Antibacterial activity and qualitative phytochemical analysis of *Vitex mollis* fruit

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The pulp of the *Vitex mollis* fruit is edible and traditionally used to treat diarrhoea. The antibacterial activity of this fruit is reported here for the first time. The fruit pulp was extracted with methanol (ME) and the extract was fractionated with solvents. ME and their fractions [hexanic (HF), chloroformic (CF), ethyl acetate (EAF) and aqueous (AqF)] were assayed against human pathogenic bacteria (microdilution test) and their phytochemicals determined (qualitative chemical determinations). The samples (i.e., ME, HE, CF, EAF and AqF) showed antibacterial activity; EAF was the most active, showing such activity against *Shigella dysenteriae* [minimal inhibitory concentration (MIC)=2 mg/ml]. Phenolics were mainly found in ME and EAF; compounds of this chemical family are well known for their antidiarrhoeal and antimicrobial activities. The reported antibacterial activity and phenolics content of *V. mollis* fruit could be associated with its use in the treatment of diarrhoea.

Key words: Antibacterial activity, diarrhoea, phytochemical analysis, *Vitex mollis*

INTRODUCTION

Around the world, infectious diseases are among the main causes of morbidity and mortality.[1] In Mexico, gastrointestinal pathogens are important morbidity agents.[2] Nowadays, the treatment with commercial antibiotics is more complicated due to the resistance phenomena and new antibacterials are required.[1] Till date, plants are the main source of bioactive compounds for drug development by pharmaceutical companies, and mostly the only option for disease treatment in rural communities characterized by low *per capita* income.[3] Plants are commonly used to treat diarrhoea where they may act as antimicrobial, slowing the gastrointestinal transit or increasing water and electrolyte reabsorption by the colon.[4]

Vitex mollis HBK (Verbenaceae) is a native plant of Sinaloa, Mexico, where it is known as "uvalamo".[5] Its fruit (uvalama) is edible and rich in dietary fibre and minerals.[6] Fruit and leaves are traditionally used to treat diarrhoea,[7] but scientifically it has not been established. The leaves of *V. mollis* have been better studied than fruit, e.g., methanolic extract was spasmolytic in guinea pig intestines and the hexanic extract did not show *in vitro* antibacterial activity at doses up to 16 mg/ml.[8] On the other hand, an aqueous extract of *Vitex doniana* sweet fruits showed antidiarrhoeic effects.[9]

The aim of this research is to demonstrate the antibacterial activity of uvalama fruit extracts against human pathogens, and also their phytochemical composition. These results could give the scientific support for the traditional use of this fruit as an antidiarrhoeal and for the future development of nutraceutical products.

MATERIALS AND METHODS

Materials

Uvalama fruits were collected from the valley of Culiacan, Sinaloa, Mexico. The plant material's identity was confirmed by Dr. Rito Vega-Aviña and a voucher specimen (VAR 2321) was deposited at the Herbarium of the Agronomy Faculty, Autonomous University of Sinaloa (UAS).

Bacterial strains were ATCC (DIFCO Laboratories, Detroit, MI, USA) and from diseased humans, kindly provided by the Instituto Nacional de Pediatría, México D.F. [Table 1].

Fruit Extract Preparation

Fruit was freeze-dried, milled in a waring blender (Waring commercial, Torrington, CT, USA), the meal was passed through a mesh 40 and stored at −20ºC until analysis.

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Extraction was carried out as described by Wall *et al*. (2006) with minor modifications.[10] Briefly, uvalama meal (200 g) was thrice extracted with pure methanol (1:5 p/v), liquid phases were mixed and vacuum concentrated (40°C) to obtain the methanolic extract (ME), the yield was 74.5% on a dry weight basis (d.w.). ME was dissolved in a minimal volume of 90% methanol solution and successively fractionated (1:1 v/v) with hexane, chloroform, ethyl acetate and water. Organic fractions were concentrated under vacuum (40°C) and the aqueous extract was concentrated by freeze-drying to obtain the hexanic (HF) (1.11% d.w.), chloroformic (CF) (0.4% d.w.), ethyl acetate (EAF) (0.38% d.w.) and aqueous (AqF) (73% d.w.) fractions. Used solvents were of reagent grade (CTR Scientific, Monterrey, Nuevo Leon, Mexico).

Antibacterial Activity

The minimal inhibitory concentration (MIC) was determined by broth microdilution.[11] This method was established in agreement with the recommendations of Cos y col., 2006.[12] Briefly, ME and AqF were dissolved in 10% dimethyl sulphoxide (DMSO) (v/v) and the organic fractions in 15% Tween 80 (v/v); these extracts were loaded in the wells of microplates at concentrations from 0.5 to 64 mg/ml and 5×10^5 UFC/ml of bacterial inoculum. Gentamicin was used as a positive control and dissolution solvents as negative controls. Microplates were incubated for 18 hours (35°C) and the MIC values were determined as the concentration at which bacterial growth was not observed. Evaluations were done in triplicate.

Phytochemical Screening

ME, HF, CF, EAF and AqF samples were analysed using the following: the reagents of Dragendorff, Mayer and Wagner for alkaloids; Keller-Kiliani, Lieberman-Burchard and Salkowski for cardiotonics; 1.0% gelatin solution and quinine sulphate solution with $FeCl_3$ for tannins; the Shinoda test for flavonoids; yellow fluorescence by reaction with NaOH and KOH for coumarins; $SbCl_3$ and lather formation for saponins; NaOH for free anthracenes and the Salkowski and Lieberman-Burchard for terpenes.[13-15]

RESULTS AND DISCUSSION

The lowest antibacterial effect was observed for the ME (MIC≥32 mg/ml) and the AqF, CF and EAF were the most active fractions with MIC values in the range of 2–8 mg/ml. In general, the active samples were more effective against Gram positive bacteria. They also showed activity against enteropathogenic bacteria. The EAF antibacterial effect was

Table 1: MIC of *V. mollis* fruit extracts against bacteria

Bacteria	MIC (mg/ml)*					
	G†	ME	HF	CF	EAF	AqF
Gram positive						
Enterococcus faecalis ATCC 29212	4	–	16	8	8	–
Staphylococcus aureus 1	4	64	8	4	8	–
Staphylococcus aureus 2	1	64	8	4	8	–
Staphylococcus aureus 3	1	64	16	4	8	–
Staphylococcus aureus 4	1	64	8	4	8	–
Staphylococcus aureus ATCC 29213	1	64	8	4	8	–
Streptococcus group A-3	4	–	16	8	8	–
Streptococcus group A-4	1	64	4	4	4	64
Gram negative						
Escherichia coli AO11	1	–	32	8	8	–
Escherichia coli AO19	1	–	32	8	8	–
Escherichia coli AO55	2	–	32	8	8	–
Escherichia coli ATCC 25922	1	–	32	8	8	–
Salmonella group A-1	1	–	32	8	8	–
Salmonella group A-2	1	–	32	8	8	–
Salmonella group PDY A-1	1	–	32	8	8	–
Salmonella group B	1	–	32	8	8	–
Salmonella group D	1	–	16	8	8	–
Salmonella typhi	1	64	16	8	8	–
Shigella dysenteriae	1	64	4	4	2	64
Shigella flexneri	2	64	8	4	4	–
Pseudomonas aeruginosa ATCC 27853	1	32	8	4	4	–

*ME, methanolic extract; HF, hexanic fraction; CF, chloroformic fraction; EAF, ethyl acetate fraction; AqF, aqueous fraction; †G, gentamicin (µg/ml); —, no activity

remarkable against *Shigella dysenteriae* (2 mg/ml) [Table 1], the causal agent of dysentery. The activities of the CF and EAF were in the range of values reported for the aerial parts of other *Vitex* species (0.02–10 mg/ml).[16-18] The MIC values of ATCC strains to gentamicin were in the range of values previously reported [Table 1].[11]

The evaluated samples showed a high content of phenolics (i.e. flavonoids and tannins), followed by terpenes [Table 2]. HF showed the highest content of terpenes but an intermediate antibacterial activity; thus, it is suggested that antibacterial activity of the most active fractions (CF and EAF) is mainly associated with phenolics [Tables 1 and 2]. Phenolics are the most common antibacterial agents isolated from plants, being mainly active against Gram positive bacteria;[19] they have been recommended as antidiarrhoeic agents.[4] The antibacterial effect of the ME could be considered low [Table 1]. Several studies have focused on antibacterial and antidiarrhoeal activity of *Vitex* spp. but not on *V. mollis* fruit. The methanolic extract of *V. mollis* leaves showed higher antispasmodic activity (37%) on guinea pig intestines than other Mexican plants, whereas the hexanic extract, evaluated up to 16 mg/ml, was not active against enteropathogenic bacteria.[8] Remarkably, aqueous extract of *V. doniana* fruit showed a significant dose–response antidiarrhoeic activity on mice (150–650 mg/kg) and it was not toxic; at the highest concentration, no significant difference was found with loperamide (5 mg/kg) (P<0.001). In addition, it was suggested that antidiarrhoeic activity could be associated with the content of phenolics.[9]

CONCLUSIONS

The antibacterial activity of the *V. mollis* fruit is reported for the first time and the fractions with highest activity (CF and EAF) have phenolics and terpenes as their constituents. Our results give scientific experimental support for the traditional use of this fruit as an antidiarrhoeic and suggest that this antibacterial activity is mainly associated with the phenolics content. Additional studies are required to characterise the chemical components of the active fractions.

Table 2: Phytochemical analysis of *V. mollis* fruit extracts

Phytochemicals	Fraction*				
	ME	HF	CF	EAF	AqF
Alkaloids	–	–	–	–	–
Free anthracenes	–	–	–	–	–
Cardiotonics	–	–	–	–	–
Coumarins	+	–	+	+	–
Flavonoids	++	+	+	++	+
Saponins	–	–	–	–	–
Condensed tannins	++	–	–	+++	+
Terpenes	+	++	+	++	–

*ME - Methanolic extract; HF - Hexanic fraction; CF - chloroformic fraction; EAF - Ethyl acetate fraction; AqF - aqueous fraction; +++ strong, ++ medium, + poor presence, and — complete absence of metabolite

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