CHEMOTAXONOMIC STUDY OF THREE MEMBERS OF THE
FAMILY ZYGOPHYLLACEAE

By:

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fulfillment of the requirements for the Degree of B.Sc. (Hons)

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Dedication

To...
My parents
My brothers and sisters
My all friends
ACKNOWLEDGEMENTS

First of all, Thanks for Allah for give me the power and willing to complete this study. After that I would like to express my sincerest gratitude to my supervisor, Dr. Ikram Madani Ahmed, for her help and advice during the supervision of this work. And I would like also to extend my thanks to ustaz Mohamed Sharaf Eldeen.
ABSTRACT

In this research three species belonging to two different genera of the family Zygophyllaceae were selected for chemotaxonomic study. These are: Tribulus terrestris, Tribulus cistoides, and Fagonia cretica in an attempt to demonstrate the possible similarities and differences between them on the basis of their proximate and phytochemical content. In the proximate content investigation, reducing sugars, amino acids, starch, and lipids were qualitatively analyzed. And for phytochemical contents, phenolic compounds were separated using thin layer chromatography (TLC).

The results of the proximate analysis showed that the three species are closely related while the phenolic content analysis which was subjected to statistical analysis (paired affinity PA, isolation value IV, and grouped affinity GA) showed that Tribulus terrestris and Fagonia cretica are the only possibly related species.
الملخص

هدف البحث القيام بدراسة تصنيفية كيميائية لثلاثة أنواع من النباتات ذوات الفلفتين تابعة لجنسين مختلفين من العائلة الرطيطية وهي: Zygophyllaceae و Tribulus cistoides و Tribulus terstris.

وذلك لتحديد التشابهات والاختلافات بينهما اعتمادًا على محتواها الكيميائي النوعي من بعض المواد البنائية الأولية وهي السكريات المختزلة و الامينات الإيضية و النشا و المحتوى الدهني.

أيضا تم القيام بتحليل كمي للمواد الفينولية باستخدام كروماتوغرافيا الطبقة الواقية. البيانات الكيميائية التي تم جمعها من دراسة المواد البنائية الأولية أوضحت درجة التقارب الكمي بين الأنواع الثلاثة. أوضحت نتائج فصل المواد الفينولية للأنواع الثلاثة لتحليل إحصائي وذلك بحساب درجة القربي و الإبعاد بينها. نتائج هذا التحليل اشارت للعلاقة الواضحة بين نوع Fagonia cretica و نوع Tribulus terstris.
CHAPTER ONE
1. INTRODUCTION AND LITERATURE REVIEW

Chemical plant taxonomy or chemotaxonomy of plants, as defined by Hegnauer, is a scientific investigation of the potentialities of chemical characters for the study of problems of plant taxonomy and phylogeny (Hegnauer, 1967). Evidence from chemical constituents has already led to the reconsideration of many plant taxa. For example, a number of taxonomically difficult families have been successfully grouped on the basis of their secondary metabolite profiles. The Bonnetiaceae, which consists of the two genera Bonnetia and Archytaceae, is in fact better associated with the Guttiferae than with the Theaceae as the presence of xanthones (Waterman, 1998). Placement of the Bretschneideraceae in the Capparales rather than in the Sapindales is supported by the occurrence of glucosinolates (Waterman, 1998). At lower taxonomic levels, several metabolites have proved useful in establishing taxonomic relationships. The distribution of indole, and carbazole alkaloids, 8-prenylated coumarins and monoterpenes or sesquiterpene dominated volatile oils, have been combined to confirm the division of the genus Murraya (Rutaceae) into two taxa (Waterman, 1998).

Zygophyllaceae, is a family of approximately 285 species subdivided into five subfamilies and about 27 genera (Sheahan and Chase, 1996, 2000). They consist of trees, shrubs and herbs mostly restricted to arid and semiarid areas in the tropics and subtropics. In Sudan Andrews (1956) reported twelve members of the Zygophyllaceae belonging to four different genera: Fagonia (F. cretica L.), Seetzenia (S. orientalis Decne), Tribulus (T. terrestris L., T. Pentandrus Forsk., T. longipetalus Viv, T. mollis Ehrenb. Ex, Schweif., T. pterocarpus Ehrenb.ex Koern, T. macropetrus Boiss)
Zygophyllum (Z. Simplex L., Z. Album L. f., Z. Coccinum L. and Z. Decumbens Del.) Most of the Sudanese species assigned to Zygophyllaceae are distributed in Northern and Central Sudan; some are restricted to the Red Sea district, others are widely distributed (Andrews, 1956).

*Tribulus* species are used in the folk medicine in many countries against various diseases such as cardiac, edema, eye trouble, skin disorders, urinary troubles and stones in the bladder, as a diuretic, aphrodisiac and anti-tumoural (Tomova et al., 1981; Shi and Kuo, 1999; Xie and Huang, 1988; and Chin et al., 2000). Among the constituents that contribute to its biological activity are steroid saponin glycosides, isoflavonoids as well as other polyphenolic compounds such as flavonols and phenolic acids, essential oils and polysaccharides (Ganzera et al., 2001). Also *Fagonia* species are reported as medicinal plants used in the indigenous system for the treatment of cancer, fever, asthma, urinary discharges, toothache, stomach troubles and kidney diseases. (Ahsan et al., 2007 and Satpute et al., 2009). Species of *Fagonia* have been found to contain saponins (Abdel-Khalik et al., 2001), alkaloids (Sharawy and Alshammari, 2009), terpenoids (Perrone et al., 2007), sterols (Shoeb et al., 1994), flavonoids (Ibrahim et al., 2008), proteins and amino acids (Sharma et al., 2010).

**Aim**

The aim of this study is to determine the chemotaxonomic variations between two members of *Tribulus* L. and *Fagonia cretica* L. of the family Zygophyllaceae on the basis of their proximate and phenolics composition.
CHAPTER TWO

2. RESULTS

2.1 Plant materials
The plant species used in this study were *Tribulus terrestris*, *Tribulus cistoides*, and *Fagonia cretica*. Leaves were used to conduct the proximate analysis while fruits were used to screen for flavonoids contents.

2.2. Methods

2.2.1. Proximate analysis
The presence of reducing sugars, amino acids, starch, and lipids in the leaves of the studied species was examined using Benedict, ninhydrin, iodine, and Sudan III reagents respectively. To three mL of water extract of the leaves three mL of Benedict, ninhydrin reagents and two drops of iodine, and Sudan III were added separately and colour change was observed.

2.2.2. Thin layer chromatography for Flavonoids:
Sample (5 g) was mixed with 50% solution of petroleum ether and aqueous methanol. It was kept for 24 h in order to get phenolic extract. The extract was evaporated to yield a sticky residue. Using a capillary tube, 3 drops of plant extract were placed on each spot at the starting point of TLC plate so that they line up with the notches etched for each plant species. The developing liquid which is a mixture of toluene, chloroform, and acetone (30:150:20) was prepared. Enough volume of the developing liquid was poured in a jar so as to reach 1 cm deep in the bottom and a piece of filter paper was placed into the jar to saturate the atmosphere with the solvent. Then the plate was dipped carefully in the developing solvent and the chromatogram was allowed to develop.
Plates were removed from the chamber and the highest solvent level on them was traced with pencil. Also the spots were traced while held under a UV lamp. Then the spots were outlined with a pencil. The $R_f$ values were then calculated for each spot.

**Calculations:**

Ratio of fronts ($R_f$) values for each spot were calculated according to the equation:

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$

2.2.3. **Paired affinity, grouped affinity, and isolation value**

To make suitable comparisons between the two species under investigation, values of paired affinity (PA) and isolation value (IV) were calculated according to Ellison *et al.* (1962) as follows:

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

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

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

3.1. Taxonomy and morphological descriptions:
Morphological descriptions of each species selected for the present study is given below:

1- Tribulus terrestris L.
Taprooted, herbaceous perennial plant that grows as a summer annual in colder climates. The stems radiate from the crown to a diameter of about 10 cm to over 1 m, often branching. They are usually prostrate, forming flat patches, though they may grow more upwards in shade or among taller plants. The leaves are pinnately compound with leaflets less than 6 mm long. The flowers are 4–10 mm wide, with five lemon-yellow petals. A week after each flower blooms, it is followed by a fruit that easily falls apart into four or five single-seeded nutlets. The nutlets are hard and bear two to three sharp spines, 10 mm long and 4–6 mm broad.

2- Tribulus cistoides L.
Closely resembling T. terrestris in general appearance, but leaves paripinnate, leaflets about 8 pairs, oblong; silky-pubescent, about 10 mm long; stipules falcate acuminate; flowers solitary, yellow, sepals 5, silky; petals 5, obovate; stamens 10, on base of annular 10-lobed disc, 5 longer ones opposite the petals, 5 shorter ones each with a dorsal gland; ovary sessile, hirsute, style short, stigmas 5; fruit of horned woody cocci, tuberculate, pubescent, partitioned within, compartments 1-seeded.

3- Fagonia cretica L.
F. cretica is widely distributed in Northern and Central Sudan. Diffuse woody spiny annual desert herb, puberulous-glandular or nearly glabrous.
Leaves opposite, very variable, 1-3-foliolate; leaflets bright-green, linear to lanceolate, acute at the apex with a callous tip, up to 18 mm. long; stipules spinescent, often longer than the leaves, very acute at the apex. Flowers solitary, axillary. Petals rose or lilac, more than twice as long as the sepals, narrowly clawed. Fruit about 6 mm long.

3.2. Chemotaxonomy:

3.2.1 Proximate analysis:

The studied species are qualitatively similar in their proximate contents. The results presented in Plates 1-4 show positive tests conducted for reducing sugars, amino acids, and lipids while simple test for the presence of starch shows negative results also for all of the species.
Plate 1: Reducing sugar test for 1: *Tribulus terrestris* 2: *Tribulus cistoides* and 3: *Fagonia cretica* fruits
Plate 2: Amino acids test for 1: *Tribulus terrestris* 2: *Tribulus cistoides* and 3: *Fagoni cretica* fruits

Plate 3: Starch test for 1: *Tribulus terrestris* 2: *Tribulus cistoides* and 3: *Fagoni cretica* fruits
Plate 4: Lipids test for 1: *Tribulus terrestris* 2: *Tribulus cistoides* and 3: *Fagoni cretica* fruits
3.2.2. Distribution of Flavonoids:

The relative distribution of all spots and their retention factor (Rf) in the fruits of the studied species separated by thin layer chromatography are shown in table 1. As shown in Table 1 the studied species are varied in their flavonoid contents analysed for the fruits. Two spots are shown for each species. No common spots are detected between the three species. Only one common spot of retention factor (Rf) value 0.83 is recorded between *Tribulus terristis* and *Fagonia cretica*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Retention factor (Rf)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tribulus terristis</em></td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>0.83</td>
</tr>
<tr>
<td><em>Tribulus cistoides</em></td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td><em>Fagonia cretica</em></td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>0.83</td>
</tr>
</tbody>
</table>

3.2.3. Paired affinity, grouped affinity, and isolation value

Paired affinity (PA) and grouped affinity (GA) based on phenolic compounds separated on TLC for the studied species are shown in Table 2. The maximum paired affinity PA and grouped affinity GA reported during the present study were 25% and 225% respectively and the minimum paired affinity PA and grouped affinity GA recorded were 0% and 200%.
respectively. The theoretical minima and maxima are 0% and 100% for PA respectively and 100% and 300% for GA respectively.

Table 2 Paired affinity (PA), grouped affinity (GA) based on phenolic compounds separated on TLC for the studied species

<table>
<thead>
<tr>
<th>Species</th>
<th>Paired Affinity</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T. terristris</td>
<td>T. cistoides</td>
<td>F.cretica</td>
</tr>
<tr>
<td>T. terristris</td>
<td>100</td>
<td>0</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>T. cistoides</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>F.cretica</td>
<td>25</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER FOUR
4. DISCUSSION

The flowering plant family zygophyllaceae has been subjected to considerable revisions during the period (1896-2009). Based mainly on morphological data the family zygophyllaceae was divided into seven subfamilies: Nitrarioideae, Peganoideae, Tetradiclidoideae, Morkillioideae, Balanitoideae, Zygophyloideae and Augeoideae (Engler, 1896a). Recent molecular work has led to a new revision to this family which is the inclusion of Augeoideae into Zygophyloideae and Balanites, the sole representative of the Englers Balanitaceae in the tribuloids group in spite of many morphological and anatomical autapomorphies (Sheahan and Chase 1996,2000).

Few works on chemical investigations have been done supporting the recent classifications. In this research since Analysis of the proximate contents selected for this study reflected 100% similarity between the three species, it is of high taxonomic value to use these contents as characters in the family level.

The analysis of the separation of the phenolic compounds on thin layer chromatography resulted in many spots of different $R_f$ values. None of them was reported as common between all of them. According to Ellison et al. (1962) PA and GA values of 50% and above are considered as marker of close relationship. In this regard none of our species is closely related. This indicates that phenolic contents is of low taxonomic value to the species level since tribulus species are already from the same genus and applying another comparative quantitative method is needed.
5. REFERENCES


