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Preliminary phytochemical screening of *Zygophyllum fabago*

The amino acid profiles and fatty acid composition of *Zygophyllum fabago* were studied to evaluate the nutritional value of this species. The most abundant essential amino acid and non-essential amino acid were leucine (4.75 *μg/g)* and glutamate (23.42 *μg/g)*, respectively. Oleic acid, a monounsaturated fatty acid, was the major component in *Zygophyllum fabago* (77.1% of total fatty acids), and the second highest component of fatty acids was linoleic acid (12.8%). The amount of MUFA (monounsaturated fatty acid) and PUFA (polyunsaturated fatty acid) was 78.0% and 13.1% of the total fatty acid content, respectively.
Keywords: Zygophyllum fabago, amino acids, fatty acids, phytochemical screening

Introduction

Zygophyllaceae are an ancient family found in arid, semiarid and saline deserts throughout the world comprising 22 genera and 230–240 species in hot dry regions of Europe, Asia, Australia, Africa and the Americas. Engler (1896a, 1931) divided the family into seven subfamilies (as well as a number of tribes and subtribes), three of which (*Nitrarioideae*, *Peganoideae* and *Tetradiclidoideae*) are now excluded. The remaining four were *Morkillioideae* (as *Chitonioideae*), *Balanitoideae*, *Zygophylloideae* and *Augeoideae* (although subsequently Engler had second thoughts about *Augeoideae*, considering that its sole representative Augea should be included in *Zygophylloideae*; Engler 1896b). The *Zygophyllum* species from Australia and southern Africa are assigned to a new genus Roepera; and two distinctive species of *Zygophyllum* found only in eastern Africa are assigned to a new genus *Melocarpum*. The remaining species of *Zygophyllum* sensu stricto, found mainly in Asia, are retained in *Zygophyllum*.

Phytochemistry: (Information taken mainly from Hegnauer 1973, 1990.) Phenolic compounds including methylated flavonoids and lignans are frequent in the family. Lignans and neolignans abound both in terms of different compounds and quantity.

Zygophyllaceae are among the relatively few families which produce steroid and triterpenoid saponins. According to Hegnauer (1990), these may also be responsible for the observed resistance to herbivore activity. The family also produces the quinazoline alkaloids Harman (e.g. in *Fagonia cretica* and *Tribulus terrestris*), harmin and harmol (in *Zygophyllum fabago*). Mucilage has been found in the leaf epidermis of *Augea* and is reported in the epidermis of *Plectrocarpa* (Castro 1981); they may also be abundant in seeds, for example, in *Augea* and species of *Zygophyllum*. Calcium oxalate crystals are frequent, sometimes very abundant; these are mostly druses but styloid, prismatic and acicular crystals also occur.

Economic Importance: Some of the South American species have been used for their timber, notably *Guaiacum* that has extremely strong, hard, resinous wood. The resin of *G. sanctum* (known as *Lignum vitae*) was also used as a treat-

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ment for rheumatism, gout and liver disorders as well as syphilis. The timber of the related genera *Bulnesia* and *Porlieria* has also been of economic importance, and polishing waxes are made from *Bulnesia* resins. The resin on *Larrea* leaves is a powerful antioxidant and has been used as a source of antiseptic, although excessive internal use can lead to liver damage. Many members of the family are poisonous to livestock, but *Augea* seeds are reported to have high protein content and are eaten by sheep. The bark of *Balanites* was used medicinally; the fruits have a high nutritional value and areal soused for soap. The buds of some species of *Zygophyllum* and *Larrea* have been used as a caper substitute, hence the name 'bean caper' which is sometimes applied to the family.

Zygophyllum L. are glabrous or pubescent spreading shrubs, sub- shrubs and herbs, rarely annuals, with fleshy articulate stems; stipules sometimes spinescent. Leaves opposite, sessile or petiolate, usually bifoliolate, rarely simple or multifoliolate, with cylindrical, fleshy or somewhat flattened lamina. Flowers yellow, cream or white, often with reddish basal spot, axillary or terminal, solitary or in pairs or few-flowered cymes. Up to 100 species, growing in warm dry regions of Africa, Asia and Australia [1].

Material and Methods

Plant material: *Zygophyllum fabago* was collected at Almaty region (Kazakhstan) in May 2012 and identified by Botanist N. G. Gemedzhieva, Head of Laboratory of Plant Resources, Sc.D. of Biology, Institute of Botany and Phytointroduction. The air dried aerial parts of *Zygophyllum fabago* (100g) was cut into small pieces and extracted by hexane (solvent extraction method). Evaporation of the hexane under reduced pressure afforded an oily residue (0.45g).

Moisture: Shredded fresh vegetable (l0g) was dried in a thermostatically controlled ventilated oven at 105°C until constant weight was obtained. The loss in weight was recorded as moisture content [2].

Ash: For the determination of ash content, dried pulverized vegetable was ashed at 550°C in a muffle furnace [2]. The data for moisture and ash contents are presented in table 1

Table 1 – Moisture and ash content of the aerial parts of *Zygophyllum fabago*

Part used	Moisture and ash content of the plant %	
	Moisture content	Ash
Aerial parts	8.26	5.43

Minerals: Minerals were analyzed by ashing 2.0g dried and ground sample in a muffle furnace at 550°C. The ash was analyzed for macro and microelements by atomic absorption spectrophotometer. The data presented in Table 2

Table 2 – Micro and macro elemental analysis of the aerial parts of *Zygophyllum fabago µg/ml*

Macro and Micro-elements											
	Ni	Mn	Pb	Cd	Cu	Zn	Fe	Mg	Ca	Na	K
Amount, µg/ml	0.0077	0.0734	0.0142	0.0026	0.0288	0.0402	0.2549	22.0580	33.7028	174.1740	72.7256

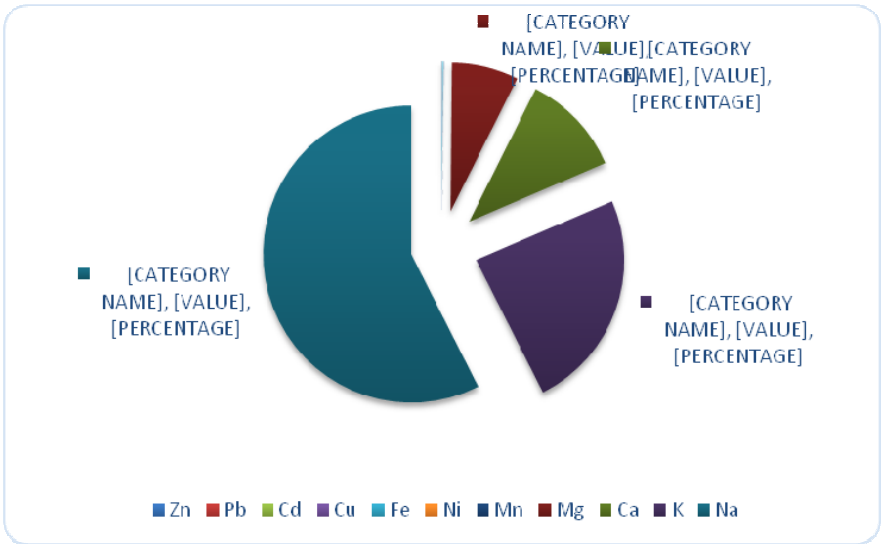

Figure 1 – Diagram of macro-and micronutrient contents of *Zygophyllum fabago*

Phytochemical screening: the air dried aerial parts of the plant were crushed into powdered form and subjected to qualitative phytochemical tests by paper chromatography using authentic reference samples and BAW (40:12.5:29) solvent system and the chromatogram sprayed with different specific reagents for determination of the different groups of naturally occurring compounds according to the reported methods [3-6].

Phenolic acids: were also tested by paper chromatography using authentic reference samples and (Benzen : Acetic acid : Water 6:7:3) solvent system and Diazotized *P*-nitroaniline as detecting reagent [3].

Fatty acid Analysis:

The composition of the saturated and unsaturated carboxylic acids (fatty acids) in plants is determined by gas-liquid chromatography apparatus «CARLO-ERBA-420" using helium as a carrier gas, flame ionization detector, carrier gas velocity 30 ml/min, detector temperature 188˚C, oven temperature 230˚C, adsorbent Cellite 545 on Chromosorb WAW. The chloroform extract of plant species is added to 10 ml of methanol and 2-3 drops of acetyl chloride and then carried out methylation at 60-70˚C in a special system for 30 minutes. Methanol was removed using a rotary evaporator, and the samples are extracted with 5 mL of hexane and analyzed by gas chromatography for 1 hour [7]. The data are presented in Table 4

Amino acids Analysis:

Analysis of amino acids was carried out chromatographically using helium as carrier gas, flame ionization detector 300˚C and condenser temperature 250˚C on Chromosorb WAW. Aqueous extract of the plant was hydrolyzed in HCl for 24 hours. The resulting hydrolyzate was evaporated to dryness in a rotary evaporator at 40˚C, after centrifugation at 2.5 thousand revolutions per minute the resulting precipitate was dissolved in sulfosalicylic acid and amino acids are eluted through an ion exchange column Dausk-50. On freshly obtained elutes 2, 2-dimethoxypropane and propanol saturated with HCl were added. The resulting mixture is heated at 110 ˚C for 20 minutes, then addition of a freshly prepared acylating reagent (1 volume of acetic anhydride and 2 volumes of triethylamine and 5 volumes of acetone), evaporation of the sample to dryness, addition of ethyl acetate and saturated aqueous solution of NaCl. Finally, the ethyl acetate layer is analyzed on the amino acid analyzer (Carlo-Erba) [8]. The data are presented in Table 5.

Identification of the compounds: Components were identified by comparing with reference standard materials while the percent composition was calculated from the peak areas without using correction factors.

Table 4 – Composition of the saturated and unsaturated carboxylic acids (fatty acids) in *Zygophyllum fabago*

Fatty acids	No. of carbon atoms	Relative percentage %	Peak No
Myristic	$C_{14:0}$	1.1	1
Pentadecanoic	$C_{15:0}$	0.4	2
Palmetic	$C_{16:0}$	4.9	3
Palmetoleic	$C_{16:1}$	0.9	4
Stearic	$C_{18:0}$	2.5	5
Oleic	$C_{18:1}$	77.1	6
Linoleic	$C_{18:2}$	12.8	7
Linolenic	$C_{18:3}$	0.3	8
Total saturated (TSF)		8.9	
Monounsaturated (MUFA)		78.0	
Polyunsaturated (PUFA)		13.1	

Table 5 – Composition of the Amino acids in *Zygophyllum fabago*

Peak No.	Amino acids	Content *µg/g*	Peak No.	Amino acids	Content *µg/g*
1	Tryptophan	1.27	11	Serine	4.28
2	Lysine	3.12	12	Methionine	1.32
3	Arginine	5.18	13	Proline	7.68
4	Ornithine	0.04	14	Threonine	2.45
5	Histidine	3.20	15	Glutamic acid	23.42
6	Tyrosine	3.32	16	Valine	2.56
7	Phenyl alanine	3.05	17	Isoleucine	3.58
8	Oxyproline	0.03	18	Leucine	4.75
9	Cysteine	0.38	19	Glycine	3.48
10	Aspartic acid	12.86	20	Alanine	10.54
Total non-essential amino acids (NEFAs)			75.68		
Total essential amino acids (EFAs)			20.83		
Total amino acids			96.51		

Results and discussion

The above results showed low moisture content (8.26%) of the aerial parts of *Zygophyllum fabago* that indicate that the possibility of microbial growth is fair. The elemental analysis showed the presence of eleven macro and microelements with the largest amount of macroelements K, Na, Mg, Ca and Fe. Potassium is essential in the maintenance of cellular water balance, pH regulation in the body and it is associated with protein and carbohydrate metabolism. The high potassium content could be utilized for the management of hypertension and other cardiovascular conditions [9].

The study also demonstrated the percentage of total extract of the powdered dry raw materials of the aerial parts of the plant with different solvent system (70% ethanol, 50% ethanol and pure ethanol) with the maximum yield (43.71%) with 50% ethanol, moderate with 70% ethanol (33.68%) and the lower extractive yield with pure ethanol (21.58%).

The phytochemical screening of the different ethanoloic (70%, 50%) and ethyl acetae extracts of *Zygophyllum fabago* by using method of paper chromatography with different specific reagents which characteristics for different groups of compounds like NH_3, Diazotized *P*-nitroaniline +15% Na_2CO_3, Ferric ammonium alum and comparing the R_f value in different solvent systems (BAW) and Acetic 25%

acid with authentic samples revealed the presence of flavonoids, flavonoid glycosides, phenolic acids and terpenoids in 70% ethanol extract and flavonoids and carbohydrates in 50% ethanol extract, while in the ethyl acetate extract found flavonoids, flavonoid glycosides and phenolic acids. Also the screening revealed the presence of *p*-coumaric acid, vanillic acid and ferulic acid which identified by authentic samples using paper chromatography with a total content of organic acids which represent 7.96% of the total extract.

Analysis of the fatty and amino acids constituents of the aerial parts of the *Zygophyllum fabago* revealed the presence of eight saturated (8.9%), monounsaturated (78.0%) and polyunsaturated (13.1%) known fatty acids and twenty amino acids. The major fatty acids were oleic (77.1 %), linoleic (12.8 %), palmetic (4.9 %), stearic (2.5 %) and myristic (1.1 %) acids, while the most abundant component of amino acids were glutamic acid (23.42 *μg/g*), aspartic acid (12.86 *μg/g*), alanine (10.54 *μg/g*), proline (7.68 *μg/g*), arginine (5.18 *μg/g*), leucine (4.75 *μg/g*) and serine (4.28 *μg/g*).

Fatty acids, especially essential fatty acids (EFAs), are of vital significance for human beings. The role of EFAs such as linoleic (18:2ω6) and γ-linolenic (18:3ω6) acids obtained from various oils (mainly evening primrose, borage, and black currant oils) in the diet is crucial. It has also been suggested that consumption of a diet enriched in EFAs confers beneficial health effects such as a protective effect on the development of cardiovascular diseases, inflammatory symptoms (rheumatoid arthritis and ulcerative colitis), atopic dermatitis, psoriasis and malignant diseases [10]. They are the constituents of all plant cells, where they function as membrane components, storage products, metabolites, and as a source of energy [11]. They are also important nutrient substances and metabolites in living organisms [12].

Plant proteins can serve as a complete and well-balanced source of amino acids for meeting human physiological requirements, also on a global basis; plants provide 65% of the world supply of edible protein. The cereal grains, in particular, account for a substantial portion of the world`s food protein and energy. On the other hand, animal products contribute 35% of the per capita availability of food protein. However, there are marked discrepancies in per capita protein supplies from animal and protein sources between the developed and developing regions[13].

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