J. Plant Production, Mansoura Univ., Vol. 4 (3): 417 - 423, 2013

CHEMOTAXONOMIC STUDY OF FIVE MONOCOT SPECIES FROM NORTH- EASTERN SUDAN

Wasfi, M. A., I. Madani and Fatima El - Mubarak
Botany Department Faculty of Science, Univ. of K. Sudan

ABSTRACT

Alkaloids, flavonoids, triterpenes, sterols and saponins were qualitatively screened in different organs of five monocotyledonous species belonging to different genera: Allium cepa L., Asparagus densiflorus (Kunth) Jessop, Pancratium tortuosum Herbert., Kniphofia nubigena Mildr., and Urginea maritima (L.) Baker. Phenolic compounds were separated using thin layer chromatography to ascertain their relative phylogenetic position. Data collected from the qualitative phytochemical analysis, paired affinity, group affinity and isolation value reflected taxonomically significant information supported the inclusion of these species in different families by many taxonomists in the most recent classification systems.
Keywords: Monocot classification, chemotaxonomy, TLC, phenolics.

INTRODUCTION

Monocot classification had undergone considerable revision in the past four decades particularly in recent years. Cronquist (1968, 1981) and Hutchinson (1973) in their systems of classification assigned the five species selected for the present study: Asparagus, Urginea, Pancratium and Allium to the family Liliaceae and Kniphofia to the family Aloaceae and both families to the order liliales . Dahlgren et al. (1985) placed Kniphofia in Asphodelaceae, Pancratium in Amaryllidaceae Urigina in Hyacinthaceae, Asparagus in Asparagaceae while Allium was placed in Alliaceae and the four families in the order Asparagales . Kubitzki (1998) agreed with Dahlgren system of classification while Takhtajan (1997) reclassified the monocots. He placed the families: Amaryllidaceae,Hyacinthaceae, and Alliaceae in the order Amaryllidales while Asphodelaceae and Asperagaceae in the order Asparagales .Again, Takhtajan in his revision of his old system of classification assigned all the families: Amaryllidaceae, Hyacinthaceae, Alliaceae, Asphodelaceae and Asperagaceae to the order Asparagales Takhtajan (2009). Recently, the last revisons based on molecular data have been done by the Angiosperm Phylogeny Group (APG 1998, APGII 2003 and APG III, 2009). APG III, placed Allium and Pancratium in the family Amaryllidaceae in the subfamilies Allioideae and Amaryllidoideae respectively, Asparagus and Urginea in the family Asparagaceae in the subfamilies Asparagoideae and Scilloideae respectively and Kniphofia in the family Xanthorrhoeaceae in the subfamily Asphodeloideae (APG III, 2009).

The aim of this study was to determine the chemotaxonomic variations within and between different monocots being historically assigned to the family Liliaceae and Aloaceae and recently reclassified into different families based on both morphological and genetical variations.

MATERIALS AND METHODS

Materials:

Leaves, roots of Allium cepa L., Asparagus densiflorus (Kunth) Jessop, Pancratium tortuosum Herbert, Kniphofia nubigena Mildr and Urginea maritima Baker(L.) and bulbs of Allium cepa, Pancratium tortuosum and Urginea maritima were collected from Erkwit area in north-eastern Sudan.

Methods:

The following phytochemical screening of the studied materials was carried out according to the method described by Sofowara (1993) and Harborne (1973):

1- **Test for alkaloids**

One gram of well ground plant material was extracted with ethanol and allowed to stand for about four days before being filtered. Small portion of concentrated extract was placed in a separate test-tube to which 2-3 drops of Mayer's reagents were added. Cream coloured precipitate observed as positive results.

2- **Test for flavonoids**

One gram of powdered material was extracted with ethanol and allowed to stay for about two days then filtered. Then, to 1ml of extract 5 drops of 2% ferric chloride were added. Formation of green colour indicated the possible presence of flavonoid.

3- **Test for Triterpenes and sterols**

One gram of powdered plant material was macerated with 20ml petroleum ether (60-80°C) for six hours then evaporated. The residue was dissolved in acetic anhydride (2ml), transferred to a test-tube and cautiously concentrated sulphuric acid was poured along the side of tube. Possible presence of sterol or triterpenes was indicated by immediate appearance of violet colour.

4- **Test for saponins**

To 0.5g powder of plant material 10 ml distilled water was added and shaked vigorously for one minute in a test tube. Permanent frothing indicated the possible presence of saponins.

TLC separation of phenolic compounds:

Plant materials was extracted with a mixture of 50% of petroleum ether and 50% aqueous methanol.. The extract was evaporated under vacuum pump to yield a sticky residue. The developing liquid used was a mixture of Toluene- Formic acid - Ethyl acetate - water (25: 5.5: 5.5: 18.8) .After the development of the chromatogram, the retention value of the separated compounds was calculated .Values of paired affinity (PA), group affinity (GA) and isolation value (IV) were calculated as follows:

$$PA = \frac{\text{Spots common in species A and B}}{\text{Total spots in A and B}} \times 100$$

$$GA = \text{Total PA value} + 100$$

$$IV = \frac{\text{Number of unique spots in a species}}{\text{Total number of spots in all species}} \times 100$$

RESULTS AND DISCUSSION

Alkaloids were detected in the leaves of all studied species except Asparagus densiflorus. This finding is in agreement with Takhtajan natural system of classification, in which he separated Asparagales from the other nine orders in the superorder Lilianae based on the lack of alkaloids as well as presence of cheldonic acid and steroidal saponins. He also mentioned these chemical characters as key characters for the family Asparagaceae and the genus Asparagus (Takhtajan, 2009).

Leaf flavonoids were also detected in all studied species except Asparagus densiflorus. This character is evaluated in Kubtzi system of classification as diagnostic for the family Hyacinthaceae in which he assigned the genus Urginea and a genus character for Allium (Kubtzi, 1998). Similarly, Takhtajan (2009) used the presence of these chemicals to describe both Hyacinthaceae and Alliaceae in which he assigned the genera Urginea and Allium respectively (Takhtajan, 2009).

The lack of saponins in Kniphofia nubigena and Pancratium tortuosum is in conformity with three systems of classifications namely those of Cronquist (1981), Takhtajan (2009) and Kubitzki (1998). Cronquist (1981) used this character as one of the important chemical features separating the family Aloaceae in which he assigned the genus Kniphofia from the family Liliaceae. Takhtajan (2009) split the superorder Lilianae into 18 orders. He placed our studied species into two orders Asparagales and Amaryllidales based mainly on presence and absence of saponins. He also reported the presence of these chemicals as a key character for Alliaceae and Hyacinthaceae in which he assigned Allium and Urginea respectively. Kubitzki (1998) used the absence of saponins as a key character for Amaryllidaceae in which he placed the genus Pancratium while he used the presence of these chemicals to describe the family Asparagaceae in which he placed the genus Asparagus. He also described roots and rhizomes as saponin rich parts of Asparagus.(Table1)

Table 1: General Phytochemical screening of the studied species

A: Allium cepa

Test	Leaves	Roots	Bulbs
Alkaloids	+	-	-
Flavonoids	++	++	-
Triterpenes and sterols	+	-	+
Saponins	+	+	+

B: Pancratium tortuosum

Test	Leaves	Roots	Bulbs
Alkaloids	+++	-	-
Flavonoids	+	-	+
Triterpenes and sterols	-	+	++
Saponins	-	-	-

C: Urginea maritima

Test	Leaves	Roots	Bulbs
Alkaloids	+	-	-
Flavonoids	++	-	-
Triterpenes and sterols	+	-	+
Saponins	-	+	-

D: Kniphofia nubigena

Test	Leaves	Roots
Alkaloids	+	+
Flavonoids	+++	++
Triterpenes and sterols	+	-
Saponins	-	-

E: Asparagus densiflorus

Test	Leaves	Roots
Alkaloids	-	-
Flavonoids	-	-
Triterpenes and sterols	-	-
Saponins	++	++

Based on the general screening for alkaloids, flavonoids, triterpenen and saponins, the following biochemical key is constructed to differentiate between the studied species:

1. Presence of leaf saponins……...2
1. Absence of leaf saponins……..……..……...3
2. Presence of flavonoids and alkaloids……...Allium cepa
2. Absence of flavonoids and alkaloids…….Asparagus densiflorus
3. Presence of root alkaloids……....Kniphofia nubigena
3. Absence of root alkaloids……4
4. Absence of leaf triterpenes .....……...Pancratium tortuosum
4. Presence of leaf triterpenes ……..……..Urginea maritima.5

Screening for phenolic compounds on TLC, results in many spots of different Retention Factor (Rf) values. The relative distribution of all spots and Retention Factor (Rf) are shown in Table 2 and 3.

Table 2: Thin layer chromatography separation of phenolics in the leaves of the studied species revealing their retention values R_f

No	R_f values	Allium cepa	Pancratium tortuosm	Urginea maritima	Kniphofia nubegina	Asparagus densiflorus
1	0.1	-	-	-	+	-
2	0.23	-	-	+	-	-
3	0.3	+	-	-	+	-
4	0.67	-	-	-	+	-
5	0.7	-	+	-	-	+
6	0.74	-	-	-	-	+
7	0.8	-	+	+	-	-
8	0.86	-	-	-	-	-
9	0.9	-	-	+	-	-
10	0.93	+	-	-	-	-

Table 3: Thin layer chromatography separation of phenolics in the roots of studied species revealing their retention values R_f

No	R_f values	Allium cepa	Pancratium tortuosm	Urginea maritima	Kniphofia nubegina	Asparagus densiflorus
1	0.1	-	-	-	-	+
2	0.2	+	+	-	+	-
3	0.3	-	-	-	-	+
4	0.4	+	-	-	+	-
5	0.5	-	+	+	-	-
6	0.7	-	+	-	+	+
7	0.8	+	-	+	-	-

Table 4: Paired affinity (PA), grouped affinity (GA) and isolation value(IV) based on phenolic compounds separated on TLC for the studied species

Species	Allium cepa	Pancratium tortuosum	Urginea maritima	Kniphofia nubigena	Asparagus densiflorus	GA	IV
Allium cepa	PA					193	0
	100	22	11	27	13		
Pancratium tortuosum	22	100	25	20	13	202	0
Urginea maritima	11	25	100	0	0	147	15
Kniphofia nubigena	27	20	0	100	30	204	8
Asparagus densiflorus	13	13	0	30	100	178	8

The maximum paired affinity PA and grouped affinity GA reported during the present study were 30% and 204% respectively and the minimum paired affinity PA and grouped affinity GA recorded were 0% and 147% respectively (table4). According to Ellison et al. (1962) PA and GA values of

50% and above are considered as marker of close relationship. In this regard our studied species are not closely related.

REFERENCES

Angiosperm Phylogeny Group (1998).An ordinal classification for the families of flowering plants. Annuals of the Missouri Botanical Garden 85: 531-553.

Angiosperm Phylogeny Group II (2003). An update of the Angiosperm Phylogeny group classification for the orders and families of flowering plants: APG II., Botanical Journal of the Linnean Society 141: 399–436.

Angiosperm Phylogeny Group III (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society 161 (2): 105–121

Cronquist, A.(1968).The Evolution and classification of flowering plants. Houghton Mifflin Company, Boston.

Cronquist, A. (1981). An integrated system of classification of flowering plants. New York: Columbia University Press.

Dahlgren, R. M. T., Clifford, H. T. and Yeo, P. F. (1985).The families of the Monocotyledons. Structure, Evolution and Taxonomy. Springer-Verlag, Berlin.

Ellison WL, RE Alston and BL Turner, (1962). Methods of presentation of crude biochemical data for systematic purposes with particular reference to the genus Bahia (Compositae). Am. J. Bot 49: 599- 604

Harbone, J.B. (1973). Phytochemical methods,London. Chapman and Hall,Ltd. pp. 49-188.

Hutchinson, J. (1973). The families of the flowering plants, arranged according to a new system based on their probable phylogeny.Vol 3. London, pp.415.

Kubitzki, K., ed. (1998). The Families and Genera of Vascular Plants, Vol. III, Monocotyledons: Lilianae (except Orchidaceae), Springer-Verlag, Berlin.

Sofowara, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd,Ibadan,Nigeria, pp. 289-300.

Takhtajan, A. (1997). Diversity and classification of flowering plants. Columbia University Press,New York, pp. 204.

Takhtajan, A. (2009). Floweing Plants. Second Edition. Springer, Berlin.

دراسة التصنيف الكيميائي لخمس نباتات من ذوات الفلقة الواحدة مأخوذة من شمال- شرق السودان

ميرغني عبد الرحمن وصفي، اكرام مدني و فاطمة المبارك
قسم النبات كلية العلوم ، جامعة الخرطوم، جمهورية السودان

تم الفحص النوعي للقلويدات والفلافينويدات والتربينات الثلاثية والصابونينات والاستيرولات من اجزاء مختلفة لخمسة انواع من النباتات تنتمي لاجناس من ذوات الفلقة الواحدة وهي:

Allium cepa L., Asparagus densiflorus (Kunth) Jessop, Pancratium tortuosum Herbert. , Kniphofia nubigena Mildr., and Urginea 423aritime (L.) Baker.

تم فصل المركبات الفينولية بإستخدام كروماتوقرافيا الطبقة الرقيقة لتحديد علاقة القربي بينهم. وقد اضافت البيانات التي جمعت من التحليل النوعي للمركبات الكيمائية وللزوج المتقارب (PA) والمجموعة المتقاربة (GA) وقيمة الإنعزال((IV)معلومات ذات قيمة تصنيفية تؤيد وضع الخمسة أنواع في فصائل مختلفة كما ذهب (أو اقترح) علماء تصنيف النباتات في غالبية نظم التصنيف الحديثة.

قام بتحكيم البحث

أ.د / زين العابدين عبد الحميد محمد — **كلية الزراعة – جامعة المنصورة**
أ.د / محمد عبد العزيز نصار — **كلية الزراعة – جامعة القاهره**