

Morphological and Anatomical Studies on Some Taxa of Sub Family Malvoideae (Malvaceae s.l)

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Abstract

Morphological and anatomical studies were carried out on four (two wild and two cultivated) species belonging to sub family Malvoideae (Malvaceae s.l) and collected from different regions in Egypt to assess the diagnostic value of these characters in taxa delimitation. The results obtained using UPGMA clustering method from analysis of 71 macro- & micro morphological characters resulting in a dendrogram which indicated a close taxonomic relationship between *Hibiscus esculentus* and *Hibiscus sabdariffa* more than *Malva parviflora* with similarity index (76% and 75% respectively). Dissimilarity was recorded in *Sida spinosa* from the other three species, it distinguished by high genetic diversity based on variation in morphological and anatomical features. The studied species were analyzed for assessment of taxonomic interrelationship among them.

Key word : Malvoideae, Morphology, Anatomy, Numerical analysis

Introduction

Malvoideae is a subfamily belongs to Malvaceae s.l (Alverson *et al.* 1999; Bayer *et al.* 1999 ; Whitlock *et al.* 2001), with Bombacoideae constitute a well supported clade named Malvatheca (Baum *et al.* 1998). Malvoideae includes the traditional Malvaceae *sensu strictu* (Mallovs or Eumalvoideae) that comprises 78 genera and 1700 species (Baum *et al.* 2004). Malvaceae was divided into five tribes Abutilae, Decaschistae, Hibisceae, Malvaceae and Ureneae (Paul and Naya, 1988) while it composed of nine tribes Abutilae, Decaschistae, Gossypieae, Hibisceae, Kydieae, Malopeae, Malva visceae, Malveae, and Ureneae (Hinsley 2006).

(Judd and Manchester 1997; Alverson *et al.* 1999; Bayer *et al.* 1999) indicated that Malvaceae s.s is the only family in core families Malvales represents a monophyletic group. putatively early-branching Malvoideae have a predominantly Australasian/Asian distribution (Pfeil *et al.* 2002). Plants of subfamily Malvoideae are herbs, shrubs or trees, erect or decumbent with stellate hairs and mucilage substance. Leaves simple, lobed or divided, Flowers solitary or in cymes, epicalyx useful characters to differentiated between genera. Five sepals and petals. five-many stamens, filaments form monadelphous, epipetalous staminal tube, anther one cell. Fruit a loculicidal dehiscent or indehiscent capsule or schizocarp, rarely a berry. Seeds reniform or ovoid (Watson and Dallwitz 1992) with high economical value; stems fibers of *Hibiscus sabdariffa* and *sida* used for making cordage, ropes, sacks and papers. roots of *Althaea*

rosea and *A. officinalis* used in medicine. *Abelmoschus esculentus*; fruits are vegetable and its stalks used as fibers . *Gossypium arboreum* , *G. hirsutum*; (fibers from the seeds are used in manufacture of textile , rubber- tyre fabrics) . Petals of *Hibiscus rosa sinensis* are used for caught and coloring . *Hibiscus sabdriffa* : red fleshy epicalyx is edible. Many species of *Malva* are edible as leaf vegetables and drugs. *sida* species ; some are barely edible, and some as herbal medicine (**Pandy 2006**)

The objective of this work is to determine the taxonomic relationships among the four studied species of subfamily Malvoideae using morphological and anatomical characteristics .

Material and Methods

Samples collection

The four plant specimens for the present study were collected from different locations in Egypt, during 2014 and 2015 seasons. Plants were identified following standard literatures (**Boulos,2000**) ,(Täckholm,1974). (Table 1) shows the list of studied taxa and place of collection.

Table (1): List of Studied Taxa and their locations

Sl.No.	Plant Name	Location
1	* <i>Hibiscus esculentus</i> L. =(Ablemoscus esculentus)	El Monofia Governorate
2	* <i>Hibiscus sabdariffa</i> L.	El Dakhlia Governorate
3	<i>Malva parviflora</i> L.	El Dakhlia Governorate
4	<i>Sida spinosa</i> L.	El Dakhlia Governorate

*cultivated species

Morphological and Anatomical studies

The macromorphology of four studied taxa including whole plant habit, stem, petiol , leaf ,flower and fruit was described from fresh specimens . the average length and width for different parts were taken in cm . For micromorphology of stems and leaves , cross microtome sections of 10-20µm were cut and stained with safranin and light green according to method described by (**Johansen ,1940**) . Photographed using LEICA dm LS2 microscope fixed with ICC50Hd camera .The anatomical descriptions were reviewed with (**Metcalf and Chalk ,1979**).

Data analysis

The presence or absence of the 90 macro-µmorphological characters were given the numerical code 0(absence) or 1(present) (Table 2) .the similarity matrix was determined using PAST software (**Hampel et al.,2001**)and phylogenetic dendrogram was obtained from it following the Unweighted Pair Group Method using Arithmetic Averages (UPGMA ;**Sneath and Sokal,1973**)

Results and Discussion

Macro and micro morphological analysis

The morphological and anatomical features were shown in Tables (2&3), plates (1 -4). Morphologically ; the four studied species have similar characters such as presence of hairs, mucilage cells , alternate leaves , inflorescence type (cymes), pedicelate ,bisexual flowers ,five gamo sepals ,five poly petals numerous and epipetalous staminal tube, dorsifixed , monothecate anthers and lobed stigma . Anatomically ; they have crystals ,glandular hairs, cortical collenchyma , chlorenchyma ,mucilaginous cells in cortex and pith ,vascular bundle arranged in one ring ,wide pith and dorsiventral leaf blade.

The cluster analysis of 71 macro and micro morphological features produce a dendogram (Fig.1) illustrating that the four species of malvoideae divided into two clusters at taxonomic level (0.54) ;cluster I contain *Sida spinosa* , it specialized than others by circular stem, simple leaves with rounded base and serrate margin, short petiole, sepals shape and absence of epicalyx . Anatomically ;stem and leaf have high density and different types of hairs (glandular multicellular , shaggy ,stellate and unicellur filiform) ,two types of crystals (druses and solitary) , stem with narrow inter vascular rays and triangular phloem medullary rays , adaxial surface of leaf was leveled , the previous observations agreed with that of the foliar trichomes however possessed a remarkable diversity and much variation is noticed in anatomical characters of stems of *Sida* (Arul & Ida ,2017) and (Shaheen *et al.*,2009). They concluded that foliar epidermal features with particular emphasis on better terminology, macro-, micromorphology and distribution of different glandular and eglandular trichomes in *Sida* may be a useful taxonomic tool . Cluster II comprises the other three species .

Hibiscus esculentus and *H. sabdariffa* were closely related to each other than *Malva parviflora* , at level (0.75) *Malva parviflora* separated in group I because it differs from them in its prostrate stem ,leaf lobes number , apex , base and shape ,carples number (9-10) , discoid fruit , high density of hairs , thick cutical layer , that in the same line with the results of morphological and microscopical studies of stem, leaf and root of *M. parviflora* which may be helpful in the identification of these parts of this species (Akbar *et al.*,2014).

Separation between *H. esculentus* and *H. sabdariffa* in group II occurred at level (0.76) ,they similar in most characters as they Short lived perennial shrubs, pulvinus petiole, pedicel length, sepals shape and capsule fruit ,but they different in color of stem ,petiole , flower ,leaf shape , venation , leaf lobe type and stigma number . Anatomically *H. esculentus* had solitary crystals in the leaf , unicellular filiform trichomes ,radial ad axial cells , vascular bundles appeared in arc shape and stem phloem dissected . Distribution and quality of the crystals on the adaxial and abaxial epidermal surfaces of the two species *H. sabdariffa* and *A. esculentus* . , the differences in trichomes observed could used to separate the two species (Olotuah 2014) . Table (4) represented the similarity index among the four species ; the highest ratio between *H. esculentus* and *H. sabdariffa* (76%) and the lowest ratio between *H. esculentus* and *S.spinosa* (49%).

Table (2): Morphological characters and character states of the studied species

No .	Characters species	state	1	2	3	4
1	Habitat	Cultivated	1	1	0	0
		wild	0	0	1	1
2	Habit	Shortlived perennial shrub	1	1	0	0
		Annual herb	0	0	1	1
3	Stem type	woody	1	1	0	0
		herbaceous	0	0	1	1
4	Stem nature	erect	1	1	0	1
		prostrate	0	0	1	0
5	Stem texture	hairy	1	1	1	1
6	Stem color	green	1	0	1	1
		red	0	1	0	0
7	Stem cross section	Irregular Circle	0	0	1	0
		circular	1	1	0	1
8	polymorphism	present	1	1	0	0
9	Petiole base	pulvinus	1	1	0	0
		normal	0	0	1	1
10	Petiole texture	Glabrous	0	1	0	0
		hairy	1	0	1	1
11	Petiole color	green	1	0	1	1
		red	0	1	0	0
12	Petiole length	Long (5-12 cm)	1	1	1	0
		Short (less than 5 cm)	0	0	0	1
13	Stipules shape	Tri angular	1	0	0	0
		lanceolate	0	1	1	0
		linear	0	0	0	1
14	Leaf composition	simple	0	1	0	1
		lobed	1	1	1	0
15	leaf shape (outline)	reniform	0	1	1	0
		Cordate	1	0	0	0
		Ovate	0	0	0	1
16	lobe type	Palmatelyfid	1	0	1	0
		Palmatelysect	0	1	0	0
17	lobe no	3-5	1	1	0	0
		5-7	0	0	1	0
18	Lobe shape	Lanceolate	0	1	0	0
		Ovate	1	0	0	0
		Reniform	0	0	1	0
19	Leaf /lobe color	Green	1	0	1	1

		Green with red veins	0	1	0	0
20	Leaf /lobe margin	Serrate	0	0	0	1
		crenate	1	0	1	0
		Serrate-dentate	0	1	0	0
21	Leaf /lobe apex	acute	0	1	0	1
		mucronate	1	0	0	0
		obtuse	0	0	1	0
22	Leaf base	Truncate	0	1	0	0
		cordate	0	0	1	0
		acute	1	0	0	0
		rounded	0	0	0	1

Continue table (2):

No .	Characters species	state	1	2	3	4
23	Leaf size	Large 8-10cm	1	1	1	0
		Small 3 cm	0	0	0	1
24	Leaf /lobe venation	Reticulate	1	1	1	1
		Pinnatly unicastate	0	1	0	1
		palmatly multiicastate	1	0	1	0
25	Inflorescence type	Cymes	1	1	1	1
26	Flower Position	axillary	1	0	1	0
		terminal	0	1	0	1
27	Flower Color	white	0	0	1	0
		pink	0	1	0	0
		Yellow- red color	1	0	0	0
		White -creamy	0	0	0	1
28	Flower Size	Moderate 2-5x1-4cm	1	1	0	0
		Small 0.5x.0.2 cm	0	0	1	1
29	Epicalyxes .	present	1	1	1	0
30	Pedicel length	short	1	1	0	0
		v. short	0	0	1	1
31	Sepals shape	triangular	0	0	0	1
		ovate	1	1	1	0
		linear	0	0	0	0
32	Texture	glabrous	0	1	1	0
		Hairy	1	0	0	1
33	Color	green	1	0	1	1
		Red	0	1	0	0
34	Petals shape	obovate	1	1	1	1
35	Texture	glabrous	1	1	1	1
36	color	white	0	0	1	0

		pink	0	1	0	0
		Yellow with red color	1	0	0	0
37	Stamens	α , epipetalous and forming staminal tub	1	1	1	1
38	Carpels no & fusion	5	0	1	0	1
		5-7	1	0	0	0
		9-10	0	0	1	0
39	Ovules no per locule	1	0	0	1	1
		many	1	1	0	0
40	Stigma no	5	0	1	1	1
		5-7	1	0	0	0
41	Fruit type	capsule	1	1	0	0
		schizocarp	0	0	1	1
42	Shape	ovoid	1	1	0	1
		disciod	0	0	1	0
43	size	Moderate 2-5x1-2.5 cm	1	1	0	0
		Small 0.2-0.4x0.3-0.7 cm	0	0	1	1
44	Seeds shape	reniform	1	1	1	0
		ovoid	0	0	0	1
45	Color	brown	1	0	1	1
		black	0	1	0	0
46	Size	Small 0.5x0.3 cm	1	1	0	0
		v. small 0.1x0.1 cm	0	0	1	1

Table (3): Anatomical characters and character state of the four studied species

NO.	Characters ↓ samples →	state	1	2	3	4
			1	Leaf adaxial shape	ridged	1
	leveled	0	0		0	1
2	Cuticle layer	thin	1	1	0	1
		thick	0	0	1	0
3	Adaxial epidermal cell	radial	1	0	1	0
		Tangential	0	1	0	1
4	Hairs	present	1	1	1	1
5	Hairs type	stellate	0	0	1	1
		Glandular m.c	1	1	1	1
		unicellar filiform	1	0	1	1
		shaggy	0	0	0	1
6	Hairs density	Many	0	0	1	1
		Few	1	1	0	0
7	Mesophyll type	dorsiventral	1	1	1	1
8	Secretory cells	Mucilaginous cells	1	1	1	1

9	Mechanical tissues	collenchyma	1	1	1	1
		parenchyma	1	1	1	1
10	Vascular bundle shape	Arc shape	1	0	0	0
		Centralgroup	0	1	1	1
11	Crystals type	Druses	0	1	1	0
		solitary	1	0	0	0
12	Outline of stem	circular	0	0	0	1
		wavy	1	1	1	0
13	Cuticle layer	thick	0	0	1	0
		thin	1	1	0	1
14	Epidermal cells shape	Tangential	1	1	1	1
15	Hairs type	Stellate	0	1	1	1
		Glandular m.c	1	1	1	1
		unicellular filiform	0	1	1	1
		shaggy	0	0	0	1
16	Hairs density	Many	0	0	0	1
		few	1	1	1	0
17	Cortical cell type	Parenchyma,Collenchymas, Chlorenchyma	1	1	1	1
18	Crystals type	Druses	1	1	1	1
		Solitary	0	0	0	1
19	Crystals density	Many	0	0	0	1
		few	1	1	1	0
20	Secretory cells	Mucilaginous cells	1	1	1	1
21	Pericycle	Continuous ring	0	0	0	1
		Dissected ring of sclernchymatos tissue	1	1	1	0
22	Phloem	Continuous	0	1	0	1
		Dissected	1	0	1	0
23	Phloem medullary rays	Radial	1	1	1	0
		Tri angular	0	0	0	1
24	Xylem vessels	Complete ring	0	0	0	1
		Dissected ring	1	1	1	0
25	Inter vascular rays	wide	1	1	1	0
		narrow	0	0	0	1

(a)

(b)

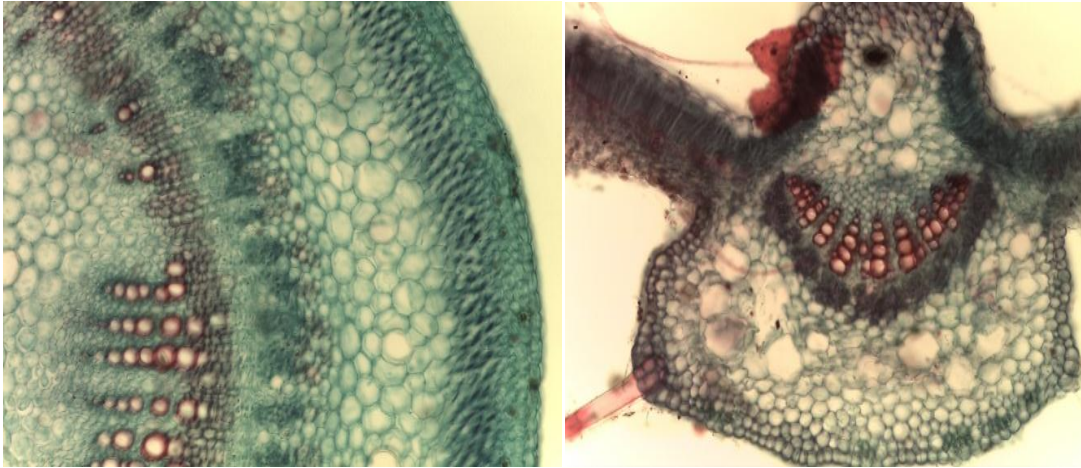


Plate (1): Anatomical characters of *Hibiscus esculentus*
(a) Cross section in stem (x40) (b) Cross section in leaf blade (x40)

(a)

(b)

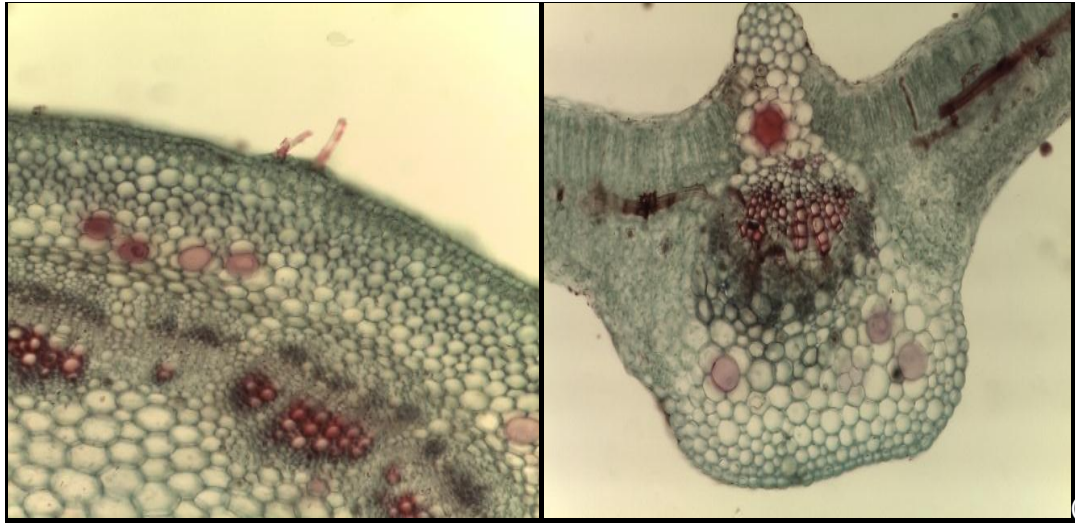


Plate (2): Anatomical characters of *Hibiscus sabdariffa*
(a) Cross section in stem (x40) (b) Cross section in leaf blade (x40)

(a)

(b)

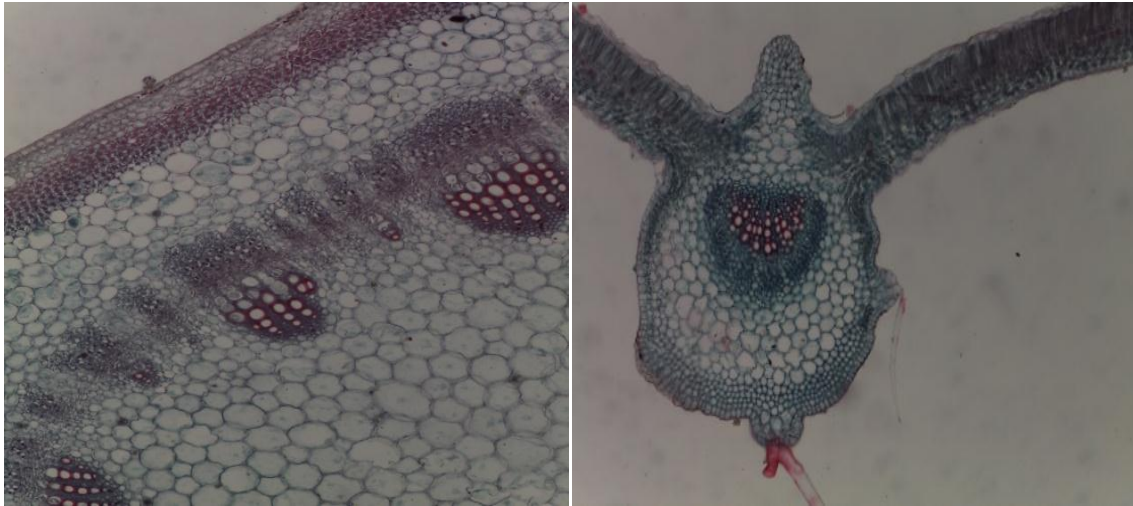
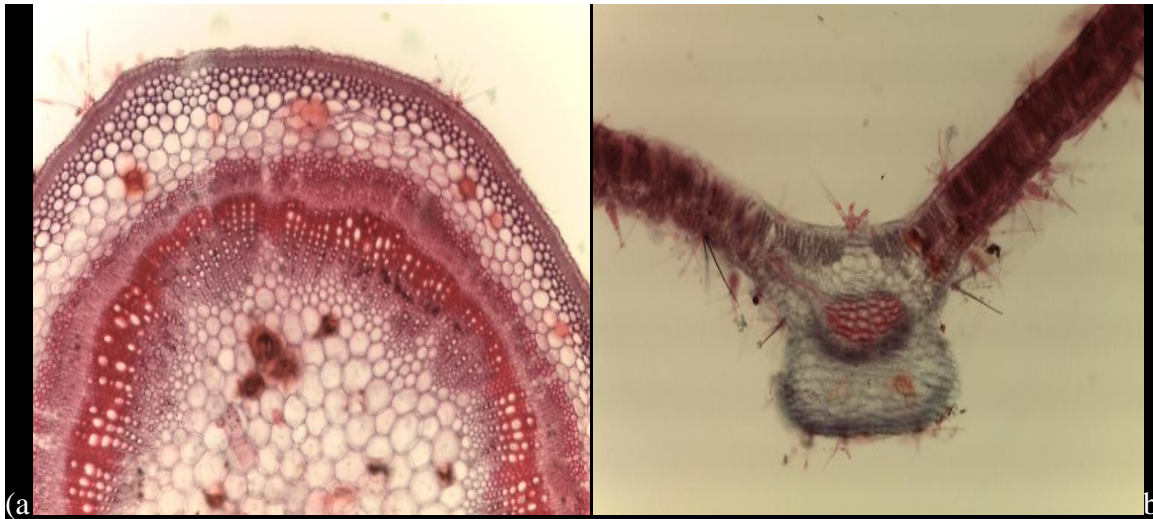


Plate (3): Anatomical characters of *Malva parviflora*

(a) Cross section in stem (x40) (b) Cross section in leaf blade (x40)

(a)

(b)



p

Plate (4) Anatomical characters of *Sida spinosa L*

(a) Cross section in stem (x40) (b) Cross section in leaf blade (x40)

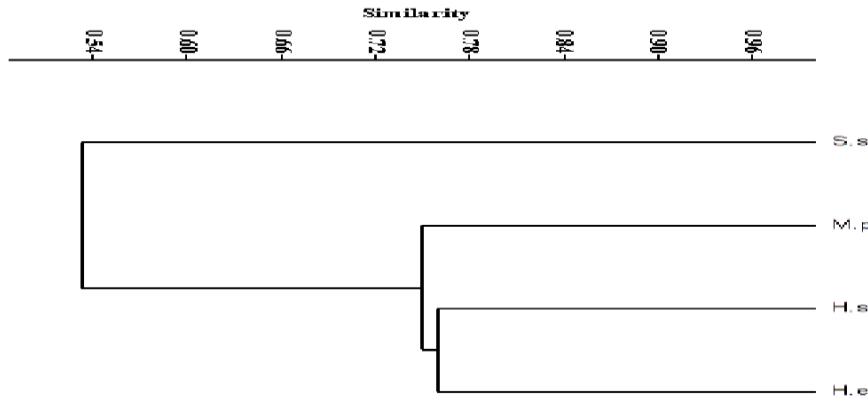


Fig.(1) :UPGMA –Dendrogram based on 90 macro and micromorphological characters illustrating average taxonomic distance between four studied species .

Table (4) Similarity index of 4 species of subfamily Malvoideae using macro and micro morphological data

	H.e1	H.s2	M.p3	S.s4
H.e1	1			
H.s2	0.76	1		
M.p3	0.75	0.75	1	
S.s4	0.49	0.58	0.52	1

CONCLUSION:

There was a close relationship between *Hibiscus esculentus* and *H. sabdariffa* (cultivated plants) more than *M.parviflora* (wild plant) . *Sida spinosa* (wild plant) which related to the same subfamily Malvoideae but it separated with single taxonomic distance from the other three species ; it has variation in many morphological and anatomical features .

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الملخص باللغة العربية

دراسة الشكل الظاهري والتركيب التشريحي لبعض الوحدات التصنيفية من تحت الفصيلة الخبازية
(للفصيلة الخبازية بالمعنى الواسع)

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تمت دراسة ستة واربعون صفة مورفولوجية للنبات الكامل وخمسة وعشرون صفة تشريحية للساق والأوراق لأربعة أنواع (اثنان منها برية وثنان منزرعة) تنتمي لتحت الفصيلة الخبازية وذلك لغرض تقييم هذه الصفات لأستخدامها فى الفصل بين الأنواع محل الدراسة وقد جمعت من أماكن مختلفة فى مصر. PAST UPGAMA, وأظهرت نتائج التحليل العددي باستخدام برامج احصائية حديثة مثل *H.esculentus* *H.sabdarrifa*, على وجود علاقة تصنيفية قريبة بين نوعى الهيببيسكس .

أما نوع *M.parviflora* من حيث التشابه فى بعض الصفات المورفولوجية والتشريحية عن فقد تميز باختلاف فى الصفات المورفولوجية والتشريحية ولذلك ارتبط بعلاقة *Sida spinosa* تصنيفية ضعيفة مع بقية الأنواع المدروسة.