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# Phytochemical study of *Kochia prostrata*

**Abstract:** In this study, complete comparative analysis of the component composition of plant of the genus *Kochia prostrata* were conducted for the first time. The data for quantitative determination of biologically active compounds and the study of amino, fatty and phenolic acids composition of plant of the genus *Kochia prostrata* family *Chenopodiaceae* were presented. 20 amino acids, 8 known fatty acids and 4 phenolic acids have been identified in the studied objects; their quantitative composition has been established. The major amino acids were glutamic acid (23.46%), aspartic acid (1.88%) and alanine (5.82%), while the major fatty acids were oleic (64.9%) and linoleic (22.0%), palmetic (5.3%) acids. Moreover, a sufficient amount of protocatechuic, vanillic, isovanillic and p-coumaric acids have been found.
**Key words:** *Kochia prostrata*, amino acids, fatty acids, phenolic acids.

## Introduction

*Kochia prostrata* Schard belongs to the family *Chenopodiaceae*, a family comprising of probably about 100 genera and 1400 species. It mostly comprises perennial herbs or shrubs mostly xerophytic or halophytic.

The genus *Kochia* is similar to *Bassia* but flowers are not hidden in hairs. *Kochia* is found in arid areas, deserts, and costal and saline habitats of Central Asia, North and South Africa, Europe, Russia. There are 10 species of the plant genus *Kochia*; 9 of these are indigenous to Kazakhstan [1,2].

The genus *Kochia* has been used in traditional Chinese medicine to treat diuresis and skin diseases. This is also used for making brooms. It has also been used for the treatment of pain in micturition, rubella, eczema and cutaneous pruritus in traditional Chinese preparations. The cardiotonic and diuretic activities of the plant have already been reported [3-6].

One of the important tasks of modern pharmaceutical science is the search for domestic sources of biologically active substances from plants to create on their basis of drugs of different pharmacological direction. These plants include the family *Chenopodiaceae.*

Object of current study is the aerial parts of *Kochia prostrata* Schard. The aim of our study was to investigate amino and fatty acids composition of aerial parts of *Kochia prostrata* Schard by gas chromatography. The isolation and identification of amino, fatty and phenolic acids composition from the aerial part of *Kochia prostrata* was the first ever to be reported from this plant.

## Materials and methods

*Analysis of amino acids.* To determine the amino acid in 1 g of substance, it hydrolyzed in 5 ml of six normal (N) hydrochloric acid at 105ºC for 24 hours in vials sealed under a stream of argon. The hydrolyzate evaporated to dryness three times on a rotary evaporator at a temperature of 40-50ºC and a pressure of one atmosphere. The resulting precipitate dissolved in 5 ml of sulfosalicylic acid. After centrifugation (1500 rev / min) for 5 minutes, the supernatant passed through a column of ion exchange resin Dowex 50, H-8, and 200-400 mesh, at a rate of 1 drop per second. Thereafter, the resin washed with 1-2 mL of deionized water and 2 ml of 0.5 N acetic acid; Resin washed to neutral pH deionized water.

Amino elution from the column passed there through 3 ml six N NH4OH solution at 2 drops per second. The eluate collected in a round bottom flask with deionized water used for washing the column to neutral pH. The flask contents evaporated to dryness on a rotary evaporator under a pressure of one atm. and a temperature of 40-50ºC.

After addition to the flask of 1 drop of a freshly prepared 1.5% solution of SnCl2, 1 drop of 2,2-di-

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methoxypropane and 1-2 ml of saturated hydrochloric acid-propanol, heated to 1100S, maintaining this temperature for 20 minutes and then the contents of the flask was evaporated again on a rotary evaporator.

In the next step in the flask, 1 ml of freshly prepared acylating reagent (1 volume of acetic anhydride, triethylamine, 2 volumes and 5 volumes of acetone) and heated at 600 ° C for 1.5-2 min. The sample re-evaporated to dryness on a rotary evaporator and added to the flask 2 mL of ethyl acetate and 1 ml of a saturated solution of NaCl. The flask contents mixed thoroughly and as two layers of liquids clearly formed – take the upper (ethyl acetate) for gas chromatography analysis, which conducted on gas-liquid chromatography «Carlo-Erba-4200» (Italy-USA).

The conditions of chromatography:

- Temperature of the flame ionization detector – $300^0$C
- Evaporator temperature – $250^0$C
- The initial temperature of the column – $110^0$C
- The final column temperature – $250^0$C
- Column temperature programming speed: from $110^0$C to $185^0$C – $60^0$C in the minutes; from $185^0$C to $250^0$C – $32^0$C min. When the temperature reaches $250^0$C column it should remain until the full release of all amino acids.

For the separation of amino acids a stainless steel column, size 400 mm 3 filled with a polar mixture of 0.31% Carbowax® 20M, 0.28% Silar® 5CP, and 0.06% on the *Lexan*® on *Chromosorb*® *W AW*, *120-140* mesh were used . Counting carried out on the chromatogram external standard of company Altex [7,8].

*Analysis of fatty acids*. Fatty acids were analyzed as methyl esters in in a Chrom-42 chromatograph using Cellite 545 adsorbent on WAW Chromosorb, He carrier gas, flame ionization detector, carrier gas flow rate 30 ml/min, detector temperature 188 ˚C and oven temperature 230 ˚C, Acids were methylated by NaOMe at 60–70°C [9].

*Analysis of phenolic acids*. The ethyl acetate extract of Kochia *prostrata* studied in phenolic content by paper chromatography in solvent systems:

I – benzene – acetic acid – water (6: 7: 3);

II – Sodium formate – formic acid – water (10: 1: 200) [10].

**Plant material**

The aerial parts of the plant were collected on September, 2015 from South Kazakhstan region of Republic of Kazakhstan. The plant material was taxonomically identified, authenticated by professors of botany at Institute of Botany and Phytointroduction, Almaty. The aerial parts of the plant were air dried, powdered to particle size in the range 6.0-8.0 mm, according to regulatory documents, sieved and weighed.

**Results and discussion**

The moisture content, total ash, qualitative and quantitative contents of biologically active constituents of *Kochia prostrata* were determined according to methods reported in the State Pharmacopoeia XI edition techniques [11].

The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, environment etc. The constituents of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant and is useful in determining authenticity and purity of sample and these values are important qualitative standards. The ash content is a measure of the total amount of minerals present within a plant, whereas the mineral contents are a measure of the amount of specific inorganic components present within it.

Moisture content is an important factor because appearance and stability of dried plants depends on the amount of water they contain and the propensity of microorganisms to grow depends on their water content.

The data quantitative determination of are shown in Table 1. From Table 1 it should be noted the predominance of saponins, tannins and flavonoids in *Kochia prostrata*.

The study revealed that *Kochia prostrata* contains 20 amino acids and 8 known fatty acids but differ in their quantitative contents (Tables 2 and 3). The major amino acids were glutamic acid (23.46%), aspartic acid (1.88%) and alanine (5.82%), while the major fatty acids were oleic (64.9%) and linoleic (22.0%), palmetic (5.3%) acids.

Eight phenolic acids were identified in *Kochia prostrata*. Of these, protocatechuic, vanillic, isovanillic and p-coumaric acids were determined by using authentic phenolic acid samples. These acids are identified for the first time from the plant genus *Kochia prostrata*.

Thus, the study of amino, fatty and phenolic acids composition of plant the genus *Kochia prostrata* is of great scientific and practical interest.

The results based for a deeper study of plants as a kind of source natural biologically active compounds.

**Table 1** – Qualitative and quantitative screening of the powdered aerial parts of *Kochia prostrata*

| Plant | Contents, % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Moisture content | Ash | Extractives substance 70% – aqueous alcohol | Flavonoids | Coumarins | Saponins | Alkaloids | Tannins | Organic acids |
| *Kochia prostrata* | 7.12 | 8.62 | 37.40 | 1.8 | 0.004 | 2.39 | 0.31 | 2.17 | 0.22 |

**Table 2 –** Composition of the saturated and unsaturated carboxylic acids (fatty acids) in *Kochia prostrata, %*

| Fatty acids | Relative percentage % | Fatty acids | Relative percentage % |
|---|---|---|---|
| $C_{14:0}$ | 1,5 | $C_{18:0}$ | 2,8 |
| $C_{15:0}$ | 2,1 | $C_{18:1}$ | 64,9 |
| $C_{16:0}$ | 5,3 | $C_{18:2}$ | 22,0 |
| $C_{16:1}$ | 1,0 | $C_{18:3}$ | 0,4 |

**Table 3 –** Composition of the amino acids contents in *Kochia prostrata, %*

| Amino acids | Relative percentage % | Amino acids | Relative percentage % |
|---|---|---|---|
| Tryptophan | 0.52 | Serine | 2.00 |
| Lysine | 2.70 | Methionine | 0.48 |
| Arginine | 3.32 | Proline | 3.35 |
| Ornithine | 0.01 | Threonine | 1.41 |
| Histidine | 1.65 | Glutamic acid | 23.46 |
| Tyrosine | 2.49 | Valine | 1.72 |
| Phenyl alanine | 2.31 | Isoleucine | 3.03 |
| Oxyproline | 0.01 | Leucine | 3.20 |
| Cysteine | 0.15 | Glycine | 2.11 |
| Aspartic acid | 1.88 | Alanine | 5.82 |

## Conclusions

- A phytochemical study of *Kochia prostrata* was carried out. The qualitative composition of amino, fatty and phenolic acids of the plant genus *Kochia prostrata* has been studied by using method of paper chromatography (PC) and *thin-layer chromatography* (TLC), their quantitative composition has been identified by gas chromatography.

– A comparative analysis of plants of the genus *Kochia prostrata* has been carried out. The major amino acids were glutamic acid (23.46%), aspartic acid (1.88%) and alanine (5.82%), while the major fatty acids were oleic (64.9%) and linoleic (22.0%), palmetic (5.3%) acids.

– By method of paper chromatography (PC) with authentic phenolic acid samples: protocatechuic, vanillic, isovanillic and p-coumaric acids have been determined from the plant genus *Kochia prostrata*.

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