

# Assessing the Antibacterial Activity and Phytochemical Screening of *Capsicum* Varieties from Côte d'Ivoire

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## ABSTRACT

Besides the use of pepper as a food ingredient, many developing countries use it as in indigenous medicine. This study was undertaken to investigate the antibacterial activities of extracts isolated from *Capsicum annum* L. and *Capsicum frutescens* fruits and to identify active compounds responsible for this activity. Three Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis*) and four Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae* and *Salmonella typhimurium*) were tested. Phytochemical screening was performed by sample adsorption on silica gels, thin-layer chromatography and compounds were identified using a standard staining procedure. The highest amount of extract and percentage of dry material were from *C. frutescens* var 'Attie' (51  $\pm$  3 mg; 10.26%) with acetone in a direct extraction method while for an exhaustive extraction method, methanol extracted the highest dry mass from *C. annuum* var 'Jaune' (1994  $\pm$  35 mg; 39.88%). Extracts tested *in vitro* against bacterial pathogens showed some antibacterial activities based on inhibition diameters: 10 to 28 mm for Gram<sup>+</sup> bacteria and 10 to 20 mm for Gram<sup>-</sup>. The exception was *C. frutescens* var 'Doux', which showed no activity against *E. coli* and *P. aeruginosa*. Phytochemical screening revealed the presence of some active compounds such as alkaloids, tannins, flavonoids, polyphenols, sterols and quinines. The activity and presence of compounds known to be biologically active are a validation for the use of *Capsicum* as a food ingredient and as a therapeutic element of traditional medicine.

Keywords: active compounds, extract, food ingredients, pathogen, pepper, traditional medicine

# INTRODUCTION

The aim of the present study was to investigate the antibacterial activities of extracts isolated from *Capsicum* fruits with two different extraction methods and also to identify the active compounds responsible for the antimicrobial activity.

Traditionally used medicinal plants have recently attracted the attention of pharmaceutical and scientific communities. Scientists are continually studying the effects of medicinal plants which may possess important therapeutic properties that can be used in the treatment of human diseases (Akinpelu *et al.* 2008). The investigation of plants for bioactive secondary metabolites is an area which most plant scientists are focusing on with the aim of discovering new clinically useful and commercially important plant products (Dewick 1997).

Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Bisignano *et al.* 1996; Lis-Balchin and Deans 1996; Maoz and Neeman 1998; Hammer *et al.* 1999). Also, the inconsistent use of these drugs could lead to microbial drug resistance. Moreover, with increasing consumer demand for natural food ingredients and minimal food processing, natural compounds from plant extracts are increasingly being tested as alternative sources of antimicrobials. Therefore, a systematic study of medicinal plants is very important for the food, cosmetic and pharmaceutical industries in order to find biologically active compounds.

Traditional Ivorian medicine uses a wide variety of

plants to treat gastrointestinal disorders such as diarrhoea. *Salmonella* spp., *S. aureus, Vibrio cholerae* and *Bacillus cereus* among others are bacterial pathogens incriminated in gastroenteritis. In rural areas of Côte d'Ivoire, infection is treated with the most accessible medicine which is traditional medicine. Among the therapeutic elements of this medicine, *Capsicum* pepper is almost always cited (Dorantes *et al.* 2000a, 2000b; Fett 2003).

Bell pepper (*Capsicum annum* L.) belongs to the Solanaceae family and is a tropical fruit, originating from South and Central America, disseminated in Europe, Africa and Asia. *Capsicum* peppers are used as food and are the best known of all household spices in tropical and subtropical areas. It is widely enjoyed and is essential for African and Ivorian dishes (Terrible 1983; Tano *et al.* 2008). *Capsicum* peppers are also widely used as folklore remedies by many tribes in Africa, especially in Côte d'Ivoire where *C. annum* and *C. frutescens*, the best known cultivated species (Terrible 1983), are used in almost all traditional medicines as drink, enema or topical treatments. Freshly harvested or dry fruit are most commonly used.

Biological and pharmaceutical activities of phytochemical compounds take into account different parameters and factors such as plant species, ecological factors and environmental conditions (Musyimi *et al.* 2008). Extraction methods are a possible source of variation for chemical composition, toxicity and antimicrobial activity of extracts (Eloff 1998).

Gastroenteritis is one of the highest causes of mortality and morbidity in Côte d'Ivoire (FAO/OMS 2005; Ouattara *et al.* 2009) where there is no available information on the antibacterial activity of *Capsicum* fruits. It is therefore important to assess the potential antibacterial activity of popular peppers from Côte d'Ivoire. To our knowledge, this is the first study of its kind from Côte d'Ivoire.

## MATERIALS

#### **Collection of plant material**

*C. annuum* vars 'Antillais' and 'Jaune', and *C. frutescens* vars 'Soudanais', 'Attie' and 'Doux' fruits were collected from four major markets in Abidjan: Koumassi, Abobo, Yopougon and Adjamé. These fruits were collected and selected to compile a representative list of those pepper fruits most used medicinally by traditional healers of Côte d'Ivoire and selection was based on the availability, accessibility and their wide use in food. These *Capsicum* fruits varieties were identified by ANADER (National Agency for Rural Development) and confirmed by the national floristic center of the University of Cocody, Abidjan, Côte d'Ivoire. 'Antillais', 'Jaune' and 'Doux' selected were fresh, ripened and firm. 'Soudanais' and 'Attie' selected were dried because they are used in this form. Fruits were carefully examined and defective fruits were discarded.

## Tests with microorganisms

Seven species of bacteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS 2003) as important causative agents of infection were used for testing: three Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* (clinical isolate) and *Bacillus subtilis* ATCC 6633) and four Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 28753, *Vibrio cholerae* (clinical isolate) and *Salmonella typhimurium* ATCC 13311). The clinical isolates were obtained from the National Public Health Laboratory (strains were confirmed by the Paris Pasteur Institute), Abidjan, Côte d'Ivoire.

#### METHODS

#### Direct plant extracts preparation

This method was adapted from Taylor *et al.* (1995), Eloff (1998) and Angeh (2006). Healthy fruits were spread out and oven dried for 72 h at 55°C and stored at room temperature. Dried fruits were finely ground to a fine powder (1 mm diameter) using a Wiley mill. 500 mg of finely ground fruit from each pepper variety was soaked in 5 mL of technical grade acetone (Merck, KGaA, Darmstadt, Germany) in a centrifuge tube and shaken for 5 min. The mixture was centrifuged at  $3000 \times g$  for 5 min. The supernatant was decanted, followed by another 5 min extraction process with 5 mL of fresh acetone and centrifuged for 5 min. This extraction process was repeated using fresh solvent for a third time. Following the final extraction, the acetone supernatant was transferred into a vial and allowed to evaporate until completely dried at 50°C. The dry matter obtained with this process was measured and expressed as a percent (w/w).

#### Serial exhaustive fruit extracts preparation

The assay was used according to the Taylor et al. (1995) and Angeh (2006) methods. 5 g of finely ground fruit was initially extracted with petroleum ether. The material was mixed with 50 mL petroleum ether (VWR Prolabo, Normapur, CE-EMB 45053) for 1 h while shaking. Following this solvent extraction, the supernatant was decanted. This process was repeated six times (from A = 1 h to F = 6 h, at 1-h intervals) with the same residue but using fresh petroleum ether. Then, the residue was allowed to dry at room temperature and the process of extraction was repeated six times each with the following solvents: dichloromethane (Merck), acetone and methanol (Merck). Each extract was filtered through Whatman filter paper No. 2. The filtrate was transferred into vials and evaporated in an oven at 50°C until completely dry. The matter extracted with each solvent was weighed. Dry matter obtained with each solvent was measured and expressed as percent dry matter.

## Antibacterial activity

Antibacterial activity was assessed by utilizing the well dif-fusion bioassay procedure as reported by Hugo and Russell (1983), Vlietinck et al. (1995) and Ncube et al. (2008). Dried mass was obtained using the direct extraction method with acetone. Pure cultures of the microorganisms were inoculated into Muller-Hinton nutrient broth (BIO-RAD, France) incubated overnight at 37°C, diluted with sterile nutrient broth to a cell density of 10° cfu/mL, equivalent to McFarland test tube number 3. The suspension was used to streak for confluent growth on the surface of Muller-Hinton agar (20 mL) plates with a sterile swab. Using a sterile cork-borer 5 mm in diameter, six wells were made into agar containing the bacterial culture. A concentration of 50 mg/mL from each pepper variety was prepared by dissolving the dry mass in sterile distilled water. The suspensions were aliquoted into the wells. Standard antibiotic ampicillin (BIO-RAD) at 10 µg/mL was used as the reference or positive control. For pre-diffusion, the agar plates were allowed to stand for 3 h at 5°C to allow proper diffusion of extract into the agar then were incubated at 37°C for 18 h. Antibacterial activity was recorded if the zone of inhibition was > 10 mm. The test was repeated twice again to insure reliability of the results.

#### Phytochemical screening

Chemical component identification was done using TLC (thin layer chromatography) according to the methods described by Nostro et al. (2000) and Abulude (2007). The extracts obtained from the serial exhaustive extraction were used for phytochemical screening. Qualitative chemical analysis of different Capsicum extracts was carried out to determine the presence of alkaloids, flavonoids, saponoside, quinines, tannins, steroids, phenol and terpenes. These compounds were determined using aluminium-backed TLC plates (Carlo Ebra reactifs-SDS, a cellulose plate for flavonoids, polyphenols, tannins and a silica plate for sterols, terpenes, quinones, alkaloids and sapronosids). In each case, 50 µg was chromatographed. The following three solvent systems were used to develop the plates: Acetic acid (VWR Prolabo, Normapur, CE-EMB 45053)/water (90: 10) (System I) used for flavonoids, tannins and phenols; Chloroform (VWR Prolabo, Normapur, CE-EMB 45053) /methanol (VWR Prolabo, Normapur, CE-EMB 45053) (98: 2) (System II) used for quinines, steroids and terpenes; Chloroform/ methanol/water (65: 25: 10) (System III) used for alkaloids. The components were visualized under visible light (254 and 366 nm) and sprayed with the following reagents in order to reveal spots of different groups: sulphuric alcohol (Panreac Quimica, PA, E-08211, Barcelona, Spain) used for sterols and terpenes, a solution of Dragendorff's reagent for alkaloids, alcoholic potash with 5% used for quinines, ferric chloride (Panreac Quimica) with 3% used for tannins, Godin's reagent and sulphuric acid (Panreac Quimica) used for saponoside.

#### Statistical analysis

All values were expressed as the mean of three measurements for each treatment. Data collected were subjected to one-way analysis of variance (ANOVA). With respect to the parameters investigated during assessment of antibacterial activity (dry mass, percentage of extraction and zone of inhibition), mean values were compared for the different quantities and percentages of dry matter extracted with each solvent. Regarding antibacterial experiments, mean values of inhibition diameters on bacteria tested were compared for the extracts from different *Capsicum* varieties. In order to determine which means for dry mass and percentage of extraction were significantly different from others, differences between means were assessed by Duncan's multiple range test at  $\alpha = 0.05$  (Musyimi *et al.* 2008).

#### **RESULTS AND DISCUSSION**

The quantity and percentage of material extracted from the five *Capsicum* varieties tested are shown in **Fig. 1**. The highest quantity of dry matter was obtained from *C. fru*-tescens varieties: 'Attie' > 'Soudanais' > 'Jaune' > 'Doux' >

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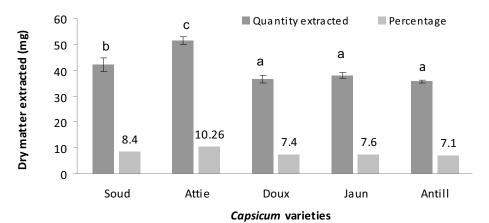
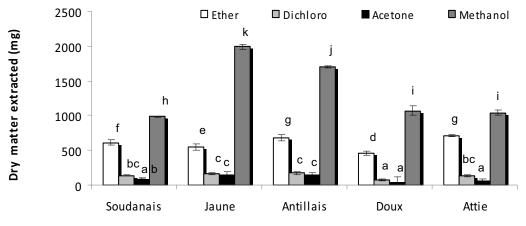


Fig. 1 Quantity (mg) and percentage of dry matter extracted from *Capsicum annuum* and *Capsicum frutescens* with direct process. Values with the same letter are not significantly different (P > 0.05).



Capsicum varieties

Fig. 2 Means values of dry matter in serial exhaustive extraction with four solvents. Values with the same letter are not significantly different (P > 0.05).

'Antillais'. The highest amount of extract was from 'Attie',  $51 \pm 3$  mg, which represents 10.26% dry matter and the lowest amount from 'Antillais' ( $35 \pm 0.5$  mg; 7.1% dry matter).

Results from the exhaustive extraction are summarised in **Fig. 2** and **Table 1**. From **Table 1** it appears that almost all the matter was extracted during the first 2 hrs. The highest dry mass and percentage of extraction were obtained with methanol and values ranged from  $1994 \pm 35$  mg (39.88%) in 'Jaune' to  $985 \pm 7$  mg (19.70%) in 'Soudanais'. The lowest dry mass (0.98 mg) was extracted with acetone.

There were significant differences between dry matter extracted from the three *C. frutescens* varieties but no significant differences were observed between the two *C. annuum* varieties.

Results of the antimicrobial activity of fruit extracts are

Table 1 Percentage and dried matter obtained per hour in the serial exhaustive extraction

Capsicum varieties	Extractants	Quantity of matter extracted (mg/500 mg)								
-		Α	В	С	D	Е	F	Total	%	
Capsicum. annuum var	Petroleum ether	291	201	99	62	17	7	667	13.34	
Antillais	Dichloromethane	101	45	18	8	0	0	172	3.44	
	Acetone	74	42	21	13	5	0	155	3.10	
	Methanol	877	431	184	106	77	24	1699	33.98	
Capsicum. annuum var	Petroleum ether	292	151	71	22	12	4	552	11.04	
Jaune	Dichloromethane	87	43	20	8	0	0	158	3.16	
	Acetone	74	42	23	11	4	0	154	3.08	
	Methanol	1013	466	275	122	84	34	1994	39.88	
Capsicum frutescens var	Petroleum ether	305	212	76	23	9	0	625	12.50	
Soudanais	Dichloromethane	74	41	15	8	0	0	138	2.72	
	Acetone	46	26	13	6	0	0	91	1.82	
	Methanol	473	294	113	65	29	11	985	19.70	
Capsicum frutescens var	Petroleum ether	358	222	94	28	10	0	712	14.24	
Attie	Dichloromethane	75	40	13	6	0	0	134	2.68	
	Acetone	31	16	9	0	0	0	56	1.12	
	Methanol	592	256	114	47	20	9	1038	20.76	
Capsicum frutescens var	Petroleum ether	227	125	72	25	10	0	459	9.18	
Doux	Dichloromethane	45	20	9	0	0	0	74	1.48	
	Acetone	28	13	8	0	0	0	49	0.98	
	Methanol	534	254	145	87	39	13	1072	21.44	

A, B, C, D, E and F: Extraction times (1h to 6h)

Capsicum varieties							
Bacteria tested	<i>C. annuum</i> var Antillais	<i>C. annuum</i> var Jaune	<i>C. frutescens</i> var Doux	<i>C. frutescens</i> var Soud	<i>C. frutescens</i> var Attie	Ampicillin	
Staphylococcus aureus	$17 \pm 00$	$15\pm01$	$12 \pm 00$	$10 \pm 01$	$12 \pm 01$	$24\pm02$	
Bacillus subtilis	$23 \pm 01$	$25\pm00$	$14 \pm 00$	$10 \pm 00$	$15 \pm 01$	$42\pm00$	
Bacillus cereus	$24 \pm 00$	$28 \pm 01$	$18 \pm 01$	$12 \pm 00$	$20 \pm 01$	$40\pm01$	
S. typhimu	$14\pm03$	$18 \pm 01$	$10 \pm 00$	$12 \pm 01$	$10 \pm 00$	$34\pm00$	
Escherichia coli	$12 \pm 00$	$12 \pm 01$	-	$10 \pm 00$	$10 \pm 01$	$24 \pm 01$	
Pseudomonas aeruginosa	$14 \pm 01$	$12 \pm 00$	-	$12 \pm 01$	$12 \pm 00$	$25\pm00$	
Vibrio cholerae	$14 \pm 00$	$20\pm01$	$11 \pm 00$	$10\pm00$	$12 \pm 01$	$40\pm01$	

- No antibacterial activity (diameter < 10 mm)

Table 3 Phytochemical composition of C. annum and	<i>C. frutescens</i> varieties from different solvent extracts.
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Capsicum varieties	Extractants				Phytochem				
-		Alkaloids	Sapronosids	Quinines	Tannins	Steroids	Terpenes	Flavonoids	Polyphenols
Capsicum annuum	P. Ether	+	-	+	-	+	+	+	+
var Antill	Dichlorom	+	-	+	-	+	+	+	+
	Acetone	+	-	+	-	+	+	+	+
	Methanol	+	-	+	+	+	+	+	+
Capsicum annuum	P. Ether	+	-	+	-	+	+	+	+
var Jaune	Dichlorom	+	-	+	-	+	+	+	+
	Acetone	+	-	+	-	+	+	+	+
	Methanol	+	-	+	+	+	+	+	+
Capsicum frutescens	P. Ether	+	-	+	-	+	+	+	+
var Souda	Dichlorom	+	-	+	-	+	+	+	+
	Acetone	+	-	+	-	+	+	+	+
	Methanol	+	-	+	+	+	+	+	+
Capsicum	P. Ether	+	-	+	-	+	+	+	+
frutescens var Attie	Dichlorom	+	-	+	-	+	+	+	+
	Acetone	+	-	+	-	+	+	+	+
	Methanol	+	-	+	+	+	+	+	+
Capsicum frutescens	P. Ether	+	-	+	-	+	+	+	+
var Doux	Dichlorom	+	-	+	-	+	+	+	+
	Acetone	+	-	+	-	+	+	+	+
	Methanol	+	-	+	+	+	+	+	+

- Absence of compound, + Presence of compound

shown in **Table 2**. The positive control (ampicilin) showed inhibition zones ranging from 24 to 42 mm against Gram<sup>+</sup> and Gram<sup>-</sup> bacteria. 'Antillais' and 'Jaune' fruit extracts showed higher positive activity against bacteria: inhibitory diameters were 10-28 mm for Gram<sup>+</sup> bacteria (*S. aureus, B. subtilis* and *B. cereus*) and 10-20 mm for Gram<sup>-</sup> bacteria (*S. typhimurium, E. coli, P. aeruginosa* and *V. cholerae*). No activity was found by 'Doux' extract against Gram<sup>-</sup> *E. coli* and *P. aeruginosa*, which were the most resistant strains to *Capsicum* extracts. The most important activity was found in *Capsicum annum* var 'Jaune' and against *Bacillus cereus* similar to results found by Ifra and Sheikh (2009) with black pepper.

Pepper fruit extracts are generally more active against Gram<sup>+</sup> than against Gram<sup>-</sup> bacteria (McCutcheon *et al.* 1992). In the present findings, direct acetone extracts showed more activity against Gram<sup>+</sup> than Gram<sup>-</sup> bacteria, supporting this view. The reason for different sensitivity between Gram<sup>+</sup> and Gram<sup>-</sup> bacteria could be ascribed to the morphological differences between these microorganisms. Gram<sup>-</sup> bacteria have an outer phospholipidic membrane carrying structural lipopolysaccharide components making the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara 1985).

*Capsicum* fruits could inhibit the activity of bacteria that can cause diarrhoea and dysentery. *E. coli* is known to cause urinary tract infections; *S. aureus*, *S. typhimurium*, *V. cholerae*, *B. cereus* are associated with diarrhoea and food infection (Pelczar *et al.* 2006; De Marino *et al.* 2008). All these pathogens were susceptible to fruit extract used in this work.

All tested bacteria had almost the same susceptibility to *Capsicum* fruit extracts, although 'Antillais' and 'Jaune' extracts were most effective against the pathogen bacteria

(Table 2), with activity comparable to the control antibiotic, ampicillin. They are the most common and widely used pepper fruits in Côte d'Ivoire. A similar report by Ifra and Sheikh (2009) about the antibacterial activities of black pepper exists in which the black pepper samples studied showed bactericidal activities; antibacterial activity of black pepper when dissolved in distilled water was better than that of black pepper dissolved in DMSO. Careaga et al. (2002) also showed antibacterial activity of Capsicum extract against Salmonella typhimurium and Pseudomonas aeruginosa inoculated in raw beef meat. However, these findings are not in accordance with those of Sema et al. (2007) on crushed red pepper. These authors found no antibacterial effect of crushed red pepper against test strains like Pseudomonas aeruginosa, E. coli and S. aureus. The difference may be due to different climates in which the plants are grown, the varying methods of extraction and Capsicum varieties.

Our finding thus supports the use of *C. annuum* and *C. frutescens* in traditional remedies in the treatment of diseases caused by microorganisms or as common household spices.

Results of the phytochemical assays on extracts from different solvents used in serially exhaustive methods are indicated in **Table 3**. Chemical tests showed the presence of flavonoids, terpenes, sterols, phenols and alkaloids. Only the methanol extract revealed the presence of tannins. Saponosides were not found in the extracts of all *Capsicum* varieties tested. Phytochemicals exert antimicrobial activity through different mechanisms; tannins act by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert 1991). Fruits that have tannins as a component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda 2003) thus exhibiting antimicrobial activity. The presence of tannins in *Capsicum* peppers support the traditional medicinal use of these fruits in the treatment of different illnesses. Li *et al.* (2003) reviewed the biological activities of tannins and observed that they have remarkable activity in cancer prevention and as anticancer agents, thus suggesting that *Capsicum* fruits are a potential source of important bioactive molecules for treatment and prevention of cancer. Tannins have stable and potent antioxidants roles.

Alkaloids, another group of phytochemical compounds observed in the stem bark, were found in the extracts of all *Capsicum* peppers. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori *et al.* 1994). In addition, alkaloids possess anti-inflammatory, anti-asthmatic, and anaphylactic properties by altering the immunological status *in vivo* (Gopalakrishnan *et al.* 1979; Ganguly and Sainis 2001; Staerk *et al.* 2002). Furthermore, alkaloids are used in powerful pain killer medications (Raffauf 1996).

Flavonoids are also constituents of stem bark extracts of both *C. annuum* and *C. frutescens* varieties; these exhibit a wide range of biological activities (antimicrobial, anti-in-flammatory, anti-angionic, analgesic, anti-allergic effects, cytostatic and antioxidant) (Hodek *et al.* 2002). Flavonoids promote health by preventing diseases associated with oxidative damage of membranes, protein and DNA (Ferguson 2001). Flavonoids in the human diet reduce the risk of various cancers and prevent menopausal symptoms (Hodek *et al.* 2002).

All these facts support the usefulness of *Capsicum* fruits in folklore remedies and are why these fruits are widely used for the treatment of many diseases among many tribes in Africa. In addition to the antimicrobial activities exhibited by flavonoids, they exhibit antitrypanosomal and antileishmanial activities (Tasdemir *et al.* 2006; De Marino *et al.* 2008).

Epidermiological studies suggest that the consumption of flavonoids is effective in lowering the risk of coronary heart disease. Furthermore, several flavonoids exhibit antiviral activities (Xu *et al.* 2000). The presence of sterol, polyphenols, terpenes and quinones has earlier been reported with antimicrobial activity (Hassan *et al.* 2006). Quinones are popular for their antiamoebal activity against the malaria parasite (Iwu 1999). Polyphenols are another group that has exhibited antimicrobial activity (Ncube *et al.* 2008). The terpenes and sterols are one of the largest and most diverse group of secondary metabolites. These studies on phytochemical compounds support our findings on the usefulness of *Capsicum* peppers in traditional remedies.

## CONCLUSION

Extracts tested *in vitro* against bacterial pathogens showed some antibacterial activities. Phytochemical screening revealed the presence of compounds such as tannins, alkaloids and Flavonoids known to be biologically active. The results of this study validate the use of *Capsicum* as a food ingredient and as a therapeutic element of traditional medicine. We conclude that the extracts of all five varieties are promising candidates as sources of new therapeutic compounds, applied as a natural preservative in the cosmetic and food industries or as an accessible and safe alternative to synthetic antimicrobial drugs.

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