ORIGINAL ARTICLE
Taylor & Francis healthsciences

Antimicrobial Activity of Nine Extracts of *Sechium edule* (Jacq.) Swartz

A. A. L. Ordoñez[1], J. D. Gomez[1], N. M. Cudmani[2], M. A. Vattuone[3] and M. I. Isla[3]

From the [1]Cátedra de Farmacotecnia II, [2]Hospital de Clínicas "Dr Nicolás Avellaneda" and [3]Cátedra de Fitoquímica, Instituto de Estudios Vegetales "Dr Antonio R. Sampietro", Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina

Correspondence to: Dra María Inés Isla, Cátedra de Fitoquímica, Instituto de Estudios Vegetales, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 461, 4000-San Miguel de Tucumán, Argentina. Fax: +54 381 4248025; E-mail: misla@tucbbs.com.ar or fitoqui@unt.edu.ar

Microbial Ecology in Health and Disease 2003; 15: 33–39

The antimicrobial properties of *Sechium edule* (Jacq) Swartz alcoholic extracts obtained according to the *Farmacopea Argentina* (6th edn) were tested against bacteria of clinical relevance as nosocomial pathogens. To evaluate antibacterial activity, the disc diffusion assay was carried out with several gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus pyogenes*, *Streptococcus agalactiae, Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212). This assay was suitable for the screening of a large number of extracts at one time. All ethanolic extracts showed activity against gram-positive bacteria. Minimal inhibitory concentration (MIC) values were determined with a microdilution assay. The highest activity was obtained with the 80% aqueous-ethanolic leaf extract (MIC values of 4.16–8.32 μg/ml against staphylococci and enterococci) and with the 96% ethanolic seed extract (MIC values of 8.32–16.64 μg/ml and >8.32 μg/ml against staphylococci and enterococci, respectively). The results indicate that both fluid extract and tincture have very good antimicrobial efficacy against all strains of multiresistant staphylococci and enterococci. In this study the minimal bactericidal concentration (MBC) values were identical to the MIC values or twofold higher than the corresponding MIC. This may indicate a bactericidal effect. Stored extracts have similar anti-staphylococcal and anti-enterococcal activity to recently obtained extracts. The results obtained might be considered sufficient to warrant further studies aimed at isolation and identification of the active principle. *Key words:* *Sechium edule*, antimicrobial activity, papa del aire.

INTRODUCTION

Sechium edule (Jacq.) Swartz (chayote or air potato) has economic and nutritional significance in Central America, Brazil and Mexico (1–9). Data compiled in recent studies highlight the use of decoctions made from leaves or fruits to relieve urine retention and burning during urination or to dissolve kidney stones, and as a complementary treatment for arteriosclerosis and hypertension (6, 7, 10, 11). The diuretic properties of the leaves and seeds, and the cardiovascular and anti-inflammatory properties of the leaves and fruits, have been confirmed by pharmacological studies (12–15). *S. edule* extracts showed inhibitory effect against the mutagenicity of 2 amino 3 methyl imido (4,5f) quinoline in *Salmonella typhimurium* TA98 and TA100 assays. The antimutagenic activity of aqueous extracts of *S. edule* was partly reduced by heating at 100°C (16). Otherwise a new type I ribosome-inactivating protein (RIP) sechiumin was purified from the seeds. The recombinant sechiumin was obtained as an insoluble protein, and the preparation of the active soluble form was achieved by renaturing the denatured protein. These studies suggest that the recombinant sechiumin could be used for the preparation of immunotoxin as a potential cancer chemotherapeutic agent (17).

At the present time, staphylococci and enterococci are recognized as important nosocomial pathogens. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have become a critical problem as an important cause of mortality among hospitalized patients (18–22). Certain antimicrobial agents are usually chosen for the treatment of MRSA infections. However, their use not only produces unexpected side effects but also reduces susceptibility to the agents by target site alteration and enzyme modification of selected resistant strains (23). Unavoidable problems associated with antibiotic therapy have led to ongoing investigations for alternative anti-MRSA agents (24, 25). The research into biologically active compounds from natural sources has always been of great interest for scientists looking for new sources of useful drugs against infectious diseases.

The purpose of the present study was to investigate the antimicrobial activity of extracts of leaf, stem and seed from *S. edule* (Jacq.) Swartz. It was conducted to find anti-MRS (methicillin-resistant staphylococci) activity and anti-enterococcal activity in ethanolic extracts of *S. edule*.

 ISSN 0891-060X

DOI: 10.1080/08910600310015583

MATERIALS AND METHODS

Plant material

Phenology: wild populations of *S. edule* flourish from April to December and give fruit from September to January. The plants were collected from September to March in San Miguel de Tucumán, Tucumán, Argentina.

Voucher specimens were deposited in the Herbarium of the Institute de Estudios Vegetales (IEV), Facultad de Bioquímica, Química y Farmacia, UNT (Tucumán, Argentina). The parts used were leaves, stems and seeds.

Preparation of ethanolic S. edule extract

Lixiviation (fluid extract): ground air-dried plant material was lixiviated in an extractor with 80% ethanol for leaves or 90% ethanol for seeds at room temperature until total extraction according to the *Farmacopea Argentina* (26).

Maceration (tincture): ground air-dried plant material was macerated in ethanol (1 g of dry tissue per 5 ml of 96% ethanol) for 7 days with shaking (40 cycles/min) at room temperature.

Alcoholature: fresh plant material was cut into small pieces and macerated in ethanol (1 g of dry tissue per 1 ml of 96% ethanol) for 8 days with shaking (40 cycles/min) at room temperature.

In all cases the preparations were filtered through Whatman no. 4 filter paper.

All reagents used were of analytical grade.

Microbiology

Bacterial strains. *Staphylococcus aureus* ($n = 23$), *Staphylococcus* spp. ($n = 4$), *Enterococcus faecalis* ($n = 29$), *Enterococcus faecium* ($n = 1$), *Streptococcus agalactiae* ($n = 1$) and *Streptococcus pyogenes* ($n = 1$) strains were obtained from clinical samples from the Hospital Nicolás Avellaneda, San Miguel de Tucumán, Tucumán, Argentina. Staphylococci were defined as MRS based on their resistance to oxacillin, according to the guidelines of the National Committee for Clinical Laboratory Standards (27). The following reference strains were included in the study: *Staph. aureus* ATCC 29213 and *Ent. faecalis* ATCC 29212.

Susceptibility screening test using agar-well diffusion method. MHA (Laboratorios Britania, Argentina) base medium (10 ml) was poured into 90-mm diameter sterile Petri dishes. Sterile 0.2% agar (3 ml) containing 30 μl of each bacterial suspension (10^5 bacteria $\times$ ml^{-1}) was poured on it to form a homogeneous layer. Five wells were made in each agar dish with a cork borer. Dilutions of known extract concentrations (1.04–133.12 μg/ml of phenolic compounds) were placed in each well. Vancomycin or oxacillin were used as a positive control and ethanol as negative solvent controls. The dishes were incubated at 35°C for 16–20 h.

Bacterial growth inhibition was determined as the diameter of the inhibition zones around the wells. The growth inhibition diameter was an average of four different measurements.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determination. Method A. A serial agar macrodilution method was used. Briefly, the antibacterial activity was determined based on the MIC values that were obtained by a twofold serial agar dilution method as recommended by the NCCLS (27). Dilutions of each extract to be tested were performed with ethanol (1 ml final volume), then 9 ml of Mueller-Hinton agar (MHA) (final concentration range of 1.04–133.12 μg/ml of phenolic compounds) were added. The control plate contained only ethanol, which did not influence bacterial growth.

These dishes were inoculated with 2 μl of each bacterial cell suspension (1×10^4 cfu) and incubated aerobically for 16–20 h at 35°C. A growth control was included for each strain tested. *Staph. aureus* ATCC 29213 and *Ent. faecalis* ATCC 29212 were used as quality controls.

The MIC was defined as the lowest concentration of *S. edule* extract at which no colony was observed after incubation. The MIC_{90} was defined as the extract concentration that produced 90% growth inhibition of the tested strains. The MIC values were also determined for oxacillin, methicillin, gentamicin and vancomycin for staphylococcal strains. The MIC values for gentamicin, streptomycin, ampicillin and vancomycin were determined for enterococcal isolates. The antimicrobial agents were supplied by Sigma Chemical Co. (USA) and Laboratorios Britania S.A. (Argentina).

Method B. A broth microdilution method was used to determine the MIC and the MBC of the different extracts against the test organisms according to the NCCLS recommendations (27). The ethanolic extract was appropriately prepared and transferred to each microplate well to obtain a twofold serial dilution of the original extract ranging from 1.04 to 133.12 μg/ml. Bacterial suspension (50 μl) was transferred to 10 ml of CAMHB (cation-adjusted Muller–Hinton broth) to a final concentration of 5×10^5 cfu/ml. Then, 100 μl of the test organism were added to each of the extract dilutions within 30 min to prevent overgrowth of the organisms. The dishes were incubated aerobically at 35°C for 16–20 h. After incubation, bacterial growth was assayed by measurement of absorbance at 625 nm.

The MIC was defined as the lowest concentration of *S. edule* extract that restricted growth to a level < 0.05 at 625 nm (no macroscopically visible growth).

To determine MBC, 10 μl of culture medium were removed from each well with no visible growth and transferred to blood agar dishes. After incubation for

16–20 h at 35°C, MBC was determined. MBC was defined as the lowest extract concentration at which 99.9% of the bacteria had been killed.

Phytochemical screening

Identification by chemical tests. Tests for alkaloids, coumarins, flavonoids, terpenes and sterols were carried out according to the methods of Harbone (28).

Identification by thin-layer chromatography. The components of the different extracts (10 μg) were separated by TLC (Merck Kieselgel 60 F254) with chloroform:methanol (9:1; v:v) as solvent. Separated components were visualized under visible and ultraviolet light (254 and 360 nm, Model UV 5L-58 Mineralight Lamp) and sprayed with 1% ferric trichloride and aluminium chloride for flavonoids, methanolic potassium hydroxide for coumarins, Dragendorff's reagent for alkaloids and anisaldehyde/sulphuric for steroids and terpenes (29).

Bioautography. Developed TLC plates were dried overnight in a sterile room. Then, 2 ml of soft medium (0.2% BHI agar) containing 10^5 cfu of *Staph. aureus* (F7) were added, incubated at 35°C for 16–20 h and sprayed with a 2.5 mg/ml MTT solution (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) in PBS (10 mM sodium phosphate buffer, pH 7, with 0.15 M NaCl). Plates were incubated at 35°C for 1 h in the dark for colour development. The areas of inhibition, coloured yellow, were compared with the Rf of the related spots on the reference TLC plate.

Phenolic compound determinations

Total phenol content was determined by absorbance at 280 nm in a Beckman DU 600 spectrophotometer with a 10-mm pathlength by the Folin-Ciocalteau method (30). Results were expressed as quercetin equivalents.

RESULTS AND DISCUSSION

Three kinds of plant materials (leaf, seed and stem) and three kinds of crude preparations (tincture, fluid extract and alcoholature) derived from each of them were investigated for their antibacterial activity. Fresh and dry material from the same plants were compared.

Table I shows the results of the extraction using 96%, 90% and 80% ethanol according to the *Farmacopea Argentina* (26). The yield of ethanol-soluble principle extractions from leaf, seed and stem was different. All extracts were analysed for total phenolic compounds content. *S. edule* fluid extract gave the best yield of ethanol-soluble principles and *S. edule* tincture gave the best yield of total phenolic compounds (expressed as quercetin equivalents) per gram of plant material.

Antimicrobial activity

The *S. edule* antibacterial activity was assayed *in vitro* by the agar diffusion method against four isolates with the largest antimicrobial resistance profiles.

Table II summarizes the means of the zones of microbial growth inhibition by nine crude ethanolic extracts of *S. edule*. All tested crude extracts were particularly active against gram-positive bacteria (*Ent. faecalis*, *Staph. aureus*, *Strep. pyogenes, Strep. agalactiae*). The most active of them were 80% aqueous-ethanolic extracts from *S. edule* leaves and 96% ethanolic extracts from *S. edule* seeds. The highest inhibition zones were shown by *Staph. aureus* followed by *Ent. faecalis*. Controls did not form an inhibitory zone with any of the tested micro-organisms.

Susceptibility of Staphylococcus species to S. edule extracts

Plants are important sources of various pharmaceutical agents and useful pharmacological activities have been widely exploited as phytochemicals. Catechins isolated from tea leaves (31), protoanemonin from *Ranunculus bulbosus* (32), chalconas (33) and isoflavanones from *Erythrina bidwilli* (34) possess anti-staphylococcal activity, although not as intensive as antibiotics (MIC values from 50 to 500 μg/ml or more).

To determine the comparative efficacy of vancomycin and the two best *S. edule* extracts against *Staphylococcus* spp., each agent was tested against 27 recent clinical isolates by an agar dilution method with an inoculum of 10^4 cfu/spot. All the isolates of gram-positive bacteria recovered between January 1999 and December 1999 in a hospital in Tucumán, Argentina were tested. These isolates included 25.92% MRSA, 59.25% MSSA (methicillin-sensitive *Staph. aureus*), 7.40% MRCNS (methicillin-resistant coagulase-negative staphylococci) and 7.53% MSCNS (methicillin sensitive coagulase-negative staphylococci). Most of the MRS clinical isolates were highly resistant not only to methicillin but also to many other antimicrobial agents, whereas the MSSA strains were susceptible to many of them. All MRSA strains were uniformly inhibited by vancomycin at ≤ 1 μg/ml.

Table III shows the *in vitro* activity expressed as minimal bactericidal activity against 27 fresh clinical isolates of *Staphylococcus*.

Aqueous-ethanolic leaf and ethanolic seed preparations inhibited the growth of both MRSA and MSSA at concentrations of 4.16–8.32 μg/ml and 8.32–16.64 μg/ml, respectively, and 8.32–16.64 μg/ml for MSCNS and MRCNS. The minimum inhibitory concentration of *S. edule* preparations were higher than that for vancomycin.

The extract concentrations required to obtain a bactericidal effect against *Staphylococcus* spp. were the same or twofold higher than the corresponding MIC value.

Table I

Ethanol-soluble principles and total phenolic compound content in Sechium edule extracts

Extracts	Plant material	Amount extracted[a] (mg/g)	Phenolic compounds[b] (mg/g)	Soluble principles[c] (mg/ml)	Phenolic compounds[d] (mg/ml)
Tincture	Leaf	56.25	2.06	3.75	0.13
	Stem	37.5	2.81	2.5	0.18
	Seed	3.56	5	17.8	0.33
Fluid extract	Leaf	90.10	0.44	106	0.50
	Stem	144	1.41	180	1.66
	Seed	46.49	0.56	54.7	0.66
Alcoholature	Leaf	40	0.15	40	0.15
	Stem	16	0.06	16	0.06
	Seed	43	0.13	43	0.13

[a]Soluble principles per gram of dry material.
[b]Phenolic compounds per gram of dry material.
[c]Soluble principles per ml of extract.
[d]Phenolic compounds per ml of extract.

Table II

Activity of different extracts of Sechium edule against some microbial agents

S. edule preparation	Plant parts	Gram-positive bacteria			
		Ent. faecalis	*Staph. aureus*	*Strep. pyogenes*	*Strep. agalactiae*
Tincture	Leaf	+	+	++	++
	Stem	+	+	++	++
	Seed	+++	+++	+++	+++
Fluid extract	Leaf	+++	+++	+++	+++
	Stem	+	++	++	++
	Seed	++	++	++	++
Alcoholature	Leaf	0	+++	+++	+++
	Stem	0	+	++	+++
	Seed	+	++	+++	+++

The inhibition is reported as 0, without inhibition; +, dr < 1.00; ++ dr $> 1.00 < 1.50$; +++ dr > 1.50, where dr is the diameter of the inhibition zone (cm) at the same phenolic compound concentrations for all *S. edule* preparations.

Table III

Antimicrobial activity of leaf fluid extract and seed tincture against several methicillin-resistant Staph. aureus strains and other strains of Staphylococcus species

Staphylococcus species	Leaf fluid extract		Seed tincture	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
MRSA ($n = 7$)	4.16–8.32	8.32–16.64	8.32–16.64	33.28–66.46
MSSA ($n = 16$)	4.16–8.32	8.32–16.64	8.32–16.64	33.28–66.46
MSCNS ($n = 2$)	8.32–16.64	8.32–16.64	8.32–16.64	33.28–66.46
MRCNS ($n = 2$)	8.32–16.64	8.32–16.64	8.32–16.64	33.28–66.46
Staph. aureus ATCC 29213	6.3	8.32	11.2	24.3

MRSA, methicillin-resistant *Staph. aureus*; MSSA, methicillin-sensitive *Staph. aureus*; MSCNS, methicillin-sensitive coagulase-negative *Staph. aureus*; MRCNS, methicillin-resistant coagulase-negative *Staph. aureus*.

MIC values were re-determined after 6 weeks or 1 year of storage at 7°C because it is desirable for extracts to retain their activity over time. Stored and recently prepared extracts had generally similar antibacterial activity.

Susceptibility of enterococcal species to S. edule extracts

To determine the comparative efficacy of vancomycin, ampicillin, gentamicin, streptomycin and two *S. edule* extracts against *Enterococcus* spp., each agent was tested

Table IV

Antimicrobial activity of leaf fluid extract and seed tincture against several Enterococcus strains

Enterococcus species	Leaf fluid extract		Seed tincture	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
Ampicillin-susceptible ($n = 27$)	4.16–8.32	4.16–8.32	> 8.32	> 8.32
Ampicillin-resistant ($n = 2$)	4.16–8.32	4.16–8.32	> 8.32	> 8.32
Ent. faecalis ATCC 29212	> 8.32	8.32	> 8.32	> 8.32

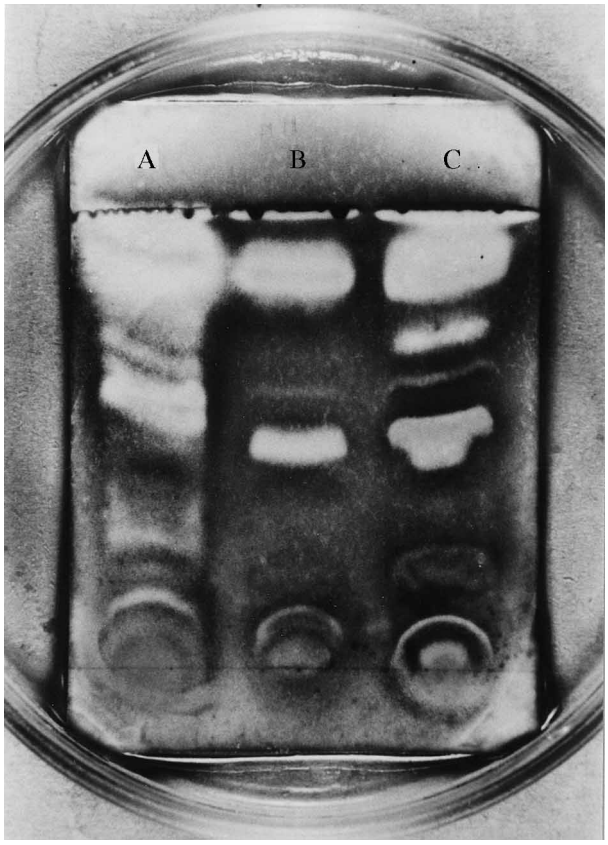

Fig. 1. Comparative bioautography of *S. edule* leaf extracts. Lane A, fluid extract; B, tincture; C, alcoholature (10 μg of phenolic compounds of each extract/plate) was separated by TLC (Merck Kieselgel 60 F254) using chloroform:methanol (9:1) as eluant. Then 2 ml of soft medium (0.2% BHI agar) containing 10^5 cfu/ml of *Staph. aureus* (F7) were added and incubated at 35°C for 24 h. The plates were then developed.

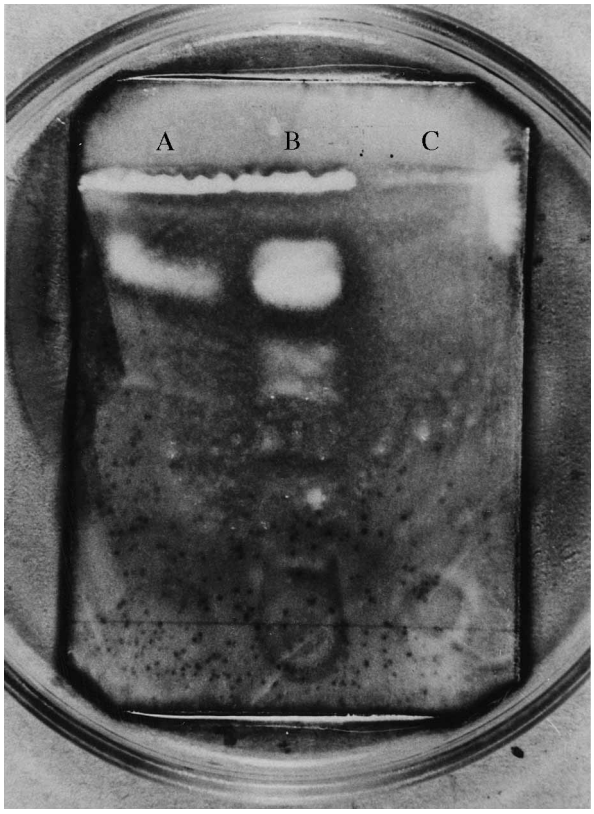

Fig. 2. Comparative bioautography of *S. edule* seed extracts. Lane A, fluid extract; B, tincture; C, alcoholature (10 μg of phenolic compounds of each extract/plate) was separated by TLC (Merck Kieselgel 60 F254) using chloroform:methanol (9:1) as eluant. Then 2 ml of soft medium (0.2% BHI agar) containing 10^5 cfu/ml of *Staph. aureus* (F7) were added and incubated at 37°C for 24 h. The plates were then developed.

against 29 recent clinical isolates by an agar dilution method. All the enterococcal isolates were recovered between January 1999 and December 1999 in a hospital in Tucumán, Argentina. Among them, 96.7% were *Ent. faecalis* and 3.3% were *Ent. faecium*. All *Ent. faecalis* isolates were susceptible to vancomycin and 99% of them were also susceptible to ampicillin, 46.6% and 40% were resistant to a high level of gentamicin and streptomycin, respectively. There were no β-lactamase producers.

Table IV shows the *in vitro* activity of leaf and seed extracts expressed as MIC and MBC against fresh isolates of 30 different strains of *Enterococcus* spp. Leaf fluid extracts inhibited the growth of ampicillin-susceptible and ampicillin-resistant enterococci at a concentration of 4.16–8.32 μg/ml and seed extract at a concentration of > 8.32 μg/ml.

The cumulative percentage of irreversibly killed bacterial strains was determined using five different dilutions of extracts: 100% of the bacterial inoculum was killed in the

range 4.16–8.32 μg/ml and >8.32 μg/ml for leaf fluid extract and seed maceration, respectively (Table IV).

Bioautographic assays

Chemical tests and TLC analysis showed the presence of flavonoids in all the extracts. The bioautography revealed clear zones of bacterial growth inhibition. Leaf alcoholic extracts showed three major antibiotic components (Rf 0.5, 0.82 and 0.98) with fresh and dry material (Fig. 1) and two inhibition zones (Rf 0.8 and 0.98) for dry seed extracts (Fig. 2) in correspondence with those of flavonoids. On the other hand, bioautography of fresh seed extracts did not show inhibition zones.

This is the first communication showing the antimicrobial activities of *S. edule* extracts. The results show that such extracts could have a potential clinical use and could be a natural source of new powerful antimicrobial compounds.

Further studies, including the isolation, structure-function relationships of the antimicrobial natural products and toxicity of these extracts are now in progress.

ACKNOWLEDGEMENTS

This research was partially supported by the Consejo de Investigación de la Universidad Nacional de Tucumán, Argentina, and by the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

REFERENCES

1. Baral BH, Thapa MP, Sherchan DP, Khanal R, Duwadi VR, Rai UP, Waldie K, Sharrock GO, Acharya LN. Traditional vegetables in the eastern hills of Nepal. PAC Working Paper. Pakhribas Agricultural-Centre 1994; 96: 1–34.
2. Cheng ZH, Meng HW, Cui HW. The production of Buddha's Hand in China. Cucurbit Genet Coop Rep 1995; 18: 64–5.
3. Lira R, Bye R. Las cucurbitáceas en la alimentación de los dos mundos. In: Simposio 1492. El Encuentro de Dos Comidas. México: Puebla, 1992: 1–38.
4. Lira R, Torres R. La familia Cucurbitaceae en el Estado de Oaxaca. In: Resúmenes XII Congreso Mexicano de Botánica (Sociedad Botánica de México Ed.). Yucatán: Mérida, 1993: 144.
5. Sharma MD, Newstrom-Lloyd L, Neupane KR. Nepal's new chayote genebank offers great potential for food production in marginal lands. Diversity 1995; 11: 7–8.
6. Yang SL, Walters T. Ethnobotany and the economic role of the Cucurbitaceae of China. Econ Bot 1992; 46: 349–67.
7. Flores E. El chayote *Sechium edule* Swartz (Cucurbitaceae). Rev Biol Trop 1989; 37(Suppl 1): 1–54.
8. Liebrecht S, Seraphine M. The edible potion and waste in foodstuffs consumed in a Hospital in Southern India (Pondicherry). Nutrition 1964; 18: 19–22.
9. Silva CR, Silva HC, Dutra JE. Conteudos de celulose, hemicelulose e lignina em dieta hospitalar hipocalórica. Alimentos e Nutricao 1990; 2: 65–71.
10. Gordon EA, Guppy LJ, Nelson M. The antihypertensive effects of the Jamaican Cho-Cho. West Indian Med J 2000; 49: 27–31.
11. Lira R. Cucurbitaceae de la Península de Yucatán: Taxanomía y Etnobotánica. Tesis Maestría en Ciencias (Ecología y Recursos Bióticos). Instituto Nacional sobre Recursos Bióticos, 1988: 1–329.
12. Bueno RR, Moura S, Fonseca OM. Preliminary studies on the pharmacology of *Sechium edule* leaves extracts. An Acad Cien Brasil 1970; 40: 285–9.
13. Lozoya X. Mexican medicinal plants used for treatment of cardiovascular diseases. Am J Chin Med 1980; 8: 86–95.
14. Ribeiro R, de Barros F, Fiuza de Melo MMR, Miniz C, Chieia S, Wanderley G, Gomes C, Trolin G. Acute diuretic effects in conscious rats, produced by some medicinal plants used in the state of Sao Paulo, Brasil. J Ethnopharmacol 1988; 24: 9–29.
15. Salama AM, Polo NA, Enrique M, Contreras CR, Maldonado RL. Preliminary phytochemical analysis and determination of the antiinflammatory and cardiac activities of the fruit of *Sechium edule*. Rev Colombiana Cienc Químic Farmac 1986; 15: 79–82.
16. Yen GC, Chen HY, Peng HH. Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of emerging edible plants. Food Chem Toxicol 2001; 11: 1045–53.
17. Wu TH, Chow LP, Lin JY. Sechiumin, a ribosome inactivating protein from the edible gourd *Sechium edule* Swartz – purification, characterization, molecular cloning and expression. Eur J Biochem 1998; 255: 400–8.
18. Eykin SJ. Staphylococcal sepsis. The changing pattern of disease and therapy. Lancet 1988: 100–4.
19. Maple PA, Hamilton-Miller JM, Brumfitt W. World-wide antibiotic resistance in methicillin-resistant *Staphylococcus aureus*. Lancet 1989: 537–40.
20. Mulligan ME, Murray-Leisure KA, Ribner BS, Standiford HC, John JF, Korvick JA, Kauffman CA, Yu VL. Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis and epidemiology with implications for prevention and management. Am J Med 1993; 94: 313–28.
21. Thompson RL, Cabezudo I, Wenzel RP. Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. Ann Intern Med 1982; 97: 309–17.
22. Townsend DE, Ashdown N, Bolton S, Bradley J, Duckworth G, Moorhouse EC, Grubb WB. The international spread of methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 1987; 9: 60–71.
23. Brumfitt W, Hamilton-Miller J. Methicillin-resistant *Staphylococcus aureus*. N Engl J Med 1989; 320: 1188–96.
24. Gorzynsky EA, Amsterdam D, Beam Jr TR, Rostein C. Comparative in vitro activities of teicoplanin, vancomycin, oxacillin and other antimicrobial agents against bacteremic isolates of Gram-positive cocci. Antimicrob Agents Chemother 1989; 33: 2019–22.
25. Funabashi Y, Tsubotani S, Koyama K, Katayama N, Harada S. A new anti-MRSA dipeptide, TAN-1057 A. Tetrahedron 1993; 49: 13–28.
26. Farmacopea Nacional Argentina. Codex Medicamentarius Argentino, 6th edn. Bs As Editorial Codex SA, 1978.
27. National Committee for Clinical Laboratory Standards, Performance standards for antimicrobial susceptibility testing. 11th International Supplement; MS100–S11. Wayne, PA: NCCLS, 2001.
28. Harbone JB. Method of plant analysis. In: Phytochemical Methods. London: Chapman and Hall, 1973: 1–32.
29. Krebs KG, Heusser D, Wimmer H. In: Stahl E, ed. Spray Reagents in Layer Chromatography: A Laboratory Handbook. Berlin: Springer-Verlag, 1969: 854–905.
30. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. Methods Enzymol 1999; 299: 152–78.

31. Toda M, Okubo S, Hara Y, Shimamura T. Antibacterial and bactericidal activities of tea extracts and catechins against methicillin-resistant *Staphylococcus aureus*. Jpn J Bacteriol 1991; 46: 839–45.
32. Didry N, Dubreuil L, Pinkas M. Microbiological properties of protoanemonin isolated from *Ranunculus bulbosus*. Phytother Res 1993; 7: 21–4.
33. Szajda M, Kedzia B. New N-substituted derivated of 3-azachalcone of potential antimicrobial activity. Pharmazie 1991; 46: 745–6.
34. Iinuma M, Tanaka T, Mizuno M, Yamamoto H, Kobayashi Y, Yonemori S. Phenolic constituents in *Erythrina bidwilli* and their activity against oral microbial organisms. Chem Pharm Bull 1992; 40: 2749–52.