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Articles

Toxicological Effects of Aqueous Leaf Extract of Dinya (*Vitex Dodianna*) on Liver Enzymes of Albino Rats

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Abstract

The research was piloted to establish the hepatotoxic potentials of the leaf extract of Vitex dodiana on liver enzymes of apparently healthy albino rats. A total of sixteen (16) albino rats were clustered into four (4) groups of four (4) rats each designated as group A – D, Group A served as control while groups B, C and D were treated with 200 mg/kg, 300 mg/kg, and 400 mg/kg aqueous leaves of extract of Vitex dodiana respectively for a period of two weeks. The liver enzymes were determined using spectrophotometric methods. The activity of AST was slightly decreased to 6.5±0.20 in the rats treated with 200 mg/kg body weight of the extract and slightly decreased to 5.2±0.12 and 5.0±0.33 in the rats treated with 300 and 400 mg/kg body weight of the extracts respectively when compared with untreated group (5.6±0.15) with no significant ($P > 0.05$) differences. The activity of ALT was slightly decreased to 2.5±0.11 in the rats treated with 300mg/kg body weight of the extracts and slightly increased to 2.64±0.17 and decreased to 2.4±0.04 in the rats treated with 200 and 400 mg/kg body weight of the extracts respectively when compared with untreated group (2.8±0.31) with no significant ($P > 0.05$) difference. The result of ALP also showed no significant ($P < 0.05$) difference of serum ALP activity, though it was observed in the rats treated with 400 mg/kg body weight of the extracts the serum concentration decreased to 100.06±0.66, and 102.44±2.34 at 300 mg/kg body weight and 104.56±1.20 at 200 mg/kg body weight of the extracts, but no significant ($P > 0.05$) difference was observed when compared with untreated group (106.26±8.51). The results revealed no significant ($P < 0.05$) decrease in the activity of serum liver enzymes of the rats treated with the three different doses of Vitex dodiana extract when compared with control rats. In conclusion, acute oral administration of aqeous extract of Vitex dodiana was found to be relatively safe.

Keywords: Vitex dodiana, hepatic, ALP, AST, ALT, liver and enzymes.

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1. Introduction

Vitex doniana belongs to the family of verbenaceae. It is widely distributed in the Northern parts of Nigeria (Tadzabia et al., 2013; Jima, Megersa, 2018). It is called Dinya in the native Hausa language of the North. It is a medium-sized deciduous tree, 8-18 m high, with a heavy rounded crown and a clear bole up to 5 m. It has a rough bark, pale brown or greyish-white, rather smooth with narrow vertical fissures. The leaves are opposite, glabrous, 14-34 cm long, usually with 5 leaflets on stalks (Bello et al., 2018). It is dark green above and pale greyish-green below. The flower petals are white except on the largest lobe, which is purple. The flowers are small, blue or violet, 3-12 cm in diameter (Bello et al., 2018; Oyeyemi et al., 2018). The fruits are oblong, about 3 cm long. They are green when young and purplish black when ripe (Oyeyemi et al., 2018).

The plant has been used in the management of many diseases by traditionalists. Some of these ailments include, diabetes, cancer, hypertension, gastrointestinal disorders, rheumatism, jaundice, leprosy and many more (Ozkaya et al., 2013; Ibisi et al., 2017).

Plant leaves are generally eaten as vegetables or salad in many African countries. They are eaten as a part of staple food daily in many areas and are quite rich in nutrients (Beyene et al., 2016; Olufunmilayo, 2017).

Though many studies have been conducted on the medicinal uses of the plant, little have been reported on its toxicological effects (Billah, Kabir, 2015).

This study was designed to investigate the toxicological effects of Vitex dodiana with the intention of providing valuable data which may lead to the development of alternative drugs and therapeutic strategies with little or no side effects.

2. Materials and methods

Plant Materials

The fresh leaf of *Vitex dodiana* was purchased from Muda Lawal market in Bauchi State, Nigeria and was taken to the Biological Science Department, Abubakar Tafawa Balewa University Bauchi.

Preparation of the Extract

The leaves were sorted out separately to obtain only fresh leaves and washed with distilled water without squeezing to remove debris and dust particles. They were air-dried and ground into coarse powder using pestle and mortar and sieved to fine powder. 150 g of the fine powder was extracted or cold macerated into 900ml of distilled water for 24 hours and the macerated mixture was then filtered through muslin cloth. It was then filtered to obtain the *Vitex dodiana* and mixture aqueous extract through filter paper. The filtrate was concentrated in an electric oven at 50^{o}C until a semisolid residue dark solid extract was obtained.

Experimental Animals

Sixteen (16) white albino rats weighing between 80-100 g were purchased from National Veterinary Research Institute (NVRI) Vom, Plateau state. The animals were placed in cages and fed appropriately at the biological science department, Abubakar Tafawa Balewa University Bauchi.

Experimental Design

At the end of the seven days' acclimatization period, the animals were randomly assigned into four different groups of four rats each, designated as groups of A – D. Group A received water and feed only and serves as control, group B were administered orally with 200 mg/kg, group C were administered orally with 300 mg/kg and group D were administered orally with 400mg/kg doses of the extract for the period of fourteen days. On the 15th day all the rats were sacrificed and blood samples collected.

Administration of the Extract

Administration of the extract was done via oral route with the aid of oral cannula and syringe. Animals received their doses once per day for the period of two weeks. They were observed daily for clinical signs of toxicity or pharmacological signs, throughout the period of study.

Collection of Blood

At the end of the two weeks of extract administration, the albino rats were slaughtered to obtain blood from the jugular vein. The collected blood samples from each rat were allowed to clot and then centrifuged at 3000 rpm for 10 minutes. Serum was obtained for the assay of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP).

Blood Analysis

Hepatic analysis of the serum enzymes for ALT and AST was done by the method of Reitman and Frankel (1957), ALP was assayed according to the method of Rec (1972).

Estimation of Parameters

Aspartate Aminotransferase (AST) assayed using the Colorimetric method of Reitman and Frankel, 1957.

Alanine Aminotransferase (ALT) assayed by Colorimetric method of Reitman and Frankel, 1957.

ALKALINE PHOSPHATASE (ALP) assayed by method of Rec, 1972.

3. Results and discussion

From the results it appears that the extract had no significant effect ($P < 0.05$) on the activity of the liver enzymes assayed at all the doses when compared with control rats.

Table 1. Effect of aqueous leaf extract of *Vitex dodiana* on liver enzymes in normal albino rats

Grouping	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
Group A **(Control)**	5.6±0.15	2.8±0.31	106.26±8.51
Group B (**200 mg/kg)**	5.5±0.20	2.5±0.11	104.56±1.20
Group C (**300 mg/kg)**	5.2±0.12	2.6±0.17	102.44±2.34
Group D (**400 mg/kg)**	5.0±0.33	2.4±0.04	100.06±0.66

Table 1 showed the effect of aqueous leaf extract of *Vitex dodiana* on liver enzymes in normal albino rats. The activity of AST was slightly decreased to 6.5±0.20 in the rats treated with 200 mg/kg body weight of the extract and slightly decreased to 5.2±0.12 and 5.0±0.33 in the rats treated with 300 and 400 mg/kg body weight of the extracts respectively when compared with untreated group (5.6±0.15) with no significant ($P > 0.05$) differences. The activity of ALT was slightly decreased to 2.5±0.11 in the rats treated with 300 mg/kg body weight of the extracts and slightly increased to 2.64±0.17 and decreased to 2.4±0.04 in the rats treated with 200 and 400 mg/kg body weight of the extracts respectively when compared with untreated group (2.8±0.31) with no significant ($P > 0.05$) difference. The result of ALP also showed no significant ($P < 0.05$) difference of serum ALP activity, though it was observed in the rats treated with 400 mg/kg body weight of the extracts the serum concentration decreased to 100.06±0.66, and 102.44±2.34 at 300 mg/kg body weight and 104.56±1.20 at 200 mg/kg body weight of the extracts, but no significant ($P > 0.05$) difference was observed when compared with untreated group (106.26±8.51).

4. Conclusion

Acute oral administration of the extracts was found to be relatively safe at all dosage levels. Hence no alteration in activity was observed.

5. Recommendations

Further studies should be carried out by increasing the number of experimental animals, so that larger data could be obtained so as to reach a better conclusion. Biochemical parameters associated with liver function tests such as bilirubin, albumin and total protein should also be analyzed so as to find out the detailed hepatotoxic effect of *Vitex dodiana*.

Histological analysis of the liver of albino rats should also be conducted.

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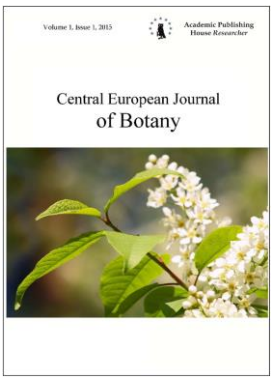

Phytochemical Screening of Selected Varieties of Okro (*Abelmochus Esculentus)* Fruit

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Abstract

Abelmoschus esculentus (Okra) is a plant with several varieties which are consumed for nutritional and medicinal purposes. Its medicinal significance has been reported particularly on diabetes mellitus. This study aimed at investigating the phytochemical quantities of selected varieties of *Abelmoschus esculentus* fruit extracts. Five varieties of the okra plant fruit were each extracted with methanol (80 %) using Soxhlet extractor. The extracts were concentrated at 30°C under reduced pressure in a rotary evaporator to a semi solid extract and finally air dried. Phytochemical contents of each of the methanol extracts of selected okra fruit varieties were evaluated. The results of the study showed different yields of extract where *NHB-AI-B* and *Yar kolon* okra fruit varieties recorded the highest % yield (22.85 and 17.11 %) respectively. Presence of phytochemicals like phenolics, saponins, Tannins, Glycosides and flavonoids were detected in all the varieties. In conclusion, the study showed that selected varieties of okra fruit extract varied in their quantities of phytochemicals and extract yields.

Keywords: *Abelmoschus esculentus,* phytochemical, nutritional, phenolics, saponins, tannins, glycosides, flavonoids.

1. Introduction

One of the most popular vegetable crop in the Indo-Pak subcontinent is Okra (*Abelmoschus esculentus*) (Kumar et al., 2010). It belongs to the Malvaceae family and the most important due to its nutritional and medicinal uses. It is widely distributed from Africa to Asia (Kumar et al., 2010), southern Europe and America (Khosrozadeh et al., 2016). It is a tropical to subtropical crop and is sensitive to frost; low temperature, water logging and drought conditions, and it is adapted to conditions of its cultivation from country to country (Khatun et al., 2011).

Its green tender fruits is the favorite for consumption purposes as a vegetable (Chekole, 2017). Okra fruits are rich in vitamins, calcium, potassium and other minerals (Ülger et al., 2018). The mature okra seed is a good source of oil and protein has been known to have superior nutritional quality (Fekadu Gemede, 2015). Okra seed oil is rich in unsaturated fatty acids such as

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linoleic acid, which is essential for human nutrition (Ndangui et al., 2010). Its mature fruit and stems contain crude fiber, which is used in the paper industry (Chanchal et al., 2018).

Phytochemical studies exhibited that polysaccharides, polyphenols, flavonoids, tannins, sterols and triterpenes are the major components of *A. esculentus* with various biological activities (Jima, Megersa, 2018). It has been reported that the okra powder plays antidiabetic and antihyperlipidemic roles in diabetic rats (Chikezie, 2015). Dietary fibers and polyphenols which are abundantly found in *A. esculentus*, may contribute to the hypoglycemic and hypolipidemic effects of *A. esculentus* (Nazar et al., 2016). The aim of the study is to evaluate and quantify the phytochemical contents of selected varieties of *Abelmoschus esculentus* Fruit.

2. Materials and methods

Plant Collection/Identification

Abelmoschus esculentus fruit varieties were obtained from a farm located at the Faculty of Agricultural Sciences, Abubakar Tafawa Balewa University, Bauchi. The plant material was identified and authenticated at the Department of Biological Science, Abubakar Tafawa Balewa University, Bauchi.

Plant Extraction

Okra fruit varieties were each extracted following the method described by Doreddula *et al* (2014) with modification in extraction time (12 h). The okra was grounded using pestle and mortar. The powdered material was then sonicated in 80 % methanol for 1 h and then extracted using Soxhlet for 12 h at room temperature. The extract was filtered, and the filtrate was concentrated at 30^{o}C under reduced pressure in a rotary evaporator until a crude solid extract was obtained which was then air dried. The dried extract was put in an air-tight container and kept in a refrigerator at 4^{o}C until used.

Phytochemical Screening

Preliminary qualitative phytochemical tests for the detection of phenols, flavonoids, alkaloids, tannins, saponins, and glycosides was conducted using methods descried by AOAC (1984).

Quantitative phytochemicals Screening

Determination of total phenolic content

The total phenolic content of extract was measured using Folin–Ciocalteu reagent. The extract was solubilized in distilled water. After that 1 ml of sample was mixed with Folin–Ciocalteu reagent (5 ml), sodium carbonates (4 ml) and distilled water (5 ml). This solution was kept at room temperature for 30 min, and the absorbance of solution was measured at 760 nm in a spectrophotometer. A set of reference standard solutions of Gallic acid (1, 2, 3, 4 and 5 ppm) were prepared. Total phenolic content was calculated using Gallic acid as standard (Madaan et al., 2011).

Determination of total flavonoid content

Aqueous extract (500 µl) was mixed with ethanol (1.5 ml), aluminum nitrate (100 ml, 10 %), potassium acetate (100 ml, 1 M) and water (2.8 ml). The solution was kept at ambient temperature for 40 min and the absorbance of solution was measured at 425 nm using a spectrophotometer. Total flavonoid content was recorded according to a standard established curve with quercetin (Mohsen, Ammar, 2008).

Determination of tannin content

Stock solution of 1 mg/ml of tannin acid was prepared by dissolving 10 mg of accurately weighed tannic acid in water. Aliquots (10 ml) were taken in clear test tube and 1 ml of Folin-Denis reagent, 1 ml of sodium carbonate solution was added to each test tube. Each tube was made up to 10ml with distilled water. All the reagents in each tube were mixed well and kept undisturbed for about 30 min and read at 760 nm against blank reagent in a spectrophotometer. A set of reference standard solutions of Tannic acid (1, 2, 3, 4 and 5 ppm) were prepared in the same manner as described earlier (Polshettiwar et al., 2007).

Determination of total Saponin

Crude saponin extracts (10ml) were dissolved in 5 ml of 50 % aqueous methanol. 2.5 ml of aliquot was transferred to test tubes into which an equal volume of vanillin reagent (8 %) was added followed by 72 % (v/v) sulphuric acid. The mixture was mixed and placed in a water bath adjusted at 60 °C for 10 min. The tubes were cooled on an ice-cold water bath for 3 to 4 min and absorbance of yellow color reaction mixture was measured at 544 nm using a UV–Vis

spectrophotometer (UV–1800 Shimadzu) against a blank containing 50 % aqueous methanol instead of sample extract. A set of reference standard solutions of Diosgenin (1, 2, 3, 4 and 5 ppm) were prepared in the same manner as described earlier. The saponin concentrations were calculated from standard curve and expressed as mg Diosgenin equivalents (DE) per g crude extract (Hiai et al., 1976; Baccou et al., 1977).

3. Results

Table 1. Percentage Yield of Methanol Extracts of Abelmochus esculentus Fruit Varieties

Sample	Weight (g)	Yield	% Yield
Clemson Spinless	240.00	38.00	15.83
LD-88	255.00	29.00	11.37
NHB-AI-B	254.00	58.03	22.85
NHAE-47-4	285.00	40.36	14.04
Yar Kolon	282.00	48.24	17.11

Table 2. Phytochemical Profile of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties

Samples	**Saponins**	**Tannins**	**Flavonoids**	**Alkaloids**	**Steriods**	**Glycosides**	**Phenolics**
CLEMSON SPINLESS	++	++	++	-	+	++	+
LD-88	++	+++	+	-	-	+++	++
NHB-AI-B	++	++	++	-	-	++	++
NHAE-47-4	+	++	-	-	-	+	+
YAR KOLON	+	+	++	+	-	+	+++

Notes: +: Present - : Absent

Table 3. Phytochemical Content of Methanol Extracts of Abelmochus Esculentus Fruit Varieties

	Phytochemicals			
Samples	Saponins (mg/g of Diosgenin)	Tannins (mg/g of tannic acid)	Flavonoids (mg/g of Quercetin)	Phenolics (mg/g of Gallic acid)
CLEMSON SPINLESS	132.8	3.3	0.0041	1.685
LD-88	164.0	2.1	0.0017	3.432
NHB-AI-B	151.1	2.3	0.0017	3.475
NHAE-47-4	156.6	2.4	0.0052	2.142
YAR KOLON	115.1	2.4	0.0067	4.066

4. Discussion

Table 1 shows percentage yield of selected varieties of *Abelmochus esculentus* fruit after extraction with methanol. *Clemson spinless* had a percentage yield of 15.88 %, *LD-88* (11.37 %), *NHB-AI-B* (22.85 %), while *NHAE-47-4* and *Yar Kolon* had yields of 14.04 % and 17.11 % respectively, this can be attributed to the fat that the plans adaptation varies from variety to variety

and also to location peculiarities as reported by Gemede (2015). From the result of the study, *NHB-AI-B* had the highest yield while *LD-88* had the lowest yield.

Table 2 shows the qualitative phytochemical analysis of selected varieties of *Abelmochus esculentus* fruit extracts. The result identified the presence of phytochemicals like saponins, tannins, flavonoids, and phenols in all the selected varieties , this is in consonance with the findings of Kumar et al. (2010).

Quantitative phytochemical analysis showed that selected okra varieties varied in their quantities of phytochemicals. Highest quantity of phenolic compounds and flavonoids was found in *Yar Kolon* variety (4.066 mg/g and 0.0067 mg/g) respectively. *LD-88* had the highest quantity of saponins (164.0 mg/g). Higher quantities of Tannins were found in *Clemson spinless* (3.3 mg/g) as shown in Table 3, attributed to varying degree of adaptation to environment, specie and cultivation as stated by Bello, Ayanda, Aworunse, Olukanmi (2018).

5. Conclusion

Based on the findings of this study, it is concluded that the methanol extracts of selected varieties of *Abelmoschus esculentus* fruit contain various phytochemicals constituents but their quantities vary hence, they have the capacity to exert some biological activity and will be useful in disease intervention.

6. Recommendations

Further research to identify and isolate all the bioactive components of different okra fruit varieties is recommended.

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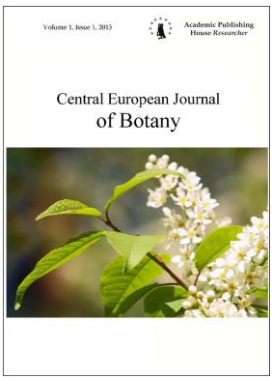

Taxonomic Study of Some Rare Species of Vitaceae from Pakistan by Foliar Micro-Morphological Approach

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Abstract

The present study is insight into foliar epidermal anatomy for characterizing species and their utility in the taxonomic separation of certain taxa of family of Vitaceae from Pakistan. The studied foliar micromorphology of 5 species; *Parthenocissus semicordata* (Wall.) Planch, *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch, *Ampelopsis vitifolia* (Boiss.) Planch. subsp. *hazaraganjiensis* Nazim & Qaiser, *Cissus trifoliata* (L.) L., and *Cissus quadrangularis* L. was analysed and documented using Light microscopy (LM) for both qualitative and quantitative characteristics. Epidermal cells observed were either polygonal or irregular shaped, with straight or undulate anticlinal walls. Two types of stomata observed were paracytic and anomocytic with elliptical shaped guard cell all the studied species except adaxial surface of *Parthenocissus semicordata*, *Parthenocissus tricuspidata* and *Ampelopsis vitifolia* subsp. *hazaraganjiensis*. Trichomes were conical shaped, non-glandular either unicellular or multicellular. The quantitative parameters studied were size (length and width) of stomata, subsidiary cell, guard cells, stomatal pore, stomatal complex, trichomes. There was much variation in the size of all the parameters investigated. These anatomical features may help in the discrimination of studied taxa and are of much significance for the plant taxonomist in the correct identification of taxa.

Keywords: taxonomy, anatomical features, light microscopy, taxonomic implication.

1. Introduction

Vitaceae is an important family, well known among commercial fruits for the economic importance of the grapes (Karkamkar et al., 2010). The family is treated under order Rhamnale, divided into two major groups: 4-merous group and 5-merous group, comprised of 14 genera and 900 species distributed widely in tropical, subtropical regions and partially in temperate region. Mostly characterized by woody creepers or lianas, few are herbaceous or erect shrubs, rarely succulent trees categorized primarily as a source of food, wine, resins and also as ornamental (Manchester et al., 2013; Lu et al., 2013). A collection of 6 genera and 12 species are represented in the Flora of Pakistan, traced in wide range of habitat with dispersed location from coastal areas to higher altitude in north. The major genera of the family in Pakistan are *Vitis*, *Parthenocissus*, *Ampelocissus*, *Ampelopsis*, *Cissus* and *Leea*. Leaves are opposite, petiolate, stipulate or extipulate, pinnately compound, lobed or palmately compound, mostly unisexual apetalous flowers, few are bisexual, superior ovary, fruit is seeded or seedless berry (Perveen, Qaiser, 2008).

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Anatomical characteristics using Light microscopy (LM) are crucial to identify and resolve the problematic taxa at species level as well as generic level. Series of investigations are processed on genera of Vitaceae on the basis of morphological and anatomical features to confirm the placement of the family in plant phylogenetic tree (Gerrath et al., 2004). Present study is the first detailed investigation on foliar epidermal anatomy of Vitaceae from Pakistan based on light microscopy. The main objective of the study is to provide fine micro-morphological characteristics including both qualitative and quantitative features of family Vitaceae. This study may help in identifying problematic taxa to be placed in correct taxonomic rank.

2. Materials and methods

A total of 5 species belonging to 3 genera were collected from different regions of Pakistan, i-e Kaghan, Mansehra, Abbottabad, Ayubia and Islamabad during May 2017 to September 2017. Plants were photographed in field for identification purpose (Figure 1). Plants collected were identified with the help of Flora of Pakistan (http://www.efloras.org) and other available literature. Names of the plants were verified from Kew's Vascular Plants database-The Plant List (http://www.theplantlist.org/). Light microcopy was done by following the method adopted by Ullah et al. (2018) with little modification. Fully grown, undamaged leaves were selected from dried plant samples and dipped in lukewarm water for half an hour to avoid damage from drying. Then few leaves were boiled in 70 % lactic acid and 30 % nitric acid till the leaves become transparent. Excess of chemical was drained and then leaves were put in petri dish. Washed with water to remove debris separated from leaves. Both the abaxial and adaxial surfaces were taken carefully with the help of needle and placed on clean glass slide. Treated with lactic acid and cover slip was placed carefully to avoid air bubble. Cover slips were fixed at corners by using transparent nail polish. A total of 6-8 samples of both the surfaces of each plant species were prepared and studied using light microscope and photo-micrographs were also taken at different resolutions using Leica D-20. Epidermal cells shape and size (length and width), anticlinal walls, stomata type, size, stomatal complex, stomatal pore size, trichome type and size were measured (Tables 1, 2).

Fig. 1. Field photographs (A) *Parthenocissus semicordata* (B) *Parthenocissus tricuspidata* (C) *Ampelopsis vitifolia* subsp. *hazaraganjiensis* (D) *Cissus trifoliata* (E) *Cissus quadrangularis*

To find average, five consecutive values were noted. Mean value and standard for each feature was calculated by using statistical software IBM SPSS Statistics 20. Values are presented as

mean (minimum-maximum) ± Standard error in tabular form. Stomatal index was calculated using formula adopted by Shah et al. (2018) (Shah et al., 2018).

$$S.I = \frac{S}{S+E} \times 100$$

S.I = Stomatal index, S = number of stomata per unit area, E = number of epidermal cells per unit area.

3. Results and discussion

In the present study, 5 species belonging to 3 genera were investigated for the qualitative and quantitative micro-morphological features of leaf epidermis by using Light Microscopy (LM) (Tables 1, 2). Epidermal cells on both the adaxial and abaxial surfaces were noted in all the species. Significant difference in epidermal cell size was recorded on both the surfaces (Table 2). Epidermal cells of all the studied species appeared to be polygonal and irregular shaped (Table 1). On both the surfaces, the epidermal cells displayed similar dimensions all over the leaf, yet the adaxial epidermal cells were recorded comparatively larger than those of abaxial surface (Table 2). The maximum epidermal cell length was recorded for *Cissus trifoliata* on abaxial side (53.5 ± 2.8 µm) and on adaxial surface (60.5 ± 4.7 µm), and lowest for *Parthenocissus tricuspidata* adaxial surface (19.2 ± 0.4 µm). In this context, the largest sized epidermal cells were observed in *Cissus trifoliata*. Three types of anticlinal walls (Straight, slightly undulate, slightly undulate) were observed in the investigated plants. Majority of the species had epidermal cells with straight anticlinal walls, except *Cissus trifoliata* (undulate-both surfaces), and *Parthenocissus tricuspidata* (slightly undulate-adaxial). The highest number of epidermal cells counted per unit area were noted in the abaxial surface of *Parthenocissus semicordata* (435 cells/unit area). Among the studied anatomical characters, the most valuable one for distinguishing the species of different genera was the type and density of stomata. Stomata were observed to be arranged randomly throughout the epidermis on both the surfaces for *Cissus trifoliata* and *C. quadrangularis*, while in rest of the species stomata were either invisible or absent (Table 1 & Figure 1). The highest number of stomata were recorded for *Cissus trifoliata* (61), while lowest for the adaxial surface of *Cissus quadrangularis* (7). Stomatal index (SI) was found to be highest in *C. trifoliata* (42.9 %). Guard cells were elliptical shaped in all the species. According to the previous reports, stomatal parameters are influenced by the effect of ecological factors as light and temperature (Dickison, 2000).

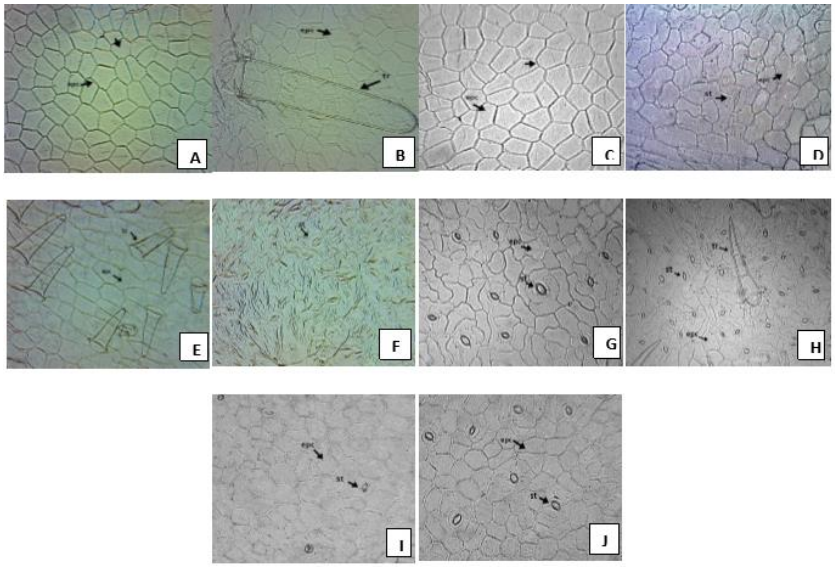

Fig. 2. Light Micrographs (LM) of Foliar epidermis, *Parthenocissus semicordata* (A) Adaxial (B) Abaxial; *Parthenocissus tricuspidata* (C) Adaxial (D) Abaxial; *Ampelopsis vitifolia* subsp. *hazaraganjiensis* (E) Adaxial (F) Abaxial; *Cissus trifoliata* (F) Adaxial (G) Abaxial; *Cissus quadrangularis* (H) Adaxial (I) Abaxial

Trichome micromorphology have been reported to be valuable for comparative systematic studies at every level of taxonomic hierarchy, because of their various shapes, ease of observation and commonly occurrence in various plant families (Mannethody, Purayidathkandy, 2018). Trichomes were present on both the surfaces in *Parthenocissus semicordata* and *Vitis trifoliata* and adaxial surface of *Ampelopsis vitifolia* subsp. *hazaraganjiensis*. While trichomes were absent on both the surfaces of *P. tricuspidata*, and *C. quadrangularis*. Two types of conical shaped trichomes were recorded, (i) unicellular non-glandular, (ii) multicellular non-glandular. Anatomical features are usually as helpful as morphological features for plant diagnostics, and they often are useful in the separation of closely related taxa (Esfandani-Bozchaloyi, Zaman, 2018; Karamian et al., 2012).

Table 1. Foliar epidermal characters of family Vitaceae-Qualitative

Taxa	Ab×Ad	Epidermal cell shape	Anticlinal walls	Stomata P/A	Type of Stomata	Shape of Guard cell	Trichome P/A	Type of Trichome
Parthenocissus semicordata (Wall.) Planch.	Ab	Polygonal	Straight	P	Anomocytic	Elliptic	P	Conical, Unicellular, Non-glandular
	Ad	Polygonal	Straight	A	-	-	P	Conical, Unicellular, Non-glandular
Parthenocissus tricuspidata (Siebold & Zucc.) Planch.	Ab	Irregular	Slightly undulate	P	Anomocytic	Elliptic	A	-
	Ad	Polygonal	Straight	A	-	-	A	-
Ampelopsis vitifolia subsp. *hazaraganjiensis* Nazim & Qaiser	Ab	Polygonal	Straight	P	Paracytic	Elliptic	A	-
	Ad	Polygonal	Straight	A	-	-	P	Conical, Unicellular, Non-glandular
Cissus trifoliata (L.) L.	Ab	Irregular	Undulate	P	Anomocytic	Elliptic	P	Conical, Multicellular, Non-glandular
	Ad	Irregular	Undulate	P	Anomocytic	Elliptic	P	Conical, Multicellular, Non-glandular
Cissus quadrangularis L.	Ab	Polygonal	Straight	P	Paracytic	Elliptic	A	-
	Ad	Polygonal	Straight	P	Paracytic	Elliptic	A	-

Notes: Ab = abaxial; Ad = adaxial; P = present; A = absent

Table 2. Foliar epidermal characters of family Vitaceae-Quantitative

Botanical Name	Ab/ Ad	Epi cell		Stomata		Subsidiary cell		Guard cell		Stomatal pore		Stomatal complex		Trichome		Avg. No. of Epi cells	Avg.No. of stomata	Avg. No. of trichomes	SI
		L	W	L	W	L	W	L	W	L	W	L	W	L	W				
		Mean (min-max) ±SE (µm)																	(%)
Parthenocissus semicordata	Ab	34 (27.5-40) ± 2.3	25.5 (20-37.5) ±3.1	32.4 (29-39) ±1.9	31.2 (26-37) ± 1.9	22.6 (21-26) ± 1.1	13.8 (15-17) ± 1.3	35.2 (32-40) ± 1.3	10.6 (10-12) ± 0.4	26 (24-28) ± 0.7	9.6 (8-12) ± 0.7	45 (37-51) ± 2.8	32.5 (21.5-35.5) ±2.1	360 (312-400) ±17.4	71 (65-75) ±1.9	435	27	2	5.8
	Ad	53 (37.5-62.5) ± 4.2	38 (30-47.5) ± 3.5	-	-	-	-	-	-	-	-	-	-	288 (280-347) ±17.5	61.5 (47.5-75) ±5.4	223	-	1	-
Parthenocissus tricuspidata	Ab	38.5 (32.5-42.5) ±2.0	28 (25-30) ±0.9	24 (22.5-27.50) ±1	7.5 (5-10) ±0.8	33 (30-37.5) ±1.5	9 (7.5-10) ±0.6	22.5 (20-27.5) ±1.4	2.5 (2.5-2.5) ±0.0	16 (12.5-20) ±1.5	3 (2.5-5) ±0.5	31.5 (22.5-37.5) ±2.9	24.5 (20-27.5) ±1.5	-	-	424	27	-	6
	Ad	19.2 (18-20) ±0.4	13.2 (9-17) ± 1.4	-	-	-	-	-	-	-	-	-	-	-	-	97	-	-	-
Ampelopsis vitifolia subsp. *hazaraganjiensis*	Ab	35.83 (30-42.5) ±3.63	19.17(17.520) ±0.83	26.67 (25-27.5) ±0.83	1.67 (1-2.5) ± 0.42	30 (27.5-32.5) ±1.44	0.5 (0.5-0.5) ± 0	26.67(25-27.5) ±0.83	0.58 (0.5-1) ± 0.08	1.08 (1-1.5) ± 0.08	0.33 (0.2-0.5) ± 0.08	29.17(27.5-32.5) ±1.67	3.5 (2.5-5) ± 0.75	-	-	187	23	-	11
	Ad	36.7 (32.5-40) ± 2.2	20 (17.5-22.5) ±1.44	-	-	-	-	-	-	-	-	-	-	48.3 (37.5-62.5) ±7.41	28.33(25-30) ± 1.67	230	-	4	-

Cissus trifoliata	Ab	53.5 (47.5-62.5) ±2.8	29.5 (25-37.5) ±2.4	19.5 (15-25) ±2	9 (7.5-12.5) ±1	31 (27.5-45) ±3.5	7.5 (7.5-7.5) ±0.0	19.5 (15-25) ± 2	2.5 (2.5-2.5) ± 0.0	13.5 (10-20) ± 2.2	6 (5-10) ± 1	31 (27.5-45) ± 3.5	22.5 (20-27.5) ±1.4	206.5 (137-292) ±32.1	42.5 (30-55) ± 4.9	245	61	11	15.3
	Ad	60.5 (50-75.5) ±4.7	45 (40-52.5) ±2.2	20 (15-25) ±1.8	8 (7.5-10) ± 0.5	32.5 (27.5-37.5) ±1.8	7 (5-7.5) ± 0.5	20 (15-25) ± 1.8	2.5 (2.5-2.5) ± 0.0	15.5 (12.5-20) ± 1.5	4.5 (2.5-7.5) ± 0.9	33.5 (30-37.5) ±1.3	22 (20-25) ± 0.9	148.5 (122-175) ±8.6	51.5 (47.5-55) ± 1.8	117	12	9	42.9
Cissus quadrangularis	Ab	45.83 (37.5-55) ± 5.07	28.33(22.5-37.5) ±4.64	2.08 (2-2.5) ±0.08	1.33 (1-1.5) ± 0.08	10.42(8.5-12.5) ±1.1	6.5 (6.5-6.5) ±0	2.25 (2-2.5) ± 0.14	0.58 (0.5-1) ± 0.08	2.08 (2-2.5) ± 0.08	0.67 (0.5-0.7) ±0.08	10.42(8.5-12.5) ±1.1	13.83(13.5-14) ± 0.08	-	-	314	20	-	6
	Ad	47.5 (37.5-55) ± 5.2	28.33(20-35) ± 4.41	2.08 (1.5-2.5) ± 0.17	1.17 (1-2) ±0.08	10.42(7.5-12.5) ±1.5	6.67 (6.5-7.5) ± 0.42	2.08 (1.5-2.5) ± 0.17	0.67 (0.5-1.2) ±0.08	0.85 (0.7-1.5) ±0.2	0.5 (0.5-0.5) ± 0	10.42(7.5-12.5) ±1.5	14.5 (13.5-16.5) ±0.88	-	-	230	7	-	3

Notes: Epi= epidermal cell, Ab= abaxial, Ad= adaxial, L= length, W= width, SI= stomatal index, Min= minimum, Max= maximum, SE= standard error, µm=micrometer

Anatomical studies have demonstrated that leaf epidermal characters are comparable and quite reliable in taxonomic studies (Yasmin et al., 2010). In the 20th century, various studies were conducted on the anatomy and morphology of individual species of Vitaceae, even fruit development (Hardie et al., 1996). The micro-morphological features of these species play significant role in identification of various anatomical features of leaf epidermis which may lead to the comparative study of the same species in other regions of the world and may provide aid to the unresolved phylogenetic status of Vitaceae. Taxonomic key was constructed on the basis of variation in microscopic characteristics observed under light microscope. Based on variations observed, taxonomical keys may be constructed so that one species can easily be identified and differentiated from the other.

4. Conclusion

Light microscopy of leaf and scanning electron microscopy of seeds of family Vitaceae has a distinguished role in identification at genus and species level and may provide evidence in the determination of taxonomic rank in the phylogenetic tree. Varied characters among species of seeds and diverse epidermal features such as shape and wall margins, stomata size and type, trichome size and type investigated in this study and their statistical analysis may possess great potential for plant taxonomist to further evaluate the chemical nature, vegetative growth and tissue development which is needed particularly for grape vine to increase the fruit.

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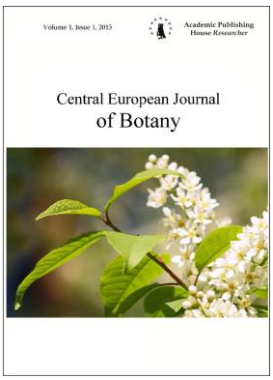

Morphological Description of Some Forage Legumes of Pakistan

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Abstract

Legumes are one of the most important forages in the world. Many Leguminosae members have economic importance, ethnobotanical and medicinal values. The aim of this study was to describe the morphological parameters of twenty five forage legume species. In total, 16 vegetative and reproductive characters have been studied. The morphological features were studied directly from the fresh specimens by using hand lens and dissecting microscope. Quantitative data obtained was analyzed using SPSS software. The most variable characters observed were; type of leaf lamina, shape of leaf apex and base, stem type and texture, flower color and size, fruit type and size, fruit indumentum. This study concluded that morphology of legumes is not just a biological pursuit but can aid in forage managing systems. In addition, more research should take into consideration the ecological forces on these Fabaceae taxa, which deserve care with regard to administration issues and sustainability.

Keywords: forage, legumes, morphology, pastures, reproductive, vegetative.

1. Introduction

Forage legumes belong to family Fabaceae (Leguminosae); Fabaceae is a cosmopolitan family include more than 19.000 taxa, after Asteraceae and Orchidaceae in the world; this family recognized for its ecological and economic potential (Lpwg, 2017). Fabaceae taxa have economic importance and have ecological attention because of adaptations their association with nitrogen fixing bacteria or with ectomycorrhizal (Lewis, 1987). Fabaceae taxa contain plant parts other than separated grain that are employed for ruminant livestock feed (Graham, Vance, 2003). They are usually grazed as fodder or silage and can be developed as monocultures or combination with other species, most frequently grasses. According to Food and Agriculture Organization of the United Nations there are 153 different taxa of legume being used as forage but still worldwide the number of these species used as forage is unknown. This list provides some idea of the variety, together with plants ranging in size from small herbs to large shrubs with temperate, tropical and arctic distributions.

In Pakistan, shortage of green forage is one of the restraining issues to uphold present livestock population. This scarcity is about 40-50 % which attains up to 75 % on May-June and November-December (Sarwar et al., 2002). Pakistan has 21 million hectares of land area that is cultivable but these cultivable lands cannot be shifted permanently to forage crops (Iqbal et al.,

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1998). Under these conditions the evolution of high yielding and first-rate quality forage crops particularly the forage legume crops is dire requirement in bridging the production and demand breach especially during the scarcity periods. Leguminous forages rich in protein, minerals and vitamins B (Hill, Curse, 1992). Forage legumes not only add to fertility, manage soil erosion but also used as covers crops to cut down erosion (Ahmed, Anwar, 1986). Intensive cultivation and higher crop yields are likely to influence the soil nutrients status; Fabaceae taxa maintaining soil fertility particularly due to its nitrogen-fixing bacteria (Khan et al., 1986). Forage performance of a crop be based on plant height, pods, weight, and number of leaves (Ahmed, Anwar, 1986). Various characters used in morphological investigation are of a continuous nature and show considerable variation even between plants of an accession. The accuracy of the data obtained through morphological description will determine the subsequent grouping of the accessions.

The aim of this study was to determine accurate assessment of the morphological variation in forage legume species for their proper identification.

2. Materials and methods

Legume species with vegetative and reproductive parts were collected. For identification and authentication, herbarium specimens as well as flora of Pakistan (Nasir, Ali, 1971) and flora of China (Bentham, Hooker, 1873). Each specimen was labeled and numbered properly with all necessary details; collection date, place of collection, name of the collector and flowering and fruiting period. Voucher specimens were deposited in the Herbarium of Pakistan, Department of Plant Sciences, Quaid-i-Azam University Islamabad.

2.1. Morphological Study

To study the morphological characters of plant specimens, a hand lens or simple binocular light microscope with a magnification of 10X and 20X was used. The vegetative and reproductive parts were described according to terminology adopted by Bentham and Hooker (1873) and Prain (1903). For reliability in terminology and variety of characters examined, a standardized format was employed to enlist the characteristic features. A binocular stereo zoom light microscope with eyepiece WF10 × 10/20 was used to investigate surface hairs and reproductive parts. To explore fresh parts of flower, needle and razor blades were used. Both qualitative and quantitative features plus some diagnostic characters for each species were examined.

3. Results and discussion

In total, 25 legume species belonging to 11 genera are studied for morphological characters (macro and micro). The detailed morphological description of each species is given in Tables 1, 2. Morphological characters exhibit certain inconsistency in studied forage legume species.

Table 1. Morphological Features of Forage Legume Species

No	Plant Species	Period	Leaf	Leaf lamina	Leaf apex	Leaf base	Type of Stem	Stem	Inflore scence	Fruit type	Diagnostic Features
1.	*Crotalaria medicaginea*	Perennial	Trifoliate	Oblanceolate	Obtuse	Subtruncate	Branched	Pubescent	Raceme	Subglobose	Leaf trifoliate, stipules filiform, bracts subulate, corolla yellow.
2.	*Desmodium triflorum*	Perennial	Trifoliate	Obovate	Acuminate	Cuneate	Rough	Glabrous	Fasicle	Curved	Inflorescence axillary fascicle having 1-5 flowers, corolla pink or white, fruit upper suture straight and lower indented.
3.	*Lathyrus aphaca*	Annual	Alternate	_	_	_	Soft	Glabrous	Raceme	Long	Stipules foliaceous, leaf reduced to a tendril, peduncle as long as stipule and corolla yellow in color.
4.	*Lathyrus pratensis*	Perennial	Paripinnately compound	Linear-lanceolate	-	-	Erect	Pubescent	Raceme	Long	Stem scrambling, peduncle longer than leaf, lower calyx tooth longer than tube and fruit 4-8 seeded.
5.	*Lens culinaris*	Annual	Paripinnately compound	Oblong-linear	Obtuse	Mucronate	Slender/ Angular	Pubescent	Raceme	Oblong	Rachis ending in a short bristle or in a tendril, corolla pale purple and cotyledons orange red.
6.	*Lespedeza juncea.*	Perennial	Trifoliate	Obovate	Acute	Mucronate	Erect	Pubescent	Raceme	Long	Branches much pubescent, calyx teeth much longer than the cup and corolla pink.
7.	*Lotus corniculatus*	Perennial	Compound	Oval-linear	Acuminate	Obtuse	Prostate	Glabrous-pilose	Umbel	Cylindrical	Bracts sessile leaf like and corolla yellow.
8.	*Medicago falcata*	Perennial	Alternate	Obovate-lanceolate	Obtuse	Cuneate	Erect/Procumbent	Glabrous	Raceme	Straight-crescentic	Stipules toothed fruit straight to crescentic.
9.	*Medicago laciniata*	Annual	Trifoliate	Obcordate	Acute	Cuneate	Procumbent	Pubescent	Raceme	Globose to ellipsoid	Leaf margins toothed and stem creeping.
10.	*Medicago lupulina*	Annual	Trifoliate	Oval	Obtuse	Cuneate	Prostate	Pubescent	Raceme	Curved	Stipules cordate and dentate, corolla yellow, fruit curved.
11.	*Medicago minima*	Annual	Trifoliate	Obovate	Acute	Cuneate	Procumbent	Pubescent	Raceme	Curved	Peduncle longer than petiole, fruit having 3-4 coils and spiny.
12.	*Medicago monantha*	Annual	Trifoliate	Obovate	Obtuse	Cuneate	Erect	Pubescent	Raceme	Long	Stipules semisagittate, calyx campanulate and fruit reticulately netted.
13.	*Medicago polymorpha*	Annual	Alternate	Renniform	Obtuse	Cuneate	Herbaceous	Glabrous	Raceme	Spiny, hooked	Stipules laciniate, corolla yellow, fruit spiral and spiny.
14.	*Medicago sativa*	Perrenial	Trifoliate	Obovate-sublinear	Obtuse	Cuneate	Erect	Pubescent/subglabrous	Raceme	Falcate	Peduncle longer than petiole, corolla violet and fruit falcate.
15.	*Melilotus indicus*	Annual	Trifoliate	Oblong-lanceolate	Obtuse	Retuse	Erect	Pubescent	Raceme	Reticulate	Inflorescence 10-16 flowered, corolla yellow, fruit with prominent veins on surface.
16.	*Melilotus officinalis*	Annual-biennial	Trifoliate	Ovate	Obtuse	Retuse	Erect/Decumbent	Pubescent	Raceme	Striated	Leaflets of lower leaves obovate to ovate, stipules of lower leaves entire and corolla yellow.
17.	*Trifolium alexandrinum*	Annual	Alternate	Oblong-lanceolate	Mucronate	Retuse	Erect	Pubescent	Globose head	Subglobose	Inflorescence oblong-conical head, in fruit minute bracts making an involucre.
18.	*Trifolium pratense*	Perennial	Alternate	Obovate-elliptic	Mucronate	Obtuse	Erect-decumbent	Pubescent	Globose head	Broad	Inflorescence globose head, rarely pedunculate, calyx pubescent, the lowermost tooth longer than all other teeth and calyx tube and corolla purple or pink.
19.	*Trifolium repens*	Perennial	Alternate	Obovate	Retuse	Cuneate	Prostate	Glabrous	Raceme	Linear	Stipules sheathing, flowers scented, calyx having nerves and corolla white.
20.	*Trifolium resupinatum*	Annual	Alternate	Oval-oblong	Obovate	Cuneate	Procumbent	Glabrous	Peduncle head	Ovoid	Inflorescence peduncled head and calyx inflated in fruit.
21.	*Trigonella gracilis*	Perennial	Compound	Obovate	Acute	Cuneate	Trailing	Glabrous	Raceme	Long	Petiole usually shorter than leaflets, flowers 1-5 in the form of raceme and corolla yellow.
22.	*Vicia hirsuta*	Annual	Paripinnate	Linear	Obtuse	Mucronate	Trailing	Pubescent-glabrascent	Raceme	Broad	Stipules lanceolate, tendril mostly branched, flowers 2-7 having peduncled raceme.
23.	*Vicia sativa*	Annual	Pinnately compound	Obovate	Acute	Obtuse	Erect	Pubescent-subglabrous	Raceme	Oblong	Tendril branched, flowers 1-2, corolla pale pink.
24.	*Vicia tetrasperma*	Annual	Paripinnate	Linear-oblanceolate	Acute	Obtuse	Decumbent	Pilose-glabrous	Raceme	Linear-oblong	Stipules hastate, flowers 1-3 in the form of peduncled raceme.
25.	*Vicia tenuifolia*	Perennial	Pinnately compound	Oblong-linear	Obtuse	Mucronate	Erect	Subglabrous	Raceme	Oblong-lanceolate	Flowers 20-40 in the form of axillary raceme.

Table 2. Quantitative Morphological Features of Forage Legume Species

S. No	Plant species	Length of Leaf [cm]	Width of Leaf [cm]	Length of Petiole [cm]	Size of Flower [cm]	Length of Pedicel [cm]	Size of Fruit [cm]
1.	*Crotalaria medicaginea*	0.6-1.4	0.3-0.5	0.2-0.5	1-2.8	0.1-0.4	2-4
2.	*Desmodium triflorum*	0.5-1.5	0.4-0.7	0.3-0.7	1.5-3	0.5-1.0	3-6.5
3.	*Lathyrus aphaca*	0.8-4.5	0.2-0.9		2.5-6.5	0.2-0.8	1.8-4
4.	*Lathyrus pratensis*	1-4.5	0.5-1.5	0.3-0.6	2-6.5	0.3-1.2	2-4.5.2
5.	*Lens culinaris*	0.5-1.5	0.2-0.5	0.1-0.4	2.5-5	0.4-1.6	0.8-1.4
6.	*Lespedeza juncea*	0.8-2.6	0.3-0.9	0.2-0.5	2-4.5	0.4-1	0.3-0.8
7.	*Lotus corniculatus*	2-4.5	1-2	0.3-0.6	1.5-2.9	0.3-0.9	2-4
8.	*Medicago falcata*	0.5-2.5	0.2-0.8	0.2-0.4	1.5-4	0.2-0.5	1-2.7
9.	*Medicago laciniata*	0.2-1.2	0.1-0.3	0.1	0.4-1.2	0.1-0.2	1-2-3.5
10.	*Medicago lupulina*	0.5-2.5	0.3-1	0.1-0.3	0.5-2.7	0.2-0.4	1.2-5.4
11.	*Medicago minima*	0.5-1.4	0.3-0.9	0.1-0.3	0.6-1.2	0.1	1-3.4
12.	*Medicago monantha*	1-2.5	0.8-1.8	0.2-0.6	2-4.5	0.1-0.5	1-2.8
13.	*Medicago polymorpha*	0.8-2.5	0.3-1.2	0.1-0.2	0.4-1.8	0.1-0.2	0.2-1.6
14.	*Medicago sativa*	1.2-2.4	0.7-1.3	0.1-0.3	0.5-2.1	0.1-0.3	0.3-2
15.	*Melilotus indica*	1-2.8	0.7-1.6	0.2-1	4-10.6	1-3.5	0.2-0.5
16.	*Melilotus officinalis*	1.2-8.6	0.5-1.7	0.2-1	3.8-12.4	1.3-4.8	0.3-0.6
17.	*Trifolium alexandrianum*	1.5-4	0.5-1	0.2-0.5	3-7.5	0.5-2.5	1-2
18.	*Trifolium pratense*	1-3	0.4-0.8	0.2-0.7	1.5-3	0.2-0.9	0.7-1.1
19.	*Trifolium repens*	1.2-4.5	0.3-0.6	0.2-1	1.8-3.6	0.2-1.7	0.3-1.8
20.	*Trifolium resupinatum*	0.7-3	02-.0.8	0.1-0.6	1-2.4	0.2-0.8	0.4-1
21.	*Trigonella gracilis*	0.5-1.2	0.3-0.7	0.2-0.6	0.4-1.9	0.2-0.4	1.5-6.5
22.	*Vicia hirsuta*	0.4-2.5	0.2-1.5	0.2-0.4	1.2-3.6	0.2-0.4	5-12.6
23.	*Vicia sativa*	0.8-4	0.1-1	0.5-1	1-3.5	0.2-0.4	2-6
24.	*Vicia tetrasperma*	0.6-2.2	0.3-1.2	0.1-0.4	0.5-1	0.1-0.3	0.6-1.3
25.	*Vicia tenuifolia*	1.2-4.5	0.7-1	0.9-1.5	0.8-1.8	0.2-0.3	2-5

In total, 16 vegetative and reproductive features have been examined. The morphology of forage species show variability in; shape of leaf lamina, leaf apex and base, type of stem and texture, flower size, type of fruit & size. Morphological characters have been studied as a significant taxonomic tool in earlier studies (Adedeji, 2006). The forage species of legumes are widespread in distribution, with greater range in subtropical regions (Singh, 2016). This study showed that most of plant species possess pinnately or imparipinnately compound and trifoliate leaves. The genus *Lathyrus* (*Lathyrus aphaca*) is distinguished from other forage species by possessing sessile leaves. Consequently, Mohammed (2014) revealed that *Lathyrus aphaca* has reduced tendril that supports the present results. Similarly, *Medicago laciniata, Medicago minima* and *Trifolium resupinatum* have been observed with procumbent type of stem, therefore separating these from rest of the species. Quantitatively, the highest leaf size (length and width) is recorded for *Lotus corniculatus* (2-4.5 cm × 1-2 cm) whereas lowest is recorded for *Medicago laciniata* (0.2-1.2 cm × 0.1-0.3 cm). The forage species of genus *Trifolium* are differentiated on the basis of trifoliate leaves, dentate margins and pubescent or glabrous leaf surfaces. These results are in agreement

with earlier study conducted on leaf characters of *Trifolieae* by Taia (2004). The results provided in this study are generally in accordance with the morphological description of leguminous plant species in Flora of Pakistan and Flora of China, but some sort of variations exist regarding the morphological description and some morphological characters are complemented in the light of this study.

4. Conclusion

This study concluded that the morphological description of forage legumes is of significant importance particularly in forage managing systems. Furthermore, attention should be paid on the ecological pressures and management issues of these fodder species, as they deserve greater attention with regard to these aspects for their sustainability.

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