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Phytochemical Circumscription of Hibiscus Collections in Nigeria

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Abstract
This work undertook a comparative chemosystematics study of identified Hibiscus species collected from different parts of Northern Nigeria. Whole plants of H.sabdariffa (SAB), H.rosa-sinensis (ROS), H.syriacus (SYR) and H.rosa-sinensis variegated (ROSV) were collected. Ten (10) qualitative phytochemicals were evaluated in ethanolic extracts of the flower, leaf, bark and root of each Hibiscus species while quantification of phytochemical was carried out on SAB alone. Minitab 16.0 was employed in data analysis.ROSvariegated contained 60% of the phytochemicals investigated in flower while SABand ROSflower contained 50% but it was 40% in ROSV. The leaf of ROSVcontained 100% of all phytochemicals with double presence of tannin and alkaloid. ROSleaf contained 90% of the phytochemicals investigated (lacking only glycoside) while SABleaf contained 80% but leaf was highly concentrated in tannin and flavonoid. ROSVand ROSvariegated contained 70% of the phytochemicals screened in stem bark while SABand ROScontained 50%. All species contained 60-70% of phytochemicals investigated in the roots. InSAB, tannin ranged from 0.3% in the flower to 17.4% in the leaf while flavonoid level ranged between 10.31% in the root and 27% in the flower, thus tannin and flavonoid were present in high concentration in SAB leaf and flower respectively. Phytochemicals such as saponin, phenol, alkaloid and steroid in flower as well as anthraquinone and glycoside in leaf, stem and bark are systematically valuable in the circumscription of the species of Hibiscus studied. Thus, SABand ROSare closely related but distant from ROSvariegated type which could be a hybrid of ROSand any other Hibiscus species. ROSVis very divergent from other species in chemical composition as shown by the dendrogam. This outcome suggests the creation of a good conservation program for this divergent species in order to conserve the unique genes present. Quantitative phytochemical study of SAB has confirmed its medicinal values.

Key Words*: Chemosystematics, Circumscription, Conservation, Hibiscus, Phytochemicals*

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I. Introduction

The Genus *Hibiscus* belongs to the family Malvaceae, a large family and source of many morphologically different ornamental species (Tang*et al.,* 2007). *Hibiscus* species are native to the tropical and subtropical parts of the world, are easily identified by marvellous blooms and showy floral structures (Wong *et al.,* 2009; Maganha*et al.,* 2010).In Nigeria, two popularly cultivated species are: *H.rosa-sinensis* and *H.sabadariffa.*The genus has now been revised and about 197 species were recognized and subdivided into 12 sections(Singh, 2016).The infrageneric classification of genus *Hibiscus* is a problem and needs much attention (Akpan and Hossain, 1998).

With the level of morphological diversity being reported at intra and inter specific levels of this plant, it is of essence to apply the use of taxonomic evidences for detailed systematics studies. These are limited and grossly insufficient in Africa. In Plant systematics, evidentiary characters from many sources help to determine character states for a more comprehensive characterization, delimitation, classification and natural interpretation of phenetic and phylogenetic relationships of taxa among plants (Pfeil*et al.,* 2002; Singh, 2016). Such sources of information may include chemosystematics studies. Chemotaxonomy or Chemosystematics is one of the most significant tools used by modern taxonomist to identify, differentiate, classify, and position closely related taxa systematically (Ankanna*et al*., 2012; Akesa, 2016). It is an approach in taxonomy in which chemical nature of plants are used in developing classification or in solving taxonomic problems. The system of chemotaxonomic classifications relies on the chemical similarity of taxa; it is based on the existence of relationship between constituents and among the plants (Ankanna*et al*., 2012; Akesa, 2016). It gives the close relationship between chemical constituents of plants and their taxonomic status. Chemotaxonomy establishes relationship between the position of the plant and exact understanding of biological evaluation and natural relationship (Anukul, 2011).

Anukul (2011) divided the chemical characters into three categories: directly visible characters such as starch grains, raphides, silica, gypsum; chemical test characters such as phenols, betalains, oil fats, waxes,

alkaloids; and Proteins. The natural characters of plant products are also divided into two groups on the basis of molecular weight: low molecular weight compounds i.e, molecular weight of 1000 or less called micromolecules such as amino acids, alkaloids, fatty acids, terpenoids, flavonoids; and the high molecular weight compounds with molecular weight of more than 1000 called macromolecules such as proteins, DNA, RNA, and complex polysaccharide. Based on taxonomic and chemical knowledge, chemotaxonomy is classified into descriptive, dynamic, and serotaxonomy. Descriptive taxonomy deals with the classification of plants and secondary metabolite and other products like sugar and amino acids. It is also concerned with evolutionary change, chemical convergence and divergence in the plants (Singh, 2016). Dynamic taxonomy is based on biosynthetic pathway while serotaxonomy or semantics is based on pathway of specific proteins and amino acids sequencing in proteins. Serotaxonomy is further classified as: DNA-primary semantics; RNA-secondary semantics; and proteins-tertiary semantics(Bhargava*et al.*, 2013; Reynolds, 2007). Plant parts used for extracting crude extract for chemical analysis are roots, leaves, or bark. **S**ome authors use methods such as paper, thin layer, gas or high pressure liquid chromatography for separating a crude plant extract. One or more types of spectroscopy are used to elucidate the structure of chemical compounds (Singh, 2016; Parimalan*et al.*, 2014).

Phytochemical studies are largely carried out for pharmaceutical values while little is done on the use of chemical components as taxonomic evidences. In plants, popular families that have been studied through chemotaxonomy are Malvaceae, Ranunculaceae, Magnoliaceae, Polygonaceae, and Solanaceae(Singh, 2016). Results from chemotaxonomic studies are helpful to taxonomist, phytochemists and pharmacologists to solve problems in pharmacology and plant systematics (Ankanna*et al.*, 2012; Reynolds, 2007; Singh, 2016) Application of chemicals in the biosystematics study of *Hibiscus* species cannot be over-emphasized. The present study has combined both morphological study with the use of chemicals to reveal the similarities and differences among collected *Hibiscus* species (family Malvaceae) in the Northern part of Nigeria.

There is dearth of systematic information on *Hibiscus* species in Nigeria. Morphological description is confusing and the data cannot be reliably used in the plant's phylogenetics whereas knowledge on the evolutionary relationship among the various species of *Hibiscus* is needed. Apart from those currently exploited for different purposes (as ornaments, food and drinks), many species are unknown and under-utilized.Comparative chemosystematics as a taxonomic evidence is a new development that can help to separate/group taxa on the basis of the type of chemicals they possess or lack, at the same time, helping to determine the therapeutic values of the plant species. The aim of this work was to undertake comparative chemosystematics studies of *Hibiscus* species found in the Northern part of Nigeria.

II. Materials And Methods

Preliminary Collection and Identification

Plant materials were collected from three different sources (bush, farmland and horticulturists) across different northern States. Whole plants were collected and identified by taxonomists in the Department of Botany, Federal University of Agriculture Makurdi. Flora Album of West Africa, Internet aid and gardeners were also consulted for this purpose. Identified species were; *H. sabdariffa*, *H. rosa-sinensi*, *H.syriacus* and *H.rosa-sinensis* variegated.

Sample Collection and Preparation for Phytochemical Studies

Samples were collected in sufficient quantity and dried at room temperature in the FUAM Chemistry Laboratory Dried samples was macerated into powder and the known amount was weighed into 1.5 liter beaker, 1000ml of ethanol was added each sample and allowed to stay for 24 hours using the procedures outlined in Gosain *et al.* (2010). The solutions were filtered through silk wool on funnel separately and were allowed to dry at ambient temperature in the Laboratory. Extracts were obtained for analysis.

Qualitative phytochemical analysis

Qualitative phytochemical analysis was carried out in 4 Parts: Flower, Leaf, Bark and Root of each of the four *Hibiscus* species: *H. sabdariffa, H.rosa-sinensis, H.syriacus* and *H.rosa-sinensis* variegated.Ten (10) phytochemicals were tested on each of the four parts from each *Hibiscus* species. They are: Tannins, Phlobatannins, Saponin, Steroid, Terpenoids, Flavonoids, Phenols, Alkaloid, Glycoside, Anthraquinones. This amounted to 40 phytochemical tests carried out on each species and a total of 160 tests analysed on the four *Hibiscus* types. Methodologies for the determination of phytochemical used in this research were adapted from those reported by Barve*et al.* (2010) and Tambe and Bhambar (2014).

Quantitative Phytochemical analysis

This was carried out on all phytochemicals common to all the four parts of *H. sabdariffa*only. Gravimetric and spectroscopic methods of quantification were adopted (Harbone, 2011; Kumar*et al.*, 2012). The following formula were employed in the determination of % of some phytochemicals as stated below:

% Tannin= $\left(\frac{mg}{100g}\right) = \frac{C\ X\ extract\ volume}{Aliquot\ Volume\ X\ Weight\ of\ sample} X\ 100$

% Alkaloids = $\frac{Weight\ of\ flavonoid}{Weight\ of\ sample}\ X\ 100$

% Flavonoid = $\frac{Weight\ of\ flavonoid}{Weight\ of\ sample}\ X\ 100$

% Saponin=:$\frac{Weight\ of\ Saponin}{Weight\ of\ sample}\ X\ 100$

% Steroid $\left(\frac{mg}{100g}\right) = \frac{C\ X\ extract\ volume}{Aliquot\ Volume\ X\ Weight\ of\ sample} X\ 100$

.% Phenols $\left(\frac{mg}{100g}\right) = \frac{C\ X\ extract\ volume}{Aliquot\ Volume\ X\ Weight\ of\ sample} X\ 100$

C = concentration read off the graph.

Data Analysis

Data analysis was done using Mintibab 16.0 and Excel workbook. Cluster analysis was employed in the generation of dendrogram to show the degree of relationship among the *Hibiscus* varieties. Radar plot displayed the amount of phytochemicals common to the four organs in *Hibiscussabdariffa.*

III. Results And Discussion

Table 1 presents the results of qualitative phytochemical screening in *Hibiscus* flower. Tannin was present in all but glycoside and phlobatannin were lacking in all flowers. Saponin and phenol were present in all flowers except in *H. syriacus.* Terpense was present only in *H. rosa-sinensis* variegated flower. All flowers possessed flavonoid but more concentrated in *H. sabdariffa.* Alkalod was present only in the flower of *H. rosa-sinensis* and the variegated type while steroid was present only in the flower of *H. sabdariffa*and *H. syriacus*. From the analysis, *H.rosa-sinensis* variegated contained 60% of the phytochemicals investigated while *H. sabdariffa*and *H. rosa-sinensis* contained 50% but it was 40% in *H. syriacus*.

Table 2 presents the results of qualitative phytochemical screening in *Hibiscus* leaf. Saponin, flavonoid, phenol and terpense were present in all leaves. Tannin was present in all but lacking in *H. rosa-sinensis* variegated. Alkaloid, steroid and Phlobatannin were present in all leaves except in *H. rosa-sinensis* variegated. Anthraquinone was present in all leaves but lacking in *H. sabdariffa*leaf. Only *H. syriacus*possessed glycoside. From the results, *H. syriacus* leaf contained 100% of all phytochemicals with double presence of tannin and alkaloid. *H.rosa-sinensis* leaf contained 90% of the phytochemicals investigated (lacking only glycoside) while *H. sabdariffa*leaf contained 80% of the phytochemicals (lacking only anthraquinone and glycoside) but leaf was highly concentrated in tannin (+++) and flavonoid (+++). Meanwhile, *H. rosa-sinensis* variegated leaf contained 50% of the phytochemicals investigated.

Table 1: Qualitative Phytochemical Screening in *Hibiscus* Flower

Phytochemicals in Flower	SAB	ROS	SYR	ROSV	Remarks
Saponin	+	+	-	+	Abs in SYR
Tannin	+	+	+	+	Pre in all
Anthraquinone	-	-	+	-	Pre in SYR
Glycoside	-	-	-	-	Abs in all
Flavonoid	+++	+	+	+	Pre in all, more in SAB
Phenol	+	+	-	+	Abs in SYR
Alkaloid	-	+	-	+	Pre in two, abs in two
Steroid	+	-	+	-	Pre in two, abs in two
Phlobatannin	-	-	-	-	Abs in all
Terpense	-	-	-	+	Pre in ROSV
% Presence	**50%**	**50%**	**40%**	**60%**	

SAB= *H. sabdariffa*

ROS= *H. rosa-sinensis*

SYR= *H. syriacus*

ROSV= *H. rosa-sinensis* variegated

Table 2: Qualitative Phytochemical Screening in *Hibiscus* Leaf

Phytochemicals in Leaf	SAB	ROS	SYR	ROSV	Remarks
Saponin	+	+	+	+	Pre in all
Tannin	+++	+	++	-	Abs ROSV, more in SAB
Anthraquinone	-	+	+	+	Abs in SAB
Glycoside	-	-	+	-	Pre in SYR
Flavonoid	+++	+	+	+	Pre in all, more in SAB
Phenol	++	+	+	+	Pre in all, more in SAB
Alkaloid	+	+	++	-	Pre in all, more in SYR
Steroid	+	+	+	-	Abs in ROSV
Phlobatannin	+	+	+	-	Abs in ROSV
Terpense	+	+	+	+	Pre in all
% Presence	**80%**	**90%**	**100%**	**50%**	

SAB= *H. sabdariffa*
ROS= *H. rosa-sinensis*
SYR= *H. syriacus*
ROSV= *H. rosa-sinensis* variegated

Table 3 presents the results of qualitative phytochemical screening in *Hibiscus* stem bark. All barks contained saponin, tannin, flavonoid, phenol and alkaloid. These phytochemicals were present in varying degrees among the species. For instance, tannin was more concentrated in the stem bark of *H. sabdariffa*while alkaoild was more concentrated in the stem bark of *H. rosa-sinensis*and *H. syriacus.* Other phytochemicals were remarkably distinguishing. For instance, anthraquinone and glycoside were present only in *H. syriacus* stem bark but lacking in other species. Phlobatannin was present only in *H. rosa-sinensis* and the variegated type while steroid was present only in the stem bark of *H. rosa-sinensis* variegated. Terpense was lacking in all. From this result, *H. syriacus*and *H. rosa-sinensis* variegated contained 70% of the phytochemicals screened while *H. sabdariffa*and *H. rosa-sinensis*contained 50%.

Table 4 presents the results of qualitative phytochemical screening in *Hibiscus* root. All roots contained saponin, tannin, flavonoid, phenol, alkaloid and steroid. The available phytochemicals were highly concentrated in the root with few exceptions among the species. *H. syriacus*and *H. rosa-sinensis* variegated contained triple presence of saponin while tannin was highly concentrated (+++) in the roots of all species. High presence was observed in flavonoid of *H. rosa-sinensis*and *H. syriacus*; and alkaloid of *H. rosa-sinensis*. Only *H. syriacus* root contained glycoside. All species contained 60-70% of phytochemicals investigated in the roots. Figure 1 shows the dendrogram of phytochemical data obtained from the flower, stem bark, leaf and root. Similarity level was 79%. *H. syriacus*was divergent among the species followed by *H. rosa-sinensis* variegated whereas *H. sabdariffa*and *H. rosa-sinensis*were similar in phytoconstituents (90%).

Table 3: Qualitative Phytochemical Screening in *Hibiscus* Bark

Phytochemicals in Bark	SAB	ROS	SYR	ROSV	Remarks
Saponin	++	+	+	+	Pre in all, more in SAB
Tannin	+++	++	++	+	Pre in all, more in SAB
Anthraquinone	-	-	+	-	Pre SYR
Glycoside	-	-	+	-	Pre SYR
Flavonoid	++	+	+	+	Pre in all, more in SAB
Phenol	++	+	+	+	Pre in all, more in SAB
Alkaloid	++	+++	+++	+	Pre in all, more in ROS and SYR
Steroid	-	-	-	+	Pre in ROSV
Phlobatannin	-	+	-	+	Pre in two, abs in two
Terpense	-	-	-	-	Abs in all
% Presence	**50%**	**50%**	**70%**	**70%**	

SAB= *H. sabdariffa*
ROS= *H. rosa-sinensis*
SYR= *H. syriacus*
ROSV= *H. rosa-sinensis* variegated

Table 4: Qualitative Phytochemical Screening in *Hibiscus* Root

Phytochemicals in Root	SAB	ROS	SYR	ROSV	Remarks
Saponin	++	++	+++	+++	Pre in all, more in SYR and ROSV
Tannin	+++	+++	+++	+++	Pre in all
Anthraquinone	-	-	-	-	Abs in all
Glycoside	-	-	+	-	Pre in SYR
Flavonoid	++	+++	+++	++	Pre in all, more in ROS and SYR
Phenol	+	+	++	++	Pre in all
Alkaloid	++	+++	++	++	Pre in all, more in ROS
Steroid	++	++	+	++	Pre in all
Phlobatannin	-	-	-	-	Abs in all
Terpense	-	-	-	-	Abs in all
% Presence	**60%**	**60%**	**70%**	**60%**	

SAB= *H. sabdariffa*
ROS= *H. rosa-sinensis*
SYR= *H. syriacus*
ROSV= *H. rosa-sinensis* variegated

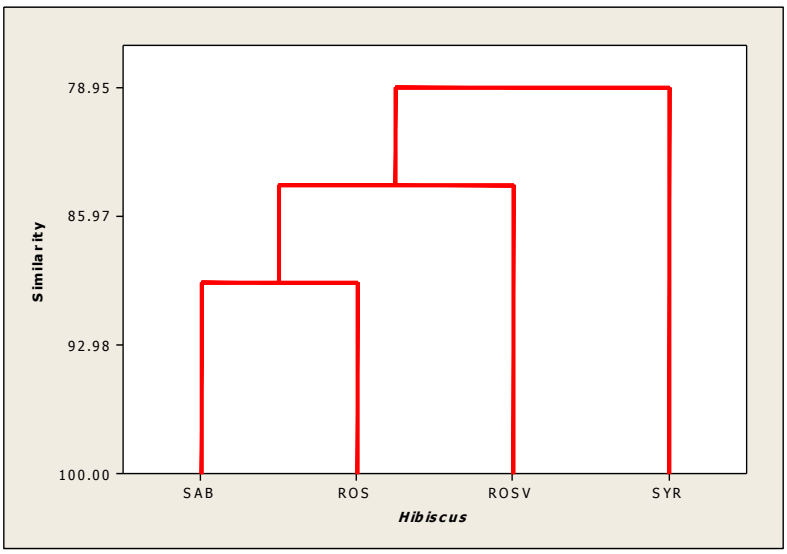

Figure 1: Dendrogram of *Hibiscus* species based on phytochemical data
SAB= *H. sabdariffa*
ROS= *H. rosa-sinensis*
SYR= *H. syriacus*
ROSV= *H .rosa-sinensis* variegated

Table 5 gives the results of quantified phytochemicals in all parts of *H. sabdariffa*. Saponin varied from 0.2% in the root to 0.92% in the leaf. Tannin ranged from 0.3% in the flower to 17.4% in the leaf. Flavonoid level ranged between 10.31% in the root and 27% in the flower. Phenol had its maximum level in the leaf (1.08%) while alkaloid was highest in the stem bark (2.1%). Steroid level was highest in the flower (0.9%) but undetectable in leaf and stem bark. Radar plot (figure 2) shows that tannin and flavonoid were present in high concentration in *H. sabdariffa*. Also, tannin was highest in the leaf as indicated with red line while flavonoid was highest in the flower as indicated with blue line. It also indicates that flavonoid had the highest value of phytochemicals quantified and it was present in the flower.

Table 5: Quantity of Phytochemicals in Four parts of *H. sabdariffa*

	Flower	**Leaf**	**Bark**	**Root**
% Saponin	0.5	0.92	0.6	0.2
% Tannin	0.3	17.4	10.41	16.2
% Flavonoid	27.01	20.09	11.2	10.31
% Phenol	0.41	1.08	0.7	0.47
% Alkaloid	0.00	0.00	2.10	2.01
% Steroid	0.9	0.00	0.00	0.73

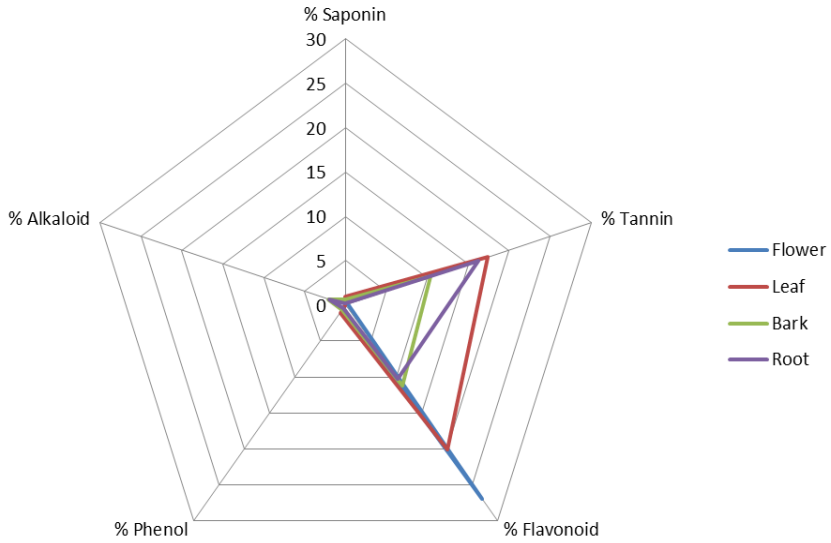

Figure 2: Radar Plots of Phytochemicals in *H. sabdariffa*

Chemosystematics information obtained has provided excellent taxonomic resolution of the *Hibiscus* species studied. Results agree with earlier submission that occurrence of secondary metabolites in plants help in classification, delimitation and circumscription of taxa (Singh, 2016). From this work, it could be inferred that chemosystematics data provide better resolution than morphological evidence because infraspecific taxon can be circumscribed using chemical evidence beyond the limit of comparative morphology. The presence of common chemicals in the *Hibiscus* species indicate convergence at the generic level while differences in chemical composition indicate divergence, a view widely supported in literatures (Ankanna*et al.*, 2012; Singh, 2016; Vidita *et al.*, 2013).

In the present work, any of the plant organs (flower, leaf, bark and root) could form the basis of separating *Hibiscus* species based on disparity in chemical composition. Analysis of chemicals in flower has shown that saponin and phenol were present in all species except in *H.syriacus* while only H.rosa-sinensispossess alkaloid in the flower, lacking in other species. Steroid was present in *H.sabdariffa*and *H.syriacus* flower but absent in *H.rosa-sinensis*. Thus, saponin, phenol, alkaloid and steroid content of flower have separated the *Hibiscus* species. Meanwhile, *H.rosa-sinensis*flower possessed more chemical types than in other species.

Chemicals in the leaf are systematically valuable. *H.rosa-sinensis* variegated type has been circumscribed from the *H.rosa-sinensis* ancestor since the former lacked tannin, alkaloid, steroid and phlobatannin but these chemicals are present in the latter. Anthraquinone is present in all *Hibiscus* species but lacking in *H.sabdariffa* while glycoside was found only in *H.syriacus.* It seems that anthraquinone and glycoside have separated the species based on the foliar chemical composition. *H.syriacus* possessed all chemicals in the leaf whereas other species lacked one or two in the leaf. However, the variegated type of *Hibiscus* lacked five of the chemicals investigated. This may be due to deficiency in pigmentation in this type of leaf. Chemicals in the stem bark have also provided good resolution. Anthraquinone and glycoside were present only in *H.syriacus* bark, lacking in other species while phlobatannin was only present in *H.rosa-sinensis*. From this report, *H.sabdariffa* is more chemically deficient in stem bark than in other species. Anthraquinone and glycoside are the two chemicals of systematic values in the stem bark whereas glycoside contained in the root of *H.syriacus*has helped distinguished this species from others.

This work has confirmed the relevance of saponin, phenol, alkaloid and steroid in flower as well as anthraquinone and glycoside in leaf, stem and bark in the systematic studies of *Hibiscus* species even to the intraspecific level. This outcome aligns with previous studies in the classification of species in family Cinnamomeae where flavonol glycosides were shown to be important chemotaxonomic markers especially in the identification of *Rosa* species. However, other chemicals such as flavonoid and terpens provided little or no systematic values. The present report did not align with some findings where flavonoid was systematically valuable. For instance, in Okeke*et al.* (2015) flavonoids and steroids content of three *Stachytarpheta*species present in Awka, South EastNigeria were tested to establish their systematic relevance. These chemicals gave good resolution and were used to delimit *S.cayannensis*from the two other *Stachytarpheta*species tested.

Similarly, root Flavonoids type of four *Convolvulus* species (*C. arvensis, C. commutatus, C. lineatus*and *C. pilosellaefolius*) in Iran were also applied to circumscribe them based on the generic type of flavonoid present based on the work done by Bahrami*et al*. (2016).

Chemosystematics data provided are less complex to interpret compared to other types of evidences. Chemicals are produced through enzyme catalyzed biosynthetic pathways, each controlled by one or more genes. Thus, phytochemicals have direct genetic basis and this makes chemosystematics data reliable. This type of evidence is not affected by the environment and therefore provides natural and phylogenetic relationship unlike in phenotypic evidence that may be subjective. Chemotaxonomy is directly related to molecular phylogeny of plants (Singh, 2016). In this work, the phylogenetic tree obtained from chemical data has shown that*H.sabdariffa* and *H.rosa-sinensis*are closely related but distant from *H.rosa-sinensis* variegated type which could be a hybrid of *H.rosa-sinensis* and any other *Hibiscus* species. The closeness between the two species stated above could be exploited in providing a successful interspecific hybridization since they are genetically compatible.

Therefore, breeding could proceed for improvement in desirable traits for specific purposes such as floral ornamentation. Meanwhile, *H.syriacus* is very divergent and dissimilar from other species in chemical composition. This outcome suggests the creation of a good conservation program for this divergent species in order to conserve the unique genes present and promote biodiversity. The extent and distribution of genetic diversity in a plant species depend on its evolution and breeding system, ecological and geographical factors. In order to manage conserved germplasm better, there is also a need to understand genetic diversity that is present in collections, thus suggesting further genetic analysis of *H.syriacus.* Through improved characterization and development of core collections based on genetic diversity information, it will be possible to exploit the available resources in more valuable ways (Demir*et al.,* 2010).

Quantitative phytochemical study of *H.sabdariffa* has confirmed the nutritional and medicinal values of the plant as reported in other work (Ali *et al.,* 2014; Farombi and Ige, 2007; Saxena*et al.,* 2013). Tannin and flavonoid, the two phytochemicals found in sufficient quantities in the leaf and flower respectively, are useful phytochemicals.Today, flavonoids are used for making anticancer, antibacterial, antiviral and antifungal drugs (Chan *et al.,* 2016; Singh *et al.,* 2016). Thus, the popular "zobo" drink made from the flower may be rich in flavonoid hence the reported medicinal values of this drink are supported based on this outcome.

IV. Conclusion

Phytochemicals such as saponin, phenol, alkaloid and steroid in flower as well as anthraquinone and glycoside in leaf, stem and bark are systematically valuable in the circumscription of the species of *Hibiscus* studied. Thus, *H.sabdariffa* and *H.rosa-sinensis*are closely related but distant from *H.rosa-sinensis* variegated type which could be a hybrid of *H.rosa-sinensis* and any other *Hibiscus* species. *H.syriacus* is very divergent from other species in chemical composition. This outcome suggests the creation of a good conservation program for this divergent species in order to conserve the unique genes present. Quantitative phytochemical study of *H.sabdariffa* has confirmed its medicinal values.

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