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Full Length Research Paper

Acute toxicity, antinociceptive and anti-inflammatory activity of the essential oil of fresh fruits of *Piper guineense* Schum & Thonn (Piperaceae) in rodents

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Piper guineense **is a popular herbal medicine used to manage pains and arthritis among other indications in South-West Nigeria. Previous biological studies report anti-oxidant, anti-microbial and antidiabetic activities for the essential oil of the plant while studies on its acute toxicity profile, potential analgesic and anti-inflammatory activities were unavailable. This study investigated the antinociceptive and anti-inflammatory effect of the plant fruit volatile component and determines its acute toxicity profile in rodents in an attempt to rationalize the use of the plant in folkoloric medicine. Essential oil of fresh fruits of *P. guineense* obtained by hydrodistillation was emulsified with Tween 80 and evaluated for acute toxicity test (LD_{50}) through the oral (p.o.) and intraperitoneal (i.p.) routes in mice. The oil (50 to 200 mg/kg, i.p.) was tested for anti-nociceptive activity on the hot plate and acetic acid-induced writhing models in mice, while the anti-inflammatory activity was assessed on the egg albumin-induced rat paw oedema. The LD_{50} values obtained were 693 mg/kg, i.p. and 1265 mg/kg, p.o. The oil dose-dependently caused significant ($p<0.01$) prolongation of reaction time on the hot plate comparable to positive control, morphine signifying central antinoceceptive effect, significantly ($p<0.01$) inhibited writhings induced by acetic acid analogous to diclofenac suggesting peripheral mechanism and caused significant ($p<0.01$) reduction in egg albumin-induced rat paw oedema comparable to dexamethasone, indicating anti-inflammatory activity. This study shows that the essential oil of *P. guineense* was moderately toxic, possessed significant antinociceptive and anti-inflammatory activities which can be used to rationalize the use of the plant in ethnomedicine.**

Key words: *Piper guineense,* volatile oil, acute toxicity, hot plate, egg albumin.

INTRODUCTION

Piper guineense otherwise known as West African Black Pepper is a herbaceous climber commonly found in African tropical forest zone, with more than 700 species found in many tropical and sub-tropical regions of the world (Olonisakin et al., 2006). In Nigeria, it is known with different vernacular names such as *Uziza* and *Iyere* among

the Igbos and Yorubas, respectively. The fruits or berries and leaves are usually sold in Southern Nigerian markets as condiments and for food flavouring agent. The plant is one of the highly valued spices across West African countries where its fruits and leaves (Figure 1) form important ingredients in domestic cooking and commercial cuisines. In Southern Nigeria, *P. guineense* is popular in folkmedines for the management of several health-related conditions including respiratory disorders, infections, infertility, pain, rheumatism and as an aphrodisiac (Ekundayo et al., 1988; Burkill, 1995; Ekanem et al., 2010; Tankam and Ilto, 2013).

Chemical composition of *P. guineense* varies from one geographical region to another and even within the same region. For example, Ekundayo et al. (1988) reported myristicin, safrole, sarisan and elemicin as major components of its fruits while Oyedeji et al. (2005) reported β-pinene, α-pinene and germacrine-B as the major components. Further studies also reported β-pinene, D-Limonene, caryophyllene and car-z-ene as the main constituents (Olonisakin et al., 2006), yet another report indicate β-pinene, α-pinene, 1,8-cineole and γ-terpinene as major constituents of the plant fruit essential oil from Nigerian species (Oboh et al., 2013).

Biological studies on the essential oil of *P. guineense* include fertility enhancing (Mbongue et al., 2005), anti-fertility (Ekanem et al., 2010), anti-oxidant (Etim et al., 2013), hypolipidemic and hypokalaemic (Nwaichi and Igbinobaro, 2012), insecticidal (Madubuike et al., 1990; Adewoyin et al., 2006), anti-microbial (Oyedeji et al., 2005), anti-diabetic and antioxidant (Oboh et al., 2013), sedative (Tankam and Ito, 2013) and larvicidal (Ohaga et al., 2007) among numerous activities.

Preliminary enquiries (field study) from herbalists in some communities within Ondo State (South West Nigeria) indicate that fruits of *P. guineense* are indicated for muscular pain, rheumatism and as an aphrodiasc either as a single agent or in combination with other herbal agents (oral communication). Since essential oil constitute a major active components of medicinal plants with considerable bioactivities, the essential oil of this plant was evaluated for antinociceptive and anti-inflammatory activity as a preliminary screening process for validation of the folkolic use of the plant in managing pain-related ailments. Furthermore, there is no data available to our knowledge concerning the acute toxicity profile of the essential oil of the fresh fruits of the plant; hence we also evaluated the oil for acute toxicity (LD_{50}) test in order to determine its relative toxic profile orally and parenterally.

MATERIALS AND METHODS

Plant identification and authentification

The Fruits of *P. guineense* were authenticated by Mr. G. Ibhanesebor, the Herbarium Officer of the Department of Botany, Faculty of Science, Obafemi Awolowo University (OAU) Ile-Ife, Osun State. The voucher specimen of the plant and its fruits were deposited at the herbarium unit of the Department of Botany, Faculty of Sciences, OAU, Ile-Ife. The Voucher specimen of the plant and its fruits were deposited at the Herbarium Unit of the Department of Botany, Faculty of Sciences, OAU, Ile-Ife, as voucher NO. IFE 16772 was issued. Fresh fruits were purchased from the Central Market, Ondo Town, Ondo State between October and December, 2012.

Extraction of the essential oil

Hydrodistillation of the fruit of *P. guineense* was carried out using clevenger-like apparatus (BP 1988). Fresh fruits of *P. guineense* (400 g) were slightly crushed to open the fruits before hydrodistilled for 4 h. The essential oil obtained was dried over magnesium sulphate crystal and stored in an air-tight bottle, refrigerated until use.

Laboratory materials and equipment

Plexiglass cage, hot plate machine, digital thermometer, fresh egg, methylated spirit, Tween 80 and other reagents were of analytical grade.

Drugs

Morphine (Sigma, St. Louis, USA), acetic acid (BDH Chemicals Ltd, Poole, England), diclofenac (Supreme Pharm. Nig. Ltd., Lagos, Nigeria), dexamethasone (Hubei Tianyo pharmaceuticals, China) and other chemicals and drugs used were of analytical grade.

Laboratory animals

Swiss albino mice (18 to 25 g) of both sexes and rats (150 to 250 kg) of both sexes were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were kept under standard laboratory conditions and fed with animal pellets and they had free access to water *ad libitum*. The study was approved by the Faculty of Pharmacy Postgraduate Committee and all animal experiments were carried out in strict compliance with the guideline of the National Institute of Health (NIH, 1985) as being implemented by the Obafemi Awolowo University Research Committee.

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Acute toxicity study

The method used was described by Lorke (Lorke, 1983). This involved using the 13-animal model for rapid determination of LD_{50}. The method involves two phases. In the first phase of both the oral and i.p. route, three increasing doses of 10, 100 and 1000 mg/kg of emulsified EOPG were administered to three different groups of mice (n = 3). In the second phase, doses of 1000, 1600, 2900 and 5000 mg/kg were used for the oral route, while doses of 400, 600, 800 and 1000 mg/kg were used for the i.p route in 4 groups of mice (n = 1). The animals were monitored for 2 h and mortality was recorded after 24 h.

Pharmacological study

Expermental design

In all the tests, animals were randomly selected into 5 different groups (n=5) as follows:

Group I: Nagative control treated with vehicle (5% Tween 80, 10 ml/kg).
Group II-IV: test grougs treated with the essential oil 50, 100 and 200 mg/kg, respectively.
Group V: positive control treated with appropriate standard drug.

All treatments were by intraperitoneal route.

Antinociceptive test

Hot plate test

Vehicle, essenial oil (50, 100 and 200 mg/kg) and morphine (10 mg/kg) were administered to different groups of mice and after 30 min, each mouse was placed on the hot-plate pre-set at 55°C and the time taken by the mouse to lick the fore/hind paw was taken as the reaction time in s. The cut off time was set at 15 s to avoid tissue damage. The test was repeated and reaction time recorded at 60, 90 and 120 min post-treatment (Silva et al., 2003).

Acetic acid-induced writhings

Different groups of mice were pretreated for 30 min with vehicle, essenial oil (50, 100 and 200 mg/kg) and diclofenac (100 mg/kg) before intraperitoneally administration with 10 ml/kg of 1% acetic acid (Hajhashemi et al., 2003). The number of writings (abdominal constriction) displayed by each mouse was counted and recorded over a period of 20 min starting from 5 min post-acid injection (Yin et al., 2003).

Antiinflammatory test

The anti-inflammatory activity was studied using egg albumin-induced paw oedema acute inflammation methods in rats previously described by Olajide et al. (2000) with minor modification. Different groups of rats were pretreated with vehicle, essenial oil (50, 100 and 200 mg/kg) and dexamethazone (1 mg/kg) for 30 min prior to injection of 100 µl of undiluted fresh egg albunin into the sub-planter surface of right hand paw of the rats. Measurement of the rats' paw sizes were carried out by measuring the circumference of the odematous paw with thread wrapped round the paw and then placing the thread on a meter ruler to determine the diameter in mm. The circumference (mm) of the odematous paw equates inflamed paw of the rat (Olajide et al., 2000). The measurement of paw size was repeated at 1, 2 and 4 h post injection of the egg albumin and the % inhibition calculated for each treatment group using the formula:

$$\% \text{ inhibition} = \frac{[(Ct - C_o) \text{ control} - (C_t - Co) \text{ treated})]}{(C_t - C_o) \text{ control}} \times 100$$

Where Ct and Co are paw sizes (mm) at different time after and before egg-albumin injection (Olajide et al., 2000).

Statistical analysis

Results are expressed as mean ± standard error of mean (SEM) and analysed by one way analysis (ANOVA), followed by Dunnett's post-hoc test with level of significance set at $p < 0.05$.

RESULTS

Essential oil obtained

The yield of the essential oil was 1.25% w/w. The oil was colourless with characteristic pungent aromatic odour and its relative density was determined to be 833 mg/L (0.88 g/L). The essential oil was readily soluble in Tween 80 and was therefore used to emulsify the oil prior to each test at concentration ≤5% v/v.

Acute toxicity (LD_{50})

The results of the acute toxicity study indicate that the LD_{50} of the oil was calculated to be 693 and 1265 mg/kg for the intraperitoneal and oral routes, respectively.

Effect of the essential oil on the hot plate and acetic acid-induced writhings

The result of the hot plate test is presented in Table 1. Essential oil of *P.guineense* (50, 100 and 200 mg/kg) and morphine induced significant ($p < 0.01$) increase in reaction time to thermal stimulation on the hot plate test at 30 [$F_{(4,20)} = 13$], 60 [$F_{(4,20)} = 3.5$], 90 [$F_{(4,20)} = 6$] and 120 [$F_{(4,20)} = 54$] min post-treatment. The mean reaction time

(s) at 30 min for the oil (50, 100 and 200) were 9.64, 9.80 and 16.35, respectively compared to 5.7 for vehicle and 16.4 for morphine; at 60 min, for the oil (50, 100 and 200) were 12.9, 15.8 and 13.8, respectively compared to 9.7

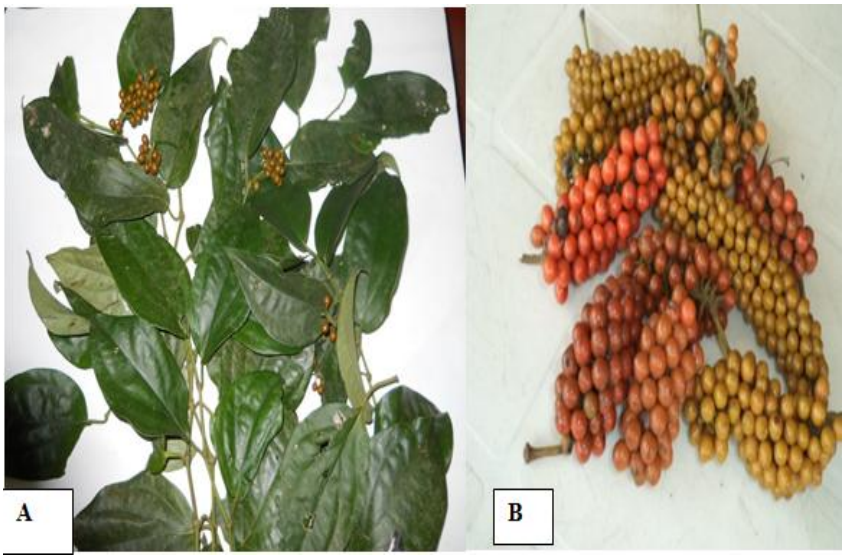

Figure 1. *Piper guineense* plant. Panel A shows the leaves and unripe fruits and panel B the ripe fruits.

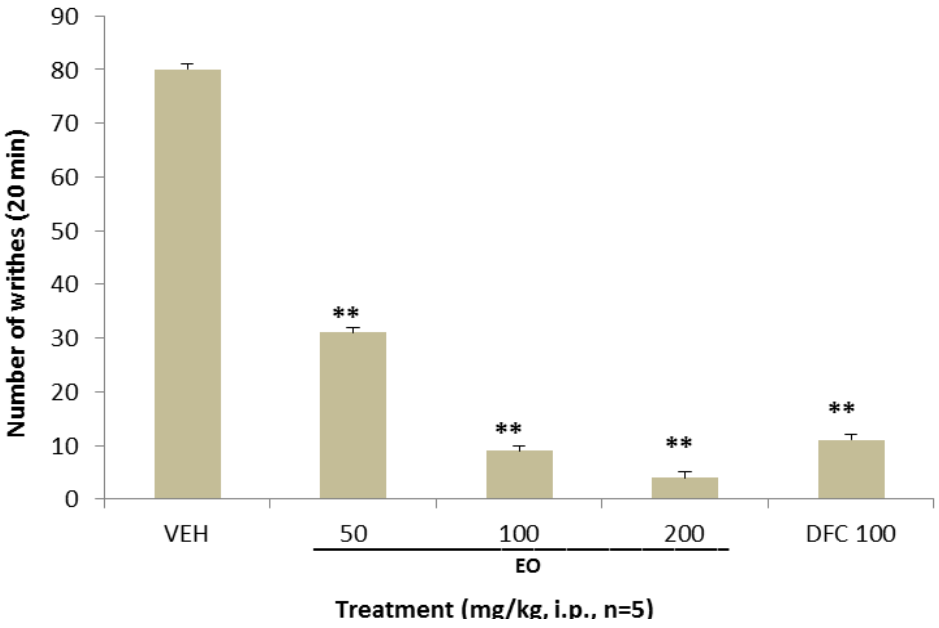

Figure 2. Effect of the essential oil of P.guineense on acetic acid-induced writhings in mice. VEH, EO and DFC represent vehicle (5%Tween 80), essential oil of P.guineense and diclofenac respectively. **p<0.01, statistically lower than vehicle (ANOVA, Dunnett's test).

for vehicle and 21.2 for morphine; at 90 min, the oil (50, 100 and 200) were 14.8, 22.2 and 17.6, respectively compared to 10.1 for vehicle and 25.2 for morphine; and at 120 min, for the oil (50, 100 and 200) were 18.4, 16.8 and 23.0, respectively compared to 10.7 for vehicle and 13.6 for morphine. All values above 15 s were equalized

to 15 as the maximum cut-off point for all reaction time. The result of the acetic acid induced writhings (Figure 2) showed that the oil (50, 100 and 200 mg/kg) and diclofenac (100 mg/kg) significantly ($p < 0.01$; $F_{(4,20)} = 90$)

Table 1. Effect of essential oil of *P. guineense* on the hot plate test in mice.

Treatment (i.p.) (n = 5)	Reaction time in seconds after			
	30 min	60 min	90 min	120 min
Vehicle	5.68±0.22	9.70±1.09	10.20± 0.38	10.72±0.40
Essential oil, *P. guineense* 50 mg/kg	9.64 ±1.55*	12.92±1.96*	14.8±1.97**	15.00 ±0.00**
Essential oil, *P. guineense* 100 mg/kg	9.80±0.66*	15.00±0.00*	15.00±0.00**	15.00±0.00**
Essential oil, *P. guineense* 200 mg/kg	12.10±1.26**	13.80±1.32	15.00±0.00**	15.00±0.00**
Morphine 10 mg/kg	15.00±0.00**	15.00±0.00*	15.00±0.00*	13.6±0.40**
P value	*P<0.05,**p<0.01	*p<0.05	**p<0.01	**p<0.01
F value	F (4,20) = 13	F (4,20) = 3.5	F (4,20) = 6	F (4,20) = 54

Vehicle is 5% Tween 80. Each value represents mean ± SEM of reaction time (s). *p<0.05, *p<0.01; statistically different from vehicle (ANOVA, Dunnett's test).

reduced the number of writhes induced by acetic acid. The writhes was reduced from 84 (vehicle) to 31, 9 and 4 by the oil (50, 100 and 200 mg/kg) representing 62, 89 and 95%, respectively, while diclofenac reduced writhes to 11, producing 87% inhibition.

Effect of the essential oil on egg albumin-induced inflammation

Rats in the negative control group showed progressive increase in paw sizes throughout the observation period of 4 h while the essential oil of *P. guineense* significantly inhibited the oedema induced by the egg albumin in a dose-dependent manner which was comparable to the potent steroidal anti-inflammatory drug, dexamethasone (1 mg/kg). The reduction in oedema sizes varies from 46 to 89% for the oil and 77 to 94% for demxamethasone from half ½ to 4 h post induction of inflammation. The reduction in oedema sizes expressed in mean ± SEM and percentage inhibition by the oil and dexamethasone with their statistical values were presented in Table 1.

DISCUSSION

Antinociceptive and antiinflammatory activities of the essential oil of *P. guineense* was evaluated in addition to determination of its acute toxicity profile in this study. The antinociceptive test was evaluated on two models (the hot plate test and the acetic-acid induced writhings) in mice, while the antiinflammatory test was evaluated on egg albumin-induced rat paw oedema in rats. Results obtained from this study showed that the oil of this plant fruits demonstrated significant effects in all the models used while the acute toxicity results indicate different toxicity outcome depending on the route of administration.

The Lorke's method (Lorke, 1983) has gained wide acceptance in preliminary screening of new agents for acute toxicity evaluation due to the use of minimal number of animals, its reliability and rapidness, and generally more economical than the previously used methods. Furthermore, the protocol is likely to be more tolerable by the Animal Rights groups agitating against the arbitrary use of animals in experiments. The LD_{50} values obtained for the oil was 1265 and 696 mg/kg for oral and intraperitoneal routes, respectively indicating that the oil may be moderately toxic orally and more toxic parenterally (Rodricks, 1992). The LD_{50} of the oral route of administration was quite higher than that of i.p. route due majorly to first pass effect that is, hepatic metabolism of drug when absorbed and delivered through portal blood (Pond and Tozer, 1984; Gavhane and Yadav, 2012). Metabolism of drug in the gastrointestinal tract (GIT) by the acidic and enzymatic contents and slower absorption rate can lead to lower bioavailability (Tracy et al., 2004). The intraperitoneal route gave faster and more consistent results which are readily reproducible (de Carvalho et al., 2001); hence it was used in this study. The LD_{50} values obtained in this study show that this oil is relatively safer than those obtained from *Eucalyptus* species which have been reported to possess analgesic acitivities and whose LD_{50} ranges from 190 to 353 mg/kg (Silva et al., 2003).

The hot plate test measures the complex responses to a non-inflammatory, acute nociceptive impulse and is one of the models normally used for studying central antinociceptive activity (Ranjit et al., 2006). In all the groups including vehicle, the reaction time on the hot plate were progressively prolonged with time (Table 1). This is in consonance with previous reports (Imam and

Sume, 2014) which confirm prolongation of reaction time on the hot plate with subsequent tests probably due to Oyemitan et al. 1196 adaptation or learning during observational periods of 120 or 240 min (Espejo et al., 1994; Casarrubea et al.,

Table 2. Effect of essential oil of *P. guineense* on egg albumin-induced paw oedema in rats.

Treatment i.p.,	Variation in paw size (mm) after			
	30 min	60 min	120 min	240 min
Vehicle	2.6±0.3 (0)	3.8±0.2 (0)	3.6±0.3 (0)	3.4±0.3 (0)
Essential oil, 50 mg/kg	1.4±0.3* (46)	1.2±0.4** (68)	1.8±0.2* (50)	2.1±0.6 (38)
Essential oil, 100 mg/kg	0.8±0.2** (69)	1.2±0.4** (68)	0.8±0.2** (78)	1.4±0.4** (59)
Essential oil, 200 mg/kg	0.8±0.2** (69)	1.0±0.0** (74)	0.4±0.3** (89)	1.0±0.0** (71)
Dexamethasone 1 mg/kg	0.6±0.2** (77)	0.8±0.1** (79)	0.2±0.2** (94)	0.4±0.2** (88)
P value	*p<0.05, **p<0.01	**p< 0.01	*p<0.05, **p < 0.01	**p< 0.01
F value	F(4,20) = 10	F(4,20) = 17	F(4,20) = 11	F(4,20) = 10

Vehicle is 5% Tween 80. Values are expressed as Mean±SEM and % Inhibition in parenthesis. *p<0.05, **p<0.01; statistically lower than vehicle (ANOVA, Dunnette's test).

2006). The results of the hot plate test show that the essential oil dose-dependently caused prolongation in the reaction time compared to vehicle at 30, 60 and 90 min post treatment (Table 1) almost comparable to morphine (potent opioid agonist), signifying central antinociceptive activity (Silva et al., 2003; Al-Nagger et al., 2003).

Pain sensation in acetic acid-induced writhing model is elicited by the triggering of local inflammatory responses leading to the release of free arachidonic acid from tissue phospholipid via cyclooxygenase and prostaglandin biosynthesis and has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products (Al-Nagger et al., 2003; Riberio et al., 2000), thereby facilitating inflammatory pain arising from enhanced capillary permeability. The effect of the essential oil on the acetic acid-induced writhing test (Figure 2) showed that it dose-dependently caused significant ($p < 0.01$) reduction in the number of writhes with the highest dose (200 mg/kg) producing 95% analgesia as against 87% for the standard nonsteroidal anti-inflammatory drugs (NSAIDs), diclofenac (100 mg/kg, i.p.) indicating peripheral antinociceptive activity (Silva et al., 2003), however the mechanism is not specific because acetic acid-induced writhings has been reported to lack specificity for either central or peripheral mechanism (Melo et al., 2013).

The egg albumin-induced paw oedema model has been used extensively for evaluating antiinflammtory effect of medicinal plants (Anosike et al., 2012; Singh et al., 2012). The oil at all the doses used significantly inhibited the paw oedema significantly ($p < 0.05$ to 0.01) throughout the experimental period of 4 h (Table 2). The decrease in oedema varies between 46 and 89%. The highest inhibition caused by the oil (200 mg/kg) was comparable to the potent steroidal drug, dexamethasone (1 mg/kg), which caused 94% inhibition. The molecular mechanisms of dexamethasone have been proposed to be inhibition of leukocyte infiltration into the inflammatory site (Tsurufuji et al., 1984), thus it can be suggested that the oil's antiinflammatory activity may be mediated similarly to dexamethasone or through inhibition of pro-inflammatory mediators or inhibition of prostaglandin synthesis at the level of phospholipase A2 and cyclo-oxygenase/PGE isomerase (Goppelt-Struebe et al., 1989; da Silva et al., 2014). These results serve as the preliminary screening data and further studies are imperative to isolate the active components of the oil in subsequent study in order to decipher the mechanism(s) involved in these activities.

In summary, results obtained in this study suggested that essential oil of *P. guineense* exhibits significant analgesic property (centrally and peripherally) in mice and displayed impressive antiinflammatory effect on the egg albumin-induced oedema in rats comparable to the standard drugs used in these models. The results of the acute toxicity profile of oil showed that it is moderately toxic when administered orally but more toxic when administered intraperitoneally. These results provide scientific and pharmacological basis for the use of *P. guineense* in ethnomedicine to manage pains, rheumatism and related ailments.

Conclusion

From the results obtained in this study we conclude that essential oil of *P. guineense* demonstrated significant antinociceptive and antiinflammatory activities in rodents, thus providing supporting evidence for the potential use of the plant in ethnomedicine as well as serving as a clue in drug discovery.

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Conflict of interests

The authors declare no conflict of interest.

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Phenolic contents and antioxidant activities *in vitro* of some selected Algerian plants

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In order to find new sources of natural antioxidants, the methanolic extracts of eleven Algerian medicinal plants from eight botanical families were investigated for their *in vitro* antioxidant activity using total antioxidant capacity (TAC), reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical scavenging and β-carotene–linoleate bleaching. The total phenolic, flavonoid and proanthocyanidin contents were also measured. Most of these plants were analyzed for the first time for their antioxidant activities. Results showed that the plants *Pistacia atlantica*, *Thymelaea microphylla* and *Marubium deserti* exhibited higher phenolic content (133.74 to 285.95 mg gallic acid equivalents/g dry matter). The flavonoid and proanthocyanidin contents varied from 0.56 to 12.44 mg catechin equivalents/g dry matter and 1.42 to 25.02 mg catechin equivalents/g dry matter, respectively. The extracts were found to have different levels of antioxidant properties in the test models used. The medicinal plants with the highest antioxidant activities were *P. atlantica*, *Helianthemum lippii* (stem, leaf, and fruit), *Inula montana, Anabasis articulata* and *Sedum villosum*. A positive correlation, R^2 = 0.906, was observed between total flavonoid contents and TAC values, reflecting a high involvement of flavonoids in antioxidant activity, but no correlation was established between the five tests and the total phenolic and proanthocyanidin contents.

Key words: Antioxidant properties, *in vitro* tests, phenolics, flavonoids, proanthocyanidin, Algerian plants.

INTRODUCTION

Currently, the scientific society (biologists and chemists alike) highlights the tragic role of the uncontrollable oxidative processes, induced by the reactive oxygen species (ROS) formed *in vivo.* These oxidants are directly responsible for various pathological states of degenerative diseases such as atherosclerosis, coronary heart diseases, aging, cancer (Finkel and Holbrook, 2000), and other disorders, for example, Alzheimer and Parkinson's diseases, Down's syndrome, inflammation, viral infection, autoimmune pathology and digestive ulcers (Atawodi, 2005). ROS are indirectly implicated in lipid peroxidation in foodstuffs. Whatever the case, the risk is worsened as

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risk is worsened as these molecules accumulate in the human body, leading to a radical chain reaction which degrades vital biological molecules such as DNA, proteins, sugars and membrane lipids; this results in cell and tissue damages (Abdi and Ali, 1999).

Actually, natural antioxidants are the subject of much research and a new breath towards the exploitation of polyphenols in health and pernicious diseases (cancer), as well as in food industry in order to lengthen the shelf life of food products by reducing the harmful substances formed (Han et al., 2008). These compounds include flavonoids which are required for their biological properties, that is antioxidant, anti-inflammatory, anti-allergic and anticarcinogenic. It is worth noting the powerful effectiveness of these substances to stop the reactions, which generate the radical species and neutralize the reactive oxygen species, by one or more of the mechanisms like reducing the activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen (Ali et al., 2008). All these activities are mainly due to their phenolic structures with the presence of hydroxyl groups. In recent years, there has been a growth of interest in natural antioxidants, whence the number of commercially available drugs derived from plant sources is increasing.

The aim of this study was to evaluate, for the first time, the *in vitro* antioxidant properties of eleven medicinal plants from Algeria, using different methods, including the total antioxidant capacity, reducing power, β-carotene bleaching test and radical scavenging activities against DPPH and hydroxyl radicals. We also wanted to determine their total phenolic compounds, flavonoid and proanthocyanidins and investigate the relationship between these contents and the antioxidant activity.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu, sodium carbonate, sodium nitrate, aluminium chloride, sodium hydroxide,vanillin, sulfuric acid, sodium phosphate, ammonium molybdate, phosphate buffer, potassium ferricyanide, trichloracetic acid, disodium hydrogen phosphate, sodium dihydrogen phosphate, 3-t-butyl-4-hydroxyanisole (BHA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxyribose, hydrochloric acid, thiobarbutiric acid, ethylenediaminetetraacetic acid (EDTA), iron (III) chloride ($FeCl_3$), β-carotene, linoleic acid and tween 40 were obtained from Sigma-Aldrich Chemie (Germany). Gallic acid, catechin, ascorbic acid, chloroform and methanol were from Merck (Darmstadt, Germany).

Sample preparation and extraction

The plant materials were collected from different localities of Algeria based on the information provided in the ethnobotanical survey and their abundance in nature. For each plant, the various data (scientific name, family, used organs, original habitat location and harvest period) are given in Table 1. The collected plants were identified by the Vegetable Ecological Laboratory, University of Tlemcen, Algeria. Voucher specimens for each plant have been deposited in the Herbarium of the Biology Department, University of Tlemcen, Algeria. One gram of plant material was ground to fine powder and extracted with 20 ml of methanol at room temperature for 48 h. After filtration through Whatman no. 0.45 filter paper, the solvent was evaporated under vacuum at 60°C. The residue was weighed and dissolved in methanol for further analysis. Eventually, the solutions were stored at -20°C (Benhammou et al., 2009).

Determination of total phenolic content

The total phenolic content of methanolic extracts was determined by spectrometry using Folin-Ciocalteu reagent assay (Singleton and Rossi, 1965). A volume of 200 µl of the extract was mixed with 1 ml of Folin-Ciocalteu reagent (diluted 10-fold in water) and 0.8 ml of a 7.5% sodium carbonate solution in a test tube. The absorbance was read at 765 nm after 30 min on a Jenway 6405 UV-vis spectrophotometer. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM) through the calibration curve with gallic acid ($y = 2.916x$, $R^2 = 0.997$). All the tests were carried out in triplicate.

Estimation of total flavanoid content

Total flavonoid content was determined by a colorimetric assay using a method described by Zhishen et al. (1999). Briefly, 500 µl of catechin standard solution with different concentrations or methanolic extracts was mixed with 1500 µl of distilled water in a test tube, followed by addition of 150 µl of a 5% (w/v) sodium nitrate solution at time zero. After 5 min, 150 µl of aluminium chloride solution to 10% (m/v) was added. After the incubation of 6 min at the ambient temperature, 500 µl of sodium hydroxide (1 M) was added. Immediately, the mixture was completely agitated in order to homogenize the contents. The absorbance was read at 510 nm and concentrations of flavonoids were deduced from a standard curve ($y = 5.140x$, $R^2 = 0.991$) and calculated in mg catechin equivalent (CE)/g dry matter (DM). Data are the mean ± SD results of triplicate analyses.

Quantification of proanthocyanidins

Proanthocyanidins were measured using the vanillin assay described by Julkunen-Titto (1985). To 50 µl of methanolic extract, 1500 µl of vanillin/methanol solution (4%, w/v) was added and the contents mixed. Then, 750 µl of concentrated hydrochloric acid was added and allowed to react at room temperature for 20 min. The absorbance at 550 nm was measured against a blank. The amount of proanthocyanidins was expressed as milligrams of catechin equivalents per gram of dry matter (mg CE/g DM) from the calibration curve ($y = 0.116x$, $R^2 = 0.996$). All the tests were carried out in triplicate.

Total antioxidant capacity

The total antioxidant capacity (TAC) of the plant extracts was evaluated by the phosphomolybdenum method of Prieto et al. (1999). An aliquot (0.3 ml) of plant extract was combined to 3 ml of

Table 1. Botanical (scientific name, family) data and harvest characteristics (location, period and plant parts) of eleven plants studies.

Botanical name	Family	Site of collection	Harvest period	Plant parts
Anabasis articulata Moq	Chenopodiaceae	Bechar	November, 2007	S
Atriplex halimus L	Chenopodiaceae	Bechar	April, 2008	S/L
Cotula cinerea Del	Asteraceae	Adrar	December, 2007	FP
Helianthemum lippii (L) Pers	Cistaceae	Aïn Ben Khelil (Naâma)	May, 2007	S/L/F
Inula montana L	Asteraceae	Oum El Alou (Tlemcen)	November, 2008	FP
Marrubium deserti De Noé	Lamiaceae	Aïn Ben Khelil (Naâma)	May, 2007	S/L
Pentzia monodiana Maire	Asteraceae	Ladjdar	November, 2007	FP
Pistacia atlantica Desf	Anacardiaceae	Aïn Fezza (Tlemcen)	May, 2007	F
Sedum villosum L	Crassulaceae	Woued Al Akhdar (Tlemcen)	April, 2007	FP
Thymelaea microphylla Coss et Dur	Thymelaeaceae	Aïn Ben Khelil (Naâma)	May, 2007	S/L
Zygophylum album L	Zygophyllaceae	Adrar	December, 2007	S

Stem: S; Leaf: L; Fruit: F; Flower part: FP.

the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. Later, the samples were allowed to cool to room temperature. The absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity of the samples was expressed as milligrams of ascorbic acid equivalents per gram of dry matter (mg AAE/g DM) from the calibration curve ($y = 4.671x$, $R^2 = 0.9869$).

Reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Various concentrations of the extracts (mg/ml) in distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% of potassium ferricyanide water solution (2.5 ml, $K_3[Fe(CN)_6]$). The mixture was incubated at 50°C for 20 min. After that, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared $FeCl_3$ solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm and ascorbic acid was used as a positive control. A higher absorbance indicates a higher reducing power. IC_{50} value (mg/ml) is the effective concentration giving an absorbance of 0.5 for reducing power and was obtained from linear regression analysis.

DPPH free radical scavenging activity

The free radical scavenging activity was measured by a modified $DPPH^{\bullet}$ assay (Sanchez-Moreno et al., 1998). A solution of the extract prepared as describe above (50 µl) was added to 1950 µl of methanolic DPPH solution (0.025 g/L). The decreasing absorbance at 515 nm was monitored in order to reach constant values. The $DPPH^{\bullet}$ concentration in the reaction medium was calculated from the following calibration curve determined by linear regression:

$A_{515nm} = 24.41 \times [DPPH^{\bullet}]t + 0.0022$ $R^2 = 0.999$

Where $[DPPH^{\bullet}]t$ was expressed as mg/ml at *t* time.

The percentage of the remaining $DPPH^{\bullet}$ (% $DPPH^{\bullet}_{REM}$) at the steady state was calculated as follows:

$\% DPPH^{\bullet}_{REM} = 100 \times [DPPH^{\bullet}]_t / [DPPH^{\bullet}]_{t=0}$

Where $[DPPH^{\bullet}]t = 0$ and $[DPPH^{\bullet}]t$ are concentrations of $DPPH^{\bullet}$ at t=0 and t= t, respectively.

Using various antioxidant concentrations, it was possible to determine the amount of antioxidant necessary to halve the initial $DPPH^{\bullet}$ concentration (EC_{50}). EC_{50} is expressed in mg of dry extract per g of DPPH. The time needed to reach the EC_{50} concentration, noted T_{EC50}, was graphically determined. The antiradical efficiency (AE) was calculated as follows:

$AE = 1 / (EC_{50} \times T_{EC50})$

Hydroxyl radical scavenging assay

The effect of extracts on hydroxyl radicals was assayed by using the deoxyribose method (Halliwell et al., 1987). The reaction mixture contains the following reagents: 0.4 ml of phosphate buffer saline (50 mmol/L, pH 7.4), 0.1 ml of extracts solution, 0.1 ml of EDTA (1.04 mmol/L), 0.1 ml of $FeCl_3$ (1 mmol/L) and 0.1 ml of 2-deoxyribose (60 mmol/L). The reaction was started by the addition of 0.1 ml of ascorbic acid (2 mmol/L) and 0.1 ml of H_2O_2 (10 mmol/L). After incubation at 37°C for 1 h, the reaction was stopped by adding 1 ml of thiobarbutiric acid (TBA) 10 g/L follow-up by 1 ml of hydrochloric acid (HCl) (25%), then heating the tubes in a boiling water bath for 15 min. The contents were cooled and absorbance of the mixture was measured at 532 nm against reagent blank. Decreased absorbance of the reaction mixture indicates decreased oxidation of deoxyribose. The percentage inhibition of deoxyribose oxidation was calculated using the following equation:

Hydroxyl radical scavenging activity (%) $= [A_0 - (A_1 - A_2)] \times 100 / A_0$

Where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract and the deoxyribose, A_2 was the absorbance in the presence of the extract without deoxyribose.

β-Carotene bleaching method

The antioxidant activity of methanolic extracts was evaluated using β-carotene-linoleate model system, as described by Moure et al. (2000). Two milligrams of β-carotene were dissolved in 10 ml chloroform and 1 ml β-carotene solution was mixed with 20 µl of purified linoleic acid and 200 mg of Tween 40 emulsifier. After evaporation of chloroform under vacuum, oxygenated distilled water (100 ml) were added by vigorous shaking. To an aliquot of 4 ml of this emulsion, 200 µl of extracts or the BHA were added and mixed well. The absorbance at 470 nm, which was regarded as t = 0 min, was measured immediately, against a blank consisting of the emulsion without β-carotene. The capped tubes were placed in a water bath at 50°C for a period of 2 h. Thereafter, the absorbance of each sample was measured at 470 nm (A_{120}). For the positive control, sample was replaced with BHA. A negative control consisted of 200 µl methanol instead of extract or BHA. The antioxidant activity (AA) was calculated according to the following equation:

$$AA = [(A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)}] \times 100$$

Were $A_{A(120)}$ is the absorbance of the sample at t = 120 min; $A_{C(120)}$ is the absorbance of the control at t = 120 min and $A_{C(0)}$ is the absorbance of the control at t = 0 min.

Statistical analysis

The results were analyzed using the Microcal Origin 6. All the data are expressed as means ± standard deviation (n = 3).

RESULTS

Extraction yields - total phenolic compounds, flavonoid and proanthocyanidins contents

The yield extracted from different parts of Algerian plants is reported in Table 2. In this experiment, the yields of extracts ranged from 3.43 to 33.43%. The highest extract yield was obtained by PAF extract, followed by *Atriplex halimus* (AHL) (24%) while the lower yield was recorded in *Helianthemum lippii* (HLS) (3.43%). These yields were higher in leaves compared to the other parts of the plant. The total phenolic contents of 11 medicinal plants were measured using the Folin-Ciocalteu method. The results were shown in Table 2. There is a large variation in total phenolic content of the plant species examined. The values varied from 3.77 ± 0.06 to 285.95 ± 10.25 mg GAE/g DM. The highest total phenolic content was observed in *Pistacia atlantica* (PAF), followed by the leaves of *Thymelaea microphylla* (TML) (257.40 mg/g) and *Marrubium deserti* (MDL) (235.18 mg/g) and their stems (201.64 and 133.74 mg/g, respectively). The total flavonoids and proanthocyanidins of the plant extracts were also measured (Table 2). For flavonoid content, the values ranged from 0.56 ± 0.03 mg CE/g for *Atriplex halimus* (AHS) to 12.44 ± 0.25 mg CE/g for PAF, whereas the levels of proanthocyanidins varied from 1.42 ± 0.17 to 25.02 ± 3.44 mg CE/g.

Assessment of antioxidant activities

In this study, we combined five complementary assays: TAC, reducing power, scavenging activity on DPPH and hydroxyl radicals and inhibition of β-carotene bleaching to evaluate the antioxidant activities of selected plant extracts.

Total antioxidant capacity (TAC)

The TAC of plant extracts was expressed as ascorbic acid equivalents. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex at acid pH. The PAF showed the highest TAC (45.51 ± 1.63 mg AAE/g DM) while the lowest capacity was found in AHS (1.64 ± 0.80 mg AAE/g DM). For the other extracts, the values were different, ranging from 6.14 ± 0.10 to 20.46 ± 0.06 mg AAE/g DM (Table 2).

Reducing power

The reducing properties are generally associated with the presence of reductones, which have capacity to donate an electron to free radicals and convert them into more stable. In Figure 1, all the extracts showed some degree of electron-donating capacity in a linear concentration-dependent manner. The best total reduction capability was observed for PAF extract (Abs $_{700nm}$ 0.88 at 0.25 mg/ml). All other extracts showed weak activities compared to the ascorbic acid (Abs $_{700nm}$ 0.81 at 0.1 mg/ml). As shown in Table 3, PAF extract possessed the strongest reducing power (0.13 ± 0.001 mg/ml), followed by HLF (0.25 ± 0.004 mg/ml), HLS (0.35 ± 0.009 mg/ml), SVFP (0.42 ± 0.004 mg/ml) and HLL extracts (0.47 ± 0.009 mg/ml). IC_{50} values of other extracts ranged from 0.52 ± 0.007 mg/ml for AAS to 4.56 ± 0.79 mg/ml for AHL. However, the ascorbic acid (0.06 ± 0.002 mg/ml) required to reduce the ferric iron was lower than the other phenolic extracts, indicating superior activity. The

Table 2. Extract yield, total phenolic (as gallic acid equivalents), total flavonoids (as catechin equivalents) and proanthocyanidins contents (as catechin equivalents) in investigated plants expressed in mg/g of dry matter.

Sample	Yield (%)	Total phenolics (mg GAE/g DM)	Flavonoids (mg CE/g DM)	Proanthocyanidins (mg CE/g DM)	TAC (mg AAE/g DM)
PAF	33.43 ± 2.70	285.95 ± 10.25	12.44 ± 0.25	3.06 ± 0.15	45.51 ± 1.63
TML	9.53 ± 2.34	257.40 ± 89.33	4.18 ± 0.04	6.91 ± 0.44	14.56 ± 0.69
TMS	7.45 ± 0.63	201.64 ± 4.65	2.98 ± 0.13	4.13 ± 0.31	16.85 ± 0.80
MDL	10.88 ± 0.17	235.18 ± 6.11	5.87 ± 0.20	25.02 ± 3.44	20.46 ± 0.06
MDS	7.18 ± 1.04	133.74 ± 27.35	3.17 ± 0.12	13.94 ± 1.37	13.41 ± 0.28
HLL	10.45 ± 0.71	60.95 ± 2.55	4.18 ± 0.20	10.37 ± 1.08	13.30 ± 0.59
HLF	9.20 ± 0.69	74.21 ± 1.18	3.57 ± 0.15	4.67 ± 0.34	17.41 ± 2.54
HLS	3.43 ± 0.56	46.09 ± 6.45	1.67 ± 0.02	9.29 ± 1.39	7.72 ± 0.39
AHL	24 ± 1.41	10.12 ± 2.24	2.48 ± 0.01	9.11 ± 0.68	11.51 ± 0.60
AHS	7.5 ± 0.70	3.77 ± 0.06	0.56 ± 0.03	1.42 ± 0.17	1.64 ± 0.80
AAS	9.36 ± 2.66	43.14 ± 0.63	4.85 ± 0.21	7.26 ± 0.36	13.99 ± 0.62
ZAS	14.30 ± 3.81	6.92 ± 0.63	1.61 ± 0.02	4.35 ± 0.57	6.14 ± 0.10
IMFP	10.19 ± 1.80	20.24 ± 1.06	6.52 ± 0.11	9.29 ± 0.69	18.99 ± 0.44
CCFP	15.79 ± 4.54	22.22 ± 0.41	3.93 ± 0.06	8.61 ± 0.18	17.19 ± 1.27
PMFP	6.93 ± 2.29	9.87 ± 0.82	2.29 ± 0.04	4.48 ± 0.47	10.45 ± 0.15
SVFP	6.76 ± 0.73	27.09 ± 0.97	2.96 ± 0.13	7.52 ± 1.87	13.01 ± 0.67

PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract. Values were the means of three replicates ± standard deviation (SD).

efficiency of iron reduction is inversely proportional to the IC_{50} value; it is in the ascending order, according to the following ranking: AA > PAF > HLF > HLS > SVFP > HLL > AAS> IMFP > MDS > TML > CCFP = TMS > AHS > PMFP > ZAS > AHL.

DPPH free radical scavenging activity

The scavenging effect of phenolic extracts on the DPPH radical expressed as EC_{50} values varied widely from 47.39 to 6310.04 mg antioxidant/g DPPH (Table 3). PAF was most efficient, with the lowest EC_{50} value of 47.39 mg/g, followed by HLF (66.28 mg/g), IMFP (66.96 mg/g), HLS (97.73 mg/g) and SVFP (99.02 mg/g). In contrast, ZAS has the lowest activity. However, the DPPH radical scavenging activity of different extracts was inferior to that of ascorbic acid (39.5 mg/g) and BHT (13.47 mg/g). To classify the antioxidant capacities of the various extracts, we used the antiradical efficiency (AE), a new parameter which involves the potency ($1/EC_{50}$) and the reaction time (T_{EC50}) (Sanchez-Moreno et al., 1998). The lower the EC_{50}, the lower the T_{EC50} and the higher the AE. As shown in Table 2, the ascorbic acid (4174.46×10^{-5}) was more powerful antioxidant than the investigated extracts, trolox (260.43×10^{-5}) and BHT (45.97×10^{-5}). The classification order of AE for the tested antioxidant was: AA > Trolox > BHT > MDS > PAF > CCFP > MDL > TMS > HLF > IMFP > SVFP > HLL > TML > AAS > HLS.

Hydroxyl radical scavenging assay

The scavenging abilities of different extracts and BHA on hydroxyl radical inhibition by the 2-deoxyribose oxidation method are shown in Figure 2. Each extract showing hydroxyl radical-scavenging activity was increased with increasing concentration of the extract sample. At a concentration 0.5 mg/ml, all the extracts showed good hydroxyl radical-scavenging activities above 35.60%. These percentages of inhibition exceeded 50%, except for ZAS (40.40%) and AHL (37.27) at 1 mg/ml. As shown in Table 3, HLS showed the highest hydroxyl radical scavenging activity ($EC_{50} = 0.20 \pm 0.08$ mg/ml) compared to BHA ($EC_{50} = 0.30 \pm 0.05$ mg/ml), while AHL showed the lowest activity ($EC_{50} = 3.07 \pm 1.68$ mg/ml). For all the plant extracts, EC_{50} values varied from 0.30 ± 0.04 to 1.02 ± 0.12 mg/ml. The scavenging abilities on hydroxyl radical are in the following descending order: HLS > PMFP = BHA > AAS > HLF > SVFP > MDL > TMS > MDS

Table 3. Antioxidant properties of the extracts of the studied plants on reducing power, DPPH radical-scavenging assay, hydroxyl assay and β-carotene-linoleic acid assay.

Sample	Reducing power	DPPH assay			Hydroxyl radical scavenging assay	β-Carotene-bleaching method
	IC_{50} (mg/ml)	EC_{50}	Temps (T_{EC50}) (min)	AE $\times 10^{-5}$	EC_{50} (mg/ml)	EC_{50} (mg/ml)
PAF	0.13 ± 0.001	47.39[a]	255.93	8.24	0.83 ± 0.14	-
TML	0.73 ± 0.04	512.75[a]	1468.13	0.13	0.50 ± 0.06	1.18 ± 0.13
TMS	1.17 ± 0.08	830.15[a]	153.91	0.78	0.43 ± 0.06	2.95 ± 0.07
MDL	0.66 ± 0.007	396.33[a]	63.84	3.95	0.4 ± 0.11	-
MDS	0.71 ± 0.01	366.36[a]	18.57	14.70	0.46 ± 0.07	-
HLL	0.47 ± 0.009	144.10[a]	2766.49	0.25	0.46 ± 0.14	1.02 ± 0.15
HLF	0.25 ± 0.004	66.28[a]	1971.34	0.76	0.35 ± 0.02	3.07 ± 0.25
HLS	0.35 ± 0.009	97.73[a]	40330.40	0.02	0.20 ± 0.08	0.76 ± 0.13
AHL	4.55 ± 0.79	31.83[b]	-	-	3.07 ± 1.68	-
AHS	3.24 ± 0.23	20.58[b]	-	-	0.66 ± 0.43	-
AAS	0.52 ± 0.007	297.74[a]	10927.27	0.03	0.33 ± 0.06	2.60 ± 0.18
ZAS	3.95 ± 0.77	6310.04[a]	-	-	1.02 ± 0.12	-
IMFP	0.64 ± 0.004	66.96[a]	2017.09	0.74	0.52 ± 0.05	-
CCFP	1.17 ± 0.05	462.19[a]	50.73	4.26	0.66 ± 0.12	-
PMFP	3.64 ± 0.51	10.04[b]	-	-	0.30 ± 0.04	-
SVFP	0.42 ± 0.004	99.02[a]	1897.42	0.53	0.39 ± 0.16	0.97 ± 0.04
AA	0.06 ± 0.002	39.53[a]	0.61	4174.46	-	-
BHT	-	13.47[a]	161.52	45.97	-	-
Trolox	-	49.21[a]	7.80	260.43	-	-
BHA	-		-	-	0.30 ± 0.05	0.03 ± 0.005

PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract, AA, ascorbic acid. [a]EC_{50} concentration expressed as mg Antioxidant/ g DPPH. [b]EC_{50} concentration expressed as mg/ml after 30 min.

= HLL > TML > IMFP > AHS = CCFP > PAF > ZAS > AHL.

β-Carotene bleaching method

The results of the antioxidant effect of various extracts of plants on the autoxidation of linoleic acid are shown in Figure 3. At a concentration 3 mg/ml, HLL (97.64 %) exhibited the best efficiency in inhibiting the oxidation of linoleic acid. At 4 mg/ml, SVFP registered higher inhibition of bleaching of β-carotene (91.13%), followed by TML (88.09%) and HLS (86.82%); whereas the inhibition percentages of AAS, HLF and TMS were, respectively 65.13, 62.94 and 59.52%. For the other extracts, the percentages of inhibition varied between 8.61 and 40.05%, except for the AHS, which showed a very weak activity, lower than 5% at 4 mg/ml. According to EC_{50} showed in Table 3, HLS exhibited an interesting antioxidant activity (0.76 ± 0.13 mg/ml), followed by the SVFP (0.97 ± 0.04 mg/ml), HLL (1.02 ± 0.15 mg/ml) and TML (1.18 ± 0.13 mg/ml). EC_{50} values of AAS, TMS and HLF were 2.60 ± 0.18, 2.95 ± 0.07 and 3.07 ± 0.25 mg/ml, respectively. All the extracts were less effective compared to BHA (0.03 ± 0.005 mg/ml).

DISCUSSION

Our results are a contribution to the valorization of some medicinal plants from Algeria. According to our best knowledge, this is the first report focusing on the evaluation of antioxidant capacities *in vitro* and the quantification of total phenolics compounds, flavonoids and proanthocyanidins from different parts of selected medicinal plants. In this study, total phenolics were higher

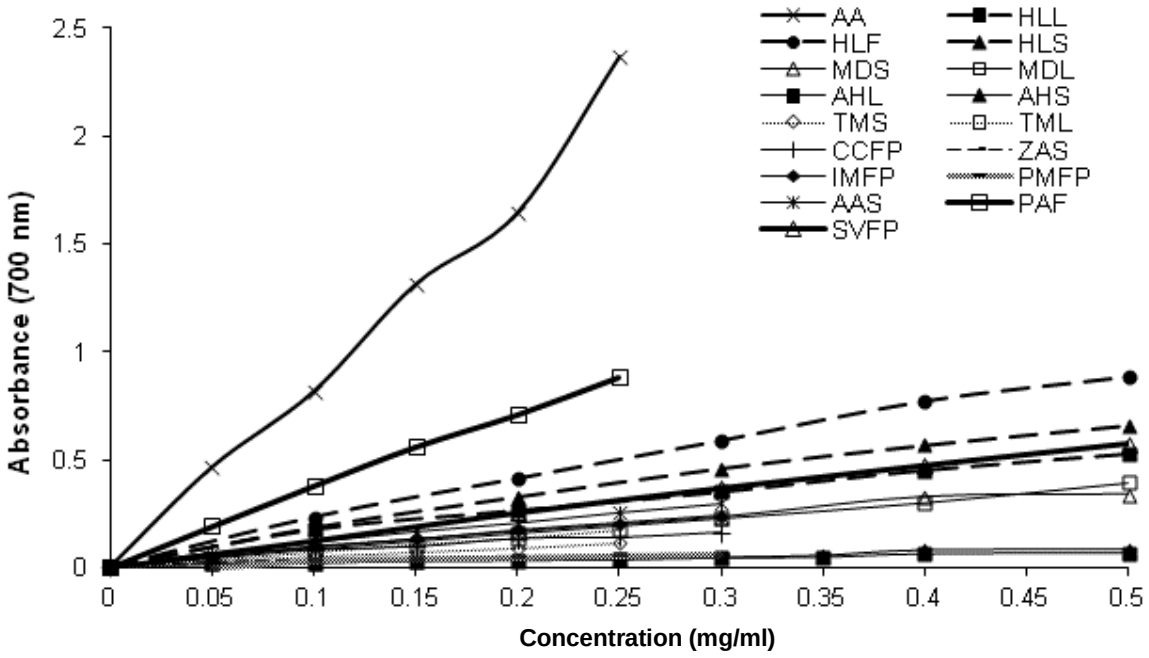

Figure 1. Reducing power as a function of methanolic extracts concentration.
PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract.

in fruits of *Pistacia atlantica*, followed by *Thymelaea microphylla* (leaf, stem) and *Marubium deserti* (leaf, stem). In comparative studies with other plant extracts, *M. deserti* (3.67 mg GAE/g DM) and *T. Microphylla* (10.80 mg GAE/g DM) showed the lowest phenolic contents (Djeridane et al*.,* 2010). These contents found in our study were also higher than those reported for others species of Jordanian plants (2.1 to 52.8 mg/g) (Alali et al., 2007) and some Algerian medicinal plants like *Thymelaea hirsute* (6.81 mg/g) (Djeridane et al., 2006).

For *Helianthemum lippii* extracts, the total phenolic contents obtained in the leaves, stems and fruits were higher than those deferred by Tawaha et al. (2007) in the methanolic (17.3 ± 1.0 mg GAE/g DW) and aqueous extracts (8.7 ± 3.0 mg GAE/g DW) from *H. ledifolium*. Alali et al. (2007) found the phenolic contents of 25 and 30.5 mg GAE/g DW for the methanolic and aqueous extracts of *H. lippii*. For the other extracts tested (*Atriplex halimus*; *Anabasis articulata; Zygophylum album; Inula montana*; *Cotula cinerea* and *Pentzia monodiana*), the contents ranged from 3.77 ± 0.06 to 43.14 ± 0.63 mg GAE/g DM. Our results were within the range of the values reported by Li et al. (2008) for 45 Chinese medicinal plants and Tawaha et al. (2007) for 51 Jordanian plants. These differences in total phenolic contents could be due to genotypic and environmental variations (climate, location, temperature, fertility and diseases) within species, plant part tested, harvesting time and extraction procedure (Shan et al., 2005).

According to the experimental data, PAF revealed a higher TAC, a strong activities to reduce iron and to scavenge DPPH and OH radicals but a very weak activity to inhibit the oxidation of β-carotene. This strong activity of *P. atlantica* extracts might be attributed to the inductive effect of the three hydroxyl groups in the gallic acid structure and other phenolic acids, such as the ρ-coumaric acid (Sanchez-Moreno et al., 1998; Benhammou et al., 2008). Moreover, the Anacardiaceae family and the Pistacia kind are characterized by the existence of myricetin and gallic acid-derived (5-O- galloyl; 3,5-O-digalloyl; 3,4,5-tri-O-galloyl) (Baratto et al., 2003). It has also been shown that vanillic, syringic, ferulic and ρ-coumaric acids as well as catechin show a strong positive correlation with the scavenging effects on DPPH and $ABTS^{+}$ radicals and the iron reducing power. Moreover, the gallic acid and catechin are narrowly correlated with the

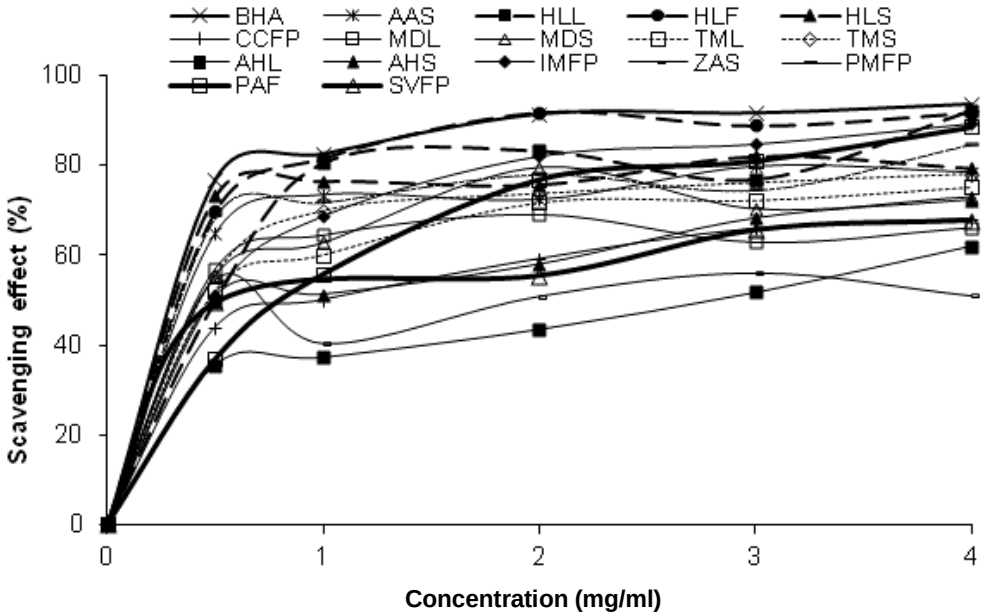

Figure 2. Scavenging effect of methanolic extracts of studied plants on hydroxyl radical compared to that of BHA.
PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract.

metal chelating activity and the inhibition of lipidic peroxidation (Tsai et al., 2007).

Considering its low TAC content, the leaves extract of *A*. *halimus* showed very low antioxidant activities to reduce the ferric iron and to quench DPPH and OH radicals. According to Benhammou et al. (2009), the ethyl acetate fraction of *A*. *halimus* exhibited an interesting antioxidant activities to scavenge DPPH (EC_{50} = 2.04 mg/ml) and to reduce iron (IC_{50} = 1.51 mg/ml). These capacities are attributed to the abundance of flavonols (kaempferol, quercetin) which constitute the main class of Atriplex species (Bylka et al., 2001).

In our study, the methanolic extracts of *H*. *lippii* also exhibited high antioxidant potency, which might be due to presence of flavonols. Similarly, Calzada et al. (1999) reported the presence of kaempferol and quercetin in methanolic extract of *H. glomeratum.* However, all other extracts presented different antioxidant capacities. Overall, antioxidant activities are more variable between species of a plant (inter-species) than within the same species (intra-species) (Ksouri et al., 2008).

The results obtained from the present study suggested that there was a negative correlation between phenolic contents and different antioxidant activity assays. The relationship between total flavonoid contents and total antioxidant capacity of extracts of different plants shows a significant relationship with coefficient correlation R^2 = 0.906 (Figure 4). It is well known that these compounds have powerful antioxidant capacities. The number and position of hydroxyl groups on the flavonoid nucleus enhances antioxidant activity. Substitution patterns in the B-ring and A-ring as well as the 2,3-double bond (unsaturation) and the 4-oxo group in the C-ring also affect antioxidant activity of flavonoids (Cai et al., 2006).

Conclusion

The antioxidant capacities and the total phenolic, flavonoid and proanthocyanidins contents of eleven selected medicinal plants growing in Algeria were evaluated. The results obtained showed that *P. atlantica*, *T. microphylla* and *M. deserti* could become a promising source of phenolic compounds. It has been found that plants like *P. atlantica, H. lippii, I. montana, Anabasis articulata* and *S. villosum* present the highest antioxidant capacities and may serve as valuable sources of natural antioxidants for further isolation and purification of

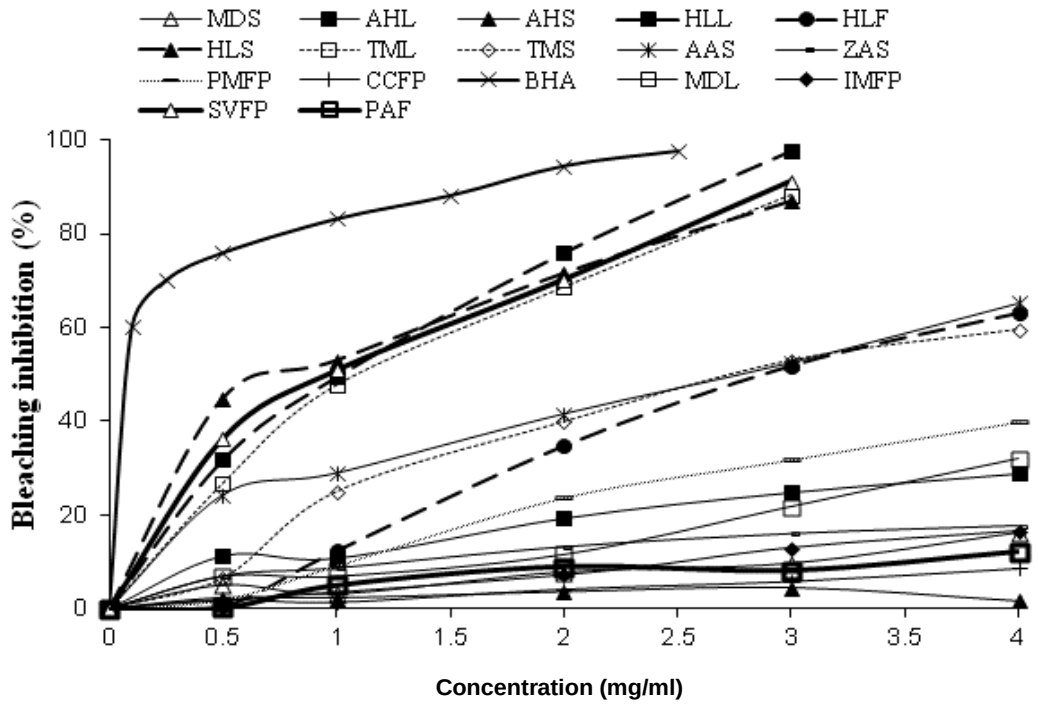

Figure 3. Inhibition (%) of lipid peroxidation of methanolic extracts of studied plants by the β-carotene bleaching method.
PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract.

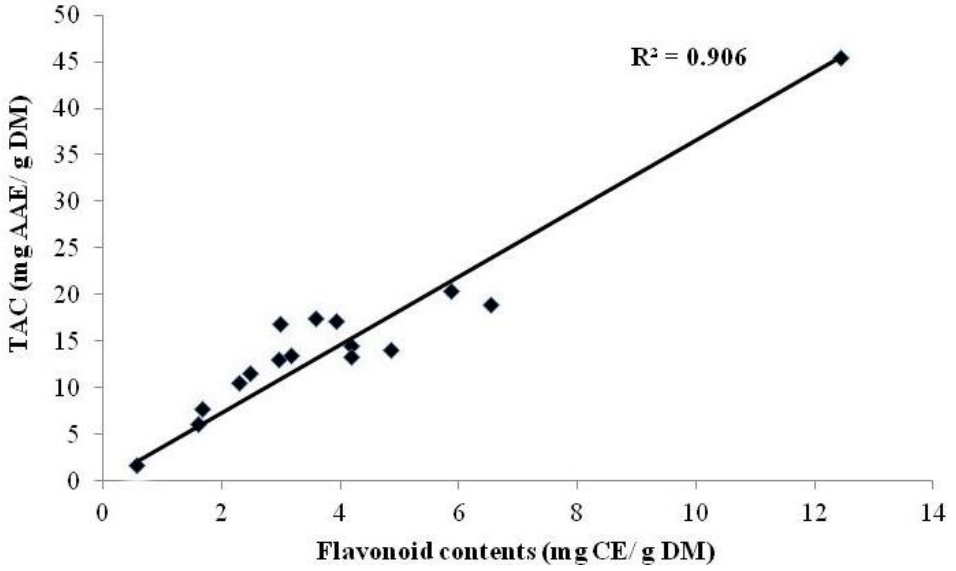

Figure 4. Correlation between the total antioxidant capacity expressed as ascorbic acid equivalent and flavonoids content.

antioxidant compounds. A strong correlation has been reported between TAC and the flavonoid contents, indicating that these compounds are the major contributor of antioxidant capacities of these plants.

Conflict of interests

The authors have not declared any conflict of interests

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Full Length Research Paper

Essential oil, fatty acids and anti bacterial activity of *Sesbania punicea* from north of Iran

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Essential oil composition of the leaves and fatty acids from the seeds of *Sesbania punicea* (rattlebox) were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Forty components (91.35%) of the essential oil were identified with the major compounds: 1,8-cineole (47.58%) and α-pinene (7.30%). The main compounds from thirty seven identified components comprising 89.28% of the seeds (hexane extract) were: linoleic acid (ω-6) (6.08%) and oleic acid (2.65%). The more abundant compounds in hexane extract of the seeds were hydrocarbons (77.32%). Antibacterial activity of ethyl acetate extract from the leaves was also evaluated by disc diffusion method against 8 gr (+/-) bacteria from which, *Salmonella parathyphi* B was the most sensitive one, even more than chloramphenicol as a standard antibiotic.

Key words: 1,8-Cineole, linoleic acid, *Salmonella parathyphi* B, *Sesbania punicea.*

INTRODUCTION

Sesbania punicea (Fabiaceae) is an ornamental shrub which has a high demand for water and thrives in high moisture areas. It has been widely distributed from its native range in South America (Hoffmann and Moran, 1991). This species has been reported as an invasive species in many of the southern United States. The plant is actively replacing nature species of riparian areas, which is taking food resources away from the local wild life and contributes to riverbank erosion and flooding in areas where it persists. *S. punicea* has been declared a noxious weed and/or seed. Any animal or human that ingests this plant or seed can become very sick and may experience symptoms such as vomiting, diarrhea, respiratory failure or fatalities (Russell, 2012). The compounds contained in this plant that makes it so toxic are saponic glycosides (Graaf, 1986).

There is a few phytochemical and pharmacological study on *Sesbania* species in the literature. Although *S. punicea* has no known industrial or medicinal use, some other species of the genus have been reported for their

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biological activities. For instance, *Stephanomeria virgata* which is a close relative to *S. punicea* has been shown to reduce the response to painful stimulation as well as inflammatory edema in mice (Russell, 2012). One of the most useful species of the genus is *Sesbania grandiflora*, which has been the subject of many projects. Sterols, saponins and tannins have been isolated from different parts of the plant (Fojas et al., 1982). These compounds are known for their biological activities such as: antibacterial and antifungal (Goun et al., 2003); antioxidant and anti urolithiatic (Doddola et al., 2008); hepatoprotective properties (Pari and Uma, 2003) and anti-tuberculosis activity (Noviani et al., 2012). Previous studies on *Sesbania* species also revealed the presence of components which possess antitumor activities. Sesbanimide, a novel anti-tumor component was isolated from the methanol extract of *Sesbania drummondii* (Hui et al., 1986) and *Sesbania vesicaria* (Kim et al., 1992). Sesbanine, a cytotoxic alkaloid with highly unusual spirocyclic structure was also isolated from the ethanol extract of *S. drummandii* seeds (Powell et al., 1979). Three new triterpenoid saponins were isolated from the seed extract of *S. vesicaria* which showed cytotoxic effect (Yuan et al., 2013). Ethanolic seed extracts of *S .vesicaria, S. punicea* and *S. drummondii* revealed significant anti tumor activities against lymphocytic leukemia P–388 (PS) in mice (Powell et al., 1976).

S. punicea (rattlebox) is cultivated in North of Iran as an ornamental plant because of beautiful reddish-orange flowers and has not been previously mentioned for chemicals and biological activities. In this project, we identified volatile compounds of leaves and fatty acids from the seeds of *S. punicea*. We also tested antibacterial activity of ethyl acetate extract of the leaves. To the best of our knowledge, this is the first report on essential oil composition and antibacterial activity of *S. punicea* leaves.

MATERIALS AND METHODS

Plant

The leaves and seeds of *S. punicea* were collected from the seaside area in Roodsar (North of Iran) in July, 2012 and were dried in shade. Identification of the plant was done by using the identification key in the Manual of Cultivated Trees and Shrubs (Bailey, 1975) and confirmed by Research Institute of Forests and Rangelands, Tehran, Iran (TARI).

Essential oil isolation

Air dried and powdered leaves (100 g) were subjected to a Clevenger-type apparatus for hydrodistillation. Then the distillate was isolated and dried over anhydrous sodium sulfate and the oil was stored at 4°C until analysis by GC and GC–MS.

Preparation of ethyl acetate extract

Ethyl acetate extract was prepared by a classic maceration method. For this purpose, air dried and powdered leaves (5 g) were soaked in ethyl acetate (30 ml) for one week at room temperature. Vigorous stirring was done during the extraction. After filtration through filter paper (Watman No. 41) the filtrate was used for antibacterial assay.

Preparation of hexane extract

The seeds were shade dried at room temperature and then were milled to a fine powder in an electrical mill and stored in the dark at room temperature for further use. 10 g of dried powdered seed was extracted by n-hexane (200 ml) (Merck, Germany) using a soxhlet apparatus (70°C, 4 h) to obtain fatty acids and the other non-polar compounds. The hexane extract was concentrated below 40°C by a rotary evaporator (Heidolph, Germany).

Methylation of hexane extract

After removing hexane using rotary evaporator, the oily mixture was derived to their methyl esters by the International Olive Oil Council (IOOC, 2001) reports by trans-esterification process. In this process, dried hexane extract was dissolved in hexane and then extracted with 2 M methanolic KOH at room temperature for 1 min. The upper phase which included fatty acid methyl esters and other non-polar compounds was analyzed by GC and GC/MS systems.

GC analyses

Gas chromatograph (GC) analyses were performed on a Shimadzu 15 A GC equipped with a split/split less injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 ml/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. The relative percentages of the characterized components are given in Table 1 (essential oil) and Table 2 (hexane extract).

GC/MS analyses

GC/MS analyses were performed using a Hewlett-Packard 5973 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C for 5 min. The flow rate of Heliumas carrier gas was 1 ml/min mass spectrometer (MS) were taken at 70 ev.

Identification of constituents

The constituents in the essential oil were identified by comparing their retention indices (RI) relative to n-alkanes (C_9-C_{22}), computer matching with the Wiley library and confirmed by comparing their mass spectra with those of authentic samples (Adams, 2000) or with data already available in the literature. The fatty acid methyl esters were identified by comparing their retention times and mass peaks with those of standard methyl ester mixtures and by NIST-Wiley library data search. Relative percentage amounts for both

Table 1. Essential oil composition of the leaves of *Sesbania punicea*.

Compound	[a]RI	Percentage (%)
α - Pinene	939	7.30
Camphene	953	0.08
β - Pinene	980	0.85
δ - 2- Carene	1007	0.15
1,8 – Cineole	1033	47.58
γ - Terpinene	1062	0.22
Terpinolene	1088	0.05
n - Nonanal	1098	0.30
endo-Fenchol	1112	0.06
trans-Pinocarveol	1139	0.09
Terpinen – 4 – ol	1177	0.49
α-Terpineol	1189	1.06
Trans-Carveol	1217	0.11
Cis-Myrtanol	1252	0.08
Trideane	1299	0.06
Methyl Geranate	1323	0.28
Benzyl Butyrate	1345	0.08
Tetradecane	1399	0.22
Armoadendrene	1439	0.08
Geranyl acetone	1453	0.59
Ionone	1485	1.33
δ - Cadinene	1524	0.11
Dodecanioc acid	1568	0.17
Caryphyllene oxide	1581	0.12
n - Hexadecane	1600	0.40
10-epi- γ -Eudesmol	1619	0.09
n – Penptadecane	1700	0.31
n – Octadecane	1800	0.38
Cyclohexadecane	1879	0.11
Nonadecane	1900	0.22
Farnesylaceton	1916	0.26
Phytol	1947	0.07
Henicosane	2100	11.93
Docosane	2200	0.20
Tricosane	2300	0.35
Tetracosane	2400	0.88
Pentacosane	2500	1.61
Bis(2-ethylhexyl) phetalat	2550	5.82
Hexacosane	2600	2.08
Heptacosan	2903	4.36
		91.35

[a]RI = Relative retention indices as determined onHP5-MS column using the Homologous of n-alkanes

essential oil components and fatty acid methyl esters were calculated from peak area using a Shimadzu C-R4A Chromatopac without the use of correction factors.

Antibacterial activity

The ethyl acetate extract of *S. punicea* leaves were tested against 4

Table 2. Chemical composition (%) of the hexanoic extract from the seeds of *Sesbania punicea.*

*Compound (related fatty acids)	Rt (min)	Percentage (%)
1,2-dimethyl- Benzene	5.17	0.37
Nonane	5.27	1.34
4-ethyl Octane	6.23	0.82
5-methyl Nonane	6.31	0.81
3-methyl Nonane	6.50	1.30
1-hexyl-3-methyl Cyclopentane	6.78	0.79
1-ethyl-3-methyl Cyclopentane	6.80	0.71
1,2,4-trimethyl benzene	6.90	0.64
Decane	7.00	16.63
2-ethyl-1-Hexanol	7.46	4.25
Indene	7.62	1.54
Tricyclo[5,2,1,0(2,6)]dec-3-ene	8.18	6.54
Methyl dicyclopentadiene	8.47	0.41
Tricyclo[5,2,1,0(2,6)]dec-4-ene	8.52	3.01
Undecane	8.64	0.57
Azulene	10.01	4.37
Dodecane	10.18	3.97
Tridecane	11.62	0.54
4-methyl tridecane	12.43	0.45
2-methyl Tridecane	12.50	1.15
3-methyl Tridecane	12.59	1.24
2,6,10-trimethyl Dodecane	12.68	2.11
Cyclotetradecane	12.90	1.06
Tetradecane	12.99	9.21
4.8-dimethyl tridecane	13.04	0.97
1,7-dimethyl Naphthalene	13.15	1.32
1,5-dimethyl Naphthalene	13.35	1.37
1,6-dimethyl Naphthalene	13.39	2.21
3-methyl tetradecane	13.89	0.73
Pentadecane	14.26	4.69
Hexadecane	15.47	1.41
Octadecane	17.70	0.41
Hexadecanoic acid, methyl ester (Palmitic acid)	19.00	2.20
9,12-Octadecadienoic acid, methyl ester (Linoleic acid)	20.65	6.08
9-Octadecenoic acid, methyl ester(Oleic acid)	20.70	2.65
Octadecanoic acid, methyl ester(Stearic acid)	20.93	0.66
1,2-benzene dicarboxylic acid, methyl ester	21.59	0.37
Total		88.90

*The composition of the extracts was determined by comparison of the mass spectrum of each component with Wiley GC/MS library data and also from its retention times(Rt).

gr(+) bacteria: *Stereptococcus agalactiae*, *Stereptococcus mutans*, *Staphylococcus saprophytycous, Staphylococcus aureus* and 4 gr(-) bacteria: *Salmonella typhi, Salmonella para typhi* B*, Shigella phlexneri, Esheichia coli.* Microorganisms were identified by Research Center of Biotechnology and Industrial Center of Fungi and Bacteria collections, Iran. The *in vitro* antibacterial activity was evaluated by the disc diffusion method (DDM) according to the standard method by Bauer et al. (1966) to assess the presence of antibacterial activities of the plant extract. A bacteria culture (which has been adjusted to 0.5 McFarland standard) was used to lawn Mueller-Hinton agar plates evenly using a sterile swab. The plates were dried for 15 min and then used for the sensitivity test. The discs which had been impregnated with the plant extract were placed on the Muller-Hinton agar surface. Each test plate comprised

Table 3. Antibacterial activity of ethyl acetate extract of the leaves of *Sesbania punicea.*

Microorganism	PTCC	Gr(+/-)	Zone of inhibition (mm)*			
			Ethyl acetate extract	Antibiotics		
				C**	P**	A**
Salmonella paratyphi B	1231	-	24	22	[b]NT	NT
Stereptococcus agalactiae	1768	+	14	25	22	NT
Staphylococcus saprophytycus	1440	+	16	NT	28	NT
Salmonella typhi	1609	-	14	19	NT	NT
Shigella phlexneri	1234	-	11	NT	NT	26
Stereptococcus mutans	1683	+	[a]NA	22	26	NT
Staphylococcus aureus	1431	+	NA	NT	30	27
Escherichia coli	1395	-	NA	NT	NT	15

*Inhibition zone diameter (mm), **C: Chloramphenicol; **P: Penicilline; **A: Ampicilline; [a]NA: not active; [b]NT: not tested, PTCC: Persian type culture collection.

of 3 discs, one positive control, which is a standard antibiotic disc, one negative control and one treated disc. The standard antibiotics were chloramphenicol, penicillin G and ampicillin (10 µg) (Hindi Co., India) and the negative control was ethyl acetate (Merck, Germany). The plates were then incubated at 37°C for 24 h. After incubation, the growth inhibition zones were measured. Each test was carried out in duplicate and the average was calculated for inhibition zone diameters.

RESULTS AND DISCUSSION

The results obtained in the GC and GC/MS analyses of essential oil of leaves and hexane extract of the seeds of *S. punicea* are listed in Tables 1 and 2, respectively. Forty components comprising 91.35% of the essential oil were identified (Table 1). The oil was dominated by monoterpenes (60.60%) from which 1,8-cineole (47.58%) and α-pinene (7.30%) were the main components. Non terpenoids including hydrocarbons (23.11%), esters (5.9%) and aldehyde (0.3%) were also found in the oil, while sesquiterpenes were found too low (0.73%) in the essential oil. Essential oil composition of the aerial part of *Sesbania* species has not been investigated before and we did not find any report in the literature.

In analysis of hexane extract from the seeds of *S. punicea*, thirty seven components (88.90%) were identified from which fatty acid methyl esters (11.96%) including unsaturated fatty acids (UFAs): linoleic acid (ω-6) (6.08%), oleic acid (2.65%) and 1,2-benzene dicarboxylic acid (0.37%) were predominated to saturated fatty acids (SFAs): palmitic acid (2.20%) and stearic acid (0.66%). Hydrocarbons (77.32%) were the main components in the hexane extract of *S. punicea* seed. Seeds of the plants have always been the subject of projects in phytochemistry field (Faroog et al., 1954; Pokharkar et al., 2008; Arekemase et al., 2013). Investigations of natural products isolated from seeds have resulted in a remarkable variety of compounds having unusual structures (Powell, 2009). Seeds of many species contain uncommon fatty acids and lipids, some of which have found applications in the cosmetic industry or as renewable (non-petroleum based) industrial raw materials. In addition to protein and energy, storage substances such as carbohydrates and lipids, seeds generally contain or have the ability to produce protective compounds that are active as plant growth regulators (Meudt, 1983), fungicides (Abad et al., 2007), insecticides (Jbilou et al., 2006) and repellants of herbivores (Degenhardt, 2009). Previous study on fatty acid composition of *S. aegyptica* seed, as determined by the thiocyanometric and fractionation methods, showed oleic, linoleic, linolenic, palmitic, stearic and lignoceric acids (Faroog et al., 1954). Quantitative evaluation of the nutritional constituents of *S. sesban* seeds has been studied and the most important part of the seed were carbohydrate, protein, fiber and moisture and the results obtained from vitamin analysis revealed that the seeds of *S. sesban* are excellent sources of B vitamins and vitamin E (Arekemase et al., 2013).

In the next part of our research, we studied anti-bacterial activity of ethyl acetate extract (0.167 mg/ml) of *S. punicea* leaves aginst 8 gr(+/-) bacteria. The results presented in Table 3 showed that the extract exhibited strong inhibition activity against the gram-negative bacteria: *S. parathyphi* B (Inhibition zone diameter 24 mm), even more than chloramphenicol (Inhibition zone diameter 22 mm) as a standard antibiotic. The extract also showed moderate antibacterial activity against two gram-positive: *Streptococous* B *and Staphylococcus saprophyticus* and two gram-negative bacteria: *Salmonella typhi* and *Shigella flexneri*.

Biological activities of the leaves, flowers and bark

extracts of *Sesbania* species have been studied before. The seasonal variation of alkaloids has been investigated in leaf, bark and wood of *S. rostrata, S. exaltata* and *S. sesban*, which are medicinally important. The leaves of *S. rostrata* showed high level of lipid and alkaloid contents more than the other two species (Kadam et al., 2013). Novel chemical constituents, isolated from the leaves of *S. aculeuta* showed anti-inflammatory activity (Sharma et al., 2014). The antibacterial activity of fatty acid methyl esters from synthesis of *S. rostrata* seed by *in situ* trans esterification reaction was evaluated and the results indicated that the fatty acid methyl esters of *S. rostrara* seed was too active against the gram-negative microorganism *Pseudomonas pseudoalcaligenes* (Pokharkar et al., 2008). Anti-microbial activity of the crude extract of *S. grandifolia* flower polyphenol extract has been studied and the gram-positive bacterium, Staphylococcus aureus was reported as the most sensitive microorganism (China et al., 2012; Krasaekoopt and Kongkarnchanatip, 2005).

Conclusion

As the result, the high amount of 1,8- cineole in the essential oil of the leaves can make the plant a good natural source of this compound. The low amount of fatty acids indicates that the seed has no significant nutritional capacity. Ethyl acetate extract of the leaves showed strong antibacterial activity especially on gram-negative bacteria. The gram-negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics, so the plant can be potent for antibacterial activity against the gram-negative bacteria: *Salmonella parathyphyi* B. Further studies are needed to confirm the *in vivo* antibacterial activity and subsequent isolation and chemical characterization of the active molecules.

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Conflict of Interest

Authors have not declared any conflict of interest.

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