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GC-MS analysis and antifungal activity from galls of *Guiera senegalensis* J.F Gmel (Combretaceae)

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ABSTRACT

Guiera senegalensis is a well known medicinal plant which is used as a drug in Burkina Faso. The aim of this study was to investigate the chemical composition and antifungal activity from *galls* of *Guiera senegalensis* against different kinds of fungi *in vitro*. The chemical composition of the *Guiera senegalensis* volatile compounds obtained from the galls was analysed using gas chromatograph (GC)-flame ionization detectors (FID) and GC-MS. Thirteen (13) components were identified for hexane-acetone (50:50) column fraction of hydroacetone extract and twenty one (21) compounds for hexane-acetone (50:50) column fraction of aqueous decoction extract. This composition differed according to the kind of extract. The ethyl acetate fraction extract from hydroacetone extract (EAF/HAE) exhibited the highest of flavonol content (0.56 ± 0.01 mg QE/100 mg of fraction). The *G senegalensis* exhibited an interesting antifungal activity against all strains tested.

INTRODUCTION

Guiera senegalensis specie (family Combretaceae) has been used traditionally in Burkina Faso for therapeutic purposes. In centre of Burkina, the Mossis call it Willinwiga while the Dioula in the south west call it Fufanikay, Bufuluk, Efunuk or Bubuun and the Peuhl in the north of Burkina Géloki (Nacoulma, 1996). The galls of *G. senegalensis* possess effective antiacetylcholinesterase, antilipid peroxidation in rat brain homogenates and erythrocytes hemolysis inhibitory activities (Sombié *et al.,* 2011 a). The galls of *Guiera senegalensis* demonstrated pronounced antioxidant potential, showed high polyphenols, totals tannins and totals flavonoids contents (Sombié *et al.*, 2011a, b). The flavonoids are not only present in plants as constitutive agents but have also accumulated in plant tissues in response to microbial attack (Galeotti *et al.*, 2008). Opportunistic fungal infections have increased over the past several years. Leaves, young shoots and galls of *G senegalensis* are used in Burkinabe folk medicine for their antibacterial and antifungal properties (Nacoulma, 1996). It has also been reported that crude methanolic extracts of *G senegalensis* exhibit antimicrobial properties on bacteria and fungi (Bassene *et al.*, 1995). Plant-derived antimicrobial are always a source of novel therapeutics. The aim of this work is to determine the volatile compounds by GC-MS, the flavonol content and the potentiality of galls from *Guiera senegalensis* against the growth of fungi.

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MATERIAL AND METHODS

Phytochemistry analysis

Sample preparation

The plant material is constituted of galls from *Guiera senegalensis*. The galls were dried and grounded to powder. The obtained powder was extracted with acetone 80% via soaking (hydroacetone extract) and distilled water via decoction (aqueous decoction extract). The hydroacetone and the aqueous decoction extracts obtained were respectively dissolved in distilled water and successively extracted with ethyl acetate and butanol. Each extract was dried to give: ethyl acetate fraction (EAF), butanol fraction (BF) and final water fraction (WF). Obtained fractions were evaporated to dryness and stored in a refrigerator at +4°C until use.

The crude hydroacetone and aqueous decoction extracts were fractionated again by Silica gel column chromatography. The elution procedure is based on the use of hexane-acetone (50:50).

Gas Chromatography (GC)–Mass Spectrometer (MS) Analysis

Shimadzu-GC-9A gas chromatograph, FID at 220, N 2 at 1.0 ml/min, SPB-5 capillary column (30 m × 0.53 mm ID; 0.3 µmdf), split ratio 1:30 injector temperature 240°C, column temperature maintained at 50°C for the first five minutes and then raised to 235°C (5°C per minute) followed by five minutes at 235°C. GC-MS: Hewlett-Packard 5890 gas chromatograph, combined with a Jeol JMS-HX 110 mass spectrometer with source at 270°C at 70 eV. Injector was set at 270°C with splitting ratio 1:30. The analysis was performed on the aforementioned programme on equivalent column HP-5 (25 m × 0.22 mm and 0.25 µmdf). A mass spectral survey was performed using the NIST mass spectral search program 1998.

Flavonols content

Flavonols content was determined according to the method of (Abarca *et al.*, 2004) using quercetin as standard. 1.5ml of extract was mixed with 1.5 ml of aluminium trichloride. The absorbance of the mixture was measured at 425 nm. The results were expressed as of mg of quercetin equivalents (QE) per 100 mg of lyohilised extract or fraction (mg QE/100 mg extract).

Antifungal activity

Microorganisms

Five serotype strains of fungi used in this study are: *Aspergillus niger*, *Mucor rouxii*, *Penicillium roquefortii*, *Fusarium oxysporum* and *Rhizopius nigricans*. The fungi strains were all obtained from the Charles Degaulle pediatric hospital of Ouagadougou and maintained in the Laboratory of Biochemistry and Chemistry Applied (LABIOCA), Formation and Research unit, Ouagadougou University (Burkina Faso).

Preparation of microbial inoculums

Fungal strains grown on nutrient agar at 37 °C for 24 hours were suspended in a saline solution (0.9% NaCl) and adjusted to the turbidity of the 0.5 MacFarland standard (0.5 ml of BaCl 2, 5H2O, 11.7 mg/ml mixed with 99.5 ml of H2SO4 1%) to obtain approximately 10^6 colony-forming units/ml.

Preparation of culture medium and inoculation

Thirty-eight grams of Mueller-Hinton agar was mixed with 1000 ml of sterile distilled water. The mixture was then sterilized by autoclaving at 120°C for 20 minutes. Under aseptic conditions in the laminar flow hood, 15 ml of agar medium was uniformly dispensed into sterilized Petri dishes. They were then covered and allowed to cool at room temperature until the culture medium hardened. The inoculation of the microbial culture on the agar surface was done by the spread plating technique.

Disc application and incubation

Discs (6 mm in diameter) were prepared from Whatman No.1 filter paper and were sterilized by autoclaving. The sterile discs were placed on the Mueller-Hinton agar surface with flamed forceps and then impregnated with 10 µl of the extract solution (25 mg/ml in water containing 10% DMSO) before being gently pressed down to ensure contact with the agar surface. Prepared solution of nystatin (5 mg/ml) were used as positive controls while a water solution containing 10% DMSO was used as negative control. The Petri dishes were finally incubated for 96 h at 37 °C in an inverted position for optimal growth. Then, the diameter (in mm) of the inhibition zone around each disc was measured. Antifungal activity was indicated by a clear zone of growth inhibition. Each test was repeated three times.

Statistical Analyses

Experimental data were analyzed using XL stat software. The mean values were calculated and reported as the mean standard deviation (n = 3). The one way analysis of variance procedure followed by Fisher test was used to determine the significant difference of flavonol content and in vitro antifungal activities. $P < 0.05$ was considered significant

RESULTS

Chemical analysis

Gas chromatography-mass spectrometry analysis

The results of GC-FID and GC-MS analyses of the column fractions from galls of *G senegalensis* of the plant are presented in **Table 1**. **Fig 1 to Fig 4 show** the GC and GC-MS chromatograms of aqueous decoction extract (ADE) and hydroacetone extract (HAE). Gas chromatography-mass spectrometry analysis of column fraction from hydroacetone extract of the plant have resulted in the identification of thirteen (13) compounds and twenty one (21) compounds for column fraction from aqueous decoction extract with spectral match factor at least 90%. Eight (8) common compounds have been identified in both extracts.

Flavonol content

The **Table 2** shows the totals flavonols content of the extracts and fractions from galls of *G Senegalensis*. The ethyl acetate fraction from hydroacetone extract (EAF/HAE) contains the highest amount of flavonol (0.56±0.01mg QE/100 mg of fraction) and the ethyl acetate fraction from aqueous decoction extract (EAF/ADE) the lowest amount (0.0142 ± 0.00025 mg QE/100 mg of fraction).

Antifungal activity from galls of *Guiera senegalensis*

The galls extracts and fractions of this plant exhibited the antifungal activity against five fungi and the results were expressed as mean ± standard deviation (n=3). **Table 3** summarizes the fungal growth inhibition by extracts and fractions from galls of *G. senegalensis*. The mean zones of inhibition against fungi were ranging from 00 to 52 mm. The hydroacetone

extract and aqueous decoction showed antifungal activity against all the fungal strains used with respective inhibition zone of 10 to 39 mm and 10 to 52 mm.

The hydroacetone extract (HAE) showed strong inhibition capacity against *Mucor rouxii* and *Fusarium oxyporum* while aqueous decoction (ADE) significantly inhibited *Mucor rouxii* and *Rhizopus nigricans* when compared to hydroacetone extract (HAE) and the different fractions. The ethyl acetate fraction from hydroacetone extract (EAF/HAE) showed inhibition activity of the growth of *Penicillium roqueforti* and *Rhizopus nigricans* while ethyl acetate fraction from aqueous decoction (EAF/ADE) significantly inhibited *Aspergillus niger*, *Penicillium roqueforti* and *Mucor rouxii* when compared to the others extracts and fractions. The butanolic fraction from hydroacetone extract (BF/HAE) significantly inhibited *Aspergillus niger* and *Penicillium roqueforti*, the water fraction from aqueous extract (WF/ADE) significantly inhibited *Aspergillus niger*, *Penicillium roqueforti* and *Rhizopus nigricans* when compared to the activity of the extracts and the others fractions. Fungal strains have been less sensitive to Butanol fraction from aqueous decoction extract (BF/ADE) and the water fraction from Hydroacetone extract (WF/HAE) when compared to others extracts and fractions.

The combination of the ethyl acetate fraction (EAF) and butanol fraction (BF) from the hydroacetone extract (HAE) and aqueous decoction extract (ADE) did not involve an improvement of their antifungal activity against most of fungi species tested. We note a resistance of *Aspergillus Niger* to the combination of ethyl acetate fraction (EAF) and butanol fraction (BF) from hydroacetone extracts (HAE) whereas this fungi strain is sensitive to the fractions taken separately. The compounds present in ethyl acetate fraction (EAF) and butanol fraction (BF) from hydroacetone extract (HAE) would act in antagonism on the inhibition of the growth of *Aspergillus Niger*. We note, however, a synergistic or additive effect of the combination of the ethyl acetate fraction (EAF) and butanol (BF) from the aqueous decoction extract (ADE) on the growth inhibition of *Fusarium oxysporum*. The both fractions gave respectively the inhibition zones of 11 and 10 mm while the combination gave an inhibition zone of 29 mm on the same fungal strain. The combination of the ethyl acetate fraction (EAF) and butanol fraction (BF) from the aqueous decoction extract (ADE) involve an improvement of their antifungal activity against *Fusarium oxysporum*.

Nystatin, a reference antifungal drug was used at a concentration of 1UI. The inhibition zones of this compound vary from 12.67 mm for *Penicillium roqueforti* to 52 mm for *Rhizopius nigricans*. It gave strong inhibition activity on the growth of *Aspergillus niger* and *Fusarium oxysporum* when compared to the inhibition activity of the extracts and fractions. On the contrary, it has been less active in the inhibition of the growth of *Penicillium roquerforti* than all the extracts and fractions except for the inhibition of aqueous decoction extract (ADE) and butanol fraction from the aqueous decoction extract (BF /ADE). The inhibition zone of nystatin on the growth of *Mucor rouxii*, which is 18 mm, showed that it is less active than the activity of hydroacetone extract (HAE) and water fraction from aqueous decoction extract (WF/ADE) on the same fungal strain. Nystatin showed a good inhibition of the growth of *Rhizopius nigricans* (52mm). This inhibition is not significantly different from those obtained by the ethyl acetate fractions from aqueous decoction extract and hydroacetone extract (EAF/ADE, EAF/HAE), the aqueous decoction (ADE), and the water fraction from aqueous decoction extract (WF/ADE).

Table. 1: GC/MS composition from galls of *Guiera senegalensis.*

Extract	Compound name	Retention time
ADE	1-methylpropylbenzene	9.2964/9.5614
	2-(4'-methylphenyl)-propanal	11.30
	2-(Dimethyl)phenylpropanal	12.46
	3-methylnonane	12.68
	1,7-dimethylnaphtalene	14.57
	1,2-dimethylnaphtalene	14.76
	1,6,7-trimethylnaphtalene	16.16
	Phenanthrene	19.03
	1-Tetradecanol	19.917
	1-Eicosanol	21.88
	Di-(2-ethylhexyl)phthalate	25.816
ADE and HAE	2-methoxyfuran	4.54/4.58
	Limonene	8.89/8.91
	1,2,3,5-tetramethylbenzene	9.41/9.86/9.87
	1,2,3,4-tetramethylbenzene	10.43/10.45/10.96
	1,3-diethyl-5-methylbenzene	11.10/11.11
	Benzofulvene	11.45/11.46
	Dodecane	11.62/11.64
	Tetradecane	14.40/14.43
	Hexadecane	16.86/16.89
	Eicosane	17.99/19.06/20.09/21.07/23.77
HAE	P-cymene	9.75
	Tridecane	13.06
	Pentadecane	15.66
	3-[4'-(t-Butyl)phenyl]furan-2,5-dione	19.84
	Heneicosane	22.01/22.91/24.59/25.39

ADE: Compounds present only in aqueous decoction extract**, HAE**: Compounds present only in Hydroacetone extract, ADE and HAE: Compounds present both in aqueous decoction extract and Hydroacetone extract.

Table. 2: Totals flavonols content from galls of *Guiera senegalensis.*

Extract/Fraction	Flavonols (mg QE/100 mg of extract)
HAE	0.15 ± 0.04^{d}
EAF/ HAE	0.56 ± 0.01^{a}
BF/ HAE	$0.207\pm0.01^{c,d}$
WF/ HAE	0.15 ± 0.003^{d}
ADE	0.393 ± 0.027^{b}
EAF/ ADE	0.0142 ± 0.00025^{e}
BF/ ADE	$0.24\pm0.01^{c,d}$
WF/ ADE	$0.12\pm0.0069^{b,c}$

Table. 3: Inhibition zone of extracts/fractions from galls of *Guiera senegalensis* on the fungi strains.

	AN	PR	MR	RN	FO
HAE	10.67 ± 0.58 c,d	15 ± 2 c,d	39 ± 1 a	20 ± 0 d	33 ± 3 b
EAF/HAE	11 ± 1c,d	20 ± 0 a	14.7±0.58 c,d	52± 0 a	13 ± 1 f
EAF1/BF1	0.0 ± 0 e	13± 1 d,e	10± 0 e	42.7±2.52 b	11± 1f,g
BF/HAE	13.7± 0.58 b	19.7± 0.58 a	15.7±0.58 b,c,d	39± 1 b,c	20± 0 d
WF/HAE	11± 1 c,d	14± 2 c,d,e	9± 1 e	41± 5 b,c	19± 1 d
ADE	12.7 ± 2.5 b,c	11.7±1.53e,f	10 ± 2 e	52 ± 0 a	11 ± 1 f,g
EAF/ADE	13.7± 1.53 b	18± 0a,b	17± 1 b,c	52± 0 a	11± 1 f,g
BF/ADE	9± 1 d	10± 0 f	14± 0 d	42± 5 b,c	10± 0 g
EAF2/BF2	11.7 ± 0.58 b,c	16± 0 b,c	10.7± 0.58e	34± 1c	29± 1c
WF/ADE	12.7± 2.52 b,c	14± 1c,d,e	38± 4 a	51± 3 a	16.7± 1.5 e
Nystatin	29.7 ±1.53a	12.7±0.58d,e,f	18±2 b	52±0 a	36.7±1.53a

Data are mean ± SEM (n = 3).
Values showing the same letter are not significantly different($P > 0.05$) from one other in the same columns.
The extracts and fractions were tested in the concentration of 25 mg/ml.

Table . 4: Mean inhibition by fungus strain of extracts and fractions.

Fungi strains	A niger	P roqueforti	M rouxii	R nigricans	F oxyporum
Mean/fungi	10.6	15.13	17.8	42.57	17.37

Table. 5: Mean inhibition of fungi strains by extract/fraction.

Extract	Mean /Sample
HAE	23.53 c
EAF/HAE	22.13 c,d
BF/HAE	21.6 d,e
EAF 1/BF 1	15.33 h
WF/HAE	18.8 f,g
ADE	19.47 f
EAF/ADE	22.33 c,d
BF/ADE	17 g,h
EAF2/BF2	20.27 e,f
WF/ADE	26.47 b
Nystatin	29.80 a

Data are mean ± SEM (n = 3).
Values showing the same letter are not significantly different ($P > 0.05$) from one other in the same columns.

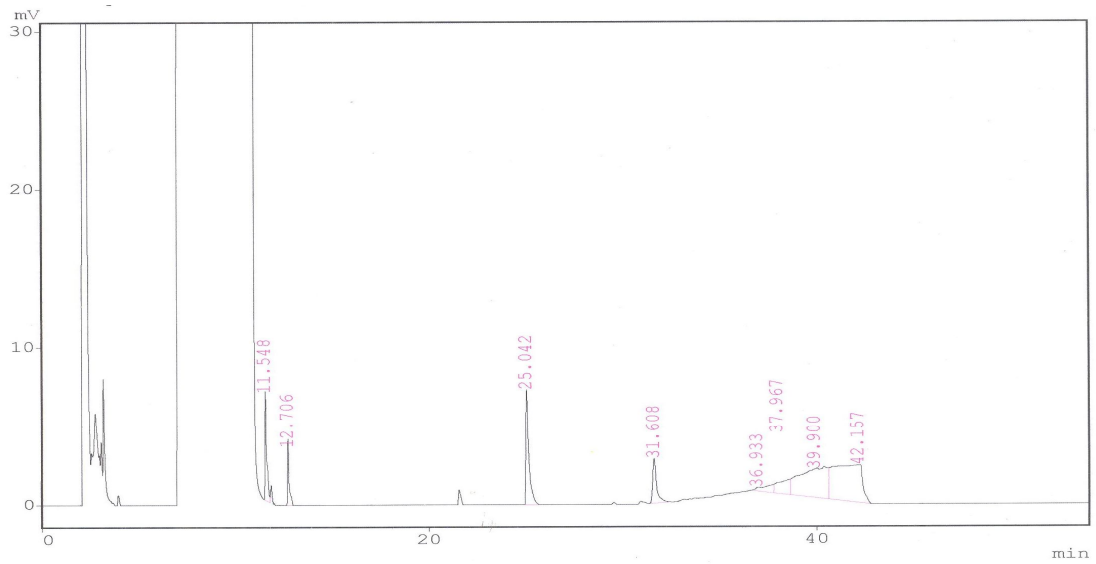

Fig. 1: Gaz chromatogram of Aqueous decoction Extract.

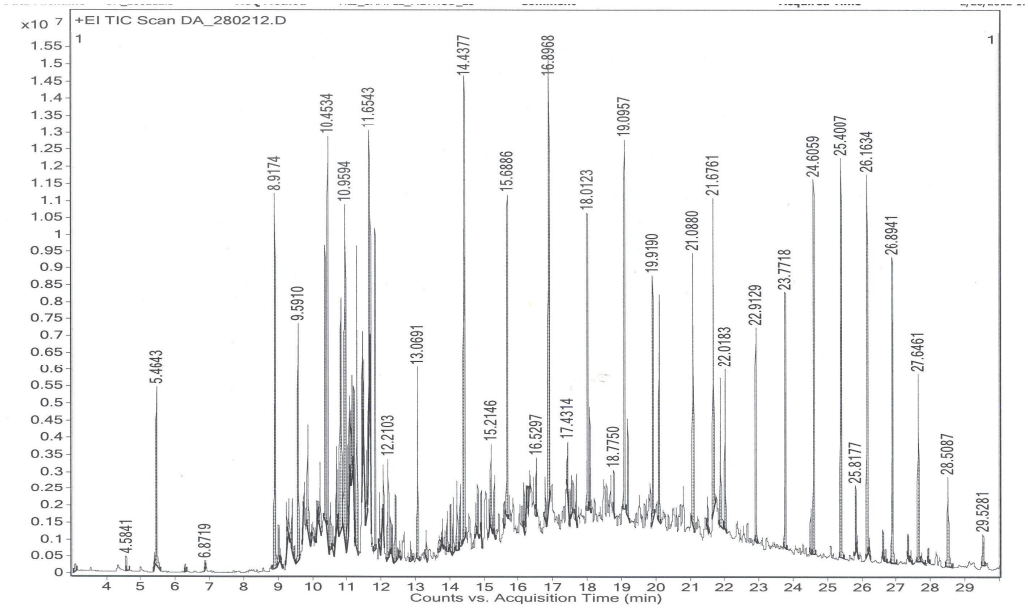

Fig. 2: GC-MS chromatogram of Aqueous decoction Extract.

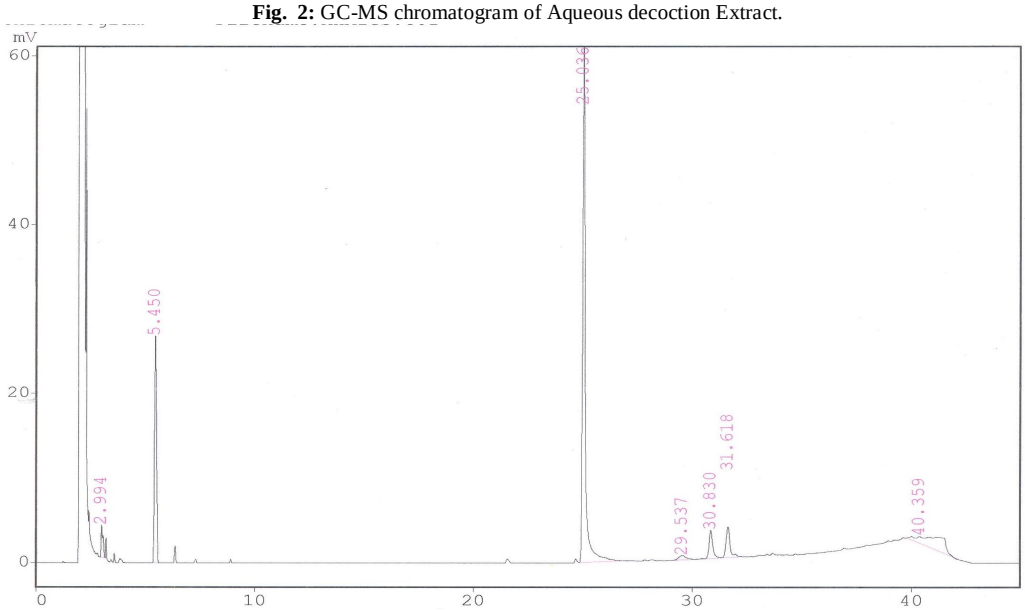

Fig. 3: Gaz chromatogram of Hydro-acetone extract.

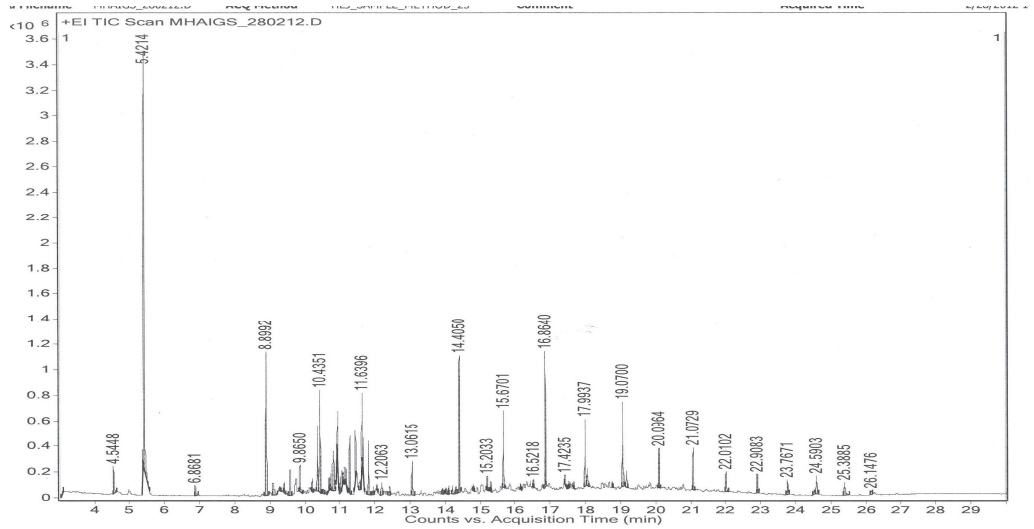

Fig. 4: GC-MS chromatogram of Hydro-acetone extract.

DISCUSSION

Now a days, endemic infections and opportunist fungal become an important medical problem. The usage of *Guiera senegalensis* for medicinal purpose was reported by several researchers. The filamentous fungi tested with the disk diffusion assay showed variable sensitivity to the extract of *G senegalensis*. The intensity of antifungal activity depended on the type of fungus and extracts or fractions used. The most inhibited filamentous fungi species by fractions and extracts was *Rhizopius nigricans* with a mean of 42.57 **(Table 4).**

The study of mean inhibition of fungi strains by extracts and fractions showed that the water fraction from aqueous decoction extract (WF/ADE) had the strong activity on the growth of the strains fungi (Table 5). This report suggests that the active biomolecule of this plant against the growth of the fungi strains is soluble in aqueous solvents. In contrast the aqueous decoction extract did not show a good mean inhibition against the fungi strains (Table 5). It contains some compounds which would act in antagonist (Yen and Chang, 2008) showed that the mixture of cinnamaldehyde with natural antioxidants has synergistic, additive or antagonistic effects against wood decay fungi.

The biological activities of some major bioactive volatile components in relation to parasitism apoptosis and antimicrobial activity have already been established. Possible synergistic and antagonistic effects of compounds also play an important role in fungi inhibition. Previous papers showed that the antifungal activities of essential oils of *Cotula coronopifolia* may be attributed to the presence of 1-eicosanol, hexacosane and heptacosane (Naz *et al.*, 2001). Carvacrol and p-cymene were found to be the most active compounds, which helps to explain the stronger activity of *Thymus vulgaris* and *Thymus zygis* (Pina-Vaz *et al.*, 2004).

Omrana *et al* (2001) showed that the limonene had an inhibitory effect against *Candida albicans*, *Aspergillus niger*, *Aspergillus* sp. and *Penicillium* sp. The antifungal activity of galls from *Guiera senegalensis* could be explained by the presence of some volatiles compounds identified by GC/MS in the extracts like 1-eicosanol, p-cymene and limonene. The antifungal property of *G senegalensis* could be due to flavonols present in the galls. Galangin, a flavonol commonly found in propolis samples, has been shown to have inhibitory activity against *Aspergillus tamarii, A. flavus, Cladosporium sphaerospermum*, *Penicillium digitatum* and *Penicillium italicum* (Cushie and Lamb, 2005).

The mechanisms of flavonoids that are antimicrobial can be classified as the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (Hendra *et al.,* 2001). The kaempferol, myricetin, naringin, quercetin and rutin have antimicrobial activity against human pathogenic microorganisms with some mechanisms of action such as inhibition of nucleic acid synthesis, cytoplasmic membrane function and energy metabolisms (Hendra *et al.,* 2001). Antimicrobial activity of galls from *Guiera senegalensis* might be due to the presence of kaempferol, myricetin, quercetin and rutin in the extracts (Lamien *et al.,* 2005).

CONCLUSION

This investigation is, for some fungi species, a first approach to study the galls of *Guiera senegalensis* as potential natural fungicides.

The compounds identified by GC-MS analysis like 1-eicosanol; p-cymene and limonene could have been responsible for the antifungal activity of the extracts of *G senegalensis*. No general rule can be proposed yet to explain the antifungal activity and more studies about their activity and action mechanism should be carried out. The results show that the galls of *Guiera senegalensis* could be used as a potential candidate for drug development for the treatment of ailments caused by these pathogens.

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