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Full Length Research Paper

Studies on morphology and anatomy of *Strychnos spinosa* Lam. (Loganiaceae)

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***Strychnos spinosa* Lam. belongs to the family Loganiaceae. It is a small tree. The morphological and anatomical features of the leaves, stem, flower and fruit were studied. *S. spinosa* has a cylindrical stem with a pair of spines at irregular spaces. The leaves are opposite, decussate, petiolate with palmate venation. The petals are greenish in colour, tubular with five lobes. There are numerous white hair-like appendages at the neck of the corolla tube. The androecium is epipetalous and attached close to the bottom of the corolla tube. The flower is a cyme, occurring in threes, with the middle one being the oldest. The fruit is a berry with very hard pericarp. It turns from light green when unripe to bright yellow when ripe. The anatomy of the leaf revealed presence of rosette crystals and intraxylary phloem which is more pronounced in the mid rib and petiole. "Kranz" structure was observed in the transverse section of the leaf. Anisocytic stomata are numerous in the abaxial (lower) epidemis but absent in the adaxial epidemis. The anatomy of the stem showed the presence of crystals in the cortex. Both solitary and multiple vessels are found in the transverse section. In the tangential longitudinal section, most of the rays were four to seven cells wide, but a few were uniseriate, biseriate and triseriate. Most of the pollen grains were triangular in the polar view but a few were quadrangular. Those that were triangular exhibited tricolporate aperture while those that were quadrangular had tetracolporate aperture. The sculpturing was perforate and were not very compact.**

Key words: *Strychnos spinosa,* rosette crystals, intraxylary phloem, pollen dimorphism, heterogenous, rays.

INTRODUCTION

Strychnos spinosa Lam. belongs to the genus *Strychnos*, in the family Loganiaceae of the order Loganiales (Hutchinson, 1967). The delimitation of the family has been a matter of debate (Leeuwenberg, 1962, Backlund et al., 2000).

Strychnos spinosa is a small, medium-sized, spiny, deciduous tree. The leaves turn yellow in the dry season before they fall off. It also grows singly in well drained soil. It is found along river fringes and sandy forests from East Africa to South Africa (Watt and Breyer - Brandwijk, 1962). In Nigeria, it occurs in the savanna wood land (Keay, 1989).

The generic name is derived from the Greek word "strukhnos" that refers to the properties present in the

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poisonous night-shade. Many species of *Strychnos* produce alkaloids like strychnine and resinous substances used in preparing the arrow poison "curare" in South America. In other places like Malaysia and Central Africa, species of *Strychnos* have been used to prepare dart poison for hunting wildlife (Mwamba, 2006). The specific name *spinosa* refers to the spines characteristically found on some nodes (Watt and Breyer - Brandwijk, 1962; Mwamba, 2006).

The circumscription of Loganiaceae varies significantly among different taxonomists. Bremer and Struwe (1992) and Backlund et al. (2000) all reported that the debate has trailed the circumscription and the intra-familial relationship of this family. While Hutchinson (1967) placed Loganiaceae in the order Loganiales, Backlund et al. (2000) placed Loganiaceae in the order Gentianales. Metcalfe and Chalk (1989) however, rightly pointed out that opinions about the relative merit of the different systems notably differ. They remarked that there is not at present and probably there will never be, an all embracing system that will generally be accepted in any taxonomic work. They concluded that it is necessary to choose a sequence in which the families will be described. The system of Hutchinson (1967) for the family Loganiaceae has been adopted in this work. Mathé and Craker (1995), De Carvatha and Ferreira (2001) observed that despite the modern advances achieved in synthetic chemistry the most efficient drugs available have their origin directly or indirectly related to the plant kingdom. Leu and Amar (2000) reported that 74% of all plant-derived drugs in clinical use world wide have been discovered through follow-up investigation of the ethnobotanical uses of plants. They noted that from medicinal literature survey, the trend has been that plants represent most of all the traditional medicinal substances. They opined that since plants represent an important source of drug discovery and the search for new biologically active compounds usually starts in the field, there should be accurate, detailed and specific ethnobotanical information obtained from local practitioners. De Carvatho and Ferreira (2001) observed that indigenous communities have long used plant extracts to treat illnesses and many of such extracts have shown effective action, resulting in screening and extraction of new bioactive compounds every year. In line with the above, Ndubani and Höjer (1999) listed the root of *S. spinosa* as one of the plants used by traditional healers in conjunction with other plants to treat sexually transmitted diseases in rural Zambia. Kokwaro (1976) reported that juice from the root is dropped into the ear as remedy for ear ache. He also remarked that decoction of the root is used as a head wash for cold. The same decoction taken with milk is used as a cure for dropsy. Chewing of the roots or drinking the boiled root decoction effects healing from snake bite. The roots and leaves are used for treating veneral diseases and as a febrifuge in Zambia, while a decoction of the leaf or root is used as an analgesic in Central Africa (Mwamba, 2006). Oguakwa et al. (1980) isolated three alkaloids from the leaves of *S. spinosa*. They reported that the crude extracts from the leaves and stem bark have muscle relaxant effects.

This study was undertaken in cognizance of the great medicinal potentials of this plant and the scanty literature available on the accurate and detailed description of its morphology and anatomy. The aim of this study is to carefully examine the morphology and anatomy of *S. spinosa*.

MATERIALS AND METHODS

Ten stands of *S. strychnos spinosa* were randomly collected from Kuje village near Abuja and Igbeji along Lagos-Ilorin road (Guinea savanna). The species was authenticated by Prof. Dr. M.O Nwosu of the Department of Plant Science and Biotechnology UNN, Mr. A. Ozioko of Bioresource Centre Nsukka in conjunction with the type specimen accessed from the website of Royal Botanical Garden Kew. Voucher specimens were deposited at the Herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Detailed examination of the morphological features of twenty five leaves, petioles, flowers and seeds was done with the help of hand lens and dissecting microscope. Measurement of the dimensions of leaves, petiole, stem, flowers, fruit and seeds was done with a meter rule.

Anatomical studies

Freshly collected leaves were washed with distilled water to remove dirt and dust and allowed to dry under a fan. They were further soaked in domestic bleach for 24 h to digest the mesophyll layer according to the method of Olowokudejo (1993). The leaves were washed in water twice and the abaxial and adaxial epidermises teased off carefully with fine forceps and dissecting needles. The membranes were stained with safranin, mounted in glycerine and viewed under the microscope.

The transverse sections of the median part of lamina, midrib and petiole were cut with the aid of sliding sledge microtome mounted on slides for microscopic examination. Transverse section (TS), transverse longitudinal section (TLS) and radial longitudinal section (RLS) of the root and stems were also made using a sliding sledge microtome. The sections were stained with safranin and counter stained with Fast green. The materials were washed in a series of alcohol and with 50/50 alcohol and xylene. They were picked with brush and mounted on slides with Canada balsam as mountant. Photomicrographs of the good slides were taken with a Sony digital camera fitted on a Zeiss microscope.

Pollen study

Anthers were teased off from mature buds of flowers of *S. spinosa* and squashed with a glass rod to release pollen grains. This was sieved with fine mesh of wire gauze, washed twice with distilled water and centrifuged at 2000 revolutions per minute for 5 min. The resulting precipitate was acetolysed using a modified version of Erdtman (1971) acetolysis method. Pollen grains were mounted on slides and examined with Zeiss microscope fitted with Sony digital camera at X 400 and X1000 magnifications. Terminologies for pollen descriptions are adapted from Eidtman (1971) and Punt et al.

Plate 1a. Inflorescence of *S. spinosa***.**

Table 1. Reproductive characteristics of *Strychnos spinosa.*

Sepal	Petal	Anther	Filament	Ovary
5 Green in colour Persistent. Almost linear in shape. Acuminate tip.	5 Tubular with five lobes White hair - like projections at neck of corolla tube Light green in colour Petals fall off easily from flower	Has two conspicuous lobes with two chambers each Anther lobes covered with hair-like projections Bright orange in colour Dorsifixed and intorse	Five Epipetalous Inserted at lower part of corolla tube Filament and anther fall off with corolla.	Small Round Has numerous ovules
Style	**Stigma**	**Fruit**	**Seed**	**Embryo**
Short	Single Orange-yellow in colour	Round berry Apple green in colour when unripe Bright yellow when ripe Very hard pericarp Light brown pulp inside Plate 1b	Light brown in colour Measure 22-24mm in length and 6-18mm in width Thin testa Fleshy endosperm	Small Embedded in endosperm Thin cotyle- dons with distinct veins like those of leaves.

(2007).

The primitive and advance features of the species were deduced from the morphological and anatomical features examined.

RESULTS

The leaf is obovate with the broadest part near the apex. The margin is entire, the base is rounded and apex has a little projection that is acute. The colour of the leaf is light green above and lighter green beneath. The venation is palmate with 2 pairs of veins on both sides of the midrib, all emanating from the petiole at the base and terminating at the apex. The two veins closest to the midrib on the right hand and on the left hand, run parallel to the midrib for a distance of 0.4 to 0.6 cm before curving and terminating at the apex. The outermost veinlet to the margin is joined by a number of arches. Leaf has no odour and is smooth (glabrous). Leaf arrangements are opposite and decussate.

The petiole is rounded at the abaxial (lower) side and grooved at the adaxial (upper) side with some hairs in the groove. It is greenish in colour and measured 0.5 to 0.9 cm in length.

The stem is fissured, the degree of fissuring increases with the age of the plant. The nodes on the young stem are swollen. Axillary spines occur but not in any particular sequence. The spine measures 0.4 to 0.7 cm in length. A black line that circles the stem exist just above the point of attachment of the leaves to the node.

The inflorescence is a cyme and terminal and appears soon after the new leaves form between March and June. The main axis ends in a flower and produces two lateral branches. These two lateral branches also behave in the same manner. The terminal flower is older than the lateral flowers (Plate 1a and b). The details of the floral parts, fruits, seeds and embryo is summarised in Table 1.

Anatomical study

Leaf

The transverse section of the leaf revealed a single layer

of upper epidermis that consisted of compactly arranged rectangular cells with the upper part that is concave. A thin layer of cuticle covered the epidermis (Plate 1e).

Palisade mesophyll consists of compactly arranged rectangular shaped cells with numerous chloroplasts. It is single-layered in some places and double at other places. The upper most palisade is longer than wide. The lower palisade consists of cells that are nearly square in shape.

The spongy mesophyll contains loosely arranged parenchyma cells thus creating numerous air spaces. Rosette crystals are found in the spongy mesophyll. Bundle sheaths consisting of a single layer of compactly arranged parenchyma cells in a circle with few vascular bundles that are enclosed in the circle occur in the spongy layer. The lower epidermis is less compactly arranged having numerous stomata for gaseous exchange.

In the midrib, the upper epidermal cells are composed of compactly arranged rectangular cells that are barrel shaped. The ground tissue immediately below the epidermis is made up of 5 to 7 layers of thin walled parenchyma cells that lack chlorophyll. The vascular bundle clearly shows the bicollateral arrangement where internal and external phloems sandwich the xylem (intraxylary phloem). Rosette crystals are present in the ground tissue. Three to four layers of ground tissue occur at the lower portion of the vascular bundle (Plate 1e). The latter resemble the cells at the upper side of the vascular bundle. The lower epidermis is single layered (Plate 1f). Examination of epidermal strip, revealed that stomata is absent on the adaxial surface. On the abaxial epidemis, the stomata are numerous and are of the anisocytic type. The epidermal cells have walls that are curved (Plate 1c and d)

The upper epidermis of the petiole is made up of compactly arranged cells with an undulating surface. There are two small projections at the ends of the upper surface (Plate 1g). The vascular bundle is arranged in form of an arc and this affects the number of cells in the ground tissue. At the adaxial side, the cells range in number from nine - eleven at the distal ends to about twenty-three at the median part. The cells range in number from nine at the distal ends to sixteen at the median part on the abaxial side. The cells in the ground tissue are parenchymatous, round or hexagonal in shape and compactly arranged. The lower epidermis consists of closely packed cells with an outer convex surface.
There are seven separate vascular bundles. In the middle is the biggest, flanked on either side by three bundles in decreasing order of sizes. The bicollateral arrangement is very pronounced with the xylem in the middle and the phloem on both the abaxial and adaxial sides.

Transverse section (T.S.) of stem: The periderm contains eighteen - twenty-six layers of rectangular shaped cells with their long axis oriented horizontally and compactly arranged. The cortex contains twenty seven – thirty one layers of closely packed, thin walled parenchyma cells. The endodermis consisting of thick walled sclerenchyma cells form a continuous ring that is

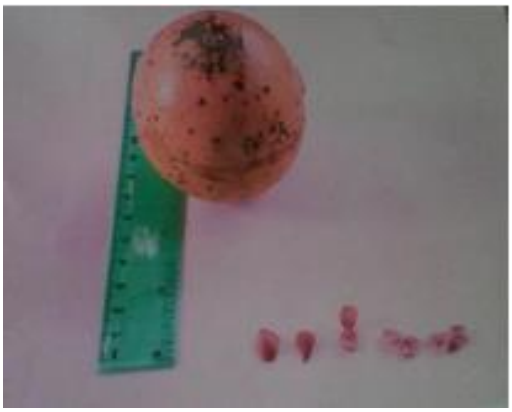

Plate 1b. Fruit of *S. spinosa.*

Plate 1c. Adaxial leaf surface of S. *spinosa* with no stomata present x400.

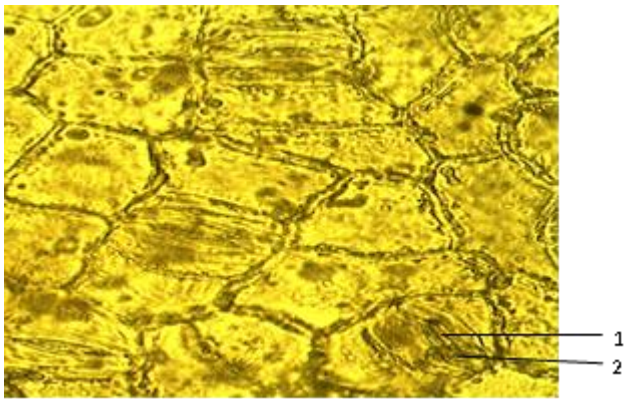

Plate 1d. Abaxial view of leaf of *S. spinosa* x400; 1, stomatal pore; 2, guard cell.

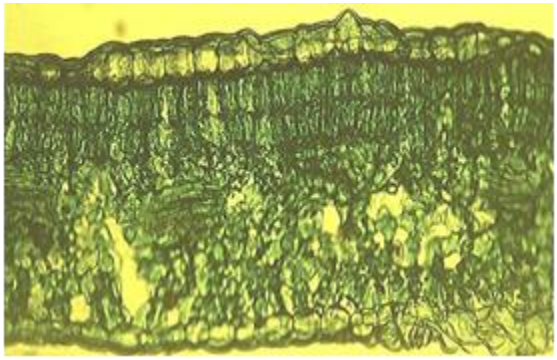

Plate 1e. TS leaf lamina *S. spinosa* x 200. **1**, Adaxial epidermis; **2**, Palisade mesophyll; **3**, Spongy mesophyll.

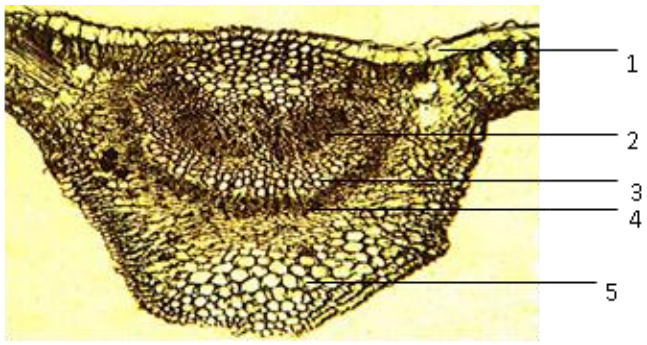

Plate 1f. Midrib of leaf*S.spinosa*x40; 1, adaxial epidermis; 2, internal phloem; 3, xylem elements; 4, external phloem; 5, ground tissue.

Plate 1g. petiole *of S. spinosa* x 40; 1, Adaxial epidermis; 2, vascular bundle; 3, ground tissue.

one or two layers, about eight cells away from the phloem. The pericycle is made up of a discontinuous

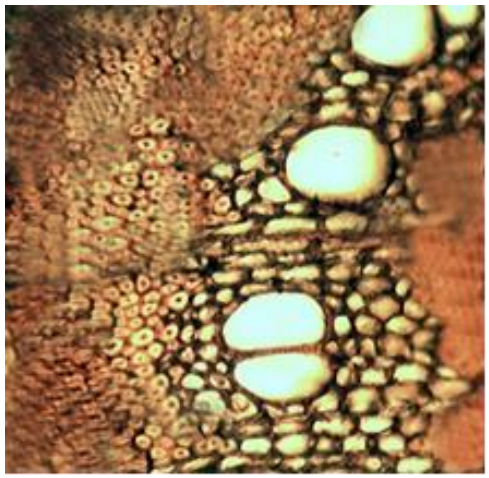

Plate 2a. TS stem of *S. spinosa* x100.

Plate 2b. TLS stem of *S. spinosa* x 200.

band of sclerenchyma that goes round. Vessels are of various shapes, while most are spherical, some have egg shape and a few have hexagonal shapes. The sizes of vessel also vary with both big and small ones. Vessel diameter measures 0.03 and 0.8 mm (at x 100). Both multiseriate and uniseriate rays occur and bands of fibers form a pattern in the section (Plate 2a).

Transverse longitudinal section (TLS): There are numerous rays of varying sizes and heights. Uniseriate rays are numerous, while multiseriate rays are few and vary from seventy - ninety cells high. Biseriate and triseriate rays are twelve - forty-five cells high (Plate 2b).

Vessels are of varying lengths ranging from 90 to 160 µ and vessel members measure between 12 to 15 µ, each. Vessel walls have simple pitting. Numerous fibers that are variously branched ramify the section.

Radial longitudinal section (RLS): The rays are hetero-

cellular, containing both upright and procumbent cells (Plate 2c). The procumbent cells have shapes that vary from square to rectangular, a few are somewhat round in shape. The height of the procumbent cells range from thirteen - twenty. The upright rays consist of cells that are all rectangular with their long axis oriented vertically and their height range from six - fifteen cells. Thus the procumbent cells outnumber the upright ones. Vessels are of varying lengths. Some vessels have one - four members and others have ten - fifteen members. There are numerous fibers that ramify the section (Plate 2c).

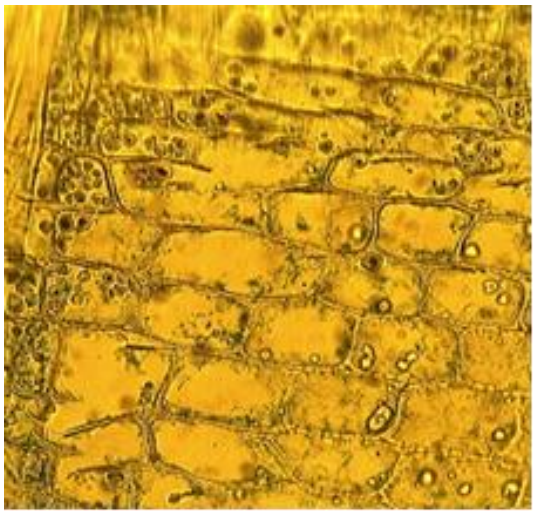

Plate 2c. RLS stem of *S. spinosa* x 200.

Root

Transverse section (TS): The cork cells contain eleven - fifteen layers of thin-walled rectangular shaped cells that are compactly arranged. The cortex contains ten - eighteen layers of thin-walled parenchymatous cells of various shapes. The endodermis consisting of a continuous ring of two to four layers of thick-walled sclerenchymatous cells is conspicuous and occurs close to the vascular tissue. Other patches of sclerenchyma are scattered in the cortex. There are numerous vessels and most of them are single though vessel multiples of twos and threes also occur (Plate 3a). Thick fibers forming bands abound and numerous rays transverse the section.

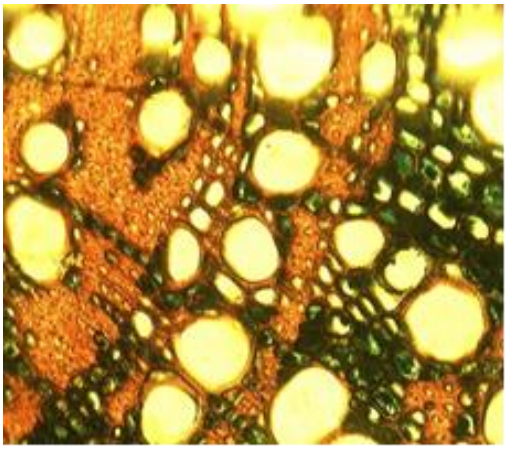

Plate 3a. TS root of *S. spinosa* x 200.

Tangential longitudinal section (TLS): There are very few uniseriate rays. Most rays are multiseriate varying in width from four - eight cells and in height from eleven - fifty four cells (Plate 3b). There are numerous fibers and vessel walls have simple pitting.

Pollen study: Pollen grains exhibit dimorphism in shape in *S. spinosa* with occurrence of 70% of pollen with triangular shape while 30% has quadrangular shape (Plate 4a and b).

Shape: The triangular ones are between oblate and subspheroidal. The quadrangular ones are oblate.

Aperture: The pollen that are triangular exhibit tricolpate aperture; while those that are quadrangular exhibit tetracolpate aperture Exine is tectate, sculpture is perforate. The perforation is irregularly arranged and not very compact.

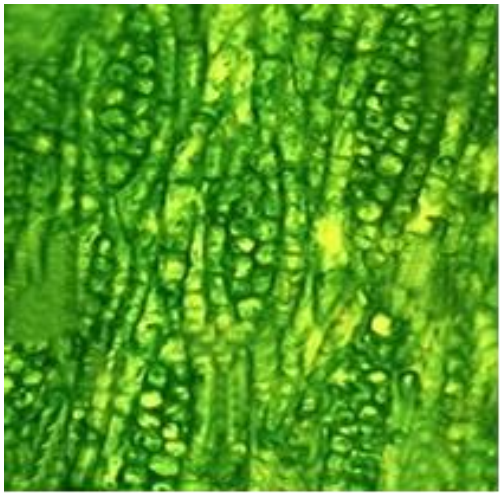

Plate 3b. TLS root of *S. spinosa* x 100.

Primitive and advanced features of *S. spinosa*

Summary of primitive and advanced features of *S. spinosa* are presented in Table 2.

DISCUSSION

The description of leaf morphology in the present study

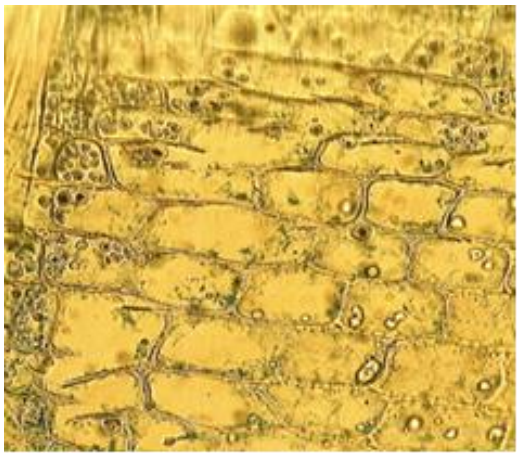

Plate 3c. RLS root of *S. spinosa* x 200.

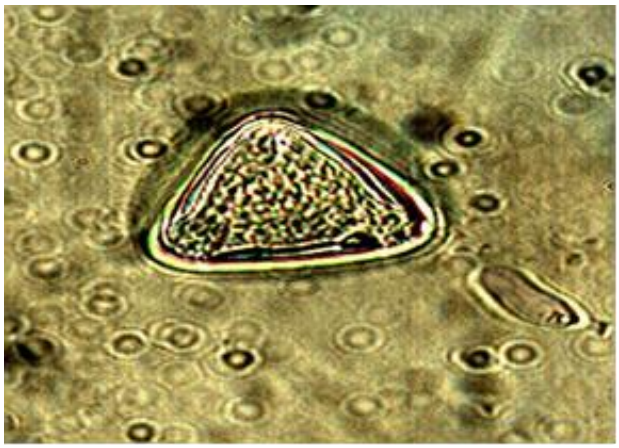

Plate 4a. Tricolporate pollen of *S. spinosa*. Triangular pollen in equational view showing perforate sculpturing x 400.

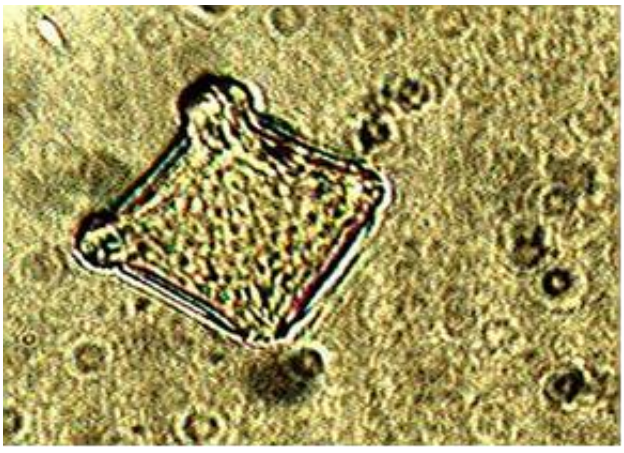

Plate 4b. Quadrangular pollen of *S. spinosa*. Quadrangular pollen in equational view showing perforate sculpturing x 400.

was found to be similar to the description of the family Loganiaceae made by Keay (1989).

The venation is diagnostic having all the veins emanating from the base or a little distance from the base (that is, palmate) and terminating at the apex. It can be used to spot the species in the field. Oduoye et al. (2013) reported the occurrence of similar venation in some Nigerian *Strychnos* spaces, they studied.

The occurrence of spines on the stem is another diagnostic feature that can be used to spot the tree in the field. Watt and Breyer Brandwijk (1962) noted that the specific name 'spinosa' is derived from the presence of spines and are often in pairs thus confirming the observation of Keay (1989).

The fragile nature of the petals was also observed in *Spigelia anthelmia,* another member of the family Loganiaceae (Asuzu and Nwosu, 2009). The transverse section of the leaf revealed a single layered, compactly arranged epidermis. This, together with the tough cuticle offers mechanical protection and reduction of water loss by transpiration (Evert, 2006).

The occurrence of "kranz" structure observed in *S. spinosa* was reported by Metcalfe and Chalk (1989) as a feature with restricted taxonomic distribution among the dicotyledon. It was suggested by Metcalfe and Chalk (1989) that plants with kranz structure probably arose in tropical surroundings. The occurrence of kranz structure in family Loganiaceae was observed by Asuzu and Nwosu (2009) in *Anthocleista djalonensis.*

Crystals were found in the mesophyll cells of the leaf and ground tissue of the petiole confirming the listing of the family Loganiaceae as one of the families where crystals occur (Metcalfe and Chalk, 1989).

In the midrib and petiole, intravascular phloem was observed. Metcalfe and Chalk (1989) reported that intravascular phloem is an anomalous structure and has restricted occurrence in plants. Evert (2006) remarked that intravascular phloem is found in families like Apocynaceae, Asclepiadaceae, Convolvulaceae, Cucurbitaceae, Mrytaceae, Solanaceae and Asteraceae. Bremer and Struwe (1992) observed that the families, Apocynaceae, Asclepiadaceae and Gentianaceae have close affinity and share some features in common with the Loganiaceae. The transverse section of the stem and root revealed vessels of varying sizes and numbers. Evert (2006) noted that wide vessels are more efficient in water conduction than narrow vessels. He postulated that the hydraulic conductivity of a vessel is roughly proportional to the fourth power of its radius or diameter. If the diameter of vessel is 2 for example, the relative volume of water flowing through it under normal conditions would be 2^4 (16). Though increased vessel diameter increases efficiency of water conduction, it also decreases safety. Vessels with larger size tend to be more vulnerable to water stress-induced cavitations. Cavitation is the formation of cavities filled with air within the conduits resulting in breakage of water columns

Table 2. Primitive and advance features of *S. spinosa*.

Primitive features	Advance features
Shrub	Opposite leaf arrangement
Perennial	Flowers arranged in an inflorescence
Simple leaves	Connate petals
Bisexual flower	Few carpels
Flowers with many parts	Fruit a berry
Petaliferous	Estipulate leaves
Hypogyny	Spine present
Endospermous seed with small embryo	
Separate stamens	
Taproot present	
Glabrous condition	
Seed present	

(Evert, 2006).

Both solitary vessels and vessel multiples were found in the sections. Evert (2006) reported that vessel multiples are safer than solitary vessels as they provide alternative paths for the xylem sap to bypass embolism or blockage of the conduit with air. When this kind of blockage occurs in a single vessel element, the wide vessel may soon be filled with water vapour and air and consequently lose the ability to conduct water.

There are many rays of varied sizes and heights. Rays in angiosperms occupy about 17% of the volume of wood and contribute substantially to the radial strength of the wood (Evert, 2006). On the issue of phylogeny, the specie *S. spinosa* has both primitive and advanced features. Jones (1979) opined that the rate of evolution is not the same for all structures of the plant. He noted that some parts of a plant may become more specialized than others leading to a situation where both advanced and primitive features occur in a taxon. The primitive features of this species outnumber the advanced features.

In conclusion, the study revealed that *S. spinosa* has the following features namely: stomata of the anisocytic type only in the abaxial section and the absence of stomata in the adaxial section; the presence of crystals in the anatomical section of leaf and stem; the presence of both solitary vessels and vessel multiples and pollen that are predominantly tricolporate with a few that are quadrangular.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Asuzu CU, Nwosu MO (2009). Morphology and Anatomy of *Anthocleista djalonensis A. Chev. (Loganiaceae).* Niger. J. Bot. 22(1):103-109.

Backlund M, Oxelman B, Bremer B (2000). Phylogenetic Relationship within the Gentianales based on NDHF and RBCL sequences with particular reference to the Loganiaceace. Am. J. Bot. 87(7):1029-1043.

Bremer B, Struwe l (1992). Phylogeny of the Rubiaceae and the Loganiaceae: Congruence or Conflict between morphology and molecular data?. Am. J. Bot. 79(10):1171-1184.

De Carvatha PB, Ferreira EJ (2001). Leishmaniasis phytotherapy. Nature's leadership against an ancient disease. Fitoterapia 72:599-618.

Erdtman G (1971). Handbook of palynology, morphology, taxonomy, ecology. Munksguaard Copenhagen. 204 p.

Evert RF (2006). Esau's plant Anatomy. John Wiley & Sons Inc., New Jersey. 601p.

Hutchinson J (1967). Key to the families of flowering plants of the world. Clarendon Press London. 828 p.

Jones SB (1979). *Plant Systematics*. McGraw-Hill Book Company, New York. 369p.

Keay RWJ (1989). Trees of Nigeria. Clarendon Press, Oxford. 476p.

Kokwaro JO (1976). Medicinal Plants of East Africa. East African Literature Bureau, Kampala. pp. 146-147.

Leeuwenberg AJM (1962). The Loganiaceae of Africa IV *Strychnos1*. Acta Botanica Neerlandica 11:45-50.

Leu E, Amar Z (2000). Ethnopharmacological survey of traditional drugs sold in Israel at the end of the 20th Century. J. Ethno Pharmacol. 72:191-205.

Mathé A, Craker LE (1995). From Medicinal Plants to Phytomedicines. J. Herbs Spices Med. Plants 3(3):3.

Metcalfe CR, Chalk L (1983). Anatomy of the dicotyledons. Vol II 2nd Edition, Clarendon Press, Oxford. 297 p.

Mwamba C (2006). Monkey Oranges: Strychnos Cocculoides (fruits for the future). University of Southampton International Centre for under utilized crops. 81p.

Ndubani P, Hojer B (1999). Traditional healers and the treatment of sexually transmitted illnesses in rural Zambia. J. Ethnopharmacol. 67:15-25.

Oduoye OT, Ogundipe OT, Olowokudejo JD (2013). Leaf epidermal analysis for diagnosis of enigmatic *S. strychnos* species in Nigeria. Int. J. Eng. Sci. 2(7):109-119.

Oguakwa JU, Galeffi C, Nicoletti M, Messana I, Patamia M, Marini-Bettolo GB (1980). On the alkaloids of *Strychnos* XXXIV: the alkaloids of *Strychnos spinosa* Lam. Gazz. Chim. Ital. 110:97-110.

Olowokudejo JD (1993). Comparative epidermal morphology of West African species of *Jatropha* L. (Euphobiaceae). Bot. J. Linn. Soc. 111:139-154.

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007). Glossary of pollen and spore terminology. Rev. Palaeobot. Palynol. 143(1-2):1-81.

Watt JM, Breyer- Brandwijk MG (1962). The medicinal and poisonous plants of Southern and Eastern Africa. E and S. Livingstone Ltd., London. 727 p.

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Genetic diversity assessment of *Guzoita abyssinica* using EST derived simple sequence repeats (SSRs) markers

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Niger (Guzoita abyssinica) **is traditionally very important in the production of oil and as an oilseed crop in Ethiopia. The level of *Niger* genetic diversity is not studied at molecular level particularly with microsatellite markers which are cost effective, informative and co-dominant. In this study, contrasting samples of 65 Ethiopian *Niger* germplasm accessions collected from diversified eco-geographic regions were studied with eleven polymorphic EST derived SSR markers. Based on dissimilarity matrix ranging from 0.056 to 0.75, the accessions were grouped into three major and six sub clusters, showing the wealth of genetic diversity for exploitation in future breeding programs. The EST derived SSR markers used in this study also revealed high polymorphic information content (PIC) ranging from 0.2624 to 0.3677, the average being 0.3308 which indicate the usefulness of the primers in *Niger* germplasm characterization in the future by providing basic breeding information for breeders. The mean number of major allele frequency, gene diversity and heterozygosity using power marker v3.25, showed a value of 0.69, 0.42 and 0.50, respectively. The number of private alleles using GenAlEx 6.41 is 66 alleles with mean of 9 alleles per locus. Thus, utilizing EST SSR marker for diversity study lays basic foundation to understand the genetic distance of crop verities and greatly contributes for further improvement and preservation.**

Key words: Alleles, dendrogram, diversity, EST-SSR markers, heterozygosity, locus, *Niger* and polymorphic information content (PIC).

INTRODUCTION

Niger (Guzoita abyssinica, 2n=30) is belongs to the plant family “Asteraceae”. It is one of the most valuable oil-seed crops in Ethiopia (Getinet and Sharma, 1996). *Niger* grows in water-logged soils where other oil-seed crops fail, and its cultivation is beneficial for soil conservation and rehabilitation. Scientific community gives a little

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attention for *Niger* and hence *Niger* is renowned as "neglected and underutilized oil crops" (Getinet and Sharma, 1996). It is widely cultivated in Ethiopia today but has received only limited attention from agricultural scientists and modern breeding techniques have yet to be applied to its improvement. *Niger* (*G. abyssinica*) is traditionally very important in the production of oil and as an oilseed crop in Ethiopia, less systematically in India, Africa and Asia (Getinet and Sharma, 1996). It has been grown in Ethiopia since at least the 2nd millennium BC and probably much earlier (Boardman, 1999; Boardman, 2000). It is indigenous to Ethiopia, a center of its diversity.

In most places, *Niger* is widely cultivated in an intercropping condition which may render an opportunity for morphological variation. Consequently, it hides from improvement using modern breeding techniques. Any crop improvement program starts with identification of variability among the genotypes. *Niger* has been characterized morphologically (Bulcha et al*.,* 2005). It was hypothesized that the domesticated crop, *G. abyssinica* probably evolved from the wild type Guizotia *scabra ssp. Schimperi* (Petros et al*.,* 2007). Besides the morphological based characterization of *Niger*, studies using molecular markers such as ISSR (Petros et al*.,* 2007) and RAPD (Geleta et al*.,* 2007) have also been conducted to assess the genetic diversity existing among *Niger* accessions. According to (Dempewolf et al*.,* 2010a) identification of SSR from EST library of *G. abyssinica* was in place. This led to the first insight in developing SSR, which was not known before.

Microsatellites (SSRs) are quite many in eukaryote genomes. They offer supreme information about the genetic diversity of the crop; they are hypervariable, locus specific, multi-allelic, co-dominant, rapid, low cost and independent from environmental factors (Pejio et al*.,* 1998). They have wide genetic application in the study of genetic diversity, genotyping, finding connections among collections emanating from diverse geographical origin and gene pool, and identifying core collections to launch easy management and effective utilization of genetic resources (Alemayehu, 2007).

This study was, therefore, aimed at studying the genetic diversity of 65 *Niger* accessions using 11 EST-SSR markers to improve its production and productivity, and conservation as assessing genetic diversity of *Niger* at molecular level is a paramount importance for future breeding program. Previous efforts to characterize diversity of *Niger* populations using anonymous genomic SSR markers was not informative to reveal the levels of intra- and inter-population diversity (Geleta et al., 2007) and the major disadvantage associated with the anonymous genomic SSR is the high cost of development and time- consuming process. In the present study, 11 EST derived SSR markers were used to trace the genetic diversity and evolutionary patterns of 65 *Niger* accessions to reach at better understanding of the origin.

MATERIALS AND METHODS

The detailed material and methods used to evaluate the genetic diversity of 65 *Niger* germplasms using 11 EST- SSR markers is clearly described as follows.

Germplasm collection/study area

In this study, 65 accessions of *Niger* were collected from different agro ecologies of Ethiopia (Table 1). The places of sampling area were systematically selected. Most of the samples were collected from North Gondar, South Gondar, West Gojjam, East Gojjam, West Showa, and North showa, South Wello, Bale, West Wellega, East Wellga, Awi and West Harergia (Table 1).

Selection of markers

There is inconsistency between the choice of markers in terms of cost, time, inheritance of marker, quantity of DNA required, technical labour and degrees of polymorphism, precision of genetic distance estimates and the statistical power of tests. The SSRs were still the preferable markers in assessing genetic diversity, genetic mapping and marker-assisted selection of important traits as SSRs are multi-allelic, easily detectable by PCR, abundantly distributed in genome and codominantly inherited (Gupta and Varshney, 2000).Thus, the certain number and length of SSRs were probable to reflect the evolutionary history in particular species and its relatives. EST derived SSRs markers have higher rate of transferability across related species and genera than anonymous genomic SSRs (Guo et al., 2006, Mishra et al., 2012), and hence are preferable for phylogenetic studies and QTL mapping. In this study, based on the above mentioned promising property SSR markers are selected. Markers that are used in this study are described below in the table.

Sample preparation

The collected planting materials were planted in green house for two weeks. Fresh young leaf materials from three to five leaf numbers were selected to extract DNA. The leaves that were targeted for DNA extraction were taken from young seedlings. The leaf sample was taken from the most upper part, which are active regions undergoing mitosis. The dirty materials and fungal infection or any other contaminant on the surface of leaf materials were removed using 70% ethanol.

DNA extraction

After ten days of collection, DNA was extracted from each fresh and dried leaf following modified CTAB method (Doyle and Doyle, 1990). The dried leaf was grinded using Geno Grinder (MM-200, Retsch). Based on the procedures, cells were fractionated and DNA was extracted. The presence of the genetic material was checked via running the mixes of 5 µL of gDNA and 3 µL of 6X loading dye containing gel red in 0.8% of agarose gel for 30 min at 100 volt, in 1xTAE buffer using Junyl electrophoresis apparatus. The presence and absence of extracted genomic DNA was checked via transilluminator (3uv bench top, M-20 transilluminator). The quantity and purity of DNA was confirmed by Nano drop spectrophotometer (ND-8000, Thermoscitnific). Following the amount purified, proper

Table 1. List of geographical area for sample collection.

S/N	Acce.	Zone	Latitude	Longitude	Altitude
1	241142	Mirab Harerge	08-52-33-N	40-39-90-E	1660
2	243793	Agew Awi	11-19-00-N	36-47-00-E	1890
3	208391	Bahir Dar Special	11-30-00-N	37-18-0 -E	1920
4	243789	Debub Gondar	12-08-00-N	37-50-00-E	2135
5	208391	Bahir Dar Special	11-30-00-N	37-18-0 -E	1920
6	243778	Semen Wello	11-50-00-N	39-32-00-E	1835
7	243796	Agew Awi	10-56-00-N	36-36-00-E	1835
8	208384	Debub Gondar	12-00-00-N	37-39-00-E	1850
9	237518	Mehakelegnaw	14-10-00-N	38-45-00-E	2200
10	243772	Debub Wello	11-24-00-N	39-11-00-E	2060
11	235781	Semen Gondar	12-07-00-N	37-01-00-E	2080
12	238286	Mirab Gojam	10-09-00-N	36-09-00-E	2000
13	243784	Semen Gondar	13-59-00-N	37-47-00-E	2555
14	208946	Misrak Wellega	09-33-00-N	37-12-00-E	2450
15	215681	Debub Wello	11-19-00-N	39-44-00-E	2050
16	238292	Semen Shewa	10-01-00-N	38-02-00-E	2500
17	243775	Debub Wello	11-29-00-N	39-22-00-E	2390
18	243785	Semen Gondar	12-21-00-N	37-31-00-E	1920
19	208394	Agew Awi	11-20-00-N	37-00-00-E	1950
20	243777	Debub Wello	11-45-00-N	39-41-00-E	2020
21	237515	Mehakelegnaw	14-12-00-N	38-56-00-E	2010
22	208947	Misrak Wellega	09-30-00-N	37-03-00-E	2470
23	243786	Semen Gondar	12-21-00-N	37-31-00-E	1920
24	243791	Mirab Gojam	11-38-00-N	37-20-00-E	1870
25	243771	Debub Wello	10-57-00-N	39-47-00-E	1780
26	243783	Semen Gondar	13-02-00-N	38-05-00-E	1310
27	212492	Semen Shewa	09-56-00-N	38-54-00-E	1610
28	243770	Debub Wello	11-10-00-N	39-54-00-E	2070
29	235780	Semen Gondar	12-02-00-N	45-11-00-E	1830
30	243773	Debub Wello	11-20-00-N	39-15-00-E	2400
31	238291	Semen Shewa	10-01-00-N	38-02-00-E	2490
32	15191	Agew Awi	10-58-00-N	36-33-00-E	2080
33	15034	Mirab Shewa	09-01-00-N	38-25-00-E	2340
34	15059	Mirab Shewa	09-00-00-N	38-17-00-E	2140
35	238288	Misrak Gojam	10-02-00-N	37-09-00-E	2180
36	229989	Bale	06-36-00-N	39-35-00-E	1430
37	15136	Mirab Shewa	10-05-00-N	39-00-00-E	1800
38	15135	Semen Shewa	10-02-00-N	38-52-00-E	1600
39	15125	Mirab Shewa	08-37-00-N	38-12-00-E	2240
40	234144	Misrakawi	14-11-00-N	38-37-00-E	2210
41	15002	Mirab Gojam	10-42-00-N	37-03-00-E	2100
42	15162	Debub Wello	11-15-00-N	39-45-00-E	1964
43	15196	Mirab Shewa	08-58-00-N	37-36-00-E	2100
44	15055	Mirab Shewa	08-58-00-N	37-52-00-E	2091
45	15167	Mirab Shewa	09-04-00-N	38-30-00-E	2390
46	15154	Mirab Wellega	09-47-00-N	35-08-00-E	1650
47	234135	Mirabawi	14-09-00-N	38-16-00-E	1900
48	15062	Mirab Shewa	09-09-00-N	37-10-00-E	1700
49	234136	Mirabawi	14-07-00-N	38-20-00-E	2930
50	15198	Mirab Shewa	09-00-00-N	38-09-00-E	2240
51	234133	Mirabawi	14-06-00-N	38-14-00-E	1850

Table 1. Contd.

52	15031	Mirab Shewa	09-01-00-N	38-20-00-E	2160
53	15159	Misrak Wellega	08-50-00-N	36-29-00-E	2250
54	235399	Semen Gondar	36-32-00-N	37-20-00-E	1850
55	15134	Semen Shewa	07-33-00-N	37-35-00-E	2350
56	15010	Bahir Dar Special	11-34-00-N	37-23-00-E	2000
57	15161	Debub Wello	11-18-00-N	39-40-0 -E	2000
58	15144	Mirab Shewa	08-58-00-N	37-36-00-E	2340
59	15079	Bale	07-01-00-N	39-24-00-E	2430
60	15142	Mirab Shewa	08-58-00-N	37-52-00-E	2010
61	15081	Mirab Shewa	08-59-00-N	37-22-00-E	1800
62	15004	Mirab Gojam	10-34-00-N	37-29-00-E	2050
63	15129	Mirab Shewa	09-01-00-N	38-20-00-E	2200
64	15037	Mirab Shewa	08-08-00-N	38-01-00-E	2400
65	235877	Semen Gondar	12-32-00-N	37-32-00-E	2050

Table 2. List and characteristics of EST-SSR markers used to assess the genetic diversity of *Niger.*

Locus	Repeat motifs	Forward primer(F, 5'-3') Sequence Versus Reveres primers (R, 3'-5') Sequence	Expected allele size	Linked Loci
GA003	$(gat)_2$	CGCCCTAAAGCTACTTTCTTCC-F CACACTCGCACTAGGA-R	399-402	GA127,GA238
GA012	$(gat)_2$	CAGTAAGCTCGGTATCTCCAAGTT-F AGAAGATCTCGTCAGCAGAAACAG-R	263-275	GA107,GA138
GA013	$(ctt)_9$	GGTAATGGTAATGGAGGTTCTGG-F CCTCATCAGAGTTCTTCGGGTTAT-R	424-455	None
GA018	$(agc)_2$	GTTCCAGCCCATGAGTCATAA-F CTATCTCTTATCTCGTGGGGTTTTG-R	353-358	GA 183,GA186
GA029	$(atc)_3$	CCATCATCAATGGCGTTACTC-F GTCTCGTTCTAGAAGCTTCATCCT-R	270-276	GA108,GA143,GA186
GA035	$(tga)_3$	GATTTCTCAGGTGAAGGA-F GCCCTCCCTACAACATACTTGATA-R	301-307	GA107,GA144,GA217
GA037	$(ta)_2$	GGTGTTTTTGTGTAGTGGTCTGTC-F GACTAGCCAGAAACCGAAGAATC-R	347-350	GA081
GA055	$(ct)_3$	CCTGAAACAAACCCCAACAA-F CAGTACATCGCGGAGAGAGG-R	194-200	GA191,GA205
GA127	$(cct)_2$	CAATCTGCAACTACTGCCATACC-F CCAGTCAGAACCCTTGATCACTA-R	213-216	GA003,GA117
GA138	$(aag)_5$	ATCAACTTCCCCATATACCTCTGG-F CTTCCTCTGTCACTTCTTTTGGAC-R	363-378	GA018,GA0035GA108, GA183
GA139	(gaa)7	GTACATCCCAACTTTACCATCCAC-F CTCTACAACCAACACCACTTTCC-R	223-241	GA077,GA238

Source: Dempewolf et al. (2010a).

concentration of DNA was determined for further use. DNA was stored in the refrigerator till the next use. The DNA extracted from different samples was coded carefully. DNA sample from the autonomous organelles, nuclear and chloroplast was included for the case of tracing evolutionary origin of *G. abyssinica.*

Polymerase chain reaction and acquisition of markers

The primers (Table 2) were obtained from published article (Dempewolf et al., 2010a). It was ordered and purchased from companies. The SSR region was amplified using proper PCR condition and reaction mixtures by using master cycler (Pros, eppendorf). A proper 96 well PCR plate with an allowed reaction mixture was in place. The touchdown PCR techniques were used with a program of 94°C for 4 min, 94°C for 45 s, 60 to 55°C for 1 min and 72°C for 1 min 30 s running for 9 cycle, and 94°C for 45 s, 55°C for 1 min and 72°C for 1 min 30 s running for 26 cycle and eventually, 72°C for 5 min final extension was suggested for all

Table 3. List of polymorphic SSR markers, allele frequency, gene diversity and PIC.

Locus	Major allele frequency	Gene Diversity	Heterozygosity	PIC
GA003	0.5846	0.4857	0.7692	0.3677
GA012	0.6308	0.4658	0.6154	0.353
GA013	0.6769	0.4374	0.4923	0.3417
GAO18	0.6615	0.4478	0.4615	0.3475
GA029	0.7231	0.4005	0.4923	0.3203
GA035	0.7538	0.3711	0.3692	0.3023
GA037	0.8077	0.3107	0.3231	0.2624
GA055	0.7000	0.4200	0.5692	0.3318
GA127	0.7154	0.4072	0.3231	0.3243
GA138	0.7077	0.4137	0.4615	0.3281
GA139	0.6385	0.4617	0.6615	0.3551
Mean	0.6909	0.4201	0.5035	0.3308

EST-SSR markers used (Cubry et al*.,* 2008). The total reaction volume and volume for each reactant including the volume of Taq DNA Polymerase was determined following the most promising PCR reaction mixture that used in article (Cubry et al*.,* 2008).

Gel electrophoresis

Gel electrophoresis was used to analyze the presence and absence of genomic DNA and PCR product. Simple sequence repeat were analyzed using polyacrylamid gel electrophoresis, to compute the size and number of alleles (Wang et al*.,* 2009). The accuracy of the PCR experiment was checked and yield of the PCR product was properly quantified in 1.2% agarose gel. 40% of (29:1, acrylamid: bis acrylamid solution) Polyacrylamid gel was used to count alleles and see patterns of SSR in the genome of the entire varieties using vertical electrophoresis apparatus (Cleaver, CS500 volt).

Scoring and data analysis

The clear and visible amplified PCR products were scored for presence (1) and absence (0) and the data were subjected to analysis using diverse tools of statistics and bioinformatics softwares. Dendrogram was constructed using Darwin 6.0 software (Perrier et al., 2003; 2006). A dendrogram was constructed using neighbour joining (NJ) as implemented in the same software. The number of alleles, gene diversity and polymorphism information content (PIC) were calculated using Power Marker V3.25 (Liu and Muse, 2005), Observed heterozygosity was calculated using Arlequin V3.1, and the number of private alleles were calculated using GenAlEx 6.41.

RESULTS

SSR amplified product and PIC statistics

In this study, the genetic relationships of 65 *Niger* germplasm were analyzed using 11 Expressed Sequence Tagged derived Simple Sequence Repeats (EST-SSRs) markers. 19 SSR markers were obtained from published articles (Dempewolf et al*.,* 2010a), and screened to select 11 of them which were polymorphic with understandable and solid band for statistics on 65 germplasm, and produced 66 total numbers of alleles with an average of 9 alleles per locus. Locus GA037 showed greater allele frequency, gene diversity and PIC. Whereas GA003 showed lower allele frequency, gene diversity, heterozygosity and PIC (see Table 3).

Dendrogram constructed using EST-SSR markers

Based on the dissimilarity matrix the entire accessions were grouped in to three major clusters and six sub clusters. The minimum and maximum dissimilarity values for the analyzed accessions were 0.056 and 0.75 respectively. The phylogenetic tree constructed using Jaccard coefficients (Figure 1). The dendrogram was shown with three different colours (Red= for accessions collected from Tigray regions, Green= from Amhara and Black= from oromia region) which is used to indicate the location of major *Niger* growing area in Ethiopia.

DISCUSSION

All the 11 EST-SSR markers used in this study are polymorphic (100%). Gupta et al. (2003) identified only 55% of 20 EST-SSR markers used were polymorphic among 52 wheat accessions. Eujayl et al*.* (2002) reported a lower level of polymorphism (25%) when 42 EST-SSR markers screened against 64 durum wheat germplasm lines. This clearly indicates that the percentage of polymorphism depends on number and nature of the material used under analysis.

In the present study, data analysis was carried out with 11 EST-SSR markers by following SMM model which assumes that each mutation (insertion and deletion) creates a new allele. A similar pattern of allele scoring was observed with genomic SSR markers in Folkertsma et al*.* (2005).

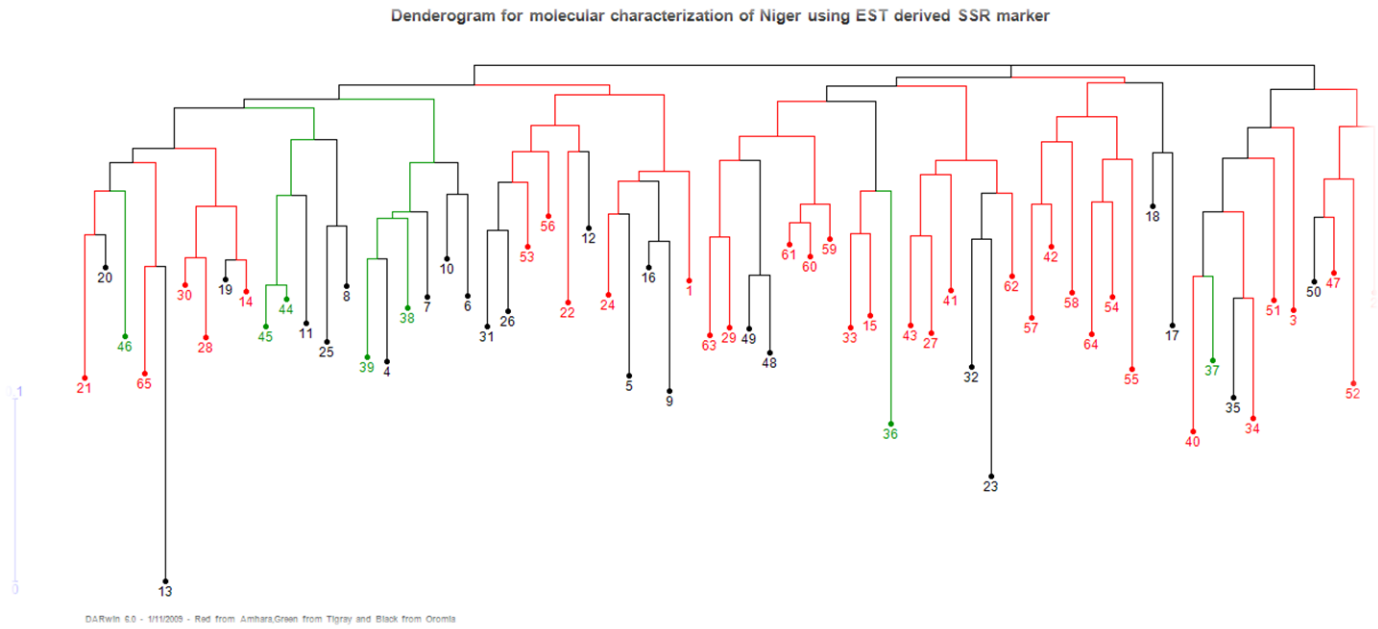

Figure 1. Dendrogram showing the genetic diversity of 65 *Niger* germplasm using SSR markers.

In total, 11 EST-SSR markers produced 66 alleles with an average of 9 alleles per locus (Table 3). This is the maximum number of alleles per markers reported using EST-SSR markers in any cereals to date. In case of tall fescue grass, an average of 2.78 alleles/marker were reported (Saha et al*.,* 2004), while 1.8 alleles/marker in bread wheat (Gupta et al*.,* 2003) with 20 EST-SSRs, 4.5 alleles/markers in durum wheat with 42 EST-SSRs (Eujayl et al., 2002), 3 alleles/markers in 54 barley accessions using 38 EST-SSR markers (Thiel et al*.*, 2003), and 4.6 alleles/marker in *Crotalaria* species (Wang et al*.,* 2006). Compared to results obtained with neutral genomic SSRs, the average number of alleles per marker detected in this study is comparable to that found in limited size core collection (Caniato et al*.,* 2007), or on geographically limited studies (e.g. Barnaud et al*.,* 2007; Deu et al*.,* 2008), but is lowest than found on the same material (Billot et al*.,* 2013).

The PIC values of markers can provide an estimate of discrimination power in a set of accessions by taking not only the number of alleles, but also the relative frequencies of each allele (Smith et al*.,* 2000). The average PIC value of EST-SSR markers (0.3308) was a bit higher in this references set of *Niger* in comparison with previous studies using EST-SSR markers for genetic diversity analysis in other crops, e.g., 0.443 in bread wheat (Gupta et al., 2003), 0.45 in barley (Thiel et al*.,* 2003). However, the average PIC value was lower compared to PIC values of genomic SSR markers in sorghum [0.62 in both studies of Agrama and Tuinsta (2003), and Caniato et al*.* (2007)]. However, this is higher than PIC value reported by Folkertsma et al*.* (2005) using 100 guinea race accessions and 21 genomic SSR markers and Ali et al*.* (2008) using 72 sorghum accessions with 41 SSR markers. This is on par with PIC (0.54) reported by Wang et al*.* (2009) in a study involving 96 sweet sorghum lines and 95 SSRs. High PIC values and large number of alleles per markers can also be attributed to the nature of the *Niger* materials that is studied. SSR markers containing dinucleotide repeats produced more alleles and hence, greater PIC values (Table 3). These results were in harmony with previous studies by Smith et al*.* (2000), Agrama and Tuinstra (2003), Casa et al. (2005), and Deu et al*.* (2008).

Conclusion

Genetic diversity analysis with proper genotyping using EST-SSR marker will help the breeders to mine trait-specific alleles and facilitate an effective way of identifying the gene for different agronomic traits. The present study showed the presence of considerable variations among *Niger* genotypes. The presence of this considerable variation among *Niger* genotypes has great promise as parents to obtain promising heterotic expression in F1's and may create considerable variability in the segregating populations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Agrama HA, Tuinstra MR (2003). Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. Afr J. Biotechnol. 10:334-340.

Alemayehu TN (2007). Genetic diversity of *Coffea arabica L.* collections using Microsatellite (SSRs) Markers. EJAST 1(1): 63-79.

Ali ML, Rajewski JF, Baenziger PS, Gill KS, Eskridge KM, Dweikat L (2008). Assessment of genetic diversity and relationship among a collection US sweet sorghum germplasm by SSR markers. Mol. Breed. 21:497-509.

Barnaud A, Deu M, Garine E, McKey D, Joly H (2007). Local genetic diversity of sorghum in a village in northern Cameroon: structure and dynamics of landraces. Theor. Appl. Genet. 114:237-248.

Billot C, Ramu P, Bouchet S, Chantereau J, Deu M, Gardes L, Noyer JL, Rami JF, Rivallan R, Li Y, Lu P, Wang T, Folkertsma RT, Arnaud E, Upadhyaya HD, Glaszmann JC, Hash CT (2013). Massive sorghum collection genotyped with SSR markers to enhance use of global genetic resources. PLoS ONE. 8:e59714.

Boardman SJ (1999). The agricultural foundation of the Aksumite empire, Ethiopia. Plants and People in Africa. Recent Archaeobotanical Evidence. New York. Plenum Publishing Corp. Pp.137-147.

Boardman SJ (2000). Archaeobotany. Archaeology at Aksum, Ethiopia, 1993-7. (ed D.W. Phillipson) . The British Institute in Eastern Africa and the Society of Antiquaries, London. Pp. 363-368.

Bulcha W, Misteru T, Nigussie A, Adunga W, Million F, Adefris T (2005). Review of Genetics and Breeding Research on Noug (*Guzoita abyssinic (L.f) Cass.)* undertaken over the period of 1999 – 2003. Holetta Agricultural Research Center. Highland Oil Crops Improvement Program.

Caniato FF, Guimaraes CT, Schaffert RE, Alves VM, Kochian LV, Borem A, Klein PE, Magalhaes JV (2007). Genetic diversity for aluminum tolerance in sorghum. Theor. Appl. Genet. 114(5):863-876.

Casa AM, Mitchell SE, Hamblin MT, Sun H, Bowers JE, Paterson AH, Aquadro CF, Kresovich S (2005). Diversity and selection in sorghum: simultaneous analyses using simple sequence repeats. Theor. Appl. Genet. 111(1):23-30.

Cubry P, Musoli P, Legnate H, Pot D, De Bellis F, Poncet V, Anthony F, Dufour M, Leroy T (2008). Diversity in coffee assessed with SSR markers: structure of the genus *Coffea* and perspectives for breeding. Genome 51:50-63.

Dempewolf H, Nolan C, Katherin L, Mulatu G, Mickale S, ZHAO L, Megan L, Endeshaw B, Johansen M, Quantin C, Lorin H (2010a). Establishing genomic tool and resource for guzotia abyssinica (L.f) Cass, the development of a library of expressed sequence tags, microsatellite loci, and the sequencing of its chloroplast genome. Mol. Ecol. Resour. 10:1046-1058.

Deu M, Sagnard F, Chantereau J, Calatayud C, Hérault D, Mariac C, Pham J-L, Vigouroux Y, Kapran I, Traore PS, Mamadou A, Gerard B, Ndjeunga J, Bezancon G (2008). *Niger*-wide assessment of in situ sorghum genetic diversity with microsatellite markers. Theor. Appl. Genet. 116:903-913.

Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Eujayl I, Sorrells ME, Wolters P, Baum M, Powell W (2002). Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. Theor. Appl. Genet. 104:399-407.

Folkertsma RT, Rattunde FH, Chandra S, Soma Raju W, Hash CT (2005). The pattern of genetic diversity of Guinea-race Sorghum bicolor (L.) Moench landraces as revealed with SSR markers. Theor. Appl. Genet. 111:399-409.

Geleta M, Bryngelsson T, Bekele E, Dagne K (2007). Genetic diversity of *Guizoita abyssinica (L.f.)* Cass. (Asteraceae) from Ethiopia as revealed by random amplified polymorphic DNA (RAPD). Genet. Resour. Crop Evol. 54:601-614.

Getinet A, Sharma SM (1996). *Niger* (Guizotia abyssinica) (L.f.) Cass. In: Promoting the Conservation and Use of Underutilized and Neglected Crops. Rome, Italy. IPGRI. 5:59.

Guo W, Wang W, Zhou BA, Zhang T (2006). Cross-species transferability of Garboreum-derived EST-SSRs in the diploid species of Gossypium. Theor. Appl. Genet. 112:1573-1581.

Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS (2003). Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol. Gen. Genomics 270:315-323.

Gupta PK, Varshney R (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113(3):163-185.

Liu K, Muse SV (2005). PowerMarker: Integrated analysis environment for genetic markers data. Bioinformatics 21(9):2128-2129

Mishra RK, Gangadhar BH, Nookaraju A, Kumar S, Park SW (2012). Development of EST-derived SSR markers in pea (Pisum sativum) and their potential utility for genetic mapping and transferability. Plant Breed. 131:118-124.

Pejio I, Ajmone-Marsan P, Morgante M, Kozumplic V, Castiglione P, Taramino G, Motto M (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. Theor. Appl. Genet. 97:1248-1255.

Perrier X, Flori A, Bonnot F (2003). Methods for data analysis. In. Hamon P, Seguin M, Perrier X, Glaszmann JC (eds) Genetic diversity of cultivated tropical plants. Science Publishers, Montpellier, Inc. and CIRAD. Pp. 31-63.

Perrier X, Jacquemoud-Collet JP (2006). DARwin software. http://darwin.cirad.fr/darwin

Petros Y, Merker A, Zeleke H (2007). Analyses of genetic diversity of Guizotia abyssinica from Ethiopia using inter simple sequence repeat markers. Hereditas 144:18-24.

Saha MC, Mian MAR, Eujayl I, Zwonitzer JC, Wang L, May GD (2004). Tall fescue EST-SSR markers with transferability across several grass species. Theor. Appl. Genet. 109:783-791.

Smith JSC, Kresovich S, Hopkins MS, Mitchell SE, Dean RE, Woodman WL, Lee M, Porter K (2000). Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Aust. J. Crop Sci. 40:226-232.

Thiel T, Michalek W, Varshney RK, Graner A (2003). Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (Hordeum vulgare L.). Theor. Appl. Genet. 106:411-422.

Wang ML, Mosjidis JA, Morris JB, Dean RE, Jenkins TM, Paderson GA (2006). Genetic diversity of*talaria* germplasm assessed through phylogenetic analysis of EST-SSR markers. Genome. 49:707-715.

Wang ML, Zhu C, Barkley NA, Chen Z, Erpelding JE, Murray SC, Tuinstra MR, Tesso T, Pederson GA, Yu J (2009). Genetic diversity and population structure analysis of accessions in the US historic sweet sorghum collection. Theor. Appl. Genet. 120:13-23.

Wang X, Rinehart TA, Wadl PA, Spiers JM, Hadziabdic D, Windham MT, Trigiano RN (2009). A new electrophoresis technique to separate microsatellite alleles. Afr. J. Biotechnol. 8(11):2432-2436.

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Effect of inter and intra row spacing on the yield and yield components of Onion (*Allium cepa*L.) at Mehoni District, Northern Ethiopia

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Onion is one of the most important high market value bulb crops cultivated commercially in most parts of the world. It is the most cultivated and high market value of vegetable crops In Tigray Northern Ethiopia. However, bulb yield of onion is limited due to improper use of plant spacing. Therefore, field experiment was conducted to determine the optimum planting spacing of Onion to maximize its productivity in 2014 and 2015 under irrigation conditions. The trial contains treatment of singe planting pattern of 20 x 10, 40 x 10, 30 x 5, 30 x 7.5, 30 x 10 and 30 x 12.5 cm inter and intra spacing respectively and double planting pattern combinations of four intra plant spacing (5, 7.5,10 and 12.5 cm) and two levels of inter spacing (40 and 50 cm) by means of total fourteen treatments were carried out in Randomized Complete Block Design (RCBD) with three replications. The current findings showed that plant height, bulb size, bulb weight, total bulb yield and marketable bulb yield were significantly influenced by planting spacing. Accordingly, the tallest plant height (37.70 cm) was obtained at a spacing of single planting of 40 cm inter row and 10 cm intra row while the maximum total bulb yield of 27.01 ton ha^{-1}) was recorded at a spacing of double planting pattern of 40 cm inter and 5 cm intra row in both years. Therefore, 40 cm inter row and 5 cm intra row spacing in double row planting manner is recommended for the growers to improve onion productivity in the study area.

Key words: Inter, intra, row, onion, spacing, yield.

INTRODUCTION

Onion (*Allium cepa* L.) belongs to the genus Allium of the family *Alliaceae* (Hanelt, 1990; Griffiths et al*.,* 2002). Onion is the most important bulb crops cultivated commercially in most parts of the world. The crop is grown for consumption both in the green state as well as in mature bulbs. Onions exhibit particular diversity in the

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eastern Mediterranean countries, through Turkmenistan, Tajikistan to Pakistan and India, which are the most important sources of genetic diversity and believed to be center of origin (Brewster, 2008). Onion is one of the most popular and the most cultivated vegetables in Ethiopia in general and in Tigray region in particular (Hailu et al., 2015). So far, research in the country was mainly focused on the identification of superior cultivars of onions and adopting improved management practices. Different cultural practices and growing environments are known to influence yield and quality of dry bulbs. Spacing has effect on different varieties as their root and leaf growth habits differ (Yemane et al., 2013).

Onion is considerably important in the daily Ethiopian diet, mostly used as seasonings or as vegetables in stews (MoARD, 2009). It is one of the richest sources of flavonoids in the human diet and flavonoid consumption has been associated with a reduced risk of cancer, heart disease and diabetes. In addition, it is known for anti bacterial, antiviral, anti-allergenic and anti-inflammatory potential. Onions are spread throughout the country being cultivated under both irrigated as well as rain fed conditions in different agro-climatic regions due considerably increasing its important in the daily Ethiopian diet. Onions are spread throughout the country being cultivated under both irrigated as well as rain fed conditions in different agro-climatic regions due considerably increasing its important in the daily Ethiopian diet (Lemma and Herath, 1992)

According to Geremew et al. (2010), yield and quality of dry bulbs can be influenced by cultural practices and growing environments. So far, research in the country was mainly focused on the identification of superior cultivars of onions and adopting improved management practices for better yield. The control of plant spacing is one of the cultural practices to control bulb size, shape and yield. The higher yield and better control of over or under bulb size could be obtained if plants are grown at optimum density. Bulb neck diameter, mean bulb weight and plant height decreased as population density increased. Total bulb yield can be increased as population density increases (Kantona et al., 2003).

Although the focus of research in the country was mainly on the identification of superior cultivars of onions and adopting, yield and quality of dry bulbs influenced by different cultural practices and growing environments. Spacing has effect on different varieties as their root and leaf growth habits differ (Yemane et al., 2013).

One of the major problems in onion production in the area is lack of proper agronomic practice and improved variety 10 cm spacing between plants is the nationally recommendation of onion, in central rift valley condition of the country some years back. Nevertheless, farmers did not adopt the recommendation in the real situation; they practice it a bit narrower or wider spacing. In Ethiopia, the crop is more intensively consumed than any other vegetable crops produced in the country. Despite that, the productivity of the crop remains with the average productivity of (10.02 $tonha^{-1}$ which is very low compared to world average yield of 19.7 $tonha^{-1}$ (Negasi, 2014).

Farmers and investors have been producing onion widely in Raya Azebo and Raya Alamata districts. Current market preference on bulbs size and variety of onion necessitate an evaluation of the response of yield and bulb size to plant populations. Results obtained from these evaluations can aid growers to decide the optimum spacing of onion. However, a research has not been conducted on improved agronomic practices like plant spacing to improve the productivity of onion in the study area. Therefore, the present study was conducted to investigate the effects of inter intra and row spacing for the productivity of onion in the study area.

Appropriate spacing enables the farmers to keep appropriate plant population in the field. Therefore, it can avoid excess or less population in a given plot of land which has negative result on yield and quality of onion. Spacing of 40 × 20 × 10 cm between furrow, row and plants, respectively has been used for onion production in Ethiopia. But producers complain 10 cm intra row spacing produces large bulb size which is not preferred by consumer for home consumption. (Habtamu et al., 2016). The present study was therefore undertaken to investigate the effects of different intra-row and inter row spacing for onion productivity in the study area.

MATERIALS AND METHODS

Description of the study site

The experiment was carried out at Mehoni Agricultural Research Center testing site in 2014 and 2015 under irrigation conditions .The site is located 12°41’50” N and longitudes of 39°42’08” E. It is 678 km north of Addis Ababa. The area is situated at an altitude of 1578 m above sea level (m.a.s.l) with mean annual rainfall of 750 mm and minimum and maximum annual temperature of 18 and 25°C, respectively. The soil is clay-loam texture f with a pH value of 7.9 at the soil depth of 0-30 cm (Haileslassie et al., 2015).

Experimental materials, treatment and design

The trial contains treatment of singe planting pattern of 20 x 10, 40 x 10, 30 x 5, 30 x 7.5, 30 x 10 and 30 x 12.5 cm inter and intra spacing respectively and double planting pattern combinations of four intra plant spacing (5, 7.5, 10 and 12.5 cm) and two levels of inter spacing (40 and 50 cm) by means of total fourteen treatments were carried out in Randomized Complete Block Design (RCBD). The experiment contains three replications having a plot size of 2.4m x 2 m for each experimental unit. Bombay Red variety was used as test crop for the experiment to all treatments.
The treatment (T) combination (Table 1) comprised:

Experimental procedure

UREA and DAP were used as the sources of Nitrogen and Phosphorous respectively.46 kg P_2O_5 ha^{-1} was applied in band application method at transplanting time. Similarly, 23 kg ha^{-1} of N was applied in two splits 20 days after transplanting and the 30

Table 1. Treatment combination.

Single planting pattern	Double planting pattern
T1. Flat planting of 20x10 cm	T7. 40 x5 cm
T2. 40x10 cm	T8. 40 x7.5 cm
T3.30x5 cm	T9. 40 x10 cm
T4. 30x7.5 cm	T10. 40 x12.5 cm
T5.30x10 cm	T11. 50 x5 cm
T6. 30x12.5 cm	T12. 50 x7.5 cm
	T13. 50 x10 cm
	T14. 50x12.5 cm

days after the first nitrogen applied. Onion was transplanting when 3-4 true leaves emerged that was in 55 days from the time of sowing. The transplanting time was in the morning in order to decrease the shocking of the plant. Ridomil was applied as a protective spray before the outbreak of the fungal disease at nursery and after transplanting in the mid stage. Other cultural management practices were done according to the national recommendation for all experimental units.

Data collection and analysis

Data collection: Important morphological, phenological and yield components of onion was collected.

Morphological and phenological traits

Plant height (cm): Five plants from the net plot area were pre tagged to collect data of plant height. It was measured using ruler from the soil surface up to the tip the leaves at bulb development stage.

Days to maturity (days): The actual number of days from seedling transplanting to the field to a day at which of the plants in a plot showing yellowing of leaves was recorded to determine the days to physiological maturity.

Bulb yield and yield components

Average bulb weight (g): Five plants from the net plot area were pre tagged to collect data of average bulb weight and expressed in gram.

Bulb size (cm): Five plants from the net plot area were pre tagged to collect data of average bulb size and expressed in centimeter.

Marketable bulb yield (MBY) (t ha^{-1}): Total weight of clean, disease and damage free bulbs were measured per net plot and converted to t ha^{-1}.

Unmarketable bulb yield (MBY) (t ha^{-1}): Total weight of decay, physiological disorder such as thick necked, split and bolters were measured per net plot and converted to t ha^{-1}.

Total bulb yield (TBY) (t ha^{-1}): Total weight of marketable and unmarketable bulbs were measured per net plot and converted to t ha^{-1}.

The data were collected from middle rows of a net plot area where the two outer most rows of each treatment were left as border effects. In addition, 0.05, 0.075, 0.1 and 0.12 m length in both ends for 5, 7.5,10 and 12 cm intra row spacing, respectively, of each harvestable row were also left as border effects.

Statistical analysis

All data were subjected to the analysis of variance(ANOVA)using the SAS (9.1)software computer package (SAS Institute, 2004) and significance difference among the treatment means was computed with Duncan's Multiple Range Test(DMRT) at 5% probability level (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Plant height

Plant height was significantly affected by planting space in both years. Tallest plant height of 37.70 cm was recorded at single planting spacing of 40 x 10 cm in 2014 where as the shortest plant height of 28.03 cm was recorded at double spacing of 40 x 5 cm. In 2015 irrigation season, the tallest plant height of 53.67 cm) was also recorded at single spacing of 40 x 10 cm while the shortest (40.33 cm) was obtained at double spacing of 40 x 5 cm (Table 2). The greatest plant height may be due to the availability of free access of environmental resources (water, nutrient and light) for the plants in the wider plants. In line with this result, Tesfalegn (2015) reported that plant height of onion plants was significantly affected by intra spacing of cultivars. The highest plant height was obtained at 10 cm and the lowest plant height at 4 cm. Similarly, Jilani et al. (2009) and Sikder et al. (2010) also reported the closer intra-row spacing resulted short plant height than wider plant spacing.

Days to 90% physiological maturity

Days to 90% physiological maturity showed no significant (P>0.05) variation to planting spacing (Table 1). This might be due to the less computation of plants for growth resources. Generally, it matured at a range of 82.33 to 88.00 starting from its transplanting time (Table 1).

Mean bulb size

There was significance difference (P<0.5) among the treatments of spacing on bulb size of onion. The highest bulb size was recorded at the spacing of of 50 x 12.5 cm followed by 50 x 10 cm in double planting manner. The smallest bulb size was also recorded at double planting of 40 x 5 cm in both years (Table 3). In agreement with the present result, Yemane et al. (2013) reported that as the intra-row spacing increased from 5 to 10 cm, the percentage of large size bulbs increased from 9.3 to 20.3. Similarilly, Jilani et al. (2009) reported that significant

Table 2. Mean number of days required for physiological maturity and plant height of onion under Irrigation.

Treatment	Plant height (cm)		Days to maturity		Mean
	2014	2015	2014	2015	
Single planting					
20 x 10 cm	33.18^{a-e}	44.00^{cde}	86.33	83.00	84.7
40 x 10 cm	37.70^{a}	53.67^{a}	85.33	83.67	84.5
30 x 5 cm	31.20^{c-f}	48.00^{a-d}	86.00	84.00	85.0
30 x 7.5 cm	35.70^{abc}	49.67^{abc}	86.00	84.00	85.0
30 x 10 cm	33.33^{a-e}	44.67^{cde}	87.00	83.33	85.2
30 x 12.5cm	34.27^{a-d}	43.00^{cde}	85.67	83.67	84.7
Double planting					
40 x 5 cm	28.03^{f}	40.33^{e}	86.33	83.00	84.7
40 x 7.5 cm	29.90^{def}	42.67^{cde}	86.33	83.00	84.7
40 x 10 cm	30.97^{cdef}	43.33^{cde}	86.33	84.00	85.2
40 x 12.5 cm	36.17^{ab}	52.33^{ab}	87.33	83.33	85.3
50 x 5 cm	29.20^{ef}	42.00^{de}	86.33	83.00	84.7
50 x 7.5 cm	31.60^{c-f}	42.67^{cde}	86.67	82.33	84.5
50 x 10 cm	33.83^{a-e}	46.67^{a-e}	88.00	82.33	85.2
50 x 12.5 cm	34.60^{a-d}	45.33^{b-e}	87.67	83.00	85.3
CV (%)	7.57	8.45	1.90	1.72	
Level of significance	*	*	NS	NS	

Means followed by the same letter (s)in the same column are not significantly different at 5% probability level according to Duncan's Multiple Range Test; NS= non-significant, *=significance at 5%.

Table 3. Mean bulb size and bulb weight for onion as influenced by plant spacing under irrigation.

Treatment	Bulb size (cm)			Average bulb weight (g)		
	2014	2015	Mean	2014	2015	Mean
Single planting						
20 x 10 cm	5.27^{bcde}	5.2^{bcdef}	5.24	76.00	73.8^{de}	74.90
40 x 10 cm	5.73^{abcd}	5.57^{bcd}	5.65	80.67	87.2^{ab}	83.94
30 x 5 cm	5.43^{bcd}	4.9^{defg}	5.17	74.67	74.9^{cde}	74.79
30 x 7.5 cm	5.33^{bcde}	5.3^{bcde}	5.32	73.33	70.9^{e}	72.12
30 x 10 cm	5.47^{bcd}	5.0^{cdefg}	5.24	82.00	76.9^{bcde}	79.45
30 x 12.5 cm	5.80^{abc}	5.63^{bc}	5.72	86.33	83.7^{abcd}	85.02
Double planting						
40 x 5 cm	4.47^{e}	4.36^{g}	4.42	78.33	75.43^{cde}	76.88
40 x 7.5 cm	5.17^{bcde}	4.60^{efg}	4.89	74.00	74.43^{cde}	74.22
40 x 10 cm	5.57^{abcd}	4.83^{efg}	5.20	79.00	76.8b^{cde}	77.90
40 x 12.5 cm	5.40^{bcd}	4.83^{efg}	5.12	78.67	77.8b^{cde}	78.24
50 x 5 cm	4.83^{de}	4.53^{fg}	4.68	77.33	71.20^{e}	74.27
50 x 7.5 cm	5.13^{cde}	5.67^{b}	5.40	81.33	85.33^{ab}	83.33
50 x 10 cm	6.06^{ab}	6.3^{a}	6.18	87.67	88.13^{ab}	87.90
50 x 12.5 cm	6.37^{a}	6.4^{a}	6.39	82.00	89.67^{a}	85.84
CV	8.58	7.08		8.68	7.77	8.23
Level of significance	**	*		NS	**	

Means with the same letter (s) in the same column are not significantly different; *= Significant at 5% probability level; **= significant at 1% probability level; NS= non-significant.

Table 4. Mean total bulb yield, marketable bulb yield and un marketable bulb yield for Onion as affected by plant spacing under Irrigation.

Treatment	Total bulb yield (t ha^{-1})			Marketable bulb yield (t ha^{-1})			Unmarketable bulb yield (t ha^{-1})		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
Sin planting									
Flat 20 x 10 cm	20.99^{a}	28.80^{abc}	24.90^{ab}	19.07^{abc}	26.09^{a-c}	22.58^{a-d}	1.92^{a}	2.72^{a}	2.32^{a}
40 x 10 cm	16.72^{abc}	19.72^{de}	18.22^{de}	15.73^{bcd}	17.78^{d}	16.75 ^{e}	0.99^{d}	1.95^{b}	1.47^{bcd}
30 x 5 cm	19.17^{abc}	30.47^{ab}	24.82^{ab}	18.02^{a-d}	28.61^{ab}	22.32^{ab}	1.15^{cd}	1.86^{b}	1.5^{bc}
30 x 7.5 cm	18.03^{abc}	24.93^{cde}	21.48^{b-e}	16.30^{a-d}	23.69^{bcd}	20.00^{b-e}	1.72^{bcd}	1.24^{cd}	1.48^{bc}
30 x 10 cm	16.18^{bc}	20.07^{de}	18.13^{de}	15.13^{cd}	19.08^{cd}	17.11^{e}	1.05^{cd}	0.99^{d}	1.02^{de}
30 x 12.5 cm	14.65^{c}	19.37^{de}	17.01^{e}	13.79^{d}	18.42^{cd}	16.10^{e}	0.87^{d}	0.96^{d}	1.91^{e}
Dou planting									
40 x 5 cm	21.28^{a}	32.92^{a}	27.01^{a}	20.60^{a}	31.30^{a}	25.95^{a}	0.67^{d}	1.62^{bc}	1.15^{cde}
40 x 7.5 cm	20.96^{a}	27.26^{a-d}	24.11^{abc}	19.69^{ab}	25.91^{abc}	22.80^{abc}	1.27^{bcd}	1.35^{cd}	1.31^{b-e}
40 x 10 cm	18.13^{abc}	22.50^{cde}	20.31^{cde}	16.23^{bcd}	21.38^{bcd}	18.81^{cde}	1.9^{ab}	1.16^{d}	1.51^{bc}
40 x 12.5 cm	20.56^{ab}	20.42^{de}	20.49^{b-e}	19.32^{abc}	19.41^{cd}	19.37^{b-e}	1.24^{cd}	1.00^{d}	1.12^{cde}
50 x 5 cm	20.86^{a}	22.85^{b-e}	21.86^{bcd}	18.61^{abc}	21.70^{bcd}	20.16^{b-e}	2.25^{a}	1.14^{d}	1.7^{b}
50 x 7.5 cm	18.89^{abc}	20.56^{de}	19.72^{cde}	17.74^{a-d}	19.54^{cd}	18.64^{de}	1.15^{cd}	1.02^{d}	1.08^{cde}
50 x 10 cm	19.10^{abc}	19.72^{de}	19.41^{de}	17.97^{a-d}	18.75^{cd}	18.36^{e}	1.13^{cd}	0.97^{d}	1.05^{cde}
50 x 12.5 cm	17.01^{abc}	18.68^{e}	17.85^{de}	16.00^{bcd}	17.76^{d}	16.88^{e}	1.01^{cd}	0.92^{d}	0.97^{e}
CV	12.45	11.5		12.8	17.9	11.00	27.4	19.2	18.0
Level of significance	*	**		*	**	*	*	*	*

Means with the same letter (s) in the same column are not significantly different; *= Significant at 5% probability level; **= significant at 1% probability level; NS= non-significant. Sin planting =single planting pattern, dou planting = double planting manner.

difference with the plant spacing appeared bigger at the wider spacing.

Average bulb weight

As indicated in Table 2 spacing did not significance affected the bulb weight of onion in the year 2014 but significantly influenced by plant spacing in the year 2015. Accordingly, the highest average bulb weight (87.90 g) was produced at double spacing of 50 x 10 cm, while the lowest average bulb weight (72.12 g) was observed in single planting of 30 x 7.5 cm. similarly Yemane et al. (2013) founded that the highest average bulb weight increase as the interspacing increases from 5 cm to 10 cm. This result also confirms Aliyu et al. (2008) who noticed that densely populated plants produced lower bulb weight as compared to thinly populated plants. Similarly, Jilani et al. (2009) also reported that the highest fresh bulb weight was observed at the wider intra spacing.

Total bulb yield and marketable bulb yield

Effect of the intra and inter spacing showed significance (P< 0.05) difference on the bulb yield of onion in both years (Table 3). Accordingly, the highest total bulb yield of 21.28 ton ha^{-1} and marketable bulb yield of 20.60 ton ha^{-1} was recorded at the spacing of 40 x 5 cm followed by single planting 20 x 10 cm (20.99 ton ha^{-1}) for total bulb yield and 40 x 7.5 cm (19.69 ton ha^{-1}) for the marketable bulb yield in 2014 in double planting manner (Table 4).

Bulb onion produced with a spacing of 40 x 5 cm was increased by 45.25% as compared to bulb yield produced with spacing of 30 x 12.5 cm (14.65 ton ha^{-1}). Similarly, the highest total bulb yield and marketable bulb yield 32.92 and 31.30 ton ha^{-1} was recorded at the spacing of 40 x 5 cm in 2015 with double planting manner. Minimum total bulb yield (18.68) was obtained from double planting of 50 x 12.5 cm in 2015. The highest total bulb yield gave 76.23% yield advantage over the lowest ones. The lowest total bulb yield production would be due to extreme wider spacing at which the required population ha^{-1} could not be accommodated; and this result in low production of total bulb yield because of low population density. Moreover, the pooled mean result indicated that double planting inters and intra spacing of 40 and 5 cm, respectively gave high yield of onion in both the total and marketable bulb yield. The current result was found consistent with Jan et al. (2003) reported the highest total bulb yield (549.90 kg ha^{-1}) was obtained at a closer spacing, whereas the lowest total bulb yield was recorded from a wider spacing. Similarly, Yemane et al. (2013) reported that as intra-row spacing increased from 5 to 10 cm, total bulb yield in tons ha^{-1} decreased. Significantly,

the highest total bulb yields of 36.14 and 33.82 tons ha^{-1} were recorded at 5 and 7.5 cm intra-row spacing, respectively. An intra-row spacing of 10 cm showed the lowest total bulb yield (28.51 tons ha^{-1}).

This finding was in agreement with similar study of Habtamu et al. (2016) concludes that yield increases significantly as population density increases.

Unmarketable yield

Significance difference was observed on unmarketable yield ($P<0.05$) by the inter and intra row spacing in both 2014 and 2015 irrigation season. The highest unmarketable yield (2.25 ton ha^{-1}) was recorded at 50 x 5 cm in double planting manner in 2014 cropping season. Whereas the lowest unmarketable yield (0.87 ton ha^{-1}) was recorded at 30 x 12.5 cm in single planting manner. In 2015 cropping season the maximum unmarketable bulb yield (2.72 ton ha^{-1}) was observed at the flat bed of 20 x 10 cm. Moreover, the pooled mean result indicated that the highest unmarketable yield was observed at flat planting of 20 x 10 cm inter and intra row spacing (Table 3). This is for the reason that flat bed planting resulted in decayed bulbs and large sized bulbs which was unwanted in the local market.

This finding was contrast with the result of other workers (Yemane et al., 2013; Habtamu et al., 2016) who concluded that plant density has an impact on unmarketable bulb size.

Conclusions

Optimum plant spacing has a promising impact in increasing the productivity and production of Onion. The findings of the present experiment showed that plant height, bulb size, total bulb yield and above ground dry biomass yield were significantly affected by inter and intra spacing of Onion. The tallest plant height was obtained at spacing of single planting 40 x 10 cm. The maximum total bulb yield and above ground dry biomass yield were obtained at the double planting of 40 x 5 cm in both years as compared to the other treatments. It is, therefore, concluded that spacing of double planting 40 x 5 cm can be recommended for the growers in the study area as the total bulb and marketable bulb was high compare to the other treatments to increase Onion productivity. Moreover further investigations need on different varieties, soil types, quality aspects together with other agronomic management practices to identify best technology on productivity and production of onion.

Conflict of Interests

The authors have not declared any conflict of interest.

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REFERENCES

Aliyu U, Dikko AU, Magaji MD, Singh A (2008). Nitrogen and intra-row spacing effect on growth and yield of onion (*Allium cepa*L.). J. Plant Sci. 3:188-193.

Brewster JL (2008). Onions and other Vegetable Alliums.2nd ed. CAB International. North America.

Geremew A, Tesheme A, Kassaye T, Amenti C (2010). Effect of intra row spacing on yield of three onion (Allium cepa L) varieties at Adamitulu Agricultural research center (Mid rift valley of Ethiopia). J. Hortic. 2(1):7-11.

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research, 2nd edition. John Wiley and Sons Inc., New York.

Griffiths G, Trueman L, CrowtherT, Thomas B, Smith B (2002). Onions a global benefit to health. Phytother. Res. 16:603-615.

Habtamu T, Minuyelet J, Esmelealem M, Alebachew E (2016). Influences of inter and intra row spacing on yield, yield component and morphological characteristics of onion (*Allium cepa* L.) at Western Amhara region. Afr. J. Agric. Res. 11(20):1797-1804.

Haileslassie G, Haile A, Wakuma B, Kedir J (2015). Performance evaluation of hot pepper (*Capsicum annum* L.) varieties for productivity under irrigation at Raya Valley, Northern, Ethiopia. Basic Res. J. Agric. Sci. Rev. 4(7):211-216.

Hailu DK, Aklilu MM, Mikael TY (2015). An investigation on the influence of intra-row spacing and variety on yield and shelf life of onion. Int. J. Agric. Sci. 5(3):559-567.

Hanelt P (1990). Taxonomy, Evolution, and History. In. Onions and Allied Crops, edited by Haim D. Rabinowitch and James L. Brewster, Boca Raton, Fla.: CRC Press. pp. 1-26.

Jilani MS, Khan MQ, Rahman S (2009). Planting densities effect on yield and yield components of onion (*Allium cepa*l.). J. Agric. Res. 47(4):397-404.

Kantona RA, Abbey L, Hilla RG, Tabil MA, Jan ND (2003). Density affects plant development and yield of bulb onion (*Allium cepa*L.) in Northern Ghana. J. Veg. Crop Prod. 8(2):15-25.

Lemma D, Herath E (1992). Agronomic Studies on *Allium*. In. Horticultural Research and Development in Ethiopia. 1-3 December, 1992 Institute of Agricultural research and food and Agricultural Organization. Addis Ababa, Ethiopia. pp.139-145.

MoARD (Ministry of Agriculture and Rural Development) (2009). Animal and Plant Health Regulatory Directorate: Crop Variety Register. Issue No. 12, Addis Ababa, Ethiopia.

Negasi T (2014). Onion production practices and response of the crop to fertilizer application in central rift valley of Ethiopia. Ph.D. Dissertation submitted to the school of graduate studies, Haramaya University, Ethiopia.

SAS (Statistical Analysis System) Institute (2004). SAS user guides, version 9.1.SAS Inc. Cary. North Carolina, USA.

Sikder M, Mondal F, Mohammed D, Alam MS, Amin MB (2010). Effect of spacing and depth of planting on growth and yield of onion. J. Agroforestry Environ. 4(2):105-108.

Tesfalegn JA (2015). Response of onion (*allium cepa*l.) varieties to intra-row spacing at Kobo, Northern Ethiopia. M.Sc. Thesis, Haramaya University, Haramaya, Ethiopia.

Yemane K, Fetien A, Derbew B (2013). Effect of intra-row spacing on yield and quality of some onion varieties (*Allium cepa* L.) at Aksum, Northern Ethiopia. Afr. J. Plant Sci. 7(12):613-622.

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Full Length Research Paper

Detection of genetic diversity among some species of *Anthemis* L. (Asteraceae) in Saudi Arabia by using RAPD-PCR analysis

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The aim of the present study was to determine the unique molecular markers among, three species of the genus *Anthemis* and the construction of phylogenetic tree using the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. Genetic diversity was analyzed among 15 populations of three species of *Anthemis* (*Anthemis melampodina*, *Anthemis*, *pseudocotula* and *Anthemis*, *bornmuelleri*), collected from different locations at Saudi Arabia by using RAPD primers. Pairwise genetic distance was calculated based on Nei and Li coefficient. Unweighted pair group method with an average (UPGMA) was used for construction of dendrogram, based on the similarity matrix data. Results showed wide variations among *A. bornmuelleri* and other two species. A wide close genetic relation was observed between *A. melampodina* and *A. pseudocotula*. RAPD-PCR technique was shown to be an accurate tool, in other to ascertain plant relationships among species of genus *Anthemis*.

Key words: Genetic diversity, random amplified polymorphic DNA-polymerase chain reaction (RAPD- PCR), *A. melampodina*, *A. pseudocotula*, *A. bornmuelleri*.

INTRODUCTION

Anthemis L. is one of the largest genus of family Asteraceae, including more than 210 described species (Oberprieler et al., 2007). They are widely distributed across Europe extending into extreme southern Arabia and tropical east Africa and other parts of the world (Oberprieler, 2001).

The main center of biological diversity or species diversity is located in Mediterranean region (Lo Presti, 2010) and southwestern Asia with 150 to 210 species, including all of the presently accepted subgenera and sections (Kilica et al., 2011). Some species inhabit northern America and southern hemisphere as well (Oberprieler, 2001). *Anthemis* is a diverse group that can be easily distinguished by the paleaceous receptacle of

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its mostly radiate capitula and the achene morphology. Some species are widely used in pharmaceutical, cosmetics, and food industry. They are also used as herbal tea for treatment of anxiety, flatulence, stomach disorders, insomnia and toothache (Vaverkova et al., 2001).

Saudi Arabia contains a large number of wild plants, both in arid and high lands. The greatest plant diversity was recorded in Asir, Hijaz and the western area, bordering the Red Sea (Collenette, 1998). Many previous studies showed that landscape of an area and the climatic influences are the main factors affecting the degree of species diversity (El-Kady et al., 1995; Shaltout et al., 1997). Some species of *Anthemis* are distributed depending on the chemical nature of bedrock and climate of geographic area. Four species of *Anthemis* were illustrated by Migahid (1996), 12 species by (Ghafoor and Al Turki, 1999) and 19 species by (Ghafoor, 2010).

Several studies on the genus *Anthemis* were focused on ecology and taxonomy (Ghafoor, 2010), biochemistry and toxicity (Uzel et al., 2004; Grace, 2002; Pavlović et al., 2010), therapeutic (Jafari et al., 2003; Darriet et al., 2009; Jaradat et al., 2016) and molecular studies (Oberprieler, 2001; Oberprieler, 2002; Lo Presti, 2010**)**. Reports dealing with diversity using molecular studies on species of *Anthimus* in Saudi Arabia, in particular *A. melampodina*, *A. pseudocotula* and *A. bornmuelleri*) are scanty.

Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Variable Number of Tandem Repeats (VNTR) and Restriction Fragment Length Polymorphism (RFLP) have been established as useful markers, for discovering genetic diversity of floras (Wang, et al., 1996). RAPD is a PCR-based technique developed by (Williams et al., 1990) and (Welsh and McClelland, 1990) which employs decamer random primers, for amplifying random DNA fragments from genomic DNA without any prior knowledge of the genomic sequence of any organism. Recently, several studies have used this tool to measure the levels and patterns of variations within different plants (Abd El-Ghani and El-Sawaf, 2004; Sarwat et al., 2008; Thendral et al., 2010; Hammadi and Qari, 2012; Ismail et al., 2016; Lu et al., 2016; Patil et al., 2016).

The present study aimed to use DNA (RAPD) markers to investigate genetic diversity, genetic relationships and polymorphism in natural populations of three species of *Anthemis* in Saudi Arabia.

MATERIALS AND METHODS

Plant materials

During the period of March to May 2015, 15 plant samples of three species of *Anthemis* were collected from different geographic regions in Saudi Arabia through Jizan, Makkah, Hail and Asir (Figure 1, Table 1). Samples comprise of 9 specimens of *A. melampodina,* 3 specimens of *A. pseudocotula* and 3 specimens of *A. bornmuelleri.* The plants were identified by the Plants Taxonomist at the Herbarium of the Faculty of Science, Umm Al-Qura University, Makkah, Saudi Arabia. Young leaves were harvested and preserved in sealed bags with suitable label. Leaves were used immediately for DNA extraction, while excess leaves were stored in -80°C for subsequent use.

Chemicals

Reagents including Taq DNA polymerase (TaKaRa), dNTPs (Boehringer Mannheim), DNA extraction kit, and agarose gel (Qiagen), oligonucleotides as random primers (Operon technologies, USA), Egypt), DNA Marker for agarose gel electrophoresis (Gibco BRL), loading dye solution (Fermentas, Lithvuania) were used. All other chemicals were obtained from Sigma Aldrich (USA).

DNA extraction

Total genomic DNA extraction of plant leaves was performed using a modified CTAB method according to the protocol of (Doyle and Doyle, 1990). Quantitative estimation of total genomic DNA in each sample was confirmed spectrophotometericaly at 260 and 280 nm, Whereas, quality was checked by running samples on 1.2% agarose electrophoresis with DNA ladder and visualized under UV light in gel documentation system.

RAPD analysis

Polymorphic primers were identified by screening fifteen random decamer primers (Table 2) with DNA of *Anthemis* sp. Out of 15 primers, only 5 gave precise and stable PCR-production for RAPD analysis (Table 2). DNA amplification was performed in a Perkin ElmerCetus 480 DNA Thermal Cycler programmed for 45 cycles as follows: 1st cycle of 3.5 min at 92°C, 1 min at 35°C, 2 min at 72°C; followed by 44 cycles each of 1 min at 92°C, 1 min at 35°C, 2 min at 72°C followed by one final extension cycle of 7 min at 72°C.

The amplification products were resolved by agarose gels electrophoresis. Controls lacking template DNA were included. Amplified PCR products were resolved on 1% agarose gel electrophoresis, visualized under UV light and photographed with gel documentation system. Each band was considered as RAPD marker. All the reactions were repeated for at least twice.

Data analysis

A binary matrix was prepared by manually scoring of photographed bands on gels, where 1 or 0 represent. The data were used for similarity – based analysis using the software program NTSYS (2.20). RAPD analyses were analyzed using the Nei genetic similarity index (Nei and Li, 1979). On the basis of the similarity matrix data, a dendrogram was constructed by unweighted pair group method with average (UPGMA) cluster analysis (Figure 2).

RESULTS AND DISCUSSION

Different approaches in genetic diversity analyses, reveal the different level of polymorphism (Porter and Smith, 1982) and also, DNA markers are independence of environmental or localities factors which show a greater level of polymorphism (Heywood, 2002). Therefore, they

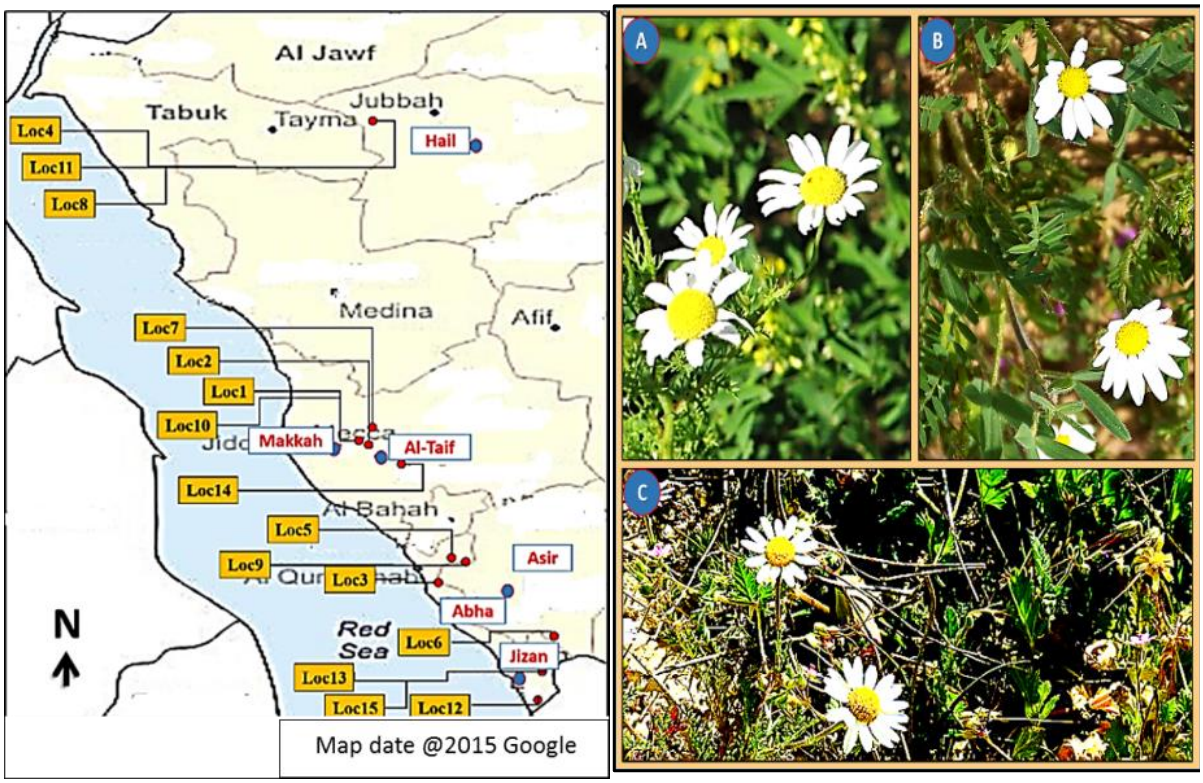

Figure 1. (Left said) map of Saudi Arabia indicating the localities where *Anthemis* populations of the three species were collected in March-May 2015, (Right side) Photos of *Anthemis* species (from field) as A: *Anthemis melampodina* (Loc6), B: *Anthemis pseudocotula* (Loc11) and C: *Anthemis bornmuelleri* (Loc15).

Table 1. Locations of plant collection.

Population ID	Scientific name	Location	
		Loci name	Description
Loc1	*A. melampodina*	Wadi Zimmah,	60km Makkah – Al-Taif road, Makkah Region.
Loc2	*A. melampodina*	Wadi Al-Madik	70km Makkah – Al-Taif road, Makkah Region.
Loc3	*A. melampodina*	Khawr 'Amiq	30 km northwest of Al-Birk, Asir Region.
Loc4	*A. melampodina*	Al-Odair valley	Hail in the Shammar Mountain, Hail region
Loc5	*A. melampodina*	Jabal Alswda	40 km west of Abha City, Asir Region.
Loc6	*A. melampodina*	Jabal Fayfa	90 Km Northest Jazan, Jizan region
Loc7	*A. melampodina*	Wadi Saulah	80 km Makkah – Al-Taif road
Loc8	*A. melampodina*	Al-Odair valley	Hail in the Shammar Mountain, Hail region
Loc9	*A. melampodina*	Jabal Alswda	30 km west of Abha City, Asir Region.
Loc10	*A. pseudocotula*	Wadi Zimmah,	60km Makkah – Al-Taif road
Loc11	*A. pseudocotula*	Al-Odair valley	Hail in the Shammar Mountain, Hail region
Loc 12	*A. pseudocotula*	Khawr Wahlan	35 km south of Jizan, Jizan Region.
Loc 13	*A. bornmuelleri*	Malaki Dam	15 km east of Abu Arish, Jizan Region.
Loc 14	*A. bornmuelleri*	Wadi Ze Ghazal	Al-Shafaa, 37Km southeast Al-Taif
Loc 15	*A. bornmuelleri*	Malaki Dam	15 km east of Abu Arish, Jizan Region

are considered as valuable tools for determining genetic relationships. Among various molecular markers, Random amplified polymorphic DNA (RAPD) markers have proved to be a very useful tool, providing a convenient and rapid

Table 2. Sequences of used (15) primers.

Primers		Sequences of primers (5′ → 3′)	Primers		Sequences of primers (5′ → 3′)
No.	Name		No.	Name	
1	OPB-16	TTT GCC CGG A	9	OPE-05	TCA GGG AGG T
2	OPA-04	GTC GAA CGA G	10	OPG-18	GGC TCA TGT G
3	OPC-20	ACT TCG CCG A	11	OPZ-13	GGG TCT CGG T
4	OPE-03	CCA GAT GCA C	12	OPD-07	TTG GCA CGG G
5	OPA-02	AGC CTT CGC T	13	OPD-03	GTC GCC GTC A
6	OPB-03	CAT CCC CCT G	14	OPC-13	AAC CCT CGT C
7	OPA-01	CAG GCC CTT C	15	OPB-10	CTG CTG GGA C
8	OPB-08	GTC CAC ACG G			

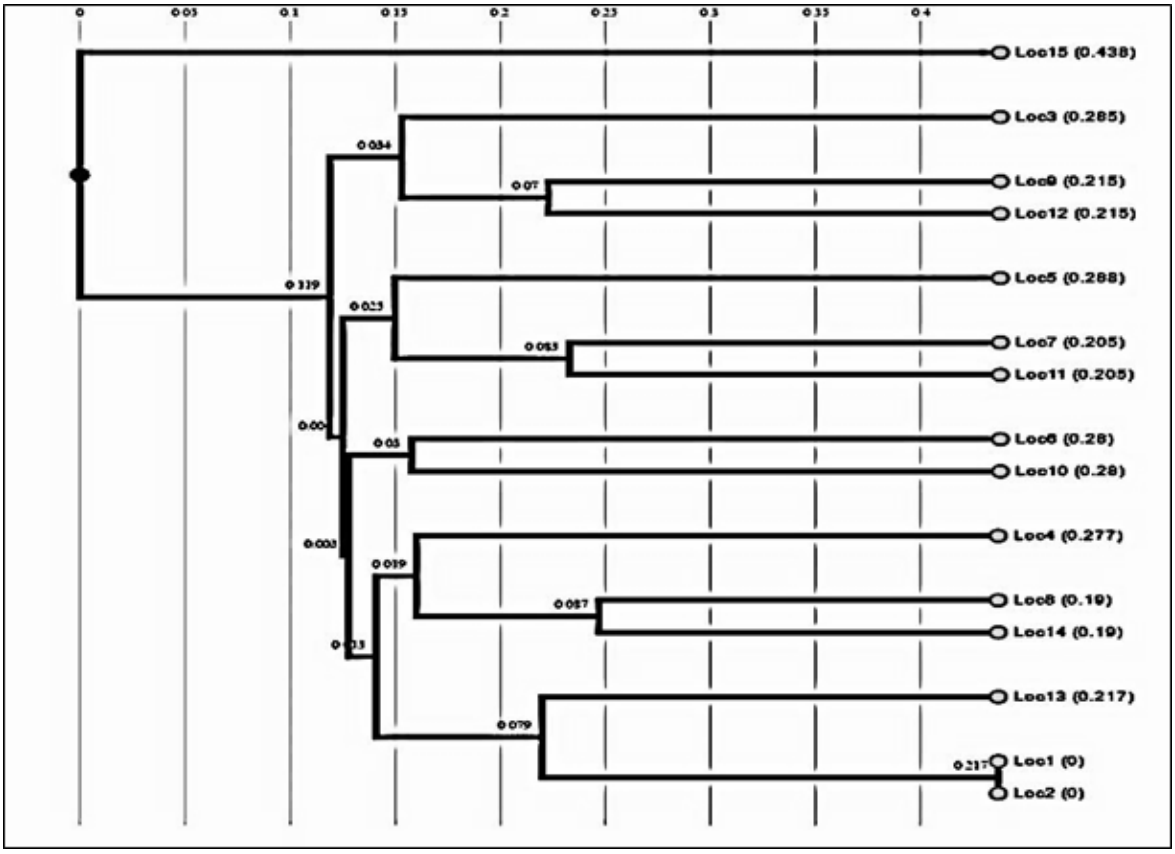

Figure 2. Dendrogram obtained from RAPD analysis using UPGMA.

assessment of the genetic differences between genotypes (Williams et al., 1990). Meanwhile, polymorphism detected by RAPD markers has proven to be useful for discrimination of genetic diversity and relationships in several plant species.

The present work is a preliminary study, aim to use DNA (RAPD) markers to investigate genetic diversity, genetic relationships and polymorphism in natural populations of only three species of this genus (*A. melampodina*, *A. pseudocotula* and *A. bornmuelleri*) collected from Saudi Arabia. In this study, a total of 15 RAPD-PCR primers were used to test 15 samples (Table 1). Out of these, only 5 primers showed reproducible results and they were chosen to amplify the whole 15 samples (Figure 3). RAPD-PCR assay had been positively used in several taxonomical and genetic diversity studies (Hammadi and Qari, 2012; Alam et al., 2009).

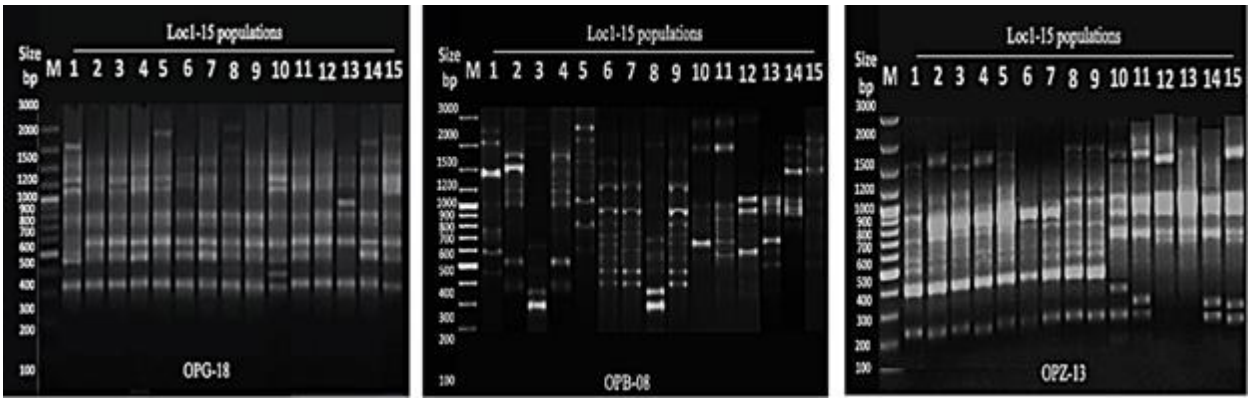

Figure 3. Polymorphic bands generated by some different RAPD primers, M: DNA stander; Numbers 1-15: Loc1- Loc15 population.

Table 3. RAPD primers data and the percentages of polymorphic bands.

Primer code	Size of fragments(bp)	Polymorphic bands	Monomorphism %	Polymorphism %
OPA-02	341-1670	11	14.2	85.8
OPB-08	310-2791	15	39.6	60.4
OPC-20	315-2105	8	31.8	68.2
OPG-18	292-2684	8	59.2	40.8
OPZ-13	263-1870	9	8.6	91.4

Table 4. Number of amplified fragments and specific markers of the three species of *Anthemis* (*A. melampodina*, *A. pseudocotula* and *A. bornmuelleri*) using RAPD analysis with five primers.

Primer	TFA	*A. melampodina*		*A. pseudocotula*		*A. bornmuelleri*		TSM
		AF	SM	AF	SM	AF	SM	
OPA-02	7	7	3	8	1	8	0	4
OPB-08	12	8	4	6	2	7	2	8
OPC-20	18	11	3	10	3	7	1	7
OPG-18	16	13	0	5	3	7	3	6
OPZ-13	10	6	3	8	1	5	0	4
Total	63	45	13	37	10	34	6	29

TFA, Total fragments amplified; AF, amplified fragments; SM, specific markers; TSM, total specific markers.

The percentages of polymorphism and monomorphism of the obtained bands are presented in (Table 3). A total of 63 bands were produced for 15 samples, 51 bands of them were polymorphic and present one or more but not all of them. Mehetre et al., (2004) reported that monomorphic bands should present in all producible bands, and the unique ones should present in at least one producible band, not in any others. The mean percentage of polymorphic bands was 69.32% with molecular sizes ranged from 263 up to 2791 bp; approximately, 18 bands of the 63 were commonly detected in all the samples, so it could be the recognized genus bands of *Anthemis*.

The results of the total fragments amplified (TFA), amplified fragment (AF) specific markers (SM) for each species of *Anthemis*: (*A. melampodina*, *A. pseudocotula* and *A. bornmuelleri*) are presented in Table 4 and the genetic distance matrix for indices of the studied samples are presented in Table 5. Welsh and McClelland (1990) found that, reproducible fingerprints of genomes could be generated using arbitrary primers with PCR technique. Species-specific markers varied between three types of *Anthemis* species (TSM=29 markers) and are clearly shown in (Table 4). *A. melampodina* revealed 13 specific bands, while *A. pseudocotula* and *A. bornmuelleri* showed 10 and 6 specific bands (SM), respectively.

Table 5. Genetic distance matrix for indices of 15 samples of the three species of *Anthemis*.

Locations	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8	Loc9	Loc10	Loc11	Loc12	Loc13	Loc14	Loc15
Loc1	0	0.81	0.75	0.74	0.72	0.70	0.60	0.59	0.64	0.58	0.58	0.55	0.51	0.51	0.58
loc2		0	0.76	0.75	0.74	0.67	0.57	0.58	0.55	0.57	0.55	0.36	0.36	0.50	0.57
loc3			0	0.78	0.79	0.7.5	0.56	0.56	0.56	0.60	0.60	0.58	0.57	0.57	0.98
loc4				0	0.75	0.71	0.70	0.61	0.62	0.59	0.53	0.51	0.50	0.50	0.76
loc5					0	0.68	0.58	0.62	0.63	0.59	0.57	0.53	0.53	0.55	0.82
loc6						0	0.74	0.62	0.66	0.56	0.60	0.57	0.56	0.53	0.78
loc7							0	0.73	0.72	0.44	0.41	0.56	0.52	0.52	0.92
loc8								0	0.68	0.43	0.43	0.41	0.41	0.38	1.00
loc9									0	0.55	0.43	0.43	0.68	0.59	0.78
loc10										0	0.69	0.73	0.70	0.70	0.79
loc11											0	0.69	0.70	0.76	0.86
loc12												0	0.79	0.70	0.95
Loc13													0	0.76	0.92
Loc14														0	1.03
Loc15															0

These bands may be possible specific markers after verifying that, individuals of each species appear the same description (Roman et al., 2003; Manen et al., 2004).

Results of monomorphism and polymorphism of *Anthemis* using RAPD-analysis might be precise findings for the genetic diversity of genus *Anthemis,* to specify each species with the appearance of specific markers and definitive bands. Many studies conducted on other genus revealed similar results with insufficient molecular studies of Anthemideae (Matousek et al., 2007; Garcia et al., 2010; Riggins and Seigler, 2012). Similar results were obtained by other authors (Riggins and Seigler, 2012; Patil et al., 2016; Higgins et al., 2016; Kumar et al., 2016).

Conclusion

A high level of polymorphism and establishment of genetic diversity among three species of *Anthemis* was detected by using RAPD markers that could be a suitable tool for understanding the aspects of divergence, to solve a lot of taxonomical problems.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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REFERENCES

Abd El-Ghani MM, El-Sawaf N (2004). Diversity and distribution of plant species in the agro-ecosystem of Egypt. Syst. Geogr. 74:319-336.

Alam MA, Pallavi G, Aswini KG, Gyan PM, Pradeep KN (2009) Assessment of genetic diversity among *Podophyllum hexandrum* genotypes of assessed by random amplified polymorphic DNA. Afr. J. Biotechnol. 7(24):4407-4414.

Collenette S (1998) Checklist of botanical species in Saudi Arabia. International Asclepiad Society. 1:80.

Darriet F, Desjobert JM, Costa J, Muselli A (2009). Identification of chrysanthenyl esters from the essential oil of *Anthemis maritima* L. investigated by GC/RI, GC-MS (EI and CI) and 13C-NMR spectroscopy: chemical composition and variability. Phytochem. Anal. 20(4):279-292.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13-15.

El-Kady HF, Ayyad M, Born Karnum R (1995). Vegetation and recent land-use history in the desert of Maktala, Egypt. Adv. Geoecol. 28:109-123.

Garcia S, Panero JL, Siroky J, Kovarik A (2010). Repeated reunions and splits feature the highly dynamic evolution of 5S and 35S ribosomal RNA genes (rDNA) in the Asteraceae family. BMC Plant Biol. 16(10):176.

Ghafoor A, Al-Turki TA (1999). A new Anthemis (Asteraceae)

from Saudi Arabia. Edinb. J. Bot. 56(1):55-59.
Ghafoor A (2010). The Genus Anthemis L. (Compositae-Anthemideae) In Arabian Peninsula: A Taxonomic Study. Pak. J. Bot. 42:79-98.
Grace MH (2002). Chemical composition and biological activity of the volatiles of *Anthemis melampodina* and *Pluchea dioscoridis*. Phytother. Res. 16(2):183-185.
Hammadi I, Qari SH (2010). Genetic Diversity Among *Zygophyllum* Populations based on RAPD analysis. Genet. Mol. Res. 9(4):2412-2420.
Heywood VH (2002). The Conservation of Genetic and Chemical Diversity in Medicinal and Aromatic Plants. In. Biodiversity: Biomolecular Aspects of Biodiversity and Innovative Utilization. Sener, B. (Ed.). Springer, Berlin, Heidelberg, pp. 13-22.
Higgins CM, Chang WL, Khan S, Tang J, Elliott C, Dietzgen RG (2016). Diversity and evolutionary history of lettuce necrotic yellows virus in Australia and New Zealand. Arch. Virol. 161(2):269-277.
Ismail NA, Rafii MY, Mahmud TM, Hanafi MM, Miah G (2016). Molecular markers: a potential resource for ginger genetic diversity studies. Int. J. Mol. Sci. 17(8):120-128.
Jafari S, Massoud A, Katayoun B, Hassan F (2003). Comparartive Study of Zataria Multiflora and *Anthemis Nobelis* Extracts with Myrthus Communis Preparation in The Treatment of Recurrent Aphthous Stomatitis. DARU J. Pharm. Sci. 11(1):23-27.
Jaradat NA, Ayesh OI, Anderson CJ (2016). Ethno-pharmacological survey about medicinal plants utilized by herbalists and traditional practitioner healers for treatments of diarrhea in the West Bank/Palestine. J. Ethnopharmacol. 182:57-66.
Kilica O, Alpaslan K, Eyup B (2011). Composition of the volatile oils of two Anthemis L. taxa from Turkey. Z. Naturforsch. C 66(11-12):535-540.
Kumar H, Priya P, Singh N, Kumar M, Choudhary BK, Kumar L (2016) RAPD and ISSR Marker-Based Comparative Evaluation of Genetic Diversity Among Indian Germplasms of uryale ferox: An Aquatic Food Plant. Appl. Biochem. Biotechnol. 30:280-294.
Lo Presti RM (2010). Geological vs. Climatological Diversification in the Mediterranean Area: Micro- and Macro-evolutionary Approaches in Anthemis L. (Compositae, Anthemideae). Logos Verlag: Berlin: GmbH. P.186.
Lu PL, Yorkson M, Morden CW (2016). Population Genetics of the Endemic Hawaiian Species Chrysodracon hawaiiensis and Chrysodracon auwahiensis (Asparagaceae): Insights from RAPD and ISSR Variation. Int. J. Mol. Sci. 17(8):129-137.
Manen JF, Habashi C, Jeanmonod D, Park JM, Schneeweiss GM (2004). Phylogeny and intraspecific variability of holoparasitic Orobanche (Orobanchaceae) inferred from plastid rbcL sequences. Mol. Phylogenet Evol. 33(2):482-500.
Matousek J, Orctová L, Ptácek J, Patzak J, Dedic P, Steger G (2007). Experimental transmission of pospiviroid populations to weed species characteristic of potato and hop fields. J. Virol. 81(21):11891-11899.
Mehetre S, Gomes M, Eapen S (2004). RAPD analysis of hybrid nature of the offspring of Gossypium hirsutum x G. raimondii. Curr. Sci. 84:24-28.
Migahid AM (1996). Flora of Saudi Arabia. 4th ed. Vol. 2, Riyadh: King Saud University Press. Saudia Arabia. pp. 1-150.
Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 74:5267-5273.
Oberprieler C, Himmelreich S, Vogt R (2007). A new subtribal classification of the tribe Anthemideae (Compositae). Willdenowia 37:89-114.
Oberprieler C (2002). A phylogenetic analysis of *Chamaemelum* Mill. (Compositae: Anthemideae) and related genera based upon nrDNA ITS and cpDNA*trn*L/*trn*F IGS sequence variation. Bot. J. Linn. Soc. 138(3):255-273.
Oberprieler CH (2001). Phylogenetic relationships in Anthemis L. (Compositae, Anthemideae) based on nrDNA ITS sequence variation. Taxon 50:745-762.
Patil RR, Pawar KD, Rane MR, Yadav SR, Bapat VA, Jadhav JP (2016). Assessment of genetic diversity in Mucuna species of India using randomly amplified polymorphic DNA and inter simple sequence repeat markers. Physiol. Mol. Biol. Plants 22(2):207-217.
Pavlović M, Lakusić D, Kovacević N, Tzakou O, Couladis M (2010). Comparative analysis of essential oils of six Anthemis taxa from Serbia and Montenegro. Chem. Biodivers. 7(5):1231-44.
Porter WM, Smith DH (1982). Detection of identification errors in germplasm collections. Crop Sci. 22:701-703.
Riggins CW, Seigler DS (2012). The genus Artemisia (Asteraceae: Anthemideae) at a continental crossroads: molecular insights into migrations, disjunctions, and reticulations among Old and New World species from a beringian perspective. Mol. Phylogenet. Evol. 64(3):471-490.
Roman B, Alfaro C, Torres AM, Moreno MT, Satovic Z, Pujadas A (2003). Genetic relationships among Orobanche species as revealed by RAPD analysis. Ann. Bot. 91(6):637-642.
Sarwat M, Das E S, Srivastava EP (2008). Analysis of genetic diversity through AFLP, AMPL, ISSR and RAPD markers in *Tribulus terrestris*, a medicinal herb. Plant Cell Rep. 27:519-528.
Shaltout KH, El-Halawany EF, El-Garawany MM (1997). Coastal low land vegetation of eastern Saudi Arabia. Biodivers. Conserv. 6:1027-1040.
Thendral HB, Premalakshmi V, Sekar T (2010). Genetic diversity in *Azima tetracantha* (Lam) assessed through RAPD analysis. Indian J. Sci. Technol. 3(2):300-309.
Uzel A, Guvensen A, Cetin E (2004). Chemical composition and antimicrobial activity of the essential oils of *Anthemis xylopoda* O. Schwarz from Turkey. J. Ethnopharmacol. 95(2-3):151-154.
Vaverkova S, Haban M, Eerna K (2001). Qualitative properties of *Anthemis tinctoria* and *Anthemis nobilis* (Chamaemelum nobile) under different environmental conditions. Ecophysiology of plant production processes in stress conditions. Abstracts of the Fourth International Conference, Račkova dolina, Slovakia. 2: 1-2.
Wang XP, Zou YP, Zhang DM (1996). Problems in the use of RAPD to the study of genetic diversity and systematic. Acta Bot. Sin. 38(12):954-962.
Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.
Williams JG, Kubelik AR, Livak KJ, Rafalski JA (1990). Tingey SVDNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

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Phytochemicals, antioxidant potentials and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *Piper guineense* (Schumach & Thonn) seed

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The study involved preparation, phytochemical screening and evaluation of antioxidant potentials of the extract of *Piper guineense*. This was with a view to investigating the possibility of using the plant in the treatment and management of oxidant and inflammatory related disorders. Spices are herbs that contain complex mixtures of phytochemicals which give characteristic odours, flavours and protect living systems from deleterious effect of reactive oxygen species (ROS). Phytochemical screening of the plant revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, triterpenoids and saponins. The extract exhibited DPPH-free radical scavenging activity which was concentration dependent. The results revealed that the extract is rich in antioxidant components which make the seed useful in the prevention and management of various forms of diseases.

Key words: Phytochemicals, antioxidant potentials, *Piper guineense*, methanolic extract.

INTRODUCTION

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (ROS), free radicals and antioxidant defences (Eboh, 2014). This imbalance leads to damage of important molecules such as proteins, lipids, nucleic acids and is also involved in the pathogenesis of various forms of diseases including cardiovascular diseases; cancer etc. Moreover, antioxidants are different group of chemicals that inhibit the oxidation of other molecules, neutralising free radicals and protect specific organs against deleterious effect of dangerous xenobiotics (Durackova, 2010; Sachdeva et al., 2014). The harmful effects of ROS are eliminated by antioxidants which are of two types, enzymatic and non-enzymatic (Eboh, 2014). Despite the presence of the endogenous antioxidant defense systems, ROS still accumulate during the life cycle. They are implicated in a number of diseases, aging and age dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders and other chronic conditions (Eboh, 2014; Sachdeva et al., 2014). Accumulation of ROS may also be triggered from compromised homeostatic pathways which normally result in the damage of cellular components such as proteins, lipids and deoxyribonucleic acids (Sachdeva et

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al., 2014).

Medicinal plant extracts (including herbal/spices) contain different phytochemicals with varieties of biological activities of valuable therapeutic indices. The protective effects of plant are attributed to the activities and potentials of phytochemicals (Poongothai et al., 2011; Etim et al., 2013), which are the non-nutrient compounds. They are classified into three main groups namely alkaloids, isoprenoids (terpenoids and steroids) and phenols (phenolic acid, flavonoids, tannins and coumarin). The plant derived phytoconstituents are capable of terminating free radical reactions and prevent the body or biological systems from oxidative damage (Saikat et al., 2010) and protect against chronic diseases (neurodegenerative and cardiovascular) (Prior et al., 2005).

Piper guineense commonly known as West African black pepper is a climbing perennial plant belonging to the family Piperaceae. It is found in the tropical regions of Central and Western Africa. It is very similar to cubeb pepper in terms of flavour, but is much less bitter and has a fresher, more herbaceous flavor. It is used as spices whose fruits contain pungent piperine an essential oil, which constitutes 5 to 8% of the weight of black pepper. It is used in the beverage and pharmaceutical industries as flavouring and preservative agent (Oyemitan et al., 2014; Opara, 2014; De LaTorres et al., 2015). The pungency of the pepper is due to the presence of resins particularly chavicine and a yellow alkaloid. The dried fruits of *P. guineense* is usually ground and used as spices in food (Hassan et al., 2010). It has also been reported to have medicinal and health benefits including treatment and prevention of morning sickness, allergy, keep the body warm and prevent cold (Ekanem et al., 2010; Tankan and Ito, 2013; Etim et al., 2013; Opara, 2014). This paper sought to evaluate the phytochemical constituents, the antioxidant potentials and DPPH-radical scavenging activity of defatted methanolic extract of *P. guineense* seeds with a view to studying the possibility of utilizing the plant in the treatment and management of disorders.

MATERIALS AND METHODS

Reagents and chemicals

All the reagents and chemicals used in this study are of analytical grade and were procured from British Drug House (BDH) Chemicals Limited, Poole, England. 2, 2'- α-bipyridyl, 2, 2' -diphenyl-1-picrylhydrazyl (DPPH), L-Ascorbic acid and Folin - Ciocalteu's phenol reagent were obtained from Sigma-Aldrich Laboratories, Switzerland. All the solutions and buffers were prepared with glass distilled water.

Plant material: Collection and identification

Seeds of *P. guineense* were purchased from a local market in Ile-Ife, Osun State, Nigeria. The identification and authentication were carried out at IFE Herbarium, Department of Botany where specimen copy with specimen number (17480) was deposited. The seeds were sun-dried, carefully cleaned and ground to fine powder using manual grinder.

Preparation of methanolic extract of *P. guineense*

The methanolic extract of *P. guineense* (MEPG) was prepared by suspending 100 g defatted powdered seed in 500 ml of methanol-water mixture (3:1 v/v) for 72 h at room temperature with intermittent shaking. The suspension was filtered using Whatman no. 1 filter paper. The filtrate was concentrated *in vacuo* to dryness under reduced pressure at 40°C on Buchi (Switzerland) Vacuum Pump V-700 rotary evaporator. The dark brown residue was kept at 4°C for further analysis.

Phytochemical screening

Phytochemical screening of the extract was carried out according to earlier standard methods (Sofowora, 2006; Tiwari et al., 2011; Prohp and Onoagbe, 2012) with the following specific detecting chemicals and reagents. Alkaloids were screened with 0.5 M Picric acid, Mayer's reagent, Wagner's reagent and Dragendorff's reagent; flavonoids [1% (w/v) Lead acetate solution and 0.5 N ethanolic KOH]; Tannins [0.1% (w/v) Ferric chloride in glacial acetic acid]; saponins (Frothing and haemolytic tests); cardiac glycosides (chloroform/conc. sulphuric acid); anthraquinone (benzene/10% ammonia solution); triterpenoids (chloroform/concentrated sulphuric acid); steroids (concentrated sulphuric acid); and xanthoproteins (concentrated nitric acid/ammonia solution).

Determination of total phenolics and flavonoids levels in MEPG

The concentration of phenolics in the extract was determined spectrophotometrically according to the method of Singleton et al. (1999). The reaction mixture consisted of 0.5 ml (1 mg/ml stock) of the extract, 0.5 ml of distilled water, 1.5 ml of 7.5% (w/v) sodium carbonate (Na_2CO_3) and 1.5 ml of 10% (v/v) Folin-Ciocalteu's phenol reagent and followed by incubation at room temperature for 1 h 30 min. The absorbance was read at 725 nm against the reagent blank on vis - spectrophotometer. The concentration of total phenolics was extrapolated from the standard calibration curve and expressed in milligram tannic acid equivalent per gram of extract (mg TAE/g extract).

The concentration of flavonoids in the extract was determined spectrophotometrcally according to the method of Sun et al. (1999) with slight modification. The analysis was based on the formation of yellow-coloured flavonoid-aluminum complex. The reaction mixture consisted of 0.5 ml (1 mg/ml) of the extract, 1.5 ml of distilled water, 0.3 ml of 5% (w/v) sodium nitrite ($NaNO_2$) freshly prepared, 0.3 ml of 10% (w/v) aluminum chloride ($AlCl_3$) and 2 ml of 4% (w/v) sodium hydroxide (NaOH). The reaction mixture was incubated at room temperature for 10 min and the absorbance taken at 500 nm against the reagent blank. The concentration of total flavonoid was extrapolated from the standard calibration curve and was expressed in milligram rutin equivalent per gram of extract (mg RE/g extract).

Determination of total vitamins C and E levels in MEPG

The vitamin C concentration in the extract was quantified using Folin-Ciocalteu's phenol reagent reaction method according to procedure that was based on the methods of Omaye et al. (1979); and Japota and Dani, (1982). 0.5 ml of the extract was mixed with 1.5 ml of 10% (v/v) acetic acid and 0.5 ml of Folin-Ciocalteu reagen t (1:10 dilution). The mixture was incubated at room temperature

Table 1. Phytochemicals of methanolic extract of *Piper guineense* (MEPG) seed.

Phytochemical constituents		Results
Alkaloids	Mayer	+
	Wagner	+
	Picric	+
	Dragendoff	++
Flavonoids		+
Tannins		+
Triterpenoids		+
Cardiac glycosides		++
Saponins		+

Table 2. Antioxidant potentials of the methanolic extract of *P. guineense.*

Property	Level
Total phenolics (mgTAE/g)	141.50 ± 1.60
Flavonoids (mgRE/g)	86.09 ± 3.10
Vitamin C (mg/g)	11.85 ± 0.10
Vitamin E (µgTE/g)	18.95 ± 0.05
DPPH scavenging activity extract of *P. guineense* (IC_{50} µg/ml)	74.00
DPPH scavenging activity of ascorbic acid (IC_{50} µg/ml)	31.17

Results were expressed as the mean ± SD, n=5 readings. TAE, Tannic acid equivalent; RE, rutin equivalent; TE, Trolox equivalent.

for 10 min. The absorbance was read at 760 nm against the reagent blank. The concentration of vitamin C was obtained from the standard calibration curve and expressed in milligram per gram of extract (mg/g extract).

The total vitamin E in the extract was determined according to a procedure that was based on the earlier methods of Baker and Frank (1968) and Santhosh et al. (2013). The analysis was based on the reduction of ferric to ferrous ion by vitamin E and the formation of red coloured complex with 2, 2'- bipyridyl. Vitamin E (0.5 ml) extracted in heptane was mixed with 0.5 ml of 2′, 2′, α-bipyridyl reagent (0.12% w/v) and 0.5 ml of ferric chloride (0.12% w/v). 0.5 ml of Trolox (25 µg/ml) in ethanol was treated similarly. The absorbance was read at 492 nm against the reaction blank. The vitamin E concentration was expressed in milligram Trolox equivalent per gram (mg TE/g) using the expression:

$$\text{Concentration of Vitamin E} = \frac{Abs\ of\ extract\ X\ Conc.of\ Std}{Abs\ of\ Std}$$

where Abs = absorbance, Conc. of Std = concentration of Trolox.

Assay of DPPH radical scavenging activity of MEPG

The free radical scavenging activity of the extract was measured according to the modified method of Blois (1985). To 1 ml each of the different concentrations of extracts (62.5, 125, 250, 500 and 1000 µg/ml) or standard (vitamin C) (7.8125, 15.625, 31.25, 62.5 and 125 µg/ml) in a test tube, 1 ml of 0.3 mM DPPH in methanol was added. The mixture was mixed and incubated in the dark chamber for 30 min. The absorbance was measured at 517 nm against a blank that contained methanol in place of the extract. The percentage DPPH radical scavenging activity was calculated using the expression:

$$\text{Percentage scavenging activity} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (µg/ml) of extract that scavenged 50% the DPPH radical.

Statistical analysis

Data were expressed as mean ± SD of n = 5 readings, mean and standard deviation were processed using Ms Excel.

RESULTS

Phytochemical constituents and antioxidant potentials

The methanolic seed extract of *P. guineense* (MEPG) gave positive reactions for the presence of phenolics, flavonoids, alkaloids, cardiac glycosides, tannins, triterpenoids and saponins. Further analysis showed that it contained total phenolics (141.50 ± 1.60 mgTAE/g of extract), flavonoids (86.09 ± 3.10 mgRE/g of extract), vitamin C (11.85 ± 0.10 mg/g of extract) and vitamin E (18.95 ± 0.05 µgTE/g of extract) (Tables 1 and 2). The results revealed that the plant is very rich in phenolic compounds as well as in non-enzymatic antioxidants.

DPPH radical scavenging activity of the extract

The extract exhibited DPPH radical scavenging activity with IC_{50} of 74.0 µg/ml, and ascorbic acid 31.17 µg/ml as positive control. The radical scavenging activity of the extract is low (42%) when compared with that of ascorbic acid. This might be due to the interference with or presence of other phytochemicals.

DISCUSSION

The study evaluated the phytochemicals, antioxidant potentials and DPPH-radical scavenging activity of methanolic seed extract of *P. guineense*. The extract weighed 12 g which was 12% of the starting material which was considerably high when compared with extraction with other solvents. The observations agreed with that of Udoh et al. (2012). It implied that methanol could be regarded as a suitable solvent for the extraction of phytochemicals for biological studies. Phytochemical screening of the extract revealed that it contained alkaloids, cardiac glycosides, flavonoids, tannins, triterpenoids and saponins. Further analyses of the extract revealed that total phenolics and flavonoids contents of *P. guineense* extract as measured spectrophotometrically were 141.50 ± 1.60 mg/g expressed as an equivalent of tannic acid and 86.09 ± 3.10 mg/g expressed as an equivalent of rutin.

Phenolic compounds exhibit strong anti-oxidant activity, protect cells against the oxidative damage, are well-known scavengers, metal chelators, reducing agents, hydrogen donors and single oxygen quenchers (Ghasemzadeh and Ghasemzadeh, 2011). Studies have revealed that phenolics are ubiquitous groups of plant metabolites with varying biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammatory, anti-atheroscelerosis and cardiovascular protection (Aiyegroro and Okon, 2010; Akinpelu et al., 2012).

Naturally occurring antioxidants are mainly synthesized in plants in form of phenolics such as flavonoids, phenolic acids, tocopherols, tannins etc. (Ali et al., 2008). Flavonoids constitute one of the most important and widely distributed plant phenolics and are hydroxylated substances known to be synthesized by plants in response to microbial infections found to elicit antimicrobial activity against wide array of microorganisms *in vitro* (Yadav and Agarwala, 2011). The biological activity of flavonoids could probably be due to their ability to complex with extracellular soluble proteins and bacterial cells (Yadav and Agarwala, 2011; Duraikannu et al., 2014). Tannins on the other hand bind to proline-rich proteins and interfere with protein synthesis, contributing to the acceleration of healing in ulcer patients and wound cuts (Yadav and Agarwala, 2011; Ekpo et al., 2013).

Moreover, saponins possess the ability of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, and cholesterol binding properties and in the management and treatment of hypercholeostreolemia which implies that saponins might be acting by interacting with intestinal absorption of cholesterol (Malinow et al., 1977; Malinow, 1984; Yadav and Agarwala, 2011). Saponins have also been reported to possess hypoglycemic and anti-infammatory activities (Poongothai et al., 2011). The presence of these compounds explains the ability of the seed extract to exhibit antioxidant properties. The results agreed with the essential observations of Ekpo et al. (2013) and Oyemitan et al. (2014) that ethanolic extract seed and essential oil of *P. guineense* contained alkaloids, flavonoids, tannins, saponins, terpenes, phenols and cardiac glycosides and exhibited potent and appreciable biological activities.

Vitamins are chemically unrelated organic entities that cannot be synthesized in right quantities by humans, as such must be supplied in the diets. They are classified into two major classes, (water and lipid soluble), based on their solubility and perform specific cellular functions (Champe et al., 2008). The water soluble vitamins include non-B complex which is L-ascorbic acid (vitamin C) and B-complex (energy releasing, heamopoetic and others) while the fat soluble vitamins include A, D, E and K (Bellows and Moore, 2012).

Ascorbic acid (vitamin C) is one of the most powerful antioxidants that scavenges harmful free radicals, reactive oxygen species (ROS) and regenerates tocopherol to its functional state (Eboh, 2014). It has a 6-carbon lactone ring structure with 2, 3–enediol moiety with its antioxidant mechanisms based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen. Gayathri et al. (2010) reported that vitamin C can prevent the oxidation of lipids by trapping water soluble peroxyl radicals before their diffusion into lipid membranes. Denre (2014) reported that high intake of ascorbic acid has been linked to lower risk of cardiovascular diseases and several types of cancers by stimulating the immune system.

On the other hand, vitamin E is a fat-soluble vitamin with its main function to protect against lipid peroxidation (Pryor, 2000). Vitamin E is the major powerful membrane bound antioxidant employed by the cell in humans (Hensley et al., 2004). Antioxidant mechanisms of tocopherols include the transfer of a hydrogen atom at 6-hydroxyl group on the chroman ring, and scavenging of singlet oxygen and other reactive species (Eboh, 2014). In the present study, the concentrations of vitamin C and vitamin E were observed to be 11.85 ± 0.10 mg/g of extract and 18.95 ± 0.05 µg/g trolox equivalent of extract respectively which is quite high when compared with other sources.

Anti-oxidants (exogenous and endogenous) are vital substances which possess the ability to protect the body

from damage caused by free radical induced oxidative stress (Ghasemzadeh and Ghasemzadeh, 2011). Naturally occurring antioxidants have attracted considerable interest among nutritionists, food manufacturers and consumers due to their presumed safety and potential therapeutic values (Kumar and Pandey, 2013). Fruits and vegetables contain many different antioxidant components which are related to three major groups-vitamins, phenolics and carotenoids (Baladi et al., 2014). The consumption of fruits and vegetables has been associated with low incidences and mortality rates of cancer and heart diseases (Thaipong et al., 2006; Doss and Pugalenthi, 2012). Eating fruits and vegetables reduces blood pressure, boosts the immune system, detoxifies contaminants, pollutants and reduces inflammation.

The antioxidant potential assays in the study showed that the plant possess antioxidant activities. Anyasor et al. (2011) reported that plant phenolics are the major group of compounds acting as primary free radical scavengers or antioxidants. 2, 2′-Diphenyl-1-picrylhydrazyl (DPPH) assay is a well-known method for the evaluation of free radical-scavenging activity (Rasooli, 2007). It is a stable nitrogen centred free radical, the colour changes from violet to yellow to colourless on reduction by either the process of hydrogen or electron-donation. Molecules or compounds which are capable to perform the above reactions are considered as antioxidants and therefore radical scavengers (Brand-Williams et al., 1995; Hinneberg et al., 2006; Egemole et al., 2007). DPPH stable free radical method is an easy, rapid and sensitive method employed to analyze the antioxidant potential of a specific compound or plant extracts (Koleva et al., 2002). A potent scavenger of free radicals may serve as a possible preventive intervention for the diseases (Gyamfi et al., 1999).

The present study showed that the free radical scavenging power of the extract of *P. guineense* increased in a concentration dependent manner, though significantly low compared to standard ascorbic acid (21.17 µg/ml). The result of the study is in close agreement with the observation of Ayoola et al. (2008) who observed a dose-dependent scavenging activities of some medicinal plants used for malaria therapy in south-western Nigeria.

Conclusion

The results of this study suggest that the defatted methanolic leaf extract of *P. guineense* has potential as an antioxidant agent. However, further investigation on the isolation and characterization of the antioxidant components is required.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Aiyegroro OA, Okoh AI (2010). Preliminary phytochemical screening and *in vitro* antioxidant activities of aqueous extract of *Helichrysum longifolium* DC. BMC Complement. Altern. Med. 10(1):21.

Akinpelu BA, Dare CA, Adebesin FI, Iwalewa EO, Oyedapo OO (2012). Effect of stem - bark of *Erythrophleum suaveolens* (Guill. & Perri.) saponin on fresh water snail (*Lanistes lybicus*) tissues. Afr. J. Environ. Sci. Technol. 6(11):446-451.

Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A, Bora U (2008). Indian medicinal herbs as source of antioxidants. Food Res. Int. 41:1-15.

Anyasor GN, Aina DA, Olushola M, Aniyikaye AF (2011). Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of *Chromolaena Odorata*. Ann. Biol. Res. 2(2):441-451.

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Trop. J. Pharm. Res. 7(3):1019-1024.

Baker H, Frank O (1968). Tocopherol. In. Clinical vitaminology, methods and interpretation. New York Interscience Publishers, John Wiley and Sons Inc., Pp.172-173.

Baladi RM, Moghaddaszadeh-Ahrabi S, Afrouziyeh M (2014). Extraction of phenolic composition and quantification of the total phenolic of pomegranate pomace. Eur. J. Zool. Res. 3(1):133-135.

Bellows L, Moore M (2012). Water-Soluble Vitamins: B-Complex and Vitamin C. Food and Nutrition Series. Fact Sheet No. 9: pp. 312.

Blois MS (1985). Antioxidant determination by use of stable free radicals. Nature 29:1199-1200.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT Food Sci. Technol. 28:25-30.

Champe PC, Harvey RA, Ferrier DR (2008). Vitamins. In. Lippincotts Illustrated Reviews: Biochemistry, Fourth Edition, Pp. 373-394.

De LaTorres JE, Gassara F, Kouassi AP, Brar SK, Belkacemi K (2015). Spice Use in Food: Properties and Benefits. Crit. Rev. Food Sci. Nutr.

Denre M (2014). The determination of vitamin C, total phenol and antioxidant activity of some commonly cooking spices crops used in West Bengal. Int. J. Plant Physiol. Biochem. 6(6):66-70.

Doss A, Pugalenthi M (2012). Evaluation of Antioxidant Activity and Phytochemical Screening of *Malus domestica* Borkh (Apple) and *Phaseolus vulgaris* L. (Green beans). J. Pharm. Sci. Innov. 3:1-4.

Durackova Z (2010). Some current insights into oxidative stress. Physiol. Res. 59:459-469.

Duraikannu D, Chandrasekaran SD, Selvakumar JN, Vaithilingam M (2014). A preliminary study of *in vitro* Antioxidant and Antibacterial Activity of *Streptomyces gancidius* VITSD1 isolated from Marine soil. Curr. Bioact. Compd. 10:292-297.

Eboh AS (2014). Biochemistry of Free Radicals and Antioxidants. Scholars Acad. J. Biosci. 2(2):110-118.

Egemole AN, Odekanyin OO, Akinpelu BA, Akinwunmi KF, Oyedapo OO (2007). An examination of the extracts of *Icacina tricantha* Oliv. Niger. J. Biochem. Mol. Biol. 22:39-42.

Ekanem AP, Udoh FV, Oku EE (2010). Effects of ethanol extract of *Piper guineense* seeds (Schum. & Thonn.) on the conception of mice (*Mus musculus*). Afr. J. Pharm. Pharm. 4(6):362-367.

Ekpo IA, Osuagwu AN, Agbor RB, Okpako EC, Ekanem BE (2013). Phytochemical composition of *Aframomum melegueta* and *Piper guineense* seeds. J. Curr. Res. Sci. 1(1):24-27.

Etim OE, Egbuna CP, Odo CF, Udoh NM, Awah FM (2013). *In Vitro* Antioxidant and Nitric oxide Scavenging Activities of *Piper*

guineense seeds. Glob. J. Res. Med. Plants Indig. Med. 2(7):485-494.
Gayathri K, Jayachandran KS, Vasanthi HR, Rajamanickam GV (2010). Cardioprotective effect of lemon grass as evidenced by biochemical and histopathological changes in experimentally induced cardiotoxicity. Hum. Exp. Toxicol. 30(8):1073-1082.
Ghasemzadeh A, Ghasemzadeh N (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J. Med. Plants Res. 5(31):6697-6703.
Gyamfi MA, Yonamine M, Aniya Y (1999). Free- radical scavenging action of medicinal herbs from Ghana Thonningia sanguinea on experimentally- induced liver injuries. Gen. Pharm. 32:661-667.
Hassan DI, Ogah DM, Yusuf ND, Musa MM, Saidu GM (2010). The effect of acute and chronic (short and long term) oral administrations of black pepper (*Piper guineense)* aqueous extract on the body weight and haematological values of albino-Wistar rat. J. Med. Plants Res. 4(12):1122-1125.
Hensley K, Benakass EJ, Bolli R (2004). New perspectives on vitamin E: α-tocopherol and carboxyethylhydroxychroman metabolites in biology and medicine. Free Rad. Biol. Med. 36:1-15.
Hinneberg L, Drmen HJD, Hiltumen R (2006). Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem. 97:122-129.
Japota SK, Dani HM (1982). A new Colorimetric Technique for the estimation of vitamin C using Folin-CIocalteau's phenol reagent. Anal. Biochem. 127:178-182.
Koleva II, Van Beek TA, Linssen JPH, de Groot A, Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 13:8-17.
Kumar S, Pandey AK (2013). Chemistry and Biological Activities of Flavonoids: An Overview. Sci. World J. 2013, Article ID 162750, 16 pages.
Malinow MR (1984). Saponins and Cholesterol Metabolism. *Atheros* ***50***, 117 – 119.
Malinow MR, Mehaughlin P, Kohler GO, Livingstone AC (1977). Prevention of elevated cholesterolemia in Monkeys. Steriods 29:105-110.
Omaye ST, Turnbull JD, Sauberlich HE (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. In: McCormick D.B. and Wright D.L. (eds). Methods of Enzymology New York: Academic Press. Pp. 3-11.
Opara MN (2014). Some Edible Spices of Southeastern Nigeria with Antiparasitic Properties: A Review. Am. J. Ethnomed. 1(6):384-392.
Oyemitan IA, Kolawole F, Oyedeji AO (2014). Acute toxicity, antinociceptive and anti-inflammatory activity of the essential oil of fresh fruits of *Piper guineense*, Schum & Thonn (Piperaceae) in rodents. J. Med. Plants Res. 8(40):1191-1197.
Poongothai A, Sreena KP, Sreejith K, Uthiralingam M, Annapoorani S (2011). Preliminary phytochemicals screening of *Ficus racemosa linn.* bark. Int. J. Pharma Bio Sci. 2(2):431-434.
Prior RL, Wu X, Schaich K (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53:4290-4302.
Prohp TP, Onoagbe IO (2012). Determination of phytchemical composition of the stem-bark of *triplochiton seleroxylon* K. Schum (Sterculiaceae). Int. J. Appl. Biol Pharm. Technol. 3(2):68-74.
Pryor WA (2000). Vitamin E and heart disease: basic science to clinical intervention trials. Free Rad. Biol. Med. 28:141-64.
Rasooli I (2007). Food preservation: A Biopreservation Approach. Food 1:111-136.
Sachdeva M, Karan M, Singh T, Dhingra S (2014). Oxidants and Antioxidants in Complementary and Alternative Medicine: A Review. Spatula DD 2(1):1-16.
Saikat S, Raja C, Sridhar C, Reddy YSR, Biplab DE (2010). Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. Int. J. Pharm. Sci. Rev. Res. 3(1): 91-100.
Santhosh KS, Samydurai P, Ramakrishman R, Nagarajan N (2013). Polyphenols, Vitamin-E Estimation and *in vitro* Antioxidant Activity of *Adriantum capillu-veneris*. Int. J. Innov. Pharm. Res. 4(1):258-262.
Singleton VL, Orthofera R, Lamuela RRM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu's reagent. Methods Enzymol. 299:152-179.
Sofowora A (2006). Phytochemical screening. In. Medicinal Plants and Traditional Medicine in Africa. Second Edition, Spectrum Books Limited, Ibadan, Nigeria. pp. 150-153.
Sun PX, Yie LK, Zhang ZL, Hu M, Lu L (1999). Colometric determination of the total content of the flavonoids in epimedium capsules. J. Shenyang Pharm. Univ. 16:68-70.
Tankan JM, Ito M (2013). Inhalation of the Essential oil of *Piper guineense* from Cameroon shows Sedative and Anxiolytic-like Effects in Mice. Biol. Pharm. Bull. 36(10):1608-1614.
Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallose L, Byrne DH (2006). Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compost. Anal. 19:669-675.
Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H (2011). Phytochemical screening and extraction: A review. Int. Pharm. Sci. 1(1):98-106.
Udoh FV, Ekanem AP, Eyo VO (2012). Pharmacodynamic Effect of Methanolic Extract of *Piper guineense* Leaf on Uterine Physiology. Pharmacologia 3(7):200- 203.
Yadav RNS, Agarwala M (2011). Phytochemical analysis of some medicinal plants. J. Phytol. 3(12):10-14.

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