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Chemical Composition, Free Radical Scavenging And Antifungal Activity of Zanthoxylum Leprieurii Essential Oils Against Epidermophyton Floccosum And Microsporum Gypseum, two Most Prevalent Cutaneous Mycosis

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Abstract
Background: *These investigations were carried out to analyze the chemical composition, to evaluate the radical scavenging, and the anti-fungal activities of essential oils from leaves and fruits of Zanthoxylum leprieurii.*
Methods: *Oils were obtained by hydro distillation using a Clevenger type apparatus. The compounds were identified by gas chromatography and gas chromatography coupled with Mass Spectrometry. Antifungal activity of the essential oils was tested in vitro against Epidermophyton floccosum and Microsporum gypseum while free radical scavenging activity evaluation was done using 2,2 diphenyl picrylhydrazyl method.*
Results*: Results showed that, the major components of the leaves oil were (E)-ß-ocimene (91.5 %) while the most abundant components from fruits oil were also (E)-ß-Ocimene (90.3 %) with pinocarveol (2.8%) and myrcene (2.3 %). The total growth inhibition of the pathogens by fruits essential oil occurred at 4000 ppm and 3000 ppm respectively, for Epidermophyton floccosum and Microsporum gypseum. The leaves oil exhibited total growth inhibition at 4000 ppm against both pathogens. These oils also showed antioxidant activities with SC_{50} values at 0.77 g/L and 1.80 g/L respectively, for fruits and leaves. There were positive and significant correlations between mycelia growth inhibition of both pathogens and the antioxidant activities of the tested oils.*
Conclusion: *These results showed that, Z. leprieurii essential oils could be used as a source of antioxidant and antidermatophitic compounds which may find applications in pharmaceutical industries.*
Keywords*: Essential oil, Antidermatophytitic activity, Antioxidant activity, Zanthoxylum leprieurii*

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I. INTRODUCTION

Cutaneous mycosis is a human dermatophytosis which is the most common infection in skin and nail worldwide especially in Sub-Africa countries including Cameroon. This infection is often caused by filamentous fungi belonging to the genera Epidermophyton, Trichophyton and Microsporum [1]. Among them, E. floccosum and M. gypseum are widespread in most countries of the world, accounting for 5 % of all dermatophytes isolated [2]. Some preliminary works in Cameroon found that these two fungi were the most prevalent cutaneous mycosis. These fungi are anthropophilic dermatophytes that are transmitted between individuals by contact, particularly in community swimming pool areas, common showers, and gym facilities. The clinical presentation predisposing factors for mycosis include immunosuppression, diabetes mellitus, advanced age nail trauma, and poor peripheral circulation [3]. Free radicals are produced in normal and or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in triggering many diseases including dermatosis. Exogenous chemical and endogenous metabolic processes in the human body might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage [4].

The clinical care of cutaneous mycosis is based on combination of a topical and systemic chemical antifungals treatment. However, synthetic antifungals suffer from various weaknesses in terms of toxicity, lack of fungicidal efficacy, cost and emergence of resistant strains caused by the frequent use of some of them [5]. In

spite of the recent introduction of new antifungal drugs, they are still limited in number. Hence, there is a great demand for novel antifungal agents, justifying the intense search for new drugs that are more effective and less toxic than those already in use [6, 7].

An alternative should be use of plant derivate products such as the essential oils (EO) to control dermatophytes infections. Essential oils are the odorous, volatile products of plant secondary metabolism normally formed in special cells or groups of cells or as glandular hair, found on any organ of the plant including roots, stem, leaves, flowers and fruits. EO have demonstrated various biological activities including anticancer, antiviral, antibacterial, antifungal and antioxidant activities [8,9,10]. As well as their ability to facilitate the passage of active molecules through the human tissues [11]. Zanthoxylum leprieurii is a large species of the family of Rutaceae which is geographically distributed across Africa. In traditional Cameroon medicine, this plant is used for treatment of intestinal worms, abdominal pain, hyperhermia, diarrhea and certain mycosis [12]. Previous works have demonstrated the antimicrobial activities of Zanthoxylum leprieurii essential oil from Cameroon [13]. However, as far as we know, there is no report on the antifungal properties of this oil against E. floccosum and M. gypseum.The aim of this study was to analyze the chemical composition of Zanthoxylum leprieurii essential oils and to evaluate their antioxidant and antifungal activities against E. floccosum and M. gyseum, the two most common causal agents of cutaneous mycosis.

II. MATERIALS AND METHODS

a) Plant material

Leaves and fruits of Z. leprieurii were collected at Bamena (West Region of Cameroon) on July 2014. The herbarium/plants were identified at the National Herbarium where voucher specimen was deposited under the reference 2713/SRFK/CAM.

b) Essential oils extraction

About 200-400 g of air-dried leaves and fruits (after grounded) were coarsely crushed. Each sample was distillated for about 4 h using Clevenger-type apparatus. Oils collected were dried over anhydrous sodium sulphate, stored in an amber-colored flask and kept at 4°C until use.

c) Essential oils analysis

Essential oils were analysed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS).

d) Gas chromatography

Each oil was analysed on a Varian CP-3380 GC with flame ionisation detector fitted with a fused silica capillary column (30 m x 0.25 mm coated with DB-5, film thickness 0.25 µm); temperature program 50°C-200°C at 5°C/min, injector temperature 200°C, detector temperature 200°C, carrier gas N_2 1 ml/min. The linear retention indices of the components were determined relatively to the retention times of a series of n-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors.

e) Gas chromatography coupled with mass spectrometry

GC-MS analyses were carried out using a furnace Focus GC (Thermo) apparatus equipped with a TG-5MS5MS column (30 m x 0.25 mm, film thickness, 0.25 µm) and interfaced with a quadruple detector (DSQ II). Column temperature was programmed from 60 to 200° C at 10° C/mn; injector temperature was 220° C. The injections were carried out in mode SPLIT (Ratio: 1/100). Helium was used as carrier gas at a flow rate of 1.2 ml/min; the mass spectrometer was operated at 70 eV.

The components were identified based on the comparison of their retention indices and their mass spectra with those given in the literature [14] .

f) Fungal pathogen

The references of fungal pathogen used for the study were E1420 for Epidermophyton floccosum and BD 023 for Microsporum gypseum. They were obtained from the Laboratory of the Ecole National Vétérinaire d'Alfort in France. After 21 days of incubation at 28 °C, the mycelia emerging from tissues were transferred into fresh Sabouraud medium. The operation was repeated many times to obtain pure culture of each isolate. Identification of both fungi isolate was based on macroscopic and microscopic criteria according to the method described by [15,16]. The cultures were stored at 4°C and sub-culture every three month.

g) Antifungal assay

The inhibition of mycelia growth was performed using agar incorporation method [17]. The test was carried out in 90 mm Petri dishes containing SDA medium supplemented with chloramphenicol. Four millimeters diameter disc was taken from the margin of 9 days old pure culture of each fungus, seeded at the center of Petri dishe and incubated at 28±2°C. Oils were previously mixed with DMSO (Dimethylsulfoxide) to obtain a final concentration of 10 % (v/v) and added into the medium to produce final concentrations of 0.5, 1, 2, 3, 4 and 5 g/L. Plate containing SDA with DMSO was negative control while griseofulvine served as positive control. Each treatment consisted of four Petri plates and the experiment repeated twice. After 14 days of incubation, the antifungal activity of each essential oil was evaluated by calculating the percentage of inhibition (% I) of mycelia growth using the following formula: % I = ((Do – De)/Do) x 100 where Do is the diameter of the mycelia growth in the negative control and De the diameter of the mycelia growth in each oil supplemented plates. Fungistatic or fungicide effect of oils was determined by transferring the discs from the plate where no growth occurred in to the new SDA medium.

h) Antioxidant assay

This was carried out by scavenging of the stable 2,2-diphenylpicrylhydrazyl (DPPH) radical according to the method describe by Brand-William et al [18]. Ten microlitres of various concentrations (1 to 5 g/L) of each essential oil in methanol was added to 1990 µl of a 10 g/L methanol solution of DPPH. After 60 min incubation period at room temperature, the absorbance was read at 517 nm. Percent of inhibition of the DPPH free radical (I %) was measured using the following equation: (%) $I = (A_{DPPH} - A_{sample} / A_{DPPH}) \times 100$, where A_{DPPH} is optical density of the positive control, A_{sample} is optical density of the essay (essential oil). Butyl Hydroxy Toluene (BHT) was used as a standard chemical reference antioxidant at the various concentrations ranging from 0.04 to 0.4 g/L.

i) Statistical analysis

Data were analyzed using SPSS version 16.0. Results were presented in term of means. Multiple comparisons of mean values were set up using one-way parametric ANOVA. The DUNCAN test was used to appreciate the differences between the means at $p<0.05$. The relationship between the different parameters was done using Pearson correlation.

III. RESULTS AND DISCUSION

Chemical composition of the essential oils

Essential oils obtained by hydrodistillation, yielded 0.025 % and 1.45 % respectively, for leaves and fruits (Table 1). Twenty one and twenty six compounds were identified representing 96.0% and 100 % of volatile oil from leaves and fruits respectively. The major components of the leaves oil were (E)-ß- Ocimene (91.5 %) while (E)-ß-Ocimene (90.3 %), Pinocarveol (2.8 %) and Myrcene (2.3 %) were the major components of the fruit oil. However, some minor components were present only in the leaf oil (α-Pinene, Limonene, (2E)-Octen-1-ol, Linalol, Nobornyl acetate, Octyl acetate, Neryl acetate and Germacreme B) and fruit oil (α-Terpinene, ß-Phellandrene, ϒ-Terpinene, Cis-Linalool axide, α-Pinene oxide, Cis-para-menth-2-enol, Allo-ocimene, Myrtenol, α-Terpineol, Myrtenal, Pinocarvone, ϒ-Curcumene and Trans ß-Guiaene).

The essential oils of Z. leuprieurii from different countries including Cameroon have been previously studied. The analysis reveals a great degree of variability and plasticity in Zanthoxylum essential oil yield and composition [19, 20]. From leaves oil of Costa Rica, [20] obtained 1.35% yield, and a total of 12 components. The major components were citronellal (23.3%), limonene (16.7%), trans-pinocamphone (11.6%), and β-pinene (10.8%). Fogang et al (2012) obtained (E)-β-ocimene (29.4%), myrcene (28.6%), limonene (13.6%) and α-pinene (8.1%) as the major compounds in leaves oil from Cameroon. Lamary et al., (1989) studied Z. leprieurii volatile oils from leaves and fruits of Cameroon. Their results showed that the oil distilled from the leaves contained only hydrocarbons. Among them, α-pinene (40.4%), myrcene (27.1%) and trans-β-ocimene (22.4%) were the most important. The composition of the fruit oil is more diverse, with a fairly large proportion of oxygenated products (46%) among which citronellol, geraniol and their acetates are the most abundant. Globally, these components were quantitatively and qualitatively different from those obtained in our study. In general, it is well demonstrated that, variation in yield and chemical composition could by explain by the extrinsic and intrinsic parameters such as the age of the plant, organ, soil, climate and ecological factors [21, 22].

Table 1: Chemical composition of leaves and fruits essential oils of Zanthoxylum leprieurii
*KI Kovats index was determined by Gas Chromatography on a DB_5 column

N°	Compounds	*KI	Relative Percentage (%)	
			Leaves	**Fruits**
1	α-Thujiene	925	0.3	0.2
2	α-Pinene	943	0.1	-
3	Camphene	954	0.1	0.1
4	β-Pinene	977	0.1	0.1
5	Octanol	983	0.1	0.3
6	Myrcene	990	-	2.3
7	α-Phellandrene	1000	0.8	0.1
8	α- Terpinene	1004	-	0.1
9	p-Cymene	1022	0.1	0.8
10	β-Phellandrene	1029	-	0.1
11	Limonene	1032	0.2	-
12	**(E) -β-Ocimene**	1039	**91.5**	**90.3**
13	(2E)-Octen 1-ol	1059	0.4	-
14	ϒ-Terpinene	1055	-	0.5
15	Terpinolene	1087	0.2	0.1
16	Cis-Linalool oxide (furanoid)	1084	-	0.1
17	Linalol	1090	0.1	-
18	α-pinene oxyde	1093	-	0.5
19	Pinocarveol	1109	0.4	2.8
20	cis-p-Menth-2-en-1-ol	1118	-	0.1
21	α-Campholenal	1124	-	0.3
22	Norbornyl acetate	1127	0.2	-
23	Allo-ocimene	1128	-	0.2
24	t-Pinocarveol	1132	0.6	0.2
25	Acetate	1186	0.1	0.1
26	Myrtenol	1190	-	0.1
27	α-Terpineol	1192	-	0.1
28	Myrtenal	1194	-	0.2
29	Octylacetate	1196	0.1	-
30	Pinocarvone	1343	-	0.1
31	Neryl acetate	1345	0.2	-
32	□ □ Curcumene	1472	-	0.1
33	Germacrene B	1475	0.2	
34	Trans-β-guaiene	1512	-	0.1
35	(2E, 6E)- Farnesyl acetate	1840	0.1	-
36	Farnesyl acetone	1850	0.1	-
Total			**96.0**	**100**
Yield (%)			**0.025**	**1.45**

2.Effects Of Essential Oils On Mycelia Growth Of E. Floccosum And M. Gypseum

Essential oils from leaves and fruits of Z. leprieurii showed activity in inhibiting the growth of E. floccosum and T. rubrum (Table 2). In fact, results showed that, there are significant differences ($p<0.05$) in the mycelia growth of each EOs supplemented samples compared with the negative control. Total inhibition of mycelia growth of M. gypseum occurred at 3000 ppm and 4000 ppm by Eos from fruits and leaves respectively while E. floccosum was completely inhibited at 4000 ppm by the oils. Maximal inhibition by Gliseofulvin was observed at 2000 ppm for M. gypseum and E. floccosum.

Many studies have been reported on the antifungal activities of Zanthoxylum essential oil [23, 24, 25, 26]. The antifungal properties of our samples could be due to the complexity of their chemical composition and structure of their different constituents. It could also be due to the presence of the main compound such as ocimene or to a possible synergistic effect of the minor or major compounds in the essential oil [27, 28]. The antifungal activities of compounds present in our tested essential oils may be explained to their lipophilic character and ability to disrupt the cellular membrane permeability and lead to the death of the cell [11]

Table 2 Mycelia growth inhibition of M. gypseum and E. floccosum by leaves and fruits essential oil of Z. leprieurii

Oils	M. gypseum			E. floccosum		
	Leaves	Fruits	Griseofulvine	Leaves	Fruits	Griseofulvine
Conc (ppm)	Inhibition percentage (%)					
0.0	0.0^{f}	0.0^{f}	0.0^{d}	0.0^{f}	0.0^{f}	0.0^{c}
500	15.0^{e}	16.0^{d}	56.0^{c}	24.0^{d}	21.66^{d}	50.0^{b}
1000	30.33^{d}	43.33^{c}	58.0^{c}	37.0^{c}	41.0^{c}	52.0^{b}
2000	69.0^{c}	64.66^{b}	100.0^{a}	37.33^{c}	41.0^{c}	100.0^{a}
3000	88.0^{b}	100.0^{a}	100.0^{a}	58.66^{b}	56.33^{b}	100.0^{a}
4000	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
5000	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}

In the same column, values followed with the same letter are not significantly different ($p \leq 0.05$) according to Duncan test.

Antioxidant activities of essential oils

Oils from leaves and fruits of Z. leprieurii showed effective free radical scavenging in DPPH assay (Figure 1). The antioxidant activities increase significantly with the concentration of each essential oil. The oils from fruits exhibited a remarkable antioxidant effects at low concentration with SC_{50} at 0.77 g/L, while SC_{50} from leaves oils was 1.80 g/L. Nevertheless, at all concentration tested, the chemical standard antioxidant BHT had a great effect compare to our oils sample and exhibited the most less SC_{50} (0.05 g/L).

It has been demonstrated that plant extract and volatile essential oil can exhibited significant antioxidant activities [29, 30, 31]. This property could be assigned to many compounds such as aromatic and oxygenated monoterpenes [32, 33, 34] which are presents in our essential oils. Similarly, [11] showed that, antioxidant and antimicrobial activities of essential oil could be related to the presence of a variety of volatile molecules such as terperne and terpenoid, phenol derived aromatic components and aromatic components.

Free-radicals are generated continuously in the body due to metabolism and diseases [35]. In order to protect themselves against free radicals, organisms are endowed with endogenous (catalase, superoxide dismutase, glutathione peroxidase/reductase) and exogenous (C and E vitamins, carotene, uric acid) defense systems. These defense systems are not sufficient in critical situations such as oxidative stress, contamination, microbial infections, where the production of free radicals significantly increases [36]. In our study, results showed that there are positive and significant correlations between the mycelia growth inhibition of E. floccosum and antioxidant activity of fruits (r^2= 0.524; p = 0.025) and leaves Eos (r^2= 0.734; p = 0.01). Positive correlation were also observed between M. gypseum growth inhibition and antioxidant properties (r^2= 0.959; p = 0.000; r^2= 0.974; p = 0.000) respectively, from fruits and leaves Eos. [37] studied the oil from Zanthoxylum sp. and found that, it had antifungal and strong antioxidant activity. There is increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants.

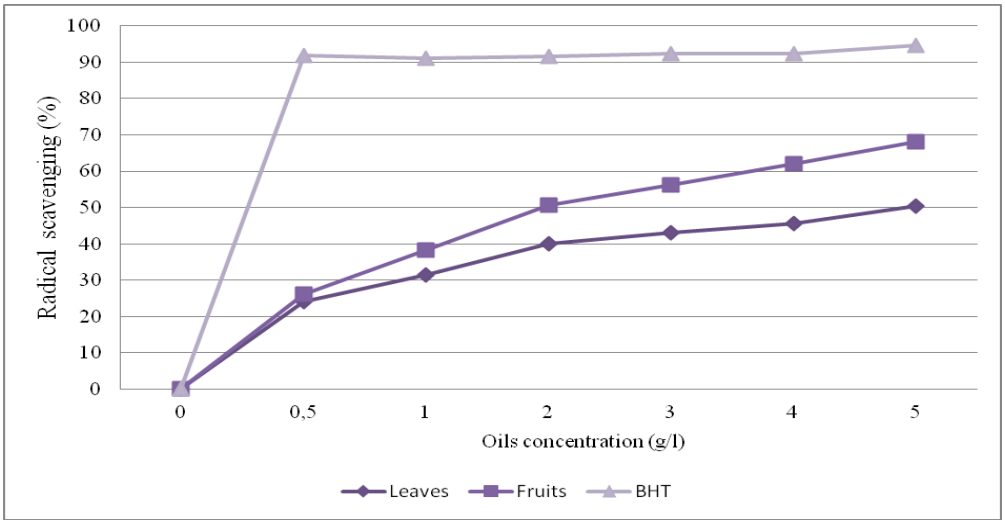

Figure 1: Antioxidant activities of leaves and fruits essential oils of Z. leprieurii

IV. CONCLUSION

According to our results, leaves and fruits of Z. leprieurii essential oils significantly inhibit the mycelia growth of E. floccosum and M. gypseum and also have antioxidant properties. These could be assigned to the synergic effects of minor and major components in the oils. This finding demonstrated that, Z. leprieurii essential oils could be served as alternative to chemical fungicide to fight against dermatophytosis. Moreover, these oils are potential natural antioxidant. However, in vivo tests are needed.

REFERENCES

[1]. Canizares, O. and Harman, R. Clinical tropical dermatology. 1992; 2nd ed. Boston: Blackwell Scientific Publications 859p

[2]. Summerbell, R.C., Weitzman I. and Padhye A. A. Trichophyton, Microsporum, Epidermophyton and agents of superficial mycoses. In PR Murray et al. (eds) 2007; Manual of Clinical Microbiology. Ch. 124. 9th ed. ASM Press. Washington, DC. p1874-1897.

[3]. Elewski, B.E. Onychomycosis: Pathogenesis, Diagnosis, and Management. 1998; Clin. Microbiol. Rev. 11(3): 415-429.

[4]. Mau, J.L., Chao, G.R., and Wu, K.T. (2001). Antioxidant properties of methanolic extracts from several mushrooms. 2001; J. Agric. Food Chem. 49(11):5461–5467.

[5]. Pinto, E., Silva, L.V., Cavaleiro, C., and Salgueiro, L. Antifungal activity of the clove essential oil from Syzygium aromaticum on Candida, Aspergillus and dermatophyte species. 2009; J. Med. Microbiol. 58:1454-1462.

[6]. Kauffman, C.A. Fungal infections. 2006; Proc. Am. Thorac. Soc. (3): 35-40.

[7]. Rapp, R. P. Changing strategies for the management of invasive fungal infections. 2004; Pharmacotherapy 24: 4S–28S.

[8]. Sameza, M.L., Bedine, B.M.A., Tchameni, N.S., Nguemnang, M.L.C., Jazet, D.P.M., Fekam, B.F., and Menut, C. (2014). Potential use of Eucalyptus globulus essential oil against Phytophthora colocasiae the causal agent of taro leaf blight. 2014; Eur. J. Plant Pathol. 140:243-250.

[9]. Delazar, A., Yari, S.M., Chaparzadeh, N., Asnaashari, N., Nahar, L., Delazar, N. and Sarker, D.S. Chemical composition, free-radical-scavenging and insecticidal properties, and general toxicity of volatile oils isolated from various parts of Echinophora orientalis. 2015; J. Essent. Oil Bearing Plants 18(6):1287-1297.

[10]. Afshar H.E., Delazar, A., Nazemiyeh, H., Asnaashari, S., Nahar, L., and Sarker, S. D. Chemical composition, free-radical-scavenging and insecticidal properties, and general toxicity of volatile oils of two Artemisia species growing wild in Iran.2015; J. Essent. Oil Bearing Plants 18(6) 1406-1416.

[11]. Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. Biological effects of essential oils: A review. 2008; Food Chem. Toxicol. 46: 446-475.

[12]. Wouatsa, N.A.V., Misra, L., Kumar, S., Prakash, O., Khan, F., Tchoumbougnang, F. and Venkatesh, R.K. Aromatase and glycosyl transferase inhibiting acridone alkaloids from fruits of Cameroonian Zanthoxylum species. 2013; Chem. Central J. 7:1-25, 576:180-188.

[13]. Tatsadjieu, L.N., Essia Ngang, J.J., Ngassoum, M.B. and Etoa, F.X. Antibacterial and antifungal activity of Xylopia aethiopica, Monodora myristica, Zanthoxylum xanthoxyloides and Zanthoxylum leprieurii from Cameroon. 2003; Fitoterapia 74: 469-472.

[14]. Adams, R. P. Identification of essential oil components by gas chromatography mass spectrometry. 2007; Illinois: Allured Publishing Corporation. 495p.

[15]. Rippon, J.W. Medical Mycology.The Pathogenic Fungi and the Pathogenic Actinomycetes. 1988; 3rd ed., WB Saunders, Philadelphia, 797 pp.

[16]. Emmons, C.W., C.H. Binford, and Utz, J.P. 1970; Medical mycology, 2nd ed. Lea & Febiger, Philadelphia.

[17]. Lahlou, M. Methods to study the phytochemistry and bioactivity of essential oils. 2004; Phytotherapy Res., 18,435–448.

[18]. Brand-William, W., Curvelier, M.E. and Berset, C. Use of a free radical method to evaluate antioxidant.1995;Lebensmittel-Wissenschaf und-Technologie 28(1):25-30

[19]. Jazet, D.P.M., Tatsadjieu, N.L., Manedong, K.S., Ndongson, D.B., Amvam, Z.P.H. and Menut, C. Inhibitory effect of essential oils of some cameroonian Zanthoxylum (Rutaceae) Against Fusarium solani. 2009; Eur. J. Sci. Res. 34(1): 34-45.

[20]. Miller, S.L., Haber, W.A. and Setzer, W.N. Chemical composition of the leaf essential oil of an undescribed species of Zanthoxylum from Monteverde, Costa Rica. Am. J. Essent. Oils Nat. Prod. 2013; 1(2): 38-40.

[21]. Vila, R., Iglesias, J., Cañigueral, S. and Cicció, J.F. Composition of the essential oil from leaves of Zanthoxylum procerum from Costa Rica. J. Essent. Oil Res. 2002; 14:44-46.

[22]. Oyedeji, A.O., Lawal, O.A., Adeniyi, B.A., Alaka, S.A., and Tetede, E. Essential oil composition of three Zanthoxylum species. J. Essent. Oil Res. 2008; 20: 69-71.
[23]. Silva, D.S.L., Figueiredo, P.M.S. and Yano, T. Chemotherapeutic potential of the volatile oils from Zanthoxylum rhoifolium Lam leaves. Eur. J. Pharmacol. 2007; 576:180-188
[24]. Jazet, D.P.M., Tchoumbougnang, F., Ndongson, B., Agwanande, W., Sandjon, B., Amvam, Z.P.H. and Menut, C. Chemical characterization, antiradical, antioxidant and anti-inflammatory potential of the essential oils of Canarium schweinfurthii and Aucoumea klaineana (Burseraceae) growing in Cameroon. Agri. Biol. J. North Am. 2010; 1(4): 606-611.
[25]. Ngoumfo, R. M., Jouda, J.B., Mouafo, F.T., Komguem, J., Mbazoa, C.D., Shiao, T.C., Choudhary, M.I., Laatsch, H., Legault, J., Pichette, A., and Roy, R. In vitro cytotoxic activity of isolated acridones alkaloids from Zanthoxylum leprieurii Guill. et Perr. Bioorg. Med. Chem.2010; 18: 3601-3605.
[26]. Patiño, O.J.R., Prieto, J.A. and Cuca, L.E. (2011). Zanthoxylum Genus as Potential Source of Bioactive Compounds 185-218. In Bioactive Compounds in Phytomedicine, Edited by Iraj Rasooli. Janeza Trdine 9, 51000 Rijeka, Croatia
[27]. Delaquis, P.J., Stanich, K., Girard, B. and Mazza, G. Antimicrobial activity of individual and mixed fraction of dill, cilantro, coriander and eucalyptus essential oil. Int. J. Food Microbiol. 2002; 74(1–2): 101–110.
[28]. Vila, R., Santana, A. I., Pérez-Rosés, R., Valderrama, A., Castelli, V., Mendonca, S., et al. (2010). Composition and biological activity of the essential oil from leaves of Plinia cerrocampanensis, a new source of a bisabolol. Bioresour. Technol. Int. J. Food Microbiol. 2010; 153(1-2): 183–191.
[29]. Tchinda, T.A., Fuendjiep, Sajjad A, Matchawe, C., Wafo, P., Khan, S., Tane, P. and Choudhary, M.I. Bioactive compounds from the fruits of Zanthoxylum leprieurii. Pharmacologyonline 2009; 1: 406-415.
[30]. Xia, L., You, J., Li, G., Sun, Z., and Suo, Y. Compositional and Antioxidant Analysis of Zanthoxylum bungeanum seed oil obtained by Supercritical CO_2 Fluid Extraction. J. Am. Oil Chem. Soc. 2011; 88 (7): 1029-1036.
[31]. Boone, G. L., Román, R. A., Aranda, R. S., Cirio, R.T., Galindo, V.R.R., Torres, W. N., González, R. and López, P.L.A. Antimicrobial and antioxidant activities and chemical characterization of essential oils of Thymus vulgaris, Rosmarinus officinalis, and Origanum majorana from northeastern México. Pak. J. Pharm. Sci.2015; 28(1):363-369.
[32]. Chatterjee, S., Gautam, S.Z.N., Adhikari, S., Variyar, S.P. and Sharma, A. Antioxidant activity of some phenolic constituents from green pepper (Piper nigrum L.) and fresh nutmeg mace (Myristica fragrans) Food Chemistry. 2007 101(2), 515-523.
[33]. Yamazaki, E., Inagaki, M., Kurita, O. and Inoue, T. Antioxidant activity of Japanese pepper (Zanthoxylum piperitum DC.) fruit. Food Chemistry. 2007; 100: 171-177.
[34]. Fogang, P.D.H., Tapondjou, A.L., Womeni, M.H., Quassinti, L., Bramucci, M., Vitali, A.L., Petrelli, D., Lupidi, G., Maggi, F. Papa, F., Vittoric, S. and Barbonid, L. Characterization and biological activity of essential oils from fruits of Zanthoxylum xanthoxyloides Lam. and Z. leprieurii Guill. & Perr: two culinary plants from Cameroon. Flavour Fragr Journal.2009; 27: 171-179.
[35]. Yeum, K.J., Aldini, G., and Chung, H.Y. The activities of antioxidant nutrients in human plasma depend on the localization of attracting radical species. The Journal Nutriment. 2003; 133: 2688-2691.
[36]. Mondon, P., Leclercq, L. and Lintner, K. Evaluation of free radical scavenger effects of Helianthus annuus extracts using new exvivotripping methods. Cosmetics Aerosols and toiletries in Australia. 1999; 12: 87-98.
[37]. Prakash, B., Singh, P., Mishra, P.K., and Dubey, N.K. Safety assessment of Zanthoxylum alatum Roxb. essential oil, its antifungal, antiaflatoxin, antioxidant activity and efficacy as antimicrobial in preservation of Piper nigrum L. fruits. International Journal Food Microbiology. 2012; 153(1-2): 183–191.

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