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RESEARCH ARTICLE

Histochemical analysis of *Xanthium strumarium* raw materials, growing on the territory of Kazakhstan

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ABSTRACT:

Histochemical analysis of medicinal plants and raw materials is designed to study the localization of various chemical substances and metabolic products in their tissues. The article presents the results of a histochemical analysis of leaves, stems, roots and fruits of a promising medicinal plant Xanthium strumarium, the herb of which is used in folk medicine to treat iodine deficiency. **Goal**. Determination of the features of localization of individual groups of biologically active substances in the aerial and underground organs of Xanthium strumarium, growing on the territory of Kazakhstan. **Materials and methods**. Materials for the study: cross sections of the stem; cross and superficial sections of leaves; cross sections of the root and the surface preparation of Xanthium strumarium's fruit. Samples of raw materials were fixed in Strauss-Fleming solution, cross sections were prepared manually, after which histochemical reactions were carried out to locate and identify the following biologically active substances: essential oil, starch, flavonoids, polysaccharides, sesquiterpene lactones and alkaloids. The study of microscopic features, as well as histochemical tests, was carried out according to the methods of the State Pharmacopoeia of the Republic of Kazakhstan using a "Biomed-4" light microscope. **Results:** By histochemical methods using light microscopy determined the localization of biologically active substances in plant raw materials. The presence of essential oil, flavonoids, alkaloids, polysaccharides and sesquiterpene lactones on cross sections of the leaf, stem, root and fruit was established. The accumulation of starch in all organs of the plant has not been established. **Conclusion:** For the first time, by histochemical tests, the localization of biologically active substances in the tissues of Xanthium strumarium were studied. The results of histochemical studies can be used to confirm the authenticity, identification and standardization of the aerial and underground parts of Xanthium strumarium.

KEYWORDS: Histochemical analysis, biologically active substances, Xanthium strumarium, medicinal plant, medicinal plant raw material.

INTRODUCTION:

The most important task for researchers is to establish the authenticity of medicinal raw materials. There are many research methods that makes it possible to assess the belonging of medicinal plants to a particular species, as well as to establish the localization of secondary metabolites.

One of the methods is histochemical analysis, which allows to determine the presence and placement of biologically active substances in tissues and organs. Medicinal plants contain many secondary metabolites, such as alkaloids, sesquiterpene lactones, flavonoids, polysaccharides, etc. Secondary metabolites are in great demand in the pharmaceutical industry and have a variety of pharmacological activities[1-6].

Histochemical studies are based on reactions between the test substance and a specially selected reagent. The result of this interaction is the formation of a colored or

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fluorescent complex. By the distribution of color (fluorescence) in the preparation, one can judge the localization, and by the intensity - the quantitative presence of the substance of interest in tissues and cells[7- 12].

The plant *Xanthium strumarium*is one of the most famous, which is widely used in folk medicine in many countries as a medicinal raw materials.

Xanthium strumarium L. is annual plant from family *Asteraceae* Dumort. (*Compositae*). *X. strumarium*grows as a weed throughout the world, except in the Far North. The homeland of the plant is Europe, Asia and North America, from there the *Xanthium* species spread to Africa, Australia, South America, India, China, Indonesia and Malaysia. The plant can be found on moist sandy soils along the banks of reservoirs, ditches, near roads and in wastelands. On the territory of Kazakhstan there are 2 species of *Xanthium* species. The most common are (especially in the southern regions) *X.strumarium* and *X.spinosum*[13-14].

The plant *X. strumarium*has a rich chemical composition and biological activity: antiseptic, anti-inflammatory, fungicidal, diaphoretic, analgesic, antipyretic, antispastic, sedative properties[15].

It is known from literary sources that, first of all, *Xanthium*species are known for helping to reduce the thyroid gland in endemic goiter. Also, decoctions and infusions of the plant have antipyretic, diaphoretic, sedative, fungicidal, antiseptic and anti-inflammatory effects. *Xanthium* oil is used externally for a number of skin diseases. The plant is an effective tool in the fight against diseases associated with iodine metabolism disorders[16-21]. It is also known that in folk medicine a plant of the genus *Xanthium* L. is used for thyroid diseases, diarrhea, gastrointestinal diseases, cholera, eczema, lichen, fungal infections of the skin and nails. Internal use of a plant of the genus *Xanthium* L. as a poisonous plant requires caution[22-27].

MATERIALS AND METHODS:

Raw materials:

The object of the study were aerial and underground organs of *X. strumarium* (Fig. 1), harvested in the fruiting phase in the vicinity of the village of Baskunchy, Panfilov district, Almaty regionof the Republic of Kazakhstan. Harvest time - August-September 2022.

Figure 1: The appearance of *X. strumarium* . Fruit-bearing phase

Histochemical study:

Freshly harvested organs (leaves, stems, fruits, roots) were fixed in a mixture of alcohol (70%), glycerol and distilled water in a ratio of 1:1:1 (Strauss-Fleming solution).

Histochemical study was carried out for cross sections of the stem, cross and superficial sections of leaves, surface preparations of fruits and cross sections of roots in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan (Vol. 1 "Methods for testing medicinal plant raw materials", "Technique for microscopic and microchemical examination of plant raw materials")[13]. When conducting histochemical analysis, we used the reagents listed in Table 1.

Table 1: Reagents for microchemical research

No.	Reagent	Defined component	Color
1	2% methylene blue solution	Essential oil	Blue
2	1% alcohol solution of $FeCl_3$	Flavonoids	Black-blue-green
3	Dragendorff's reagent	Alkaloids	Black
4	Concentrated H_2SO_4 and vanillin	Sesquiterpene lactones	Yellow
5	10% thymol solution	Polysaccharides	Orange-red, green
6	Lugol's reagent	Starch	Blue

The localization of individual groups of biological substances was judged by the change in the color of the tissues of the saltpeter. The study of anatomical preparations was carried out using a "Biomed-4" microscope with ×10, ×20 eyepieces, ×4, ×10, ×20, ×40 lenses. The photographs were taken with a Sony Siber Shot camera. The photographs were processed in the Paint 10.1 program.

RESULTS AND DISCUSSION:

As a result of the study, coloring of plant tissues was established, which showed the presence of essential oils, flavonoids, alkaloids, polysaccharides and sesquiterpene lactones. Qualitative reactions to the presence of starch were not detected. The results of histochemical analysis of aerial and underground organs of *X. strumarium* are presented in Table 2 and in Figures 2-5.

Table 2: Results of histochemical analysis of the aerial and underground parts of *X. strumarium*

No.	Reagent	Stem	Leave	Root	Fruits
1	2% methylene blue solution	+	+	-	-
2	1% alcohol solution of $FeCl_3$	+	+	+	+
3	Dragendorff's reagent	-	+	+	-
4	Concentrated H_2SO_4 and vanillin	-	+	+	+
5	10% thymol solution	+	+	+	-
6	Lugol's reagent	-	-	-	-

Note: "-"negative reaction; "+"positive reaction

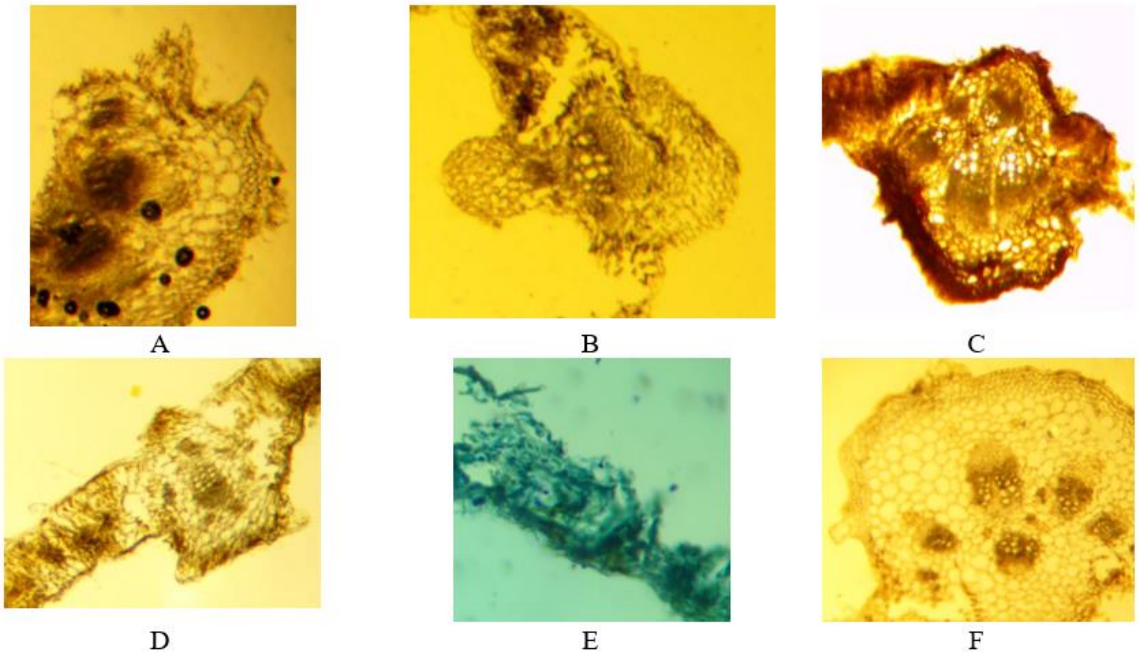

Figure 2: The result of histochemical reactions on a cross section of a X. strumarium leaf (mag. ×1). Reagent: A - concentrated sulfuric acid with vanillin, B - iron chloride, C - thymol solution, D - Dragendorff's reagent, E - methylene blue, F - Lugol's reagent

Identification of essential oil in the aerial and underground organs of *X. Strumarium:*

The results showed that the accumulation of essential oil was not recorded in all organs of the studied plant. Thus, its localization was noted on the cross section of the leaf, mainly in the epidermis and in the mesophyll; as well as on the cross section of the stem. Areas of the core's parenchyma and annular areas of the xylem were colored. The essential oil has not been identified in other organs.

Identification of flavonoids in aerial and underground organs of *X. Strumarium:*

After treatment of micropreparations of the studied samples with a 1% alcohol solution of $FeCl_3$, characteristic coloring was observed for all the studied organs. On the cross section of the leaf, the conductive bundles, sclerenchyma colored more intensely, and to a lesser extent, the mesophyll and epidermis. On the cross section of the stem and root, localization of flavonoids in the zone of the cortical parenchyma and in the conductive zone, to a lesser extent - the epidermis and rhizoderm, was noted.

Identification of alkaloids in the aerial and underground organs of *X. Strumarium:*

The presence of alkaloids was confirmed by uneven coloring in the form of black spots in the leaves and roots of *X. strumarium*. Conductive bundles and areas of the mesophyll around them were colored on the leaves; sections of the root - sections of the conductive zone. The remaining parts of the plant did not show the presence of alkaloids.

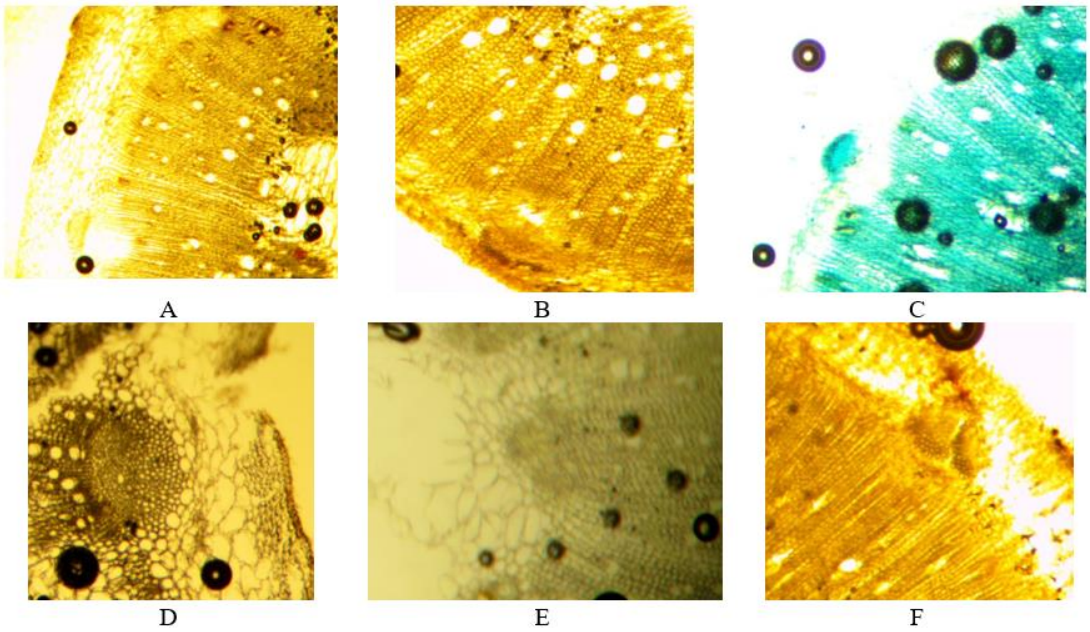

Figure 3: The result of histochemical reactions on a cross section of X. strumarium stem (mag. ×1). Reagent: A - iron chloride, B - Lugol's reagent, C - methylene blue, D - thymol solution, E - vanillin solution in concentrated sulfuric acid, F - Dragendorff's reagent

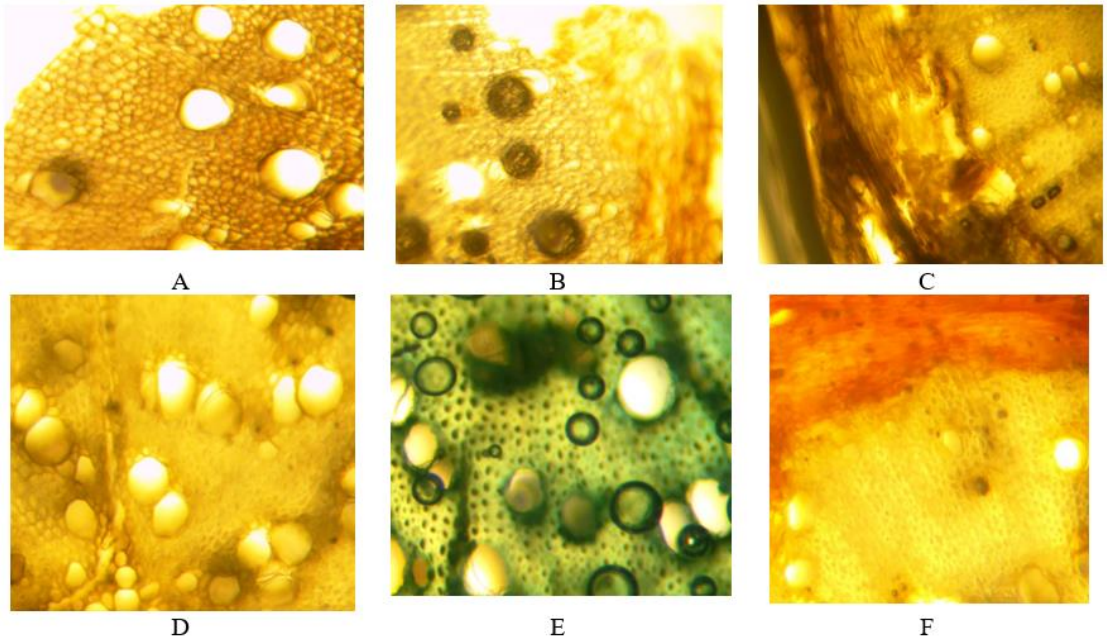

***Figure 4*: The result of histochemical reactions on a cross section of X. strumarium stem (mag. ×1). Reagent: A - vanillin solution in concentrated sulfuric acid, B - Dragendorff's reagent, C - iron chloride, D - Lugol's solution, E - methylene blue, F - thymol solution**

Identification of sesquiterpene lactones in aerial and underground organs of *X. strumarium:*

Sesquiterpene lactones were also found only for leaf and root preparations. On the ross section of the leaf, their localization in the columnar and spongy mesophyll was noted; on the root section - areas of xylem.

Identification of polysaccharides in the underground organs of *X. strumarium:*

Polysaccharides were found in all studied organs. So, on the cross section of the leaf, the epidermis and columnar mesophyll were intensively stained, and the spongy mesophyll was less intensely colored. On the cross section of the stem - the parenchyma around the xylem; on the cross section of the root, good coloring was observed for the core parenchyma, to a lesser extent - for the conducting zone.

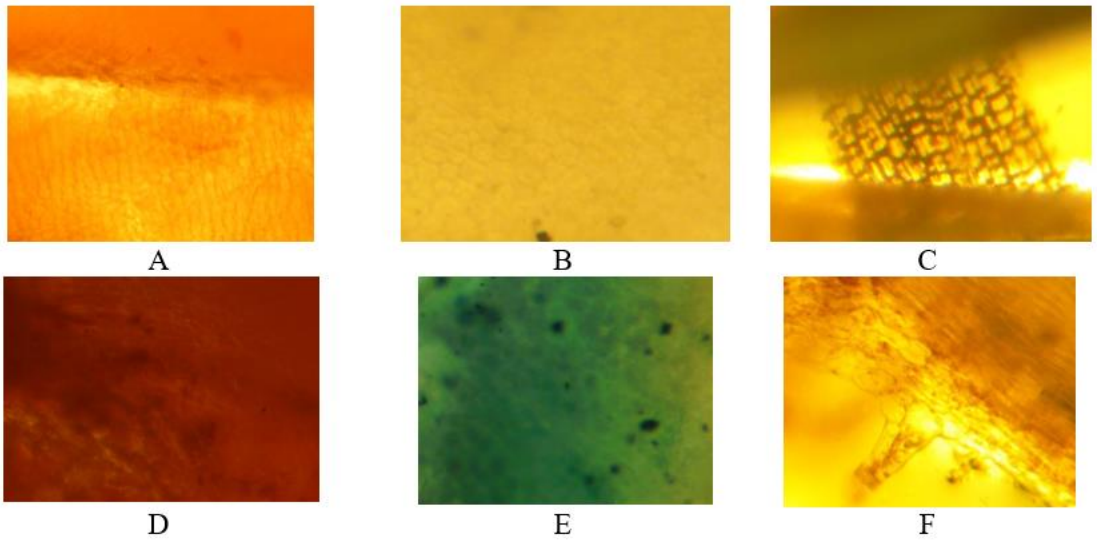

Figure 5: The result of histochemical reactions on a cross section of a X. strumarium fruit (magn. ×1). Reagent: A - concentrated sulfuric acid with vanillin, B - iron chloride, C - thymol solution, D - Dragendorff's reagent, E - methylene blue, E - Lugol's reagent.

Histochemical analysis of fruits showed the presence of such active substances as flavonoids and sesquiterpene lactones. Other biologically active substances were not identified (Table 2, Fig. 5).

Flavonoids were found in the cells of the exoderm and periderm of the fruits, sesquiterpenoids - in the cells of the exoderm.

CONCLUSION:

For the first time, the secretory structures of *X. strumarium* were studied by light microscopy in combination with histochemical tests. As a result of histochemical tests on cross sections of leaf and stem, fruit surface preparation, cross sections of roots, essential oil, flavonoids, polysaccharides, alkaloids and sesquiterpene lactones were found, and their localization was established:

- Essential oil - on the cross section of the leaf, mainly in the epidermis and mesophyll; on a cross section of the stem; areas of the core's parenchyma and ring sections of the xylem were colored;
- Flavonoids - on the cross section of the leaf, the vascular bundles, sclerenchyma colored more intensely, and to a lesser extent - the mesophyll and epidermis. On the cross section of the stem and root, localization of flavonoids in the zone of the cortical parenchyma and in the conducting zone, and to a lesser extent - the epidermis and rhizoderm, was noted. Flavonoids were found in the cells of the exoderm and periderm of the fruit;
- Alkaloids - on the leaves, vascular bundles and mesophyll areas around them were colored; cross sections of the root - sections of the conductive zone.
- Sesquiterpene lactones - on the cross section of the leaf, their localization in the columnar and spongy mesophyll was noted, on the root section - areas of xylem; sesquiterpenoids - in exoderm cells.
- Polysaccharides - on the cross section of the leaf, the epidermis and columnar mesophyll were intensively colored, less intensively - the spongy mesophyll; on the cross section of the stem - the parenchyma around the xylem; on the cross section of the root, good coloring was observed for the core parenchyma, to a lesser extent for the conducting zone.

CONFLICTS OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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