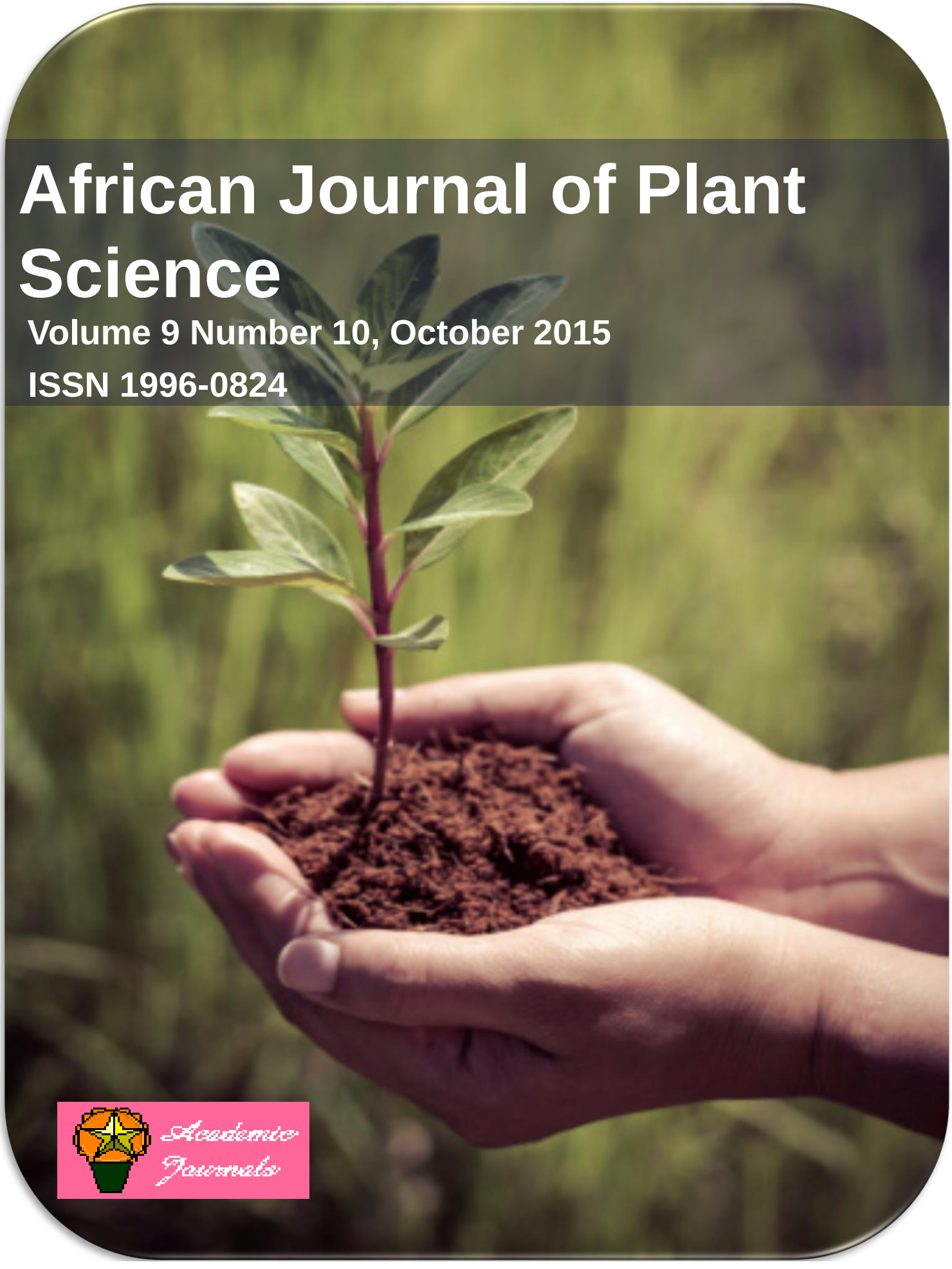
African Journal of Plant Science

Volume 9 Number 10, October 2015

ISSN 1996-0824



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Vol. 9(10), pp. 401-411, October 2015
DOI: 10.5897/AJPS2015.1334
Article Number: 87F240955772
ISSN 1996-0824
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African Journal of Plant Science

Full Length Research Paper

Morphological characteristic variation of eleven provenances of *Moringa oleifera* seedlings grown in the Northern Sudanese area of Burkina Faso

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Received 15 July, 2015; Accepted 23 September, 2015

This research investigated the morphological characteristic variation among West African provenances of *Moringa oleifera* in order to select the most suitable provenance for planting in Burkina Faso. A provenance experiment involving seven provenances from Burkina Faso, one from Mali, one Ivory Coast and one from Ghana was established in June 2014 in a randomized complete block design with three replications at Ouagadougou in the North Sudanese area of Burkina Faso. Plant growth traits (height, number of branches and pinnae, leaf length and width) and biomass production (above and underground biomass dry weight, under: aboveground ratio and total dry weight) were measured on two-week old and two-month old seedlings. The result indicates no significant correlation between morphological characteristics and agroclimatic data (longitude, altitude and annual rainfall) of the seed origin. Significant variations between provenances for morphological characteristics and biomass production (P ≤ 0.05) were observed. Two-month old *M. oleifera* exhibited significant differences between provenances (P ≤ 0.05). The average height ranged from 107-40 cm, number of branches from 15-8, number of pinnae per leaf from 12-5, leaf length from 44-16 cm and leaves width from 105-34 cm. The aboveground biomass productions ranged from 1-14 g, underground biomass production from 1-7 g, ratio of under: aboveground from 0.4-0.9 and total dry weight from 2-21 g. Five provenances from Burkina Faso (Gaoua and Dano in the South Sudanese area, Ouagadougou, Fada N'Gourma and CNSF in the North Sudanese area) and one from Ivory Coast (Niangon-Lokoua/Abidjan) in the sub equatorial area) showed superior performances. They can be recommended for planting in Ouagadougou and other areas with similar ecological conditions.

Key words: Leaves, pinnae, agroclimatic, correlation, aboveground and underground biomass.

INTRODUCTION

Moringa (*Moringa oleifera* Lam.) in the family *Moringaceae* is a plant indigenous to the sub-Himalayan regions in Northwestern India currently found in numerous countries situated in the tropical and

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sub-tropical regions across Africa, South East Asia and South America (Paliwal et al., 2011; Leone et al., 2015); where it is commonly used for animal forage, nutrition, medicine and water purification oil (Manh et al., 2005; Rashid et al., 2008). It is a fast growing, small to medium sized tree ranging between 5 and 12 m in height and tree canopy has an umbrella shaped crown with bi-(tri-)pinnate leaves, while the individual leaflets have a leaf area of one to two cm^2 (Muhl et al., 2011). Flowers are white to cream colored and zygomorphic. The tree bears 20 to 30 cm long fruit that once mature, change colour from green to brown revealing numerous round or triangular seeds with three papery wings (Arbonnier, 2002).

In West Africa, moringa leaves are mixed with other vegetables to make sauce. The use of the fresh and dried leaves as staple foods is very common in Burkina Faso. Leaves contain high amounts of total phenols and proteins and are a good source of vitamins and minerals: perhaps more than any other tropical vegetable (Ferreira et al., 2008). Moringa is used in many African feeding programs to fight malnutrition and its associated diseases, including blindness (Mulh et al., 2011). Fragrant flowers of moringa are also used as a source of vegetables and bee forage; twigs with leaves as fodder; green leaves as mulch and solid wood as energy source (Smith and Eyzaguirre, 2007).

Moringa is cultivated in tropical and sub-tropical areas with annual rainfall varying from less than 100 to over 1500 mm and different soils types (Nouman et al., 2012). It has been introduced into gardening systems because of its versatility (Palada and Chang, 2003; Nduwayezu et al., 2007).

Although moringa is grown throughout numerous agro systems, no large-scale commercial plantings have been reported, possibly as a result of the limited scientific data that is currently available on the morphological characteristics that control its survival and productivity. Therefore, further insights into the morphological characteristics of this species are needed before recommending its large scale introduction to home gardens of smallholders' growers (Scott and Sullivan, 2007). At present, data are not available to make reliable recommendations to garden landowners on the best seed sources and how the species might perform in various agroforestry systems. Therefore, the objective of this study was to evaluate the variations in morphological characteristics of the growth of *M. oleifera* at nursery stage for selection of seed sources suitable for plantation establishment under different geoclimatic environmental conditions.

MATERIALS AND METHODS

Study site

The study was conducted at the women gardening center "Amicale des Forestières du Burkina Faso (AMIFOB)" located at Ouagadougou, Burkina Faso (12°7'32"N, 01°40'24"W). The rainfall is uni-modal with a mean annual rainfall of the last 15 years data from the nearest meteorological station in Ouagadougou, of 950 mm $year^{-1}$, with the mean potential evapotranspiration of 177 mm. $month^{-1}$ and the mean temperature of 29.2°C $month^{-1}$ (Figure 1a and b). Soils are sandy clay to clay-sandy ferruginous leached with very low nutrient content according to French soil classification (Pallo et al., 2009). The common natural vegetation found at Ouagadougou is described as semi-deciduous open woodland. Main genera include, *Eucalyptus, Azadirachta, Mangifera, Vitellaria, Lannea, Piliostigma, Acacia., Ziziphus, Tamarindus* and *Combretum*.

Seed sources, experimental design and establishment

The experiment included eleven *Moringa oleifera* provenances of Ouahigouya (P1), Ségou (P2), Centre National de Semences Forestières (CNSF) (P3), Ouagadougou (P4), Koudougou (P5), Fada N'Gourma (P6), Bobo Dioulasso (P7), Dano (P8), Gaoua (P9), Tamalé (P10) and Niangon-Lokoua (P11) (Table 1). These provenances were selected from four countries, Burkina Faso, Mali, Ghana, and Ivory Coast and four climate areas according to their agro-ecological characteristics (Sahelian, Sub Equatorial, South and North Sudan). Seeds were collected in 2014 in plantation farmland from at least 12 mother trees per provenance. The experimental design was a randomized complete block design (RCBD) with three replications, and each plot represented a provenance planted at 5 x 6 rows in a contiguous arrangement of 20 x 20 cm (Figure 2). Plot measured 10 x 1 m and contained 30 trees and the distances between blocks were 2 m. Seed samples were pretreated with water for 24 h and sown in June 1st 2014. Weeding was done twice during the rainy season. The seedlings were grown without fertilizers and pesticide.

Morphological measurements

The morphological measurements were performed on two-week and two-month old *M. oleifera*. Twenty seedlings from each provenance per block were selected randomly and their heights were assessed from root collar up to the terminal shoot using a measuring tape. At the age of two months, 20 randomly selected plants from each provenance in each block were labeled for counting the total number of branches per plant and pinnae per leaf.

Dry matter production

After two months from germination, all trees were harvested for dry matter measurements using an analytical balance. Each was separated into aboveground biomass (leaves, buds and stems) and underground biomass (roots), and dried at 70°C for 48 h using an oven.

Data analysis

All statistical analysis were carried out using ANOVA performed with JMP® Pro 11.1.1 (SAS Institute, Cary, NC, USA). Normality and homoscedasticity were graphically verified on residual plots of the linear models (Quinn and Keough, 2002). When effects were significant, the Duncan test was used for multiple mean comparisons to detect the significant differences between the

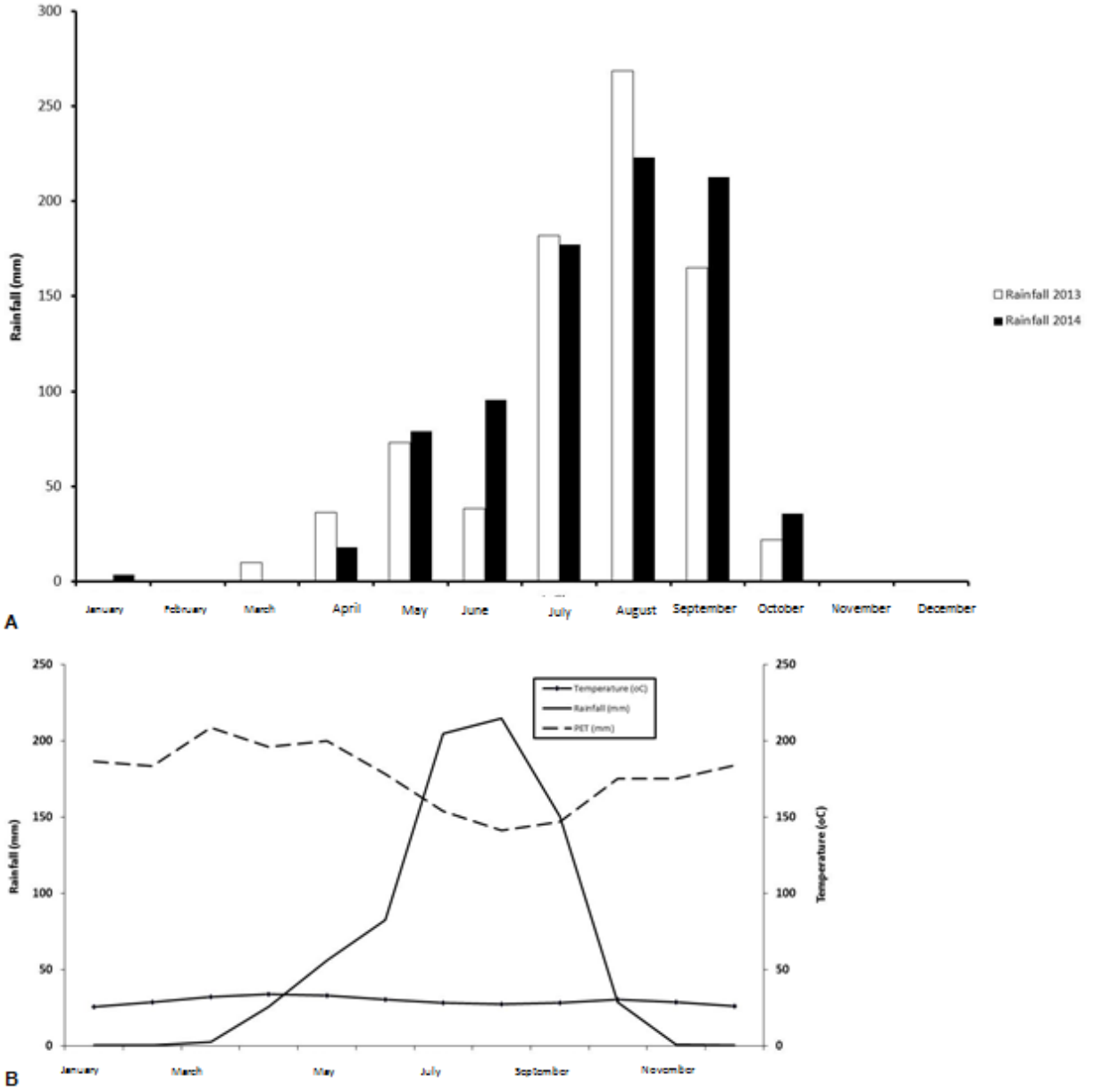


Figure 1. Mean rainfall of 2013 and 2014 (mm) (a); mean rainfall (mm), mean PET (mm) and mean temperature (°C) of the last 30 years (b) of Ouagadougou, the nearest meteorological station to AMIFOB site, Burkina Faso.

means. Statistical significance was fixed at 0.05. The correlation between the morphological characteristic variables and the geoclimatic data (altitude, longitude and average rainfall) of each provenance seed origin was assessed using scattered diagram to determine the linearity and the trend of the relationships.

RESULTS

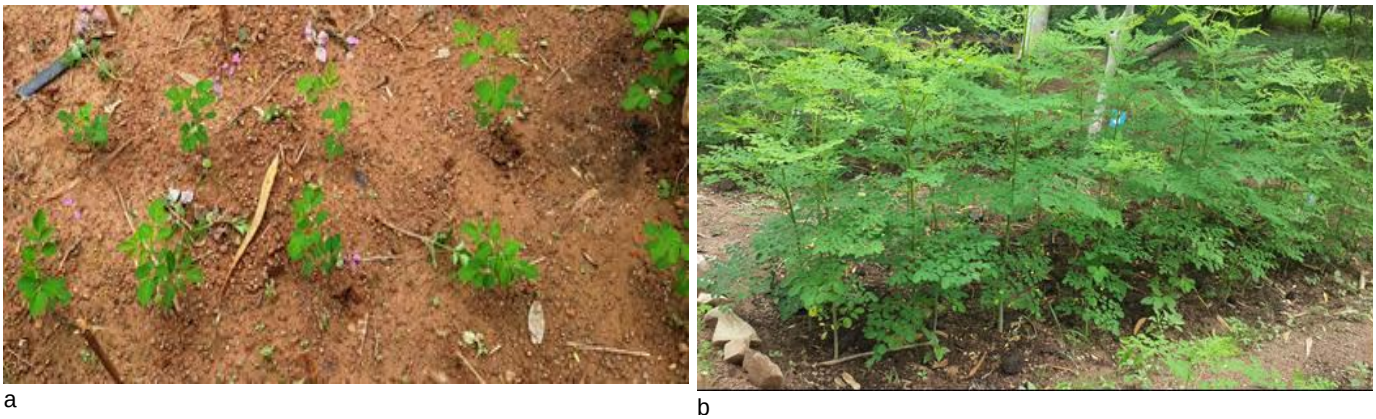
Morphological traits

The height of two-week old seedlings showed significant differences ($P \leq 0.05$) among the studied provenances (Figure 3a). The provenance P9, P3, P1 and P11 exhibited the highest shoot length (average of 22 cm), which was significantly different from P2, P4, P5, P6, P7, P8 and P10. Significant differences ($P \leq 0.05$) were also observed in the height of two-month old trees (Figure 3a), with the provenance P9 exhibiting the highest shoot length of 117 ± 4.5 cm and the provenance P7 exhibiting the lowest shoot length of 40 ± 3.3 cm. The provenance P1, P3, P9 and P11 had the greatest average number of branches per two-week old seedling (6 branches), but

Table 1. Seeds origin of provenances of *M. oleifera* used in the study.

Provenance code*	Provenance	Country	Latitude (N)	Longitude (W)	Altitude (m)	Average rainfall (mm.year^{-1})
P1	Ouahigouya	Burkina Faso	13°30'04"	02°24'31"	306	500
P2	Ségou	Mali	13°22'05"	05°16'24"	294	500
P3	CNSF	Burkina Faso	12°30'07"	02°07'34"	304	800
P4	Ouagadougou	Burkina Faso	12°21'58"	01°31'05"	315	800
P5	Koudougou	Burkina Faso	12°15'04"	02°22'28"	308	800
P6	Fada N'Gourma	Burkina Faso	12°03'41"	00°21'30"	300	900
P7	Bobo Dioulasso	Burkina Faso	11°11′00″	04°17′00″	339	950
P8	Dano	Burkina Faso	11°09'00"	03°04'00"	287	950
P9	Gaoua	Burkina Faso	10°19'12"	03°10'12"	319	1000
P10	Tamalé	Ghana	09°24'27"	00°51'12"	169	1100
P11	Niangon-Lokoua/Abidjan	Ivory Coast	05°18'28"	04°06'19"	73	2000

*: This notation sequence was considered the correct one for all the manuscript.



a b

Figure 2. *Moringa oleifera* trial in the women gardening center of AMIFOB, a = two-week old seedlings plants; b = two-month old plants; Ouagadougou. Photo MCE DAO, Ouagadougou, 2014.

was not significantly different (P ≤ 0.05) from P2, P8 and P10 (Figure 3b). However, the difference was significant when compared with P4, P5, P6 and P7. The highest number of branches of two-month old trees was observed on the provenances P3, P4, P6, P8, P9 and P11 (15 branches). Provenances P10 and P7 had the smallest number of branches estimated at 8 and 9, respectively (Figure 3b).

Significant differences between provenances were found in leaf length and width (P ≤ 0.05) (Figure 4a and b). Two-month old *M. oleifera* from provenances P3, P4, P6, P8, P9 and P11 exhibited long and large leaves. The provenance P3 had the longest and widest leaves, measuring 45 and 105 cm, respectively. The provenances P3, P4, P6, P8, P9 and P11 had significantly (P ≤ 0.05) greater number of pairs of pinnae per leaf (Figure 4c). The provenances P3, P4, P6, P8 and P9 exhibited 12 pinna per leaf.

There was no correlation between the altitude, longitude and average rainfall of the seed source origin with the morphological traits (leaf length and width, number of pinnae per leaf and number of branches per tree) (Figures 5, 6 and 7).

Dry matter productions

The provenances displayed substantial variations (P ≤ 0.05) in dry matter production and location (Table 2). Aboveground biomass dry weight of the provenances varied between 1.2 and 14.0 g. The provenance P9 produced the highest aboveground biomass dry weight, which was significantly different from the other provenances. The provenances P1, P2, P7 and P10

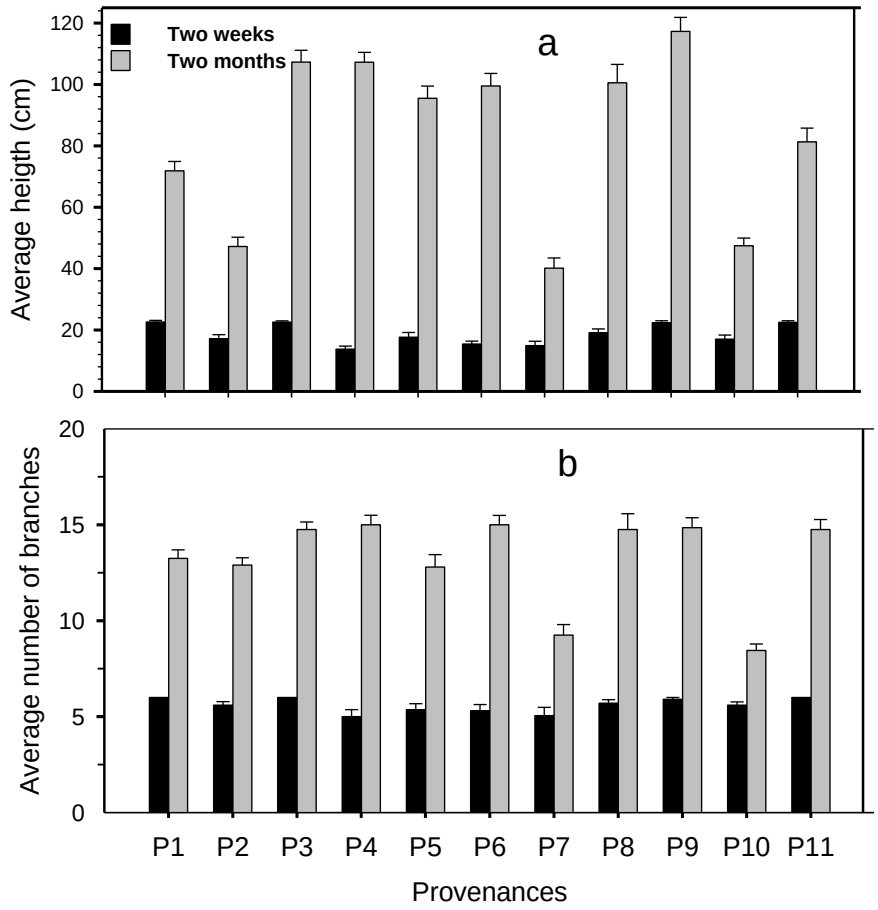


Figure 3. Morphological characteristics of *M. oleifera*: Average height of two weeks, and two months of age (a); average branch number of two weeks and two months of age (b). Values are means ± standard error.

produced the smallest aboveground biomass dry weight.

The underground biomass dry weight of the provenances varied from 1.0 to 7.4 g. The provenance P9 had the highest underground biomass dry weight, which was not significantly different from that produced by P3, P4, P5 and P6. However, significant difference appeared when they were compared with P1, P2 P7, P8 and P10, which produced the smallest underground biomass dry weight (Table 2). The underground biomass : aboveground ratio of the provenances varied from 0.4 to 0.9, and the highest ratio was shown by P10, but it was only significantly different from that of P1 and P8 (Table 2). Provenance P9 was also superior in total dry weight, which was significantly greater than the total dry weights produced by P1, P2, P5, P7, P8, P10 and P11 (Table 2).

DISCUSSION

This study evaluates the variations in morphological characteristics of the growth of 11 provenances of *M. oleifera* from different geoclimatic conditions of West Africa in order to select the seed sources suitable for plantation establishment under North Sudanese environmental conditions in Burkina Faso. Considering together all morphological characteristics and the biomass production, six provenances showed significant differences. The significant variability in the morphological characteristics was supported by previous studies on *M. oleifera* (Nduwayezu et al., 2007; Edward et al., 2014) and other species (Kozlowski and Pallardy, 1997; Ky-Dembele et al., 2014). The number of pairs of pinnae (5-12) is within the range reported by Arbonnier (2002). Moreover, the variation in number of branches may be related to genetic differences (Wright, 1976).

Despite the wide geoclimatic range of the sites sources, no correlation was found between provenances and the altitude, longitude and the annual rainfall for seedlings morphological characteristics. Thus, variations in seedling morphological traits among provenances

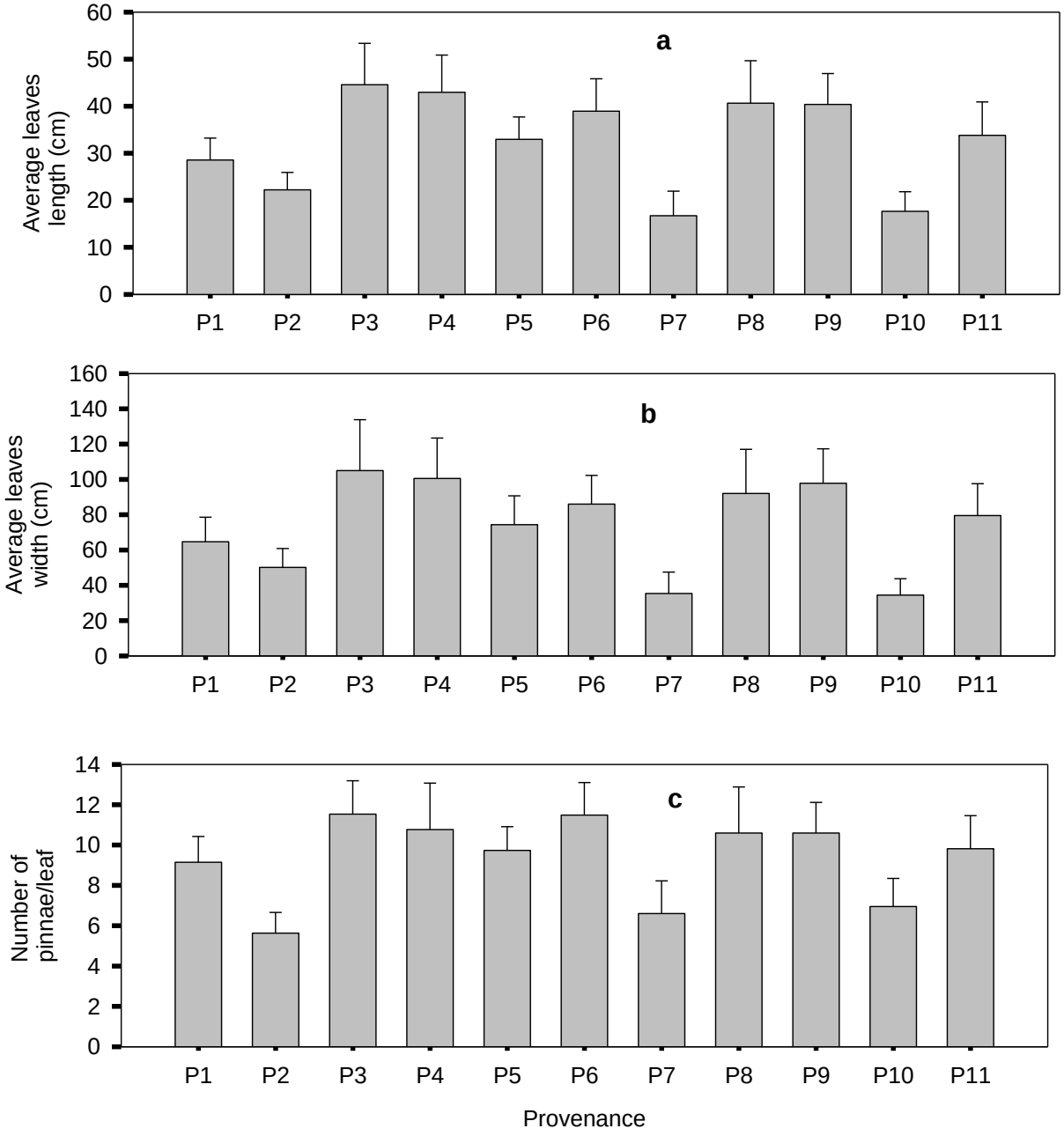
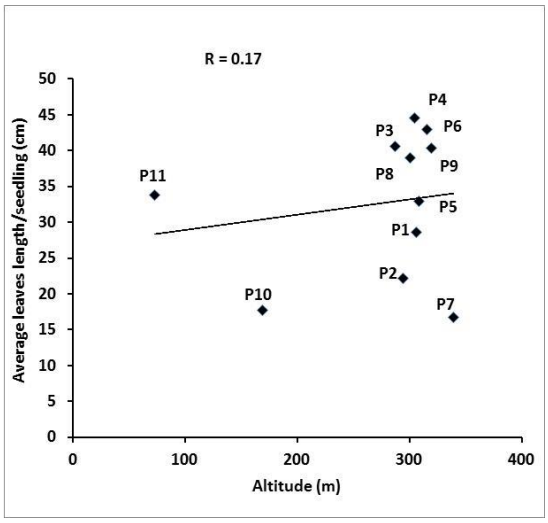


Figure 4. Morphological characteristics of *M. oleifera*, two months: leaf length average (a), leaf width average (b), number of pinnae/leaf (c). Values are means ± standard error.

cannot be explained by the average annual rainfall, altitude and longitude at the seed source origin. These results disagree with previous works reported by Abrams (1994), who showed the relationships between high rainfall areas and the plant morphological characteristics.

The significant differences in biomass production among the different parts of the provenances are consistent with the findings by Kundu and Tigerstedt (1998) in *Azadirachta indica* and Hardiyanto et al. (2004) in *A. mangium*. The good performance in above and underground biomass production of the provenances Gaoua (P9), Fada N'Gourma (P6), Ouagadougou (P4) and CNSF (P3) was attributed to its advantages in good growth in height, number of branches, pinnae and leaves at the study site.

The results of this study indicate the existence of



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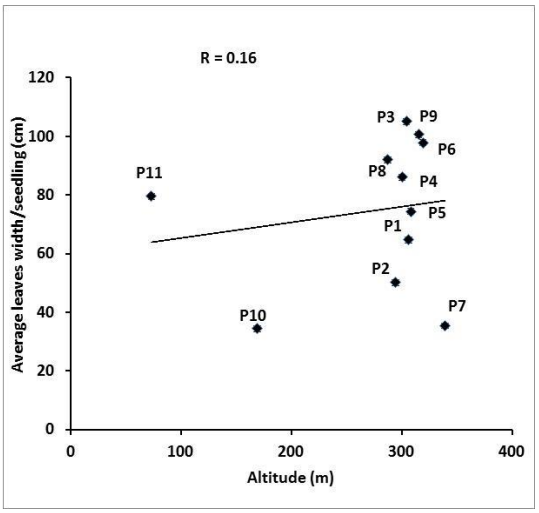

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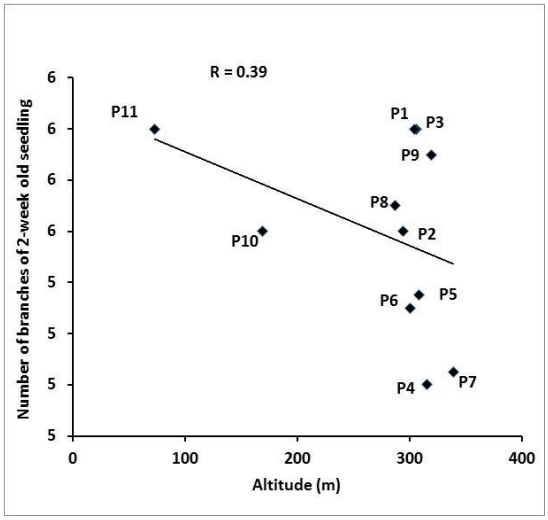

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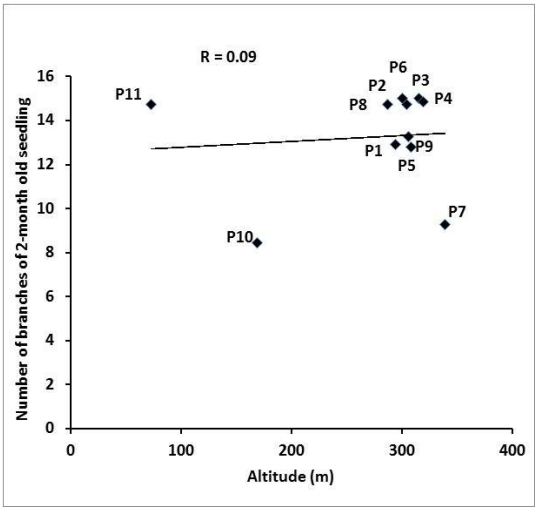

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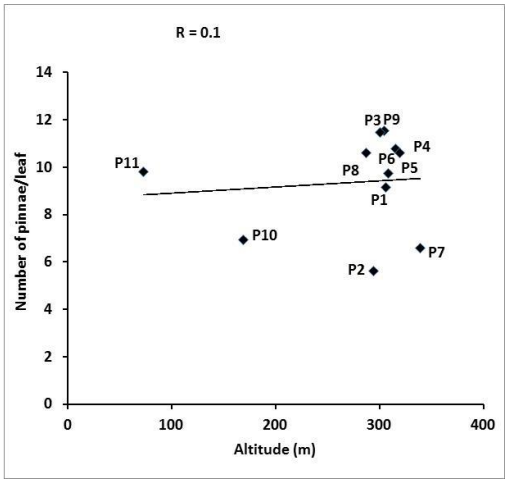
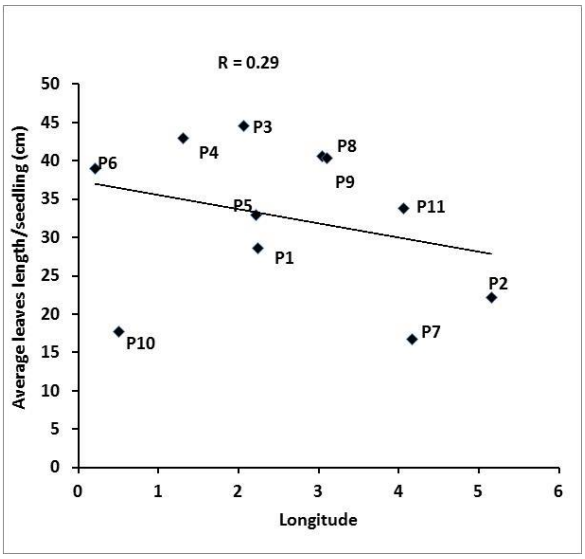


Figure 5. Correlation of altitude with leaf length average (a), leaf width average (b), average number of branches of two-weeks old plants (c), average number of branches of two-months old plants (d) and average number of pinnae per leaf (e) of eleven provenances of *M. oleifera*.



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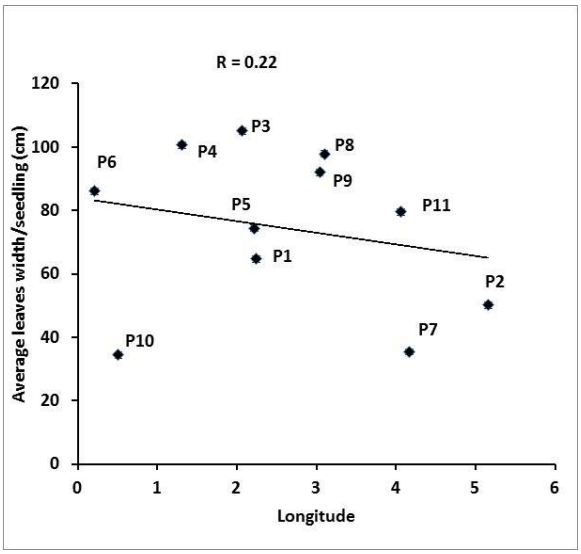

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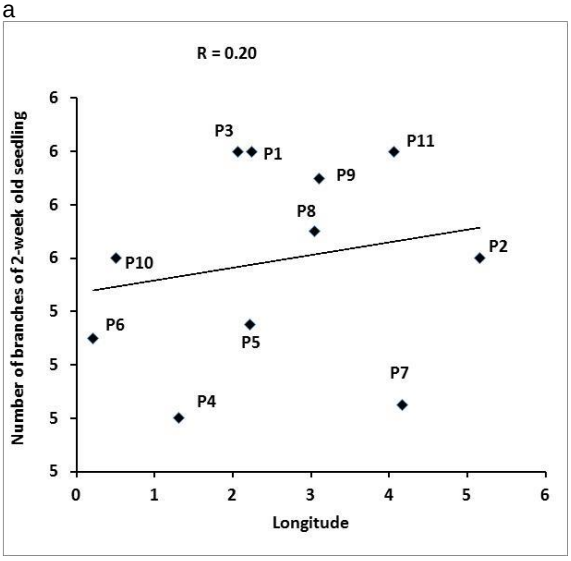

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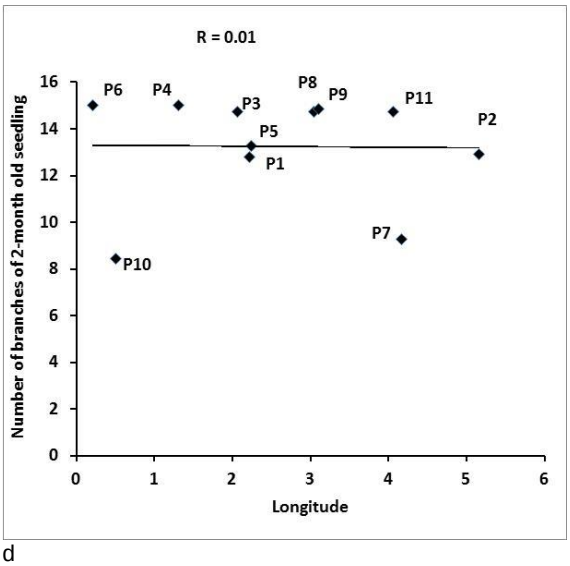

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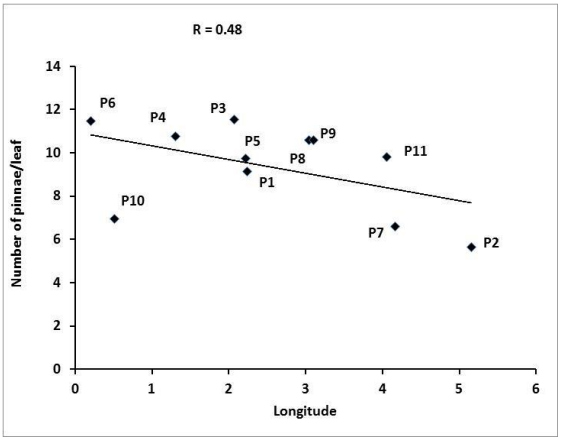
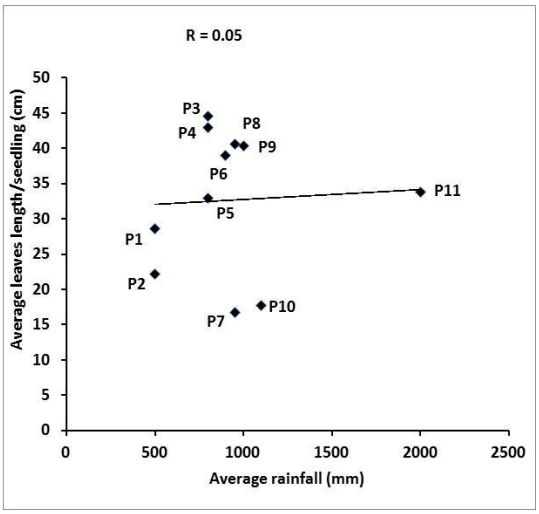


Figure 6. Correlation of longitude with leaf length average (a), leaf width average (b), average number of branches of two-weeks old plant (c), average number of branches of two-months old plant (d) and average number of pinnae per leaf (e) of eleven provenances of *M. oleifera*.



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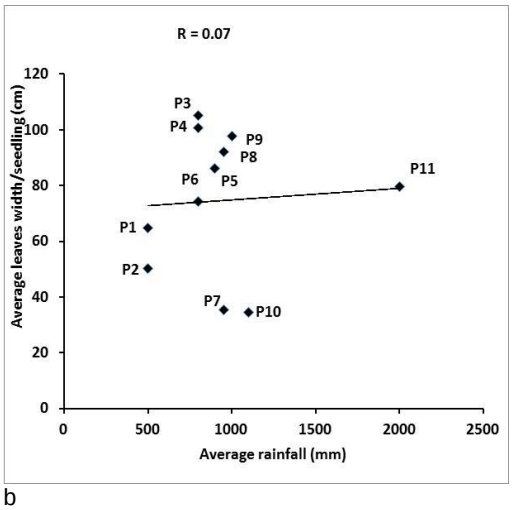

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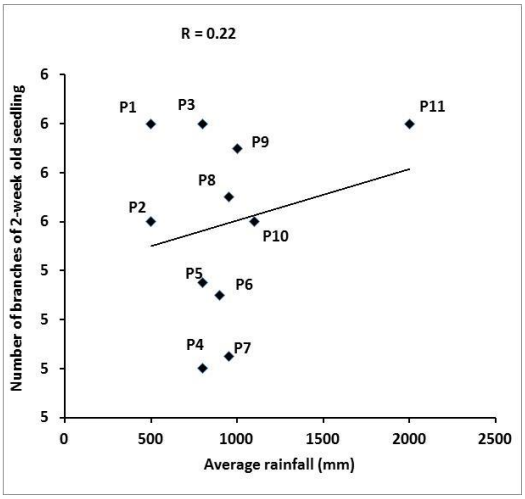

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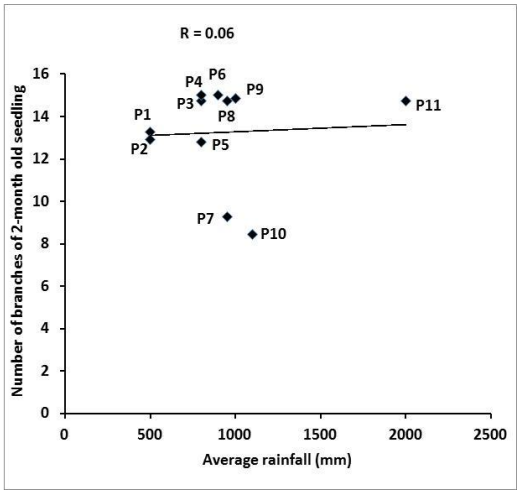

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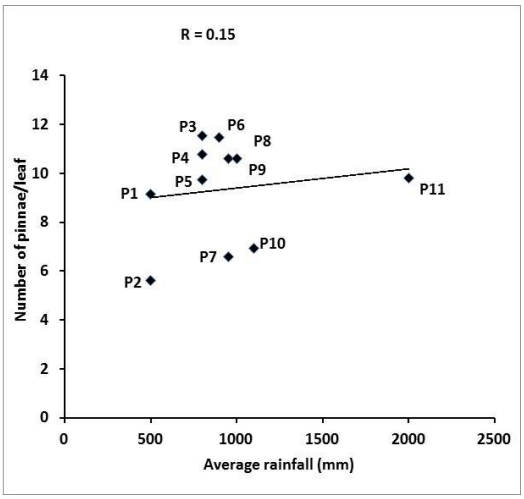

Figure 7. Correlation of average rainfall with leaf length average/seedling (a), leaf width average/seedling (b), average number of branches of two-weeks old seedling (c), average number of branches of two-months old seedling (d) and average number of pinnae per leaf (e) of eleven provenances of *M. oleifera.*

Table 2. Above and underground biomass of two-month old plants of *M. oleifera* from eleven provenances (the provenance codes indicated here is in accordance with the codes in Table 1 modified.

Provenance code	Aboveground dry weight (g)	Underground dry weight (g)	Underground biomass : aboveground ratio	Total dry weight (g)
P1	4.0 ± 0.5 de	2.2 ± 0.7 cde	0.5 ± 0.1b	6.2 ± 1.0 def
P9	3.4 ± 0.3 e	1.8 ± 0.2 cde	0.5 ± 0.1ab	5.2 ± 0.5 ef
P3	12.2 ± 1.1 ab	5.8 ± 0.9 abc	0.5 ± 0.1ab	18.0 ± 1.8 abc
P4	11.6 ± 1.6 ab	6.6 ± 1.4 ab	0.5 ± 0.1ab	18.2 ± 2.8 ab
P5	6.3 ± 0.8 cde	4.5 ± 1.0 ab	0.7 ± 0.1ab	10.7 ± 1.5 cde
P6	10.2 ± 1.1 abc	5.3 ± 0.8 ab	0.5 ± 0.1ab	15.5 ± 1.7 abc
P7	1.2 ± 0.2 f	1.0 ± 0.2 e	0.7 ± 0.1ab	2.2 ± 0.4 f
P8	7.9 ± 0.9 bcd	3.1 ± 0.3 bcde	0.4 ± 0.0b	11.0 ± 1.2 bcde
P9	14.0 ± 1.6 a	7.4 ± 1.3 a	0.6 ± 0.1ab	21.4 ± 2.4 a
P10	1.4 ± 0.1 f	1.2 ± 0.1 de	0.9 ± 0.1a	2.6 ± 0.3 f
P11	7.8 ± 1.0 bcde	5.2 ± 1.2 abcd	0.7 ± 0.1ab	12.9 ± 1.8 bcd

Values are means ± standard error. Means in the same column with the same letter (s) are not significantly different at P ≤ 0.05, according to Duncan multiple range test.

strong variations in seedling morphological characteristics and biomass production among the studied provenances. The climatic conditions prevailing in the seeds origins had no effect on the seedling's morphology and biomass production. The absence of significant correlations between the wide range of provenances from diverse geoclimatic conditions and the morphological traits and biomass production of *M. oleifera* was attributed to the ability of the species to grow on a wide variety of climate and soil types.

Conflict of interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors are very grateful to the support provided by the technician Zeba Abinatou from the Forestry Department of the Research Institute of Agriculture and Environment of Burkina Faso, Denis Walsh for the useful help in statistics and the members of women association (AMIFOB) for their tireless efforts in making this work a success.

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Vol. 9(10), pp. 412-420, October 2015
DOI: 10.5897/AJPS2015.1345
Article Number: 5FAC45455775
ISSN 1996-0824
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African Journal of Plant Science

Full Length Research Paper

Antimicrobial and antioxidant activities of extracts from medicinal plant ginger (*Zingiber officinale*) and identification of components by gas chromatography

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Received 20 August, 2015; Accepted 29 September, 2015

The present study was conducted to investigate antioxidant and antimicrobial activities of *Zingiber officinale*, and detected possible chemicals existence in the plant. Phytochemical screening was carried out using the standard test methods of different chemical groups. Investigating the antioxidant activity, one complementary test method namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out. Disc diffusion method, with minor modifications was used for the evaluation of *in vitro* antimicrobial activity. The extracts were a rich source of phytochemicals. In DPPH free radical scavenging test, the ethanol, acetone and cyclohexane extracts show the highest free radical scavenging activities, although the extracts revealed good antimicrobial activities. Finally, the bioactive compounds of *Zingiber officinale* rhizomes have been evaluated by using GC-spectroscopy.

Key words: *Zingiber officinale*, GC spectroscopy antimicrobial, antioxidant, agar-well diffusion, phytochemical screening.

INTRODUCTION

Ginger is widely used as spices in food and pharmaceutical chemistry. Ginger (*Zingiber officinale*) botanical family is Zingiberaceae and it is a flavoring agent, and herbal medicine used when fresh and dried. Ginger rhizomes are the plant part used for culinary and medicinal aims (Afzal et al., 2001). The root of ginger has been used as a spice for over 2000 years and its cultivated in many tropical and subtropical countries, including India, Nigeria, Fiji, Taiwan, Jamaica, China, Australia, and some area of Kurdistan. Ginger is one of the common additives in some foods and beverages and is valued for its aromatic volatile constituents as well as for its spicy and pungent constituents (Bartley et al., 1994). Traditional medicine in Japan, China and India uses the rhizomes of ginger as a constituent of the herbal treatment for digestive disorders (indigestion, nausea, constipation and flatulence), headaches, rheumatism, colds and cough (Mustafa,1990). A large portion of

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traditional Chinese herbal remedies contain ginger. In the Ayurvedic medical practice in India, ginger is the herbal treatment for colds and other viral infections, poor appetite, digestive problems, arthritis and headache (Ghayur et al., 2005). In Kurdistan, most ginger is marketed as whole fresh rhizomes and used as salad components as a flavored taste and extracted essential oils used as cold treatment among rural peoples.

The antimicrobial properties of ginger have been known and valued for centuries (Gupta et al., 2003). The original discovery of ginger's inhibitory effects on prostaglandin biosynthesis in the early 1970s has been repeatedly confirmed (Raju et al., 2013). This discovery identified ginger as the herbal medicinal product that shares pharmacological properties with non-steroidal anti-inflammatory drugs. Ginger is a strong antioxidant substance and may either mitigate or prevent generation of free radicals. It is considered as a safe herbal medicine with only few and insignificant side effects.

The oxidation importance inside our body and in foodstuffs has been widely recognized. Oxidative metabolism is the main source of the survival of cells. Side effects of this depend on the production of free radicals and other reactive oxygen species that cause oxidative changes. Defense mechanisms against the effects of excessive oxidations are provided by the action of various antioxidants and the need to measure antioxidant activity is well documented (Ebana et al., 1993).

The purpose of the present study was to determine the chemical composition of dry ginger as well as its antioxidant and antimicrobial activities of extracted components. An attempt was also made to investigate association between the antioxidant and antimicrobial activities of extracted components of dry ginger extracted in different solvents.

MATERIALS AND METHODS

Collection and identification of plant material

Rhizome of ginger (*Z. officinale* Rosco) was purchased from a local market of Rania, Kurditsan region-Iraq and the species was taxonomically confirmed by a taxonomist at the Biology Department of Raparin University.

Preparation of plants for extraction

The collected rhizome was separated from undesirable materials. They were dried in open air under shade for three weeks. The shade plants were grinded to coarse powder with the help of a suitable grinder (pistol and mortar). The powder was stored in an airtight container and kept in a cool, dark and dry place until extraction process started with selected solvent.

Solvent extraction

For acetone, ethanol, and cyclohexane extractions, about 1 kg of air dried, powdered sample were immersed in 150 ml of 80% acetone, 95% ethanol and 99% cyclohexane separately in a clean and sterilized glass containers separately. The glass containers with its contents were sealed and kept for maceration for 3 days accompanying occasional shaking and stirring. At the end of third day, the whole mixtures were filtered carefully using Whatman filter paper NO.1. The resultant filtrates were then allowed to evaporate in water bath maintained about 37°C to dryness and thus a greenish black semisolid masses of each extracts were obtained (yield 30, 38 and 43 g consequently). Those gummy semisolid masses were designated as crude extracts of each solvent.

GC-spectroscopy

Preparation of extract

Two microliter (2 µl) of the ethanol, cyclohexane and acetone extracts of *Z. officinale* was employed for GC/MS analysis (Shahidi et al., 1992).

Instruments and chromatographic conditions

GC-Spectroscopy was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-S) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1.2 ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 40°C (isothermal for 2 min), with an increase of 5°C/min, to 200°C/min, then 5°C/min to 250°C/min, ending with a 10 min isothermal at 250°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of components

Interpretation on mass spectrum of GC-Spectroscopy was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The retention time of the unknown component was compared with the retention time of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Phytochemical screening of extract

The method described with slight modification was used for screening of alkaloid, steroids, phlobotannins, flavonoids, glycosides, saponins, tannin and terpenoids.

Alkaloids test

One gram (1 g) of the ginger and 5 ml of honey was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. One milliliter (1 ml) of the filtrate was treated with few drops of Draggendoff's reagent. Blue black turbidity serves as preliminary evidence of alkaloids.

Saponins test

One gram (1 g) of the extracts and 5 ml of honey was shaken with distilled water in a test tube. Frothing which persists on warning was taken as preliminary evidence of the presence of saponins.

Tannins

One gram (1 g) of extracts and 5 ml of honey was stirred with 100ml distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate determines the presence of Tannins (Ahmed et al., 2013).

Phlobotannins test

Disposition of red precipitate when an aqueous extract of the test samples was boiled with 1% hydrochloric acid determines the presence of phlobotannins (Merlin et al., 2009).

Flavonoids test

One milliliter (1 ml) of diluted ammonia solution was added to aqueous filtrate of the test samples followed by the addition of concentrated H_2SO_4. A yellow coloration observation determines the presence of flavonoids.

Cardiac glycosides (keller-killiani test)

One gram (1 g) of the extracts and 5 ml of honey was dissolved in 2 ml glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1 ml concentrated H_2SO_4. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form just gradually spread throughout this layer (Sadhu et al., 2007).

Steriods

Two milliliter (2 ml) of acetic anhydride was added to 0.5 g of extract and 2 ml of sulphuric acid was added by the sides of the test tube and observed the colour change from violet or blue-green.

Terpenoids (Salkowski test)

To 0.5 g of the extracts, was added 2 ml of chloroform. Then concentration H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoid.

Determination of antimicrobial activity

In this test, two strains of Gram-positive (*Staphylococcus aureus* and *Klebsiella*) and two strains of Gram-negative bacteria (*Escherichia coli* and *Streptococcus*), were used to evaluate the Anti-inflammatory potency. These Microorganisms were sub-cultured properly in nutrient broth and nutrient agar. They were collected from the clinical central laboratory of Rania.

Test microorganisms

For the determination of antimicrobial activity, disc diffusion method is widely acceptable. In this method, antibiotics were diffused from a source through the nutrient agar and a concentration gradient was created. Dried, sterilized filter paper discs (6 mm diameter, HI-Media, China) containing the extracts of known amounts (0.1, 0.2 and 0.4 µg/ml per disc) were applied on nutrient agar medium consistently seeded with the test microorganisms (Pisoschi et al., 2009). Then standard antibiotic of ciprofloxacin (10 µg per disc) and blank discs were used as positive and negative control. For the maximum diffusion of the test materials to the surrounding media, these plates were kept at low temperature (4°C) for about 24 h. Then the plates were incubated (at 37°C) for about 24 h to allow optimum growth of the organisms. The test materials with antimicrobial potency inhibited microbial growth in plates and thereby yielded a clear, distinct zone defined as zone of inhibition (ZOI). Thus, the antimicrobial activity of the extracts was determined correctly by measuring the zone of inhibition expressed in millimeter (Zheng et al., 2001).

Determination of DPPH antioxidant activity

The antioxidant activities of plant extract and the standard antioxidant, Vitamin E were assessed on the basis of free radical scavenging effect of the stable DPPH free radical (Valadez-Vega et al., 2013). Stock solutions (10 mg/ml) of the ethanol, acetone and cyclohexane extracts of *Z. officinale* were prepared in respective solvent systems from which serial dilutions were carried out to obtain concentrations of 3, 6, 12, 25, 50 and 100 µg/ml, respectively. In this assay, an equal amount of sample solution was added to an equal amount of 0.1 mM ethanolic DPPH solution, vortexes and allowed to stand at the dark place at 25°C for 30 min for the reaction to occur (Larson, 1988). After 30 min of incubation period, the absorbance was read against a blank at 517 nm with (Jen way, UK) UV/Visible spectrophotometer. The radical scavenging activity was expressed as the percentage of inhibition (I%) and calculated as per the equation:

$$I\ (\%) = (Abs\ blank - Abs\ sample/Abs\ blank) \times 100$$

Where Abs blank is the absorbance of the control reaction (containing all reagents except the extracts) and Abs sample is the absorbance of the defined concentration of extracts with all reagents.

IC_{50} value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from the plot of inhibition (%) against logarithm of extracts concentration (Gao et al., 2000). All the tests were carried out in triplicate and average of the absorptions was noted. Vitamin E was used as positive control standard for this study (Yu et al., 2005).

RESULTS AND DISCUSSION

The phytochemical screening of *Z. officinale* extracts gave different results as shown in Table 1. Depending on test results, extracts mostly contained alkaloids followed by flavonoids and then saponins.

GS-Spectroscopy

The GC-spectroscopy study of *Z. officinale* has shown

Table 1. Results of phytochemical screening of Zingiber officinale extracts.

Bioactive principles	Ethanol extract of ginger	Cyclohexane extract of ginger	Acetone extract of ginger
Alkaloids	+++	+++	++++
Tannins	++	++	++
Glycosides	++	++	++
Saponins	+++	+++	++
Steriods	+	+	-
Flavonoids	+++	++	+++
Terpenoids	+	+	+
Phlobotannins	+	-	+

Table 2. Extracted compounds of Figure 1, Figure 2 and Figure 3 of ginger extracts.

Ethanol extract			Acetone extract		Cyclo-hexane extract	
Peaks	**compounds**	**RT**	**Compounds**	**RT**	**Compounds**	**RT**
1	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-6-pentyl)	3.37	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-6-pentyl)	3.40	1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl	3.31
2	4-(3-hydroxy-2-methoxyphenyl) butan-2-one	4.13	n-Hexadecanoic acid	5.47	trans-.alpha.-Bergamotene	3.38
3	n-Hexadecanoic acid	5.49	E-1,9-Tetradecadine	6.30	2- butanone, 4-(4-hydroxy-3-methoxyphenyl)	4.08
4	9-Octadecyne	6.33	2- butanone, 4-(4-hydroxy-3-methoxyphenyl)	7.19	Sesquirosefuran	5.47
5	2-Butanone, 4-(4-hydroxy-methoxyphenyl)	6.84	Gingerol	8.03	E-12-Tetradecenal	6.31
6	Butan-2-one 4-(3-hydroxy-2-methoxyphenyl)	7.12	Dihydrocapsacin	8.82	Capsaicin	7.2
7	Capsaicin	7.98	Furan, 2,5-dibutyl	9.11	6-(3,5-Dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one	7.61
8	Bis(2-methylphenylthio)-methane	8.03	4-Hexanoyal resorcinol	10.98	Gingerol	7.95
9	6-(3,5-Dimethyl-furan-2-yl)-6-methyl- hept-3-en-2-one	8.87	Campesterol	12.5	2H,6H-Pyrano[3,2-b]xanthen-6-one, 5,9-dihydroxy-8- methoxy-2,2-dimethyl-7-(3-methyl-2-butenyl)-	8.91
10	4-Hexanoyl resorcenol	9.14	Stigmasterol	Squalene	Squalene	9.06
11	Campesterol	12.52			Propan-2-one, 1-(4-isopropoxy-3-methoxyphenyl)-	10.47
12	Stigmasterol	12.84			5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone(Gingerol	10.97
13					Campesterol	12.49
14					Stigmasterol	12.82

RT: Retention time.

many phytochemicals (Table 2) with peaks (Figures 1, 2 and 3) which contribute to the medicinal activity of the plant. Analysis of the chemical composition of the extract by GC-spectroscopy helped in the identification of components in ethanol extract. The major compounds identified in ginger ethanol extract were Bicyclo [3.1.1]

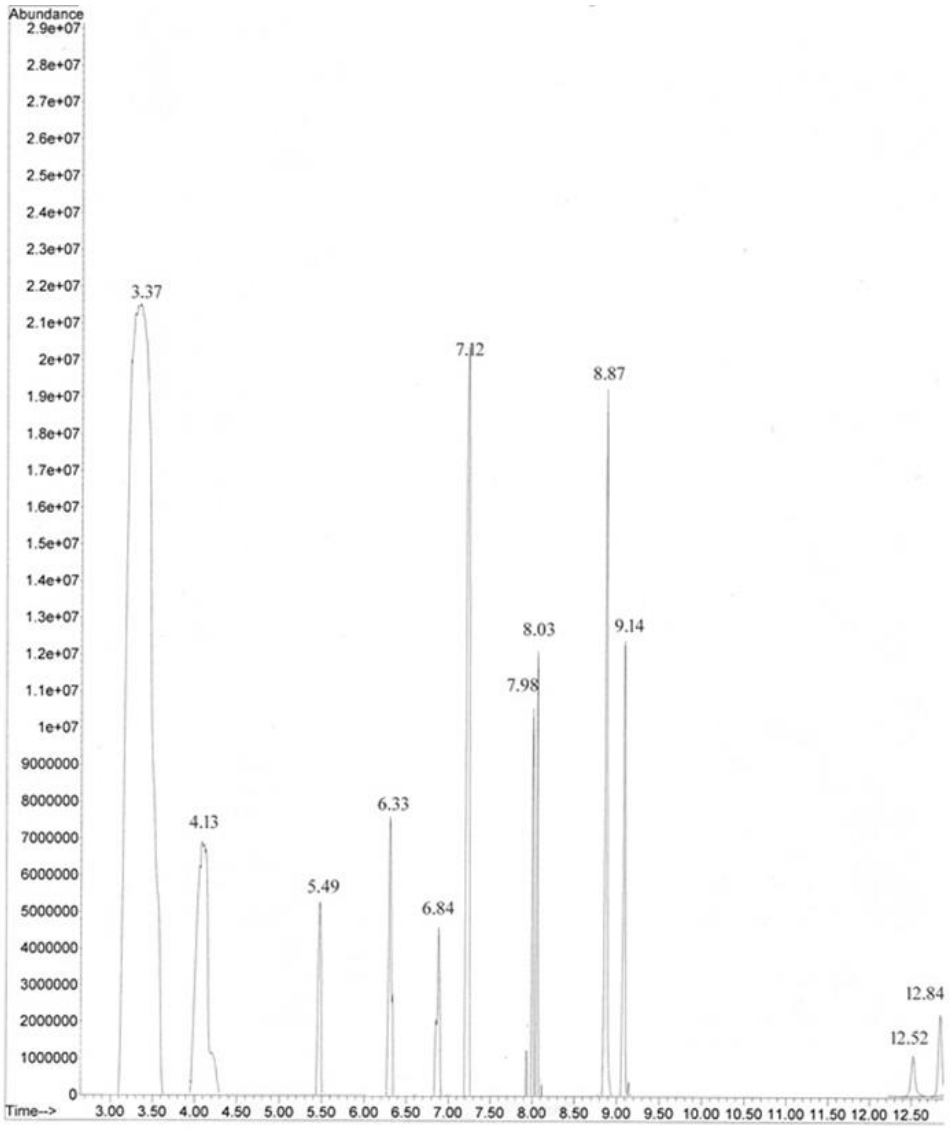

Figure 1. GC-spectroscopy of ethanol extracts.

hept-2-ene, 2, 6-dimethyl-6-(4-methyl-6-pentyl) and 4-(3-hydroxy-2-methoxyphenyl butan-2-one, 9-Octadecyne, 4-Hexanoyl resorcinol. The other compounds were Capsaicin and Campesterol (Figure 1). The acetone extract was subjected to identification of compounds in the extract. The major compounds identified in extract were Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-6-pentyl) and 4-(3-hydroxy-2-methoxyphenylbutan-2-one, Gingerol, Furan, 2,5-dibutyl,4-Hexanoyal resorcinol (Figure 2). The cyclohexane extract was performed for the identification of the compounds in the extract. The major compounds identified in this extract were 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl, trans-.alpha.-Bergamotene, Sesquirosefuran, E-12-Tetradecenal, Gingerol, 2H,6H-Pyrano[3,2-b]xanthen-6-one, 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methyl-2-butenyl) (Figure 3).

Determination of antimicrobial activity

The zones of inhibition observed in the disk diffusion bio assay are shown in Table 3. Plants with an average zone of inhibition in diameter of ≥ 2.8 mm was considered as those recording a significant antimicrobial activity. It indicates that *Z. officinale* has strong antimicrobial activity against all selected organisms. The cyclohexane extract of *Z. officinale* failed to show any activity against the selected bacterial isolates and it showed activity against only Klebsiella.

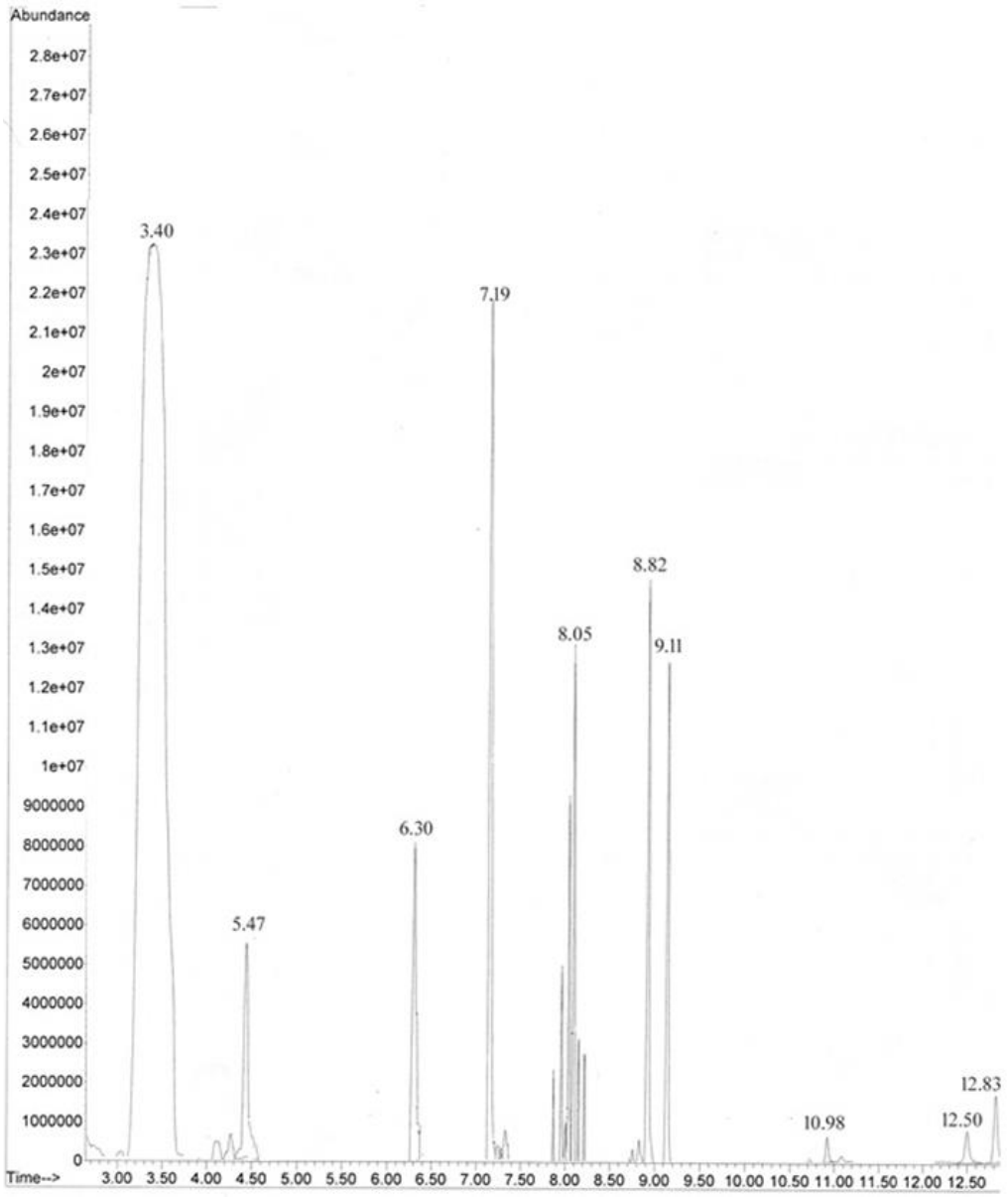

Figure 2. GC-Spectroscopy of acetone extracts.

DPPH- radical scavenging activity

Cancer, malignant tumor or neoplasm is a broad term for a large group of diseases that can affect any part of the body. 8.2 million people worldwide died from cancer in 2012 and 60% of world's total new annual cases occur in Africa, Asia and Central and South America (American Society, 2009). So, cancer and reactive species are causes of many lots of health complication and the pace is increasing in a surprisingly higher level. Studies for new source of antioxidant compounds are the major concern of the time. As certain groups of plant secondary metabolites like tannin, reducing sugar, alkaloid, flavonoid, gum, saponin and steroidal compounds are responsible for some specific pharmacological actions, the cyclohexane, ethanol and acetone extracts of *Z. officinale* were tested to determine whether these definite groups were present in the extract (Khamis et al., 1997). The study demonstrated the presence of alkaloids, tannins, gums, flavonoids and saponins as the major secondary metabolites (Newman et al., 2002). There has been a great deal of interest of late in the role of complementary and alternative drugs for the treatment of various acute and chronic diseases. Among the several

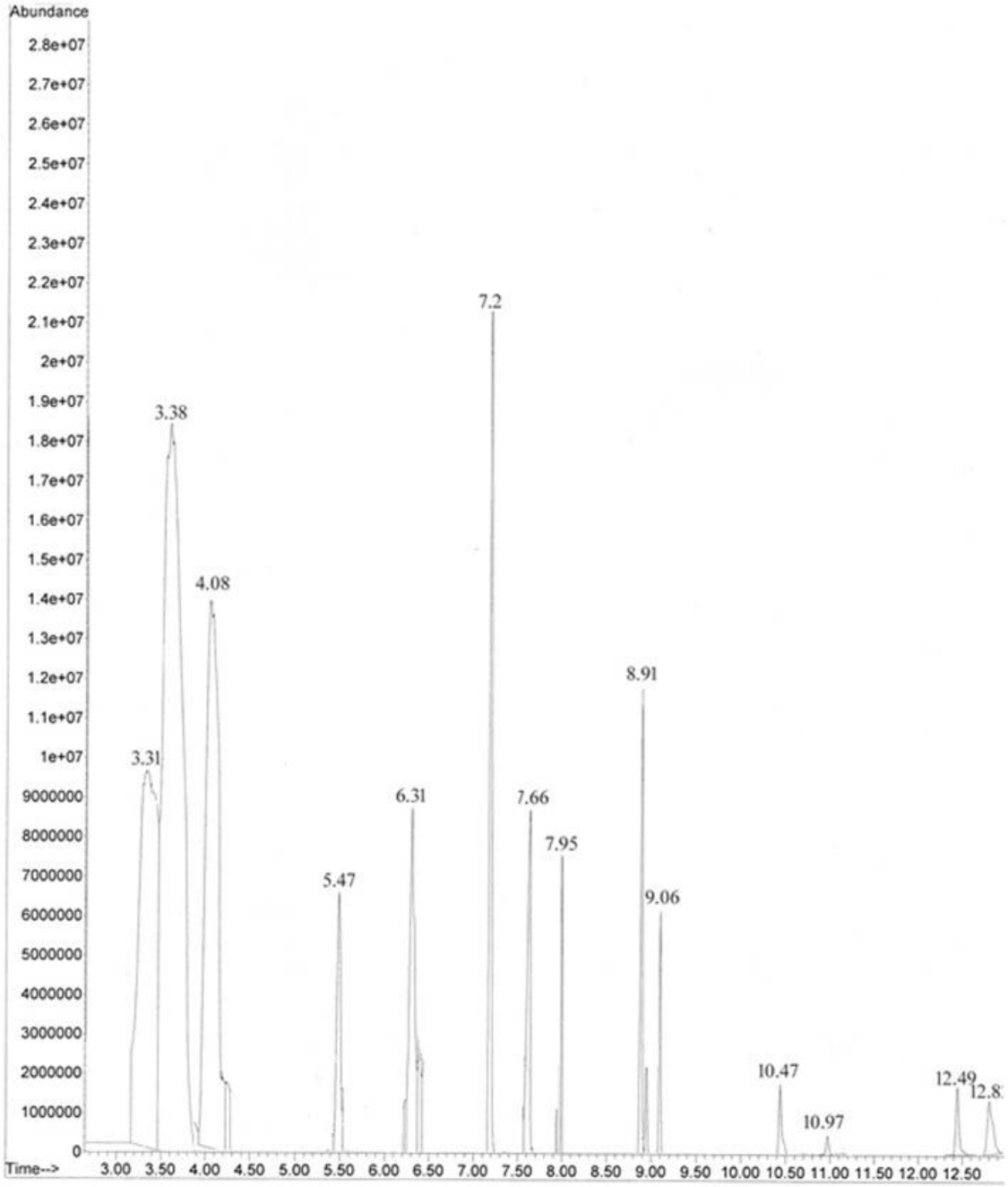

Figure 3. GC- spectroscopy of cyclohexane extracts.

classes of phytochemicals, interest has focused on the antioxidant property of the polyphenols that are found in various botanical agents. Plant vegetables and spices used in folk and traditional medicine have gained wide acceptance as one of the main sources of prophylactic and chemo preventive drug discoveries and development.

In our study to show the antioxidant activity of extracted ginger, the standard (vitamin E) showed IC_{50} 31.62 µg/ml after 30 min. In free radical scavenging assay, IC_{50} values of ethanol, acetone and cyclohexane extracts were 83, 81 and 25.11 µg/ml, respectively in comparison to the standard V. E IC_{50} 31.62 µg/ml. From this study, it was evident that, the ethanol and acetone extracts of *Z. officinale* showed lowest antioxidant activities, while the cyclohexane extract showed the highest and significant

Table 3. Antimicrobial activity extracts from *Zingiber officinale.*

Bactria species		Inhibition zone diameter in mm of Zingiber officinale extracts												Ciprofloxacin
Gram negative bacteria	Gram positive bacteria	Cyclohexane extract (µg/ml)				Ethanol extract (µg/ml)				Acetone extract (µg/ml)				(µg/ml)
		0.4	0.2	0.1	0	0.4	0.2	0.1	0	0.4	0.2	0.1	0	
	Staphylococcus aureus sensitivity ZOI (mm)	0	0	0	0	2.1	1.1	0.5	0	2.3	1.1	0.5	0	0.5
Escherichia														
coli sensitivity ZOI (mm)		0	0	0	0	2.2	2.0	0.3	0	2.1	1.2	0.2	0	1.3
Streptococcus		0	0	0	0	2.5	1.6	0.7	0	3.1	1.1	0.1	0	0.8
	Klebsiella	0.07	0.03	0.025	0	2.0	1.9	1.0	0	2.8	2.6	2.0	0	1.2

ZOI: Zone of inhibition.

Table 4. Radical scavenging activities of *Zingiber officinale* extracts determined by the reduction of DPPH free radical.

Concentration ug/ml	*Zingiber officinale*		Standard vitamin E	
	% inhibition	IC_{50} µg/ml	% inhibition	IC_{50} µg/ml
Cyclohexane extracts				
100	44.93		12.67	
75	40.56		10.15	
50	32.93		9.92	
25	29.12	25.11	8.17	
12	22.8		8.1	
6	13.2		6.8	
3	5.1		4.2	
Ethanol extract				
100	28.17			
75	6.50			
50	4.41			
25	3.78			
12	2.26	83		31.62
6	2.03			
3	1.99			
Acetone extract				
100	57.42			
75	34.71			
50	33.17			
25	31.21	81		
12	27.13			
6	18.60			
3	12.19			

antioxidant activity (Table 4).

Conclusion

Out of the three different solvent extracts of *Z. officinale* for the evaluation of antioxidant activities, ethanol and acetone extracts exhibited the greater antioxidant capacity. The antioxidant activities of medicinal plants may be due to the presence of flavonoids compounds containing the hydroxyl groups that confers the hydrogen

donating ability; the antimicrobial activity of selected plant materials against the above mentioned bacterial pathogens in the laboratory. Again Ethanol and Acetone extracts of *Z. officinale* showed significant inhibitions against all selected organisms, while cyclohexane extract showed insignificant inhibition against selected species. Thus, these products may be useful as potential sources for the future drug development.

Conflict of interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Frooq Emam of Salahaddin University for the corrections made in this paper.

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