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Phytochemical study and evaluation of the antioxidant activity of extracts of Opuntia Ficus-Indica cladodes from the Oriental of Morocco and the effect of microwave activation on the drying time of the plant

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Abstract

In the present work, the phytochemical study and evaluation of the antioxidant activities of the crude extracts of various solvents with increasing polarity (ethanol> acetone> dichloromethane > ethyl acetate > hexane) on fine dried cladode powder was studied. Each microwave oven power (500, 350, 160 and 90 W), of an *Opuntia ficus-indica* (OFI) medicinal species of the Cactaceae family collected in the rural commune of Bni Rais in Taourirt province of Oriental of Morroco. The phytochemical screening showed the presence of the studied chemical compounds (Flavonoids, Tannins, Quinones and Steroids) rich in powers of 500W and 350 W, especially in extracts with ethanol and acetone. The antioxidant activity of *OFI* cladodes extracts was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method. The results obtained show that the acetone extracts have a very important activity, especially at the power of 500 W, IC_{50} = 0.29 mg/ml is found followed by the ethanol extract with a value of IC_{50} equal to 0.34 mg/ml. After the results we realize that the power 500 W showing antioxidant activity of each solvent extract (Et, Ac, Dc, AE and Hx) greater than that of the power 350, 160 and 90 W respectively (IC50: 500 W <350W <160 W <90 W). Also, the effect of microwaves on the duration of drying of the plant was assessed (40 min under microwave activation compared to 17.5h of conventional heating). The cleaning activities of all extracts, however, were significantly low compared to the reference standard of ascorbic acid with a sequence IC_{50} = 0.114 mg/ml, used at the same dose.

Keywords: *Opuntia ficus-indica, Phytochemical screening, Cladode powder, Microwaves activation, Antioxydant activity.*

1. Introduction

The plant is indispensable for humans since its appearance. It has been too beneficial in terms of its nutritional use of clothing and especially medical. Until the beginning of the 20th century, almost all medicines were of plant origin through the centuries, human traditions have developed the knowledge and use of medicinal plants to improve the health of men [1]. The medicinal properties of plants are due to products synthesized by the plants themselves called secondary metabolites [2]. Many secondary metabolites, mainly polyphenols, are antibiotics in the broadest sense because they protect plants against fungi, bacteria, animals and even other plants [3]. Phenolic compounds are also known for their biological activities that are directly related to the health of humans. They are used in chemotherapy and in the treatment of several types of cancer [4-6]. They are present as ingredients in several cosmetic preparations used in the treatment of cellular aging and the protection of the skin [7]. The plants possess thousands of active substances inside their organs (leaves, flowers, roots...) and can, according to chemical techniques (extraction, distillation...), allow the isolation of the active principle for its use in pharmacy. These natural remedies are often very effective with few recognized side effects than many synthetic drugs, but can still be deadly or toxic to the body when misused [8]. The countries of the Mediterranean Basin in general are Morocco in particular is characterized by a mild climate with long periods of sun, which leads to a great diversity of plants in terms of aromatic and medicinal plants. Among these plants are the prickly pear or Opuntia Ficus Indica which is a plant widely used in traditional medicine in addition to being the subject of many modern medical studies [9-15]. Considering the importance of cladodes of the prickly pear, we conducted a study on phytochemical screening and evaluation of the antioxidant activity of extracts of various solvents to the increasing polarity dried by microwaves at different powers.

2. Materials and methods

2.1. ***Materials***

Whirlpool microwave oven: white color - JC 218 WH, magnetic agitator, organic solvents (ethanol, acetone, dichloromethane, ethyl acetate & hexane), $Fecl_3$, NaOH, HCl, H_2SO_4, Zinc, Wagner's reagent, acetic anhydride, Ascorbic acide, 2,2-Diphenyl-1-picryhydrazyl (DPPH), coffee grinder, Rotary evaporator (Buchi R-200), Vacuum filtration.

2.2. ***Harvest***

Samples of cladodes of *Opuntia ficus-indica* were harvested in the rural commune of Bni Rais (Taourirt Province), in the eastern region during the last week of March and the beginning of April (2019). The sampling was carried out in the laboratory of the department of Chemistry at the Multidisciplinary Faculty of Nador, University of Mohammed I, Oujda, Morocco. (X (m) : 488 880,39 ; Y (m) : 3 756 650,91 ; Z (m) : 3 756 650,91).

2.3. ***Preparation of extracts***

All the extractions were carried out under the same operating conditions. Each extraction was carried out by maceration of a mass of fine powder obtained by drying the samples of cladodes under microwave activation at different powers (500, 350, 160 and 90 W) in a volume of an organic solvent 1: 5 (m: v), and under mechanical stirring at room temperature. Using various solvents of increasing polarity (Ethanol> Acetone> Dichloromethane > Ethyl acetate > Hexane). The extract solutions were filtered with filter paper, then pooled and concentrated using a rotary evaporator in 35-55°C. The extracts obtained from various solvents of each power are then stored at a temperature of -14°C in the absence of light until they are used.

2.4. ***Phytochemical screening***

Qualitative phytochemical tests can detect different chemical families present in plant extracts by coloring and precipitation reactions [16, 17].

2.4.1. Detection of Flavonoids

To 5 ml of each extract (Et, Ac, Dc, AE and Hx), add a few drops of concentrated HCl and a few milligrams of turnings of magnesium or zinc. The presence of Flavonoids is confirmed by the appearance of a red or orange color [18].

2.4.2. Detection of Tannins

A volume of 2 ml of each extract is added 2 to 3 drops of the 1% $FeCl_3$ solution. After a few minutes of incubation, the ferric chloride develops a green-black coloration which indicates the presence of Catechin or blue-black Tannins which reveals the existence of Gallic Tannins [18].

2.4.3. Detection of Alkaloids

2 ml of Wagner's reagent (2 g of potassium iodide KI + 1.27 g of iodine I_2 + 100 ml of distilled water) is added to 2 ml of each extract. The appearance of a yellow or brown white precipitate indicates the presence of Alkaloids [19, 20].

2.4.4. Detection of Steroids

Liebermann-Burchard reaction**:** To 5 ml of our extracts, 5 ml of acetic anhydride and a few drops of concentrated H_2SO_4 are added. The Steroids give according to this reaction a red coloring [21].

2.4.5. Detection of Quinones

On a volume of each extract, a few drops of 1% NaOH are added. The appearance of a color that turns yellow, red or purple indicates the presence of Quinones [22].

2.5. ***Antioxidant activity***

The ability to trap 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined by the standard method with some modifications [23]. Briefly, 0.05 ml of different concentrations (0.01-0.3 mg/ml) of each extract are added to 1.95 ml of the methanolic DPPH solution of 6.34×10^{-5} M. After incubation for 30 minutes in the dark and at room temperature, the absorbance is measured at 517 nm against a methanol blank and the DPPH solution. The radical-sweep activity of DPPH was calculated as follows [24]:

$$\mathbf{IP}\ (\%) = (\mathbf{A}_{blank} - \boldsymbol{A}_{sample} / \mathbf{A}_{blank}) \times 100$$

IP (%) **:** percentage of inhibition, $\boldsymbol{A}_{sample}$ **:** Absorbance of the sample (with presence of the extract) and $\mathbf{A}_{blank}$ **:** negative control absorbance (without presence of the extract). The ascorbic acid is used as a positive control, and the extract concentration which inhibits 50% (IC_{50}) of the DPPH radical is calculated from the graph of the percentage of inhibition as a function of the concentration of the extracts (Et, Ac , Dc, AE and Hx) of each microwave power (500, 350, 160 and 90 W), using the exponential equation.

3. Results and Discussions

3.1. ***Humidity***

The measurement of the moisture content indicates that snowshoes of the prickly pear are generally very rich in water, which can sometimes reach the value between 88% and 95% of the total mass with a low value of dry matter. The table 1 below lists the results found for the sample in various powers (500, 350, 160 and 90 W).

Table 1. ***Humidity level***

samples	Equivalent temperature (°C)	Drying time	H (%)
A (500 W)	80	40 min	7.6
Conventional heating	80	17.5 h	7.8
B (350 W)	70	50 min	7.6
C (160 W)	58	100 min	9.6
D (90 W)	37	180 min	10.1

The highest moisture content is found in samples A and B with a value equal to 92.4%. On the contrary, the lowest values of rates are observed for the samples C and D which are respectively equal to 90.4% and 89.9%. The drying time clearly depends on the power (W) of the microwave oven with which it works. We find that the speed of drying rackets varies proportionally to the microwave irradiation power used. OFI snowshoes are characterized by the containment of a large quantity of water which represents a valuable source of liquid for animals in arid zones. The difference between conventional drying (oven) and microwave drying is quick and efficient compared to conventional heating since working in similar conditions, we found that there was a considerable reduction in the mass of the sample under the microwaves (from 100g to 7.7g) compared to the oven (from 100g to just 92.64g). It took 17.5 hours to completely dry the fresh rackets under conventional heating (at 80 °C) compared to only 40 min under microwave activation at 500 W (which corresponds to 80 °C), so there is a specific microwave effect.

3.2. Extraction yields

In this study, the maceration extraction method using five solvents with increasing polarity (Ethanol> Acetone> Dichloromethane > Ethyl acetate> Hexane). Extraction is the crucial step towards the recovery and isolation of phytochemicals from plant material of cladodes of the prickly pear. Table 2 shows the extraction yields in various powers 500, 350, 160 and 90 W, it is clear from these results that the highest yield is obtained in the ethanolic extract with the decrease of the irradiation effect of microwave oven (500 W to 90 W) value 2.75% to 9.1%, against other extracts of various powers that change randomly in varying proportions. The variation in the yield of the extracts is attributed to the polarity of the extraction solvent and its ability to solubilize the compounds.

Table 2. ***Extraction yields of various microwave oven powers***

Extraction yields (%)	Microwave oven powers (W)			
	500	**350**	**160**	**90**
Et	2.75	6.3	8.5	9.1
Ac	1.22	1.83	1.66	2.1
Dc	1.76	2.05	1.83	4
AE	1.56	1.3	0.92	3.2
Hx	1.12	0.84	0.91	2

These results are in good agreement with the fact that the extractable compounds were more pronounced with solvent polarity and confirmed the usefulness of the screening process to identify the best solvent and the best power that can be used for unnecessary antioxidant activities. So far, no literature has compared the five extracts of various solvents to different powers in this study.

3.3. ***Phytochemical screening***

Of the solvents used for the extractions, ethanol is the solvent that produced the largest amount of mass extracted in all microwave powers, and the smallest hexane. These results suggest the use of ethanol as a solvent indicated for the extraction of natural chemical compounds. The phytochemical screening of extracts obtained (Table 3) under the powers (500 W to 90 W) is the richest in chemical compounds (Flavonoids, Tannins, Quinones and Steroids) especially for the ethanolic and acetonic extracts, and that the Alkaloids have not detected in any extract.

Table 3. ***Phytochemical Screening of OFI cladode extracts under the powers of 500 W at 90 W (Et = Ethanol, Ac= Acetone, Dc = Dichloromethane, AE = Ethyl acetate, Hx = Hexane).***

Secondary metabolites	Extracts														
	Flavonoïds (red-orange)					Tannins (green-black)					Alcaloïdes (brown precipitation)				
Powers	Et	Ac	AE	Dc	Hx	Et	Ac	AE	Dc	Hx	Et	Ac	AE	Dc	Hx
500 W	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-
350 W	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-
160 W	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-
90 W	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-

Secondary metabolites	Extracts									
	Stéroids (red)					Quinones (yellow)				
Powers	Et	Ac	AE	Dc	Hx	Et	Ac	AE	Dc	Hx
500 W	+	-	+	-	-	+	+	+	+	+
350 W	+	-	-	-	-	+	+	+	+	+
160 W	+	-	+	-	-	+	+	+	+	+
90 W	+	+	-	-	-	+	+	+	+	+

(+): Presence of compounds, (-): Absence of compounds

The compounds characterized in the various tests are known for their medicinal importance. For example, phenolic compounds derived from medicinal plants show biological activities such as: antioxidant activity, antibacterial activity and antifungal activity [25–27].

3.4. ***Antioxidant Activity***

DPPH is an intense violet color radical. The measure of the effectiveness of an antioxidant (ability to fix free radicals, thus stop the propagation of the chain reaction) is achieved by measuring the decrease in violet color. The measurement of the optical density was carried out spectrophotometrically at 517 nm. Figure 1 shows the variation of the percent inhibition as a function of the concentration of *OFI* cladode extracts under different potencies (0.01-0.3 mg/ml). For comparative purposes, using ascorbic acid as a reference antioxidant, used at the same dose.

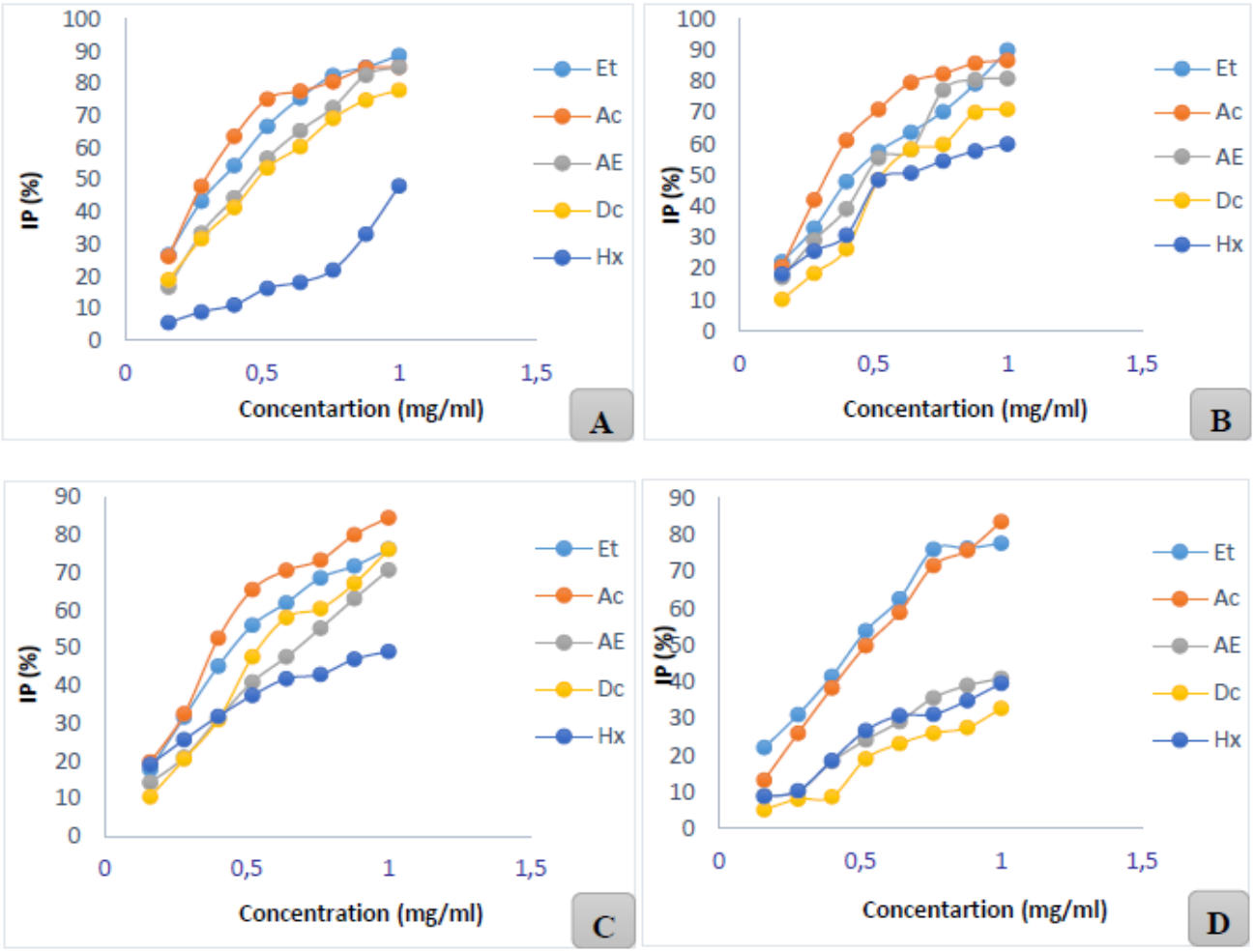

Figure 1. Percentage of DPPH radical inhibition by extracts of each power: (A) = DPPH of 500 W, (B)= DPPH of 350W, (C) = DPPH of 160 W, (D) = DPPH of 90 W.

The IC_{50} value is defined as the concentration of extract that inhibits 50% of the free radical DPPH. This value is calculated by modeling the percent inhibition as a function of the extract concentration using the exponential equation. The IC_{50}'s of each of the different extracts in different microwave oven powers (Table 4). According to Kadri et al., A lower IC_{50} value indicates higher antioxidant activity [28].

Table 4. ***IC50 inhibitory concentrations of the test from DPPH***

Extracts	IC_{50} (mg/ml)				
	Conventional drying	Microwave drying (W)			
	80°C	*90*	*160*	*350*	*500*
Ethanol	0.39	0.48	0.44	0.42	0.34

Acetone	0.31	0.52	0.38	0.32	0.29
Ethyl acetate	0.49	-	0.54	0.48	0.45
Dichloromethane	0.53	-	0.68	0.52	0.48
Hexane	0.54	-	-	0.53	0.50
Ascorbic acid	0.114 mg/ml				

These results revealed that all extracts exhibited dose dependent scavenging effects. In power 500 W, the acetone extract therefore has the highest antiradical activity (IC_{50} = 0.29 mg/ml), followed by the ethanol extract with an IC_{50} of the order 0.34 mg/ml. The lowest antiradical activity was expressed by the hexane extract (IC_{50} = 0.50 mg/ml). We find that the DPPH of the 500 W power showing antioxidant activity of each extract (Et, Ac, Dc, AE and Hx) greater than that of the power 350 W, 160 W and 90 W respectively (IC_{50}: 500 W <350 W <160 W <90 W). The cleaning activities of all the extracts, however, were significantly lower compared to the reference standard of ascorbic acid (IC_{50} = 0.114 mg/ml), And also, it can be deduced that the IC_{50} of the acetone extract in the 500 W power (microwave drying) and in the temperature 80 °C (conventional drying) are almost equal (***0.29 mg/ml ≈ 0.31 mg/ml***). Acetone extracts are a good DPPH radical scavenger. This can be explained by its richness in substances possessing hydrogen-donating capacities via their hydroxyl groups, as well as by their capacity to give electrons to act as an antioxidant. The difference observed between the DPPH scavenging capacity of OFI cladodes extracts dried in a microwave oven at 500, 350, 160 and 90 W power could probably be attributed to differences in phenolic contents due to the effect of Microwave oven irradiation (the power effect W).

4. Conclusion

Recent decades have been marked by a particular interest in the development of medicinal plants as a source of natural bioactive substances. Polyphenols are a family of compounds that are ubiquitous in the plant kingdom. They seem particularly interesting, because of their strong antioxidant power. Concerning the specific effect of microwaves activation could be demonstrated only in the case of drying time of the plant which has been considerably reduced under microwave activation compared to conventional heating (oven) from 17.5 h by conventional heating at 40 min under microwaves irradiation at a similar temperature of 80°C. However, the yield and antioxidant activity are substantially the same for both drying modes. The antioxidant activity of cladodes extracts (*OFI*) was evaluated by the DPPH free radical scavenging method. The results obtained showed that the acetone extracts have the most important activity (*IC_{50}=0.29 mg/ml*), and this activity also increases with the increase of the microwave power (90 to 500 W). The acetone and ethanol extracts of *Opuntia ficus-indica* cladodes are a natural and promising source of chemical molecules with important antiradical activities. These activities still remain significantly less than that of ascorbic acid (*IC_{50}=0.114 mg/ml*), but they are crude samples containing a large number of different compounds. It is therefore very likely that they contain compounds which can be compared to that of ascorbic acid. Further research is needed to identify, isolate and purify these constituents.

References

[1] P. Iserin, M. Masson, J.P. Restellini, E. Ybert, A. De Laage de Meux, F. Moulard, E. Zha, R. De la Roque, O. De la Roque, P. Vican, Larousse medicinal plants, identification, preparation, care, *Ed. Larousse*, (2001) 15-16.

[2] S. Pagare, M. Bhatia, N. Tripathi, S. Pagare, Y.K. Bansal, Secondary Metabolites of Plants and their Role : Overview, *Curr. Trends Biotechnol. Pharm*., 9 (2015) 293-304.

[3] B.B. Buchanan, W. Gruissem, R.L. Jones, Biochemistry and molecular biology of plants, edit. : Rockville Md. USA, *Am. Soc. Plant Physiol.*, (2000).
[4] C. Manach, F. Regerat, O. Texier, G. Agullo, C. Demigne, C. Remesy, Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids, *Nutr. Res.*, 16 (1996) 517-544.
[5] S. Rahaiee, E. Assadpour, A.F. Esfanjani, A.S. Silva, S.M. Jafari, Application of nano/microencapsulated phenolic compounds against cancer, *Advances in Colloid and Interface Science*, 279 (2020) 102153.
[6] A. Golonko, T. Pienkowski, R. Swislocka, R. Lazny, M. Roszko, W. Lewandowski, Another look at phenolic compounds in cancer therapy the effect of polyphenols on ubiquitin-proteasome system, *European Journal of Medicinal Chemistry*, 167 (2019) 291-311.
[7] F. Menaa, A. Menaa, J. Tréton, Chapter 63 - Polyphenols against Skin Aging, *Polyphenols in Human Health and Disease*, 1 (2014) 819-830.
[8] K. Benmiloud, "Criblage phytochimique, activités antioxydantes et anticandidose des extraits de Nepeta amethystina (Gouzeia)", *Thesis* (University Abu Bakr Belkaid Tlemcen), 2014.
[9] D.K. Ahn, Illustrated book of Korean medicinal herbs, Kyohaksa Seoul, (1998) 497.
[10] I. Ammar, M. Ennouri, H. Attia, Phenolic content and antioxidant activity of cactus (*Opuntia ficus-indica* L.) flowers are modified according to the extraction method, *Industrial Crops and Products*, 64 (2015) 97-104.
[11] C.E. Aruwa, S.O. Amoo, T. Kudanga, Extractable and macromolecular antioxidants of *Opuntia ficus-indica* cladodes: Phytochemical profiling, antioxidant and antibacterial activities, *South African Journal of Botany*, 125 (2019) 402-410.
[12] L. Felkai-Haddache, F. Dahmoune, H. Remini, K. Lefsih, L. Mouni, K. Madani, Microwave optimization of mucilage extraction from *Opuntia ficus indica* Cladodes, *International Journal of Biological Macromolecules*, 84 (2016) 24-30.
[13] M. Ennouri, H. Fetoui, E. Bourret, N. Zeghal, F. Guermazi, H. Attia, Evaluation of some biological parameters of Opuntia ficus indica. 2. Influence of seed supplemented diet on rats, *Bioresource Technology*, 97 (2006) 2136-2140.
[14] A. Ressaissi, N. Attia, R. Pacheco, P.L. Falé, M.L.M. Serralheiro, Cholesterol transporter proteins in HepG2 cells can be modulated by phenolic compounds present in *Opuntia ficus-indica* aqueous solutions, *Journal of Functional Foods*, 64 (2020) 103674.
[15] E. Salehi, Z. Emam-Djomeh, G. Askari, M. Fathi, *Opuntia ficus indica* fruit gum: Extraction, characterization, antioxidant activity and functional properties, *Carbohydrate Polymers*, 206 (2019) 565-572.
[16] M.I. Hernández-Urbiola, E. Pérez-Torrero, M.E. Rodríguez-García, Chemical Analysis of Nutritional Content of Prickly Pads (Opuntia ficus indica) at Varied Ages in an Organic Harvest, *Int. J. Environ. Res. Public. Health*, 8 (2011) 1287-1295.
[17] M. Saleem, H.J. Kim, C.K. Han, C. Jin, Y.S. Lee, Secondary metabolites from Opuntia ficus-indica var. saboten, *Phytochemistry*. 67 (2006) 1390-1394.
[18] Y. Karumi, P.A. Onyeyili, V.O. Ogugbuaja, Identification of active principles of M. balsamina (Balsam Apple) leaf extract, *J. Med. Sci.*, 4 (2004) 179-182.
[19] K. Benzahi, «Contribution à l'étude des flavonoides dans la Plante cynodon Dactylon-L "chiendent"», *mémoire de Magister*, Univ. Ouargla, (2001) 15-17.
[20] N. Chaouch, « Etude des Alcaloïdes dans le coloquinte colocynthis vulgaris (L) Scharad (Cucurbitacées) Région de Oued N'SA (Wilaya de Ouargla)», *mémoire de Magister*, Univ. Ouargla, (2001) 44.
[21] K. N'Guessan, B. Kadja, G. Zirihi, D. Traoré, L. Aké-Assi, Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire), *Sci. Nat.*, 6 (2009), 1-15.

[22] O.I. Oloyede, Chemical Profile of Unripe Pulp of Carica papaya, *Pak. J. Nutr.*, 4 (2005) 379-381.
[23] J. Kubola, S. Siriamornpun, Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts *in vitro*, *Food Chem.*, 110 (2008) 881-890.
[24] L. Barros, P. Baptista, I.C.F.R. Ferreira, Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays, *Food Chem. Toxicol.*, 45 (2007) 1731-1737.
[25] R.R. Espinosa, R. Inchingolo, S.M. Alencar, M.T. Rodriguez-Estrada, I.A. Castro, Antioxidant activity of phenolic compounds added to a functional emulsion containing omega-3 fatty acids and plant sterol esters, *Food Chem.*, 182 (2015) 95-104.
[26] D. Lopes-Lutz, D.S. Alviano, C.S. Alviano, P.P. Kolodziejczyk, Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils, *Phytochemistry*, 69 (2008) 1732–1738.
[27] M. Zabka, R. Pavela, Antifungal efficacy of some natural phenolic compounds against significant pathogenic and toxinogenic filamentous fungi, *Chemosphere.*, 93 (2013) 1051-1056.
[28] A. Kadri, Z. Zarai, A. Békir, N. Gharsallah, M. Damak, R. Gdoura, Chemical composition and antioxidant activity of Marrubium vulgare L. essential oil from Tunisia, *Afr. J. Biotechnol.*, 10 (2011) 3908-3914.