

## ECOLOGICAL STUDIES ON WILD MEDICINAL PLANTS IN EGYPT.

### III- *Cynanchum acutum* L.

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### ABSTRACT

The present work contributes information about morphology and anatomy of *Cynanchum acutum*. Also, it comprises a detailed ecological study on *C. acutum* community type. Phytochemically, the mean values of flavonoids and polyphenols were detected. The GLC analysis of fatty acids was carried out and the antioxidant and antimicrobial activities of methanolic extract were examined. In all the stands, *Cynanchum* was the dominant and produced the greatest part of the dense plant cover. *Chenopodium murale*, *Cynodon dactylon*, *Torilis arvensis* and *Sisymbrium irio* were common associates. *C. acutum* community comprised 45 species belonging to 17 families. These species have been distinguished into 30 annuals, 13 perennials and two biennials. Also, grouped into five life-forms; therophytes (76%), geophytes (9%), hemicryptophytes (7) and each of helophytes and nanophanerophytes (4%). The Mediterranean, Irano-Turanian and Euro-Siberian were the major chorotypes. Flavonoids and polyphenols contents of *Cynanchum* shoot were 0.313 and 0.375 g/100g, respectively. Ten fatty acids were detected in the lipoidal matter of *C. acutum*. The methanolic extract of the plant showed antioxidant and antimicrobial activities.

**Keyword:** *Cynanchum*, autecology, metabolites, antimicrobial activity.

### INTRODUCTION

The medicinal plants in Egypt represent a new promising resource as there is a relatively high representation of medicinal species in the native flora (Botanouny, 1999). The prevailing environmental conditions may induce the accumulation of a high concentration of metabolic products.

Family Asclepiadaceae is the former plant family now treated as subfamily Asclepiadoide in Apocynaceae (Jurgens *et al.*, 2008 and Kamel *et al.*, 2014 ). It comprises many medicinal plants with a wide range of therapeutic activities (Alikhan & Khanum, 2005). Most members have milky juice, pod-like follicles and tufted silky-haired seeds. *Cynanchum acutum* has been selected to be the subject of the present study. It is a wild perennial herb commonly distributed in the Nile Delta and known as mentioned (Tackholm, 1974). *C. acutum* commonly popular medicinal plant. It has been used as purgative in the French pharmaceutical codex (Garnier *et al.*, 1961). Its milky latex is used for skin and eye problems in Tunisian folk medicine (Boukef, 1986 ).

Many researches on *C. acutum* have revealed the presence of several bioactive metabolites including  $\beta$ -sitosterol, lupeol, amyryl (Halim *et al.*, 1990), sarcostine and quercetin (El-Sayed *et al.*, 1994), four flavonoid glycosides: quercetin dio-o-hexoside, quercetin 3-O- rhamnosyl glycoside, quercetin 3-O-galactoside and quercetin 3-O- xyloside (Heneidak *et al.*, 2006) and two coumarins: scopoletin and scoparone (El-Demerdash *et al.*, 2009), as well as seven other flavonoids (Ghada *et al.*, 2008). In India and equatorial parts of America continent, *C. acutum* utilized as pesticide and parasite repellent (Lewis and Lewis, 1977). In China *Cynanchum* utilize for its anti-fever and diuretic trait .

The alcoholic extract of *Cynanchum* leaves could be used as anti-inflammatory, analgesic, antipyretic, hypotensive, in treatment of cardiac arrhythmia, against skin infections, for improving respiration in asthma and anti-spasmodic (Awaad, 2000; and Atta *et al.*, 2005). El-Meligy *et al.*, (2014) concluded that *C. acutum*

possessed potent hepatoprotective activity. Golzardi *et al.*, (2015) assess the potential allelopathic effect of two *C. acutum* population on germination and shoot length of barely.

The present study aims at carrying out ecological survey of *C. acutum* community, investigation of its biological aspects, bioactive metabolic products as well as the antioxidant and antimicrobial activities.

### MATERIALS AND METHODS

To illustrate the morphological characteristics of *Cynanchum acutum*, fresh samples were described according to Forster and Gifford (1974); Heywood (1978) and LAWG (1991).

For anatomical investigation, thin sections of stem, leaf, and root were prepared according to Peacock and Bradbury (1973). Then examined by light microscope and photographed.

The floristic components of *C. acutum* community type were recorded in a series of 20 representative stands. The list of species was made including phonological aspect, cover-abundance and presence estimates as described by Mueller-Dombois and Ellenbery (1974), Werger and Sprangers (1982), and Kent and Coker (1992). Identification and nomenclature of the plants were according to Boulos (2009).

Soil samples were collected from the different stands and analysed for estimation of their physical and chemical characteristics as described by Jackson, 1967; Pansu & Gautheryous, 2006 and Carter & Gregorich, 2008.

Quantitative estimation of flavonoids and polyphenols was according to Sadasivam & Manickam, 2008. GLC analysis of fatty acids was carried out using apparatus, Agilent Technologies 6890N, as described by Finar, 1967.

The antioxidant activity of methanolic extract of *C. acutum* shoot was estimated using the method described by EbrahimZadeh *et al.*, (2009). The antimicrobial activity was investigated by the filter paper disc assay ( Sardari *et al.*, 1998).

## RESULTS AND DISCUSSION

- **Ecological characteristics**
- **Vegetation analysis**

The vegetation of twenty stands representing *Cynanchum acutum* community was analysed in detail. These stands were located at El Dakahlyia Governorate in the north east of Nile Delta.

In all stands *C. acutum* was the dominant species and present in all stands ( $P = 100\%$ ) and contributing most of the plant cover which is often dense (average 60%) with scattered individuals of the associates occupying the interspaces. *Chenopodium murale* is the most abundant associate ( $P = 55\%$ ). *Cynodon dactylon*, *Torilis arvensis* and *Sisymbrium irio* were common associates having presence value of 30%. *Silybum marianum* and *Urtica urens* have presence estimates of 25%. *Althaea ludwigii*, *Sonchus oleraceus* and *Lolium multiflorum* have presence values of 20%. There were one perennial (*Phragmites australis*) and three annuals (*Malva parviflora*, *solanum nigrum* and *Senecio aegypticus*) with presence estimate of 15%. Also, this community includes sixteen associates, each recorded in two stands ( $P = 10\%$ ), among them: *Cyperus rotundus*, *Raphanus raphanistrum*, *Medicago intertexta*, *Stellaria pallida*, *Centaurea aegyptiaca* and *Atriplex halimus*. Another fourteen associates are rarely present and each recorded in a single stand ( $P = 5\%$ ).

From the floristic components point of view, this community comprised 45 species belonging to 17 families. Among these 13 (29%) monocots and 32 (71%) are dicots. Poaceae includes 11 species (25%), Asteraceae 9 species (20%), Fabaceae 6 species (14%), Brassicaceae and Malvaceae 3 species each (7%) and Chenopodiaceae two species (5%). Also, there are eleven families are represented by a single species each, Fig. (1).

The plant assemblage of *C. acutum* community has been distinguished into: 30 annuals (76%), 13 perennials (29%), and two biennials (4%), Fig. (2).

With respect to the life-forms, the recorded species were categorized into five groups: therophytes (34 species = 76%), geophytes (4 species = 9%), hemicryptophytes (3 species = 7%). In addition to helophytes and nanophanerophytes, each represented by 2 species (4%), Fig. (3).

As shown in Fig. (4), the floristic elements were grouped into ten chorotypes, namely; Mediterranean (28 taxa), Irano-Turanian (20 taxa), Euro-Siberian (18 taxa), Cosmopolitan (8 taxa), Pantropical (3 taxa) and both of Sudano-Zamezian and Palaeotropical represented by two taxa. Also, there are two chorotypes, each includes a single taxon. Comparable plant communities have been described in the Nile Delta by Al Sodany (1992), Shaltout *et al.*, (2005) and Askar (2015).

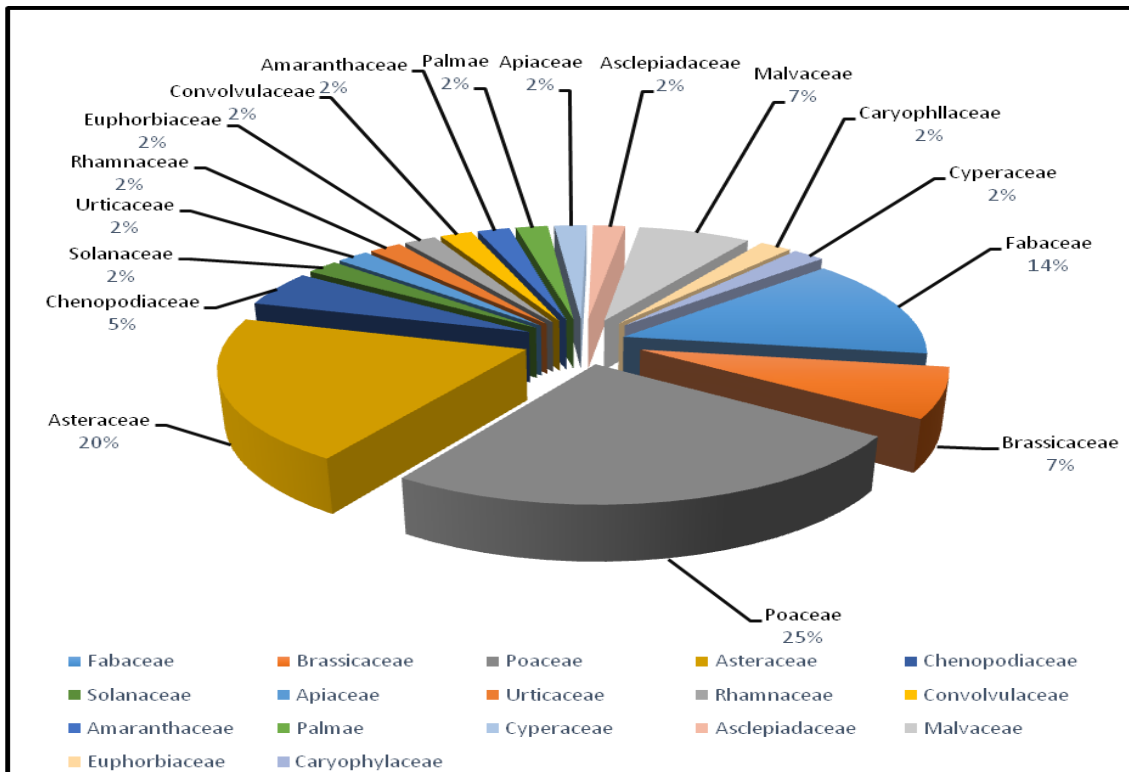


Fig. (1) : Plant families of the recorded species in *Cynanchum acutum* community type

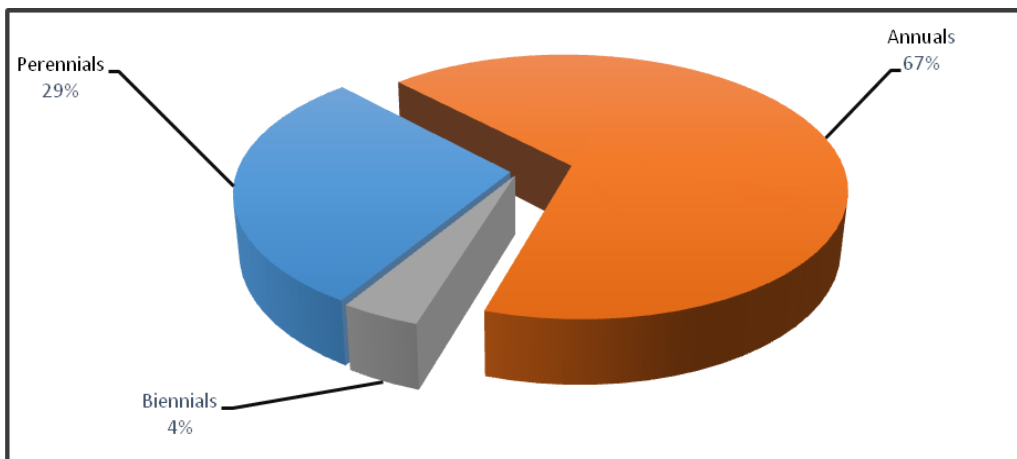


Fig. (2): Life Span of the recorded species in *Cynanchum acutum* community type.

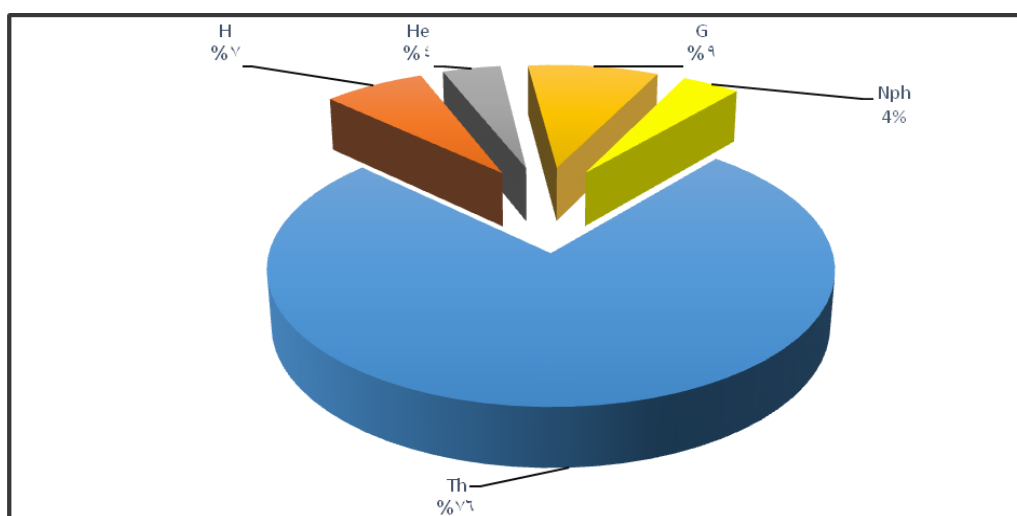


Fig. (3) Life form spectra of the recorded species in *Cynanchum acutum* community.

■ Th = Therophytes, ■ H = Hemicryptophytes, ■ He = Helophytes, ■ G = Geophytes, ■ Nph = Nanophanerophytes.

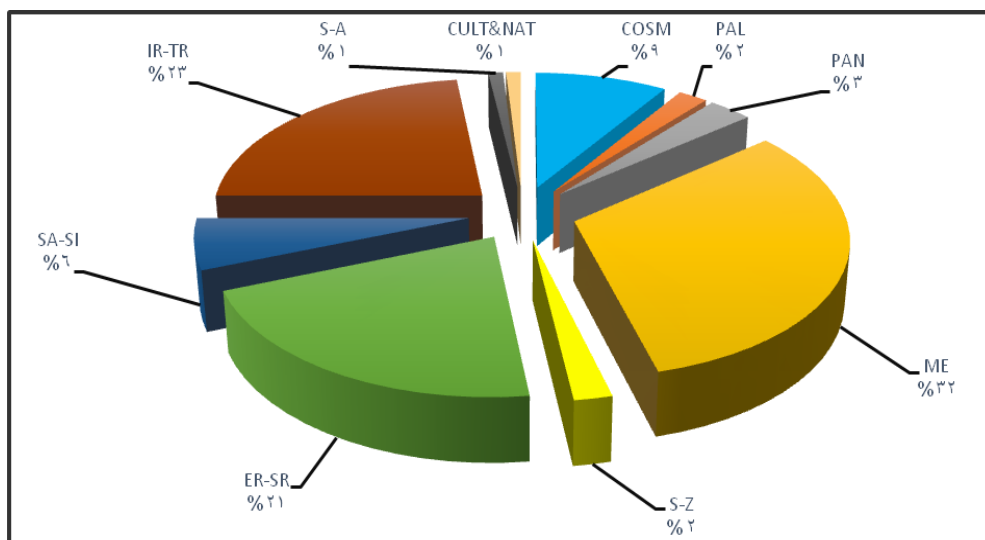


Fig. (4) Chorotypes of the recorded species in *Cynanchum acutum* community type.

■ COSM = Cosmopolitan, ■ PAL = Palaeotropical, ■ PAN = Pantropical, ■ ME = Mediterranean, ■ S-Z = Sudano-Zambeian, ■ ER-SR = Euro-Siberian, ■ SA-SI = Saharo-Sindian, ■ IR-TR = Irano-Turanian, ■ S-A = Saharo-Arabian, ■ CULT and NAT = Cultivated and Naturalized .

The soil supporting *C. acutum* was sandy textured with predominance of medium and fine sand, mean values = 32.7 and 64.8%, respectively. The percentage of silt attained highest value of 12.3%. The clay fraction ranged from 0.5 to 5.4%. Due to the high percentage of porosity (43.3 - 54.8%) and water-holding capacity (43.6 - 55.7%), moisture content was usually high (7.9 - 17.7%). Organic carbon content attained high value of 1.9%. Salinity level was low (0.1-0.8%) with electric conductivity of 145-575  $\mu$  mohs/cm. Most of soluble anions were sulphates. The soil reaction was alkaline (PH = 7.2 - 7.6). Consequently, *C. acutum* flourished in moist, sandy loam, non-saline, fertile and slightly alkaline soil (Table 1). It is intolerant to water stress and thrive in irrigated areas.

• **Biological aspects**

*Cynanchum acutum* is a prostrate or twining by supporting itself over other plant, perennial herb (Plate 1). It reproduced by seeds and suckers inserted from the non-tuberous tap root. Stem is herbaceous, has long internodes, cylindrical and sparsely covered by flexuous trichomes. Leaves are cordate-deltoid, exstipulate, and opposite-decussate. Sepals five, basally fused and companulate. Petals five rotate-infundibuliform, basally purple and apically rose. Stamens five, fused to form column, fit between the pink corona lobes. The superior ovary is bicarpellary. Follicle is long pod-like. Seeds are ovate, brown, with terminal tuft of silky hairs. This agrees with the findings of Thomas and Allen (1997).



Plate (1): A *Cynanchum* plant supporting itself by twining over other plant .Habitat conditions

• **Anatomy**

The stem is a typical dicot has epidermis of a single row of cells, do not possessed hairs, with the outer wall thickened and heavily cutinized. Cortex consists of hypodermis represented by 2-3 layered angular collenchyma cells, parenchyma zone of 4-5

layers containing chloroplasts and the inner layer of the cortex constitutes the endodermis which surrounds the stele. The vascular bundles are anomalous bicollateral and found in continuous ring. The pith is wide and formed of thin walled parenchyma cells having latex lysigenous ducts among them (Plate 2a).



**Table (1): Analysis of soil samples collected from twenty five representative stands of *Cynanchum acutum* Community . M.C.=moisture content, Por.=porosity, W.H.C.=water–holding capacity, Org. C=organic carbon, T.S.S=total soluble salts, and E.C.=electric conductivity.**

Samples No.	Physical characteristics							Chemical characteristics											
	Mechanical Analysis							M.C. %	Por. %	W.H.C. %	Org.C %	CaCO <sub>3</sub> %	Analysis of 1 : 5 water extract						pH
	Particles Size mm (%)												T.S.S %	E.C. μ mohns /Cm	Cl <sup>-</sup> %	SO <sub>4</sub> <sup>-</sup> %	CO <sub>3</sub> <sup>-</sup> %	HCO <sub>3</sub> <sup>-</sup> %	
	>2.057	2.057-1.003	1.003-0.500	0.500-0.211	0.211-0.104	0.104-0.053	<0.053												
1	0.10	8.49	21.76	34.79	29.43	2.88	2.59	10.7	54.8	44.0	0.9	0.5	0.2	253	0.06	0.25	0.0	0.09	7.6
2	0.10	5.95	23.49	34.72	27.08	6.57	2.07	17.6	53.1	47.1	1.2	2.0	0.3	145	0.05	0.41	0.0	0.06	7.2
3	0.00	4.03	22.05	35.46	33.42	4.54	0.56	7.9	53.3	44.8	0.6	1.5	0.3	180	0.06	0.37	0.0	0.06	7.2
4	0.10	10.18	20.36	33.01	31.37	2.75	2.16	8.6	52.2	44.6	0.9	3.5	0.2	165	0.09	0.25	0.0	0.06	7.3
5	0.02	3.04	18.66	33.27	36.12	4.63	4.35	9.5	53.9	43.6	0.5	5.5	0.3	168	0.06	0.41	0.0	0.03	7.3
6	0.00	1.50	22.70	24.51	43.40	4.78	2.45	8.5	52.5	45.3	0.9	0.5	0.2	188	0.06	0.21	0.0	0.03	7.5
7	0.00	6.92	20.50	18.87	46.60	4.03	2.99	8.0	52.2	45.5	1.4	1.5	0.1	180	0.05	0.21	0.0	0.06	7.4
8	0.00	5.90	21.93	21.22	40.29	5.98	2.90	11.0	52.4	46.3	0.3	3.5	0.1	260	0.06	0.61	0.0	0.06	7.5
9	0.00	5.05	21.03	33.40	31.42	6.50	2.61	8.9	52.4	52.3	1.2	2.5	0.5	325	0.07	0.20	0.0	0.09	7.2
10	0.08	6.96	20.69	34.52	31.15	4.50	2.08	8.6	53.3	49.6	1.3	5.5	0.4	337	0.04	0.20	0.0	0.12	7.6
11	0.10	7.22	21.12	34.75	29.75	4.72	2.33	14.2	54.0	45.6	1.1	1.2	0.2	199	0.05	0.33	0.0	0.08	7.4
12	0.05	7.10	26.20	34.23	27.39	3.64	1.36	8.3	52.8	44.7	0.8	2.5	0.2	172	0.07	0.31	0.0	0.06	7.2
13	0.01	2.27	24.18	25.39	39.76	4.70	3.40	9.0	53.2	44.5	0.7	3.0	0.2	178	0.06	0.26	0.0	0.03	7.4
14	0.00	6.41	20.21	24.04	40.44	5.00	2.94	9.5	52.3	45.9	0.9	2.5	0.1	220	0.05	0.14	0.0	0.06	7.4
15	0.04	6.00	20.86	33.96	31.28	5.50	2.34	8.7	52.9	51.0	1.3	4.0	0.4	331	0.05	0.20	0.0	0.11	7.4
16	0.00	2.31	13.50	42.40	27.35	11.50	2.85	17.7	50.1	51.4	1.7	5.5	0.6	341	0.12	0.16	0.0	0.21	7.4
17	0.10	1.38	15.86	37.30	31.33	8.60	4.96	10.2	46.9	47.9	1.1	6.5	0.7	376	0.13	0.29	0.0	0.15	7.3
18	0.70	2.05	22.18	33.50	29.15	9.89	2.35	9.4	50.6	48.6	1.2	4.6	0.8	498	0.13	0.21	0.0	0.18	7.4
19	1.20	2.10	28.64	30.10	28.44	7.42	1.92	9.1	42.6	55.7	1.0	4.5	0.7	417	0.14	0.21	0.0	0.15	7.5
20	0.00	1.30	11.95	38.20	30.34	12.37	5.49	10.5	51.2	50.9	1.4	5.2	0.3	552	0.08	0.08	0.0	0.24	7.4
21	0.60	1.07	19.45	37.10	29.01	10.55	2.15	11.2	44.2	53.7	1.3	4.5	0.4	575	0.10	0.16	0.0	0.21	7.3
22	0.00	2.10	26.87	31.20	28.13	9.47	2.10	10.9	51.6	55.3	1.8	9.5	0.3	416	0.15	0.21	0.0	0.18	7.4
23	1.02	2.51	22.61	43.23	23.87	4.59	2.07	16.6	43.3	52.7	1.6	8.5	0.5	347	0.16	0.08	0.0	0.18	7.5
24	0.30	2.07	21.58	35.90	32.16	5.39	2.51	8.4	52.6	54.8	1.9	7.3	0.6	216	0.16	0.04	0.0	0.18	7.4
25	1.20	3.01	29.43	33.70	24.22	5.71	2.18	12.0	48.2	49.7	1.4	6.4	0.5	196	0.13	0.15	0.0	0.19	7.3
Mean	0.23	4.28	21.51	32.75	32.12	6.25	2.63	10.6	51.1	48.6	1.1	4.1	0.4	289	0.09	0.24	0.0	0.11	7.4
S.E	0.39	2.63	4.00	5.93	5.84	2.67	1.04	2.9	3.4	3.9	0.4	2.4	0.2	12.4	0.04	0.12	0.0	0.06	0.1

The leaf has epiderms of conical shaped cells covered by thick cuticle. Stomata are of anomocytic type. Mesophyll is bifacial, clearly differentiated into palisade and spongy parenchyma. The vascular bundle of the midrib composed of lignified radiating xylem vessels in vertical rows and phloem. Also, small veins consists of few conducting cells are found (Plate 2b).

The root exhibits secondary thickening, as some herbaceous dicots produced small amount of secondary tissues (Plate 2c). It consists of periderm of few layers of cork cells, multi-layered cortex of thin-walled parenchyma cells having intercellular spaces, undulated cylinder of vascular tissues which composed of crushed primary phloem, centrifugal secondary phloem, meristematic strand of cambial cells, centripetal secondary xylem of wide vessels and abundant xylem parenchyma then inward primary xylem arches. The medulla forms the central portion. The anatomical features of *C. acutum* are similar to other Asclepiadaceae genera (Cim *et al.*, 2010).

**Phytochemical investigation**

The flavonoids content of *C. acutum* shoot was 0.313 g/100g. Previous investigations on *Cynanchum* have revealed the presence of four flavonoid glycosides (Heneidak *et al.*, 2006) as well as seven other flavonoids (Ghada *et al.*, 2008). The polyphenols content among the aerial parts of *C. acutum* was 0.375 g/100g. It has reviewed by Lou *et al.*, (1993) the presence of phenolic compounds in *Cynanchum*.

Ten fatty acids constituents of the lipoidal matter of *C. acutum* have been identified and evaluated. Myristic, lauric, palmitic, linolenic, tridecanoic and pentadecanoic were the major fatty acids (Table 2).

**Table (2): Antimicrobial activity of *C. acutum* methanolic extract.**

Tested micro-organisms	Methanolic extract
<i>Bacillus Subtilis</i>	+ve 9mm
<i>Streptococcus pyrogensis</i>	+ve 7mm
<i>Staphylococcus epidermis</i>	-ve
<i>Erwinaea rotovara</i>	-ve
<i>Klebsiella pneumonia</i>	-ve
<i>E. coli</i>	-ve
<i>Candida (fungus)</i>	+ve 8mm

The DPPH free radical scavenging activity of *C. acutum* methanolic extracts is expressed as IC<sub>50</sub>. The *Cynanchum* extract exhibited radical scavenging activity by value of 0.186 mg ml<sup>-1</sup>. The extract of the natural antioxidant catechol attained the IC<sub>50</sub> value of 0.03 mg ml<sup>-1</sup>. The obtained result is in coherence with that reported by Miser-Salihoglu *et al.*, 2013. The antioxidant activity of plant extract could be attributed to the hydroxyl groups in phenolic compounds which have a significant role in antioxidant activity (Cai, *et al.*, 2004).

*Cynanchum acutum* showed antibacterial activity against *Bacillus subtilis* and *streptococcus pyrogensis*

with inhabitation zones of 9 and 7mm, respectively. Also, *C. acutum* extract exhibited antimicrobial potency against the fungal pathogen, *Candida* (Table 3).

**Table (3): GLC analysis of fatty acids of *Cynanchum acutum* shoot.**

Fatty acid	Conc. (g/100g)
Myristic acid	0.968
Lauric acid	0.847
Palmitic acid	0.827
Linolenic acid	0.786
Linoleic acid	0.439
Tridecanoic acid	0.323
Pentadecanoic acid	0.316
Oleic acid	0.232
Stearic acid	0.176
Undecanoic acid	0.197

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### دراسات بيئية للنباتات الطبية البرية بمصر. ٣- نبات المديد محمد السيد أبو زياده ، نور كاظم الكريشي و مها الشامي قسم النبات – كلية العلوم – جامعة المنصورة (مصر)

يهدف هذا البحث إلى إجراء دراسة بيئية تفصيلية لعشيرة نبات المديد التابع للفصيلة العشارية والظروف البيئية بمواقع عديدة في محافظة الدقهلية، ووصف السمات المورفولوجية والتشريحية للنبات، والتقدير الكمي للفلافونيدات والبولي فينولات، واستجلاء الأحماض الدهنية بالأجزاء الخضيرية وأيضاً الكشف عن كفاءة المستخلصات الكحولية للمجموع الخضري كمضادات أكسدة وكمضادات ميكروبية. أوضح التحليل الفلوري للكساء الخضري في عشيرة المديد وجود ٤٥ نوع نباتي تنتمي إلى ١٧ فصيلة، وكانت النيجليات والمركبات والبقوليات والصلبية والرمامية أكثر الفصائل تواجداً. وصنفت النباتات إلى ٣٠ نوع حولي، ونباتين ثنائي الحول وثلاثة عشر نباتاً معمرأً. وكانت النباتات قصيرة الأجل أكثر صور شيوعا (٧٦%) يليها الأرضيات وشبه المختبئات، كما سجلت عشر مجموعات فلورية أهمها نباتات إقليم البحر المتوسط وإقليم إيران والأناضول وإقليم أوروبا وسببيرا. خلصت الدراسة أن المحتوى المائي والقوام والأملاح الكلية الذائبة والرقم الأيروجيني أهم خصائص التربة التي تتحكم في ظهور وانتشار نبات المديد بالإضافة إلى عناصر المناخ خاصة درجة الحرارة والأمطار.

تبين أن نبات المديد يحتوي على الفلافونيدات (٠.٣١٣ جرام/ ١٠٠ جرام) والبولي فينولات (٠.٣٧٥ جرام/ ١٠٠ جرام) كما يحتوي على عشرة أحماض دهنية منها حمض الميريستيك، واللوريك، والبالمتيك، واللينوليك. وكذلك سجل مستخلص نبات المديد نشاطا كمضاد للأكسدة، وكان للمستخلص الميثانولي تأثيرا مثبطا لبعض السلاسل البكتيرية والفطرية.