Full Length Research Paper

Phytochemical analysis of *Ziziphus mucronata* Willd. extract and screening for antifungal activity against peanut pathogens

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Chemical analysis of aqueous extract of *Ziziphus mucronata* Willd. was determined by liquid chromatography–mass spectrometry (LC-MS) analysis. Among the 11 compounds found, catechin, rutin (quercetin 3-O-rutinoside), delphinidin-3-glucoside, isoquercetin (hyperoside) and quercitrin (quercetine3,7-O-L-dirhanmopyranoside) were identified as the major phenolics components in this aqueous plant extract. To elute the target compounds, the fractionation of crude extract was carried out on solid phase extraction (SPE) columns. The different fractions (from FZ1 to FZ5) obtained after fractionation were evaluated *in vitro* against economically important foliar fungal pathogens of peanut, including *Cercospora arachidicola*, *Phaeoisariopsis personata* and *Puccinia arachidis.* The treatments with *Z. mucronata* fractions were compared with negative control (water) and standard solutions of cathechin and rutin (1 mg/mL). All the fractions recorded an inhibitory effect, firstly on conidial germination and germ tube elongation, secondly on disease evolution on peanut leaves previously inoculated by fungi; the level of efficiency of inhibition varied from 40.55 (FZ1 against *C. arachidicola*) to 57.14% (FZ2 and FZ3 against *P. arachidis*). Then, spores of *P. arachidis* seemed to be more sensitive to the treatment.

Key words: Fungal pathogens, peanut, plant extract, phenolics, *Ziziphus mucronata* Willd.

INTRODUCTION

Peanut (*Arachis hypogaea*) remains a high potential plant whose socio-economic importance is unquestionable throughout the West African sub-region. It is regarded as both food and cash crop, and the processing of products (butter, dough and cake) is an additional source of income for women.

In Burkina Faso, peanut is the second most important oily crops (DGESS, 2015). In 2014, a total production of 335.223 metric tonnes has been recorded throughout the country. However, its cultivation is facing climate changes and an intense parasitic pressure. Foliar diseases constitute a real obstacle to the growth of groundnut

(peanut). The most widespread are the early leaf spot (causal agent *Cercospora arachidicola*), late leaf spot (causal agent *Phaeoisariopsis personata*) and rust (causal agent *Puccinia arachidis*) (Subrahmanyam et al., 1995). Leaf spot are critical yield-limiting diseases of groundnut in West Africa accounting for yield reductions of 50 to 70%, where fungicides are not used (Shokes and Culbreat, 1997).

The need for applications (4 to 5 sprays) of recommended fungicides, such as chrorothalonil, mancozeb, and folicur, discouraged the extensive adoption of peanut by resource-poor farmers of the rain-fed production system (Krishna and Pande, 2005). Thus, the chemical methods are expensive and can affect the beneficial microbial population present in the ecosystem (Kagale et al., 2004). It is therefore imperative to develop and implement research programs in order to achieve better profitability in the sector to make it more attractive.

A major challenge facing crop production is to provide tools for controlling field diseases that would also be able to maintain higher quality crop production (Sarpeleh et al., 2009). Disease management by chemical treatment has shown its limits by the development of resistances in many important pathogens and environmental pollution. The use of plant extracts to control plant pathogens has been suggested as one of the sure alternatives to substitute synthetic chemical products. Natural plant products are an important source of new agrochemicals for the control of plant diseases (Nebie et al., 2002).

Pesticides of plant origin are non-phytotoxic, systemic and easily biodegradable. In the past few decades, many studies have focused on the antifungal activity of plant extracts on several phytopathogenic fungi. For example, the water extract of Neem leaf was found very effective against peanut diseases caused by the fungi *Puccinia arachidis* and *Mycosphaerella berkeleyi* (Ghewande, 1989). It was also successful in preventing fruit rottening in Cucurbitaceae caused by the fungus *Fusarrium equisitifolium* and *Fusarrium semitectum* (Krishna et al., 1986).

Zida et al. (2008) reported that, the growth of pathogenic fungi (*Fusarium moniliforme*, *Curvularia lunata*, *Colletotrichum graminicola*, *Exserotrilum rostratum*) of sorghum and millet seeds was inhibited by aqueous extracts of *Acacia gourmaensis* A. Chev. and *Eclipta alba* (L.) Hassk with inhibition rates of 27 to 72% and 56 to 86%, respectively. Linde et al. (2010) showed the efficiency of *L. rehmannii* essential oil on *Rhizoctonia solani, Fusarium oxysporum* and *Penicillium digitatum* fungi on potato, maize and orange tree pathogens, respectively.

Shakil et al. (2012) conducted experiments in which aqueous extracts of the leaves of *Calotropis procera* were as effective as the fungicide Ridomil in the control of collar decay of groundnut. Concerning the Ziziphus genre, some works have reported the anti microbial activities of certain species. So, different extracts and fractions of the leaves, fruits and seeds of *Ziziphus spina-christi* L. grown in Egypt showed a moderate *in vitro* activity against the fungus *Trichphyton rubrum* (Shahat et al., 2001).

Some recent works have shown the inhibitory effect of *Ziziphus mucronata* Willd. water extracts on rust of groundnut (Koïta et al., 2012). However, no data exists in the nature of the biochemical compounds present in the extract and active against fungi. This calls for further research to identify compounds active in this extract. The objectives of the present study are to realize phytochemical analysis of a crude extract of *Ziziphus mucronata* and, after its fractionation to evaluate the antifungal activity of the crude extract and its different fractions against *Cercospora arachidicola*, *Phaeoisariopsis personata* and *Puccinia arachidis*.

MATERIALS AND METHODS

Plant materials

Z. mucronata, a fruit crop in the Rhamnaceae family native to Asia (Guinko and Assi. 1981), was collected in the Gampêla district (12°25'N, 12°22'E) in Burkina Faso (West Africa). A botanical certification of the species was obtained from the Plant Ecology and Biology Laboratory of University Ouaga I Pr Jospeh KI-ZERBO. The fruits were selected for uniformity of color, absence of mechanical damage and disease symptoms. Using a mortar, the fruits were lightly crushed in order to separate the pulp from the seed. The pulp obtained was ground to a fine powder using an electric grinder (cyclotech sample mill, tecator, Hoganas, Sweden). During the biological test, the leaves from the peanut TS32-1 susceptible variety to late leaf spot and rust, about 30 days after seedling (DAS), were used to carry out artificial contaminations. Fungal spores of *C. arachidicola*, *P. arachidis* and *P. personata* were gathered form the Gampêla district on leaves naturally infected in fields which have not received antifungal treatments.

Extraction procedure

The phytochemistry was realized at the University of Montpellier 2 (France). 100 g of powdered sample was dissolved in 1000 ml of purified water obtained from Milli-Q plus water purification system (Millipore, Bedford, MA, USA) in a 2000 ml Erlenmeyer flask. The mixture was then left at room temperature (15 to 17°C) for at least 3 h. Supernatants obtained by centrifugation at 15,000 rpm for 15 min (centrifuge Sorvall RC 26 Plus) were evaporated completely, under reduced pressure using a rotavapor (Rotavapor R3, BUCHI). The dried material obtained was dissolved in 100 ml of purified water in order to get aqueous extract (1 g/ml), then sterilized using a 0.22 μm filter (Sartorious AG, Göttingen, Germany) before the liquid chromatography-mass spectrometry (LC-MS analysis and fractionations.

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LC-MS analysis

LC-MS analysis was performed using a Waters/Micromass ZQ equipped with an Electro-Spray Ionization source (ESI) that operated in both positive and negative ionization modes. The compounds were detected between 200 and 600 nm on 4 nm step. The mobile phase of HPLC consisted of 0.1% (v/v) formic acid in water (eluent A) and of acetonitrile: 0.1% formic acid (1:1, v/v) (eluent B). The gradient program was as follows: 0 to 8 min, 99 to 90% A; 8 to 17 min, 90 to 83% A; 17 to 26 min, 90 to 83% A; 17 to 26 min, 83 to 73% A; 26 to 55 min, 73 to 0% A and 55 to 60 min, 0 to 95% A. The column (C18 type (XTerra MS (Waters), 2.1 mm × 100 mm, 3.5 µm particle size) was operated at a temperature of 26°C, the flow rate was 1 ml/min, and the injection volume was 5 µl. All eluents used including the acetonitrile, formic acid (Carlo-Erba trademark) or methanol (VWR HiPer Solv Chroma norm trademark) was of HPLC quality. Using LC-MS, the compounds were identified on the basis of their retention time, absorbance spectrum and mass fragmentation compared to data from literature and, when possible, data from chromatography to standard.

Fractionation of *Z. mucronata* crude extract

The fractionation of crude extract was carried out on solid phase extraction (SPE) columns (Bond Elut LR C C_{18} VARIAN, 100 × 4.6 mm) to elute the target compounds. The SPE column was initially washed with 5 ml of methanol and 5 ml of purified water successively, to remove any impurities. Dynamic adsorption and desorption experiments were first carried out to determine the loading volume and the elution conditions. Then, the crude extract (25 ml) was loaded on the column and an elution gradient with increased polarity was applied to the column by adding solvents composed of a water/acetonitrile mixture (100/0; 95/5; 90/10; 85/15; 10/90). Each fraction was collected separately and the process was repeated five times. All the similar fractions were gathered and called FZ1 (100% water), FZ2 (95/5 water/acetonitrile), FZ3 (90/10 water/acetonitrile), FZ4 (85/15 water/acetonitrile) and FZ5 (10/90 water/acetonitrile). Each fraction was concentrated to dryness in a rotavapor (Rotavapor R3, BUCHI) under reduced pressure and controlled temperature (30°C), and then lyophilized. One mg of dried extract was dissolved in 1 ml purified water, then sterilized using a 0.22 µm filter (Sartorious AG, Göttingen, Germany) before assessing for antifungal activity and LC-MS analysis. FZ6 and FZ7 fractions corresponding to the two standard solutions, respectively cathechin and rutin (Karlsruhe, Germany) with a purity ≥ 98.0% at concentration of 1 mg/ml in water were used as positive controls. The use of both compounds as positive controls is explained by numerous studies have confirmed their antifungal efficiency (Gomez-Vasquez et al., 2004; Boligon et al., 2012; Panda et al., 2016).

Spore suspension preparation and antifungal activity

Fungi were isolated from naturally contaminated peanut leaves in Gampêla district. After collection, the leaves were washed with distilled water to be rid of dead spores and incubated for 48 h at 22°C in order to allow sporulation (Subrahmanyam et al., 1982). The spores of each fungal strain were collected by scraping the surface of the leaves suspended in sterile water. The spore suspension was adjusted to a concentration of approximately 100 spore/ml using Malassez counting chamber. Fractions of *Z. mucronata* crude extract (FZ1, FZ2, FZ3, FZ4 and FZ5) were prepared at a concentration of 1 mg of dry extract in 1 ml distilled water and used in the tests. A control (FZ8) consisted in a treatment with sterile distilled water (2 ml). For the spore germination test, 2 ml of each solution were placed in a test tube into which 2 ml of the adjusted spores' suspension was added. This was then incubated in complete darkness at 22°C for 8 h for rust spores and 25°C for 24 h for leaf spot spores. The fungal appressorium was then measured for 100 spores in each treatment with a microscope (Zeiss Primo Star) at ×40 magnification. A spore was considered to have germinated when germ tube length was greater than the width of the spore. The efficiency rate (E) of each extract fraction has been calculated using the formula proposed by Greche and Hajjaji (2000):

$$E(\%) = 100 \times [MLC - MLE] \div MLC$$

Where MLC is the mean length of the germ tube of the spore with the negative control and MLE is the mean length of the germinal tube of the spore with the tested plant extract.

A foliar stain inhibition test was assessed on the contaminated leaves from a susceptible variety of peanut (TS32-1). The harvested leaves undamaged were placed by ten in glass Petri dishes (90 mm × 20 mm). The infection was carried out by spraying the spore suspension on the abaxial leaf surface. The Petri dishes were placed on a shelf in a culture room at a temperature of 21 ± 2°C with a 12 h photoperiod. From 5 days after inoculation, sprays of *Z. mucronata* fractions or standards (or water for control) were carried out. This treatment was repeated every five days, in all, four treatments were applied to these contaminated leaves. The inhibition of foliar stains of leaf spot was evaluated by using the 9 classes' severity scale of ICRISAT (Subrahmanyam et al., 1982). This operation was repeated five times and observations were made during the 30 days. The treated leaves were compared to the negative control (water) and standard solutions of cathechin and rutin (1 mg/ml).

Statistical analysis

Statistical differences between the treatments and the control were evaluated by ANOVA and students-Newman-Keuls post-hoc tests using XLSTAT, 2010 version 12.5 08. *P* values of < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis of crude extract of *Z. mucronata*

The HPLC profile obtained with crude aqueous extract of *Z. mucronata* fruit pulp and peaks corresponding to compounds 1 to 11 are shown in Figure 1. Most of the compounds were eluted within the first 40 min, and showed a maximum absorption peak at 330 nm.

In this aqueous extract, five compounds were clearly identified (a large polymer (1) and peaks 2, 3, 4, 6). Others peaks are indicated (peak 5, 7, 8, 9, 10 and 11) corresponding to unidentified compounds in the present study. However, during the first 15 min of separation, the presence of a large polymer prevented individualized peaks (1+). This compound exhibited an *m/z* of 289 in negative mode and two maximum UV absorption at 207 and 277nm. With fragments ions at *m/z* 203, 125 and 108, this compound shared characteristics of catechin and / or epicatechin, flavonids monomers of the condensed tannins. The presence of these flavonoids
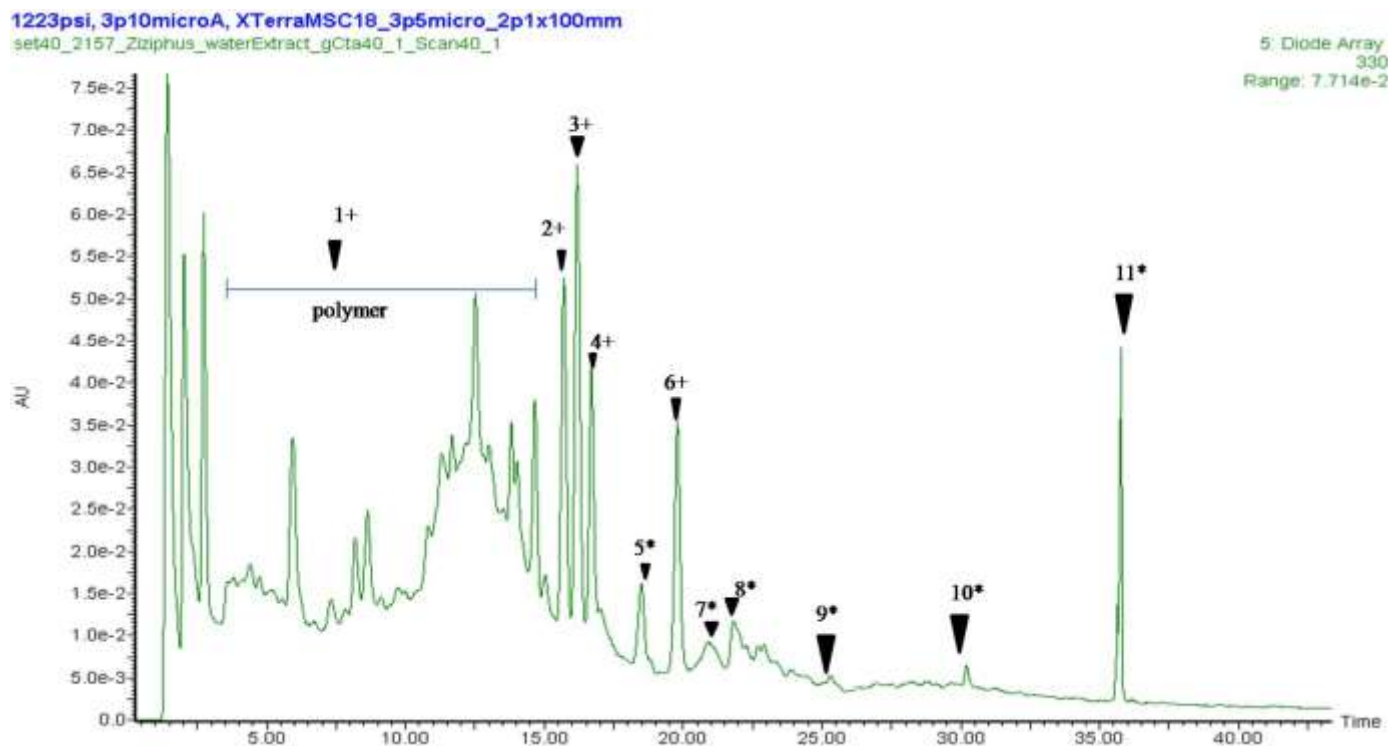
Figure 1. Chromatographic pattern of *Z. mucronata* aqueous extract. Wavelength: 330 nm, injection volume: 5 µl. Caption: The numbers followed by + correspond to identified compound peak and those followed by * are unidentified ones. 1+: catechin and / or epicatechin, 2+: rutin (quercetin 3-O-rutinoside), 3+: the delphinidin-3-glucoside, 4+: isoquercetin (hyperoside), 6+: quercitrin (quercetine3,7-O-L-dirhanmopyranoside), unidentified compounds: 5*, 7*, 8*, 9*, 10* and 11*.

has been reported previously in other species from Rhamnaceae family (Boligon et al., 2012) and particularly in the genus *Ziziphus*, for instance *Z. lotus* L. (Diallo et al., 2004; Borgi et al., 2007; Soumia, 2009). In their study, Berthod et al. (1999) stress the difficulty of precisely identifying those compounds whose polymer chains of different lengths are difficult to separate.

Compound 2 (peak 2+) was detected by UV absorption at 270 and 350 nm at retention time of 15.84 min. Its LC-MS spectrum showed fragments at *m/z* 609 in negative mode and other fragments were detected at *m/z* 301 and 300. This compound was identified as a glycosylated flavonoid namely rutin (quercetin 3-O-rutinoside) based on literature and the LC-MS profile. The presence of this compound in leaves and fruits of *Z. jujuba* and *Z. spina-christa* has been previously reported (Pawlowska et al., 2009; Guo et al., 2011).

Compound 3 corresponding to peak 3+, at a retention time of 16.38 min, included a parent ion *m/z* 464 and fragment ions *m/z* 300, 301, 255 and 299 at high fragmentation in negative mode. The UV absorbance band showed a maximum at 205 and 275 nm. This compound is the delphinidin-3-glucoside, an anthocyanidin, a primary plant pigment which has antioxidant and antimicrobial properties (Dixon et al., 2005; Panjehkeh et al., 2009). The presence of this

compound has been reported in the leaves and fruits of the species *Z. jujuba* and *Z. spina-christa* (Guo et al., 2011; Pawlowska et al., 2009).

Fragmentation in negative ion mode of compound 4 (peak 4+) presented parent ion *m/z* 463 and fragments ion *m/z* 301 and 300. It showed UV absorption band at 205 and 270 nm at retention time of 16.86 min. All these characteristics corresponded to isoquercetin, also known as hyperoside or quercetin 3-O-glucoside. This compound is a flavonoid whose presence was reported in the leaves and fruits of *Z. spina-christi* (Shahat et al., 2001) and *Z. jujuba* (Pawlowska et al., 2009).

The fifth and last compound identified corresponds to peak 6+. It was recorded at the retention time of 19.88 min. Its absorbance spectrum indicates two absorption peaks at 265 and 345 nm. The mass spectrum of this compound produced by electrospray negative ES- has a parent ion of m/z = 447. The higher energy ionization gives, in addition to the parent ion, fragments ions of m/z = 302, 301 and 300. All these characteristics correspond to quercetin 3,7-O-L-dirhanmopyranoside, one of the synonyms of which is quercitrin. This compound is a flavonoid whose presence was reported by Pawlowska et al. (2009) in the leaves and fruits of *Z. jujuba* and *Z. spina-christi*.

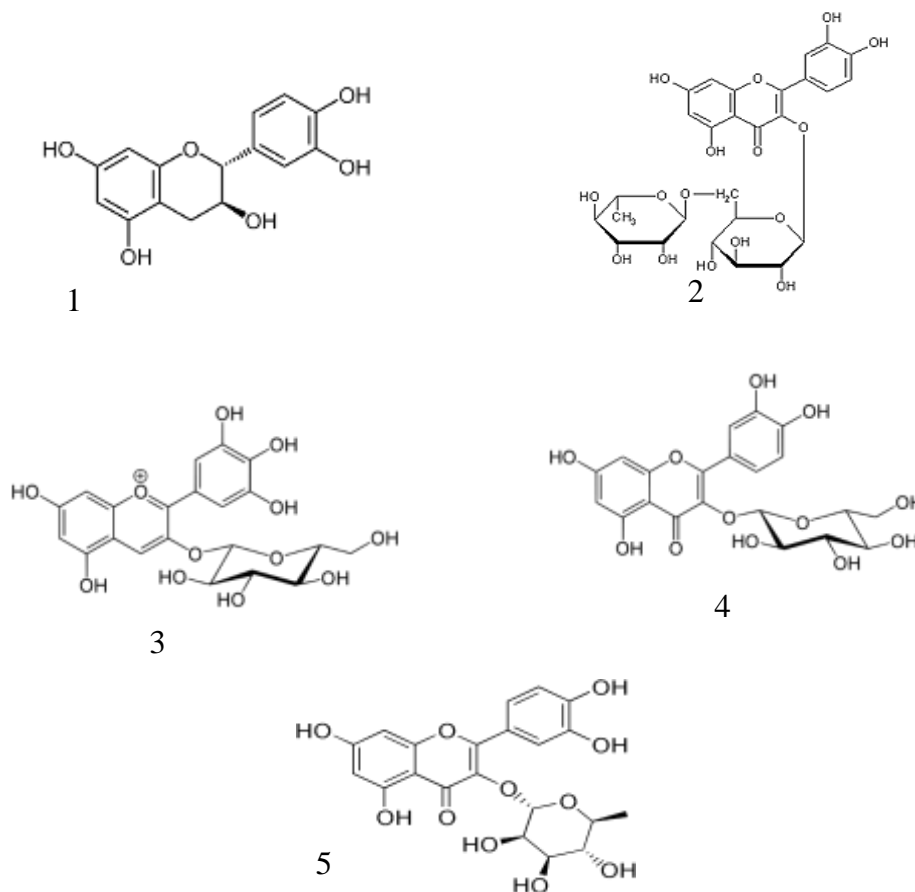The major compounds isolated and identified in our

Figure 2. Chemical structures of the major compounds isolated from *Z. mucronata* pulp. Caption. 1: catechin and / or epicatechin, 2: rutin (quercetin 3-O-rutinoside), 3: delphinidin-3- glucoside, 4: isoquercetin (hyperoside /quercetin 3-O-glucoside), 5: quercetin 3,7-O-L-dirhanmopyranoside (quercitrin).

sample are all members of the polyphenol family (flavonoids and tannins). The phenolic compounds found in the sample analyzed are, for 3 of them, quercetin derivatives (Figure 2). Many of this compounds where equally obtained by Panda et al. (2016) and Nemudzivhadi and Masoko (2015) on *Z. mucronata* and other plants species such as *Ricinus communis* L. In addition to this family of secondary metabolites, phytochemical studies by Soumia (2009) on *Z. lotus* reported the presence of unidentified triterpenes, anthraquinones, alkaloids and saponosides. It should be noted that this author used solvents much more apolar such as methanol/water (60/40). This could explain the results he got.

LC-MS analysis of *Z. mucronata* crude extracts fractions

The crude extract obtained by aqueous extraction of *Z. mucronata* pulp was separated into five fractions (FZ1,

FZ2, FZ3, FZ4 and FZ5) differing by their polarity. Around 74% of the dry mass of the crude extract was recovered in the five fractions and 80% of this total dry mass was found in FZ1, the first fraction. Then, the yield decreased with the polarity in each fraction, becoming 0.14 and 0.10% in FZ4 and FZ5 fractions, respectively (Table 1). Because of low yields from FZ4 and FZ5, the experiment was repeated 5 times to collect enough dry mass to perform the antifungal tests. The proposed gradient allowed concentrating the delphinidin-3-O-glucopyranoside in the FZ2 fraction and the other two monoglucosylated phenolics, isoquercetin and quercitrin, in the last three fractions. Only catechin polymers and rutin were noticed in all the fractions. Thus, delphinidin-3-O-glycopyranoside is observed only in fraction FZ2 in which the absence of isoquercetin and quercitrin is noted. It also appears that FZ1, the aqueous fraction which represents 80% of the total fraction weight, is essentially composed of catechin derivatives and rutin. The fractions FZ3, FZ4 and FZ5 contain the same compounds (catechin derivatives, rutin, isoquercetin and quercitrin),

Table 1. Phytochemical content of different *Z.mucronata* pulp extracts obtained before (crude extract) and after fractionation (FZ1, FZ2, FZ3, FZ4 and FZ5) on inverse solid phase.

Fraction	Dry mass (mg)	Percentage of total mass (%)	Catechin derivatives (polymers)	Rutin	Delphinidin-3-O-Glucopyranoside	Isoquercetin	Quercitrin
			Identified compounds				
Crude extract	500.0	100	+++	+++	++	++	++
FZ1	299.0	59.80	++	++	nd	nd	nd
FZ2	63.0	12.68	++	++	++	nd	nd
FZ3	7.0	1.40	++	++	nd	++	++
FZ4	0.7	0.14	++	++	nd	++	++
FZ5	0.5	0.10	++	++	nd	++	++

+++: Highly present; ++: present, +- : trace ; nd: not detected. FZ1: 100% water, FZ2: 95/5 water/Acetonitrile, FZ3: 90/10 water/Acetonitrile, FZ4: 85/15 water/Acetonitrile; FZ5: 10/90 water/Acetonitrile. Data present the values obtained after one fractionation.

Table 2. Efficiency rate of *Z. mucronata* pulp fractions (FZ1, FZ2, FZ3, FZ4 and FZ5), standards (FZ6 and FZ7) and water control (FZ8) on inhibition of *C. arachidicola, P. personata* and *P. arachidis* germination.

Treatment		Cercospora arachidicola	Phaeoisariopsis personata	Puccinia arachidis
		Inhibition of spore germination (%)		
Fractions of *Z. mucronata* crude extract	FZ1	40.55[c]	44.44[b]	52.38[ab]
	FZ2	49.05[b]	50.00[ab]	57.14[ab]
	FZ3	52.00[ab]	50.00[ab]	57.14[ab]
	FZ4	49.35[b]	44.44[b]	47.62[b]
	FZ5	50.08[ab]	50.00[ab]	47.62[b]
Standards control	FZ6	56.24[b]	55.56[ab]	61.91[ab]
	FZ7	68.22[a]	61.11[a]	66.67[a]
	FZ8	0.00[d]	0.00[c]	0.00c
Probability		<0.0001**	<0.0001**	<0.0001**

FZ1: 100% water, FZ2: 95/5 water/Acetonitrile, FZ3: 90/10 water/Acetonitrile, FZ4: 85/15 water/Acetonitrile; FZ5: 10/90 water/Acetonitrile; positive controls: FZ6 (catechin) FZ7 (rutin); FZ8: negative control. Values are expressed in percentage and correspond to the mean of 5 measurements. **: highly significant different. Means followed by the same letter were not significantly different between treatment at $p \leq 0.05$ (Newman-Keul's test).

certainly present at varying concentrations.

Antifungal activity of fractions from *Z. mucronata* extract

In vitro antifungal effect of fractions of *Z mucronata* against *C. arachidicola*, *P. personata* and *P. arachidis* was firstly evaluated by the percentage of efficiency of each extract fraction on inhibition of spore germination (Table 2). All the fractions recorded an inhibitory effect on the germ tube elongation of the spores for all the fungi. The level of efficiency of inhibition varied from 40.55 to 57.14% (Table 2), the lowest inhibitory effect being observed with the first fraction against *C. arachidicola* and the highest with second and third fractions (FZ2 and FZ3). Inhibition by solutions of pure catechin (FZ6) or

rutin (FZ7) was higher, rutin effect being superior to that of catechin (from 7 to 17.5% more active, according to fungal strain). For the three fungal species, there was a significant difference in the antifungal activity between the fractions and the negative control (FZ8). Except for the fraction FZ4 and FZ5, spores of *P. arachidis* seemed to be more sensitive to treatment. Indeed, with the FZ1 treatment, efficiency rate was 26.6 and 18% higher for *P. arachidis* than for *C. arachidicola* and *P. personata*, respectively. For all the strains, one of the highest inhibition was observed with the FZ3 extract.

Inhibition of fungal growth was also estimated looking to the evolution of fungal diseases on peanut leaves previously inoculated by *C.arachidicola*, *P. personata* and *P. arachidis* in presence or not of fractions of *Z. mucronata*. For the three fungal species (Figure 3), control leaves, which have received a water treatment
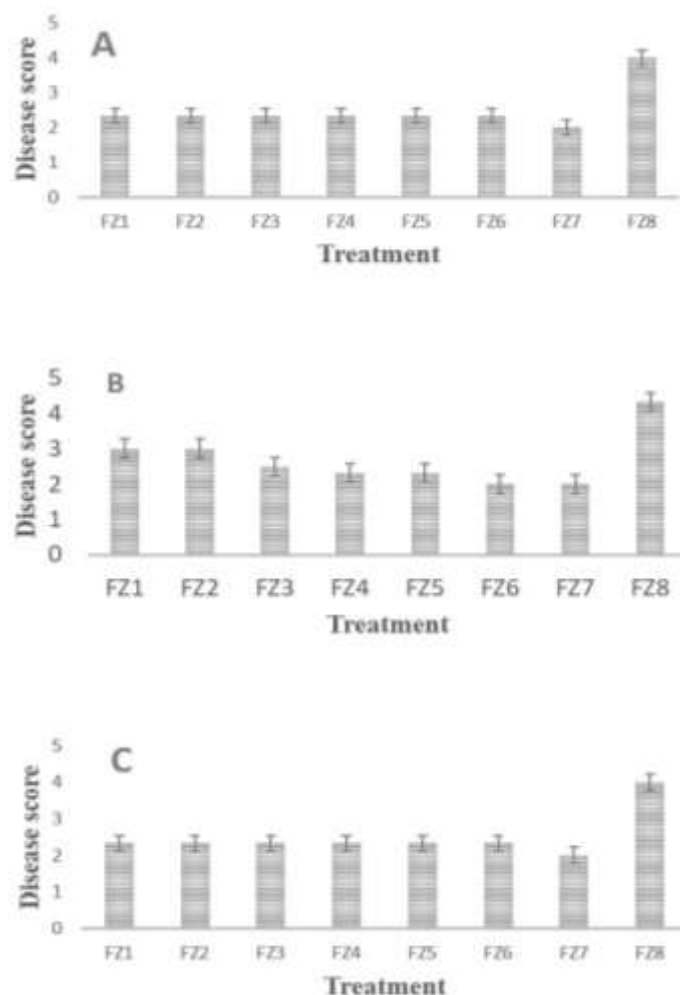
Figure 3. Extension of foliar diseases on peanut leaves artificially contaminated in presence of *Z. mucronata* extracts (FZ1, FZ2, FZ3, FZ4 and FL5), positive control (FZ6 and FZ7) and negative control (FZ8). A: *C. arachidicola*, B: *P. personata* and C: *Puccinia arachidis*. Caption: Error bars represent ± standard deviation of mean (n = 3). FZ1: 100% water, FZ2: 95/5 water/Acetonitrile, FZ3: 90/10 water/Acetonitrile, FZ4: 85/15 water/Acetonitrile; FZ5: 10/90 water/Acetonitrile; positive controls: FZ6 (catechin) FZ7 (rutin); FZ8: negative control. Extension is expressed using the 9 classes' severity scale of ICRISAT.

(FZ8), showed the highest symptom, with a score ranging from 4 to 4.33. On the spores of *C. arachidicola* (Figure 3A), the fractions of extracts (FZ1-5) and the reference solutions showed the same efficiency with a score of 2.5. Only the fraction FZ1 recorded a score of 3.5. On *P. personata* (Figure 3B), FZ6 and FZ7 noted the strongest inhibition with an evolution score of 2.0. Treatments with extract fractions did not show the same sensitivity on this fungus. Thus, the disease evolution scores varied from 2.5 to 3 for fractions FZ1, FZ2, FZ3 and from 2 to 2.33 for fractions FZ4 and FZ5. In the case of rust pustules, no differences in disease severity were observed between

crude extract fractions (Figure 3C). The FZ7 fraction had the lowest disease outcome, that is, 2.0.

The results of the screening indicated the presence of compounds which are natural bioactive substances such as tannins and flavonoids. Different biological activities such as antinociceptive and antipyretic (Adzu et al. 2001; Nisar et al. 2007), antioxidant, antilisterial (Al-Reza et al., 2009), larvicidal (De Omena et al., 2007) and antimycobacterial (Suksamrarn et al., 2006) effects have been reported for various constituents of *Ziziphus* species.

Available literature indicates that each compound when taken separately has antimicrobial activity (Boligon et al., 2012; Panda et al., 2016). This may explain the antifungal activities of crude extract fractions, as indicated by the inhibition of the fungal appressorium formation and spot leaves necrosis. The antimicrobial activity of anthocyanidins has been reported (Scalbert, 1991; Snyder et al., 1991). Dixon et al. (2005) suggested that the major function of the anthocyanidins and their derivatives present in the fruits, bark, leaves and seeds of many plants, is to protect them against microbial pathogens, insect pests and large herbivores. Susceptibility to diseases and pests in some plants has been associated with the lack or low concentration of proanthocyanidins oligomers of catechin and epicatechin. For example, susceptibility of coffee varieties to the fungal pathogen *Hemileia vastatrix* was reported to be associated with low proanthocyanidin levels (Gonzalez de Colmenares et al., 1998).

In the present study, all the fractions obtained from aqueous extract of the fruit pulp were enriched in catechin derivatives and rutin and had a relative activity against the different fungi. However, a variation in the level of activity was observable between fractions and may be attributable to the presence of other phenolics, such as quercitrin, isoquercetin or delphinidin-3-O-glycopyranoside. But rutin and catechin derivatives concentrations also varied in the different fractions and this fact could certainly play a part in the variation of the rate of inhibition. Studies have shown a positive correlation between the amount of compounds such as flavonoids and anthocyanidins in plants and their resistance to *Phytophthora* species (Panjehkeh et al., 2009).

Although this study did not determine which of the compounds is involved in inhibiting the growth of the various fungi, it has shown that the phenolic compounds of the pulp of *Z. mucronata* exhibit an antifungal action and that pulp extracts can be used for biological control of peanut fungi. However, further investigations are needed to study the role of each compound in the antifungal activity and understand the detailed mechanism.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ACKNOWLEDGEMENTS

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