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Research Article

Analysis of genetic diversity of certain species of *Aristolochia* using ISSR-based molecular markers

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ABSTRACT

Inter Simple Sequence Repeat (ISSR) polymorphism was used to determine genetic diversity and phylogenetic relationship in 4 species of *Aristolochia* viz *A. indica* Linn., *A. saccata* Wall., *A. tagala* Cham. and *A. cathcartii* Hook.f & T. A panel of 8 ISSR primers was screened representing di-nucleotide repeats, of which 57 polymorphic and informative patterns were selected to determine the genetic diversity. The consensus tree constructed using binary data from banding patterns generated by ISSR-PCR depicting that *A. indica* and *A. cathcartii* are more similar in terms of their genetic level and included in the same cluster. However, the rest of the two species *viz.*, *A. saccata* and *A. tagala* are included into two different clusters which indicate their level of polymorphisms in the genetic level. This study may be useful in identifying diverse genetic stocks of *Aristolochia*, which may then be conserved on a priority basis. The ISSR markers are thus useful in the fingerprinting of wild species germplasm, and in understanding the evolutionary relationships of *Aristolochia.*

Keywords: *Aristolochia indica*; *Aristolochia saccata*; *Aristolochia tagala*; *Aristolochia cathcartii*; ISSR; Genetic diversity.

1. INTRODUCTION

*Aristolochia (*Aristolochiaceae) is an important genus widely used in traditional medicine [1]. During the past two decades, this genus has attracted much interest and has been the subject of numerous chemical and pharmacological studies. It is a rich source of aristolochic acids, which are unique to this genus, and of terpenoids [2]. The members of the genus *Aristolochia* are mostly distributed in tropical, subtropical, and Mediterranean regions of the world [3-6]. The species of *Aristolochia* are shrubby or perennial herbs, usually climbing.

The genus *Aristolochia* consists of a large number of species are cultivated as ornamentals [7] and popularly used as sources of abortifacient, emmenagogue, sedative, analgesic, anticancer, anti-inflammatory, anti-feedant, muscle relaxant, antihistaminic, and antiallergic drugs, for intestinal worms, in the treatment of cholera, stomach ache, abdominal pain, rheumatism, malaria, wounds and skin diseases, and also useful in treatment of different types of poisonous bites and stings [1, 2].

Phylogenetic placement of the medicinal plants is an important task for their further exploitation. Differences in genetic level may not express in the phenotype because mostly gene expression is regulated by environmental parameters. Finding out the genetic relatedness among

closely related medicinal plants might be helpful for bioprospection. DNA marker based analyses is the best option so far to find out their genetic relatedness and phylogenetic placement in molecular level.

The commonly used PCR-based DNA marker systems are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and more recently simple sequence repeats (SSRs) or microsatellites [8, 9]. The major limitations of these methods are low reproducibility of RAPD, high cost of AFLP and the need to know the flanking sequences to develop species specific primers for SSR polymorphism. ISSR-PCR is a technique that overcomes most of these limitations [10-13]. It is rapidly being used by the research community in various fields of plant improvement [14]. The technique is useful in areas of genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary biology in a wide range of plant species. In this method SSRs are used as primers to amplify mainly the inter-SSR regions. SSRs or microsatellites are short tandem repeats (STRs) or variable number of tandem repeats (VNTRs) of 1-4 bases of DNA ubiquitously present in eukaryote genomes [15]. They are dispersed throughout the genome and vary in the number of repeat units. Moreover, ISSR markers offer other advantages in the detection of somaclonal variation, notably a high degree of sensitivity, reproducibility, and the dominant representation of polymorphic genetic alleles. Characterizing the types and extent of genetic variation is essential to identify genotypes sothat they can be effectively used by breeders, geneticists, and conservationists [16, 17]. Earlier classifications and evaluations of the members of *Aristolochia* were based solely on morphological and physio-logical characteristics, which are easily influenced by the environment. A reliable and consistent classification can be obtained only through genetic information. Therefore, the present study was emphasized to find out the genetic relationships among the four different species of *Aristolochia* viz. *A. indica* Linn., *A. saccata* Wall., *A. tagala* Cham. and *A. cathcartii* Hook.f &T.

2. MATERIALS AND METHODS

2.1. Collection of plants

In this study, four species of *Aristolochia viz*., *A. indica* Linn., *A. saccata* Wall., *A. tagala* Cham. and *A. cathcartii* Hook. f. & T.were used. *A. indica* and *A. saccata* were collected from Lakhimpur district (27°27′16.8″ N, 94°12′11.58″ E), *A. tagala* and *A. cathcartii* were collected from Karbi Anlong district (25°54′20.22″ N, 93°39′41.16″ E) of Assam, India. The collected experimental plants were grown and maintained in the experimental garden of Botany Department, Gauhati University.

2.2. DNA extraction

For extraction of total DNA, tender leaf samples of 4 species of *Aristolochia* used in this study were taken as young leaves are considered to be the best source for isolation of DNA, also the amount of secondary metabolites are much less in young tender leaves compared to mature leaves of the trees. Leaf tissues of 1.0 g were grounded into powder with liquid nitrogen with mortar and pestle and DNA was recovered by modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987), by adding 1% polyvinyl pyrolidone (PVP) and 1% β-mercaptoethanol (β-ME) to the extraction buffer to inhibit phenolic compound interruption, and extracted DNA was purified with RNase [18].

2.3. DNA quantification

The genomic DNA was quantified by visual comparison with a DNA standard under UV light following electrophoresis on a 1% agarose gel in 1X TAE buffer containing 0.5 mg/ml of ethidium bromide (EtBr) for 30 min by running 2 µl of DNA from each sample.

2.4. PCR amplification for ISSR analysis

PCR amplification was done for ISSR analysis using a panel of eight (8) ISSR primers obtained from Operon Technologies Inc., CA, USA. The list of the primers used in this study has been listed in the Table 1.

Table 1. List of ISSR primers and their sequences.

Serial No.	Primer	Sequence (5′-3′)
1	ISSR-UBC 812	5′ GA GA GA GA GA GA GA GAA 3′
2	ISSR-UBC 814	5′ CT CT CT CT CT CT CT CT CTA 3′
3	ISSR-UBC 818	5′ CA CA CA CA CA CA CA CAG 3′
4	ISSR-UBC 836	5′ AG AG AG AG AG AG AG AG YA 3′
5	ISSR-UBC 840	5′ GA GA GA GA GA GA GA GA YT 3′
6	ISSR-UBC 842	5′GA GA GA GA GA GA GA GA YG 3′
7	ISSR-UBC 843	5′ CT CT CT CT CT CT CT CT RA 3′
8	ISSR-UBC 848	5′ CA CA CA CA CA CA CA CA RG 3′

Where Y = C, T and R= A/G

2.5. PCR reaction mix

PCR amplification was performed in 25 ml reaction volume contained 50 ng of DNA template, 2.5 µl 1X assay buffer, 1 U *Taq* DNA polymerase, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 5 pmol of primers using a Thermal Cycler (Bio-Rad, USA) for every primer as cited on Table 1 [19]. PCR ingredients were procured from Thermo-Technologies.

2.6. PCR reaction condition

PCR amplification conditions were as follows: 94°C for 5 min followed by 35 cycles of 94°C (30 s) / 42°C (45 s) / 72°C (45 s), and a final extension at 72°C for 10 min. Separation of amplification product was carried out in 1.5 % agarose gel.

2.7. Data analysis for genetic diversity

The ISSR bands were scored manually and converted to binary data as '1' (band present) or '0' (band absent). Only distinct and unambiguous bands showing polymorphism were used in analysis. Various band features such as % polymorphism, and PIC (polymorphic information content) values for each marker were calculated [20].

The pair wise genetic similarities among accessions were calculated from ISSR marker data using Jaccard's coefficient [21]. The resulting similarity matrix data was used to construct the dendrogram using UPGMA method. All the analysis was performed using NTSYSpc version 2.2. [22].

3. RESULTS

A small aliquots of isolated genomic DNA was run along with the uncut lambda DNA as reference on a 0.8% (w/v) Tris acetate EDTA (TAE) agarose gel to check the quality of DNA sample and all the selected samples have shown high molecular weight intact genomic DNA band (Fig. 1). The DNA quantification was carried out by using UV-Vis spectrophotometer which ranged from 550-650 µg/gm.

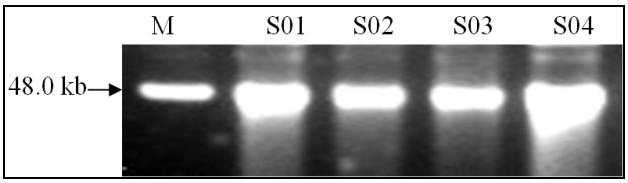

Figure 1. Genomic DNA of the four species of *Aristolochia* in 0.8% agarose gel. *M* = 100 ng lambda uncut DNA; *lane1: A. indica; lane2: A. saccata; lane3: A. cathcartii* and *lane4: A. tagala*.

Amplification of genomic DNA of 4 species of *Aristolochia* using ISSR marker analysis, yielded total amplified 66 fragments that could be scored, of which 57 were polymorphic while the remaining were monomorphic in nature (Fig. 2 and 3). In ISSR analysis number of amplified fragments ranged from 06 (UBC 812) to 10 (UBC 842 and UBC 848) which varied in size from 150 bp to 1100 bp. Of the 66 amplified bands, 57 bands (86.3%) were polymorphic with an average of 8.25 polymorphic fragments per primer (Table 2).

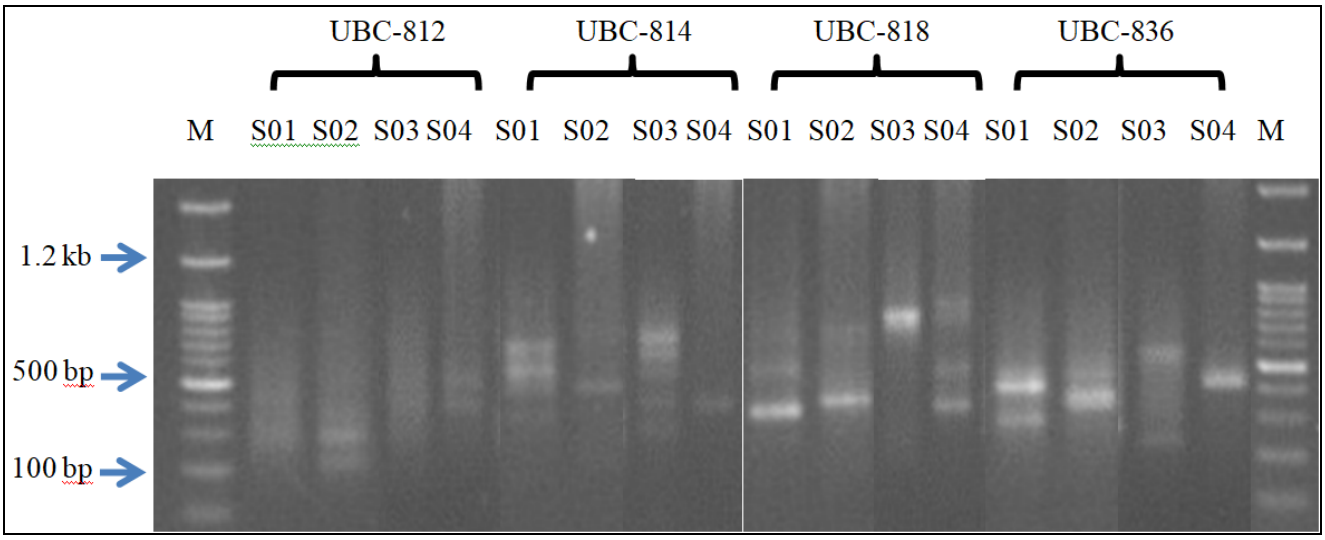

Figure 2. ISSR amplification products obtained from the 4 species of *Aristolochia* in 1.0% agarose gel. *M* = 100 bp ladder, S01*: A. indica;* S02*: A. saccata;* S03*: A. cathcartii* and *S04: A. tagala.*

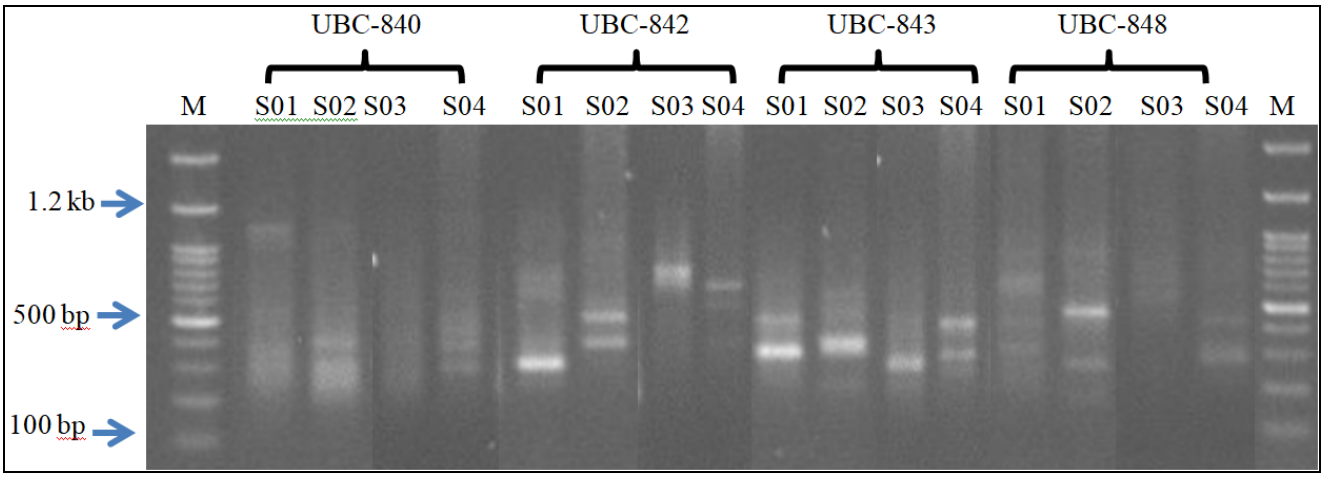

Figure 3. ISSR amplification products obtained from the 4 species of *Aristolochia* in 1.0% agarose gel. *M* = 100 bp ladder, S01*: A. indica;* S02*: A. saccata;* S03*: A. cathcartii* and *S04: A. tagala.*

Table 2. List of primers, number of amplified fragments and number of polymorphic bands generated by PCR using a panel of eight ISSR primers.

Primers	Total fragments	Polymorphic bands	Polymorphism (%)	Average PIC
UBC-812	6	5	83.33	0.43
UBC-814	9	8	88.89	0.42
UBC-818	8	6	75.00	0.46
UBC-836	7	7	100.00	0.39
UBC-840	8	7	87.50	0.43
UBC-842	10	8	80.00	0.42
UBC-843	8	7	87.50	0.41
UBC-848	10	9	90.00	0.44
Total	66	57	86.53	0.43

The percentage of polymorphism ranged from 75% (primer UBC 818) to 100% (primer UBC 836). The primers based on poly (GA with YG at 3′) and poly (CA with RG at 3′) produced maximum number of bands (10 bands) while poly (GT with A at 3′) produced minimum number of bands (06 bands). The PCR amplification using ISSR primers gave rise to reproducible amplification products. Further, the mean PIC (polymorphism information content) was 0.425 and the lowest and highest PIC value were 0.39 (UBC-836) and 0.46 (UBC-818), respectively. This suggested that all the primers used in this study were equally effective in determining polymorphisms.

A dendogram analysis was carried out by using NTSYS pc version 2.2. A total of 57 polymorphic bands were used to estimate the Jaccard's similarity coefficient and cluster analysis. Jaccard's similarity values were ranged from 0.15 to 0.4 with an average of 0.27. The details of similarity coefficient analysis have been on Table 3.

According to the genetic distances obtained and the relative position of each genotype in the UPGMA tree, using a combined data set for the eight ISSR primers used in this study revealed three major clusters. The cluster I consisted of 2 species of *Aristolochia viz.*, *A. indica* and *A. cathcartii*, whereas the cluster II and III comprised of one species each *viz.*, *A. saccata* and *A. tagala* respectively.There is great genetic variation among these 4 species of *Aristolochia* used in this study which revealed 62 percent similarity between *A. indica* and *A. cathcartii*. Again, *A. saccata* is showing 28 % similarity with cluster I and *A. tagala* is 22% similarity with cluster II (Fig. 4).

Table 3. Similarity indices on the basis of Jaccard's similarity coefficient analysis

Similarity index	S-01	S-02	S-03	S-04
S-01	1.00			
S-02	0.35	1.00		
S-03	0.40	0.24	1.00	
S-04	0.21	0.29	0.15	1.00

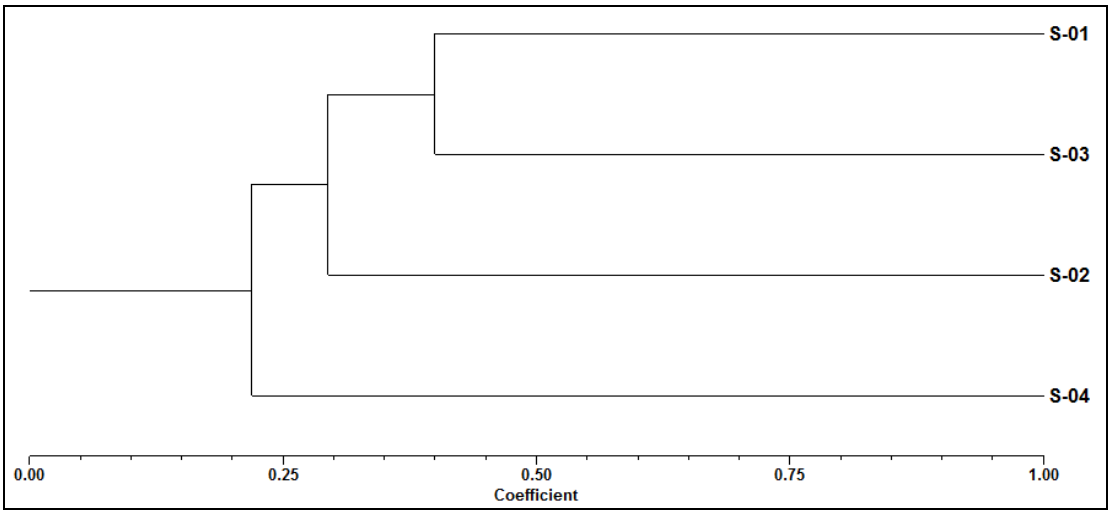

Figure 4. UPGMA dendrograms showing neighborhood joining clustering of accessions using ISSR markers data (S-01: *A. indica*; S-02: *A. saccata*; S-03: *A. cathcartii* and S-04: *A. tagala*).

4. DISCUSSION

DNA markers have proved valuable in plant breeding programme, especially in studies on genetic diversity and gene mapping. Among the different methods so far available, ISSRs have been successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of plant species including crop plants [23]. Superiority of ISSR-PCR over other marker techniques has been brought out in such investigations by various workers. ISSRs were more useful for the analysis of diversity in the genus *Eleusine* in terms of quality and quantity of data

output as compared to RFLP and RAPD [24]. Significantly, the efficiency of the technique was evident in characterization even at the varietal level of a species by different workers. For instance, three 5′ anchored primers together could distinguish 20 cultivars of *Brassica napus* [25]. ISSR is the marker of choice for assessment of genetic diversity in cocoa [26], gymnosperms [27] and even fungi [28]. In a study on white lupin it has been demonstrated that among10 primers used any two were sufficient to distinguish all the 37 accessions studied [29].The use of such highly informative primers lowers the cost, time and labour for diversity analysis.

In this study, a panel of 8 ISSR primers has been used to assess the genetic diversity among the four species of *Aristolochia* available in Assam. Nine of the 66 bands were shared by the four studied species, indicating a common evolutionary history or homoplasy, while the remaining 57 bands reflect divergence among the species. If one species was derived from the other, producing a progenitor-derivative pair and this was associated with a reduction in effective population size that would expect to observe only a subset of alleles in the derived species [30]. About 86 % of polymorphisms (57 of 66 bands) that are specific to each other among the four species of *Aristolochia* strongly suggest that though these are similar in generic level are genetically very distinct.

The dendrogram generated by ISSRs could be partially explained by the different number of PCR products analyzed reinforcing again the importance of the number of loci and their coverage of the overall genome, in obtaining reliable estimates of genetic relationships among the four species of *Aristolochia* used in the study. Here, it is clearly depicting that *A. indica* and *A. cathcartii* are more similar in terms of their genetic level and included in the same cluster. However, the rest of the two species *viz., A. saccata* and *A. tagala* are included in to two different clusters which indicate their level of polymorphisms in the genetic level.

6. CONCLUSION

ISSR marker based phylogenetic analysis clearly placed the four species of *Aristolochia* into three different clusters showing their genetic relatedness. With this study it can be concluded that the analyses of ISSR markers was useful for study the genetic relationships among the 4 different species of *Aristolochia*, providing the ISSR markers a powerful tool for the generation of potential fingerprinting diagnostic markers for these plants. Also the phylogenetic analysis on the basis of ISSR-derived phenogram would be valuable information for suitably selection of these important groups of rare and endemic medicinal plants to exploit for bioprospection.

AUTHORS' CONTRIBUTION

Both authors contributed equally for the success of this research. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this article.

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Research Article

Azadirachta indica and *Citrullus colocynthis* extracts increase defense response of wounded *Ricinus communis* and improve its growth

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ABSTRACT

The interactive effects of mechanical wounding with or without crude extracts of neem (*Azadirachta indica*) and bitter apple (*Citrullus colocynthis*, CCT) supplementation on growth, chlorophyll, carotenoids, soluble sugars (SS), soluble proteins (SP) and total free amino acids (TAA) in *Ricinus communis* plants were studied. In responses to mechanical wounding *Ricinus* plants produced more soluble sugars and soluble proteins. On the other hand, chlorophyll a, cartenoids and total free amino acids contents as well as dry mass production declined upon wounding. Neem and CCT crude extracts application, whether independently or in combination, counteracted in various degrees the deleterious effects of wounding stress on growth. Crude extracts increased SS, SP as well as Chl and cartenoids contents and improved wounded plants growth. The effect of single factors (wounding, neem and CCT extract) could be modified or reverse by the interaction between these factors when used in combination (eg. total amino acids, Chl a and carotenoids). The results clearly indicate that CCT and neem crude extracts supplementation might be beneficial in attenuating the harmful effects of mechanical wounding stress on plant growth.

Keywords: Amino acids; Carotenoids; Chlorophyll; Leaf area; Soluble sugars.

1. INTRODUCTION

Plants suffer from a biotic and biotic stress throughout their ontogeny. Recent studies have paid more attention to the abnormal variation of physiological-biochemical characteristic caused by the mechanical injures. In the vegetative phase, plants adjust the primary and secondary metabolism which may result in altered growth processes of leaves and roots upon attack by pathogens or herbivores. Wounding of plant tissues has been shown to induce complex molecular responses many of which are considered plant defense responses [1-3].

Wounding induced phenolic accumulation and browning in *Lactuca sativa* L. [4] and *Ricinus communis* plants [3] leaf tissue. It is the most effective activating the phenylpropanoid metabolism and thus promoting a higher accumulation of phenolic compounds [5], oligosaccharides, and alkaloids [2].

Wound-induced production of plant secondary metabolites is mediated by signaling molecules such as reactive oxygen species (ROS), ethylene

(ET) and jasmonic acid. Jasmonate signaling plays a crucial role in regulating responses of plants to various abiotic and biotic stresses, and plant growth and development [6- 10].

Wounding stress slows down growth of *Catharanthus roseus* [10]. Lomate and Hivrale [11] indicated that leucine amino peptidases (LAPs) are induced in response to wounding mechanical stresses have been shown to alter plant growth. Giridhar and Thiemann [12] reported that when the whole segment of oat (*Avena sativa* L.) leaf was subjected to different types of wounding, chlorophyll loss was promoted in all cases and the loss increased with increase in the intensity of wounding.

Ricinus communis (Euphorbiaceae) is a tropical plant, known as castor bean that is distributed in Egypt and widely across the world [13]. All parts of plant are important bark, leaves, flowers, seed, oil etc. The plant is reported to possess antioxidant, anti-implantation, anti-inflammatory, antidiabetic, central analgesic, antitumour, larvicidal and adult emergence inhibition, anti-nociceptive and antiasthmatic activity [14, 15].

Citrullus colocynthis L. (CCT) is one of the plants belonging to family Cucurbitaceae. It is one of the native plants of the Middle East countries , is abundantly growing plant in desert area used in traditional medicine and naturally adapted to arid environments and originally from tropical Asia and Africa. It has a fruit commonly known as bitter apple. It has been used in herbal treatment of diabetes [16]. *Citrullus colocynthis* as promising medicinal plant with wide range of pharmacological activities which could be utilized in several medical applications because of its effectiveness and safety [17].

Neem (*Azadirachta indica* A. Juss) is an evergreen tree belonging to family *Meliaceae* [18]. Neem is native of India and naturalized in most of tropical and subtropical countries are of great medicinal value and distributed widespread in the world [19]. Neem extract is cost-effective and environmentally friendly, and is therefore widely used as a means of controlling agricultural pests. It composed of a complex mixture of molecules, including normal hydrocarbons, phenolic compounds, terpenoids, alkaloids, glycosides [20], flavonoids, and saponins, which are common antibiotics found in plants [21]. Neem extracts having antimicrobial properties [22] and insecticidal activities in addition to improvement of plant seed quality and emergence of plant seedlings [23, 24]. Neem extract is also capable of controlling pathogenic microorganisms [25-27].

The effects of mechanical wounding, crude neem and CCT extracts as a bi-factorial combination have not been previously studied. Accordingly this present study aimed to evaluate the role of foliar application of crude aqueous extracts of neem (*Azadirachta indica*) and CCT (*Citrullus colocynthis*), either singly or in combination, in alleviating the deleterious effects of wounding on *Ricinus communis* plant. Therefore some aspects of a possible dual effect of mechanical wounding, neem and CCT crude extracts on growth (shoot dry weight and leaf area), photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) and some soluble carbon (soluble sugars) and nitrogen (soluble proteins, total free amino acids and proline) metabolites were investigated.

2. MATERIALS AND METHODS

Castor bean (*Ricinus communis L.*) were grown in plastic pots containing 5 kg of air dry soil (sand/clay 1:2) in the experimental outdoor green house at Botany and Microbiology Department, Faculty of Science, Assiut University (Egypt) under natural field conditions as discusses by Sayed and Gadallah [3].

2.1. Mechanical wounding induced stress

The expanded leaves of castor bean plant were damaged by puncturing leaves on the plant [28]. For the puncture treatment, the leaf was punctured 0, 10, 20, and 30 times with a sterile needle of syringe (needle diameter was 0.1 cm). Wound treatment resulted in 10, 20 and 30 uniformly spaced perforations per leaf area in addition to unwounded leaves (0 pores).

2.2. Collection and preparation of plant crude extracts

Leaves of neem (*Azadirachta indica* A. Juss) and fruits of bitter apple *(Citrullus colocynthis* L.)

plants were collected from the natural sources of Assiut University and Wadi Natash in the south Eastern Desert of Egypt, respectively. The leaves and fruits of the two plants were collected freshly and shade dried at room temperature (22-25^{o}C) in dark condition. The dried leaves and fruits were powdered to a fine powder. The extracts used for application were performed at concentration 0.25 gram of the dried powders per 100 ml of distilled water [3, 29].

2.3. Plant crude extracts application and experimental design

One set of the wounded plants (0, 10, 20 and 30 perforations) sprayed with *Citrullus* fruit aqueous extract solution (0.25%), the second set of wounded plants was sprayed with neem (*Azadirachta indica* A. Juss) leaf aqueous extract solution (0.25%), the third set of wounded plants was sprayed with a mixture of previously mentioned plant extracts (0.25 %). Control plants were sprayed with distilled water. Five pots were assigned at random to each treatment combination at each application. A week after last foliar plant extract applications, the plants were harvested and analyzed.

2.4. Plant extraction

Shoot extractions (Castor bean) were prepared using 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 g polyvinylpyrrolidone (PVP) and used for the determination of soluble carbohydrates, soluble protein, other free amino acids and proline.

2.5. Determination of photosynthetic pigments

Chlorophyll a, chlorophyll b and carotenoids, were extracted from fresh leaves samples and absorbance readings determined with a spectrophotometer (Unico UV-2100 spectrophotometer). Chlorophylls and carotenoids concentrations were calculated using equations as cited by Lichtenthaler [30].

2.6. Determination of proline content

Free proline was extracted from fresh leaves and estimated by ninhydrin reagent [31]. Proline concentration was determined using calibration curve and expressed as μg proline g^{-1} FW.

2.7. Soluble carbon and nitrogen metabolites

The contents of soluble sugars, free amino acids and soluble proteins were determined in fresh mass extracts according to the methods of Buysse and Merckx [32], Lee and Takahshi [33] and Lowry et al. [34], respectively. Calibration curves using glucose (soluble sugars), glycine (amino acids), and bovine serum albumin (soluble proteins) were constructed.

2.8. Harvesting and determination of shoot dry weight and leaf area

The dry weight (DW) was obtained after drying the plant tissues for 48 h at 72ºC. Leaf area (cm^2) was measured using the disk method [35].

2.9. Statistical Analysis

All data obtained have been subjected to one way analysis of variance (ANOVA) using the SPSS statistical package. The data were statistically analyzed using Duncan's [36] multiple range test ($p < 0.05$). Also, factorial ANOVA analysis of Ostle [37] was performed to partition the variance into the main effects of single factors as well as their interactions using the MSTATC test.

3. RESULTS

3.1. Chlorophylls and carotenoids contents

Wounding stress leads to significant decreases in Chl a and carotenoids contents as compared with unwounded plants (Table 1). On the other hand, Chl b content showed an opposite trend of response where wounding stressed plants had higher Chl b content than the unwounded ones. Application of neem extract increased Chl a, Chl b and carotenoids in wounded plants and Chl b only in unwounded plants. CCT extract cause an increase in Chl a, Chl b and carotenoids in both wounding stressed and unstressed plants except at 10 (Chl a), 20 (Chl b) perforation levels and carotenoids in unwounded plants. Treatment with both extracts in

combination resulted in significant increase in Chl a, Chl b at all wounding levels and carotenoids at moderate and high perforation levels only. On the contrary, both extracts in combination reduced carotenoids contents in unwounded and 10 perforation levels.

Table 1. Effects of leaf wounding intensity stress (pores/leaf area) and *Citrullus colocynthis* L. (CCT), *Azadirachta indica* A. Juss (Neem) and their mixture (CCT+ Neem) extracts of 0.25% (w/v) sprays on chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids contents (mg g^{-1} FW) in *Ricinus communis* L. plants. Data are means of five replicates ± SD. Different letters denote significant differences between different treatments at $p \leq 0.05$ (Duncan's test).

Parameter	Treatment				
	Wounding intensity stress	Control	CCT	Neem	CCT + Neem
Chl a	00 Pores	2.413±0.098^{b}	2.224±2.025^{ab}	2.547±0.206^{b}	1.979±0.085^{a}
	10 Pores	2.118±0.064^{b}	2.383±2.101^{c}	1.802±0.044^{a}	1.923±0.046^{a}
	20 Pores	1.322±0.052^{a}	2.357±1.682^{c}	1.745±0.080^{b}	1.838±0.097^{b}
	30 Pores	1.035±0.059^{a}	1.850±1.479^{b}	1.911±0.068^{b}	1.726±0.051^{b}
Chl b	00 Pores	0.180±0.022^{a}	0.200±0.006^{a}	0.230±0.023^{a}	0.224±0.014^{a}
	10 Pores	0.217±0.011^{a}	0.227±0.033^{a}	0.249±0.006^{a}	0.233±0.007^{a}
	20 Pores	0.241±0.011^{b}	0.257±0.005^{b}	0.121±0.013^{a}	0.253±0.008^{b}
	30 Pores	0.214±0.012^{a}	0.263±0.012^{b}	0.229±0.015^{ab}	0.246±0.014^{ab}
Carotenoids	00 Pores	1.073±0.017^{c}	0.873±0.008^{ab}	0.960±0.030^{bc}	0.810±0.067^{a}
	10 Pores	0.826±0.030^{b}	0.839±0.023^{b}	0.702±0.023^{a}	0.675±0.040^{a}
	20 Pores	0.582±0.077^{a}	0.860±0.038^{b}	0.638±0.017^{a}	0.653±0.001^{a}
	30 Pores	0.429±0.041^{a}	0.672±0.047^{b}	0.655±0.019^{b}	0.741±0.021^{b}

Table 2. Effects of leaf wounding intensity stress (pores/leaf area) and *Citrullus colocynthis* L. (CCT), *Azadirachta indica* A. Juss (Neem) and their mixture (CCT + Neem) extracts of 0.25% (w/v) sprays on soluble proteins, total free amino acids, and soluble sugars contents (mg g^{-1} FW) in *Ricinus communis* L. plants. Data are means of five replicates ± SD. Different letters denote significant differences between different treatments at $p \leq 0.05$ (Duncan's test).

Parameter	Treatment				
	Wounding intensity stress	Control	CCT	Neem	CCT + Neem
Soluble proteins	00 Pores	37.20±1.735^{a}	43.97±0.470^{b}	36.07±1.200^{a}	40.97±0.410^{b}
	10 Pores	37.63±0.939^{a}	36.23±0.433^{a}	49.93±0.617^{b}	51.67±0.821^{b}
	20 Pores	39.17±1.098^{a}	42.17±0.590^{c}	48.17±0.590^{c}	47.13±0.546^{c}
	30 Pores	57.41±2.685^{c}	45.87±0.318^{a}	46.33±0.760^{a}	51.47±1.317^{b}
Total amino acids	00 Pores	2.824±0.059^{a}	3.457±0.075^{b}	2.900±0.056^{a}	5.567±0.209^{c}
	10 Pores	3.697±0.075^{b}	2.770±0.075^{a}	4.143±0.187^{c}	3.783±0.122^{bc}
	20 Pores	2.383±0.117^{a}	2.163±0.026^{a}	4.127±0.162^{b}	5.520±0.125^{c}
	30 Pores	1.200±0.085^{a}	4.347±0.186^{d}	1.850±0.051^{b}	3.503±0.064^{c}
Soluble sugars	00 Pores	12.25±0.568^{a}	16.28±0.720^{b}	11.72±0.191^{a}	17.08±0.847^{b}
	10 Pores	15.28±0.129^{b}	13.59±0.515^{a}	18.30±0.076^{c}	22.44±0.808^{d}
	20 Pores	14.60±0.303^{b}	12.60±0.136^{a}	13.51±0.183^{ab}	26.86±1.000^{c}
	30 Pores	14.52±0.324^{a}	17.47±0.767^{b}	20.25±0.561^{c}	23.34±0.450^{d}

Table 3. Effects of leaf wounding intensity stress (pores/leaf area) and *Citrullus colocynthis* L. (CCT), *Azadirachta indica* A. Juss (Neem) and their mixture (CCT+ Neem) extracts of 0.25% (w/v) sprays on content of proline (µmol g^{-1} FW), shoot dry weight (g) and leaf area (cm^2 $plant^{-1}$) in *Ricinus communis* L. plants. Data are means of five replicates ± SD. Different letters denote significant differences between different treatments at $p \leq 0.05$ (Duncan's test).

Parameter	Treatment				
	Wounding intensity stress	Control	CCT	Neem	CCT+ Neem
Proline	00 Pores	0.100±0.003^{a}	0.113±0.002^{b}	0.098±0.003^{a}	0.124±0.003^{c}
	10 Pores	0.074±0.008^{a}	0.096±0.027^{bc}	0.110±0.004^{c}	0.093±0.002^{b}
	20 Pores	0.073±0.001^{a}	0.088±0.001^{b}	0.088±0.002^{b}	0.086±0.003^{b}
	30 Pores	0.059±0.003^{a}	0.082±0.006^{b}	0.082±0.0003^{b}	0.078±0.004^{b}
Shoot dry weight	00 Pores	6.87±0.097^{b}	5.82±0.336^{a}	7.25±0.187^{b}	6.67±0.039^{b}
	10 Pores	6.57±0.183^{a}	6.18±0.443^{a}	7.15±0.412^{a}	8.75±0.431^{b}
	20 Pores	4.92±0.146^{a}	6.82±0.286^{bc}	6.64±0.081^{b}	7.40±0.268^{c}
	30 Pores	4.63±0.337^{a}	6.41±0.195^{b}	7.75±0.069^{c}	7.45±0.162^{c}
Leaf area	00 Pores	52.75±1.435^{a}	75.05±1.155^{b}	66.27±5.774^{b}	54.38±0.577^{a}
	10 Pores	49.08±1.155^{b}	52.88±1.155^{a}	52.41±0.237^{a}	54.84±0.485^{a}
	20 Pores	42.31±0.876^{a}	51.88±0.577^{c}	49.18±0.577^{b}	43.26±0.577^{a}
	30 Pores	26.88±0.577^{a}	37.91±1.155^{b}	55.64±0.104^{c}	57.22±0.577^{d}

Table 4. Coefficient of determination (η2) values for the effects of Wounding, Neem, CCT extracts and their interactions (Wound x CCT, Wound x Neem, CCT x Neem and Wound x CCT x Neem) on contents soluble proteins, total amino acids, soluble sugars, proline, chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids, shoot dry weight, leaf area in leaves of Ricinus *communis* L. plants.

Parameter	Treatment						
	Wounding	CCT	Neem	Wound x CCT	Wound x Neem	CCT x Neem	Wound x CCT x Neem
Shoot dry weight	0.078	0.473	0.056	0.075	0.159	0.002	0.157
Leaf area	0.436	0.038	0.019	0.268	0.026	0.084	0.129
Proline	0.600	0.080	0.095	0.021	0.035	0.080	0.087
Soluble sugars	0.150	0.295	0.185	0.094	0.04	0.112	0.124
Total amino acids	0.108	0.206	0.180	0.166	0.237	0.020	0.083
Soluble proteins	0.383	0.105	0.006	0.310	0.069	0.020	0.107
Chl a	0.341	0.001	0.07	0.199	0.216	0.126	0.047
Chl b	0.116	0.001	0.167	0.306	0.192	0.003	0.216
Carotenoids	0.569	0.017	0.011	0.134	0.203	0.028	0.038

3.2. Carbon and nitrogen metabolites contents

Soluble sugars (SS) and soluble proteins (SP) contents (Table 2) increased progressively with increasing pore intensity. The reverse was true for total free amino acids (TAA) contents where their contents decreased significantly in response to wounding stress as compared with unwounded plants. Contents of SS were generally higher in un-wounded and highly wounded-stressed caster bean plants receiving neem crude extracts than in those sprayed with distilled water. The reverse held true in low and moderately wounded plants. Soluble proteins contents were higher in plants sprayed with

neem extract at 10 and 20 perforation levels. The reverse was true at 0 and 30 perforation levels.

Treatment with CCT extract resulted in an increase in SS (at low and highly wounding levels) and SP (in low and moderately wounded plant) and significant decreases at the other wounding levels. Unwounded and wounded caster bean plants receiving both extracts in combination had significantly higher SS and SP as compared with control (highly stressed plants were exceptions for SP).

Total free amino acids (Table 2) contents were generally higher in plants receiving both extracts withers independently or in combination. The effect was more pronounced in plants treated with both extracts in combination.

3.3. Proline contents

Proline contents (Table 3) decreased progressively with increasing wounding stress where its content reduced to about 58% that of the unwounded plants at high perforation level. Unwounded and wounded plants sprayed with neem and CCT extracts, had higher proline content than those sprayed with distilled water. The enhancement of proline accumulation was more pronounced when the two extracts used in combination.

3.4. Leaf area and shoot dry mass

Shoot dry mass (Table 3) reduced progressively with increasing perforation level. Spraying with neem extract increased shoot dry mass production in moderately and highly wounded plants but reduced dry mass in unwounded and low wounded plants. Application of CCT extracts enhanced dry mass accumulation over the entire wounding levels. Combination treatment of the two extracts increased dry mass content in wounded plants.

Wounding stress, at all perforation intensities, reduced castor bean leaf area than in the unstressed plants (Table 3). In general, plants sprayed with neem and CCT extract had larger leaf area than their analogous sprayed with distilled water. In this respect neem extract was more effective in increasing leaf area than CCT extracts where the largest leaf area was noticed in plants treated with neem extract.

The effects of single factors (Wounding, CCT and Neem), bi-factorial interactions (Wounding × CCT, Wounding × Neem and CCT × Neem) and three factorial (Wounding × CCT × Neem) interactions on the parameters tested were shown by analysis of variance to be statically significant. Calculation of the coefficient of determination (η^2), which indicates the relative role of each factor on the total effect of treatment combination (Table 4), pointed to that:

1 - wounding had dominant role in affecting proline, Chl a, carotenoids and leaf area.

2 - CCT has a dominant effect on changes of SS, total amino acids and shoot dry mass.

3 - The role of the interaction (Wounding × CCT) was dominant in changing chlorophyll b content.

4 - Wounding × neem interaction had dominant role in affecting soluble TAA.

The three factors and their interactions seem to have dual role in their subsidiary effect.

4. DISCUSSION

Wound signaling in plants is a complex process involving a whole array of molecules with regulatory activity [2, 38]. Wounding stress induced higher activation of the primary and secondary metabolism of carrots to prevent water loss [5, 39].

In the present work, wounding stress, at all perforation intensities, reduced caster bean leaf area than in the wound-unstressed plants (Table 3). A reduction of the leaf area following repeated wounding was reported for *Nicotiana benthamiana* [40]. Our results are in agreements with this finding where wounded plants produced less dry mass and leaf area than the unwounded ones. Growth reduction might be result of decrease photosynthesis through chlorophyll reduction (Table 1) could be another alternative explanation for growth reduction. According to Li et al. [41] reduction in growth may be attributed to reduced cell division, due to the phenolic allelochemicals which could inhibit cell division and alter the ultrastructure of the cells. These compounds interfere with various mechanisms of action and could influence a number of target sites [42]. Phenols can suppress the synthesis of protein and nucleic acids and inactivate several enzymes in the growing plants [43]. Zhang and Turner [40] suggesting that wound-induced

jasmonates suppress growth by reducing cell division. Jasmonates (JAs) act on gene expression to slow down growth and to redirect metabolism towards producing defense molecules and repairing damage [10].

Treatments with neem and CCT extracts increased leaf area and shoot dry mass content could be due to increases nitrogen assimilation where neem cake-coated urea has been produced, which increase nitrogen assimilation [44] and higher photosynthesis through enhancement chlorophyll accumulation (Table 1). This is in agreement with results of Salama and Al Rabiah [45]. Umarani and Vanangamudi [46] reported that the pre-coating of *Casuarina equisetifolia* seeds with neem leaf powder can sustain the activity of the enzymes amylase, catalase, peroxidase and superoxide dismutase, that maintain the seed germination potential.

Neem extract was more effective in increasing leaf area than CCT extracts where the largest leaf area was noticed in plants supplemented with neem extract. These results are in agreement with An et al. [47]. They showed that any secondary compound with allelochemical activity can cause both stimulatory and inhibitory effects. Neem leaves could be used as a source for the preparation of vermicomposting having both fertilizer and pesticidal potential [48]. In addition the crude extracts of neem (*A. indica*) were able to inhibit the growth of bacterial isolates *in vitro;* it therefore means that the plant has antibacterial properties [49]. Nwankwo et al. [50] reported that plant of cowpea (*Vigna unguiculata* L. Walp) treated with the crude extract of neem (*A. indica*) gave the best result in all growth parameters.

Carotenoids are bioactive compounds with remarkably special properties produced by plants in response to internal and external stresses. Mechanical injury led to dynamic changes in metabolism, especially in sugar and carotenoid contents [51]. García-Plazaola et al. [52] noticed emissions of carotenoid cleavage products upon heat shock and mechanical wounding from foliose lichen. Carotenoids are known to play important roles in plants as antioxidants [53, 54]. Carotenoid biosynthesis has regulatory mechanisms in plants [55]. Treatments of castor bean with neem and CCT extracts both singly or in combination increased carotenoid content under wounding stress compared to their control (Table 1). The results of Sayed and Gadallah [3] indicated the ability of these extracts to combat oxidative stress by quenching free radicals which reveals that, the attenuation of wounding stress effects due to their anti-oxidant property.

Wounding, can affect the carbon status of plants. Mechanical wounding induced an increase in soluble sugars with corresponding decrease in chlorophyll content of *Ricinus* plants. Giridhar and Thimann [12] reported that chlorophyll loss in oat (*Avena sativa* L.) leaves increased with increase in the intensity of wounding. The higher accumulation of SS in wounded plants with corresponding lower chlorophyll a content (Table 1 and 2) indicates that increases in SS was not the result of higher photosynthesis under wounding stress but could be due to changes in transcription of enzymes. For example sucrose synthase is a ubiquitous plant enzyme which serves a key function in plant primary carbon metabolism by its ability to synthesize and cleave sucrose, the principal form of transported carbon. Induction of sucrose synthase expression, however, has been observed to decline after wounding [56]. According to Klotz and Haagenson [57], wounding was associated with several-fold changes in sucrose synthase transcript levels. Lukaszuk et al. [51] indicated that the mechanical injury triggered an increase of the activities of sucrose hydrolysing enzymes, such as sucrose synthase (SuSy) and several types of invertases, Their results underlines important roles of SuSy and invertase in regeneration of injured tissues, most probably by providing precursors for cell wall biosynthesis and by modulating sugar-signalling in plant cells. Severe defoliation of *Casearia nitida* seedlings increased starch break-down, suggesting increased allocation of reserves towards sink organs such as new leaves [58].

Treatment with CCT extract resulted in an increase in SS (at highly wounding levels) and SP (in moderately stressed plant) and significant decreases at the other wounding levels. The same findings of stimulation carbohydrates content in *H. vulgare* and increasing the proteins content of *H. vulgare* and *Vicia. faba* by extracts of *C. colo-cynthis* compared to control was reported by Salama and Al Rabiah [45]. This may be due to interfering of allelochemicals with physiological

and biochemical processes in tested crops. Similar results were observed by El-Khatib and Hegazy [59] and El-Khawas and Shehata [60].

Unwounded and wounded caster bean plants receiving both extracts in combination had significantly higher SS and SP as compared with control. *Ricinus* plants supplied with crude extracts of neem and CCT had higher contents of chlorophyll than the control plants. This is in agreement with data of Salama and Al Rabiah [45], as pigments (Chlorophyll a, b and carotenoids) were increased in *Vicia* treated with *Citrullus colocynthis* extracts. The higher accumulation of SS in wounded plants with corresponding higher chlorophyll contents (Table 2) in plants supplemented with crude extract indicates that increases in SS was the result of higher photosynthesis and increasing metabolic activities of the tissues in response to crude extract treatments. According to Berger et al. [61], these sugars can then (i) down-regulate photosynthesis-related gene expression (feedback-inhibition) or (ii) be used to activate defense reactions in the plant.

The higher accumulation of free amino acids (Table 2) in plants supplemented with crude extract and their combination reflect the effect on ion transport across membranes, as well as enzyme activities, and can act as osmolytes or scavengers of reactive oxygen species. They may be precursors for defense-related secondary metabolites such as VOCs or alkaloids, as well [62]. The enhancement of proline accumulation was more pronounced when the two extracts used in combination (Table 3). Proline acts as an excellent compatible solute in the plant system, a metal chelator, an antioxidant, and a signaling molecule participating in the alleviation of stress sensitivity [63, 64]. Proline impacts a wide range of cellular processes, including bioenergetics, differentiation, growth, and stress adaptor [65-68].

Bi-factorial and tri-factorial interactions were mostly significant for the parameters tested as indicated by analysis of variance. These interactions between single factors could be modified or reversed their effect when used in combination. This means that the interaction between single factors (as in natural conditions) when used in combination could be modify or reverse their effects. In certain cases (e.g. TAA and Chl b contents), the relative role of the interaction between the single factors is quite large to the extent that the role of single factors could be considered minor, though even significant.

5. CONCLUSION

Results of our study clearly indicates that *Ricinus communis* plants respond to wounding stress by changes in their biochemical processes through accumulation of soluble sugars and soluble proteins. *Citrullus colocynthis and Azadirachta indica* might be beneficial in attenuating the elevated biochemical parameters induced mechanical wounding damage.

AUTHORS' CONTRIBUTION

Both authors made a significant contribution to experiment design, acquisition of data, analysis and preparing of the manuscript. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Research Article

Effects of slenderness coefficient in crown area prediction for *Tectona grandis* Linn. f. in Omo Forest Reserve, Nigeria

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ABSTRACT

Crown area which is crown size, is closely related to the photosynthetic capacity of tree and it is an important parameter to characterize biomass, leaf area and wildlife habitats. Conversely, assessment of crown dimensions still remain one of the most difficult and tedious task in forestry. The difficult measurements and the sensitivity of crown dimension on management makes it desirable to develop estimation procedures based on variables that are easier to measure than crown extension itself. The main objective of this study was to develop and observe the effects of tree slenderness coefficient in predicting crown area for *Tectona grandis* in Omo Forest Reserve. Twenty temporary sample plots of size 20 m x 20 m were randomly selected across the stand ages (9, 11, 12 and 18 years). Tree growth variables measured from each plot include diameter at breast height (Dbh) ≥ 5 cm, total height (THt) and crown diameter (CD). The data was analysed using descriptive, correlation and regression analyses. Amidst the models developed and verified, double logarithmic function with adj. R^2 = 62.0%, RMSE = 0.265% and PRESS = 0.263 gave the best fit and predictive ability. This was also supported by the undeviating bands of the graphical analyses of the residual. Conversely, the inclusion of TSC had impact on the predictive abilities of the models which implies that slenderness coefficient typically indicates the size of crown dimension, centre of gravity and a better developed root system. Therefore, it is recommended that the model can be used by forest managers for the development of stocking guideline.

Keywords: Crown area; Dbh; *Tectona grandis*; Model; Tree slenderness coefficient.

1. INTRODUCTION

The crown of tree is the centre of physiological activity, particularly gas exchange, which drives growth and development. The crown contains the foliage, the photosynthetic structure that provides carbohydrates for the growth and development of the whole tree [1]. According to Dubravac et al. [2] one of the most important elements of tree structure is the crown, where essential living processes like photosynthesis take place. The crown area also known as crown projection area, together with crown volume, also determines the amount of intercepted precipitation, and regulates the amount of precipitation that reaches the forest floor [3]. Many ecological and economic problems in forestry are approached using crown dimensional measures [4]. According to Bella [5] individual tree competition indices are derived from crown area estimates. This is because crown dimension is a result of past competition as

well as an indicator of the current growth potential [6]. Conversely, assessment of crown dimensions remain one of the most difficult and tedious task in forestry. Crown area can be estimated from stem dimensions [7]. The difficult measurements and the sensitivity of crown dimension on management makes it desirable to develop estimation procedures based on variables that are easier to measure than crown extension itself. Thus, maximum crown diameters, which can be derived from stem diameter, has been used to estimate crown area [8]. Measurement of crown dimension from either above the canopy or under the canopyare both subjected to a likely underestimation of crown width due to a limited visibility of crowns especially in a dense or mixed forest.

The size of a tree crown is strongly correlated with the growth of the trees such as diameter at breast height, slenderness coefficient, tree height [9]. The crown displays the foliage for photo-synthesis which is a key process in tree growth development. Thus, crown measurement is often done to help in the quantification of the growth of trees in the forest stand [10]. Tree slenderness coefficient often serves as an index of tree stability, or resistances to wind throw [11]. A low slenderness coefficient value usually indicates a longer crown, lower centre of gravity, and a better developed root system.

Most of forest stands in Nigeria suffer considerable losses due to action of abiotic factors, such as wind. This brings about damages in the forest structures. It is, therefore, important to know slenderness of trees which is considered to be a measure of their stability, especially of *Tectona grandis* and its relationship with crown area (CA) in Omo Forest Reserve. Tree slenderness coefficients which is defined as the ratio of total height to diameter at 1.3 m above ground, have been widely used as an index of the resistance of trees to wind throw. In earlier studies [12, 13] slenderness was usually one of the factors analyzed or it was investigated in respect of trees of a single species or it concerned several species growing in different regions. However, the suitability and effect of slenderness coefficient in predicting CA in *Tectona grandis* in Omo Forest Reserve has rarely been investigated. This study was aimed at investigating the effect of slenderness coefficient in crown area prediction models for *Tectona grandis* in Omo Forest Reserve.The findings of this study served as an aid in forest management and silvicultural practices. Hence, it would be the baseline information for forest inventory studies and determination of stand structure in pure *Tectona grandis* stands.

2. MATERIALS AND METHODS

2.1. Study area

The study was carried out in Omo Forest Reserve which is situated between latitude 6^{o} 35′ to 7^{o} 03′ North longitudes 04^{o} 9′ to 04 ^{o} 40′ East in the southwestern part of Nigeria [14]. The reserve is bounded by Benin-Shagamu expressway to the south and Omo River and Oni River to the east. The forest soils are dominantly ferralitic and highly weathered. The texture of the forest soil is usually loamy and sandy becoming heavier at greater depths, frequently with layers of gravel found between 30-60 cm. The soils tend to be acidic with low cations exchange capacity. If the soils are not maintained under forest cover surface soil erosion would set in. The Reserve lies within the tropical rainforest and has a mean annual rainfall of 1,200 mm November or part thereof is sometimes dry along with December and January each year. Sometimes, double maxima of rainfall are experienced when a dry period would exist between July and August commonly referred to as August break [14, 15]. Figure 1 shows the map of Omo Forest Reserve with a total land area of 139,100 ha.

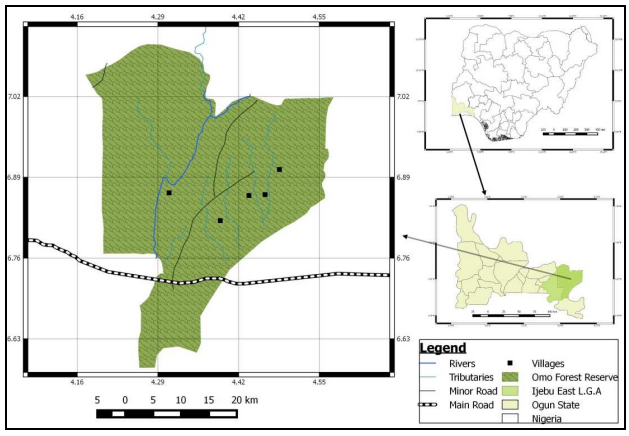

Figure 1. Map of Omo Forest Reserve, Nigeria.

2.2. Data collection

This study was carried out in temporary sample plots of *Tectona grandis* stands of different stand ages (9, 11, 12 and 18 years) using stratified random sampling technique in Omo Forest Reserve, Ogun State, Nigeria. These plantations were located at different areas in Area J4 with total land area of 39.3 hectares. In each of the selected stands, simple random sampling technique was adopted for plot location. Five temporary sample plots of size 20 m x 20 m were randomly selected from each of the stand ages. A total number of eight hundred and sixty-six (860) trees were measured in all the twenty selected plots.

2.3. Data processing and analysis

The following were computed; Basal area, tree slenderness coefficient and Crown area.

Basal area for each tree was computed using:

$BA = (\pi D^2/4)$ Equation (1)

Where: BA = Basal Area (m^2); π = Pi is constant (3.143); D^2 = Dbh (cm)

Tree slenderness coefficient was estimated for all trees using this formula:

$TSC = THt/D$ Equation (2)

Where: TSC = Tree slenderness; THt = Total height (m); D = Dbh (m)

Crown area (CA) of a tree is the area of vertical projection of the outermost perimeter of the crown on horizontal plane. Crown size, which is closely related to the photosynthetic capacity of tree, is an important parameter to characterize biomass. Crown area for each tree in the plots was estimated using the formula:

$CA = (\pi CD^2/4)$ Equation (3)

Where: CA = Crown area (m^2); CD = Crown diameter (m); π = Pi is constant (3.143).

2.4. Descriptive statistics and correlation analysis

Descriptive and inferential statistics were used in this study. The tree growth variables were described using measures of central tendency and measures of dispersion. Graphs were plotted to examine the relationship between the variables. Correlation analysis was carried out to examine the relationship between the tree growth variables. Karl Pearson's product moment coefficient of correlation was used.

2.5. Model description and fitting procedure

Descriptive, Pearson's correlations and regression analyses were carried out on the measured data. Pearson's correlation analysis was done to investigate the nature of relationship between the dependent (i.e. Crown area) and selected tree variables (i.e. CD, THT, BA, Dbh and TSC) while regression analysis was used to identify appropriate functional relationships between the dependent and independent variables.

The available fitting data set consists of measurements taken from trees located within different randomly selected sample plots. In this study, Least Square Method was used in fitting the models. Linear and logarithm models (Table 1) were proposed as candidate models for the investigation of the effectiveness of slenderness coefficient for predicting Crown area of *Tectona grandis* in the study area.

Table 1. The selected candidate models.

F/C	Model form
1	$CA = B_0 + B_1Dbh$
2	$LnCA = B_0 + B_1LnDbh$
3	$LnCA = B_0 + B_1Dbh$
4	$CA = B_0 + B_1Dbh + B_2TSC$
5	$LnCA = B_0 + B_1LnDbh + B_2LnTSC$
6	$LnCA = B_0 + B_1Dbh + B_2TSC$

F/C = Function code, CA = Crown area, Dbh = Diameter at breast height, TSC = Tree slenderness coefficient, B_0, B_1 and B_2 are parameters to be estimated.

The evaluation of the models was based on numerical and graphical analyses of the residuals. Three statistical criteria were used to examine the models' performance. These criteria are: root mean square error (RMSE), which analyses the precision of the estimates; the adjusted coefficient of determination (Adj. R^2) and Standard error of the estimates (SEE). Another important step in evaluating the models was by performing graphical analysis of the residuals.

In addition, the significance of regression coefficients (t) was observed. A model with high Adjusted R^2 and least RMSE and SEE was judged to have good fit. Residuals values were plotted against the predicted Crown area values to check the constant error assumption.

3. RESULTS

The minimum, maximum, mean, standard deviation and standard error of main measured and derived variables for the four plantations (1996, 2002, 2003 and 2005) of *Tectona grandis* established year used in the study are presented in Table 2. The distribution of diameter at breast height (Dbh) ranged from 5.9 to 37.9 cm, crown diameter ranged from 2.2 m to 10.10 m, tree slenderness coefficient ranged from 36.8 to 223.3 and CA ranged from 3.8 m^2 to 80.1 m^2.

Table 3 shows the result of Pearson's product-moment correlation analysis. In this result, fewer of the growth variables had negative correlations with each other while most of the variables had significantly positive correlations. Significant and positive correlation existed between tree diameter at breast height (Dbh) and total height (THt), crown diameter (CD), crown area (CA) with the correlation coefficient values of 0.57, 0.749, and 0.728 respectively while showing negative correlation (r = - 0.665) with slenderness coefficient (TSC). However, most of the correlations were strong. Slenderness coefficient (TSC) is negatively and significantly correlated with all other tree growth variables apart from Total height 0.148. Crown area (CA) had strong and positive correlation with CD (r = 0.987). The correlation coefficients between CA and Dbh were higher than those correlation coefficients between the other variables. Basal area (BA) had positive and significant correlation with other measured variables and negatively correlated with TSC (r = - 0.561). Thus, the bivariate correlations of the growth variables were 73% positive and 27% negative.

Table 2. Summary statistics table for growth characteristics variables.

Growth variables	Minimum	Maximum	Mean	Std. error	Variance
Diameter at breast height (cm)	5.9	37.9	17.1	0.19	29.73
Total height (m)	6	26	14.5	0.11	10.99
Crown diameter (m)	2.2	10.1	5.3	0.04	1.44
Basal area (m^2)	0.003	0.1	0.03	0.00	0.00
Crown area (m^2)	3.8	80.1	23.5	0.37	118.77
Tree slenderness coefficient	36.8	223.3	89.8	0.80	0.06
Number of observations = 860					

Table 3. Correlation matrix for the tree growth variables.

	Dbh	THT	CD	BA	CA	TSC
Dbh	1					
THt	0.570*	1				
CD	0.749*	0.325*	1			
BA	0.881*	0.468*	0.639*	1		
CA	0.728*	0.291*	0.987*	0.638*	1	
TSC	-0.665*	0.148*	-0.596*	-0.561*	-0.570*	1

* Correction is significant at the 0.05 level (2-tailed), N = 860. Where: Dbh = diameter at breast height (cm), THt = total height (m), CD = crown diameter (m), BA = Basal area (m^2), CA = crown area (m^2) and TSC = Tree slenderness coefficient.

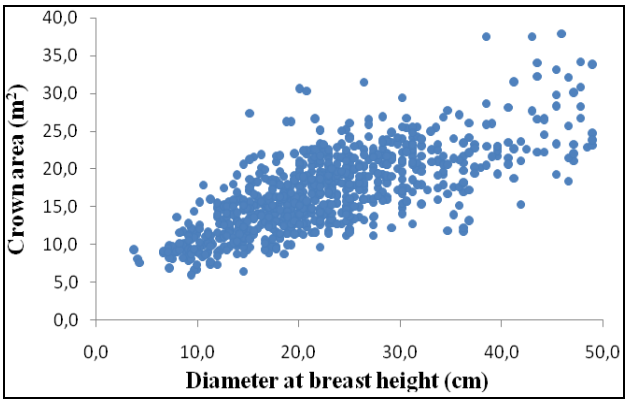

Figure 2. Relationship between crown area and diameter at breast height.

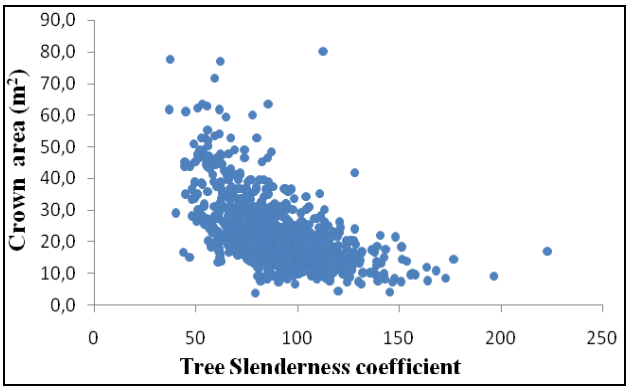

Figure 3. Relationship between crown area and tree slenderness coefficient.

It can be seen that crown area of *Tectona grandis* trees in the study area are essentially linearly related with diameter at breast height (Dbh, Figure 2) but has inverse linear relationship with the tree slenderness coefficient (TSC, Figure 3).

Model fitting and evaluation are important parts of model building. Fitting of tree crown area models were based on the total data set. A number of different regression models were developed for predicting crown area. The candidate models for predicting crown area fitted to the data with corresponding parameter estimates, fit and prediction statistics are as presented in Table 4. The candidate models were based on Dbh and TSC as explanatory variables. All the regression coefficients of the candidate models were found statistically significant at the probability level of 0.05.

On the basis of estimated Adjusted R^2 values, 0.55 to 0.62 of the total variation in observed CA values was explained by the six candidate models. From the results obtained (Table 4), double log (Model No. 5) had the highest value of Adj. R^2 of 0.62 with the least values of RMSE and SEE of 0.265 and 0.263 respectively. This was followed by model 2 with the Adj. R^2 value of 0.60, RMSE and SEE values of 0.284 and 0.84 respectively. Linear model (Model No. 1) had the lowest value of Adj. R^2 of 0.55 as well as highest values of RMSE and SEE of 7.171 and 7.159, correspondingly. All the model statistics (Adj. R^2, RMSE and SEE) for the candidate models showed that model 5 (i.e. double logarithm) was the best followed by model 2 which is also double logarithm.

Table 4. Model developed.

F/C	Model	Parameter			Model statistics		
		B_0	B_1	B_2	Adj. R^2	RMSE	SEE
1	$CA = B_0 + B_1Dbh$	-1.461	1.438		0.55	7.171	7.159
2	$LnCA = B_0 + B_1LnDbh$	0.083	1.062		0.60	0.284	0.284
3	$LnCA = B_0 + B_1Dbh$	2.002	0.061		0.57	0.295	0.294
4	$CA = B_0 + B_1Dbh + B_2TSC$	7.060	1.260	-0.061	0.56	7.101	7.082
5	$LnCA = B_0 + B_1LnDbh + B_2LnTSC$	1.545	0.925	-0.242	0.62	0.265	0.263
6	$LnCA = B_0 + B_1Dbh + B_2TSC$	2.415	0.052	-0.003	0.58	0.291	0.290

F/C = Function code, CA =Crown diameter, Dbh = Diameter at breast height, TSC = Tree slenderness coefficient, B_0, B_1 and B_2 are parameters estimated.

Figure 4 shows the distribution of residuals against the predicted natural logarithm of crown area. This model gives the impression that supports the assumption of constant error variance of a desirable model. The undeviating bands of the scatter plot shows that the model is suitable for predicting crown area.

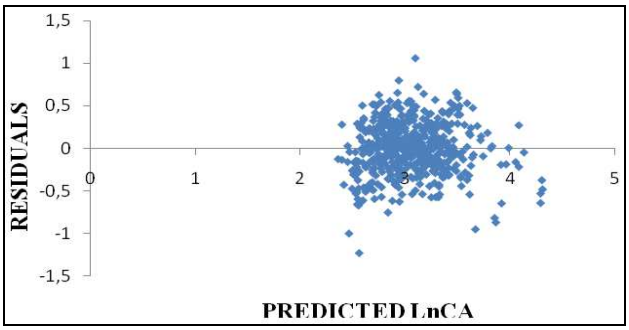

Figure 4. Residual distribution against Predicted LnCA using the best candidate Model 5 (Double log).

4. DISCUSSION

The correlation between tree basal area and slenderness coefficient was negative. This implies that the proportion of trees prone to wind-throw or damage in the area decreases with increase in tree basal area. This agrees with the finding of Martin-Alcon et al. [16] that the proportion of wind-throw and damaged trees in a stand decreases strongly at higher stand basal area for a given slenderness ratio. Slenderness coefficient is negatively and significantly correlated with most of the tree growth variables such as crown area (CA). This clarifies the significance of tree slenderness coefficient architecturally as an indicator of crown type as also reported by Wang et al. [17]. Lowest coefficient of correlation was obtained for the relationship between tree slenderness coefficient and total height. This is similar to the report of Oyebade et al. [18].

There was an improvement in the model developed upon addition of TSC after the inclusion of Dbh for the model data. Crown area would be influenced by Dbh in similar ways as TSC (i.e. competition would cause a reduction in individual tree growth, which would result in both smaller Dbh and TSC). Dbh was used in all of the models because prior studies have reported that this is the most correlated variable with tree growth characteristics including CA [19]. Factor such as highstand density influenced both Dbh and CA in similar ways by causing reduction in growth. TSC was used as measures of tree stability, crown architecture and it is related to species stand growth characteristics [20, 21]. However, it was interesting that the inclusion of TSC had impact on the predictive abilities of the models. For this double logarithm of the model 5 with TSC and Dbh showed significant improvement, as judged from reduction in RMSE, SEE or increased Adjusted R^2, over the models with Dbh as the only independent variable. This is not similar with the previous study on Dbh - CA as described by Shimano [19]. He stated that power sigmoid function was most suitable for predicting crown area. In his study, Dbh was used as the only independent variable.

5. CONCLUSION

This study found that tree crown area could be predicted from commonly measured tree variables. Based on the evaluation of the models fitted and examined in this study, the double logarithm function is recommended as crown area models for *Tectona grandis* in Omo Forest Reserve. This function has diameter at breast height and tree slenderness coefficient as independent variables. In this study, though diameter at breast height was to be common useful independent variable in all the selected models. It was observed that tree slenderness coefficient helps in improving the predictive accuracy of the crown area model developed. However, it is recommended that this model can be used in predicting crown area from forest inventories for growth models, development of stocking guideline and wildlife suitability index models that use crown characteristics.

AUTHORS' CONTRIBUTION

JUE: acquisition of data, analysis and interpretation of data, writing manuscript, material support and review of manuscript. OC: development of methodology, conception and design, interpretation of data, writing and review of manuscript and material support. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare that there is no conflict of interests.

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Research Article

Floristic composition, life-forms and biological spectrum of Toor Al-Baha District, Lahej Governorate, Yemen

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ABSTRACT

This paper enumerates 542 plant species belonging to 289 genera in 89 families of vascular plants collected from Toor Al-Baha district, Lahej governorate, Yemen, during 2008-2015. The Poaceae has the, relatively highest number of species (50 sp., 9.23%) followed by Asteraceae (38 sp., 7.01%), Euphorbiaceae (34 sp., 6.27%), Asclepiadaceae (30 sp., 5.54%), Fabaceae (28 sp., 5.17%) and Acanthaceae (26 sp., 4.80%). A genus represented by the greatest number of species is *Euphorbia* (19 species). Classification based on life form indicates that the chamaephytes (38.19%) comprise the largest proportion of the plants in the study area, followed by therophytes (28.60%) and phanerophytes (20.85%). The present results revealed that there are three parasitic species belong to two families these are: *Cistanche phelypaea* and *Cistanche rosea* (Orobanchaceae) and *Striga angustifolia* (Scrophulariaceae). Sixty three succulents taxa belong to eighteen families were recorded in the flora of the study area, among these families three are the richest ones (Asclepiadaceae 17, Euphorbiaceae 11 and Aloaceae 6).

Keywords: Floristic composition; Life form; *Euphorbia*; Succulent taxa; Yemen.

1. INTRODUCTION

The Toor Al-Baha district (Fig. 1) has a special geographical, bio-geographical and ecological position in the Lahej governorate, Yemen. It extends between latitudes 12° 58’ and 13° 20’ N. and between longitudes 44° 11’ and 44° 39’ E., with an area of about 1883 sq. km. Toor Al-Baha district is bordered by Al-Qubaytah district in the north, Al-Maqatrah district, Al-Madaribah and Ras Al-Aarah district in the west, Tuban district in the east and by Gulf of Aden and parts of Aden governorate in the south (Fig. 1). The flora of Yemen is very rich and diverse. Species diversity is a result of considerable climatic changes in former periods, which enabled different species to survive, in the different ecological habitats [1]. Previous studies reported that, there are about 2844 plant species belong to 1068 genera and 179 families in Yemen [2-12]. The system of Raunkiaer is the most and worldwide accepted, which is based upon the principle of position and degree of protection of the buds during the adverse climate condition.

There are several workers on floristic composition of different regions in Yemen [2, 13-21]. Besides, no work on life forms has so far been carried out in Yemen.

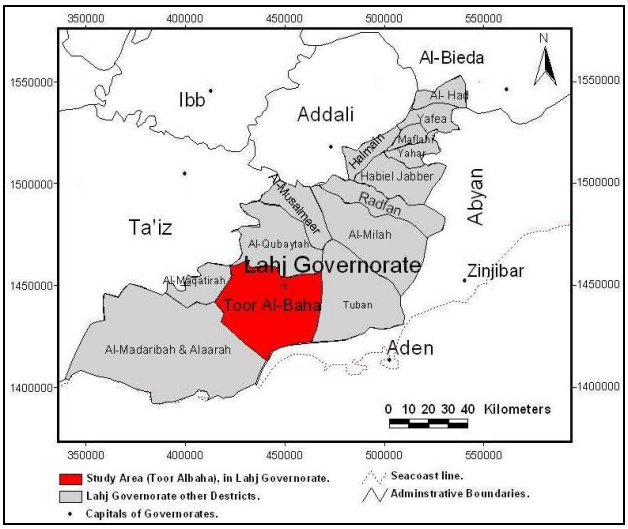

Figure 1. Map of Lahej governorate with browsing the location of study area - Toor Al-Baha district (modified after Ministry of the Local Administration).

2. MATERIALS AND METHODS

This work is based on the results of four years (2008-2015) of intensive study of the vascular plants of Toor Al-Baha district, Lahej governorate, Yemen. The plant specimens were pressed in the field and transported to be continued in the laboratory. When the species were completely dried, each individual specimen was mounted on a herbarium sheet. Specimens of all plants were identified and named with the aid of relevant floras and available revisions [3, 6, 22-54**]**. All the plants species were classified on the basis of life forms as defined by Hassib [55] and Raunkiaer [56], to determine the phytoclimate of the area. The percentage of various life form classes put together is called as the biological spectrum.

The plant taxa found in the study area are listed in Appendix. The list provides scientific name and life form.

Abbreviations

Abbreviations of life form categories used in the paper include in alphabetical order: Ch = Chamaephytes; Ep = Epiphytic; G = Geophytes; He = Hemicryptophytes; P = Parasites; Ph = Phanerophytes; Th = Therophytes; S = Succulent.

3. RESULTS

3.1. Floristic analysis

Plant species recorded in studied area (Toor Al-Baha district) with their families are listed in the Appendix. The list includes 542 species, representing 289 genera and 89 families: 81 monocots (14.94%), 452 dicots (83.39%), 8 ferns (1.48%) and one Gymnospermae (*Juniperus procera*). The richest families in terms of number of taxa are Poaceae (50), Asteraceae (38), Euphorbiaceae (34), Asclepiadaceae (30), Fabaceae (28) and Acanthaceae (26). The largest families in terms of number of genera are Poaceae (28), Asteraceae (26), Asclepiadaceae (20) and Acanthaceae (13) Appendix. The genera with the largest number of species are *Euphorbia* (19), *Acacia* (12), *Indigofera* (10), *Heliotropium* (9) and *Eragrostis* (8) (Fig. 2).

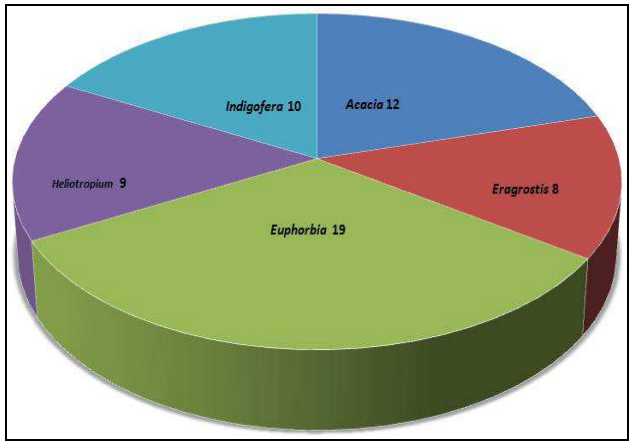

Figure 2. The richest genera in terms of number of species in studied area.

3.2. Life forms

The life form spectrum of the whole study area (Appendix), showed that the most dominant life form is chamaephytes (207 sp., with 38.19%), followed by therophytes (155 sp., 28.60%) and phanerophytes (113 sp., 20.85%). Among the flora of Toor Al-Baha the geophytes (36 sp.) were low and represent about 6.64%. On the other hand hemicryptophytes were represented by (23 sp. and

4.24%). During the field work five species were observed growing on the others plants (epiphytes or hemiparasit), these species represent 0.92% of the total recorded species. The present results revealed that there are three parasitic species belong to two families these are: *Cistanche phelypaea* and *Cistanche rosea* (Orobanchaceae) and *Striga angustifolia* (Scrophulariaceae). These three species represent 0.55% of the total collected species in the studied area (Appendix; Table 1; Fig. 3).

Table 1. Different life-forms classes of the flora of Toor Al-Baha District.

Life form classes	Abbreviation	No. of species	Percentage
Chamaephytes	Ch	207	38.19
Epiphytic	Ep	5	0.92
Geophytes	G	36	6.64
Hemicryptophytes	He	23	4.24
Parasites	P	3	0.55
Phanerophytes	Ph	113	20.85
Therophytes	Th	155	28.60
Total		542	100

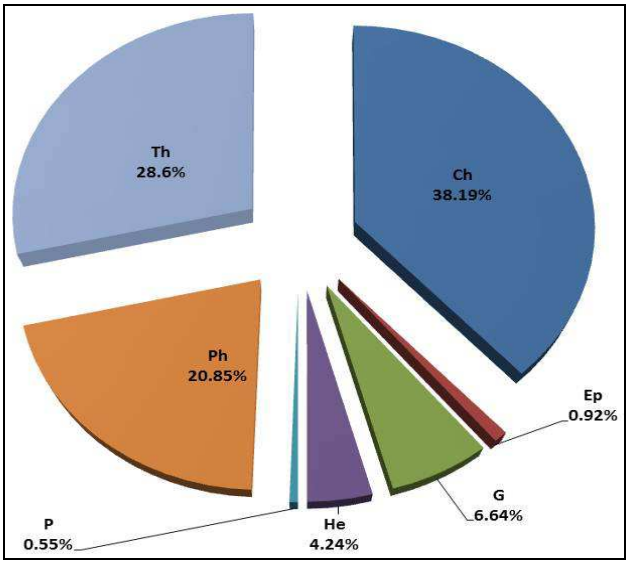

Figure 3. Biological spectrum of life-forms of present study (explanation of abbreviations in Materials and Methods).

3. 3. Succulent plants

Among the 542 species recorded in the studied area, 63 species are succulents in different parts (roots, stems and leaves). The following families comprise the high numbers of the succulent taxa: Asclepiadaceae (17), Euphorbiaceae (11), Aloaceae (6), Aizoaceae, Crassulaceae and Vitaceae (4 species for each) (Table 2).

4. DISCUSSION

The vascular flora of the Toor Al-Baha district contains a total 542 taxa (including species and infra-specific taxa) in 89 families (71 dicots, 14 monocots, three pteridophytes and one Gymnospermae) and 289 genera. The richest families in terms of number of taxa are Poaceae (50), Asteraceae (38), Euphorbiaceae (34), Asclepiadaceae (30), Fabaceae (28) and Acanthaceae (26). These families represent a high percentage (38.01%) of the total species of the studied area (Appendix). A comparison of families in terms of the number of species found in this study compared with studies of nearby regions with similar habitats [2, 14, 19, 57, 58] was done. Poaceae was the largest families in all studies except for Asteraceae in Wadi Dahr [14]. *Euphorbia* was the largest genus in terms of number of species in studied area (Fig. 2). The present results agree with the other references [2, 7, 13, 14, 58, 59].

Following the well "life form" system of [56], based on the location of renewal buds, the life form spectrum is thought to be either hereditary adjustment to environment or representing the residual effects of some historical, climatic or biotic conditions on the plant population [60]. In the present studies, the chamaephytes are the most dominant life form in the studied area. They are represented by 207 taxa which constitute about 38.19% of the recorded taxa, followed by therophytes (155 taxa, 28.60%) and the phanerophytes (113 taxa, 20.85%) (Table 1, Fig. 3).

The dominance of the chamaephytes life form, and the short life cycles plants (therophytes) may be attributed to be response to the hot dry climate, topographic variation and biotic influence, while phanerophytes provide good evidence that there abundance is fact of an expression of monsoon climate. Thus these characters of chamaephytes, therophytes and phanerophytes show dominance over the other life form. In the neighbor countries such as Taif of Saudi Arabia, Mosallam [61] reported that the dominant life form of that region

are therophytes and chamaephytes while El-Ghanim et al. [62] reported the same results from Hail region of Saudi Arabia. Abd El-Ghani and Abdel-Khalik [63] from the southeastern corner of Egypt (Elba) reported that the dominant life forms in that area are: therophytes, phanerophytes and chamaephytes. Kambhar and Kotresha [64] from Gadag district, Karnataka, India, reported that the dominant life forms of that region are (therophytes and phanerophytes).

Table 2. Numbers of succulent taxa and their families recorded in the study area (explanation of abbreviations in Materials and Methods).

Family	Life form							
	Ch (s)	G (s)	He(s)	P(s)	Ph (s)	Th (s)	Total	%
Agavaceae	-	1	-	-	-	-	1	1.59
Aizoaceae	-	-	-	-	-	4	4	6.35
Aloaceae	5	-	-	-	1	-	6	9.52
Apocynaceae	-	-	-	-	1	-	1	1.59
Asclepiadaceae	15	-	2	-	-	-	17	26.98
Asteraceae	2	-	-	-	-	-	2	3.17
Cactaceae	-	-	-	-	2	-	2	3.17
Chenopodiaceae	1	-	-	-	-	-	1	1.59
Crassulaceae	4	-	-	-	-	-	4	6.35
Dracaenaceae	-	2	-	-	-	-	2	3.17
Euphorbiaceae	8	-	-	-	3	-	11	17.46
Moraceae	1	-	-	-	-	-	1	1.59
Orchidaceae	-	1	-	-	-	-	1	1.59
Orobanchaceae	-	-	-	2	-	-	2	3.17
Passifloraceae	-	-	-	-	1	-	1	1.59
Portulacaceae	-	-	-	-	-	2	2	3.17
Vitaceae	2	-	2	-	-	-	4	6.35
Zygophyllaceae	-	-	-	-	-	1	1	1.59
Total	38	4	4	2	8	7	63	100
Percentage	60.32	6.35	6.35	3.17	12.70	11.11	100	

Epiphytic or semi-parasitic species are recorded among the flora of this region but with small percentage (0.92%). They are represented by five species. All of them are belonging to two families: Loranthaceae, four species in three genera these are: *Oncocalyx doberae* on *Dobera glabra* trees, *Phragmanthera austroarabica* on *Ficus cordata* and *Acacia asak, w*hile *Plicosepalus acaciae* on *Acacia* spp., *P. curviflorus* on *Acacia tortilis* and *Euphorbia cactus*. The fifth species is *Viscum cruciatum* which belongs to family Viscaceae was found on *Tamarix aphylla* (Table 1, Fig. 3). Another plant group, parasitic plants, the present results of Toor Al-Baha flora proved that there are three species of this group. Two species are belonging to Orobanchaceae: *Cistanche phelypaea* and *C. rosea* and only one species belonging to Scrophulariaceae (*Striga angustifolia*). This number of the parasitic species constitutes 0.55% of the total number of species recorded in the studied area. This value is insignificant. The occurrence of the parasitic species denotes the importance of water conservation. The study on the flora of Toor Al-Baha proved that there are not any aquatic plants since there are neither streams nor water bodies in the studied area. But the present studies reported of *Phyla nodiflora* (Verbenaceae) prefers the wetted soils or canal banks. Also the flora of Toor Al-Baha is very poor in the halophytes plants because its land is far away from the sea shore or costal land.

Succulent plants are of a great ecological significance, particularly in arid and semi-arid parts

of Yemen or the Arabian Peninsula in general. They store water in their stems, leaves or roots, a characteristic feature adopted by several plants to withstand high temperature and low precipitation. In Yemen, succulent plants are usually seen in, along the Aden Gulf and the Red Sea coast, shallow depressions and dry places with low altitudes. In the study area, approximately, 63 species belonging to 18 families are generally recognized as succulent. Some of the families, which are rich in succulent species, are Aizoaceae, Aloaceae, Asclepiadaceae, Crassulaceae, Euphorbiaceae, and Vitaceae (Table 2). The same results are in agreement with those of McCoy [50]. The succulent habit of the plants may be reflect the dominant climatic factors in this region since plants modify their parts leaves, stems and inflorescences to storage the available water in the wet rainy seasons to survive in the dry seasons.

All most all plants are useful in one way or the other. Economically, the flora of Toor Al-Baha comprises several plant species with high economic value. These plants can be classified into: timber plants, fibre yielding plants, edible plants and medicinal plants. People at Toor Al-Baha usually used *Juniperus, Prosopis, Tamarix, Ziziphus, Cordia, Combretum, Dracaena, Ficus, Grewia* etc. as a source of timber for construction. Pillows have been made from the inflorescence of *Aerva javanica* and mats from the fibres of *Sansevieria, Hyphaene, Phoenix* and *Dracaena.* Some other wild species are reported to be edible and are eaten in one way or the other as *Amaranthus lividus, Rumex vesicarius, Corchorus spp., Portulaca oleracea, Pulicaria jaubertii, Cissus rotundifolia, Desmidorchis awdelianus, Monolluma quadrangula, Orbea deflersiana* etc. These plants are important plants of which the fresh leaves or stem are eaten. The use of *Salvadora persica* roots as tooth-brush, myrrh from *Commiphora spp.,* henna from *Lawsonia inermis,* etc. is common even in these days. Species such as *Lawsonia inermis, Withania somnifera, Senna alexandrina, S. italica, Acacia etbaica, Jatropha curcas, Solanum incanum* etc. are good sources of medicines for treating various ailments.

AUTHOR'S CONTRIBUTION

All authors have equally contribution in fields of work, collection, identification, scrutiny of literature, manuscript preparation and editing, associated with this research article. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare that there is no conflict of interests.

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Appendix. List of plant species recorded in the studied area with their families and life-forms (explanation of abbreviations in Materials and Methods).

Taxon	Life form
Acanthaceae (26)	
Acanthus arboreus Forssk.	Ph
Anisotes trisulcus (Forssk.) Nees	Ph
Asystasia guttata (Forssk.) Brummitt	Ch
Barleria acanthoides Vahl	Ch
Barleria hildebrandtii S. Moore	Ch
Barleria hochstetteri Nees	Ch
Barleria parviflora R. Br. ex T. Anders.	Ch
Barleria prionitis L. subsp. *appressa* (Forssk.) Brummitt & J. R. I. Wood	Ch
Barleria proxima Lindau	Ch
Barleria trispinosa (Forssk.) Vahl	Ch
Blepharis ciliaris (L.) B. L. Burtt.	Ch
Crossandra johanninae Fiori	Ch
Dyschoriste radicans Nees	Ch
Ecbolium gymnostachyum (Nees) Milne-Redh.	Ch
Ecbolium viride (Forssk.) Alston	Ch
Justicia calyculata Defl.	Th
Justicia debilis (Forssk.) Vahl	Ch
Justicia flava (Vahl) Vahl	Th
Justicia heterocarpa T. Anders. subsp. *heterocarpa*	Th
Justicia ladanoides Lam.	Th
Justicia odora (Forssk.) Lam.	Ch
Lepidagathis calycina Hochst. ex Nees	Ch
Megalochlamys violacea (Vahl) Vollesen	Ch
Peristrophe paniculata (Forssk.) Brummitt	Th
Ruellia discifolia Oliv.	Ch
Ruellia patula Jacq.	Ch
Actiniopteridaceae (2)	
Actiniopteris radiata (Swartz) Link	G
Actiniopteris semiflabellata Pic.-Ser.	G
Adiantaceae (5)	
Adiantum capillus-veneris L.	He
Adiantum incisum Forssk.	G
Cheilanthes coriacea Decne.	G
Negripteris scioana (Chiov.) Pic.-Ser.	G
Onychium divaricatum (Poir.) Alston	G
Agavaceae (1)	
Agave sisalana Perrine	G (s)
Aizoaceae (4)	
Aizoon canariense L.	Th (s)
Trianthema crystallina (Forssk.) Vahl	Th (s)
Trianthema triquetra Willd.	Th (s)
Zaleya pentandra (L.) C. Jeffrey	Th (s)
Alliaceae (1)	
Allium subhirsutum L.	G
Aloaceae (6)	
Aloe inermis Forssk.	Ch (s)
Aloe irafensis Lavranos, McCoy & Gifri	Ch (s)
Aloe niebuhriana Lavranos	Ch (s)
Aloe officinalis Forssk.	Ch (s)

Taxon	Life form
Aloe rivierei Lavranos & L. E. Newton	Ch (s)
Aloe sabaea Schweinf.	Ph (s)
Amaranthaceae (12)	
Achyranthes aspera L.	Ch
Aerva javanica (Burm. f.) Juss. ex Schult. var. *javanica*	Ch
Aerva lanata (L.) Juss. ex Schult.	Ch
Alternanthera pungens Kunth	Th
Amaranthus graecizans L. subsp. *graecizans*	Th
Amaranthus lividus L.	Th
Amaranthus sparganiocephalus Thell.	Th
Amaranthus spinosus L.	Th
Digera muricata (L.) Mart. subsp. *muricata*	Th
Psilotrichum gnaphalobryum (Hochst.) Schinz	Ch
Pupalia lappacea (L.) A. Juss. var. *velutina* (Moq.) Hook. f.	Ch
Saltia papposa (Forssk.) Moq.	Ph
Amaryllidaceae (2)	
Crinum album (Forssk.) Herb.	G
Pancratium tortuosum Herbert.	G
Anacardiaceae (2)	
Pistacia falcata Becc. ex Mart.	Ph
Rhus flexicaulis Bak.	Ph
Apiaceae (Umbelliferae) (2)	
Conium maculatum L.	Ch
Oreoschimperella arabiae-felicis (C. C. Townsend) C. C. Townsend var. *laevis* C. C. Townsend	Th
Apocynaceae (2)	
Acokanthera schimperi (DC.) Schweinf.	Ph
Adenium obesum (Forssk.) Roem. & Schult	Ph (s)
Arecaceae (Palmae) (2)	
Hyphaene thebaica (L.) Mart.	Ph
Phoenix dactylifera L.	Ph
Aristolochiaceae (1)	
Aristolochia bracteolata Lam.	Th
Asclepiadaceae (30)	
Blyttia spiralis (Forssk.) D. Field & J. R. I. Wood	Ch
Calotropis procera (Ait.) Ait. f.	Ph
Caralluma subulata (Forssk.) Decne.	Ch (s)
Cynanchum viminale (L.) L. subsp. *stipitaceum* (Forssk.) Meve & Liede	Ch (s)
Desmidorchis cf. arabicus (N. E. Br.) Meve & Liede	Ch (s)
Desmidorchis awdelianus (Defl.) Meve & Liede	Ch (s)
Desmidorchis penicillata (Defl.) Plowes	Ch (s)
Duvalia sulcata N. E. Br. subsp. *seminuda* (Lavranos) Meve	He (s)
Duvalia sulcata N. E. Br. subsp. *sulcata*	He (s)
Echidnopsis scutellata (Defl.) A. Berger subsp. *scutellata*	Ch (s)
Edithcolea sp. nov.	Ch (s)
Glossonema boveanum (Decne.) Decne.	Ch
Glossonema varians (Stocks) Benth. ex Hook. f.	Ch
Huernia rubra Plowes	Ch (s)
Leptadenia arborea (Forssk.) Schweinf.	Ch
Leptadenia pyrotechnica (Forssk.) Decne.	Ph
Marsdenia schimperi Decne.	Ph
Monolluma quadrangula (Forssk.) Plowes	Ch (s)
Odontanthera radians (Forssk.) D. V. Field	Th

Taxon	Life form
Orbea chrysostephana (Defl.) Bruyns	Ch (s)
Orbea deflersiana (Lavranos) Bruyns	Ch (s)
Pentatropis nivalis (J. F. Gmel.) D. V. Field & J. R. J. Wood	Ch
Pergularia daemia (Forssk.) Chiov.	He
Pergularia tomentosa L.	Ch
Periploca aphylla Decne.	Ph
Periploca visciformis (Vatke) K. Schum.	Ph
Rhytidocaulon splendidum T. A. McCoy	Ch (s)
Sulcolluma hexagona (Lavranos) Plowes	Ch (s)
Sulcolluma shadhbana (Lavranos) Plowes	Ch (s)
Sulcolluma shadhbana (Lavranos) Plowes var. *barhana* (Lavranos & L. E. Newton) Plowes	Ch (s)
Asparagaceae (1)	
Asparagus africanus Lam.	Ch
Asteraceae (Compositae) (38)	
Acanthospermum hispidum DC.	Th
Bidens biternata (Lour.) Merr. & Sherff	Th
Blepharispermum yemense Defl.	Ph
Blumea bovei (DC.) Vatke	Ch
Echinops erinaceous Kit Tan	Ch
Eclipta prostrata (L.) L.	He
Erigeron bonariensis L.	Ch
Flaveria trinervia (Spreng.) C. Mohr	Th
Helichrysum glumaceum DC.	Ch
Iphiona scabra DC.	Ch
Kleinia odora (Forssk.) DC.	Ch (s)
Kleinia pendula (Forssk.) DC.	Ch (s)
Laggera decurrens (Vahl) F. N. Hepper & J. R. I. Wood	Ch
Launaea intybacea (Jacq.) Beauverd	Th
Launaea massauensis (Fresen.) Sch. Bip. ex Kuntze	Th
Launaea petitiana (A. Rich.) N. Kilian	Ch
Osteospermum vaillantii (Decne.) Norl.	Th
Pegolettia senegalensis Cass.	Th
Pluchea indica (L.) Less. subsp. *indica*	Ph
Pluchea indica (L.) Less. subsp. *yemenensis* King-Jones	Ph
Pluchea ovalis (Pers.) DC.	Ch
Pseudoconyza viscosa (Mill.) D'Arcy	Th
Psiadia punctulata (DC.) Vatke	Ch
Pulicaria jaubertii Gamal-Eldin	Th
Pulicaria petiolaris Jaub. & Spach	Ch
Pulicaria schimperi DC.	Ch
Pulicaria somalensis O. Hoffm. subsp. *schweinfurthii* Gamal-Eldin	Ch
Reichardia tingitana (L.) Roth	Th
Sonchus oleraceus L.	Th
Tagetes minuta L.	Th
Tridax procumbens L.	He
Vernonia arabica F. G. Davies	Ch
Vernonia cinerascens Sch. Bib.	Ch
Vernonia cinerea (L.) Less.	Th
Vernonia spatulata (Forssk.) Sch. Bip. ex Asch.	Ch
Volutaria albicaulis (Defl.) J. R. I. Wood	Ch
Xanthium spinosum L.	Th
Xanthium strumarium L.	Ch

Taxon	Life form
Balanitaceae (1)	
Balanites aegyptiaca (L.) Delile var. *aegyptiaca*	Ph
Bignoniaceae (1)	
Rhigozum somalense Hall. f.	Ph
Boraginaceae (17)	
Arnebia hispidissma (Lehm.) DC.	Th
Cordia monoica Roxb.	Ph
Cordia nevillii Alston	Ph
Cordia sinensis Lam.	Ph
Echium rauwolfii Delile	Th
Ehretia abyssinica R. Br. ex Fresen.	Ph
Ehretia obtusifolia Hochst. ex A. DC.	Ph
Heliotropium aegyptiacum Lehm.	Ch
Heliotropium bottae Defl.	Ch
Heliotropium longiflorum (A. DC.) Jaub. & Spach var. *longiflorum*	Ch
Heliotropium ovalifolium Forssk.	Th
Heliotropium pterocarpum (DC.) Hochst. & Steud. ex Bunge	Th
Heliotropium rariflorum Stocks	Ch
Heliotropium strigosum Willd. var. *bicolor* (Hochst. & Steud.) Schwartz	Th
Heliotropium strigosum Willd. var. *cordofanum* (Hochst.) Schweinf.	Th
Heliotropium zeylanicum (Burm. f.) Lam.	Ch
Trichodesma trichodesmoides (Bunge) Gürke	Ch
Brassicaceae (Cruciferae) (5)	
Diplotaxis harra (Forssk.) Boiss.	Th
Farsetia linearis Decne. ex Boiss.	Ch
Farsetia longisiliqua Decne	Ch
Farsetia stylosa R. Br.	Ch
Schouwia purpurea (Forssk.) Schweinf.	Th
Burseraceae (5)	
Commiphora gileadensis (L.) C. Chr.	Ph
Commiphora kataf (Forssk.) Engl.	Ph
Commiphora kua (Royle) Vollesen	Ph
Commiphora myrrha (Nees) Engl.	Ph
Commiphora schimperi (O. Berg) Engl.	Ph
Cactaceae (2)	
Opuntia dillenii (Ker-Gawl.) Haw.	Ph (s)
Opuntia ficus-indica (L.) Miller	Ph (s)
Caesalpiniaceae (9)	
Delonix elata (L.) Gamble	Ph
Parkinsonia aculeata L.	Ph
Senna alexandrina Mill.	Ch
Senna holosericea (Fresen.) Greuter	Ch
Senna italica Mill.	Ch
Senna obtusifolia (L.) Irwin & Barneby	Ch
Senna occidentalis (L.) Link	Ch
Senna sophera (L.) Roxb.	Ch
Tamarindus indica L.	Ph
Capparaceae (18)	
Boscia arabica Pestalozzi	Ph
Cadaba baccarinii Chiov.	Ph
Cadaba farinosa Forssk. subsp. *farinosa*	Ph
Cadaba glandulosa Forssk.	Ph

Taxon	Life form
Cadaba heterotricha Stocks	Ph
Cadaba longifolia DC.	Ph
Cadaba rotundifolia Forssk.	Ph
Capparis cartilaginea Decne.	Ch
Cleome brachycarpa Vahl ex DC.	Th
Cleome gynandra L.	Th
Cleome paradoxa R. Br. ex DC.	Ch
Cleome scaposa DC.	Th
Cleome viscosa L.	Th
Dipterygium glaucum Decne.	Ch
Maerua angolensis DC.	Ph
Maerua crassifolia Forssk.	Ph
Maerua macrantha Gilg	Ph
Maerua oblongifolia (Forssk.) A. Rich.	Ph
Caryophyllaceae (3)	
Cometes abyssinica R. Br.	Ch
Minuartia filifolia (Forssk.) Mattf.	Th
Polycarpaea repens (Forssk.) Aschers & Schweinf.	He
Celastraceae (2)	
Maytenus parviflora (Vahl) Sebsebe	Ph
Maytenus senegalensis (Lam.) Exell	Ph
Chenopodiaceae (5)	
Chenopodium carinatum R. Br.	Th
Chenopodium murale L.	Th
Halothamnus bottae Jaub. & Spach subsp. *niger* Kothe-Heinrich	Ch
Salsola spinescens Moq.	Ch
Suaeda aegyptiaca (Hasselq.) Zohary	Ch (s)
Cistaceae (1)	
Helianthemum stipulatum (Forssk.) C. Chr.	Ch
Colchicaceae (1)	
Gloriosa revoilii (Franch.) J. C. Manning & Vinn.	G
Combretaceae (2)	
Combretum molle R. Br. ex G. Don	Ph
Terminalia brownii Fresen.	Ph
Commelinaceae (6)	
Aneilema forskalei Kunth.	Th
Commelina albescens Hassk.	He
Commelina benghalensis L.	He
Commelina erecta L.	He
Commelina forsskaolii Vahl	He
Commelina imberbis Ehrenb. ex Hassk.	He
Convolvulaceae (11)	
Convolvulus arvensis L.	He
Convolvulus hystrix Vahl	Ch
Evolvulus alsinoides (L.) L.	Ch
Hildebrandtia africana Vatke subsp. *arabica* Sebsebe	Ch
Ipomoea dichroa Choisy	Ch
Ipomoea eriocarpa R. Br.	Ch
Ipomoea nil (L.) Roth	Ch
Ipomoea obscura (L.) Ker-Gawl.	Ch
Seddera arabica (Forssk.) Choisy	Ch
Seddera latifolia Hochst. & Steud.	Ch

Taxon	Life form
Seddera virgata Hochst. & Steud.	Ch
Crassulaceae (4)	
Kalanchoe alternans (Vahl) Pers.	Ch (s)
Kalanchoe bentii Hook. f. subsp. *bentii*	Ch (s)
Kalanchoe deficiens (Forssk.) Asch. & Schweinf. var. *glabra* Raadts	Ch (s)
Kalanchoe yemensis (Defl.) Schweinf.	Ch (s)
Cucurbitaceae (12)	
Citrullus colocynthis (L.) Schrad.	He
Coccinia grandis (L.) Voigt	Ch
Corallocarpus glomeruliflorus (Defl.) Cogn.	Ch
Corallocarpus schimperi (Naud.) Hook. f.	Ch
Cucumis dipsaceus Ehrenb. ex Spach	Th
Cucumis melo L. subsp. *agrestis* (Naud.) Grebensc.	Th
Cucumis prophetarum L. subsp. *dissectus* (Naud.) C. Jeffrey	Th
Cucumis prophetarum L. subsp. *prophetarum*	Th
Cucumis pustulatus Naud. ex Hook. f.	Ch
Kedrostis foetidissima (Jacq.) Cogn.	Ch
Momordica balsamina L.	Th
Zehneria anomala C. Jeffrey	Th
Cupressaceae (1)	
Juniperus procera Hochst. ex Endl.	Ph
Cyperaceae (2)	
Cyperus falcatus Nees.	G
Cyperus rotundus L.	G
Dracaenaceae (3)	
Dracaena ombet Kotschy & Peyr.	Ph
Sansevieria ehrenbergii Schweinf. ex Bak.	G (s)
Sansevieria forskaoliana (Schult. f.) Hepper & J. R. I. Wood	G (s)
Euphorbiaceae (34)	
Acalypha ciliata Forssk.	Th
Acalypha fruticosa Forssk. var. *fruticosa*	Ch
Acalypha indica L.	Ch
Chrozophora oblongifolia (Delile) A. Juss. ex Spreng.	Ch
Croton lobatus L.	Th
Euphorbia balsamifera Ait. subsp. *adenensis* (Defl.) Bally	Ch (s)
Euphorbia cuneata Vahl subsp. *cuneata*	Ph (s)
Euphorbia fractiflexa S. Carter & J. R. I. Wood	Ch (s)
Euphorbia fruticosa Forssk.	Ch (s)
Euphorbia granulata Forssk. var. *glabrata* (Gay) Boiss.	Th
Euphorbia granulata Forssk. var. *granulata*	Th
Euphorbia greuteri N. Kilian, Kürschner & P. Hein	Ch (s)
Euphorbia heterophylla L.	Th
Euphorbia hirta L.	Th
Euphorbia inarticulata Schweinf.	Ch (s)
Euphorbia indica Lam.	Th
Euphorbia longituberculosa Boiss.	Ch (s)
Euphorbia prostrata Ait.	He
Euphorbia qarad Defl.	Ph (s)
Euphorbia schimperi Presl.	Ch (s)
Euphorbia schimperiana Scheele	Th
Euphorbia serpens Kunth	Th
Euphorbia triaculeata Forssk.	Ch (s)

Taxon	Life form
Euphorbia uzmuk S. Carter & J. R. I. Wood	Ph (s)
Flueggea virosa (Roxb. ex Willd.) Voigt.	Ph
Jatropha curcas L.	Ph
Jatropha pelargoniifolia Courb. var. *pelargoniifolia*	Ch
Jatropha spinosa Vahl	Ch
Micrococca mercurialis (L.) Benth.	Th
Phyllanthus amarus Schum. & Thonn.	Th
Phyllanthus fraternus Webster	Th
Phyllanthus maderaspatensis L.	Ch
Phyllanthus rotundifolius Willd.	Th
Ricinus communis L.	Ph
Fabaceae (28)	
Cadia purpurea (Picc.) Ait.	Ph
Crotalaria incana L.	Th
Crotalaria microphylla Vahl.	Th
Crotalaria pycnostachya Benth. subsp. *pycnostachya*	Th
Crotalaria saltiana Andr.	Th
Crotalaria senegalensis (Pers.) DC.	Th
Indigofera arabica Jaub. & Spach	Th
Indigofera argentea Burm. f.	Th
Indigofera coerulea Roxb. var. *coerulea*	Ch
Indigofera coerulea Roxb. var. *occidentalis* Gillett & Ali	Ch
Indigofera colutea (Burm. f.) Merr.	Th
Indigofera hochstetteri Bak.	Th
Indigofera oblongifolia Forssk.	Ch
Indigofera semitrijuga Forssk.	Th
Indigofera spinoflora Boiss.	Ch
Indigofera spinosa Forssk.	Ch
Microcharis tritoides (Bak.) Schrire subsp. *tritoides*	Ch
Ormocarpum yemenense Gillett	Ch
Rhynchosia minima (L.) DC. var. *prostrata* (Harv.) Meikle	He
Rhynchosia pulverulenta Stocks	He
Rhynchosia schimperi Hochst. ex Boiss.	He
Sesbania leptocarpa DC.	Ph
Tephrosia heterophylla Vatke	Ch
Tephrosia purpurea (L.) Pers. subsp. *apollinea* (Delile) Hosni & El- Karemy	Ch
Tephrosia quartiniana Cuf. ex Greuter & Burdet	Ch
Tephrosia subtriflora Hochst. ex Bak.	Th
Tephrosia uniflora Pers.	Ch
Vigna aconitifolia (Jacq.) Maréchal	Th
Gentianaceae (1)	
Centaurium pulchellum (Sw.) Druce	Th
Geraniaceae (1)	
Geranium biuncinatum Kokwaro	Th
Gisekiaceae (1)	
Gisekia pharnaceoides L. var. *alata* M. Gilbert.	Th
Hyacinthaceae (4)	
Albuca abyssinica Jacq	G
Dipcadi filifolium Bak.	G
Dipcadi serotinum (L.) Medic.	G
Dipcadi viride (L.) Moench	G

Taxon	Life form
Lamiaceae (Labiatae) (17)	
Endostemon tenuiflorus (Benth.) M. Ashby	Th
Lavandula dhofarensis A. G. Miller	Th
Lavandula pubescens Decne.	Ch
Lavandula setifera T. Anders.	Ch
Leucas alba (Forssk.) Sebald	Th
Leucas glabrata (Vahl) R. Br. var. *glabrata*	Ch
Leucas inflata Benth.	Ch
Leucas urticifolia (Vahl) Sm. var. *urticifolia*	Th
Micromeria imbricata (Forssk.) C. Chr.	Ch
Ocimum filamentosum Forssk.	Ch
Ocimum forsskaolii Benth.	Ch
Ocimum spicatum Defl.	Ch
Ocimum tenuiflorum L.	Ch
Otostegia fruticosa (Forssk.) Schweinf. ex Penz. subsp. *fruticosa*	Ch
Plectranthus hyemalis J. R. I. Wood	Ch
Plectranthus montanus Benth.	Ch
Teucrium yemense Defl.	Ch
Linaceae (1)	
Linum volkensii Engl.	Th
Loranthaceae (4)	
Oncocalyx doberae (Schweinf.) A. G. Miller & J. A. Nyberg	Ep
Phragmanthera austroarabica A. G. Miller & J. A. Nyberg	Ep
Plicosepalus acaciae (Zucc.) Wiens & Polh.	Ep
Plicosepalus curviflorus (Benth. ex Oliv.) Tieghem	Ep
Lythraceae (1)	
Lawsonia inermis L.	Ph
Malvaceae (17)	
Abutilon bidentatum A. Rich.	Ch
Abutilon figarianum Webb	Ch
Abutilon fruticosum Guill. & Perr.	Ch
Cienfuegosia welshii (T. Anders.) Garcke	Ch
Gossypium arboreum L.	Ph
Hibiscus aristaevalvis Garcke	Th
Hibiscus cannabinus L.	Th
Hibiscus micranthus L. f.	Ch
Hibiscus palmatus Forssk.	Ch
Hibiscus purpureus Forssk.	Ch
Hibiscus trionum L.	Ch
Hibiscus vitifolius L.	Ch
Pavonia arabica Hochst. & Steud. ex Boiss.	Ch
Pavonia flavoferruginea (Forssk.) Hepper & J. R. I. Wood	Ch
Senra incana Cav.	Ch'
Sida alba L.	Ch
Sida ovata Forssk.	Ch
Meliaceae (2)	
Azadirachta indica A. Juss.	Ph
Turraea parvifolia Defl.	Ph
Menispermaceae (1)	
Cocculus pendulus (J. R. & G. Forst.) Diels	Ph
Mimosaceae (14)	
Acacia asak (Forssk.) Willd.	Ph

Taxon	Life form
Acacia edgeworthii T. Anders.	Ph
Acacia ehrenbergiana Hayne	Ph
Acacia etbaica Schweinf. subsp. *uncinata* Brenan	Ph
Acacia hamulosa Benth.	Ph
Acacia hunteri Oliv.	Ph
Acacia johnwoodii Boulos	Ph
Acacia laeta R. Br. ex Benth.	Ph
Acacia mellifera (Vahl) Benth.	Ph
Acacia nilotica (L.) Willd. ex Delile subsp. *indica* (Benth.) Brenan	Ph
Acacia oerfota (Forssk.) Schweinf.	Ph
Acacia tortilis (Forssk.) Hayne subsp. *tortilis*	Ph
Prosopis cineraria (L.) Druce	Ph
Prosopis juliflora (Sw.) DC.	Ph
Molluginaceae (4)	
Corbichonia decumbens (Forssk.) Exell	Th
Glinus lotoides L.	Ch
Limeum obovatum Vicary	Th
Mollugo cerviana (L.) Ser.	Th
Moraceae (8)	
Dorstenia barnimiana Schweinf.	G
Dorstenia foetida (Forssk.) Schweinf. subsp. *foetida*	Ch (s)
Ficus cordata Thunb. subsp. *salicifolia* (Vahl) C. C. Berg	Ph
Ficus glumosa Delile	Ph
Ficus ingens (Miq.) Miq.	Ph
Ficus palmata Forssk. subsp. *palmata*	Ph
Ficus sycomorus L. subsp. *sycomorus*	Ph
Ficus vasta Forssk.	Ph
Moringaceae (1)	
Moringa peregrina (Forssk.) Fiori	Ph
Nyctaginaceae (7)	
Boerhavia diffusa L.	Ch
Boerhavia elegans Choisy	Ch
Boerhavia erecta L.	Th
Commicarpus grandiflorus (A. Rich.) Standl.	Ch
Commicarpus helenae (J. A. Schultes) Meikle	Ch
Commicarpus mistus Thulin	Ch
Commicarpus plumbagineus (Cav.) Standl.	Ch
Oleaceae (2)	
Jasminum grandiflorum L. subsp. *floribundum* (R. Br. ex Fresen.) P. S. Green	Ch
Olea europaea L. subsp. *cuspidata* (Wall. ex G. Don) Ciferri	Ph
Orchidaceae (1)	
Eulophia petersii (Reichb. f.) Reichb. f.	G (s)
Orobanchaceae (2)	
Cistanche phelypaea (L.) Cout.	P (s)
Cistanche rosea Bak.	P (s)
Oxalidaceae (1)	
Oxalis corniculata L.	G
Papaveraceae (2)	
Argemone mexicana L.	Th
Argemone ochroleuca Sweet	Th
Passifloraceae (1)	
Adenia venenata Forssk.	Ph (s)

Taxon	Life form
Pedaliaceae (1)	
Pedalium murex L.	Th
Plantaginaceae (1)	
Plantago lanceolata L.	Th
Poaceae (Gramineae) (50)	
Aeluropus lagopoides (L.) Trin. ex Thwaites	G
Aristida abnormis Chiov.	Th
Aristida adscensionis L.	Th
Aristida ferrilateris S. M. Phillips	Th
Aristida mutabilis Trin. & Rupr.	Th
Arundo donax L.	G
Brachiaria lata (Schumach.) C. E. Hubb.	Th
Brachiaria leersioides (Hochst.) Stapf	Th
Brachiaria ovalis Stapf	Th
Cenchrus ciliaris L.	Th
Cenchrus pennisetiformis Hochst. & Steud.	Th
Chloris barbata Swartz	Ch
Chrysopogon plumulosus Hochst.	G
Cymbopogon schoenanthus (L.) Spreng.	G
Dactyloctenium aegyptium (L.) Willd.	Th
Dactyloctenium aristatum Link	Th
Dactyloctenium robecchii (Chiov.) Chiov.	G
Dactyloctenium scindicum Boiss.	G
Dichanthium foveolatum (Delile) Roberty	Th
Digitaria abyssinica (Hochst. ex A. Rich.) Stapf	Th
Digitaria ciliaris (Retz.) Koeler	Th
Digitaria pennata (Hochst.) T. Cooke	Th
Echinochloa colona (L.) Link	Th
Echinochloa pyramidalis (Lam.) Hitchc. & Chase	Th
Enneapogon persicus Boiss	Th
Eragrostis aspera (Jacq.) Nees	Th
Eragrostis barrelieri Daveau	Th
Eragrostis cilianensis (All.) Vignolo ex Janch.	Th
Eragrostis ciliaris (L.) R. Br.	Th
Eragrostis lepida (A. Rich.) Hochst. ex Steud.	Th
Eragrostis minor Host	Th
Eragrostis papposa (Roem. & Schult.) Steud.	Th
Eragrostis tremula (Lam.) Hochst. ex Steud.	Th
Hyparrhenia hirta (L.) Stapf	Th
Lasiurus scindicus Henrard	Th
Leptothrium senegalense (Kunth) Clayton	Th
Melinis repens (Willd.) Zizka	Th
Ochthochloa compressa (Forssk.) Hilu	G
Odyssea mucronata (Forssk.) Stapf	Ch
Panicum turgidum Forssk.	G
Paspalum dilatatum Poir.	G
Pennisetum setaceum (Forssk.) Chiov.	Ch
Setaria verticillata (L.) P. Beauv.	Th
Sporobolus angustifolius A. Rich.	Ch
Stipagrostis ciliata (Desf.) De Winter	Th
Stipagrostis plumosa (L.) Munro ex T. Anders.	Th
Tetrapogon cenchriformis (A. Rich.) Clayton	Th

Taxon	Life form
Tetrapogon tenellus (K. D. Koenig ex Roxb.) Chiov.	G
Tetrapogon villosus Desf.	G
Tragus berteronianus Schult.	Th
Polygalaceae (4)	
Polygala abyssinica R. Br. ex Fresen.	Ch
Polygala erioptera DC.	Th
Polygala irregularis Boiss.	Ch
Polygala senensis Klotzsch	Ch
Polygonaceae (2)	
Calligonum comosum Herit.	Ph
Rumex vesicarius L.	Th
Portulacaceae (2)	
Portulaca oleracea L. subsp. *oleracea*	Th (s)
Portulaca quadrifida L.	Th (s)
Primulaceae (2)	
Anagallis arvensis L. subsp. *arvensis* var. *coerulea* Gouan	Th
Anagallis arvensis L. subsp. *foemina* (Mill.) Schinz & Thell.	Th
Resedaceae (2)	
Ochradenus baccatus Delile	Ph
Reseda sphenocleoides Defl.	Ch
Rhamnaceae (2)	
Ziziphus mucronata Willd.	Ph
Ziziphus spina-christi (L.) Desf.	Ph
Rubiaceae (4)	
Kohautia aspera (Heyne ex Roth) Bremek.	Ch
Kohautia caespitosa Schnizl.	Ch
Pavetta longiflora Vahl subsp. *longiflora*	Ph
Wendlandia arabica Defl. subsp. *arabica*	Ph
Salvadoraceae (2)	
Dobera glabra (Forssk.) Poir.	Ph
Salvadora persica L.	Ph
Sapindaceae (2)	
Allophylus rubifolius (Hochst. ex A. Rich.) Engl. var. *rubifolius*	Ph
Pappea capensis Eckl. & Zeyh.	Ph
Sapotaceae (2)	
Mimusops laurifolia (Forssk.) Friis	Ph
Sideroxylon mascatense (A. DC.) Pennington	Ph
Scrophulariaceae (11)	
Anticharis arabica Endl.	Th
Anticharis glandulosa Aschers.	Th
Anticharis senegalensis (Walp.) Bhandari	Th
Campylanthus junceus Edgew.	Ch
Campylanthus yemenensis A. G. Miller	Ch
Kickxia scalarum Schweinf. ex D. A. Sutton	Ch
Kickxia woodii D. A. Sutton*	Ch
Schweinfurthia pedicellata (T. Anders.) Balf. f.	Th
Schweinfurthia pterosperma (A. Rich.) A. Braun	Th
Scrophularia arguta Sol.	Th
Striga angustifolia (D. Don.) C. J. Saldanha	P
Selaginellaceae (1)	
Selaginella imbricata (Forssk.) Spreng.	G

Taxon	Life form
Solanaceae (14)	
Datura innoxia Miller	Ch
Datura metel L.	Ch
Datura stramonium L.	Ch
Lycium shawii Roem. & Schult.	Ph
Nicotiana glauca R. C. Graham	Ph
Physalis angulata L.	Th
Solanum coagulans Forssk.	Th
Solanum cordatum Forssk.	Th
Solanum forsskaolii Dunal	Th
Solanum glabratum Dunal	Ch
Solanum incanum L.	Ch
Solanum nigrum L.	Th
Solanum villosum Miller subsp. *miniatum* (Bernh. ex Willd.) Edmonds	Th
Withania somnifera (L.) Dunal	Ch
Sterculiaceae (3)	
Melhania denhamii R. Br.	Ch
Melhania stipulosa J. R. I. Wood	Ch
Sterculia africana (Lour.) Fiori	Ph
Tamaricaceae (1)	
Tamarix aphylla (L.) Karst.	Ph
Thymelaeaceae (1)	
Gnidia somalensis (Franch.) Gilg	Ch
Tiliaceae (10)	
Corchorus depressus (L.) Stocks	Ch
Corchorus tridens L.	Th
Corchorus trilocularis L.	Th
Grewia arborea (Forssk.) Lam.	Ph
Grewia erythraea Schweinf.	Ph
Grewia schweinfurthii Burrett	Ph
Grewia tembensis Fresen.	Ph
Grewia tenax (Forssk.) Fiori	Ph
Grewia trichocarpa Hochst. ex A. Rich.	Ph
Grewia velutina (Forssk.) Vahl	Ph
Ulmaceae (1)	
Celtis africana Burm. f.	Ph
Urticaceae (3)	
Forsskaolea tenacissima L.	Ch
Forsskaolea viridis Webb	Th
Parietaria debilis G. Forster	Th
Velloziaceae (1)	
Xerophyta arabica (Bak.) N. Menezes	G
Verbenaceae (5)	
Chascanum marrubifolium Fenzl ex Walp.	Ch
Lantana camara L.	Ph
Lantana viburnoides (Forssk.) Vahl	Ch
Phyla nodiflora (L.) Greene	G
Priva adhaerens (Forssk.) Chiov.	Ch
Viscaceae (1)	
Viscum cruciatum Sieb. ex Boiss.	Ep
Vitaceae (4)	
Cissus quadrangularis L.	He (s)

Taxon	Life form
Cissus rotundifolia (Forssk.) Vahl	He (s)
Cyphostemma digitatum (Forssk.) Descoings	Ch (s)
Cyphostemma ternatum (J. F. Gmel.) Descoings	Ch (s)
Zygophyllaceae (7)	
Fagonia indica Burm. f. var. *indica*	Ch
Fagonia indica Burm. f. var. *schweinfurthii* Hadidi	Ch
Fagonia paulayana Wagner & Vierh.	Ch
Tetraena simplex (L.) Beier & Thulin	Th (s)
Tribulus macropterus Boiss. var. *arabicus* (Hosni) Al-Hemaid & J. Thomas	He
Tribulus pentandrus Forssk.	He
Tribulus terrestris L.	He