Research Article

SPECTRAL ANALYSIS AND ANTIFUNGAL ACTIVITY OF FATTY ACIDS METHYL ESTERS ISOLATED FROM ALGERIAN *CITRULLUS COLOCYNTHIS* L. SEEDSD. Chabane Sari¹, A. Amrouche^{1,2*}, H. Benmehdi^{3,4}, H. Malainine², M. Chabane Sari¹, N. Ouled Necir²¹Laboratory of Natural Products Research (LAPRONA) University of Tlemcen, Algeria²Faculty of Sciences and Technology, Department of Sciences University of Bechar, Algeria³Laboratory of Lasnabio, Department of Chemistry, University of Tlemcen 13000, Algeria⁴Faculty of Sciences and Technology, Department of Technology, University of Bechar 08000, Algeria

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ABSTRACT

The present work was aimed at evaluating antifungal effect of the fraction of fatty acids methyl esters isolated from the seeds of *Citrullus colocynthis* against two toxigenic fungal strains (*Aspergillus flavus* and *Aspergillus ochraceus*). The evaluation of antifungal activity was carried out by two methods: the radial growth solid medium and the evaluation of the biomass liquid medium. The seeds under study provided acceptable yields of oils and esters. The characterization of these oils and their corresponding methyl esters was carried out by spectroscopic analysis. The reading of the GC/MS spectrum revealed the richness of these oils in α -linolenic acid and linoleic acid. The results of the antifungal activity showed that the crude esters and their corresponding major esters have exhibited a pronounced antifungal power. This effect was very important on the solid medium and more pronounced for *A. flavus*. The major esters provided the best antifungal index [IAF = 51.67 ± 0.40 % *A. flavus*, IAF *A. ochraceus* = 35.58 ± 0.37 %] compared to crude esters [IAF *A. flavus* = 48.64 ± 0.28 % IAF *A. ochraceus* = 28.37 ± 0.17 %]. These findings suggest that *Citrullus colocynthis* L. seeds are an interesting source of fatty acids methyl esters having antifungal potentialities allowing them to be used as preservative compounds against toxigenic fungi.

Keywords: *Citrullus colocynthis*, methyl ester, antifungal activity, *Aspergillus flavus*, *Aspergillus ochraceus*.**INTRODUCTION**

Contamination by toxigenic fungi and toxin production of food takes place under specific environmental conditions¹, the presence of these fungi can affect and lead to the accumulation of toxic secondary metabolites; mycotoxins². The problem of mycotoxins is not only universal but also mysterious because of its impact on public health, agriculture and economics³. It is difficult, sometimes impossible to eliminate these mycotoxins. Prevention is undoubtedly the best way to reduce the contamination of food and feed by fungi and their toxins⁴. The issue of reducing mycotoxin remains complex, which has led researchers to investigate the use of natural substances extracted from medicinal plants that can fight against the development of fungi producing these dangerous substances^{5,6}. The use of medicinal plants with antifungal properties is one of the most interesting trails to explore⁷. Our interest has been focused on *Citrullus colocynthis* (Cucurbitaceae family) which has different properties include the antibacterial, antimicrobial and anti-candida activities⁸. Several active chemical constituents of this species as bitter substances cucurbitacine A, B, C, D and E have a great interest. Further, several studies have indicated that different cucurbitacin inhibit the proliferation of cancer cells through different mechanisms⁹. This plant has broad-spectrum antibacterial, anti-candida and antimicrobial enabled. Other biological actions such as natural insecticidal, growth regulator, used for treatment of fever and a cure for the infection of the skin^{10,11}. In this context, and for the first time, FAMES was separated from oil of *Citrullus colocynthis*. In a second one the *in vitro* antifungal effect of these esters has been investigated against two fungal strains: *Aspergillus flavus* and *Aspergillus ochraceus*.

MATERIALS AND METHODS**Collection of plant material and extraction of oil**

The *C. colocynthis* seeds used for the present study were collected in January 2012 at Abadla area, (Bechar, Algeria). The seeds were shade and dried at room temperature. Into a Soxhlet apparatus, a quantity of 100 g of powdered seeds was extracted with petroleum ether under reflux for 6 h. After evaporating the solvent, crude extract of oil (6.8 g) was obtained¹².

Saponification of fatty acids

A mixture of 3.4 g of oiliness residue, ethanol (15 mL), water (15 mL) and sodium hydroxide 3 g was refluxed for about 45 minutes. After evaporating of the ethanol under reduced pressure, the aqueous extract was extracted with 15 mL of diethyl ether. The organic layer was acidulated with concentrate acid (HCl) and extracted with 20 mL of diethyl ether. After eliminating of the solvent, fatty acids residue was obtained Yield: 3.24 g¹³.

Methylic esterification of fatty acids and purification of their corresponding esters

Concentrate sulfuric acid (1 mL) was added drop wise to a solution of 3 g of fatty acids and 25 mL of methanol. The mixture was stirred and refluxed for about 2 h and allowed to reach room temperature and to stand for 2 h. After cooling, the mixture was poured onto 300 g of crushed ice. The aqueous layer was then extracted with chloroform. The organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo to give 1.12 g of methyl esters of fatty acids^{14,15}. Purification by silica gel column chromatography, eluting with petroleum ether/chloroform (9:1, v/v), gave two fractions (Frac.1 (0.9 g) and Frac.2 (0.3 g) of title compounds.

Fungal materials and confirmation of testing strain

Phytopathogenic fungi known as *A. flavus* MTTC 2799 and *A. ochraceus* CECT 2092 collected from the microbiological laboratory at Bechar University were used for the assessing of the antifungal potency. Confirmation of genera and species of the used strains was carried out by micro culture and single spore methods according respectively to Wheelis (2008) and Dugan (2006)^{16,17} and Pitt and Hocking (2009)¹⁸. These fungi were maintained on potatoes dextrose agar acidified and stored at 4°C until time of use.

Determination of percent mycelial inhibition by growth radial technique on solid medium

Technique consists in placing simultaneously in test tubes different volumes of FAME's extracted from *C. colocynthis* seeds oil and full to 15 ml by solid medium PDAA (Potatoes Dextrose Agar acidified) to obtain concentrations: 0.7, 1.3, 2.0, 2.7, 3.3, 4.0, 4.7, 5.3, 6.0 and 6.7 µL/mL. After agitation, the content tubes were poured into dishes which were inoculated by the respective spore solution of each tested fungal strain (Agar solution at 0.2 % + 5 % tween 80 and spores of fungal strains). After 7 days of incubation at 25 ± 2 °C. Mycelial radial growth was measured from the third day of incubation^{19,20}. The inhibition percentage of mycelial growth was calculated using the following formula: $PI = ((DT-D)/DT) \times 100$ where DT is mean diameter of mycelial growth in control and D is mean diameter of mycelial growth in treatment²¹.

Determination of percent mycelial inhibition by biomass technique on liquid medium

The FAME's antifungal assessing by biomass technique on liquid medium was tested by using bottles in which we put different volumes of FAME's: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µL and complete to 50 mL by the PDBa medium (Potato Dextrose Broth acidified). After inoculation by 30 µL of spore suspension; the bottles were incubated at (25 ± 2°C) for 14 days^{22,23}. After filtration, the filter paper was dried at 60°C for 24 hours²⁴. The weight of the biomass formed (P) is determined by the formula described by Imtiaz and Lee (2007)²⁵

$$(P = P_1 - P_0)$$

Where P_0 : Weight of filter paper and P_1 : Weight of filter paper and fungal biomass formed after drying.

Statistical analysis

Results obtained were analyzed statistically by the analysis of variance using MS excel 2007. All values are expressed as Mean ± standard deviation.

RESULTS AND DISCUSSION

Currently, aromatic plants have a considerable advantage through the gradual discovery applications of their fixed oils in antimicrobial and antifungal activities mainly. Fixed oils have a very broad spectrum of action against growth of bacteria as fungi and yeasts. Their antimicrobial activity is mainly based on their chemical composition, and in particular the nature of their major compound or synergy between the phytochemicals present in the extract. In order to investigate the quality of the isolated oil, physicochemical analysis has been made and the results are given in Table 1.

Physicochemical analysis of oils

The physicochemical profile shows that the measured parameters of the oil of *C. colocynthis* are assimilate to those

described by the standard. Analysis of refractive index results showed that this oil has an index of 1.474. This value was higher than the work of Hassimi sadou *et al* (2007) has been observed a refractive index in the interval [1.4607 - 1.4620]²⁶. As seen in Table 1, high values of iodine (85.50), oil of *C. colocynthis*. This physicochemical parameter confirms the better behavior of carbon chain oils and their ability to bind to iodine molecules. As regards the acidity value, was found to be lower (3.3 %) than the value reported by the *Codex alimentarius*. While the peroxide index was found smaller compared to the results of Akpambang *et al* (2008)²⁷.

Through the results depicted in Table 1, it appears that the seeds of *C. Colocynthis* gave acceptable yields oil (18 %). On the other hand, our results obtained by isolation methods from fatty acids showed a yield of 29 % of crude esters which have provided a considerable percentage of FAMES (56.8 %). These results are in analogy with literature data which confirm the richness of this oil with fatty acids and their corresponding esters.

Spectral analysis of FAMES

Analysis by gas chromatography

The profile of the fraction of methyl esters of free fatty acids (FAMES) revealed the presence of four peaks with different retention times. The actual transit time of the different fractions generally was 10 to 11 minutes. Fraction of fatty acid methyl esters were identified by the relative time with respect to those known using a bank of NBS and WILEY data library for mass spectroscopic identification.

Figure 1

The actual transit time of FAME's different fractions isolated from oil *Citrullus colocynthis* L. was generally 11 minutes. The spectrum analysis CG Figure 3.²⁷ shows the different fractions generally peaks at 8.63, 9.48 and 9.62 minutes. These peaks correspond generally to the methyl esters of the respective fatty acids: palmitic acid, α-linolenic, linoleic and stearic.

Mass spectroscopy Analysis

Reading the mass spectrum of the peak at 8.63 minutes, revealed the presence of the molecular ion at $m/z = 270$. Moreover, the mass spectra of methyl esters of unbranched FAME (methyl ester of palmitic acid) are characterized by fragment ions at $m/z = 74$ (McLafferty rearrangement), $m/z = 87$ (cutoff β) and $[M - 31]$ (loss of OMe). Other fragment ions $[M - C_nH_{2n+1}]$ (227, 185, 143, 129, derived from the cleavage of the alkyl chain is unbranched saturated recorded Figure.

Figure 2

The methyl ester of α-linolenic acid (Figure 3) is an abundant molecular ion ($m/z = 292$) and hydrocarbon ions of general formula $[5 - C_nH_{2n}]^+$ having a tendency to dominate the spectrum of the ion at $m/z = 78.8$ as the base peak. A peak at $m/z = 150$ assigned to the methyl esters of polyunsaturated fatty acids $[C_{11}H_{13}]^+$, while the one at $m/z = 108$ is assigned to the terminal group $[C_8H_{13}]^+$. In addition, we note the presence of ions formed by a similar cleavage of the carboxyl end giving a fragment containing the first two double bonds and the second methylene group (minus one proton) that could be called off «alpha». The ion in the spectrum corresponds to the methyl 9, 12, 15 - octadecatrienoate is at $m/z = 236$.

Table 1: Mean yields of isolates esters and oil physicochemical analysis

<i>Citrullus colocynthis</i> seeds oil	
Yield (%)	18
color	yellow
Physical analysis	
Density	0.9083
refractive index (20°C)	1.4742
freezing point (°C)	-6°
Chemical analysis	
▪ Saponification index (mg KOH/g)	218.6
▪ Iodine index	85.80
▪ Insaponifiable matter (%)	2.268
Characters of alteration	
Acid index (%)	3.682
Acidity (%)	1.858
peroxide index (meq/Kg)	1.18
Esters fractions	
▪ Crude esters yield (%)	29
▪ Major esters yield	56.8

Table 2: Antifungal index of FAMES isolated from *Citrullus colocynthis* L. tested against *A. flavus* and *A. ochraceus* (Growth radial technique on solid medium) (P<0.05)

		10	20	30	40	50	60	70	80	90	100
<i>A. flavus</i>	Crude ester	47.32±0.09	47.20±0.10	47.22±0.14	45.80±0.20	46.58±0.22	39.34±0.11	45.25±0.38	48.30±0.22	47.43±0.19	48.64±0.28
	Major ester	45.56±0.11	45.69±0.37	32.53±0.15	45.74±0.18	49.60±0.18	48.461±0.19	51.65±0.22	51.67±0.40	51.32±0.32	49.50±0.45
<i>A. ochraceus</i>	Crude ester	22.48±0.21	21.54±0.14	27.49±0.18	28.18±0.24	28.37±0.17	28.32±0.21	28.11±0.14	27.06±0.44	27.49±0.44	28.08±0.45
	Major ester	29.07±0.36	32.21±0.10	33.43±0.11	28.36±0.11	25.48±0.25	24.56±0.22	27.16±0.48	26.98±0.38	35.58±0.37	35.58±0.43

Table 3: Antifungal index of FAMES isolated from *Citrullus colocynthis* L. tested against *A. flavus* and *A. ochraceus* (Biomass technique on liquid medium) (P<0.05)

		10	20	30	40	50	60	70	80	90	100
<i>A. flavus</i>	Crude ester	2.79±0.07	1.90±0.02	7.35±0.17	2.43±0.18	7.40±0.10	3.41±0.12	2.40±0.11	2.55±0.03	1.38±0.11	1.18±0.05
	Major ester	3.47±0.13	4.40±0.12	5.13±0.16	2.29±0.17	2.64±0.04	1.47±0.15	1.88±0.06	4.47±0.13	8.64±0.16	4.68±0.04
<i>A. ochraceus</i>	Crude ester	1.30±0.09	2.27±0.15	2.71±0.11	2.53±0.05	3.16±0.09	6.46±0.15	5.50±0.14	6.73±0.11	6.77±0.06	5.21±0.15
	Major ester	2.44±0.12	2.06±0.06	5.16±0.08	1.66±0.05	4.71±0.09	7.31±0.09	7.41±0.11	6.61±0.07	5.87±0.02	5.19±0.02

Figure 1: GC spectrum of the major FAMES isolated from oil *Citrullus colocynthis* L.

Figure 2: Mass spectrum of the methyl ester of palmitic acid (Peak at 8.63 minutes)

Figure 3: Mass spectrum of the methyl esters of α -linolenic and linoleic acids (peaks at 9.48-9.53 minutes)

Figure 4: Mass spectrum of the methyl ester of stearic acid (Peak at 9.62 minutes)

Figure 5: Infrared spectrum of FAMES

Figure 6: ¹H-NMR Spectrum of FAMES

Figure 7: Spectrum ¹³C-NMR of FAMES

Figure 3

The analysis of the data in Figure 3 shows that the methyl ester of linoleic acid has an abundant molecular ion ($m/z = 294$) and a residual ion due to the loss of the McLafferty ion ($m/z = 220$), although the McLafferty ion ($m/z = 74$) has a low abundance. The peak at $m/z = 263.1$ corresponds to the fragmentation $[M - 31]^+$. The hydrocarbon ions of general formula $[C_nH_{2n-3}]^+$ dominate in the lower mass range ($m/z = 67, 81, 95, 109, 123$, etc.). Reading the mass spectrum of the peak at 9.62 minutes confirmed that the corresponding compound is methyl ester of stearic acid.

Figure 4

The results in Figure 3.³² showed the peak of the molecular ion at $m/z = 298$, the fragment ions at $m/z = 74$ (McLafferty rearrangement), loss of OMe ($m/z = 267$) (the cleaving β) at $m/z = 87$ and the ion $[M - C_nH_{2n} + 1]^+$ fragments to ($m/z = 255, 241, 213, 199, 185, 157, 143, 129$ and 101).

Analysis by infrared (IR)

The IR spectrum of the FAMES displayed strong carbonyl absorption at (1743.37 cm^{-1}). Absorption at (3009.71 cm^{-1}) is characteristic to olefinic ($=C-H$) stretching. Similarly, the absorption at (1658.37 cm^{-1}) correspond to $C=C$ stretching (Figure 5).

Figure 5**RMN.¹H analysis**

NMR in chemistry becomes an increasingly important tool. Used in the characterization of compounds These developments are often conducted in specialized laboratories, especially interested in the development of methods for chemical systems which are often only "plans to test" validation of these methods. It is based on the magnetic property of certain atoms (or nuclei), the proton (1H), carbon (^{13}C). We did use this method to determine the number of protons and their distribution in the carbon chain. As seen in Figure 6, the proton NMR spectrum (400 MHz) showed a large singlet at ($\delta\ 3.6\text{ ppm}$) assigned to the ester methyl groups. Moreover, the presence of vinylic protons was confirmed by the signals between (5.25 and 5.28 ppm). Another massive signal at 0.819 ppm was assigned to the protons of methyl group CH_3-R .

Figure 6**Analysis RMN.¹³C**

The carbon-13 NMR (^{13}C or sometimes simply called carbon NMR) is the application of nuclear magnetic resonance (NMR) carbon. It allows the identification of the carbon atoms in an organic molecule. Thus, the ^{13}C NMR is an important tool in determining the chemical structure of organic chemistry. Only the carbon isotope ^{13}C spin-1/2, whose natural abundance is only 1.1 %, is detectable by NMR, because the main isotope of carbon ^{12}C has a spin zero. As depicted in Figure 7, the ^{13}C NMR spectra of FAMES showed carbonyl signals around (174.28 ppm), while signals between (130.20 and 127.90 ppm) indicated the presence of vinylic carbons ($C=C$). Furthermore, resonances corresponding to methylene carbons appeared between (34.09 and 22.56 ppm).

Figure 7**Antifungal activity of FAMES**

This part of the study aims to evaluate the antifungal activity of crude esters and major fraction of esters by two techniques namely the evaluation of the radial growth on solid medium, and the assessment of biomass liquid medium. The findings of the antifungal activity screening using the inhibition of radial growth on solid medium revealed that the FAME's exhibited a potential antifungal activity against *A. flavus* and *A. ochraceus* and inhibited the growth of fungi, even at low concentrations. The antifungal power of the methyl esters of *C. Colocynthis* was optimal on *A. flavus* than the second fungus at all dilutions (Table 2). The effect on *A. flavus* was observed especially at concentrations of 50; 80 and 90 $\mu\text{L/mL}$ with inhibition index of 46.58 ± 0.22 ; 48.30 ± 0.22 and $47.43 \pm 0.19\%$, respectively. This inhibition was also observed on *A. ochraceus* at concentrations of 80 and 100 $\mu\text{L/mL}$ with inhibition index of 37.06 ± 0.44 and $35.58 \pm 0.43\%$. As regards the esters fraction, inhibition of mycelial growth of *A. flavus* at concentrations of 70; 80 and 90 $\mu\text{L/mL}$ was observed with inhibition percentage 51.65 ± 0.22 , and 51.67 ± 0.40 and $51.32 \pm 0.32\%$, respectively. In case of *A. ochraceus* pronounced inhibition index was noted (34.06 ± 0.44 ; 35.92 ± 0.37 and $36.92 \pm 0.43\%$) at dilutions of 80; 90 and 100 $\mu\text{L/mL}$.

Table 2

Our results are in agreement with those of Lima (2011) and Canales (2011) who reported that the methyl esters of fatty acids have antibacterial and antifungal effects^{28,29}. As described above, the esters fraction provided important inhibitory effect on solid medium against the two fungal strains at tested concentrations. Several authors particularly Chandrasekaran (2011)³⁰, Nehdi *et al.* (2013)³¹ and Choi *et al.* (2012)¹² have mentioned that FAME's isolated from the seeds oil extracted from some medicinal plants developed antifungal potency against pathogenic microorganisms. These findings correlate with the studies of Amrouche *et al.* (2013)³² who indicated that the antifungal effect is probably due mainly to the presence linoleic acid in the *C. Colocynthis* oil. As regards the antifungal indices on liquid medium, the analysis of the results obtained from the effect of the crude ester against *A. flavus* has been shown that the inhibition observed at concentrations of 30 and 50 $\mu\text{L/mL}$ with a percentage of inhibition of 7.35 ± 0.17 and $7.64 \pm 0.40\%$, respectively; while the effect on *A. ochraceus* was well noted at concentrations of 60 and 70 $\mu\text{L/mL}$ with a percentage of inhibition of 6.46 ± 0.15 and $6.65.50 \pm 0.14$, respectively. In the case of the effect of the major fraction ester, the results showed that there is an antifungal activity on *A. flavus* with better inhibition of $8.64 \pm 0.16\%$ at the concentration of 90 $\mu\text{L/mL}$. For the strain *A. ochraceus*, the results showed an antifungal effect at concentrations of 60 and 70 $\mu\text{L/mL}$ with inhibition percentage of $7.31 \pm 0.09\%$ and $7.41 \pm 0.11\%$, respectively.

Table 3

The biomass on liquid medium showed that the esters presented less effect. Similar findings were reported by Ozdemir *et al.* (2012)³³ and Elsemra *et al.* (2012)³⁴. This less effect can be assigned to the used method. Indeed, many authors have suggested that incubation under stirring can provide improved homogenization culture medium and increase contact between fungus and bioactive fraction^{35,12}. The assumption that the fractions present in the FAME's may

be responsible for the antifungal activity in reducing mycelial growth on solid media and not reacted with the same manner on liquid medium³².

CONCLUSION

Analysis of the results of this study indicates that the fatty acid esters of *Citrullus colocynthis* possess antifungal properties that could be applied as a chemical agent against fungal contamination to protect public health and reduce economic losses. In addition, the phytochemical research is needed to identify the active fatty acid responsible for the antifungal effect.

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REFERENCES

- Constance Vermue. Mycotoxins: news, epidemiology, prevention and risk management; 2009. p. 182.
- Tabuc, C. Fungal flora of different substrates and optimal output of mycotoxins. PhD thesis. Unpublished, National Polytechnic Institute, University of Bucharest. Toulouse; 2007.
- Nguyen Minh Tri M. Species identification of potentially producing mold mycotoxins in rice sold in five provinces of the central region of Vietnam-study conditions that may reduce the production of mycotoxins. PhD thesis. The National Polytechnic Institute of Toulouse; 2007. p. 147.
- Pineiro M, Nagler MO, Coker R, Nicolaides L, Wareing P, Myhar R. Manual on the application of the system of risk analysis, critical points for their control (HACCP) for the prevention and control of mycotoxins. FAO Food and Nutrition Paper No. 73; 2005. p. 92.
- Canet C. Mycotoxins in food: assessment and risk management chapter XVI. Part Four: risk management. France: Technology and documentation (Lavoisier); 1999. p. 409-427.
- Benzeggouta Naïrouz. Study on the Antibacterial Activity of Infused Oils of Four Medicinal Plants Known As Food. Thesis Constantine Algeria; 2005. p. 134.
- Chebaibi A et al. Study of antimicrobial activity of the leaves of the olive tree *Olea europaea* L. Art. day. Sc Fac. Sc Kenitra; 2007.
- Belsem Marzouk Z, Décor R, Edziri H. et al. Antibacterial and anticandidal screening of Tunisian *Citrullus colocynthis* Schrad. From Medenine. Journal of Ethno pharmacology 2009; 125: 344-349. <http://dx.doi.org/10.1016/j.jep.2009.04.025>
- Sebbagh N, Cruciani Guglielmacci C, Ouali F, Sari C. Comparative effects of *Citrullus colocynthis*, sunflower and olive oil enriched diet in streptozotocin-induced diabetes in rats. Algeria. Diabetes and Metabolism 2009; 35: 178-184. <http://dx.doi.org/10.1016/j.diabet.2008.10.005>
- Dallak M, Bin Jaliah I. Antioxidant activity of *Itrullus colocynthis* pulp extract in the rbc's of alloxan-induced diabetic rats. Pak J Physiol; 2010. p. 5.
- R Savitha, T Shasthree, Sudhakar B Mallaiiah. High frequency of plantlet regeneration and multiple shoot induction from leaf and stem explant of *Citrullus colocynthis* (L) Schrad, an endangered medicinal cucurbit; 2010.
- Choi GJ, Jang KS, Choi YH, Yu JH, Kim JC. Antifungal activity of lower alkyl fatty acid esters against powdery mildews. Plant Pathol J 2012; 26(4): 360-366.
- Belsem Marzouk, Zohra Marzouk, Ehsen Haloui, Nadia Fenina, Abderrahman Bouraoui, Mahjoub Aouni. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. Elsevier Journal of ethno pharmacology; 2010. p. 15-19. <http://dx.doi.org/10.1016/j.jep.2009.11.027>
- Angé C, Bourget E. Purification and analysis of organic compound. 4th ed. Ellipses: Laboratory Techniques; 2008. p. 156.
- Peter K, Velhardt C, Neil ES. Treatise of organic chemistry. 5th ed. De Boeck; 2009.
- Wheelis M. Principles of modern microbiology. Canada: John and Barlett Publishers; 2008. p. 293-300.
- Dugan FM. The identification of fungi: an illustrated introduction with keys glossary and guide to literature. Portland: Book News, Inc.; 2006.
- Pitt JI, Hocking AD. Fungi and food spoilage. 3rd ed. London: Springer Science; 2009. <http://dx.doi.org/10.1007/978-0-387-92207-2>
- Soro S, Ouattara D, Guédé NZ, Coffi K, et al. Inhibitor *in vitro* and *in vivo* Effect of extract powder and oil Essential *Xylopia aethiopica* (Dunal) A. Rich. (Annonaceae) on *Fusarium oxysporum* f. sp *Radicis lycopersici* (Forl), Fungus Parasite Cultures of Tomato. European Journal of Scientific Research 2010; 39 (2): 279-288.
- Kra KD, Diallo HA, Kouadio YJ. Antifungal activities of the extract *Chromolaena odorata* (L.) King and Robins *Fusarium oxysporum* two isolates (EF Sm.) Responsible for lethal yellowing leaves of banana trees. Journal of Applied Biosciences 2009; 24: 1488-1496.
- Singh P, Kumar A, Dubey NK, Gupta R. Essential Oil of *Aegle marmelos* as a Safe Plant-Based Antimicrobial Against Postharvest Microbial Infestations and Aflatoxin Contamination of Food Commodities. Journal of food science 2009; 74(6): 302-307. <http://dx.doi.org/10.1111/j.1750-3841.2009.01223.x>
- Tubajika KM. Efficacy of alkyl dimethyl benzyl ammonium chloride on supression of *Physalospora vaccinii* in laboratory assays. Journal of food protection 2006; 69(10): 2460-2464.
- Hibar K, Daami Remadi M, Jabnoun Khiareddine H, Znaïdi IE, El Mahjoub M. Effect of compost extracts on mycelial growth and aggressiveness of *Fusarium oxysporum* f. sp. *Radicis lycopersici*. Biotechnol. Agron. Soc. About 2006; 10(2): 101-108.
- Dhandhukia PC, Thakkar VR. Standardization of growth and fermentation criteria of *Lasiodiplodia theobromae* for production of jasmonic Acid. African Journal of Biotechnology 2007; 6(6): 707-712.
- Imtiaj A, Lee TS. Screening of antibacterial and antifungal activities from Korean wild mushrooms. World journal of agricultural sciences 2007; 3(3): 316-321.
- Hassimi Sadou, Haoua Sabo, Mousbahou Malam Alma, Mahamane Saadou, Claude Louis Leger. Chemical content of the seeds and physico-chemical characteristic of the seed oils from *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum* of niger. Bull. Chem. Soc. Ethiop 2007; 21(3): 323-330. <http://dx.doi.org/10.4314/bcse.v21i3.21213>
- Akpambang V, Amoo IA, Izuagie AA. Comparative compositional analysis on two varieties of melon (*Colocynthis citrullus* and *Cucumeropsis edulis*) and a variety of almond (*Prunus amygdalus*). Research Journal of Agriculture and Biological Sciences 2008; 4(6): 639-642.
- Lima B, Lopez S, Luna L, Agüero MB, Aragón L. Essential oils of medicinal plants from the central andes of Argentina: Chemical composition and antifungal, antibacterial and insect-repellent activities. Chem Bio divers 2011; 8: 924-936. <http://dx.doi.org/10.1002/cbdv.201000230>
- M Canales, T Hernández, MA Rodríguez Monroy, CM Flores, M Jiménez Estrada, LB Hernández, A Durán, M Hernández Moreno, NA Trejo, AB Hernández, JJ Ramírez, J Orozco I, MG Eleno and KE Martínez. Evaluation of the antimicrobial activity of *Acalypha monostachya* Cav. (Euphorbiales: Euphorbiaceae); 2011.
- Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. Eur Rev Med Pharmacol Sci 2011; 7: 75-80.
- Nehdi IA, Sbihi H, Tan CP, Al Resayes SI. Evaluation and characterisation of *Citrullus colocynthis* (L.) Schard seed oil: comparison with *Helianthus annuus* (sunflower) seed oil. Food Chem 2013; 136(2): 348-353. <http://dx.doi.org/10.1016/j.foodchem.2012.09.009>
- Amrouche A, Benmehdi H, Dalile H, Chabane Sari M, Zaaboub I, Smahi DE, Moussaoui A and Chabane Sari D. Evaluation of antifungal activity of free fatty acids methyl esters fraction isolated from Algerian *Linum usitatissimum* L. seeds against toxigenic *Aspergillus*. Asian Pacific Journal of Tropical Bio medicine 2013; 3(6): 443-448.
- Ozdemir H, Kara MI, Erciyas K, Ozer H, Ay S. Preventive effects of thymoquinone in a rat periodontitis model: a morphometric and histopathological study. J Periodontol Res 2012; 47(1): 78-80. <http://dx.doi.org/10.1111/j.1600-0765.2011.01406.x>
- Elsenra AI, Shama SM, Akila SH, Nessrine HY, Mervat SY, Sawssan MA. Effect of treatment with mold inhibitors on plant growth of corn and some nutritional components of stored grains, infected with *Aspergillus flavus* and *Fusarium verticilloides*. eSci J Plant Pathol 2012; 01: 6-13.
- Fouzia Amamou, Daoudi Chabane Sari, Miloud Bouafia, Radjaa kaouthar Meziane, Abdelhafid Nani. *Citrullus colocynthis*: a desert plant native in Algeria, effects of fixed oil on blood homeostasis in Wistar rat (Scholars Research Library); 2011. p. 7.

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