			125	8.15 ± 0.27		56.2
			250	9.25 ± 0.30		63.7
			500	10.73 ± 0.52		74
			1000	12.20 ± 0.25		84.1
	ATCC 29213	Control	62.5	8.65 ± 0.14	14.63 ± 0.51	59.1
			125	9.71 ± 0.25		66.3
			250	10.32 ± 0.65		70.6
			500	11.54 ± 0.50		78.9
			1000	12.79 ± 0.26		87.4
	ATCC 43300	Control	62.5	6.61 ± 0.25	12.37 ± 0.40	53.4
			125	6.94 ± 0.15		56.1
			250	8.53 ± 0.50		68.9
			500	9.40 ± 0.30		75.9
			1000	10.17 ± 0.35		82.2
	MRSA1	Cattle	62.5	5.17 ± 0.38	12.43 ± 0.45	41.6
			125	7.64 ± 0.41		61.4
			250	8.20 ± 0.28		65.9
			500	9.47 ± 0.16		76.1
			1000	11.10 ± 0.26		89.3
	MRSA2	Cattle	62.5	8.73 ± 0.25	18.53 ± 0.40	47.1
			125	10.64 ± 0.15		57.4
			250	11.57 ± 0.28		62.4
			500	12.37 ± 0.24		66.8
			1000	14.40 ± 0.33		77.8
	SOSA1	Rabbits	62.5	8.10 ± 0.17	14.34 ± 0.30	56.4
			125	8.18 ± 0.28		57.1
			250	9.76 ± 0.21		68.1
			500	10.60 ± 0.20		73.9
			1000	11.33 ± 0.21		79
	SOSA2	Rabbits	62.5	7.80 ± 0.20	18.10 ± 0.51	43
			125	11.44 ± 0.21		63.2
			250	12.37 ± 0.27		68.3
			500	14.50 ± 0.21		80.1
			1000	14.60 ± 0.25		80.6
	CoNS1	Rabbits	62.5	6.99 ± 0.62	18.60 ± 0.23	37.6
			125	8.79 ± 0.23		47.2
			250	10.00 ± 0.26		53.8
			500	11.23 ± 0.11		60.3
			1000	14.63 ± 0.16		78.7
	CoNS2	Rabbits	62.5	7.68 ± 0.28	14.25 ± 0.30	53.9
			125	7.88 ± 0.10		55.2
			250	9.63 ± 0.32		67.6
			500	10.25 ± 0.51		71.9
			1000	11.43 ± 0.16		80.2
	CoNS3	Rabbits	62.5	7.92 ± 0.10	18.53 ± 0.50	42.8
			125	9.21 ± 0.09		49.8
			250	9.38 ± 0.13		50.7
			500	10.43 ± 0.30		56.2
			1000	12.30 ± 0.50		66.3

(*continued on next page*)

Table 1 (*continued*)

Plants	Strains	Strains origin	Extract activity		Oxacillin activity	RPI (%)*
			Concentrations (μg/mL)	IZ (mm)*	IZ (mm)	
P. vera	ATCC 25923	Control	62.5	9.13 ± 0.32	18.37 ± 0.40	49.8
			125	10.30 ± 0.20		56.1
			250	11.43 ± 0.25		62.2
			500	12.40 ± 0.21		67.5
			1000	14.63 ± 0.15		79.8
	ATCC 29213	Control	62.5	6.30 ± 0.20	18.33 ± 0.42	34.3
			125	8.65 ± 0.65		47.1
			250	9.63 ± 0.10		52.6
			500	10.33 ± 0.23		56.3
			1000	14.51 ± 0.58		79.1
	ATCC 43300	Control	62.5	4.85 ± 0.22	12.43 ± 0.17	39.1
			125	5.33 ± 0.58		42.9
			250	8.60 ± 0.42		69.1
			500	9.76 ± 0.65		78.5
			1000	10.37 ± 0.31		83.4
	MRSA1	Cattle	62.5	5.43 ± 0.23	12.30 ± 0.32	44.1
			125	6.58 ± 0.40		53.4
			250	8.15 ± 0.12		66.2
			500	9.33 ± 0.61		75.9
			1000	11.33 ± 0.40		92.1
	MRSA2	Cattle	62.5	6.20 ± 0.26	12.43 ± 0.23	49.9
			125	7.98 ± 0.30		64.1
			250	8.40 ± 0.13		67.6
			500	10.00 ± 0.19		80.4
			1000	11.50 ± 0.46		92.5
	SOSA1	Rabbits	62.5	7.12 ± 0.16	14.24 ± 0.41	50
			125	9.47 ± 0.25		66.5
			250	10.45 ± 0.09		73.3
			500	11.43 ± 0.26		80.2
			1000	12.30 ± 0.76		86.3
	SOSA2	Rabbits	62.5	6.13 ± 0.61	10.45 ± 0.35	58.7
			125	6.66 ± 0.30		63.8
			250	7.17 ± 0.15		68.7
			500	9.28 ± 0.57		88.9
			1000	10.32 ± 0.28		98.7
	CoNS1	Rabbits	62.5	7.13 ± 0.65	14.60 ± 0.30	48.9
			125	8.70 ± 0.20		59.6
			250	9.28 ± 0.82		63.6
			500	11.00 ± 0.25		75.3
			1000	12.30 ± 0.17		84.2
	CoNS2	Rabbit	62.5	6.67 ± 0.21	12.65 ± 0.51	52.8
			125	7.40 ± 0.40		58.4
			250	7.83 ± 0.50		61.9
			500	9.56 ± 0.60		75.6
			1000	10.78 ± 0.67		85.2
	CoNS3	Rabbits	62.5	6.32 ± 0.13	18.60 ± 0.36	33.9
			125	7.23 ± 0.61		38.9
			250	9.43 ± 0.60		50.7
			500	10.42 ± 0.26		56.1
			1000	11.56 ± 0.30		62.1

(*continued on next page*)

Table 1 (*continued*)

Plants	Strains	Strains origin	Extract activity		Oxacillin activity	RPI (%)*
			Concentrations (μg/mL)	IZ (mm)*	IZ (mm)	
Z. amole	ATCC 25923	Control	62.5	9.80 ± 0.20	14.78 ± 0.51	66.6
			125	10.48 ± 0.09		71.1
			250	11.32 ± 0.30		76.9
			500	12.65 ± 0.15		85.9
			1000	14.76 ± 0.23		99.8
	ATCC 29213	Control	62.5	5.85 ± 0.12	14.32 ± 0.60	40.9
			125	6.33 ± 0.21		44.2
			250	7.44 ± 0.21		51.9
			500	10.12 ± 0.16		70.7
			1000	12.45 ± 0.13		86.9
	ATCC 43300	Control	62.5	8.79 ± 0.20	18.10 ± 0.45	48.6
			125	9.30 ± 0.11		51.3
			250	10.15 ± 0.27		56
			500	12.32 ± 0.14		68
			1000	14.16 ± 0.34		78.2
	MRSA1	Cattle	62.5	8.42 ± 0.45	12.60 ± 0.53	66.9
			125	9.30 ± 0.22		73.8
			250	9.48 ± 0.30		75.2
			500	10.47 ± 0.21		83
			1000	11.58 ± 0.34		91.9
	MRSA2	Cattle	62.5	6.33 ± 0.14	12.32 ± 0.37	51.3
			125	8.22 ± 0.17		66.8
			250	8.56 ± 0.23		69.4
			500	9.30 ± 0.14		75.4
			1000	11.61 ± 0.18		94.2
	SOSA1	Rabbit	62.5	7.19 ± 0.20	18.42 ± 0.52	39
			125	9.06 ± 0.16		49.1
			250	10.13 ± 0.21		54.9
			500	12.44 ± 0.24		67.5
			1000	14.30 ± 0.14		77.6
	SOSA2	Rabbits	62.5	8.52 ± 0.24	14.62 ± 0.47	58.2
			125	9.40 ± 0.16		64.2
			250	10.39 ± 0.51		71
			500	11.52 ± 0.27		78.8
			1000	12.83 ± 0.31		87.8
	CoNS1	Rabbits	62.5	8.23 ± 0.32	14.63 ± 0.15	56.2
			125	9.76 ± 0.35		66.8
			250	10.15 ± 0.24		69.3
			500	12.35 ± 0.11		84.4
			1000	12.75 ± 0.15		87.1
	CoNS2	Rabbits	62.5	7.85 ± 0.23	14.16 ± 0.16	55.4
			125	8.28 ± 0.43		58.4
			250	9.75 ± 0.34		68.9
			500	10.25 ± 0.24		72.3
			1000	12.32 ± 0.15		87
	CoNS3	Rabbits	62.5	10.09 ± 0.14	18.53 ± 0.31	54.4
			125	11.59 ± 0.13		62.6
			250	12.15 ± 0.16		65.6
			500	12.92 ± 0.32		69.8
			1000	14.10 ± 0.18		76

RPI, Relative Percentage Inhibition.
IZ, Inhibition Zone.

of staphylococci. In another study, *P. vera* polyphenols were shown to exhibit bactericidal property against MRSA strains [21]. As previously stated by other authors, the activity may be due to the cell wall or cell membrane disruption together with cell enlargement [22].

Ziziphus sp. is reported to possess bioactive constituents, recognized for traditional use and therapeutic importance. Present work evaluated the antibacterial potentiality of *Z. amole* methanolic extract against pathogenic strains of staphylococci. Interestingly, the RPI value for the extract was found to be the highest against strains of cattle origin, followed by rabbits and control staphylococcal strains. Antimicrobial activity of some other species of genus *Ziziphus* has already been reported in the previous literature [23,24]. In another report, *Z. mauritiana* methanol extract showed promising antibacterial activity against *S. aureus* [25]. The variation in activities observed amongst different species might be due to the diversity of bioactive compounds under influence of genetic features and environmental aspects [26].

The phenolics are the largest known groups of secondary metabolites exhibiting antibacterial activities. The number of site(s) and phenol hydroxyl groups leads to the increased hydroxylation, causing relative toxicity to bacteria [27]. The results of the present context revealed that the total phenolics content differed significantly among the plant extracts. The total phenolics content of the extracts was compared with the standard Gallic acid and the values were found to be maximum for *P. vera* extract, followed by *Z. amole* and *E. crassipes* extract. Our findings were found to be in complete agreement with the reports of Shanab and Shalaby [28] who observed the substantial level of phenolics content in the methanolic extract of *E. crassipes*. Previously, phenolic components viz. 4-methylresorcinol, 2-

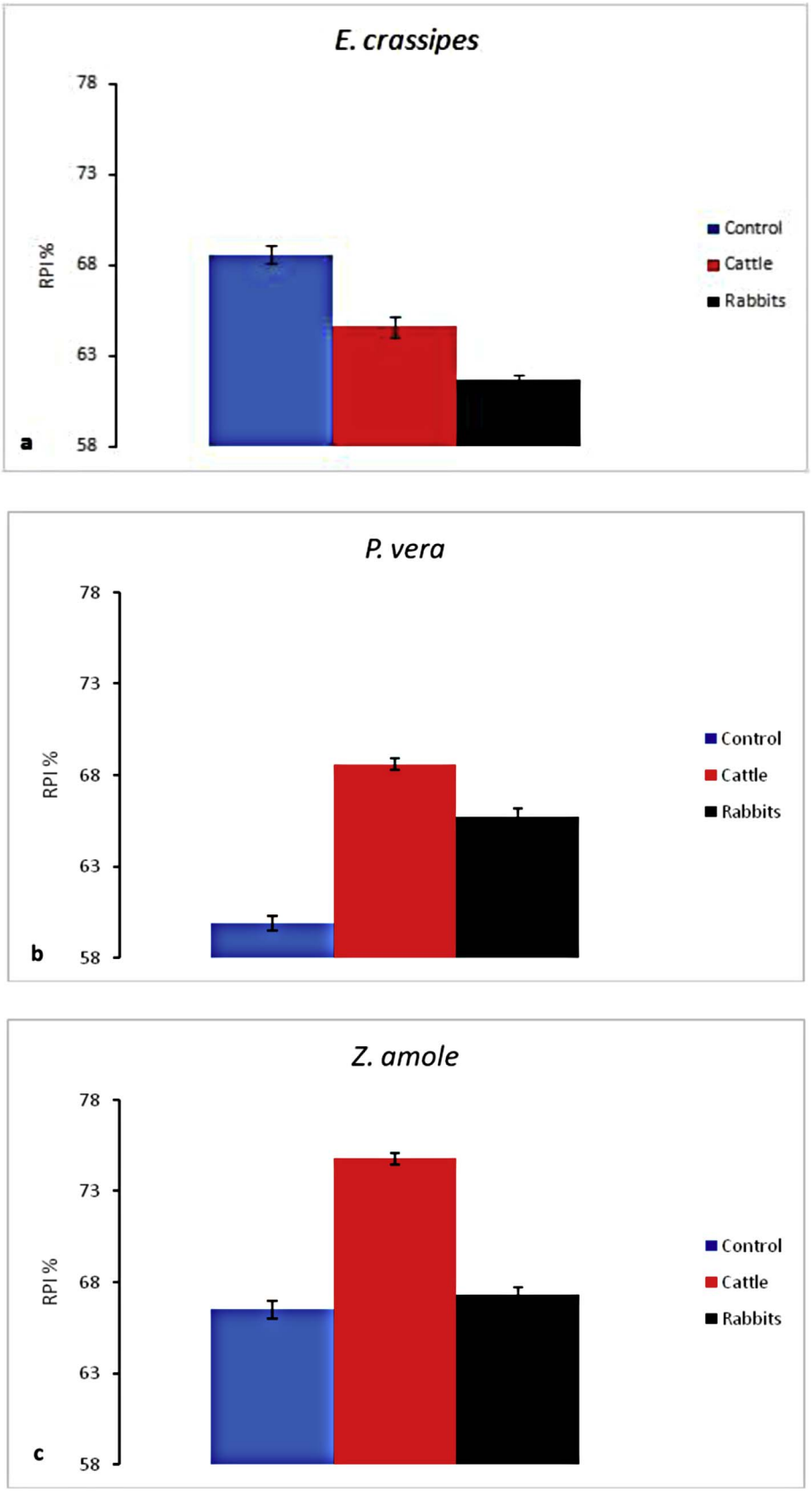

Fig. 2. Susceptibility (RPI %) of control *S. aureus* strains and other staphylococci from cattle and rabbits to the methanolic extract of (a) *E. crassipes*, (b) *P. vera*, and (c) *Z. amole*.

methylresorcinol, catechol, pyrogallol, genetisic, *p*-hydroxybenzoic, salicylic acids, and resorcinol have been reported in the various parts of *E. crassipes* [29].

Saponin has been reported to have a wide range of pharmacological and medicinal activities. The present study revealed the significant level of saponin content in the methanolic extract of *P. vera*, *Z. amole*, and *E. crassipes*. Interestingly, saponin has been reported to have nematicidal, molluscicidal, insecticidal and antioxidant properties [30];

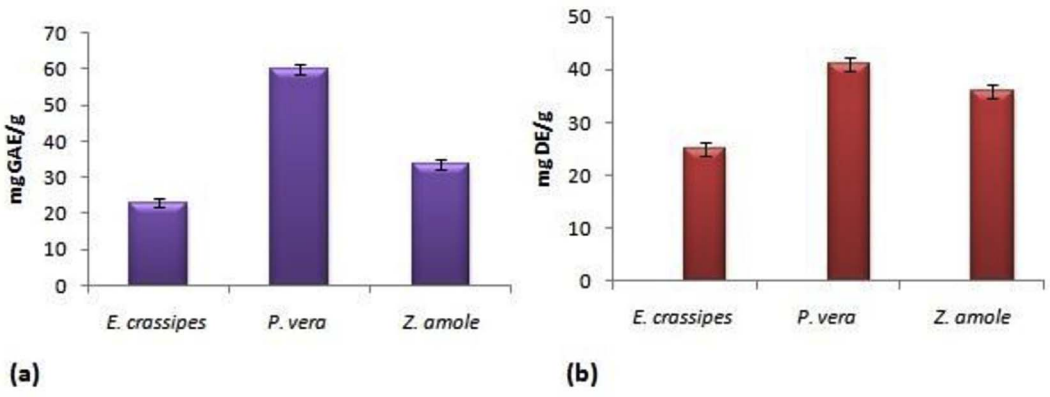

Fig. 3. Total phenolics (a) and total saponins (b) measured from the methanolic extracts.

tumoricidal activity [31], and antimicrobial characteristics [32]. The significant level of saponin in the leaves might be as a result of the necessity to protect plants against pathogens. It has been noted that many saponins are present in healthy plants in high concentrations because of their antimicrobial properties. The presence of saponin might be to serve as a natural defense mechanism. Plants need to protect themselves against herbivores and pathogens [33]. The potential anti-staphylococcal characteristics of plants studied in this investigation might be due to the efficacy of vast secondary metabolites, including phenolic compounds and saponins [34,35]. Based on the outcome of this investigation, the use of *P. vera*, *Z. amole*, and *E. crassipes* leaves in ethnomedicine as therapeutic drugs against staphylococcal infections is thus suggested.

5. Conclusions

In a nutshell, the present study demonstrated the potentiality of the methanolic extract of *P. vera*, *Z. amole*, and *E. crassipes* leaves to inhibit the growth of various staphylococci strains. Additionally, cattle were found to be the host for diversiform pathogenic strains of MRSA. Rabbits were observed as host for SOSA and CoNS strains, which indicates alarming situation for the livestock industries. Tested plants exhibited pronounced activity against all the indicator *Staphylococcus* sp. in a dose dependent manner. *E. crassipes* extract revealed promising RPI values against control strains, followed by staphylococcal strains of cattle and rabbits. In contrary to this, *P. vera* and *Z. amole* extracts showed high RPI values against staphylococci isolated from cattle, followed by rabbits and control strains. Further, the findings revealed the presence of two important groups of phytoconstituents viz. phenolics and saponin in the methanolic extract of the investigated plants in a substantial amount. This study suggests that these plants can be productively used in the pharmaceutics, particularly against staphylococcal infections because of its promising activities as well as the presence of bioactive phytoconstituents reported.

Conflict of interest

None declared.

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