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Journal of Medicinal Plants Research

ARTICLE

Full Length Research Paper

In vitro antioxidant and anticholinesterase activities and *in vivo* toxicological assessment (Zebrafish embryo model) of ethanolic extracts of *Capsicum chinense* Jacq.

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The ripe and dried fruits of organic pepper *Capsicum chinense* Jacq., obtained during planting, cultivation and harvesting, were subjected to solid-liquid extraction using a Soxhlet apparatus with hexane to remove non-polar compounds, and then with ethanol to remove the target compounds. The ethanolic extracts were characterized by gas chromatography-mass spectrometry (GC-MS) determining the composition of capsaicin (74.1%), dyhidrocapsaicin (8.9%), docosenamide (11.7%), ethyl linoleate (3.2%) and ethyl palmitate (2.1%). Three different bio-screenings: (1) the Trolox® equivalent antioxidant capacity (TEAC); (2) the inhibition of the enzyme acetylcholinesterase (AChE) and; (3) the embryo toxicity and the induction of phenotypic changes in the Zebrafish model were used. It was found that the ethanol extracts possess antioxidant activity (TAA 1800 ± 91 mmol Trolox®/kg extract) and AChE inhibition (IC_{50} 18.8 ± 0.5 µg/ml), as well as a moderate toxicity in zebrafish embryos (LC_{50} 39.7 ± 2.1 µg/ml), and good dual activity that make them extremely important not only for nutrition, but also as pharmacological substrates.

Key words: *Capsicum chinense*, capsaicinoid, acetylcholinesterase inhibitor, Trolox® equivalent antioxidant capacity (TEAC), zebrafish, developmental toxicity.

INTRODUCTION

The use of plants with medicinal properties is as old as human civilization and plant preparations were one of the main options for the treatment of different diseases (Jayaprakasha et al., 2012). Among these plants, chili

peppers (*Capsicum* spp.) are a rich source of diverse bioactive compounds with potential health-promoting properties, particularly analgesic, anti-inflammatory and antioxidant due to the presence of capsaicinoids, capsinoids, carotenoids, and phenolic compounds (Wahyuni et al., 2013a; Dubey et al., 2015; Galano and Martinez, 2012; Zimmer et al., 2012; Loizzo et al., 2015; Tundis et al., 2013). These biological effects of those bioactive compounds, in addition to the culinary properties of chili peppers (*Capsicum* spp.), make them extremely important not only for nutrition, but also as pharmacological substrates.

The genus *Capsicum* comprises more than 200 varieties, and the fruits vary widely in size, shape, flavor and sensory heat due to changeable chemical composition and content (Wahyuni et al., 2013b). Pepper used as a spice for food preparations is usually a single type or a combination of several different varieties. This choice is usually based on individual preferences, without any consideration of health benefits. Among the five most cited species of important economic value, *Capsicum annuum*, *C. baccatum*, *C. frutescens, C. pubescens* and *C. chinense*, the latter species, *C. chinense* Jacq. is often known as orange habanero peppers and is a very aromatic variety native that belongs to the Solanaceae family, characteristic of tropical and humid zones of Central and South America. A lipophilic alkaloid called capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) is the main representative pungent component of these chili peppers. Although excessive exposure to capsaicin can be toxic, causing irritation on the contact area or respiratory problems, as well as some types of cancers due to ingestion of high quantities, capsaicin has been extensively studied via experimental and clinical investigations, for its prominent pharmaceutical, neurological and antioxidant properties (Bae et al., 2012; Dubey et al., 2015; Rosa et al., 2002; Zeyrek and Oguz, 2005). A common use of capsaicin is in topical anti-arthritic and anti-inflammatory ointments.

Clinical trials have shown that capsaicin may have potential value in the treatment of rheumatoid arthritis and cluster headaches by producing discharge of a sensory neurotransmitter, causing an insensitivity to pain (Cordell and Araujo, 1993). However, consumption in great amounts of this compound over prolonged period of time can cause chronic gastritis, kidney damage, liver damage and neurotoxic effects (Johnson, 2007). Capsaicin also possesses antimicrobial properties and inhibitory effect on acetylcholinesterase (AChE) activity that open doors for exploring its potential as a natural inhibitor of pathogenic microorganisms in food (Dorantes et al., 2000; Kurita et al., 2002; Xing et al., 2006; Jones et al., 1997; Chatterjee et al., 2010) or as preventive medicine for Alzheimer's disease (AD) (Orhan et al., 2007). Recent studies indicate that inflammatory processes are directly associated with Alzheimer's disease (Kepp, 2012). Amyloid peptides in senile plaques

found in the brains of Alzheimer's patients can induce inflammatory processes, in which reactive oxygen species (ROS) are released along with other components. The ROS are responsible for cell damage at the structural level and act as secondary messengers in inflammation. Therefore, the antioxidants can "catch" ROS attenuating inflammation (Vina et al., 2004; Kontush and Schekatolina, 2004). On the other hand, AD is currently treated clinically by the use of agents, which restore the level of acetylcholine mainly through the inhibition of acetylcholinesterase (Rauk, 2009; Citron, 2010; Kar, 2002). Therefore, medicine with dual action (antioxidant and anti-AChE activities) can act as neuroprotective agents and used to prevent or treat this disease.

Capsaicinoid content in peppers increases with maturing and climacteric ripening of the fruit (Gnayfeed et al., 2001; Estrada et al., 2002). The quality of the extracted compounds as well as their antioxidant activity is affected by processing parameters such as separation, steam blanching, extraction temperature and drying (Ramesh et al., 2001). Solid-liquid extraction with organic solvents such as hexane, chloroform, and ethanol is the most commonly employed method for capsaicin recovery (Tapia et al., 1993). Although it is believed that *C. chinense* (orange habanero peppers) contains the highest concentrations of capsaicinoids compared to other varieties, the chemical composition and biological properties of *C. chinense* dried fruits and their extracts are scarce. In particular, there is a lack of information about the potential use of this material and no information exists with respect to its safety and toxicological effects. Recently, it was reported that lipophilic fractions of habanero *C. chinense* did not exhibit acetylcholinesterase inhibitory activity, but showed weak butyrylcholinesterease inhibitory properties (Menichini et al., 2009).

With these information in mind, we wanted to evaluate ethanolic extracts of *C. chinense* dried mature fruits as preventive medicine for AD. To this purpose, the antioxidant activity, using the total antioxidant activity (TAA) value, obtained by the ABTS⁺• radical-cation discoloration method, the inhibition of AChE enzyme frequently targeted for the treatment of Alzheimer's disease were investigated, relating to the chemical composition of *C. chinense* dried fruits extracts, and their toxicological assessment using for the first time zebrafish embryo model. To the best of our knowledge, this is the first report on three different bioscreenings of *C. chinense* dried mature fruits, a promising source of valuable capsaicinoids.

MATERIALS AND METHODS

Chemicals and samples

The reagents used were hexane, ethanol, dimethyl sulfoxide

(DMSO), water (JT Baker, analytical grade), sodium hydrogen phosphate, potassium hydrogen phosphate, Tween 20, acetylthiocholine iodide, dithiobisnitrobenzoic acid, capsaicin, dihydrocapsaicin, acetylcholinesterase from *Electrophorus electricus* (EC 3.1.1.7, Type VI-S), potassium persulfate, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), t-butylhydroxytoluene (BHT), t-butylhydroxyanisole (BHA), D-α-tocopherol (Vitamin E), sodium chloride, potassium chloride, potassium chloride calcium and magnesium sulfate (Sigma-Aldrich).

Plant

The plant material was grown organically at a farm in the Municipality of Piedecuesta in Santander, Colombia (geographic coordinates: latitude 7° 0.477' N, longitude 73° 3.205' W and 1020 m altitude). The *Capsicum chinsense* fruits were collected at maturity, taking into account healthy and robust appearance. Taxonomic identification (No. COL558072) was held at the Colombian National Herbarium of the Institute of Natural Sciences, National University of Colombia. Pepper fruits were examined for integrity, absence of dust and insect contamination. They were devoid of peduncles and seeds as they were cut into small pieces. Samples were dried in an oven at 40°C to constant weight, pulverized and stored at 20°C until they were analyzed.

Extraction procedure

Extracts were obtained in triplicate by solid-liquid extraction using a Soxhlet apparatus. In order to remove non-polar compounds, pulverized fruits of pepper were treated first with hexane for 12 h. Then, treated fruits were extracted with ethanol for 12 h. The ethanolic extracts were concentrated and dried in vacuum pump (5 mmHg) for four hours to obtain the capsaicinoids.

GC–MS analysis of extracts

The extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). The GC-MS analyses were carried out using a gas chromatograph Agilent Technologies 7890A coupled to a mass selective detector Agilent 5975C with split/splitless inlet (split ratio 1:10) and HP-1MS column (30 m × 0.25 mm ID × 0.25 µm df) with stationary phase 100% poly-(dimethylsiloxane). The heating rate used in the chromatographic oven was 50°C (5 min) @ 5°C/min to 200°C (5 min) and @ 10°C/min to 260°C (20 min). Mass spectra were obtained by electron ionization (70 eV) with a quadrupole analyzer mass range *m/z* 40 to 400 in full-scan mode. The temperatures of the ionization chamber and the transfer line were maintained at 230 and 285°C, respectively. Chromatographic and spectroscopic data were processed using software MSD ChemStation Agilent Technologies G1701 (EA Version E.02.02.1431). Identifying the components of the extract was performed as follows: the capsaicinoids were cross-referenced with mass spectra and retention times obtained from a registered pattern of capsaicin and dihydrocapsaicin. The other compounds were compared to the spectra obtained with those reported in the databases W8NO8 and NIST05.

ABTS$^{+\bullet}$ assay and TAA values

In order to determine the Trolox equivalent antioxidant capacity (TEAC), the method of Re et al. (1999), with the modifications proposed by Muñoz-Acevedo et al. (2011) was used. In order to generate the radical cation ABTS$^{+\bullet}$, an aqueous solution of 7 mM concentration ABTS with potassium persulfate ($K_2S_2O_8$) was allowed to incubate for 16 h at room temperature, in the absence of light. Stock solutions of the extracts at a concentration of 1000 ppm were prepared, which were diluted serially in 96 well plates. Inhibition occurred between 20 and 80% of the blank absorbance after adding 10 µl aliquots of the aqueous solutions to the ABTS$^{+\bullet}$ (200 µl). Absorbance measurements were performed on a microplate reader at 734 nm, reading 1 min after the initial mixture up to 30 min. The response-concentration of substances, as a percentage of the absorbance of radical cation ABTS$^{+\bullet}$ without inhibition was calculated using Equation (1).

Inhibition of A_{734} (%) = (1-Af/Ao) × 100 (**1**)

where: Ao: is the absorbance of radical cation without inhibition; Af: is the absorbance measured at 30 min after the addition of the antioxidant agent. The percent inhibition of the absorbance at 734 nm was calculated and plotted as a function of the sample, with the data of the reference substance (Trolox®) and the TAA was determined according to Equation (2) (Muñoz-Acevedo et al., 2011; Arnao et al., 1999):

$$TAA = \frac{\text{mmol of Trolox}}{\text{kg of substance evaluated}}$$ (2)

Solvent blanks were performed on each plate. The experiment control substance was vitamin E, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) and all assays were performed in triplicate.

Bioassay for anti-acetylcholinesterase activity

Acetylcholinesterase (AChE, EC 3.1.1.7, Type VI-S) inhibition was assessed by the Ellman method modifying by scaling microplates (Ellman et al., 1961), which is based on the reaction of released thiocholine to give a coloured product with a chromogenic reagent. Fifty (50 µl) of the extract solution (at serial concentrations from 1000 to 1 ppm), dissolved in phosphate buffered saline pH 7.5 and 50 ml of the AChE (0.25 U/ml) were placed. The plate was incubated at room temperature for 30 min and 100 ml of pH 7.5 substrate solution was added [0.04 M Na_2HPO_4, 2,2'-dinitro-5,5'-dithiodibenzoic 0.2 mM, 0.24 mM acetylthiocholine iodide]. At five minutes into the reaction, the absorbance at 412 nm in a microplate reader VERSAmax was read. The inhibition rate (%) was calculated by Equation (3):

Inh. A (%) = 100-[(AS-AB)/(AC-AB)] × 100 (3)

Where: AS is the absorbance of sample; AB is the absorbanceof blank; AC is the control absorbance to determine the enzyme activity without inhibitor. Galantamine was used as reference compound and assays were performed in triplicate. The IC_{50} calculations and graphics were performed using SoftMax Pro 5.2 software from Molecular Devices. The IC_{50} was defined as the concentration required to achieve 50% of the maximum inhibitory effect.

Toxicity assay, analysis and phenotypic changes of the ethanol extracts using the zebrafish embryonic model

The toxicity studies of ethanolic extracts using zebrafish embryos (*Danio rerio*, wild type) were performed based on the protocol developed by Ali et al. (2011) and adapted in our laboratories (Puerto and Kouznetsov, 2013). The embryos were collected, washed twice with E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, and 0.33 mM $MgSO_4$, pH 6.8 - 6.9), and transferred to a Petri dish. The embryos were examined periodically through a

dissecting microscope in order to remove dead, unfertilized, malformed or developmentally delayed embryos. During this period, the embryos were kept in an incubator at 28 ± 2°C under natural light-dark photoperiod. The selected embryos were carefully transferred from the Petri dish to a 96-well microplate, placing one embryo with E3 medium in 200 µl per well. The care and use of fish were performed according to international guidelines of the National Institute of Health in the United States for the care and use of laboratory animals, keeping them healthy and free of any sign of disease.The research ethics committee of the Heart Institute of Bucaramanga, approved this protocol in its meeting on May 26, 2012 (No.050).

Determination of LC_{50} in zebrafish embryos

Three independent experiments in three different plates, where the extract was tested three times in each plate were carried out with embryos obtained from different matings. In total, 72 embryos were employed. geometric series of concentrations, starting at 300 µg/ml and ending at 4.68 µg/ml were calculated on each plate. Determining the LC_{50} (expressed as µg of extract/ml of solution) was based on the cumulative mortality, which was monitored in a dissecting microscope (trinocular stereomicroscope OPTIKA, model version SZM-1) at 72 h after chemical exposure (96 hpf).

Analysis of phenotypic changes using embryonic zebrafish model

In order to correlate this experiment with the determined LC_{50} values, the embryos selected for the phenotypic study were exposed to the established geometric series after 24 hpf. Each extract was diluted in the medium E3 with 2% v/v of dimethyl sulphoxide (DMSO) and aliquots of 200 µl were prepared in a 96 well plate at concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 µg/ml and the appropriate aliquot of the ethanolic extract (200 µl) was transfered into the corresponding well of the embryonic plate. Two controls wells were used peer plate, each containing E3 medium with 2% v/v of DMSO. The embryonic plates were incubated at 28°C and photographed at 48, 72 and 96 h post-fertilization (hpf) using an OPTIKA zoom stereo microscope (trinocular version of model SZM-1).

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means ± standard deviation (SD). The concentration giving 50% inhibition (IC) was calculated by nonlinear regression with the use of Prism Graphpad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). The dose-response curve was obtained by plotting the percentage inhibition versus concentration. The lethal concentration (LC_{50}) is expressed as the standard error of the mean (SEM) of three different experiments in triplicate, the analysis was made using regression probit analysis with SPSS for windows version 19.0.

RESULTS AND DISCUSSION

Planting, growing and harvesting of plant material and extraction procedure

Seeds of selected native species were collected in the municipality of San Gil, Santander, Colombia (location 33'

N, 78° 8' W). No chemical weed or control pest were used during the planting of the organic chili peppers in order to ensure that the plant material was free from pesticide residues that could result in false positive tests in AChE inhibition. After planting, growing and harvesting, selected mature seedless fruits were washed and disinfected with 3% sodium hypochlorite. The material was dried in an oven at 40°C to constant weight for 96 h (humidity at 80.1 ± 0.4%), and pulverized to facilitate and streamline the process of extraction. Extraction was performed using a Soxhlet apparatus. 10 g of pepper were weighed and were added to 300 ml of hexane and extracted for 12 h, the hexane was removed and added to 300 ml of ethanol extractor and extracted for 12 h. The extractions were performed in triplicate. The dry weight yield was 7.9 ± 0.5%. The GC-MS analysis of the chemical composition (Figure 1) and the relative amount of components in the extract are shown in Table 1. Capsaicin (74.1%) was determined as a main constituent followed by docosenamide (11.7%), dihydrocapsaicin (8.9%), and two esters of fatty acids: ethyl linoleate (3.2%) and ethyl palmitate (2.1%).

Determination of the Trolox Equivalent Antioxidant Capacity-TEAC

The TEAC is widely applied to assess the amount of radicals that can be trapped by an antioxidant (Re et al., 1999). In this research, the extract solution was added to a preformed radical cation $ABTS^{+\bullet}$ and after 30 min, the remaining radical cation $ABTS^{+\bullet}$ was quantified spectrophotometrically at 734 nm. The TAA values for controls substances: vitamin E, BHA and BHT were 2659 ± 74, 6819 ± 46 and 4272 ± 38, respectively, while the value of TAA (mmol Trolox/kg of extract) for the extract was 1800 ± 91. These results showed that *C. chinense* extract had a moderate antioxidant capacity.

Study to determine the inhibitory activity of the extract against the acetylcholinesterase enzyme

The profiles of the results revealed interesting numbers, where the extract was able to inhibit the enzyme with IC_{50} 18.8 ± 0.5 µg/ml. The value for vitamin E was 42.0 ± 0.6 µg/ml. It should be noted that vitamin E is one of most studied molecules for use in neurodegenerative disorders (Singh et al., 2008; Kontush and Schekatolina, 2004; Sen and Chakraborty, 2011). While the inhibitory concentration for the control drug, galantamine was 0.30 ± 0.01 µg/ml.

Determination of LC_{50} in zebrafish embryos

In order to study the toxicity (lethal concentration - LC_{50}, µg/ml), zebrafish embryos were treated with the obtained
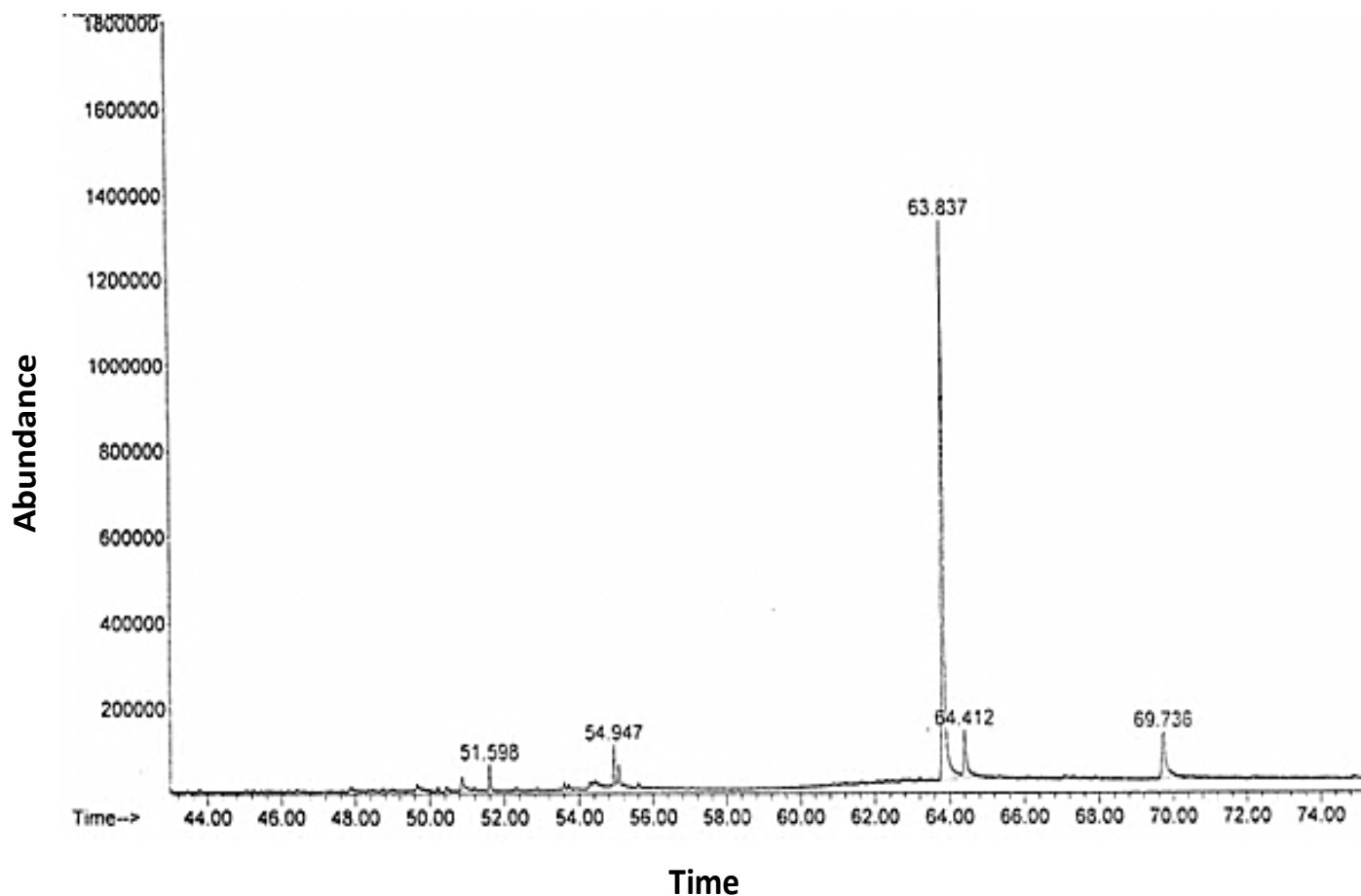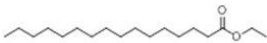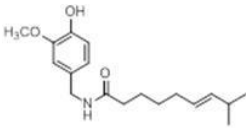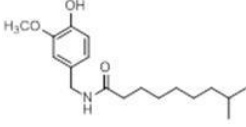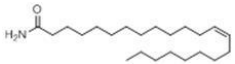
Figure 1. Chromatographic profile of the ethanolic extract of *Capsicum chinense*.

Table 1. Secondary metabolites obtained by Soxhlet extraction and analyzed by GC-MS, present in mature fruits of *C. chinense*.

Order elution	Components	Structure	T_R (min)	Relative amount (%)
1	Ethyl palmitate		51.60	2.1
2	Ethyl linoleate		54.95	3.2
3	Capsaicin		63.84	74.1
4	Dihydrocapsaicin		64.41	8.9
5	Docosenamide		69.74	11.7
	Total			100
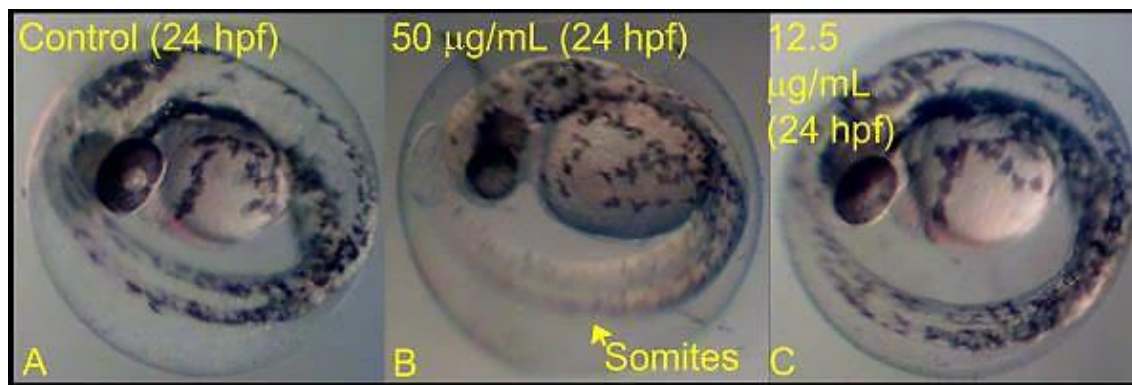
Figure 2. Photographs of zebrafish embryos treated with the extract of *Capsicum chinense* Jacq. 48 hpf. (A) Control. (B) Embryo having an abnormal tails that suggests the irregular development of the somites. (C) shows a normal embryo development compared to the control.

extracts folowed by the Ali protocol (Ali et al., 2011) with some modifications (Puerto and Kouznetsov, 2013). Data collected from three independent experiments were analyzed statistically and the extract of *C. chinense* Jacq. determined that LC_{50} value was 39.7 ± 2.1 µg/ml. From these results it can be seen that the toxicity of *C. chinense* extract is comparable to that of other bioactive substances or drugs, such as salicylic acid (LC_{50} = 46.7 µg/ml) (Ali et al., 2011).

Analysis of phenotypic changes using embryonic zebrafish model

Because the optical clarity of the embryo, a wide variety of functional and morphological changes may be observed without killing, dissecting or manipulating the embryos (Goldsmith, 2004; Eggert, 2013; Puerto and Kouznetsov, 2013; Ali et al., 2011; Peterson et al., 2000). Once the LC_{50} was determined for the extract of *C. chinense*, a range of concentrations below this value was established to evaluate the phenotypic changes induced by the ethanolic extract using the zebrafish embryo model, considering that every fertilized embryo can become a macroscopically representation of a cell. For this experiment the embryos, at 24 hpf to correlate both assays, were exposed to a geometric series of concentrations below the LC_{50} in order to observe a detailed development of each embryo until the end of the experiment.

After the chemical exposure, the morphology and development of embryos exposed to seven concentrations below the LC_{50} were observed at different stages from 48 to 96 hpf using a dissecting microscope. In general, all embryos treated with the extract, at two or three levels below the respective LC_{50}, showed no visible phenotypic change during the early hours of chemical exposure. However, at one or two concentrations close to the LC_{50} of the ethanolic extract, the embryos showed a

delayed development characterized by a lack of pigmentation in the tail and the abnormal development of the somites, evidenced by the pronounced tail curvature and its wide end, that are responsible for giving rise to most of the axial skeleton and the muscles around the cord during the first stages of development. Figure 2 shows photographs of embryos with observed normal development and the curvature that suggest the abnormal development of the somites.

After 96 hpf, the embryos treated with the ethanolic extract at concentrations above the LC_{50} and those exhibited delayed development during the first 48 hpf eventually died, confirming the toxicity of the extract. However, those embryos that showed no visual phenotypic changes during the first hours after the chemical exposure, reached later stages of development after 96 hpf with no any visual evidence of malformations, defects or injuries in comparison with the control, suggesting that their morphology and development did not differ from the embryogenesis of the control embryos, as seen in Figure 3.

The obtained biological data of ethanolic extracts of *C. chinense* Jacq are summarized in the following table (Table 2). It should be noted that obtained ethanolic fraction contains capsaicin as the main phytoconstituent (74.1%) (Table 1). Therefore, tested biological properties of this fraction can be attributed to the capsaicin.

Conclusion

It was found that the TAA value (1800 ± 91 mmol Trolox/kg of extract) and the AChE inhibitory activity of the ethanolic extract (IC_{50} 18.80 ± 0.5 µg/ml) were below its lethal concentration (LC_{50} 39.7 ± 2.1 µg/ml), suggesting that antioxidant and/or the inhibition of AChE activity is not being lethal for the embryos and it does not induce phenotypic changes, teratogenic injury or developmental malformations. Toxicity tests of *C.*

Table 2. Bioevaluation of ethanolic extracts of *Capsicum chinense* Jacq.

Parameter	Antioxidant activity, TAA (mmol Trolox/kg of extract)	Anticholinesterase activity, IC_{50} (µg/ml)	Toxicity on zebrafish embryos, LC_{50} (µg/ml)
Ethanol extract	1800 ± 91	18.8 ± 0.5	39.7 ± 2.1
Vitamin E	2659 ± 74	42.0 ± 0.6	nt
BHA	6819 ± 46	nt	nt
BHT	4272 ± 38	nt	nt
Galantamine	nt*	0.30 ± 0.01	nt
Salicylic acid	nt	nt	46.7± 1.1

*nt – not tested
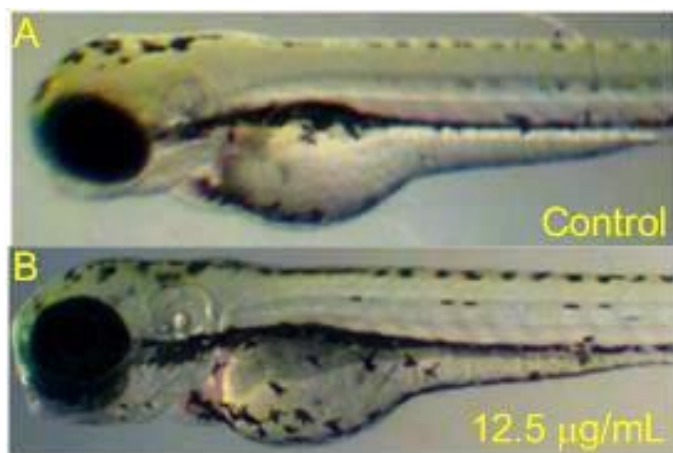
Figure 3. Photographs of zebrafish embryos treated at 96 hpf. (A) Control. (B) Embryo treated with *Capsicum chinense* Jacq. extract.

chinense using zebrafish embryo model were realized for the first time and consistent with previous studies (Wahyuni et al., 2013a). The capsaicinoids like capsaicin and dihydrocapsaicin present in plants of the genus *Capsicum* have been associated with biochemical and pharmacological effects, including antioxidant and anti-inflammatory activities. However, there are few reports on the AChE inhibitory activity, key enzyme associated with AD. It was found that the ethanolic extract of *C. chinense,* an important species with economic value, showed a high inhibitory activity of AChE.

From this work it can be concluded that moderate antioxidant capacities and high inhibitory activity of AChE of *C. chinense* extract, along with its moderate toxicity, makes the consideration of the ethonolic extract *C. chinense*, and their fruits, as a promising, recoverable source with high content of capsaicinoids, and consolidate the model as a tool with chili high usability for the development of phyto-pharmaceutical and ethnopharmacology in Colombia.

Conflicts of interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Bioactive properties of some selected Libyan plants

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The present work evaluates the antioxidant and antibacterial activities of four Libyan plants (*Arbutus pavarii*, *Pegnanum harmal*, *Pistachia atlantica*, and *Fagonia bruguieri*) using various assays including reducing power, Phosphomolybdenum, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide free-radical scavenging activity. *In vitro*, the antibacterial activity of crude and flavonoids extracts were determined against five strains, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhi*. In the extracts of all plants under investigation, the total flavonoids and polyphenols were quantitatively estimated. Leaves of *A. pavarii* possess the highest antioxidant potential with significant free-radical-scavenging effects; the highest flavonoids content (1504.28±80.89 mg Rutin g^{-1}) and a relatively high content of phenolics (1217.14±50.52 mg GAE g^{-1}) compared to the other tested plants. Regression analysis of the antioxidant assays showed a strong correlation between phenolics or flavonoids content and nitric oxide scavenging activity as well as total antioxidant activity. Also the crude extracts especially *A. pavarii* showed greater growth inhibition capacity towards tested bacterial strains. Results of the antioxidant assays and antimicrobial screening showed that, all tested plants can act as radical scavengers and antimicrobial agents to a certain extent. Future study is recommended to further purify and examine individual bioactive compounds from these extracts and evaluate their *in vivo* antioxidant and bacterial activities.

Key words: *Arbutus pavarii*, *Pegnanum harmal*, *Pistachia atlantica*, *Fagonia bruguieri*, polyphenols, antioxidant activity, antibacterial activity.

INTRODUCTION

Increasing attention has been paid to the use of natural antioxidants, such as ascorbic acid, tocopherols, phenolic compounds including flavonoids**,** phenolic acids, and volatile compounds for preventing oxidation of biomolecules which can lead to cell injury and death (Patil et al., 2003). Also alkaloids has been showed of marked anti antioxidant ability (Maiza-Benabdesselam et al., 2007). Great number of substances of plant origin has been shown to exhibit antioxidant activity (Gulcin et al., 2003; Ali et al., 2008). The use of antioxidants that scavenge reactive oxygen species (ROS) has been studied by evaluating its potential and therapeutic applications. Antiradical antioxidants act by donating hydrogen atoms to lipid radicals. Radicals obtained from

antioxidants with molecular structures such as phenols are stable species and will then stop the oxidation chain reaction (Morales-González, 2013).

The biostatic and biocidal activities of natural bioactive compounds including alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, anthraquinones, against a range of enteric pathogenic microbia were investigated (Enwa et al., 2013).

Many plant extracts have been shown to possess potent anti-oxidation activities (Tachakittirungrod et al., 2007), being able to scavenge a wide range of ROS (superoxide, hydroxyl and peroxyl radicals), reactive nitrogen species (RNS) (peroxynitrous acid) and chlorine species (hypochlorous acid) (Hernandez et al., 2009). Therefore, a number of plants have been utilized successfully for the treatment of free radical-mediated diseases in humans such as rheumatoid arthritis, atherosclerosis, cancer, Alzheimer's disease (AD), Parkinson's disease, aging and some inflammatory diseases (Das, 2002).

Plant extracts have also shown to display *in vitro* antimicrobial properties in which their bioactive constituents may exhibit different modes of action against enterotoxigenic bacterial strains. It range from interference with the phospholipoidal cell membranes which leading to an increase in the permeability profile and loss of cellular constituents, to the damage of the enzymes involved in the production of cellular energy and synthesis of structural components as well as destruction or inactivation of genetic material. In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport mechanisms, and coagulation of cellular proteins (Kotzekidou et al., 2008).

Plants are a valuable source of new products. Despite the availability of different approaches for the synthesis of therapeuticals, natural products remain one of the best supplies of new antioxidants and antimicrobial agents. Therefore, the present investigation aims to evaluate the antioxidant and antibacterial abilities of some medicinal Libyan plants.

MATERIALS AND METHODS

Plant collection and crude extract preparation

Arbutus pavarii (Ericaceae) (flowers and leaves) were collected in January 2014 from Al Marj, Libya*, Pegnanum harmal (Zygophyllaceae)* (seeds), *Pistachia atlantica (Anacardiaceae)* (Fruits) were collected in May 2014 from Nafusa Mountain, Libya, and *Fagonia bruguieri* was collected in May 2014 from Ghadames, Libya. The three plants were authenticated by the Department of Botany, Faculty of Science, and University of Tripoli, Libya. After cleaning and drying plants, samples were powdered using electric blender.

About 5 g of each sample (powdered) were soaked in 50 ml of methanol for 24 h with gentle shaking. It was then filtered using Whatman filter paper No.1 and the filtrate was evaporated to

dryness under vacuum at 40°C using a rotary evaporator (Hidolph, Germany). The obtained crude extracts were preserved at 4°C until use.

Test organisms

The bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhi*) were provided and identified by the Microbiology Laboratory, Medical Research Center, Libya. Bacterial strains were routinely grown and preserved on Nutrient broth or Nutrient agar medium (2.0% agar was added whenever needed).

Phytochemical screening

Plants extracts were subjected to phytochemical screening as described by Harborne (1992).

Determination of polyphenols and flavonoids contents

The total phenolic content was determined using the Folin-Ciocalteu reagent (Sigma, USA) according to the method of Marinova (2005). 200 µl of the sample (0.1 g/ml) in triplicate were incubated with 1 ml of two fold diluted Folin-Ciocalteu reagent for 5 min. 1 ml of 7% Na_2CO_3 was then added to the reaction mixture which was incubated again for 90 min. Thereafter, the absorbance was read at 750 nm using Jenway UV-VIS 6305 spectrophotometer. The total phenolic content is expressed as gallic acid equivalent (GAE) in milligrams per gram of dry sample.

Total flavonoid content was estimated according to (Zhishen, 1999). 1 ml of plant extract (0.1 g/ml) was diluted with 4 ml of water and was mixed with 0.3 ml of $NaNO_2$ (5% w/v). After 5 min, 0.3 ml of $AlCl_3$ (10% w/v) was added followed by the addition of 2 ml of NaOH (1 M) six minutes later. The reaction volume was increased up to 10 ml by adding 2.4 ml distilled water and the sample was incubated at RT for 15 min. The absorbance was measured at 510 nm. The assay was performed in triplicate, and the flavonoids content was determined by interpolating the absorbance of the samples against a calibration curve constructed with rutin standard (1.0 to 5.0 mg/ml) and expressed as milligrams of rutin equivalent per gram of extract (mg RE/g).

Assessment of antioxidant activity

Reducing power

The reducing capacity of crude plant extracts were investigated according to the method of Oyaizu (1986). Various concentrations of plant extracts (1.25 to 10 mg/ml) were mixed with 2.5 ml of phosphate buffer (2 M, pH 6.6) and 2.5 ml of potassium ferric cyanide (1%), and the mixture was incubated at 50°C for 20 min. After which, 1.5 ml of 10% TCA was added to the reaction mixture and then centrifuged at 1000×g for 10 min. The supernatant (0.5 ml) was mixed finally with $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 665 nm. The higher the absorbance of the reaction mixture the greater is the reducing power. Concentrations of ascorbic acid were used to obtain a standard curve and the reducing power of extracts was expressed as ascorbic acid equivalents.

Evaluation of total antioxidant capacity (TAC) by phosphomolybdenum method

The total antioxidant capacity was evaluated by the

Table 1. Preliminary phytochemical screening of crude extract of some libyan plants.

Phytochemical compounds	A. pavarii (Ericaceae)		P. harmala (Apiaceae)	P. atlantica (Euphorbiaceae)	F. bruguieri (Rhamnacea)
	Used part				
	Flowers	Leave	Seeds	Fruits	Leave
Alkaloids	+++	+++	+	++	+
Flavonoids	+++	+++	+	+++	+
Tannins	+++	++	-	++	-
Terpenes	+++	++	-	++	-
Coumarins	+++	+	+++	++	-
Saponins	+	+++	-	-	+
Anthraquinones	+++	+++	-	+	-

(+++) high, (++) medium, (+) poor, (-) no found.

phosphomolybdenum method according to the procedure described by Prieto et al. (1999). 0.3 ml of extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After that, the absorbance of the green phosphate/Mo complex was measured at 695 nm.

DPPH free radical-scavenging activity

The free radical scavenging activity of plant extracts was measured by DPPH (2, 2-diphenyl-1-picrylhydrazyl) (Sigma, USA) according to Wong et al. (2006) method. Briefly, 40 µl of methanolic extract of plant at different concentrations (5, 12.5, 25 and 50 mg/ml) or 0.165, 0.25, 0.5 and 1 mg/ml for *A. pavarii (flowers and leaves)* were added to 3 ml of DPPH (0.1 mM) in methanol solution, shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance was measured at 517 nm using a UV- visible spectrophotometer (U-6305 model, Jenway, Japan). The percent of DPPH scavenging effect was calculated as follows:

$$\% \ DPPH = ([A_C - A_S]/A_C) \times 100$$

Where A_C was the absorbance of the control reaction and As was the absorbance in the presence of the sample plants.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically according to Garrat (1964) method. 1 ml of sodium nitroprusside (10 mM) in phosphate buffer was added to 0.5 ml of sample (1.25 mg/ml) and incubated at 25°C for 150 min. Thereafter, 0.5 ml of the reaction mixture containing nitrite ions was taken; 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added. After being shaken, the samples were allowed to stand for 5 min. then 1 ml of naphthylethylene diamine dihydrochloride (0.1%) was added, mixed and allowed to stand for 30 min. The absorbance of the mixture was measured at 540 nm against the corresponding blank. The percentage of scavenging activity was measured with reference to ascorbic acid as standard.

Antimicrobial assay

The antibacterial activity of the crude and flavonoids plant extracts

was screened against some Gram-negative and Gram-positive bacteria using the Agar diffusion method (Nair et al., 2005) at a concentration of 100 mg/ml for crude extract and 5 mg/ml for flavonoids extracts. Nutrient agar (Difco™ Tryptic Soy Agar, Becton Dickinson and Company, USA) was inoculated with 250 µl the suspension of the respective 24 h cultured organism and poured into a sterile Petri dish. Ceproxazine (Merck, Germany) was used as a positive control, the solvent of each extract as a negative control. A pre-diffusion for 3 h at 4°C was guaranteed. Diameters of inhibition zones (DIZs) were measured in mm after 18 h incubation at 37°C. The inhibition zones were measured excepting the hole diameter (8 mm). The results were recorded as the mean of triplicate experiments. Inhibition zones >15 mm were declared as strong, from 8 to 15 mm as moderate and from 1 to 8 mm as weak activities. Minimal inhibitory concentration (MIC) values against selected strains were determined by standard serial broth microdilution assay (European Pharmacopoe, 1997).

Statistical analyses

The experiments were carried out in triplicate. The results are given as mean ±standard deviation (SD), Student's t-test was used for comparison between the means of samples and standards. A difference was considered statistically significant when $P<0.05$. Correlation analysis was carried out on total phenolics and flavonoids contents (TPC, TFC) and the three antioxidant models (total antioxidant activity, DPPH & Nitric oxide scavenging activity) using SPSS 12.0 for Windows (Statistical package).

RESULTS

Phytochemical screening

The phytochemical characters of the four investigated medicinal plants are summarized in Table 1. Flavonoids and alkaloids were the most common components present in investigated plants. Coumarins are found in *A. pavarii*, *P. harmal*, *P. Atlantica* while absent in *F. bruguieri*. Anthraquinones are present only in *A. pavarii*, and *P. Atlantica*, while Terpenoids are found in *A. pavarii* and *P. atlantica*. Tannins are present in *A. pavarii*, and
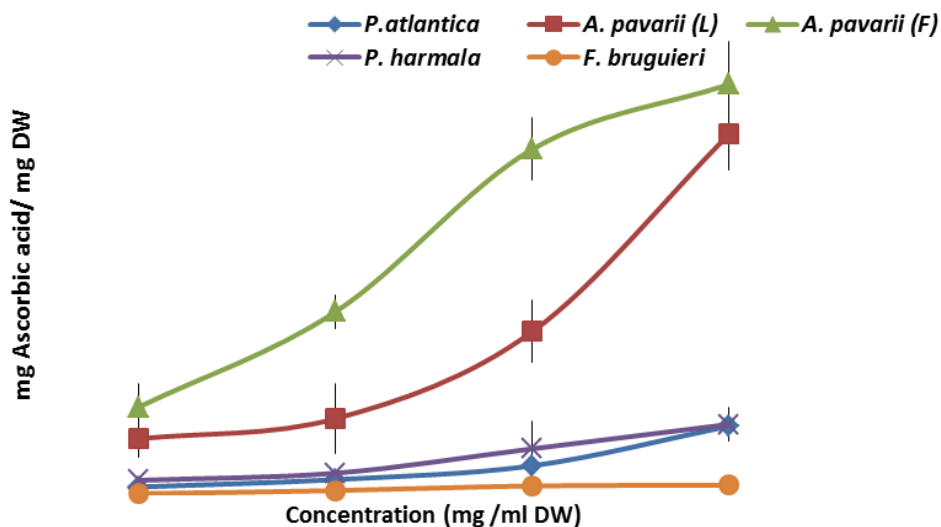
Figure 1. Total reducing power of crude extracts of the investigated plants estimated by ferrous cyanide. Each value is represented as mean ±SD (n=3).
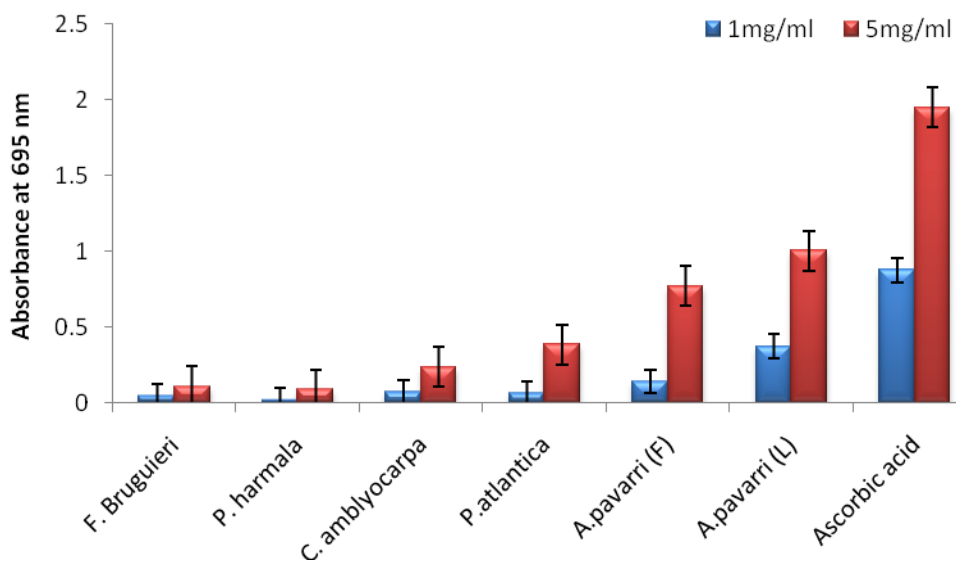
Figure 2. Total antioxidant capacities of extracts at two different concentrations (1 and 5 mg/ml) as determined by *Phosphomolybdate* method. Each value is expressed as mean ±SD (n=3).

P. harmal.

In vitro antioxidant activity

Antioxidant capacity of plant extracts were assessed and compared using four different antioxidant assays.

Reducing power activity

As shown in Figure 1, reducing power increased with the

increase of extracts concentration. Flowers of *A. pavarii* had the highest ability to reduce Fe (III) while *F. bruguieri* extract had the lowest capacity. Also the reducing power of methanolic leaves extracts of *A. pavarii* was more than that of the *P. harmala*, and *P. atlantica* extracts.

Phosphomolybdate assay

Results showed in Figure 2 revealed that the antioxidant activities of the tested plant extracts were significantly

Table 2. IC_{50} of plants extracts in comparison with ascorbic acid as standard.

Plant	IC_{50} (mg /ml)
A. pavarii (L)	0.62±0.003**
A. pavarii (F)	1.30±0.004**
P. atlantica	50±3.75
P. harmala	100.30± 10.23
F. bruguieri	12.50± 1.15*
Ascorbic acid	3.05±0.005

Each value in the table is represented as mean ±SD (n=3).* indicates significance at *P*<0.05, ** indicates significance at *P*<0.01.
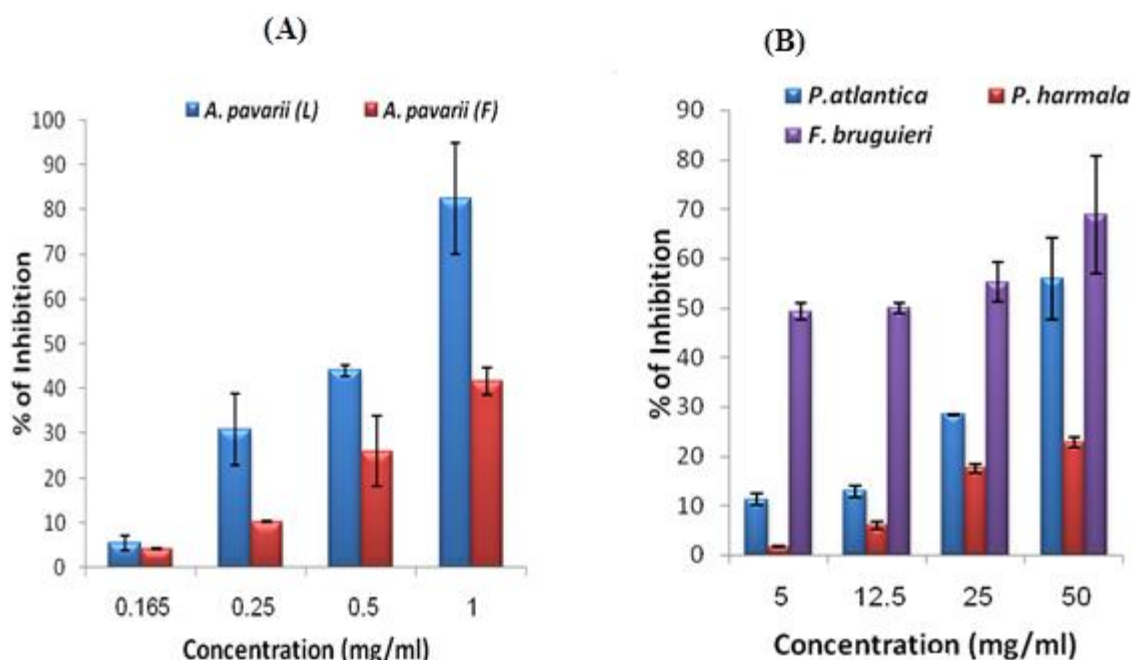
Figure 3. DPPH radical scavenging activity of plant extracts at various concentrations. Each value are represented as mean ± SD (n = 3).

different especially at high concentrations (2.5 to 5.0 mg/dL). Although *A. pavarri* (L) had the highest antioxidant capacity for phosphomolybdate reduction, its activity did not reach that obtained by ascorbic acid. *P. harmala* exhibited the lowest antioxidantion capacity for phosphomolybdate reduction.

DPPH radical scavenging activity

The results showed that *A. pavarii* possessed highest radical scavenging and reducing power activities (Table 2, Figures 1 and 3A). Leaves and flowers extracts of *A. pavarii* displayed strong radical scavenging effect compared with ascorbic acid at very low concentration

(0.165, 0.25, 0.5 and 1 mg/mL) (Figure 3A). Compared to ascorbic acid a significant difference between the IC_{50} of *A. pavarii* (leaves and flowers) and ascorbic acid (0.62±0.003, 1.30±0.004 and 3.05±0.005 respectively) (*P*< 0.001), which means that the radical scavenging activities of these two extracts were higher than ascorbic acid (Table 2). In addition, the radical scavenging activity of *F. bruguieri, P. atlantica* and *P. harmala* was substantially low as compared with ascorbic acid (*P* < 0.05) (Figure 3B).

Nitric oxide scavenging activity

Figure 4 shows the comparative NO scavenging activity
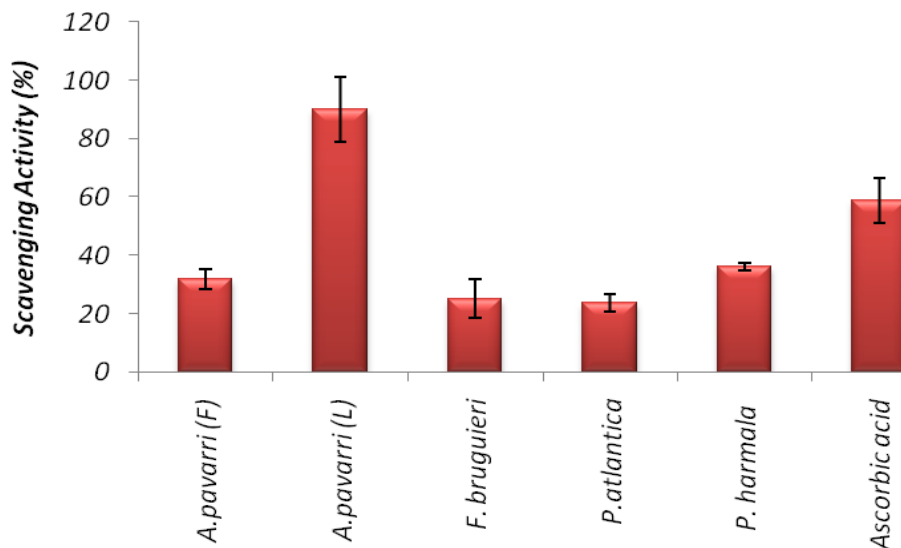
Figure 4. Nitric oxide radical scavenging activity of crude extracts under testing. Each value is represented as mean ±SD (n=3).

Table 3. Total polyphenols (TPC) and total flavonoids (TFC) concentrations of plants under investigations.

Plant	Used part	TPC (mg GA/g DW)	TFC (mg Rutin/g DW)
P.atlantica	Fruits	585.47±8.98	110.07±3.83
A. pavarii	Leaves	1217.14±50.52**	1504.28±80.89**
	Flowers	883.33±169.64*	357.66±5.79
P. harmala	Seeds	654.28±90.94*	88.95±6.69
F. bruguieri	Leaves	249.28±54.48	349.28±54.48

Each value is represented as mean ±SD (n=3). *indicates significance at $P<0.05$.** indicates significance at $P<0.01$.

of the extracts at concentration 1.25 mg/ml. *A. pavarii* leave extract showed greater NO inhibition (90.27%) as comparative to other plant extracts and ascorbic acid (Figure 4). The maximum NO scavenging of *P. atlantica, P. harmala*, and *F. bruguieri* were 23.70, 36.075, 25.25 and 35.73% respectively, which was less than ascorbic acid (45%).

Total phenolics and flavonoids contents

Table 3 shows that, the highest amount of phenolic and flavonoids compounds were in *A. pavarii* (Leaves) (1217.14±50.52, 1504.28±80.89 mg/g Dw respectively), while *F. bruguieri* had the lowest content of phenolics and flavonoids (249.28±54.48 and 6.95±0.95 mg/g Dw respectively).

Correlation of values of antioxidant activities with TPC and TFC

Table 4, shows a high correlation ($R^2 = 0.734$) between

phenolic compounds and flavonoids. The results show moderate correlation ($R^2 = 0.585$) between the DPPH radical scavenging activity and total phenolic compounds, but there was a low correlation ($R^2 = 0.420$, Table 4) with flavonoids level. Also moderate correlation was found between TP content and NO scavenging activity ($R^2 = 0.686$) whereas highly significant correlation was observed between TF and NO scavenging activity ($R^2 = 0.951$) ($P<0.01$).

Antimicrobial activity of plants

In the present study, the antimicrobial activities of crude and flavonoids extracts against six test microorganisms were examined and their potency was qualitatively and quantitatively assessed by the presence or absence of inhibition zones. The results presented in Tables 5 and 6 showed moderate antimicrobial activities for crude extracts and high antimicrobial activity for flavonoids extracts against some microorganisms tested. There is no specifically previous report on evaluation of these plants against these set of microorganisms (Table 5).

Table 4. Correlation indexes between phytochemicals (phenolics and flavonoids) and the applied three methods for determination antioxidant potential in some Libyan plants.

Assays	Correlations R^2	
	Phenolics (TPC)	Flavonoids (TFC)
Flavonoids content	0.734*	-
DPPH radical scavenging ability	0.585	0.420
Nitric oxide scavenging activity	0.686*	0.951**
Phosphomolybdate antioxidant activity	0.798*	0.712*

*significant at $P>0.05$, **significant at $P>0.01$.

Table 5. Antibacterial activity of the crude and flavonoids extracts, and positive control against some bacteria strains. Results are expressed as diameter of inhibition zone (mm)[a].

Plant name	B. sub		S. aur		S. faecalis		E. coli		P. aeruginosa		S. typhi	
	C	F	C	F	C	F	C	F	C	F	C	F
A. pavarii (L)	27	30	13	23	26	22	20	25	15	25	20	25
A. pavarii (F)	25	45	20	32	25	35	30	25	20	33	28	35
P.atlantica	15	15	na	20	15	20	15	20	10	na	15	17
P. harmala	12	25	na	25	13	30	13	23	10	40	**30**	20
F. bruguieri	22	18	na	na	15	20	15	na	30	18	18	14
Ceproxazine (5 µg)	16		20		nt		18		nt		20	

a: Diameter of inhibition zone including hole diameter of 8 mm. **na**, not active; C: crude extract; F: Flavonoids extract, *B. sub: Bacillus subtilis, E. coli: Escherichia coli, S. aur: Staphylococcus aureus, P. aer: Pseudomonas aeruginosa, S. faecalis: Streptococcus faecalis, S. typhi: Salmonella typhi.*

According to the present results, the flavonoids extract of flowers of *A. pavarii* extract were found to be active against all tested bacteria. The strongest antibacterial activity was seen against *S. typhi* with a MIC value of 0.019, followed by *B. sub* and *E.coli with* MIC 0 .156 mg/ml, followed by *S. aureus* and *Ps. Aeruginosa,* MIC 0.3125 mg/ml. Whereas, *S. faecalis, S. typhi*, *Ps. Aeruginosa* and *B.Sub* showed best susceptibility towards the methanol extract of leaves of *A. pavarii* with a MIC value of 0.156 mg/ml followed by *E.coli* and *S. aureus* MIC 0.132 and 1.25 mg/ml respectively. The flavanoids extract of *P. harmal*, and *P. Atlantica demonstrated* moderate activities against tested bacteria with MIC ranged from 0.156 to 0.132 mg/ml.

DISCUSSION

The beneficial medicinal effects of plant materials typically result from the combinations of plant specialized products. These compounds are mostly secondary metabolites such as terpenoids, alkaloids, tannins, and flavonoids which are capable to play definite biological and pharmacological activities and may have potential to be used as chemotherapeutic agents or serve as starting material in the developing of new antibiotics (Sibanda, 2007). Therefore, this study was carried out to screen the

presence of some of these phytochemical compounds in the selected four Libyan plants as well as their bioactivity. Flavonoids and alkaloids were the most common compounds present in the all plant extracts investigated. These finding correlated well with several earlier publications (Havsteen, 2002; Ncube et al., 2008; Abubakar et al., 2010).

Polyphenols which are commonly found in medicinal plants exist as a group of highly hydroxylated phenolic compounds including hydroxycinnamate derivatives, hydroxycoumarins, flavanols, flavanones, flavones, proanthocyanidins (tannins), anthocyanins, aurones, and hydroxystilbenes. Such bioactive compounds have showed many pharmacological activities (Sibanda, 2007). Tannins, saponins and terpenes are useful anti-inflammatory agents, as they have been shown to cure inflamed tissues (Li et al., 2003). Flavonoids are important constituents of plants, since they possess prominent antioxidant properties (Havsteen, 2002). Alkaloids have long history of use in pharmaceutical industry as antiallergy, analgesic, muscle relaxant and antimalarial (Yen and Chen 1995). Several studies have demonstrated a highly significant correlation between the phenolic content in a plant and the antioxidant activity (Madson et al., 2000; Jagethia et al., 2004; Abubakar et al., 2010). In this study, a high correlation ($R^2 = 0.734$) between phenolic compounds and flavonoids has been

Table 6. Antibacterial activity expressed as minimum inhibitory concentration (MIC)[a] of the crude and flavonoids extracts against some bacteria strains.

Plant name	B. Sub		S. aur		S. faecalis		E. coli		P. aeruginosa		S. typhi	
	C	F	C	F	C	F	C	F	C	F	C	F
A. pavarii (L)	1.0	0.156	8.0	1.25	0.5	0.156	0.5	0.312	2.0	0.156	0.25	0.156
A. pavarii (F)	0.25	0.156	1.0	0.156	0.5	0.156	0.25	0.156	2.0	0.156	0.25	0.019
P.atlantica	0.5	2.5	nt	0.312	4.0	0.312	1.0	0.312	4.0	nt	1.0	0.312
P. harmala	2.0	0.312	nt	0.625	8.0	0.156	0.5	0.625	1.0	0.625	4.0	0.321
F. bruguieri	1.0	2.5	8.0	5.0	8.0	nt	2.0	5.0	nt	nt	2.0	5.0

a: minimum inhibitory concentration values are given as mg/mL. nt, not tested. *B. sub: Bacillus subtilis, E. coli: Escherichia coli, S. aur: Staphylococcus aureus, P. aer: Pseudomonas aeruginosa, S. faecalis: Streptococcus faecalis, S. typhi: Salmonella typhi.*

statistically proved, which is in accordance with a previous recent study (Ibrahim et al., 2011).

Antioxidants (free radical scavengers) are substances that interact with, and neutralize free radicals, thus preventing them from causing cellular damage in the biological system (Rahman, 2007). Several methods have been used to determine the antioxidant activity *in vitro* in order to allow rapid screening of bioactive substances. Antioxidant activities have been attributed to various reactions and mechanisms such as prevention of chain initiation of lipid peroxidation, binding of transition metal ion catalysts, reductive capacity, and or radical scavenging effect (Frankel and Meyer, 2000; Huang et al., 2005).

In reducing power assay, the presence of the reductants in the solution causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by absorbance measurement at 700 nm. In this study, when the concentration of extracts was increased, the reducing ability was increased. This result is similar to that reported earlier (Noriham et al., 2004; Premanath and Lakshmidevi, 2010). Flowers of *A. pavarii* had the highest ability to reduce Fe (III) while *F. bruguieri* extract had the lowest one. Also the ability of reducing power for methanolic leaves extract of *A. pavarii* leaves were more than that of the *P. harmala*, and *P. Atlantica* extracts. Many studies suggested that the reducing power of plants might be related to the presence of reductans agents such as polyphenols (Duh, 1998; Sibanda, 2007; Knežević et al., 2011). A previous report suggested that the reducing properties have been shown to exert antioxidant action *via* donating a hydrogen atom to break the free radical chain (Gordon, 1990).

The antioxidant capacity of the extract was measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 765 nm. The present study revealed that leaves of *A. pavarri* (L) showed the highest antioxidant capacity for reduce phosphomolybdate. Previous studies have shown that many polyphenols including flavonoids contribute significantly to the phosphomolybdate scavenging character of medicinal plants (Sharififar et al., 2009; Khan et al., 2012).

In the radical scavenging assay, when the DPPH is exposed to antioxidant compounds the purple color of DPPH changes to yellow indicating the free radical scavenging ability of a sample (Ebrahimzadeh et al., 2008). The more yellowish color of DPPH observed the greater the antioxidant activity of the plants tested. In this work the results of radical scavenging activity showed that leaves and flowers of *A. pavarii* extracts possessed strong radical scavenging effect with low IC50 (0.62±0.003). Although many studies have suggested that polyphenolics and flavonoids are highly effective against free radicals (Havsteen, 2002; Amic et al., 2003), in this study a moderate correlation (R^2 = 0.585) was shown between the DPPH radical scavenging and total phenolic content of sample. Also a low correlation between plant flavonoid level and the DPPH radical scavenging (R^2= 0.420, Table 4). Therefore, it may be possible that the radical scavenging activity of a sample cannot be predicted on the basis of its total phenolic content (Kähkonen et al., 1999).

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, and is involved in the regulation of various physiological processes (Lata et al., 2003). NO scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants. In this study, a moderate correlation was found between total phenolics content and NO scavenging activity (R^2 = 0.686) whereas highly significant correlation was observed between TF and NO scavenging activity (R^2 = 0.961) (*P<0.01*). These results were in line with previous studies which revealed that the scavenging activity of plants is related to their contents of polyphenolics and flavonoids (Madson et al., 2000; Jagetia et al., 2004). Results of this study suggest that the extracts especially *A. pavarri* contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge its possible potential cellular damage in living system.

Plant extracts have been used for many years,

especially in food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997; Aboud, 2015). It has long been acknowledged that some plants exhibit antimicrobial properties, this was a motive to investigate the metabolites of those plants precisely which have been used, in traditional medicine to improve the quality of healthcare. The mode of action of natural products toward microbial infections is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Tsuchiya et al., 1996).

There are many factors such as climate, soil composition, age and vegetation cycle stage that explain the differences of antimicrobial activities of medicinal plants belonging to different regions of the world on quantity, and quality of bioactive compound and composition of extracted natural products (Masotti et al., 2003; Angioni, 2006; Noumedem et al., 2013). In addition the effect of solvent polarity used in the extraction play an important role in the amount of biologically active materials (Al-Zubaydi et al., 2009; Bakht et al., 2011; Bedi, 2010; Anmar, 2015).

In the present study A. pavarii represents a good candidate as a reliable source for the extraction of some major bioactive compounds that inhibit the growth of microorganisms, thereby proving to be very effective as alternative source of antibiotics. The continued traditional medicinal use of these plants is therefore encouraged while it is suggested that further studies should be carried out to isolate, purify and possibly characterize the active constituents responsible for the activity of these plants. Most traditional medicinal plants in use today have no scientific data on their bioactivity and levels of safety or even how they are likely to affect each other when used as combinations in medicines.   Furthermore, scanty research has been done on their mechanisms of action considering that most are orally consumed. Therefore, further studies are needed to find out the mode of action of the tested plant extracts against bacteria.

Conclusion

This research indicates that the tested parts of A. pavarii, especially leaves have prominent antioxidant and antibacterial activities. The presence of phenolic and flavonoids compounds which were found in high level could be attributable to the observed high antiradical properties of these extracts. However, further investigation is necessary to separate and characterize the component of each individual extracted sample and then evaluate the antioxidants activity of each component as well as to find out the possible mechanisms of antioxidant and antimicrobial activities in vivo. These plants could be added to the long list of promising medicinal plants that offer distinctive source of natural antioxidants that can be used on industrial or medical scales.

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