OPEN ACCESS

African Journal of **Biotechnology**

11 July 2018
ISSN 1684-5315
DOI: 10.5897/AJB
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

ABOUT AJB

The African Journal of Biotechnology (AJB) (ISSN 1684-5315) is published weekly (one volume per year) by Academic Journals.

African Journal of Biotechnology (AJB), a new broad-based journal, is an open access journal that was founded on two key tenets: To publish the most exciting research in all areas of applied biochemistry, industrial microbiology, molecular biology, genomics and proteomics, food and agricultural technologies, and metabolic engineering. Secondly, to provide the most rapid turn-around time possible for reviewing and publishing, and to disseminate the articles freely for teaching and reference purposes. All articles published in AJB are peer-reviewed.

Contact Us

Editorial Office: ajb@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJB

Submit manuscript online http://ms.academicjournals.me/

Editorial Board

African Journal of Biotechnology

Table of Content: Volume 17 Number 28 11 July, 2018

ARTICLES

Vol. 17(28), pp. 880- 884, 11 July, 2018
DOI: 10.5897/AJB2015.14847
Article Number: E7B1CF157769
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Potential evaluation of *Saccharomyces cerevisiae* strains from alcoholic fermentation of mango pulp

Cosme Damião Barbosa[1*], Inayara Cristina Alves Lacerda[2] and Evelyn de Souza Oliveira[2]

[1]Department of Technology and Inspection of Animal Products (DTIPOA), Veterinary School, Federal University of Minas Gerais, Av. Presidente Antônio Carlos, 6627, Campus Pampulha, Belo Horizonte, 31 270- 90, Brazil.
[2]Departament Food, Faculty of Pharmacy, Federal University of Minas Gerais, Av. Presidente Antônio Carlos, 6627, Campus Pampulha, Belo Horizonte, 31 270-90, Brazil.

Received 9 July, 2015; Accepted 13 October, 2015

The fermentation characteristics of different strains of *Saccharomyces cerevisiae* isolated from sugar cane musts for the production of cachaça (Brazilian sugar cane spirit) and from beer musts were analyzed based on the kinetic parameters of alcoholic fermentation in mango pulp. The commercial pressed baker's yeast was used as a standard inoculum. The results show that the yeast strains tested from sugar cane must and beer must had low fermentation performance when inoculated into mango pulp. They did not meet the selection criteria for fermentative cultures, such as productivity, efficiency, ethanol yield and ethanol production. It is observed that the commercial pressed baker's yeast showed greater adaptability in the mango must than the other yeasts.

Key words: Alcohol fermentation, industrial microbiology, mango, yeast.

INTRODUCTION

Fruit fermented beverages are promising products due to the tendency of their consumer acceptance as showed in researches, and to their contribution to the reduction in postharvest losses of perishable fruits (Sandhu and Joshi, 1995). The consumer market is becoming increasingly exigent about product quality, which places the food industry under pressure with regard to the adequacy and improvement of its products. The search for improvements becomes evident, for instance, in studies that aim to improve yeast strains in order to make the fermentation process more effective and productive. *Saccharomyces cerevisiae* used in the fermentation process to obtain alcoholic beverages must present some essential features, such as high yield, high ethanol tolerance, quick conduction of fermentation in order to prevent contamination by other micro-organisms, balanced production of secondary compounds and to be easily removed at the end of fermentation (Oliveira, 2001).

Oliveira et al. (2005) evaluated different yeast strains for the production of cachaça, a Brazilian sugar cane spirit. From their observations, it was found that there are significant differences between the fermentation potential among different strains of *S. cerevisiae* and that the ethanol yield was the most important factor in the differentiation of the strains. Brazil is the world's largest

*Corresponding author. E-mail: barbosacosme@yahoo.com.br. Tel: 55 31 9228 8302.

producer of fruits and the Northeast region is the country's biggest producer. The northern part of the state of Minas Gerais produced 41,951 tonnes of mangoes of the varieties Palmer and Tommy Atkins. However, several factors act negatively on the flow and efficient allocation of mangoes production and therefore postharvest losses represent a significant portion of this production. Various techniques are being developed and used in order to increase the postharvest life of the fruit as well as to allow its full use. Among these techniques is fermentation, which is a highly viable alternative for developing new products and adding value. It is an efficient and low-cost technology that has become one of the alternatives to the use of the fruits. It also represents a new branch for horticulture industry and a tool for the development of new beverages (Chitarra and Chitarra, 1990; Silva et al., 2007; Asquieri et al., 2008; Banco do Nordeste, 2008).

This research was carried out to evaluate and compare the potential in a laboratory-scale mango pulp fermentation of seven strains of *S. cerevisiae* (LC03, LC06, LC07, LC17, UFMG 1007, UFMG 1031 and UFMG 905) from sugar cane musts for the production of cachaça and from musts used in the production of beer, compared with *S. cerevisiae* cells from commercial pressed baker's yeast.

MATERIALS AND METHODS

Microrganisms and preservation

In the fermentation tests, eight strains of *S. cerevisiae* were used. Among these strains, one was isolated from commercial pressed baker's yeast. The other strains were isolated and identified by the Laboratory of Taxonomy, Biodiversity and Biotechnology of Fungi, Department of Microbiology, Institute of Biological Sciences, UFMG, and coded as LC03, LC06, LC07, LC017, UFMG 905, UFMG 1007 and UFMG 1031. Yeast cells of *S. cerevisiae* from commercial pressed baker's yeast were used as a standard, because they have already shown good fermentation results in fruit musts, such as banana (Lara, 2007; Alvarenga et al., 2011) and mango (Alvarenga et al., 2013) in previous studies conducted in the Laboratory of Industrial Microbiology and Biocatalysis (LAMIB), Department of Food, Faculty of Pharmacy, UFMG. All the yeast strains were maintained in GYMP Broth at -80°C covered with a thin layer of sterilized mineral oil in order to avoid air contact.

Inocula preparation

For the preparation and standardization of inocula, all of the strains were streaked on the surface of the culture medium yeast extract–malt extract - YM agar (malt extract 0.3%, yeast extract 0.3%, peptone 0.5%, 1.0% glucose, 2.0% agar) containing 0.02% of chloramphenicol in a Petri dish. After 48 h incubation at 30°C, the same cultures were resuspended in saline solution (0.85). To standardize the initial number of cells, the parameter used was inocula turbidity determined by reading absorbance at 600 nm with a spectrophotometer (Femto). The suspensions were prepared aseptically with colonies progressively diluted in saline solution until absorbance of 0.7, which corresponds to an inoculum of 10^8 cells per ml.

Must preparation

The mango pulp was enzimatically hidrolized using Pectinex Ultra SP (0,025%) with enzimatic activity of 4000 PG. The must was composed of a 1:1 mixture of sterile distilled water and pulp, adjusted to 18 ° Brix with a solution of commercial sucrose and initial pH value of 4.5. The must was pasteurized (65°C for 30 min).

Laboratory-scale fermentation

The tests were performed in triplicate in 250 ml Erlenmeyer flasks, containing 100 ml of must inoculated with 10 ml of the suspension with the concentration of yeast cells previously standardized. The flasks were incubated in orbital shaker at 30 ± 2°C for 24 h and 150 revolutions for minute. For the analyses, samples of the musts were taken immediately after inoculation and after fermentation. The samples were centrifuged at 1006 *g* for 15 min.

Analytical methods

The final product obtained from fermentation were analyzed for the determination of total reducing sugars (TRS) by the method described by Miller (1959); alcohol content (°GL) with the methodology described by Salik and Povoh (1993); total titratable acidity (TTA), density and pH according to the methodologies of Adolfo Lutz Institute (IAL, 2008).

Calculation of the kinetic parameters

In order to determine the kinetic parameters of fermentation with the use of different strains of the yeast *S. cerevisiae*, the following values were calculated: ethanol yield (%), yeast efficiency, productivity (g. L^{-1}. h^{-1}), the conversion factor of substrate into product ($Y_{p/S}$), rate of substrate consumption (g. h^{-1}) and rate of TRS conversion (%).

Ethanol yield

The ethanol yield was determined by the amount of ethanol formed in relation to the theoretical quantity, through the conversion of sugars present in the must. It obeys the stoichiometry in which the expected amount of ethanol is calculated considering that 1 g of TRS produces 0.511 g of ethanol, expressed in percentage.

Efficiency

The efficiency of the fermentation expresses the ethanol production in relation to the theoretical production according to the content of sugar determined in the must. It determines the efficiency of the conversion of fermentable sugars (TRS) into ethanol, expressed in percentage.

Ethanol productivity

The ethanol productivity expresses the mass of ethanol produced (g) volume (l) in the fermentation medium per unit of

time (h). It allows the determination of the rate of transformation of fermentable sugars (TRS) into ethanol.

Conversion factor of substrate into product ($Y_{p/S}$)

The conversion factor of substrate into product ($Y_{p/S}$) is the relationship between product formation and substrate consumption. It is based on the stoichiometry of the reaction in which 1 g of sugar will produce 0.511 g of ethanol.

Rate of substrate consumption

The rate of substrate consumption determines the rate at which the sugar is consumed by the yeast for the production of biomass and by-products such as ethanol. It is expressed in grams of sugar consumed per unit of time (g. h^{-1}).

Rate of substrate conversion

The rate of TRS conversion determines the amount of reducing sugars consumed in relation to the total of available sugars in the fermentation medium. It is expressed in percentage (%).

Data analysis

The data were statistically analyzed using the Analysis of Variance (ANOVA) followed by the Tukey Multiple Comparison test ($p < 0.05$) using the software SPSS Statistic 19®.

RESULTS AND DISCUSSION

The Table 1 presents the mean of the kinetic and physic-chemical parameters obtained for the fermentation tests. Table 2 presents data comparing three yeasts used in this study with data from the fermentation of banana pulp. The fermentation tests with different strains of *S. cerevisiae* showed low values for the parameters analyzed, except the one from the commercial pressed baker's yeast. The parameter efficiency was not statistically different from the commercial yeast for the strains LC03, LC06, LC07 and LC17. Once the yeasts were not able to fully consume the fermentable sugars by converting them to ethanol, all other parameters that are based on the conversion of substrate (sugar) contained in the must into product (ethanol) were compromised (Table 1). Fermentation carried out with the commercial yeast differed from the fermentation with the other strains of *S. cerevisiae* analyzed, presenting higher rates for ethanol content (5.81 g. L^{-1}), ethanol yield (70.20%), efficiency (92.90%), ethanol productivity (0.24 g. L^{-1}. h^{-1}) , rate of TRS conversion (78.60%) and rate of substrate consumption (0.510 g. h^{-1}). The values obtained were close to those reported by Alvarenga et al. (2011) that examined the potential of different strains of *S. cerevisiae* for the fermentation of banana pulp. The fermentation of mango pulp using the commercial pressed baker's yeast did not differ statistically from the strains LC03, LC06, LC07 and LC17 regarding the conversion factor of substrate into product. The rate of substrate consumption (0.510 g. h^{-1}) differed statistically from other strains, except for the strain UFMG1007. When comparing the rate of TRS conversion, there is no statistical difference between the strains UFMG 1007, UFMG 905 and the commercial yeast.

Oliveira et al. (2001) analyzed the fermentative capacity of different yeast strains on synthetic medium with a glucose concentration of 150 g. L^{-1}. In that study, yeasts were classified into distinct groups based on the analyzed parameters such as ethanol yield (%) rate of TRS conversion (%), efficiency (%) the conversion factor of substrate into product ($Y_{p/S}$), among others. Regarding the $Y_{p/S}$ parameter, the authors classified the yeasts into three groups: very high level when the yeasts presented $Y_{p/S}$ values from 0.491 to 0.510 g. g^{-1}; high level with values from 0.451 to 0.490 g. g^{-1}; and mid-level with $Y_{p/S}$ ranging from 0.420 to 0.450 g. g^{-1}. According to this classification, the $Y_{p/S}$ of the yeasts from the commercial pressed baker's yeast (0.470) used in the present study (0.470) is considered high.

The strains UFMG 905 and UFMG 1007 were also analyzed in other studies. Alvarenga et al. (2011) used these strains for the fermentation of banana pulp (Table 2). Oliveira et al. (2001) used the same strains while analyzing the fermentation characteristics of different yeast strains on synthetic medium containing glucose. Silva et al. (2006) studied the kinetic parameters of these strains of *S. cerevisiae*, including flocculation capacity. studied the production of volatile compounds by these yeasts isolated from cachaça distilleries. Furthermore, Marini et al. (2009) did a comparative study including these strains among others of *S. cerevisiae* as starter cultures for the traditional and industrial production of cachaça. The values for the kinetic parameters found in the present study were lower than those reported by Alvarenga et al. (2011). In general, in both the current study and the one conducted by Alvarenga et al. (2011), the parameters evaluated in the fermentation of banana pulp with yeast strains UFMG 905 and UFMG 1007 were lower when compared to commercial yeast.

The results obtained for $Y_{p/S}$ and ethanol productivity were lower than those reported by Marini et al. (2009) and Silva et al. (2006) for the strains UFMG 1007 and UFMG 905, respectively. For the strain UFMG 1007, the authors reported values of $Y_{p/S}$ of 0.391 ± 0.007 and ethanol productivity of 6.19 ± 0.00 g. $L^{-1}.h^{-1}$ and for the strain UFMG 905, $Y_{p/S}$ of 0.439 and ethanol productivity of 6.85 g. $L^{-1}.h^{-1}$. It is important to emphasize that efficiency takes into account the content of ethanol produced in relation to the amount of TRS consumed, while the yield is calculated by the ratio between the

Table 1. Mean values of physico-chemical and kinetic parameters obtained from fermentation tests.

Strains	Ethanol[1]	TRS f[2]	TA_f[3]	pH_f[4]	Yield[5]	Effic.[6]	Produc.[7]	Y p/s[8]	Conv. s/p.[9]	Conv. TRS
LC03	2.16^{bc}	10.71^{a}	0.28	3.93^{a}	25.70^{c}	74.80^{abc}	0.09^{bc}	0.380^{abc}	0.24^{c}	34.85^{c}
LC06	2.00^{c}	10.62^{a}	0.26^{d}	3.86^{b}	24.12^{c}	71.90^{abc}	0.08^{c}	0.390^{abc}	0.23^{c}	34.72^{c}
LC07	2.33^{bc}	10.81^{a}	0.27^{d}	3.71^{d}	27.27^{bc}	77.90^{abc}	0.10^{bc}	0.400^{abc}	0.25^{c}	35.50^{c}
LC17	2.20^{bc}	11.63^{a}	0.17^{f}	3.69^{d}	25.60^{c}	83.84^{ab}	0.09^{bc}	0.430^{ab}	0.22^{c}	30.83^{c}
UFMG 1007	2.93^{b}	6.11^{bc}	0.23^{e}	3.60^{e}	35.23^{b}	56.50^{bc}	0.12^{b}	0.290^{bc}	0.42^{ab}	62.43^{ab}
UFMG 905	2.21^{bc}	7.34^{b}	0.34^{a}	3.77^{c}	26.50^{c}	48.95^{c}	0.09^{bc}	0.250^{c}	0.38^{b}	55.05^{ab}
UFMG1031	2.60^{bc}	7.00^{b}	0.29^{c}	3.87^{b}	31.12^{bc}	55.05^{bc}	0.10^{bc}	0.280^{bc}	0.38^{b}	56.90^{b}
Commercial yeast	5.81^{a}	3.94^{c}	0.31^{b}	3.69^{d}	70.20^{a}	92.90^{a}	0.24^{a}	0.470^{a}	0.51^{a}	78.60^{a}

Means followed by the same letters in the same column do not differ by Tukey multiple comparison test ($p < 0.05$); 1, Ethanol (g; L^{-1}); 2, final content of total reducing sugars (g; L^{-1}); 3, titratable acidity (g; L^{-1}); 4, hydrogenic potential (pH); 5, yield in ethanol (%); 6, efficiency (%); 7, productivity in ethanol (g; L^{-1} h^{-1};); 8, conversion factor of substrate into product; 9, rate of substrate conversion (g; h^{-1}); 10, rate of TRS conversion (%).

Table 2. Comparison of parameters: ethanol production, efficiency and ethanol yield of fermentations conducted with the strains UFMG 905, UFMG 1007 and commercial pressed baker's yeasts compared with existing data on the fermentation of banana pulp.

Strains	Parameters					
	Ethanol (g. L^{-1})		Efficiency (%)		Yield in ethanol (%)	
	Banana pulp	Mango pulp	Banana pulp	Mango pulp	Banana pulp	Mango pulp
UFMG 905	5.64	2.21	76.82	48.95	73.69	26.46
UFMG 1007	5.68	2.93	76.53	56.74	73.90	35.23
Commercial yeast	7.84	5.81	96.41	92.89	94.06	70.17

ethanol obtained and the ethanol expected considering the initial TRS content in the must. This explains why the efficiency values are higher than yield values. The parameters ethanol production, efficiency and ethanol yield in fermentations conducted with the strains UFMG 905 and UFMG 1007 in this study were lower than the ones reported by Alvarenga et al. (2011). These results may be due to differences between the musts since the author used banana musts in his tests, while in this study the mango must was used. However, the fermentation conducted with commercial pressed baker's yeast showed similar values to those reported by the author.

The yield is based on the hourly quantity of ethanol produced, reflecting directly on the efficiency of the yeast in transporting the carbohydrates found in the must (glucose, fructose, sucrose and others) to the interior of its cell. Alvarenga et al. (2011) points out that productivity is excellent aspect for selection of yeasts, the higher the values, the more suitable the yeast is for the fermentation process. The strains from sugar cane must and beer must used in the present study had low fermentation performance when inoculated into mango pulp and did not meet the selection criteria for fermentative cultures, such as productivity, efficiency, ethanol yield and ethanol production. The results showed that the commercial pressed baker's yeast had greater adaptability in mango must than the other strains. Due to this fact, the commercial pressed baker's yeast was selected for obtaining mango wine.

CONFLICT OF INTERESTS

The authors declared that there have no conflict of interests.

ACKNOWLEDGMENTS

The authors give thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG) for the financial support.

REFERENCES

Alvarenga LM, Alvarenga RM, Dutra MBL, Oliveira ES (2013). Avaliação de aguardente de banana e manga. Alimentos e Nutrição 24(2):195-201.

Alvarenga RM, Geocze AC, Silva CM, Oliveira ES (2011). Potential application of *Saccharomyces cerevisiae* strains for the fermentation of banana pulp. African Journal of Biotechnology 10:3608-3615.

Asquieri ER, Rabelo AMS, Silva AGM (2008). Fermentado de jaca: estudo das características físico-químicas e sensoriais. Ciência e Tecnologia de Alimentos 28(4):881-887.

Banco do Nordeste (2008) Disponível em:< http://www. bnb. gov. br>. Acesso em 13.

Lara CA (2007). Produção de aguardente de banana: emprego de enzimas pectinoliticas e efeito de fontes de nitrogênio e quantidade de inóculo na formação de álcoois superiores. Dissertation in Food Science, Federal University of Minas Gerais P 74.

Marini MM, Gomes FCO, Silva CLC, Cadete RM, Badotti F, Oliveira ES, Cardoso RC, Rosa CA (2009). The use of selected starter *Saccharomyces cerevisiae* strains to produce traditional and industrial cachaça: a comparative study. World Journal of Microbiology and Biotechnology 25:235-242.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31(3):426-428.

Oliveira ES (2001). Características fermentativas, formação de compostos voláteis e qualidade da aguardente de cana obtida por linhagens de leveduras isoladas de destilarias artesanais. Tese (Doutorado em Tecnologia de Alimentos), Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas (UNICAMP) Campinas.

Oliveira ES, Cardello HM, Jeronimo EM, Souza EL, Serra GE (2005). The influence of different yeasts on the fermentation, composition and sensory quality of cachaça. World Journal of Microbiology and Biotechnology 21(5):707-715.

Vol. 17(28), pp. 885-891, 11 July, 2018
DOI: 10.5897/AJB2017.16112
Article Number: DEEF4BC57771
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effects of aqueous seed extracts of *Mucuna sloanei* (Fabaceae) on body weight and some biochemical parameters of *Rattus novergicus*

Ugwu, Godwin C.[1*], Ejere, Vincent C.[1], Okanya, Chinagorom L.[1], Omeje, Joy N.[2], Egbuji, Jude V.[1], Onu, Martina C.[3] and Chukwuka, Christian O.[1]

[1]Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.
[3]Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Received 14 June, 2017; Accepted 14 December, 2017

Mucuna sloanei **is an annual leguminous plant widely used among the various ethnic groups in Nigeria. The effects of aqueous *M. sloanei* seed extract on the body weight and some biochemical parameters of 48 normal male *Rattus novergicus* (albino rats) were investigated for 28 days. The rats were divided into control group (A) which received distilled water and treatment groups (B, C and D) that received oral administration of 100, 200 and 400 mg/kg body weight of the seed extract, respectively. Each group was further divided into three replicates of four rats each. Blood samples were collected before the experiment started (week 0) and at weekly interval from one rat per replicate. The biochemical profiles were determined using bioassay. The lethal dose (LD_{50}) of the aqueous seed extracts of *M. sloanei* may be above 5000 mg/kg, since no death occurred at that dose. The overall change in body weights of treated rats did not differ significantly (P>0.05) from those of the control and were not dependent on treatment duration. However, there was a significant decrease (P<0.05) in alanine aminotransferase (ALT) level at the lowest dose of 100 mg/kg when compared with the control. Also, there was no significant difference (P>0.05) in the mean values of AST from weeks 1 to 4 when compared with the control except at the dose level of 400 mg/kg which showed a significant decrease (P<0.05) at week 4. Similarly, a significant decrease (P<0.05) was observed in the mean serum urea at the dose levels of 100 and 200 mg/kg and BUN at 200 and 400 mg/kg at week 1, and creatinine at dose levels of 200 and 400 mg/kg in the third week of administration when compared with the control. This study indicates that the aqueous *M. sloanei* seed extract could have some hepato and nephro-protective properties.**

Key word: *Mucuna sloanei*, aqueous seed extracts, liver markers, kidney markers, albino rats.

INTRODUCTION

Mucuna sloanei, commonly called the 'Horse-eye' or 'Hamburger' bean, is an annual leguminous plant widely used among the various ethnic groups in Nigeria (Obute, 2010). It is known in various places as, 'ukpo' by the Ibos; 'karasuu' by the Hausas; 'yerepe' by the Yorubas (Nwosu, 2011) and 'ibabat' by the Efiks of Nigeria (Obochi et al., 2007). The seeds are used as source of vegetable oil, condiment or thickener of soup by Igbo communities in sub-Saharan Africa (Afolabi et al., 1985; Ukachukwu et al., 2002). Ukachukwu and Obioha (1997) stated that some rural populations of Nigeria consume seeds of *M. sloanei* during the period of scarcity of other

common legumes. The *M. sloanei* seeds have protein, carbohydrate, crude fat and fiber contents (Akpata and Miachi, 2001) as well as a very rich amino acid content (Ojiako et al*.,* 2012). They also contain many important bioactive substances such as L-3, 4-dihydroxyphenylalanine (L-DOPA) (Rai and Saidu, 1977; Adebowale et al*.,* 2005), which has been reported to be a potent precursor of the brain neurotransmitter, dopamine (Hornykiewicz, 2002; Kostrzewa et al*.,* 2005*;* Nagatsua and Sawadab, 2009). They have also been reported to contain important phytochemicals such as alkaloids, phytic acid, tannins, flavonoids, haemoglutinin and oligosaccharides (Obute and Adubor, 2007). Similarly, lectin from *M. sloanei* seeds has been reported to have an effective and suitable cell receptor signal inducer due to its ability to agglutinate blood cells of humans, goat, cow and chicken (Obochi et al., 2007). Whereas the Efiks in Nigeria claim that the consumption of seeds of *M. sloanei* lowers libido in men (Obochi et al., 2007), a recent study has shown that the methanolic extract of *M. sloanei* seeds has positive effects on sex hormones and sperm count in males (Egwurugwu et al*.,* 2012). Nutritional and anti-nutritional characteristics and metabolisable energy of *M. sloanei* seeds has also been reported (Ekwe et al., 2016). Ejere et al. (2015) evaluated the effects of aqueous extracts of *M. sloanei* seed on haematological parameters of normal Wistar rats and discovered that the plant seed has no harmful effects on the haematological parameters investigated.

Despite the aforementioned medicinal values of this important food condiment, there is paucity of information regarding its effects on liver and renal function of experimental animals as well as the possible risks associated with its consumption by humans. The present study was therefore initiated to provide information on the effects associated with the oral consumption of aqueous extracts of shade dried de-hulled *M. sloanei* (Fabaceae) on body weight and some biochemical profile of albino rats (*Rattus novergicus*).

MATERIALS AND METHODS

Collection and preparation of *M. sloanei* crude seed extract

Dried and mature nuts of *M. sloanei* were purchased from local markets around Nsukka metropolis. The seeds were identified at the herbarium of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, and a voucher specimen (UN/PCOG/12/1164) was deposited in the same department. They were de-hulled, dried at room temperature and pulverized into fine powder using a milling machine. The method of extraction followed that of Akintayo et al. (2000). A total of 100 g of the powdered sample was introduced into 2000 ml flat bottom flask and 1500 ml of distilled water was added. The content was mixed thoroughly and left for about 24 h with an occasional shaking to increase the extraction capacity. Thereafter, the soaked substance was filtered with a muslin clothe (number 60 mesh size) and concentrated to dryness. The solid extract was weighed and re-dissolved in normal saline according to the body weights of the animals for oral administration.

Procurement and management of experimental animals

Forty-eight adult male albino rats weighing 167.3 to 189.0 g were obtained from Genetics and Animal Breeding Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats had no history of drug consumption. They were kept in stainless wire-rat cages equipped with drinkers and fecal collecting trays, in a clean and fly proof experimental animal house. The rats were fed commercial growers chick mash (18% crude protein) made by Vital Feeds Nigeria Limited and clean drinking water, and acclimatized for 14 days before the start of the experiment. All the animals were maintained under the standard laboratory condition for temperature, humidity and light throughout the experiment and were allowed free access to food and water. The fecal droppings in the tray were removed daily. The experimental rats were handled in strict compliance with international guidelines as prescribed by the Canadian Council on the Care and Use of Laboratory Animals in Biomedical Research (1984).

Experimental design

The procured rats were assigned into four groups (A, B, C and D) of 12 rats per group. Each group was further replicated 3 times comprising of 4 rats each. The rats in group A (Control) were fed normal rat feed and 1 ml/kg body weight of normal saline *ad libitum*. On the basis of the toxicity result, the treatment groups B, C and D were administered in addition to the normal rat feed and water, 100, 200 and 400 mg/kg body weight of the aqueous seed extract, respectively. All the doses were administered once daily orally for 28 days (four weeks) for all the groups using 1 ml syringe without needles.

Collection of blood sample

About 5 ml of the blood samples was collected from each of the anaesthetized rats using the retro orbital plexus as described by Hoff (2000) and allowed to clot for about 30 min and centrifuged at 2000 rpm for 10 min. This was done at baseline (Week 0) and at weekly intervals during treatment (weeks 1 to 4).

Determination of body weights

The body weights of the individual rats were determined before the beginning of the experiment (day 0) and subsequently during treatment before collection of blood samples on days 7, 14, 21 and 28 using an electronic balance (Metller, PC 2000).

Determination of LD_{50}

This was determined according to the method of Lorke (1983). Three groups (A, B and C) of 3 mice each were used for this

*Corresponding author. E-mail: godwin.ugwu@unn.edu.ng. Tel: +234 8064005944.

Table 1. Effects of different treatments of the aqueous seed extract of *M. sloanei* on body weight, BW (g) of albino rats.

Concentrations (mg/kg)	Duration (days)				
	0	7	14	21	28
Control	181.7±11.85^{a1}	197.7±19.34^{a1}	213.0±16.09^{a1}	213.3±20.67^{a1}	244.7±21.06^{a1}
100	167.3±6.70^{a1}	185.0±11.59^{a1,2}	203.0±9.64^{a,2}	207±19.43^{a,2}	236.0±18.88^{a,3}
200	189.0±15.10^{a1}	227.0±32.90^{a1}	220.0±23.76^{a1}	234.3±17.03^{a1}	210.7±19.41^{a1}
400	181.3±16.37^{a1}	205.7±20.54^{a1}	218.7±23.92^{a1}	220.0±12.34^{a1}	236.0±32.30^{a1}

Values with different alphabetic (lower case) superscripts differ significantly (P<0.05) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly (P<0.05) between different exposure periods within the same concentration.

experiment. The plant seed extract dissolved in normal saline (0.85 g/mol) was orally administered to the mice in doses of 1000, 3000 and 5000 mg/kg in groups A, B and C, respectively. The number of animals dead within 24 h after oral administration was recorded for each group. The lethal dose was calculated as the arithmetic mean of the dose that killed the least number of animals and the one next to lower dose that did not kill any animal.

Determination of biochemical parameters

Alanine transaminase (ALT) and aspartate aminotransferase (AST) activities were determined using the standard method described by Reitman and Frankel (1957). The blood urea, blood urea nitrogen (BUN) and creatinine (CREAT) levels were determined according to the methods of Weatherburn (1967), Bartels and Bohmer (1972) and Kaplan (1965), respectively.

Ethical approval

The experimental animals were handled in strict compliance with international guidelines as prescribed by the Canadian Council on the Care and Use of Laboratory Animals in Biomedical Research (1984).

Statistical analysis

Data accumulated was analyzed using the GENSTAT (VSN International, Hemel Hempstead, Herts, UK). One-way ANOVA was used to test the effect of treatment and two-way ANOVA was used to determine the interactive effects of treatment and duration. Fisher's least significant difference (F-LSD) was used in the separation of means of the different treatment groups. All results were expressed as mean ± standard error of mean (SEM), while values were considered significant at P< 0.05.

RESULTS

Acute toxicity test

The oral LD_{50} of the aqueous seed extract in the rats showed no mortality at the different doses of 1000, 3000 and 5000 mg/kg. However, in the rats administered 5000 mg/kg dose, physiological side reactions such as shivering, bulging of eyes and dullness were observed.

Effects of aqueous extracts of *M. sloanei* on body weight of albino rat

Table 1 shows the weekly effects of the seed extracts of *M. sloanei* on the body weight (BW) of the albino rats. There was no overall significant difference (P>0.05) in the BW of the treated rats when compared with the control. It was also observed that this non-significant difference (P>0.05) was independent of the dosage and duration of treatment. However, the body weights of the treated animals increased minimally from the value at day 0 as the duration of treatment increased, while that of the rats administered 100 mg/kg increased significantly (P<0.05) from days 14 to 28 when compared with the control.

Effects of aqueous extracts of *M. sloanei* on ALT and AST of albino rats

The results of the weekly effects of the aqueous seed extracts of *M. sloanei* on some hepatic enzymes of the albino rats are presented in Table 2. There was no significant difference (P>0.05) in overall dose and duration in the serum ALT and AST levels of the rats administered the various doses when compared with the control. However, wavelike variations were noticed in the serum activities of both enzymes among the dose levels in some weeks. Whereas the ALT serum levels of the rats administered 200 and 400mg/kg significantly increased (P<0.05) in days 21 and 28, the serum AST levels of the rats that received 200 and 400 mg/kg significantly decreased (P<0.05) in day 28 from the value in day 21 when compared with the control.

Effects of aqueous extracts of *M. sloanei* on kidney markers of albino rats

The results of the analyses carried out on the blood samples obtained from adult albino rats for the determination of urea, blood urea nitrogen (BUN) and creatinine levels before and on weekly intervals during the seed extract administration are shown in Table 3.

Table 2. Effects of different treatments of the aqueous seed extract of *M. sloanei* on ALT and AST of Albino rats on weekly basis.

Parameter	Treatment (mg/kg)	Duration (days)				
		0	7	14	21	28
ALT (U/L)	Control	41.3±3.32^{a1}	58.0±8.02^{a1}	65.0±4.73^{a1}	72.7±5.78^{a1}	87.7±5.36^{b1}
	100	49.9±7.51^{a1}	45.7±9.13^{ab1}	65.7±7.36^{a1}	62.7±6.77^{a1}	61.3±8.57^{a1}
	200	40.6±0.67^{a1}	39.7±5.67^{a1}	49.3±4.41^{a1}	89.3±7.54^{a2}	90.0±1.53^{b2}
	400	46.4±1.16^{a1}	65.7±8.41^{b1}	51.7±5.61^{a1}	77.7±11.68^{a2}	81.3±7.84^{ab2}
AST (U/L)	Control	11.93±1.17^{a1}	20.17±3.44^{a1}	24.43±0.47^{a1}	32.00±3.04^{a1}	40.67±9.24^{b1}
	100	19.13±0.32^{b1}	15.33±2.49^{a1}	21.83±4.48^{a1}	25.00±2.57^{a1}	24.67±2.59^{ab1}
	200	12.60±1.55^{a1}	13.00±5.80^{a1}	18.33±4.28^{a1}	43.17±6.34^{b2}	32.17±4.92^{ab1}
	400	10.27±0.09^{a1}	18.17±3.61^{a1}	19.33±3.01^{a1}	30.33±4.21^{a2}	21.33±3.84^{a1}

Values with different alphabetic (lower case) superscripts differ significantly (P<0.05) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly (P<0.05) between different exposure periods within the same concentration. Results are expressed as Mean ± SEM.

Table 3. Effects of the aqueous seed extract of *M. sloanei* on some nephrotic enzymes of Albino rats on weekly basis.

Parameter	Treatment (mg/kg)	Duration (days)				
		0	7	14	21	28
UREA (mg/dl)	Control	21.2±0.44^{b1}	26.5±2.01^{b1}	35.0±7.88^{a1}	42.1±5.63^{a1}	34.2±5.21^{a1}
	100	20.7±0.30^{ab3}	18.1±1.32^{a4}	36.5±9.50^{a2}	40.9±8.31^{a2}	32.9±1.66^{a23}
	200	19.2±0.19^{a1}	20.9±0.89^{a1}	30.0±3.79^{a1}	39.5±6.20^{a2}	26.8±3.11^{a1}
	400	20.1±0.29^{a1}	28.5±2.46^{b1}	35.2±6.42^{a2}	32.6±2.40^{a1}	29.3±3.50^{a1}
BUN (mg/dl)	Control	11.93±1.17^{a1}	20.17±3.44^{a1}	24.43±0.47^{a1}	32.00±3.04^{a1}	40.67±9.24^{b1}
	100	9.93±0.20^{ab1}	12.37±0.94^{a12}	16.33±3.69^{a2}	19.97±2.90^{a2}	16.00±2.43^{a2}
	200	10.20±0.66^{b1}	8.47±0.61^{b1}	17.00±4.41^{a2}	19.73±3.21^{a2}	15.37±0.77^{a2}
	400	8.97±0.07^{a1}	9.77±0.42^{b1}	14.00±1.77^{a1}	19.27±3.72^{a2}	12.57±1.45^{a1}
CREAT (mg/dl)	Control	2.30±0.40^{a1}	0.28±0.12^{ab1}	0.67±0.24^{a1}	2.46±0.96^{b1}	0.68±0.27^{a1}
	100	2.37±0.70^{a1}	0.24±0.11^{ab1}	0.50±0.30^{a1}	2.50±0.57^{b1}	0.90±0.85^{a1}
	200	2.60±0.21^{a1}	0.43±0.12^{a1}	0.42±0.29^{a1}	0.27±0.12^{a1}	6.30±4.14^{b2}
	400	2.90±0.79^{a12}	0.09±0.03^{b1}	1.03±0.49^{a12}	0.36±0.22^{a1}	4.07±2.52$^{b2\ 3}$

Values with different alphabetic (lower case) superscripts differ significantly (P<0.05) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly (P<0.05) between different exposure periods within the same concentration. Results are expressed as Mean ± SEM.

There was no significant difference (P>0.05) in overall dose dependence observed in the serum urea, blood urea nitrogen and creatinine levels in all the weeks when compared with the control. However, some forms of minimal variations were observed at certain dose levels of the nephrotic enzymes on a weekly basis. Whereas a significant decrease (P<0.05) was obtained in the serum urea levels of the rats at dose levels of 100 and 200 mg/kg in week 1, the effects of duration of treatment of the varying doses on the mean urea values showed a marked decline in the fourth week of treatment. In the same vein, a significant decrease (P<0.05) in the BUN levels was observed in the rats given the dose level of 100 mg/kg in week 1 when compared with the control. The effects of duration of treatment of the varying doses on the mean BUN values of those animals treated with aqueous *M. sloanei* seed extracts also showed a marked decline in the fourth week of treatment. Similarly, a significant decrease (P<0.05) was observed in the serum levels of creatinine at the dose levels of 100 and 400 mg/kg in week 3 when compared with the control. The effects of duration of treatment of the varying doses on the mean creatinine values of the animals treated with 200 and 400 mg/kg also exhibited some fluctuations from weeks 1 to 4.

DISCUSSION

The possible effect of aqueous extracts of *M. sloanei*

seed on body weights and some biochemical parameters of normal albino rats were investigated for a total of 28 days. LD_{50} of aqueous *M. sloanei* seed extract showed that although at a dosage of 5000 mg/kg no death occurred, though cage side behaviors such as shivering, bulging of eyes as well as dullness were observed in the rats. This present report suggests that the extract was tolerated and is therefore relatively safe for consumption. This observation corroborated that of Ejere et al. (2015) who observed no lethal effects of *M. sloanei* aqueous extract on albino rats after 24 h at a high dose of 5000 mg/kg. Contrarily, it disagreed with Egwurugwu et al. (2012) who recorded lethal effects of the methanolic *M. sloanei* seed extract at a dose level of 3,872.98 mg/kg in experimental rats. This discrepancy aptly calls for more studies to fully unravel the true identity of the *Mucuna* species involved.

Data obtained in the present work showed that the aqueous *M. sloanei* seed extracts exhibited a dose and duration independent non-significant difference in the body weights of the treated animals in all the weeks (Table 1). In addition, there was a progressive minimal non-significant increase in the body weight of the rats administered the various doses of the extract as the duration of treatment progressed. The absence of a significant effect on body weights of the animals is an indication that the extract did not adversely affect the body size of the animals and as such may not be considered a good anti-obesity agent (Ashafa et al., 2011). On the other hand, the ability of the seed extract to minimally increase the body weights of the treated animals must be taken seriously. This is indicative of the seed extract's tendency to cause increases in weight with increasing dosage and duration of administration. Therefore, there is need to further investigate the safe concentration levels of this important food condiment in Nigeria as several studies have indicated the possibility that high doses of plant extracts could lead to life threatening public health diseases (Ene-Ojo et al., 2013; Ijeh and Agbo, 2006; Ijeh and Ukweni, 2007; Mehrdad et al., 2011).

The importance of blood chemistry profiles in relation to nutrient intake has been reported (Church et al., 1984). Serum levels of ALT and AST which are the two diagnostically important transaminases have not only been used as good bio-indicators of the functionality and cellular integrity of the liver but as well, to assess the functional health status and the internal environment of the organism (Rehman et al., 2006; Sood, 2006; Lavanaya et al., 2011). Normally, an elevation in their serum levels may be indicative of an inflammation or damage to the hepatocytes or liver dysfunction (Edwards et al., 1995; Sood, 2006) especially whenever the liver undergoes such pathological conditions as cirrhosis or subjected to abnormal onslaught that accompany the presence of toxins or usage of some drugs (Nyblom et al., 2004; Crook, 2006). The fact that the extract had no significant effect on the serum levels of these liver marker enzymes (Table 2) is an indication that it had no negative interaction on the hepatocytes and as such did not increase the activities of the lysosomes. In addition, the extract interaction with the animal appeared to have not caused any damage to the mitochondria nor did it affect the membrane permeability of the liver cells, thus not inducing any form of damage to the morphology of the liver (Crook, 2006). This view corroborates an earlier one on another *Mucuna* species at Nsukka. Odoh and Osadebe (2010) reported that aqueous extracts of *Mucuna flagellipes* seeds did not have any significant effect on the serum chemistry of albino rats. Nevertheless, the extract's ability to cause minimal non-significant increases in the serum levels of both enzymes is very instructional. This may also be an indication of the extract's potentiality to gradually manifest its harmful tendencies in a dose and duration dependent fashion. This is believable because the difference in value between the observed least ALT (39.7±5.67 U/L) and the highest (90.0±1.53 U/L) level in the treated rats was greater than the normal ALT range (10 to 40 U/L) in man (Chernecky and Berger, 2008). Similarly, the observed difference in value between the least (13.00±5.80 U/L) and highest serum AST (43.17±6.34 U/L) level was greater than the AST range (14 to 20 U/L) obtained for human males (Chernecky and Berger, 2008).

The minimal non-significant reduction in AST activity at week 4 in all the treatment groups is nonetheless very important. This may be an evidence of the extract's ability to improve hepatic functions following prolonged administration or, it could be an indication that the effect of the extract may be self-limiting. On the other hand, it may be that the extract is metabolizable into less toxic substances by the liver or as a result of the extracts high content of bioactive constituents like flavonoids which have been reported to have anti-oxidative effects (Middleton, 1996).

Furthermore, many dietary supplements may not be harmful; some have been associated with nephrotoxicity while others have the potential to do so (Thomson et al., 2002). Usually, an elevation in serum urea and BUN levels presupposes renal dysfunction as a result of kidney damage. The observed lack of overall dose dependent significant difference in the serum urea and blood urea nitrogen (BUN) levels (Table 3) when compared with the control, suggested that the *M. sloanei* seed extract was not harmful to the kidney function, but may in some degree confer positive effect on waste excretion in the rats. It seems plausible that the seed extract on reaching the end tract of the collecting tubules minimally decreased urea re-absorption. Similarly, it is possible that there was no increased tissue protein catabolism or excess breakdown of blood protein resulting in increased urea excretion by the kidney (Nduka, 1999; Adepoju and Odubena, 2009). Since urea is the major nitrogen-containing metabolic product of

protein catabolism, the significant reduction in the mean serum urea at the dose levels of 100 and 200 mg/kg and BUN at 200 and 400 mg/kg of *M. sloanei* at week 1 may be attributed to an impairment in the urea cycle leading to reduced production of the metabolic product (Yakubu et al*.,* 2003). This is indicative of an abnormality in the physiological excretion of urea caused by a non-renal factor which is the seed extract in this study. This observation corroborates the findings of past reports using other *Mucuna* species. Adepoju and Odubena (2009) working on *Mucuna pruriens* reported a significant decrease in the serum urea levels of rats fed different doses of the plant extract. Odoh and Osadebe (2010) also observed that the aqueous seed extracts of *M. flagellipes* had no significant effect on the serum chemistry of albino rats when compared with the control.

Creatinine is a major catabolic product of protein metabolism in the muscle tissue usually excreted by the kidneys. Serum urea is also used as an indicator of the renal functional ability (Aliyu et al., 2006). The lack of an overall dose dependent significant difference coupled with a dose independent significant decrease in the serum creatinine levels at 200 and 400 mg/kg in the third week of administration when compared with the control suggests that the aqueous extract may have therapeutic effects on the renal system. That is, the *M. sloanei* seed extract did not impair the functioning renal tubular mass vis-à-vis its regulatory functions. Nevertheless, the observed sudden sharp increases in the fourth week of the rats administered 200 (6.30±4.14 mg/dl) and 400 mg/kg (4.07±2.52 mg/dl) far above the serum creatinine value (0.5 to 1.3 mg/dl) for humans (Mehrdad et al., 2011) is very worrisome. Although, an experimental default in the analysis is strongly suspected, it may nonetheless tend to suggest the possibility of some kinds of renal toxicity which may be exacerbated with increase in the dosage and duration of administration. However, since several studies (Ene-ojo et al*.,* 2013; Ijeh and Agbo, 2006; Ijeh and Ukweni, 2007; Mehrdad et al*.,* 2011) have indicated the possibility that the usage of high doses of plant extracts could lead to acute renal failure, there is need to further investigate safe concentration levels of this important food condiment in Nigeria.

Conclusion

Conclusively, in as much as the domestication and utilization of underutilized legumes as inexpensive and elegant source of protein is commendable in developing countries, care must be taken in their consumption. Data collated and reported in the present study tend to show that moderate consumption of *M. sloanei* seeds could be beneficial to human health. Although, the seed extract could have hepato-protective ability in the long run, it is doubtful if it can be a good anti-obesity agent. It is also noteworthy that its nephro-protective ability may be fully realized when moderately consumed. This calls for further studies to fully harness the beneficial properties of this food condiment which is a delicacy in most homes in developing countries. Nevertheless, further work should be done to ascertain the safe concentration of this important food condiment for consumption in Nigeria following prolonged administration. This will go a long way to assess its stability and suitability in clinical trials.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adebowale YA, Adeyemi A Oshodi AA (2005). Variability in the physicochemical, nutritional and anti-nutritional attributes of six *Mucuna* species. Food Chemistry 89(1):37-48.

Adepoju GKA, Odubena OO (2009). Effects of *Mucuna pruriens* on some haematological and biochemical parameters. Journal of. Medicinal Plants Research 3(2):73-76.

Afolabi OA, Oshuntogun BA, Adewusi SR, Fapojuwo OO, Ayorinde FO, Grisson FE, Oke OL (1985). Preliminary nutritional and chemical evaluation of raw seeds from *Mucuna solanei*: An underutilized food source. Journal of Agricultural and Food Chemistry 33:122-124.

Akintayo ET, Oshodi AA, Adebowale KO (2000). Physicochemical Properties of Lima bean *(Phaseolus lunatus*) unmodified and modified starches. Nigeria Journal of Science 34(2):187-193.

Akpata AO, Miachi EU (2001). Chemical composition and selected functional properties of sweet oranges and legumes flours. Plant Foods for Human Nutrition 54:353-362.

Aliyu R, Adebayo AH, Gatsing D, Garba IH (2006). The Effects of ethanolic Leaf extract of *Commiphora africana*(Burseraceae) on rat liver and kidney functions. Journal of Pharmacological and Toxicological 2:373-379.

Ashafa AOT, Sunmonu TO, Afolayan AJ (2011). Effects of leaf and berry extracts of *phytolacca dioca L*. on haematological and weight parameters of wistar rats. African Journal of Pharmacy and Pharmacology 5(2):150-154.

Bartels H, Bohmer M (1972). Clinical chemistry. Acta Tropica 37:193-198.

Canadian Council of Animal Care (1984). Guide to the Handling and Use of Experimental Animals. Ottawa, Canada.

Chernecky CC, Berger BJ (2008). Laboratory Tests and Diagnostic Procedures. Philadelphia, PA: Elsevier Saunders.

Church JP, Young JT, Kebay JL, Kin WW (1984). Relationship among dietary constituent and specific serum clinical components of subjects eating self-selected diet. American Journal of Clinical Nutrition 40:1338-1344.

Crook MA (2006). Clinical Chemistry and Metabolic Medicine. Edward Arnold (Publishers) Ltd, London.

Edwards CRW, Bouchier IAD, Haslet C, Chilvers ER (1995). Davidson's principles and practice of medicine. Churchill, Livingstone.

Egwurugwu JN, Nwafor A, Olorunfemi OJ, Anaduaka SC (2012). Effects of methanoic seed extracts of *Mucuna sloanei* on male sex hormones and sperm quality in rats. Journal of Medicinal Plants Research 6(22):3919-3922.

Ejere VC, Ugwu, GC, Chukwuka CO, Nnamonu EI (2015). Effects of aqueous extracts of *Mucuna sloanei* (Fabaceae) seed on haematological parameters of albino rats (*Rattusnovergicus*). African Journal of Biotechnology 14(25):2088-2094.

Ekwe CC, Ukachukwu SN, Akinmutimi AH, Ekwe KC (2016). Determination of nutritional and anti-nutritional characteristics and metabolisable energy of *Mucuna sloanei* seeds. Nigeria Agricultural Journal 46(2):17-24.

Ene-ojo AS, Ene AC, Fatihu MY (2013). Toxic effects sub-chronic administration of chloroform extract of *Artemisia maciverae* Linn on the kidney of Swiss Albino Rats. Int. J. Biochem. Res. Rev. 3(2):119-

128.
Hoff J (2000). Methods of blood collection in the mouse. Laboratory Animals 29(10):50-51.
Hornykiewicz O (2002). L-DOPA: from a biologically inactive amino acid to a successful therapeutic agent. Amino Acids 23(1-3):65-70.
Ijeh II, Agbo CA (2006). Body organ weight changes following the administration of aqueous extracts of *Ficus exasperate Vahl*. Journal of Animal and Veterinary Advances 5:277-279.
Ijeh II, Ukweni AI (2007). Acute effect of administration of ethanol extracts of *Ficus exasperate* Vahl on Kidney function in albino rats. Journal of Medicinal Plants Research 1(2):27-29.
Kaplan A (1965). Urea Nitrogen and Urinary Ammonia. In: Meites, S. (Editor). Standard Methods of Clinical Chemistry. Academic Press, New York.
Kostrzewa RM, Nowak P, Kostrzewa JP, Kostrzewa RA, Brus R (2005). Peculiarities of L-DOPA treatment of Parkinson's disease. Amino Acid 28(2):157-164.
Lavanaya S, Ramesh M, Kavitha K, Malarvizhi A (2011). Haematological, biochemical and ion regulatory response of Indian major Carp Carta catla during chronic sublethal exposure to inorganic arsenic. Chemosphere 82(7):977-985.
Lorke D (1983). A new approach to practical acute toxicity testing. Archives of Toxicology 54:275-289.
Mehrdad M, Mozhgan GP, Sayed AT, Alireza J (2011). Study of Histopathologic changes of *Zingiber* extract on mice kidneys. International Conference on Food Engineering and Biotechnology – IPCBEE, 9:16-20.
Middleton E (1996). Biological properties of plant flavonoids: An overview. International Journal of Pharmaceutics 34(95):344-348.
Nagatsua T, Sawadab M (2009). L-dopa therapy for Parkinson's disease: Past, present, and future. Parkinsonism and Related Disorders. 5(1):S3-S8.
Nduka N (1999). Clinical Biochemistry for Students of Pathology. Animo Press Ltd, Nigeria.
Nwosu J (2011). The effect of storage condition on the rheological/functional properties of soup thickner, *Mucuna sloanei* (Ukpo). Researcher 3:6-10.
Nyblom H, Berggren U, Balldin J, Olsson R (2004). High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. Alcohol and Alcoholism 39(4):336-339.
Obochi GO, Malu SP, Effiom EO, Bassey GA (2007). Efficacy and suitability of lectin from *Mucuna sloanei* seeds extracts as a cell receptor signal inducer. Research Journal of Biological Sciences 2(6):667-669.
Obute GC (2010). Horse eye bean, *Mucuna sloanei* fauw.and rendle (Fabaceae): An underutilized agro biodiversity resource in South Eastern Nigeria. Ethnobotanical Leaflets 14:864-874.
Obute GC, Adubor GO (2007). Chemicals detected in plants used for folk medicine in South Eastern Nigeria. Ethnobotanical Leaflets 11:173-194.
Odoh UE, Osadebe PO (2010). Effects of *Mucuna flagellipes* (Leguminosae) seeds on haematology and serum chemistry of rats. Journal of Tropical Medicinal Plants 11(1):35-38.
Ojiako OA, Anugweje K, Igwe CU, Alisi CS (2012). Evaluation of the amino acid profile and haemoglobin polymerization inhibition potential of some Nigerian legumes. British Journal of Pharmaceutical Research 2(2):80-88.
Rai PP, Saidu M (1977). Characterization of L-DOPA in seeds of *Mucuna sloanei*. Current Science 46:722-778.
Rehman H, Ali M, Atif F, Kaur M Bhatia K, Raisuddin S (2006). The modulatory effect of deltamethrin on antioxidants in mice. Clinica Chimica Acta 369(1):61-65.
Reitman S, Frankel S (1957). A colourimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruivic acid transaminase. American Journal of Clinical Pathology 28:56-63.
Sood R (2006). Medical Laboratory Technology, New Delhi, India: Jaypee Brothers Medical Publishers Limited.
Thomson M, Al-Qattan KK, Al-Sawan SM, Alnaqeeb MA, Khan I, Ali M (2002). The use of ginger (*Zingiber officinale* ROSC.) as a potential anti-inflamatory and anti-thrombotic agent. Prostaglandins, Leukotrienes and Essential Fatty Acids 67(6):475-478.
Ukachukwu SN, Obioha FC (1997). Chemical evaluation of *Mucuna cochinchinensis* as alternative protein feedstuff. Journal of Applied Chemistry and Agricultural Research 4:33-38.
Ukachukwu SN, Ezeagu IE, Tarawali G, Ikeogu JEG (2002). Utilization of Mucuna as a Food and feed in West Africa. In. Flores, B. M., Eilittä, M., Myhrman, R., Carew, L. B. and Carsky, R. J. (Editors). Food and Feed from Mucuna: Current Uses and the Way Forward Workshop. CIDICCO, CIEPCA and World Hunger Research Center, Tegucigalpa, Honduras.
Weatherburn MW (1967). Nutrient analysis of GJ-nature. Analytical Chemistry 39:971-974.
Yakubu MT, Bilbis LS, Lawal M, Akanji MA (2003). Evaluation of selected parameters of rat Liver and kidney function following repeated administration of yohimbine. Biokemistri 15:50-56.

Vol. 17(28), pp. 892-897, 11 July, 2018
DOI: 10.5897/AJB2018.16482
Article Number: 3C1BE0B57773
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effect of seasonal variations on the yield of essential oil and antioxidant of *Achillea fragrantissima* (Forssk) Sch. Bip

Eman Elsharkawy[1,2*] and Nour El-Din M. Nahed[1]

[1]Department of Plant Ecology and Range Management, Ecology and Dry Lands Agriculture Division, Desert Research Center, Mathef El-Mataria, 15753 Egypt.
[2]Department of Chemistry, Science Faculty for Girls, Northern Border University-ARAR, North Region, Saudi Arabia.

Received 15 April, 2018; Accepted 15 June, 2018

Temperature stress is becoming the major concern for plant scientists worldwide due to climate change. Temperature stress has devastating effects on plant growth and metabolism. The aim of the study was to investigate the effect of climatic seasonal change on the yield and composition of essential oil of the plant, *Achillea fragrantissima*. The gas chromatography-mass spectrometry (GC-MS) used to analyze the essential oil collected during dry and wet seasons revealed it has 25 compounds. The major compounds were santolina alcohol (5.31), camphor (4.3), and cedrene (9.01) during winter months, while the percentages of α-cubebene (17.1%), spathulenol (1.54) and globulol (5.2) were highest during summer season. The analysis of essential oil in the two seasons revealed that there are different amounts and composition of essential oils. The antioxidant activity of the essential oil of the plant shows higher activity of IC_{50} 0.11±0.01g/L and Ec_{50} 0.25±0.02 g/L during winter than in summer. However, the reducing capacity of standard substances used (ascorbic acid and α-Tecopherol) were 0.033±0.001 and 0.93±0.07 g/L for DPPH and 0.091±0.002 and 0.026±0.002 g/L for FRAP method, respectively. These results showed that *A. fragrantissima* is a natural source of active compounds, and antioxidant properties, and the difference in chemical composition leads to changes in the antioxidant activity of the plant, which contributes to seasonal change.

Key words: *Achillea fragrantissima*, antioxidant, seasonal vibration, essential oil.

INTRODUCTION

Gebel Elba is a mountain of south-eastern Egypt. This mountain range is considered a continuation of the granitic formation of the Red Sea highland complex between Egypt and Sudan. It is situated between 36 and 37° of the eastern longitudes and about 22° of the northern latitude. The flora of this area comprises hundreds of species of plants.

Gebel Elba receives a vast majority of its precipitation as mists that form the Oasis on the upper areas of the mountains. The mountain tops get 400 mm of rainfall during the year and the surrounding area gets notably less. To cope with this problem some of the plants have evolved to uptake water from their leaves from the mists that lay over the area. There is no real watershed in

*Corresponding author. E-mail: elsharqawyeman@hotmail.com.

Gebel Elba because any water that escapes the oasis quickly evaporates or soaks into the sands. In aromatic crops photoperiod, intensity of light, temperature and season of harvesting have profound influence on terpenoid composition of those crops (Voirin et al., 1990; McGimpse et al., 1994)

According to Brant et al. (2008), plant species belonging to the same botanical family do not present a similar behavior based on the environmental conditions. As one of the many factors that may influence the characteristics of essential oils, climatic variations that occur in a year have been the focus of many researchers attempting to identify the most appropriate time of the year for optimal extractions in terms of yield and/or compound concentration. When the set of climatic factors in seasonal climates with two well-defined seasons is modified, these variations act on the plants and generally alter their metabolism (Scherer, 2007).

Lawlor (2002) reported that the growth and metabolism of plants have optimum temperature limits for every species. The growth, development and productivity of plants that are continuously exposed to environmental stimuli are affected. High temperature, insufficient light, and water deficiency all are factors that negotiate plants' productivity (Lawlor, 2002).

El Zalabani et al. (2017) reported that the essential oil yield of *Artemisia monosperma* during summer was higher than that detected in plants grown in Libya during winter, where the essential oil yield was 0.16% v/w. This higher yield of essential oil is related to plant growth and its ability to survive under drought condition. Plants which grow under this condition can adapt due to their ability to accumulate chemical compounds (El Zalabani et al., 2017).

Achillea fragrantissima, Asteraceae family is a common plant in the Mediterranean region and easily found growing in fields and on roadsides. It contains a high percentage of flavonoids, tannins, volatile oils, sterols and triterpenes; it contains unsaturated amides and sesquiterpene lactones. *Achillea* was highly valued as a medicinal plant for its antiseptic properties. It was used to cover cuts and sores and hasten scar tissue formation. Its clinical uses are not described (Nemeth and Bernath, 2008).

The present work attempts to study the effects of seasonal variation on biomass, essential oil yield, chemical composition, and antioxidant of essential oil of the plant, *A. fragrantissima* which grows widely in Gebel Elba, in 2017.

MATERIALS AND METHODS

Area of study

The Gebel Elba mountainous group is one of 3 coastal mountains in the south-east corner of Egypt that faces the Red Sea, extending between latitude 24° 50'N and 22° N on the Sudano-Egyptian border. A wide coastal desert plain separates the Gebel Elba mountain range from the Red Sea coast. Although not the highest in its group, Gebel Elba is nearest to the sea (20-25 km), as described by Monier and Kadry (2006).

Climate of the study area

Monier and Kadry (2006) reported that the area of the study lies in the arid climatic province; its rainfall ranges between 50 and 10 mm year^{-1} in spring; it has mild winter (18-22°C) and hot summer (28-33°C). As for its geographical position and peculiar set of environmental conditions, Gebel Elba receives greater water revenue from orographic precipitation than the other northern blocks.

Plant material

Flowering aerial parts of *A. fragmentissima* (Figure 1) were collected from wild population in Gebel Elba, Hlayeb Region, in 2017, during two seasons: dry season (summer) and wet season (winter). The plants were identified in Desert Research Center, Cairo Egypt; voucher specimens were deposited in the Herbarium of Desert Research Center.

Sample preparation

The fresh aerial parts of *A. fragmentissima* (50 g plant powder) were extracted by percolation with a mixture of n hexane- ether (1:1, v/v). The solvents were removed subsequently under reduced pressure (Elsharkawy et al., 2013).

Fresh and dry weight analysis

For fresh weight the plants were uprooted and washed to remove surface adhered soil particles and wrapped in blotting papers. Dry weight of the plants was recorded after drying them at 80°C for 24 h in hot air oven.

GC-MS analysis

The constituents of the volatile oils obtained from the n-hexane-ether extracts were analyzed by GLC and GC-MS as reported previously (El-Shazly et al., 2002). Compounds were identified by comparison of their retention indices (RI) (C9 to C24 n-alkane mixture) and mass spectra with those reported in the literature (Merfort et al., 1994; Cavalieri et al., 2004; Adams, 1995).

Antioxidant activity

To evaluate the antioxidant activity of essential oil of *A. fragmrntissima* in the two seasons (summer and winter), its scavenging activities on DPPH radicals were tested. DPPH test is a direct and reliable method for determining radical scavenging action. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515 to 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, that can be quantitatively measured from the changes in absorbance. According to Abdallah et al. (2016):

$$\% \text{ Inhibition} = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \times 100$$

Figure 1. *Achillea fragrantissima* plant.

Ascorbic acid and α-tocopherol were used as positive control and the concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted.

Antioxidant capacity: Fe (III) to Fe (II) reduction capacity

One milliliter of each concentration was mixed with 2.5 mL of potassium hexacyanoferrate $K_3Fe(CN)_6$ solution and 2.5 mL of phosphate buffer (0.2 mol/L, pH 7.0). It was incubated at 50°C for 30 min. Later, 2.5 mL of trichloroacetic acid (10%) was added to the mixture. Then, 2.5 mL of this solution was homogenized with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm and the concentration of the samples at the absorbance of 0.5 (EC_{50}) was determined. Ascorbic acid and α-tocopherol were used as positive control for comparison.

RESULTS

Seasonal climatic changes

Annual climatic variation for the last years in Gabel Elba region was described by Monier and Kadry (2006). The study area has arid climate, its rainfall ranges between 50 and 10 mm $year^{-1}$ in spring, has relatively low temperatures in winter season (18-22°C); its sky is cloudy and sunshine hours are minimal during these seasons. Temperature rises from spring and reaches maximum during summer (28-35) in some days of the month; in May, June and finally August. For the previous three years, there has been increase in temperature during summer month and decrease in rainfall. These drastic conditions (hot and dry summer and cold winter) change the flora of the region and lead to low vegetation. Some plants adapt to these conditions by increasing the concentration of some bioactive compound, like essential oils.

Effect of climate changes on plant growth and physiological attributes

It is evident from the results that *A. fragrantissima* plant,

Table 1. Essential oil composition % of Achelia fragmentissima during different seasons.

S/N	Chemical compound	Summer	Winter	RI
1	Eucalyptol	0.21	0.31	883
2	Thujone	**9.93**	9.98+	928
3	Benzyl alcohol	0.12	0.31	1026
4	limonene	1.52	-	1029
5	Camphor	2.5	4.3	1123
6	limonene oxide	2.4	0.23	1137
7	α-Terpineol	-	0.4	1169
8	trans-p-Mentha-2,8-dienol	0.3	0.25	1160
9	Gerinol	**0.79**	0.09	1253
10	Lavandulyl-acetate	0.25	0.15	1264
11	B-Caryophyllene	0.41	0.30	1408
12	Eugenol	0.02	0.06	1440
13	Cedrene	3.34	9.01	1421
14	Caryophyllene oxide	-	0.51	1568
15	Isocalamendiol	-	0.05	1545
16	Globulol	5.2	3.2	1578
17	Cis-Farnesene	-	0.74	1697
18	Santolina Alcohol	0.46	5.31	1690
19	α –Tocopherol	**0.08**	0.6	
20	Cederan-diol	-	0.14	1597
21	α-Cubebene	17.1	0.11	1560
22	Spathulenol	1.54	0.50	1573
23	beta-Sesquiphellandrene	1.48	0.56	1525
24	Santanol acetate	-	0.18	1771
25	Farnesol	0.27	-	1722
	Total identified (%)	98	93	
	Monoterpene (%)	4.76	4.34	
	Oxygenated monoterpene (%)	33.33	34.78	
	Sesquiterpenoids (%)	23.80	21.73	
	Oxygenated sesquiterpenoids (%)	19.04	26.08	
	Other (%)	14.85	13.04	

collected in two different seasons, exhibited diverse pattern of growth parameter and metabolite. Plants collected in winter season have highest values for plant height (50.32 cm); significant reduction in plant height was recorded in summer (45.35 cm); highest fresh weight (40.54 g) per plant was recorded in winter, but was lowest (25.67g) in summer.

Plants exhibited maximum (23.63 g) dry matter accumulation and minimum (17.61 g) during April and July, respectively. Winter season has high rainfall, short photoperiods, increased metabolic process and fresh weight.

Essential oil composition

Results of statistical analysis indicate that environmental factors significantly affect the concentration and the essential oil yield *of A. fragmrntissima* ranging from 0.02 to 0.4% during summer and winter season, respectively. The difference in essential oil yield and composition of *A. fragmentissima* in response to seasonal changes was investigated under semi-arid tropical climatic conditions of Gabel Elba region. The results showed that essential oil concentration during the winter months is higher than that of summer season in some compounds (Table 1). Evaluation of terpenoid compositions showed minimum concentrations of mentha-2,8-dienol, geraniol and maximum concentrations of santolina alcohol (5.31), camphor (4.3), and cedrene (9.01) during winter months, while percentages of α-cubebene (17.1 %), spathulenol (1.54) and globulol (5.2) were highest during summer season. The plants grown under these hard conditions are tolerant to this abiotic stress due to their accumulation of some compounds or by accumulation of some compounds like acetate or alcohol, santanol acetate and

Table 2. Antioxidant activity of essential oil during different seasons.

Antioxidant assay	Summer	Winter	Ascorbic acid	α-Tocopherol
IC_{50}(g/L)	0.95±0.03	0.11±0.01	0.033±0.001	0.93±0,07
EC_{50} (g/L)	2.11±0.05	0.25±0.02	0.091±0.002	0.026±0.002

lavandulyl acetate, cis-farnesene found in winter season and converted to farnesol in summer season.

Antioxidants activity

In the present study, the FRAP method and DPPH scavenging capacity were used to determine the antioxidant capacity of *A. fragmentissma* essential oil, by reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), and reduction of DPPH. The results of this study show that *Achillea* has higher antioxidant activity of IC_{50} 0.11±0.01 and Ec_{50} 0.25±0.02 during winter than summer (Table 2). However, the standard substances used in this study (Ascorbic acid and Tecopherol) present an antioxidant activity of 0.033±0.001 and 0.93±0.07 g/L for DPPH and 0.091±0.002 and 0.026±0.002 g/L for FRAP method, respectively.

DISCUSSION

In this research we studied the growth, physiological and biochemical attributes of *A. fragmentissima* widely distributed in Gabel Elba, Egypt in response to varying temperature conditions of Gabel Elba. The plant has optimum plant height, fresh weight, dry weight, yield of essential oil accumulation during winter season, while the same parameters significantly decrease in the plant samples investigated in July and January (summer season). This agrees with the study of Prakash et al., (2011, 2011), who observed an increase in the morphological characteristics of plants in suitable environmental conditions and a decrease in this parameter observed beyond a certain limit. Sudden and extreme increase in temperature is accompanied with more stressful conditions which affect growth and development of plant species.

While some compounds of essential oil show opposite trend, the major compounds, camphor (4.5%), α-cubebene (17.1%) and globulol (6.51%) had more concentration during dry season. This is because plants grown under stressful condition can adapt due to their ability to accumulate secondary metabolites.

The results agree with those of Mozaffari et al. (2000) that myrcene and alpha-pinene are synthesized and released in higher amounts under stress. Drought stress causes induction of essential oil of monoterpene carvacrol in *Neigella sativa* (Mozaffari et al., 2000; Costa et al., 2010).

The results revealed that essential oil compositions are sensitive to seasonal climatic changes. Many studies support our results (Castelo et al., 2012; Hazzoumi et al., 2015). The yield of essential oils changes in a major way during the growth period since they are found between January and early February, a period that corresponds to dormancy phase; levels of essential oils decrease from 0.8% to 0.4% and then increase slightly during the growth phase between March and April (0.6%). However, the yield increases very well from mid-April until the end of June and reaches a maximum value of 1.2%. This period corresponds to the blooming phase of the plant. Hazzoumi et al., (2015) studied *Pelargonm graveolens; when* the plant was subjected to moisture stress, essential oil was rich with citronellol which was accumulated as a mechanism to adjust the thermal stress (Hazzoumi et al., 2015)

Antioxidant of essential oil at different seasons of DPPH shows the essential oil exhibits highest radical scavenging activity during winter season than in summer. Essential oil containing cederan-diol, santolina alcohol, caryophllene oxide and cederen with different chemical compositions had the highest levels of yield during winter. The results are in agreement with those of other studies, where essential oil of the leaf of *Artemisia absinthium* showed radical scavenging activity of DPPH in flowering stage during winter season (Canadanovic-Brunet et al., 2005; Mohammadi et al., 2015).

Conclusion

The results of antioxidant and chemical composition during two seasons (wet season and dry season) reflect the effect of seasonal change on the growth and behavior of plant to adapt to dry and harsh condition in Gabel Elba region. The analysis of the essential oil in the two seasons revealed there are differences in the composition and amount of essential oil. Some compounds are accumulated in high concentration in dry season, which reflects the method of tolerance to stress. By accumulation of these compounds plant can grow and survive. The results of antioxidant activity are compatible to the composition of essential oil. This study has shown that some compounds such as santolina alcohol, camphor, and cedrene were responsible for antioxidant activity in essential oil in preflowering stage during winter season.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors are grateful to Northern Border University and Desert Research Center for financial support and giving us the facilities used for the work.

REFERENCES

Lawlor DW, (2002). Limitation to Photosynthesis in Water-stressed Leaves: Stomata *vs.* Metabolism and the Role of ATP. Annals of Botany 89:871-885.

Abdallah EM., Elsharkawy ER, Ed-dra A (2016). Biological activities of methanolic leaf extract of *Ziziphus mauritiana*. Bioscience Biotechnology Research Communications 9(4):605-614.

Adams RP (1995). Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. Texensis Publishing.

Brant RS, Pinto, JP, Bertolucci SV, Albuquerque CB, (2008). Essential oil content of *Aloysia triphylla* (L'Her.) Britton in function of seasonal variation. Revista Brasileira de Plantas Medicinais 10:83-88.

Canadanovic-Brunet JM, Djilas SM, Cetkovic GS (2005). Free-radical scaveng-ing activity of wormwood (*Artemisia absinthium*) extracts. Journal of the Science of Food and Agriculture 85:265-72.

Castelo AM, Del Menezzi CS, Resck IS (2012). Seasonal Variation in the Yield and the Chemical Composition of Essential Oils from Two Brazilian Native Arbustive Species. Journal of Applied Sciences 12:753-760.

Cavalieri E, Mariotto S, Fabrizi C (2004). Alpha-bisabolol, a nontoxic natural compound, strongly induces apoptosis in glioma cells. Biochemical and biophysical research communications 315:589-594.

Costa LB, Pinto José EP, Evaristo MC (2010) Yield and Composition of the Essential Oil of *Ocimum selloi* Benth. Cultivated Under Colored Netting. Journal of Essential Oil Research 22:34-39.

El Zalabani SM, Tadros SH, El Sayed AM, Daboub AA, AmenSleem A (2017). Chemical Profile and Biological Activities of Essential oil of Aerial parts of *Artemisia monosperma* Del. Growing in Libya. Pharmacognosy Journal 9(4):577-586.

Elsharkawy ER, Elshathely MR, Abdel Jaleel G, Al-Johar HI (2013). Anti-inflammatory effects of medicinal plants mixture used by Bedouin people in Saudi Arabia. Herba Polonica 59(3):67-87.

El-Shazly A, Dorai G, Wink M (2002). Chemical composition and biological activity of the essential oils of Senecio aegyptius var. discoideus Boiss. Zeitschrift fuer Naturforschung C. 57:434-439.

Hazzoumi Z, Moustakime Y, Amerani Joutei K (2015). Variation in Yield and Composition of Essential Oil in Pelargonium sp During the Vegetative Stage, International Conference on Chemical, Environmental and Biological Sciences (CEBS-2015) March 18-19, 2015 Dubai (UAE).

McGimpse YA, Douglas MH, van Klink JW, Beauregard DA, Perry NB (1994). Seasonal variation in essential oil yield and composition from naturalized *Thymus* vulgaris L. in New Zealand. Flavour and Fragrance Journal 9:347-352.

Merfort I, Heilmann J, Hagedorn-Leweke U, Lippold BC (1994). In vivo skin penetration studies of camomile flavones. Die Pharmazie 49:509-511.

Mohammadi A, Ahmadzadeh Sani T, Ameri AA, Imani M, Golmakani E, Kamali H (2015). Seasonal variation in the chemical composition, antioxidant activity, and total phenolic content of *Artemisia absinthium* essential oils. Pharmacognosy research 7(4):329-334.

Monier MG, Kadry NA (2006). The Floristic Diversity and Phytogeography of the Gebel Elba National Park, South-East Egypt. Turkish Journal of Botany 30:121-136.

Mozaffari FS, Ghorbanli M, Babal A, (2000) The effect of water stress on the seed oil of *Nigella sativa* L. Journal of Essential Oil Research 12:33-35.

Nemeth E, Bernath J (2008). Biological activities of yarrow species (Achillea spp.) Current Pharmaceutical Design 14:3151-3167.

Prakash V, Bisht H (2011). Prasad P. Altitudinal variation in morpho-physiological attributes in Plantago major: selection of suitable cultivation site. Research Journal of Medicinal Plant 5:302-311.

Scherer LM, (2007). Effect of season and vegetal hormones on the *in vitro* multiplication and yield of essential oil. M.Sc. Thesis, Western State University of Parana, Marechal Candido Rondon, Brazil.

Voirin B, Brun N, Bayet C (1990) Effects of day length on the monoterpene composition of leaves of Mentha x piperita. Phytochemistry 29:74S-755.

Related Journals: