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RESEARCH ARTICLE

PROFILES OF BIOACTIVE COMPOUNDS OF SOME PEPPER FRUIT (*CAPSICUM L.*) VARIETIES GROWN IN CÔTE D'IVOIRE

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Abstract
Besides the use of pepper as a food ingredient, many developing countries use it in indigenous medicine. The aim of this work was to isolate bioactive compounds from some varieties of pepper (*Capsicum*) grown and consumed in Côte d'Ivoire, and to assess the ability of organic solvent to extract these compounds. Crude acetone extract contained 35 mg *C. annuum* antillais and 53 mg *C. frutescens* attie material in 0.5 g of pepper powder. The dry matter obtained from the petroleum ether extract (lipid compounds) ranges from 459 mg (*C. frutescens* doux) to 712 mg (*C. frutescens* attie). That from the methanol extract varies from 985 mg to 1994 mg versus 74 mg to 172 mg for the dichloromethane, and 49 mg to 155 mg for the acetone in the successive extraction. Polar, non polar and acid compounds of intermediate polarity range from 33 to 34 compounds for *C. annuum* and from 27 to 33 for *C. frutescens*. The number of lipid compounds isolated from *C. annuum* varies from 32 to 33 and 24 to 29 in *C. frutescens*. The total non-lipid compounds ranges from 88 to 103 for *C. annuum*, and 84 to 96 for *C. frutescens*. The number of lipid compounds was higher for *C. annuum* than for *C. frutescens*. Methanol has the best extraction profile. The number of compounds isolated was highly variable depending on the variety of *Capsicum*. Identification and characterization of the compounds isolated are needed for their used in the food and pharmaceutical industry and also in medicinal therapy.

Keywords: *Capsicum* varieties, bioactive compounds, degree of polarity, extract, profile

Introduction

The importance of fruit and vegetables in human diet is very widely recognized especially in tropical countries (Gockowski *et al.*, 2003; Ekué *et al.*, 2010). Bell peppers (*Capsicum L.*) are the most important vegetable spice grown in the tropical and sub-tropical regions of the world because of their color, taste, pungency, flavor and aroma (Nwokem *et al.*, 2010; Mueller *et al.*, 2010; Kouassi and Koffi-Nevry, 2012). They are used as an exhauster of peppery taste and the *capsicum* fruit are known for their flavor and burning sensation in the mouth. They are used as food flavouring or food seasoning (Batchelor and Bradley, 2000; Snitker *et al.*, 2009; Kouassi and Koffi-Nevry, 2012).

The cultivation of *Capsicum* (*Solanaceae*) originated in Central and South America, with the *Capsicum* annuum, *C. frutescens*, *C. baccatum*, *C. pubescens* and *C. Chinense species* (Menichini *et al.*, 2009; Zimmer *et al.*, 2012). Pepper (*Capsicum*) is a tropical and an important agricultural crop and one of the popular vegetables, not only because of its economic value, but also for the combination of color, taste and nutritional values of its fruit (Barham *et al.*, 2010; Kouassi and Koffi-Nevry, 2012).

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Fruit and vegetables are important sources of bioactive compounds (such as phenolic compounds, terpenoids, steroids and alkaloids) known for their health-promoting effect against degenerative diseases (Meghvansi *et al.*, 2010; Mueller *et al.*, 2010; Zimmer *et al.*, 2012). The pepper is also used in traditional medicine to treat some diseases. The presence of bioactive compounds in *Capsicum* peppers supports the traditional medicinal use of these fruits for the treatment of different illnesses. They are used to treat intestinal disorders such as diarrhea and dysentery and have an antimicrobial activity. The challenge of food security, the constant increase of microbial infections, and diseases related to malnutrition led scientists to investigate new bioactive molecules (Al Bay *et al.*, 2008; Kappel *et al.*, 2008; Martin *et al.*, 2011). These bioactive compounds are the active principles found in plants and have many pharmaceutical and therapeutic applications. These compounds are vitamins and other secondary metabolites such as phenolic compounds, terpenoids, steroids and alkaloids (Eloff, 2004; Epifano *et al.*, 2007; Zimmer *et al.*, 2012).

It is therefore necessary to investigate the phytochemical profile (polar compounds, non-polar compounds, acidic compounds of intermediate polarity) of organic extracts isolated from *Capsicum* fruit. The aim of this study is to identify the groups of bioactive compounds responsible for the therapeutic properties of the C*apsicum* varieties grown and most used in Cote-d'Ivoire according to their degree of polarity and also to determine the ability of various organic solvents to extract those bioactive compounds.

Materials and methods

Collection of plant materials

The study was carried out on five varieties of fresh commercial pepper samples, *Capsicum annuum* var. antillais, *Capsicum annuum* var. jaune, *Capsicum frutescens* var. doux, *Capsicum frutescens* var. soudanais and *Capsicum frutescens* var. attié at maturity stage of full size fruits. These varieties were obtained from four local wholesale markets (Abobo, Adjamé, Treichville and Koumassi) in Abidjan, Côte d'Ivoire. These fruit were surveyed and selected to compile a representative list of these Bell pepper fruit mostly used by traditional healers of Côte d'Ivoire for their availability, accessibility and wide utilization in food. Two *Capsicum* fruit varieties were identified by the *ANADER* (The National Agency for Rural Development) and confirmed by the national floristic center of the University of Cocody, Abidjan, Côte d'Ivoire. *Capsicum annuum* selected were fresh, ripened and firm. *Capsicum frutescens* selected were dried because they are used in this state.

Preparation of organic extracts of pepper

The method is adapted from Angeh (2006). Fresh, unblemished fruit were washed then oven dried separately for 5 days for the less fleshy fruit such as *Capsicum frutescens*, and 8 to 10 days for the fleshy fruit such as *Capsicum annuum*. The dried fruits were crushed in an electric blender (Warring Commercial 8010E Model 38BL40, France) at 3000 rpm/min. The mixture obtained was studied using a sieve of about 1-2 mm mesh.

Crude extract with acetone

The direct extraction with acetone (Merck, Germany) was done according to the method described by Eloff (2004), Angeh (2006) and also Kouassi *et al.* (2010). The dry matter obtained was measured in mg and repeated 3 times.

Successive fruit extracts preparation

The assay was used according to the Eloff (2004) and Angeh (2006) methods. Five grams of powder for each variety of *Capsicum* was submitted to successive extractions using solvents of increasing polarity such as petroleum ether (VWR Prolabo, Normapur, CE-EMB 45053), dichloromethane, acetone (Merck, Germany) and methanol (VWR Prolabo, Normapur, CE-EMB 45053) until complete exhaustion. For the same solvent, the process of extraction was repeated six times with the same residue but using a fresh solvent each time. The organic solvents were evaporated under reduced pressure to dryness to obtain the respective residues from the fruits named CPE (crude petroleum ether extract), I (dichloromethane extract), ACT (acetone extract) and MET (methanol extract). The extracted mass was expressed in mg, and done in triplicate.

Phytochemical screening

The chemical components identification was done using TLC (thin layer chromatography) according to the methods described by Lagnina (2005) and Mamyrbékova-Békro *et al.* (2008). The extracts obtained from the successive extractions were used for phytochemical screening. Different systems (solutions) resulting from several solvents for different classes of compounds determination were used according to the methods described by Eloff *et al.* (2005), Angeh (2006) and Suleimana *et al.* (2010). The system I, a mixture of acetic acid (VWR Prolabo, Normapur, CE-EMB 45053) and water (90:10) was used to isolate the polar compounds (flavonoids, tannins and phenols). The system II, composed of chloroform (VWR Prolabo, Normapur, CE-EMB 45053) and methanol (VWR Prolabo, Normapur, CE-EMB 45053) (98:2) was used for the acid compounds of intermediate polarity (quinines, steroids and terpenes) and the system III, which is a mixture of chloroform, methanol and water (65:25:10), for the non polar compounds (alkaloids).

The components were visualized under ultraviolet light (254 and 366 nm) (Chromato-view Cabinet Model CC-20) in order to reveal fluorescent spots of different groups. The TLC plates (Carlo Ebra reactifs-SDS) were then sprayed with the sulphuric vanilla developer which revealed compounds not detected under ultraviolet light. The plates were then oven dried at 80°C for 5 min and a new reading was done in the visible light. The total of all the spots obtained according to the different readings was counted and correlated to the number of bioactive compounds extracted by each solvent. These compounds were then classified into polar compounds, non polar compounds and acidic compounds of intermediate polarity.

Statistical analysis

All the values were expressed as the mean of three measurements for each treatment. The data collected were subjected to one way analysis of variance (ANOVA). The mean values were compared for the different quantities and percentages of dry matter extracted with each solvent. In order to determine which means for dry mass and percentage of extraction were significantly different from which others, means were calculated using the test of Duncan at $\alpha = 0.05$ (Musyimi *et al.* 2008).

Results and discussion

Extraction of the dry matter by the organic solvents

The amount of dry material extracted from the five *Capsicum* varieties tested with direct and successive extraction is shown in figure 1.

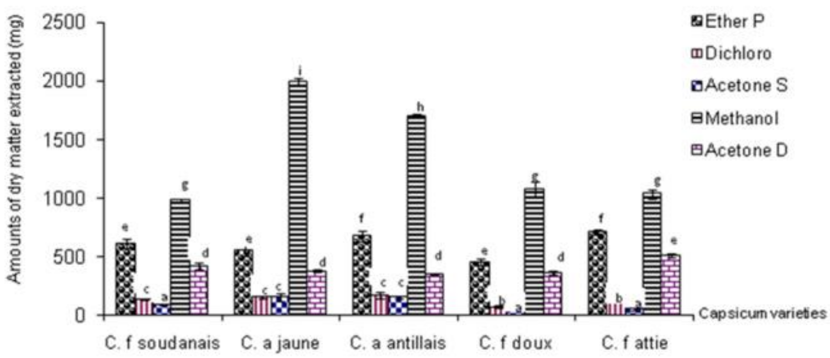


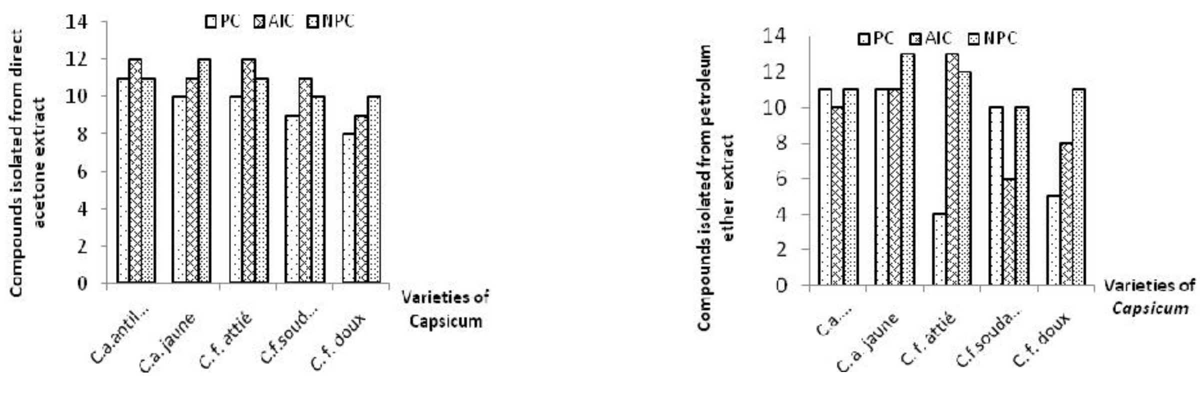
Figure 1. *Amount of material obtained with the direct and successive extraction from the different varieties of Capsicum studied. (Values with the same letters are not significantly different (p> 0.05))*

The values obtained using only acetone (direct extraction) ranged from 360 mg for *C. annuum* antillais to 530 mg for *C. frutescens* attie (from 7.1 to 10.26% respectively). There were significant differences (P<0.05) among the dry matter extracted from the three *C. frutescens* varieties but none (P>0.05) between the two *C. annuum* varieties. The amounts of dry matter obtained by petroleum ether during the successive extraction methods ranged from 459 mg for *C. frutescens* doux to 712 mg for *C. frutescens* attie. There are significant differences (P<0. 05) between the two varieties of *C. annuum* and also among the 3 *C. frutescens* varieties. The use of petroleum ether led to the defatting of the extract. The amount of extractible dry matter with dichloromethane (74 mg to 172 mg) and acetone (49 mg to 155 mg) is lower than the one with methanol (985 mg to1994 mg). The materials extracted with methanol are those that were not solubilised in the previous organic solvents (petroleum ether, dichloromethane and acetone).

The highest amount of extract was obtained with methanol from *C. annuum* jaune, 1994 mg, and the lowest with acetone from *C. frutescens* doux, 49 mg. It turns out that no significant differences (P>0.05) were observed between the amount of the dry matter obtained from the dichloromethane and the acetone regarding the varieties of *Capsicum* studied. The quantities of material extracted by the methanol are not significantly different (P>0.05) among the varieties of *C. frutescens* (Figure 1). The quantities of material extracted by the methanol are not significantly different (P>0. 05) among the varieties of *C. frutescens*. However, there are significant differences (P<0.05) between the two varieties of *C. annuum*. According to the work of Eloff *et al*. (2008), petroleum ether has a better extractible ability on plant materials. The large amount of materials dissolved by the petroleum ether in this work shows that *Capsicum* fruits grown in Côte d'Ivoire are rich in lipid substances. This result goes along with that of Yang *et al*. (2011) who showed that pepper varieties contain large amounts of lipid compounds.

Phytochemical profile

The phytochemical profiles of the *Capsicum* extracts, divided into polar, non polar and acidic compounds of intermediate polarity vary from one species of *Capsicum* studied to the other and within the varieties of the same species (figure 2a).



(a) **(b)**

Figure 2*. Phytochemical profile of Capsicum varieties extracts by crude acetone (**a**) and petroleum ether (**b**)*

Capsicum annuum antillais has the same number (11) of polar and non polar compounds. *C. annuum* jaune and *C. frutescens* doux have more non polar compounds (12 and 10 respectively) than the other varieties while, the acidic compounds of intermediate polarity (11and 12) are predominant in the attie variety. The number of extractible compounds by petroleum ether (lipid compound) ranges from 32 to 35 for C. *annuum* antillais and *C. annuum* jaune respectively; this number was 29, 24 and 24 for *C. frutescens* attie, soudanais and doux respectively (figure 2b). The difference between the profile of *C. annuum* antillais and *C. frutescens* soudanais was in the number of acidic compounds of intermediate polarity which was 10 and 6 respectively.

The screening of the dry materials obtained from the crude acetone and petroleum ether extracts showed less variability of the compounds obtained among the varieties of *Capsicum*. This consistency among the compounds isolated could be explained by the extraction method. However, the number of substances dissolved in petroleum ether revealed a difference in the composition of the lipid compounds of the *Capsicum* varieties. *Capsicum annuum* antillais contains 11 polar compounds which mean at least 11 lipid compounds that could be 11 different free fatty acids. According to Tani *et al*. (2004), the non polar and polar lipid compounds derived from extracts of plant materials were generally neutral lipid compounds, which could be identified as unsaturated fatty acids or fatty substances (phospholipids, mono and triglycerides).

Table 1. *Number of bioactive compounds isolated from defatted Capsicum extract*

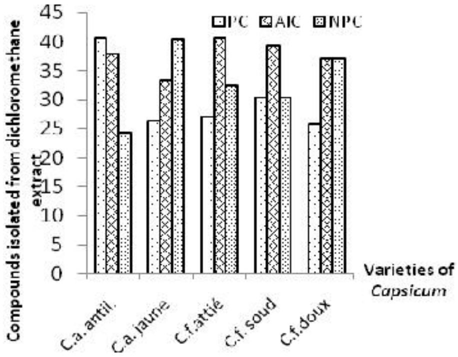
	Bioactive compounds			
	DCM	**ACT**	**MET**	**Total compounds**
C. annuum antillais	38	26	24	88
C. annuum jaune	42	35	26	103
C. frutescens attié	37	30	29	96
C. frutescens soudanais	33	28	34	95
C. frutescens doux	35	23	26	84

DCM :dichloromethane extract, ACT: acetone extract; MET: methanol extract

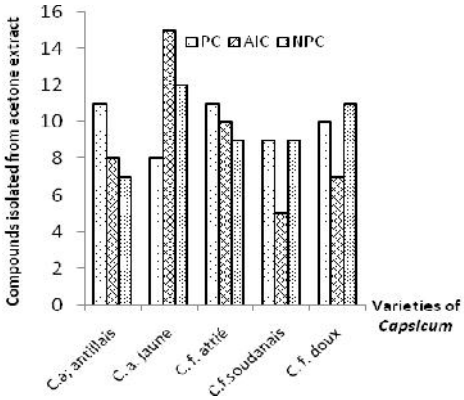
The total number of non-lipid compounds, obtained successively with dichloromethane, acetone and methanol is given in table 1. These compounds were 88 and 103 for *C. annuum* antillais and jaune respectively, and vary between 84 and 96 for *C. frutescens* varieties. The phytochemical profile of dichloromethane extracts vary among the varieties of the same species. The screening of the pepper dry materials in extracts from the successive extractions after defatting with the petroleum ether has shown a large variability of the number of compounds among the species and varieties of *Capsicum*. Shahverdi *et al.* (2007) and Lytovchenko *et al.* (2009) showed that depending on the method used, many different compounds can be isolated by thin-layer chromatographic (TLC) study. Faizi *et al.* (2003) and also Mamyrbekoa-Békro *et al.* (2008) stated that compounds isolated by TLC are numerous. According to the work of Samyand and Gopalakrishnakone (2010), these compounds can be proteins, glycosylated compounds and other bioactive compounds.

It can be seen in figure 3a that *C. annuum* antillais is characterized by a high number of polar compounds (15 compounds), *C. annuum* jaune by the non polar compounds (17) while the acidic compounds of intermediate polarity predominate in *C. frutescens* attie and soudanais (15 and 13). *Capsicum frutescens* doux contains as many non polar compounds (13) as acidic compounds of intermediate polarity. In general, for all the varieties of *Capsicum* studied, the dry materials extracted with petroleum ether and methanol has revealed the highest number of compounds. Indeed, petroleum ether being the most non polar solvent, has the ability to absorb more non polar compounds and methanol, the most polar and the last solvent used in the successive extractions, dissolves more polar compounds.

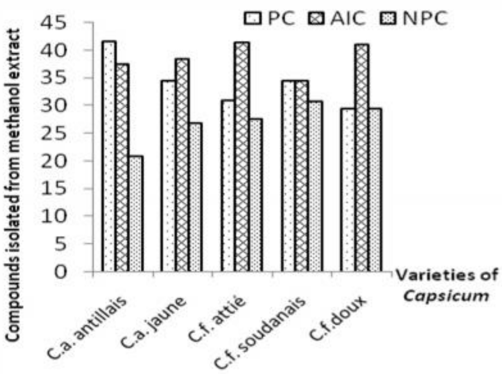
The results of this work confirm those of Lagnika (2005) and Mamyrbekoa-Békro *et al*. (2008) on the affinity of solvent absorption. The variation of phytochemical profiles among *Capsicum* species and also among the varieties of the same species using different organic extracts was noted in similar studies (Angeh, 2006; Lytovchenko *et al*., 2009). In *C. frutescens* attie, the polar compounds (free fatty acids) were poorly diversified (4 different fatty acids), but the neutral lipid substances (triglycerides) and the acidic substances of intermediate polarity (phospholipids) were numerous. The results showed that the qualitative composition of the lipids varies considerably among the varieties of the capsicum species, and therefore the bioactive properties provided by the lipid compounds may vary in quantity and quality depending on the varieties of *Capsicum*. The number of non-lipid compounds varies also according to the *Capsicum* varieties. Ming-Chih *et al*. (2011) indicated that the variability of the quantitative and qualitative compounds isolated from the plant varieties in a thin-layer chromatographic study is related to either, the composition or the season when the plant materials were harvested, or on the part of the plant material used for the analysis. After defatting, the successive extractions using solvents of increasing polarity showed that the amount of dry material extracted was high (20.76 to 39.88%) (from 138 to 1994 mg of dry matter in 5000 mg of powder of *Capsicum*) for *Capsicum frutescens* attie and *Capsicum annuum* jaune respectively. These results showed that the different varieties of *Capsicum* studied were also rich in soluble polar substances.



(a)



(b)



(c)

Figure 3*. Phytochemical profile of Capsicum varieties extracts by dichloromethane (**a**), acetone (**b**) and methanol (**c**)*

The compounds that were not soluble in dichloromethane but soluble in acetone and methanol gave the characteristic profiles of each variety of *Capsicum*. The profiles of acetone extracts are different for the two species of *Capsicum* studied and also for the varieties of the same species. *Capsicum annuum* jaune is characterized by a high number of acidic compounds of intermediate polarity (15 compounds); polar compounds (11compounds) predominate in antillais and attie varieties (figure 3b), while non polar compounds predominate in *C. frutescens* doux (11 compounds). On the other hand, there were as many polar compounds as non polar compounds (9 compounds) in *C. frutescens* soudanais. According to Eloff (2004) and Angeh (2006), the methanol has the ability to extract polar compounds soluble in water. In this study, the best profile of extraction was obtained with methanol; this result could be explained by the fact that the varieties of Capsicum are rich in soluble polar substances. Our results are in accordance with those of Suleimana *et al*. (2010) who indicated that methanol is very well known as having excellent extraction ability for many plant materials. The quantities of soluble intermediates in solvents such as acetone and dichloromethane are negligible. These quantities vary from 1.48 to 3.44% (from 74 to 172 mg of dry matter in 5000 mg of powder of *Capsicum*) for dichloromethane extract and from 0.98 to 3.10% (from 49 to 155 mg of dry matter in 5000 mg of powder of *Capsicum*) for acetone extract.

The profiles of the methanol extracts profile are also different for the two species of *Capsicum* studied and for the varieties of the same species. There are as many polar compounds as acidic compounds of intermediate polarity (10) in *C. annuum* antillais and *C. annuum* jaune (Figure 3c). But the acidic compounds of intermediate polarity predominate in *Capsicum annuum* jaune. However, for *Capsicum frutescens*, the acidic compounds of intermediate polarity dominate in the attie and doux varieties (12 and 14 compounds respectively). *Capsicum frutescens* soudanais contains as many polar compounds as acidic compounds of intermediate polarity (10).

The differences observed in the non polar, polar and acidic compounds of intermediate polarity could be explained in part by the successive exhaustive extraction methods used. All the different types of *Capsicum* extracts analyzed were rich in phytochemicals with high bioactive potential. These compounds detected could be some secondary metabolites such as alkaloids, flavonoids, saponins, phenolic, tannins, phenolic compounds also known for various other biological properties (N'guessan *et al*., 2009; Zimmer *et al*., 2012). Previous studies on these compounds have revealed some important pharmacological and medicinal properties such as antimicrobial, antioxidant, anti carcinogenic, analgesic properties (Evans and Johnson, 2010; Kouassi *et al*., 2010; Samyand and Gopalakrishnakone, 2010; Koffi-Nevry *et al*., 2012).

Conclusion

Methanol has the best ability to extract non-lipid compounds. The number of bioactive compounds obtained in this study show that *C. annuum* and *C. frutescens* are a great source of potential bioactive compounds. From this study, it seems important to identify and characterize the compounds isolated for their use in the food, pharmaceutical, medicinal and therapeutic industries. We conclude that the extracts of all the *Capsicum* varieties studied 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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