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Anti-conjunctivitis effect of fresh juice of *xGraptoveria* (Crassulaceae): A phytochemical and ethnobotanical study

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ABSTRACT

Aim: The parent of x*Graptoveria*, *Graptopetalum paraguayense*, is used in Chinese folk medicine for alleviating hepatic disorders, detumescence and detoxication, lowering of blood pressure, inhibition of cancer cells, exerting diuretic effects, relieving pain and infections. No data are available regarding its anti-conjunctivitis effect. The aim of this preliminary study is to test the anti-conjunctivitis properties of x*Graptoveria* (Crassulaceae) and to identify its bioactive constituents. **Materials and Methods:** Fresh watery juice of leaves of x*Graptoveria* was extracted with *n*-butanol and the extract was analyzed using gas chromatography-mass spectrometry (GC/MS). The ethnobotanical appraisal of the anti-conjunctivitis properties of x*Graptoveria* was based on 11 interviews about the symptoms against which this plant demonstrated positive effect. **Results:** Fresh juice of x*Graptoveria* leaves applied directly to the irritated eye 2 times per day cured conjunctivitis in all reported cases. The main groups of organic compounds identified by GC/MS analysis in the fresh extracted leaf juice of x*Graptoveria* were: Alkylamines, hydroxycarboxylic acids, aliphatic and aromatic carboxylic acids, amino acids, alcohols, aromatic and aliphatic hydrocarbons. **Conclusion:** In this preliminary study, it is suggested that x*Graptoveria* exerts anti-conjunctivitis activity, through synergistic effect of different chemical compounds, most probably alkylamines and mainly hydroxycarboxylic, aliphatic, and aromatic carboxylic acids.

KEY WORDS: Alkylamines, conjunctivitis, ethnobotanical, gas chromatography-mass spectrometry, x*Graptoveria* leaf juice

INTRODUCTION

Conjunctivitis can be caused by viruses, bacteria or fungi, exposure to chemicals or irritants or long-term presence of a foreign body such as hard or rigid contact lenses [1-3].

Plants have shown considerable activity against various microbes [4-7]. It is considered that plants are a source of a wide variety of bioactive molecules that can be used for the development of new medicines with a wider spectrum of activities and with less adverse effects than those produced by the drugs currently in use [7-9].

Bulgarian folk medicine treats conjunctivitis by several plants. Most popular are species of the genus *Euphrasia*, and even their common names are related to that use "ochanka" (in Bulgarian Ochi: eyes) [10-13]. There are empirical data for a therapeutic effect of *Geumurbanum*, *Althea officinalis*, *Pimpinela saxifraga*, *Anagalis arvensis*, in cases of conjunctivitis [11-13].

Members of the Crassulaceae family are known for their antiseptic and antibacterial properties. Particularly, leaves of *Echeveria gigantean* Rose and Purpus are used for eye illness treatment [8], but there are no data about the chemical or biological studies.

The object of this study, *xGraptoveria*, is an intergeneric hybrid between *Graptopetalum paraguayense* and *Echeveria* sp. div. It is a succulent, drought-resistant perennial grown as ornamental house plant in temperate regions, as it cannot survive winter outside. The parenting taxa belong to the Crassulaceae family. They are native to Mexico and are distributed widely in tropical and subtropical countries where they are mainly cultivated as ornamental plants, but are popular in Chinese herbal medicine.

To the best of our knowledge, there is no information in the literature on the application of *G. paraguayense* juice for treatment of conjunctivitis.

The aim of this study is to test the anti-conjunctivitis properties of x*Graptoveria* (Crassulaceae) and to identify its bioactive constituents.

MATERIALS AND METHODS

xGraptoveria

We have grown x*Graptoveria* as an ornamental house plant at the Institute of Organic Chemistry with Center of Phytochemistry, Sofia, Bulgaria for more than 10 years. The vegetative reproduction of this plant can be easily induced by separating offsets and leaf cuttings. Rosettes and fine wax cover are visible external features of the leaves [Figure 1].

Ethnobotanical Study

Since its introduction in Bulgaria, x*Graptoveria* has also found its place in folk medicine. Some anecdotal data have been reported for the use of freshly obtained leaf juice for treatment of eye problems, mainly forms of conjunctivitis. We managed to collect eye healing information for x*Graptoveria* via interviewing 11 herbalists that had already been treated with the same plant. A semi-structured questionnaire [14] was constituted using the following questions: (1) What is the plant used for; (2) How is it used; (3) What part of the plant is used; (4) What is the dose used for eye treatment; (5) How long does the treatment take; (6) How many applications are needed for eye improvement?

Treatment of Volunteers with Conjunctivitis by *xGraptoveria* Leaf Juice

Volunteers

Four women and four men of age between 33 and 60, volunteered to have their eyes treated by freshly prepared *xGraptoveri*a Leaf Juice. All of them suffered from conjunctivitis as estimated by an ophthalmologist. The symptoms are described in Table 1.

Method of treatment

Fresh juice obtained directly by pressing out *xGraptoveria* leaf was immediately dropped in the irritated eye without any dilutions. Treatment of volunteers was performed in clean eyes as follows: 1 drop per eye; 2 times a day (in the morning and in the evening). The applications were done by the volunteers themselves at the Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria.

Phytochemical Analysis of the Fresh Juice used for the Eye Treatment

Preparation of extracts of xGraptoveria

Fresh leaves of *xGraptoveria* were picked and pressed out immediately to give watery juice. The juice was then extracted with *n*-butanol. The butanol extract (xGBE) was evaporated and analyzed by gas chromatography mass spectrometry (GC/MS).

GC/MS analysis

The analysis of xGBE was performed with a Hewlett Packard 6890 GC System Plus MS 5973 (Hewlett Packard, Palo Alto, CA, USA) equipped with capillary column HP5-MS (30 cm, 0.25 mm, 0.25 mm film thickness, Agilent Technology, USA). The carrier gas was helium with flow rate 0.8 mL/min. The following temperature program was used: 100-300°C (10 min isotherm) at 5°C/min. The method of electron-impact ionization was utilized. The ion source was set at 230°C and the ionization voltage was 70 eV. Because the ion current generated depends on the characteristics of the investigated compounds and is not true quantification, GC/MS analyses do not give exact quantitative data.

Preparation of the sample for GC/MS analyses

The sample of about 5 mg of xGBE was prepared. It was silylated prior to GC/MS measuring via mixing with 75 mL of dry pyridine and 25 mL of bis(trimethylsilyl)trifluoroacetamide and heating at 80°C for 20 min.

Identification of compounds

The GC/MS identification was based on the interpretation of the mass spectral fragmentation facilitated by HP Mass Spectral Library NIST98 (Hewlett Packard, Palo Alto, CA, USA). Not all, but the main components were identified.

RESULTS

Eleven interviews were performed according to a semi-structured questionnaire (Section 2.2.) [14]. The informants were chosen

Figure 1: *xGraptoveria* (Crassulaceae)

Table 1: Conjunctivitis symptoms, diagnosis and number of applications leading to improvement and complete cure of the volunteers

Gender	Age	Symptoms	Diagnosis	Improvement	Complete cure
Female	33	Redness in the area of sclera caused by visible capillaries; pain	Allergic conjunctivitis	3	6
Female	34	Redness in the area of sclera caused by visible capillaries	Allergic conjunctivitis	3	6
Female	45	Redness in the area of sclera caused by visible capillaries; stinging and itching eyes; purulentdischarge	Bacterial conjunctivitis	2	6
Female	57	Redness in the area of sclera caused by visible capillaries	Allergic conjunctivitis	3	6
Male	34	Redness in the area of sclera caused by visible capillaries; mucoid discharge; stickyeyelids	Adenoviral conjunctivitis	2	6
Male	35	Redness in the area of sclera caused by visible capillaries; purulentdischarge; crustyeyelids	Bacterial conjunctivitis	3	6
Male	40	Redness in the area of sclera caused by visible capillaries; mucoiddischarge	Bacterial conjunctivitis	2	6
Male	60	Redness in the area of sclera caused by visible capillaries	Allergic conjunctivitis	2	6

amongst herbalists that had been treated by *xGraptoveria*. All of them gave the same answers concerning the plant and described it to have strong healing effect on human conjunctivitis. This information encouraged us to try out the effect of the plant grown in our laboratory following the treatment conditions described during the interviews (Section 2.3.2.).

Eight volunteers were diagnosed by an ophthalmologist with various types of conjunctivitis (chronic allergic, adenoviral, and bacterial conjunctivitis) [Table 1]. Redness in the area of the sclera caused by visible capillaries was the symptom observed in all cases. Allergic conjunctivitis was the case for three of the women (age 33, 34 and 57) and one of the men (age 60). Redness and pain accompanied the symptoms of the 33-old woman. Volunteers with bacterial conjunctivitis suffered from redness, mucoid or purulent discharge and crusty eyelids. The symptoms diminished the 1st day (2-3 applications) and completely disappeared the next 3 days in all described cases. The symptoms, diagnosis and number of applications leading to improvement and complete cure of the volunteers are given in Table 1.

To study the components of *xGraptoveria* leaf juice that may be responsible for the curative effect on conjunctivitis, the juice was subjected to phytochemical analysis. In order to maximize the identification of constituents, the juice was extracted with *n*-butanol and the resulting extract (xGBE) was investigated by GC/MS. This method allowed for analysis of complex mixtures as described in 2.4.2. The GC/MS chromatogram of xGBE is presented on Figure 2. The extract investigated could contain a significant number of metabolites, including some minor compounds, which cannot be identified by other methods.

The main groups of organic compounds identified by GC/MS analysis in xGBE are presented in Table 2: Alkylamines (ethylamine and butylamine), hydroxycarboxylic acids (hydroxypropenoic, hydroxybutanoic, malic, dihydroxybutanoic and methylhydroxybenzoic acids), aliphatic and aromatic carboxylic acids (malonic, pentadecanoic, oleic, hexanedioic, palmitic, stearic, benzoic, and 3-pyridinecarboxylic acids), amino acids (tryptophane), alcohols (dihydroxyethane and glycerol), aromatic and aliphatic hydrocarbons (pentamethylheptane, ethyl-dimethylbenzene and tetramethylbenzene), and sugars. Some of them (hydroxypropenoic acid, pentamethylheptane, ethyl-dimethylbenzene, butylamine, dihydroxyethane, tetramethylbenzene, hydroxybutanoic acid, dihydroxybutanoic acid and methylhydroxybenzoic acid) can exist in several isomers. However, the method did not allow for identification of the existing forms.

Table 2: GC/MS data for the main organic compounds identified in xGBE, R_t (min), TIC (%)

R_t	Compound	TIC
8.6	Ethylamine	2.3
9.0	Hydroxypropenoic acid (3-Hydroxypropenoate)	0.7
9.2	Pentamethylheptane*	0.3
11.3	Ethyldimethylbenzene*	1.1
11.8	*n*-Butylamine	19.4
12.2	Tetramethylbenzene*	1.2
12.7	Dihydroxyethane*	0.8
13.0	Hydroxybutanoic acid*	0.6
15.2	Benzoic acid	0.9
16.1	Glycerol, 3-pyridinecarboxylic acid	2.0
17.2	Malonic acid	0.7
20.2	Dihydroxybutanoic acid*	0.3
21.6	Malic acid	1.2
25.0	Methylhydroxybenzoic acid (3-methylsalicylic acid)	0.7
29.4	Pentadecanoic acid	0.4
31.6	Oleic acid	0.5
31.9	Tryptophane	1.0
32.9	Hexanedioic acid (adipic acid)	0.4
33.4	Palmitic acid	0.7
	Sugars	8.8

*Compound can exist in several isomers. Rt: Retention time, TIC: Total ion current, GC/MS: Gas chromatography-mass spectrometry, xGBE: x*Graptoveria* butanol extract

DISCUSSION

Fresh juice of *xGraptoveria* leaves was used successfully for the complete cure of various types of conjunctivitis in 8 volunteers [Table 1]. The treatment conditions were taken from the informants who had recovered from conjunctivitis by treatment with the same plant. Since this is a very preliminary study it was defined between a small set of volunteers and no clinical and pharmacological research was embarked. However, we still aimed at finding a strong motivation for further profound study leading to relevant exploitation of *xGraptoveria*. For this reason, we analyzed the phytochemical composition of the fresh juice, which was exactly the curative part used for the treatment of conjunctivitis.

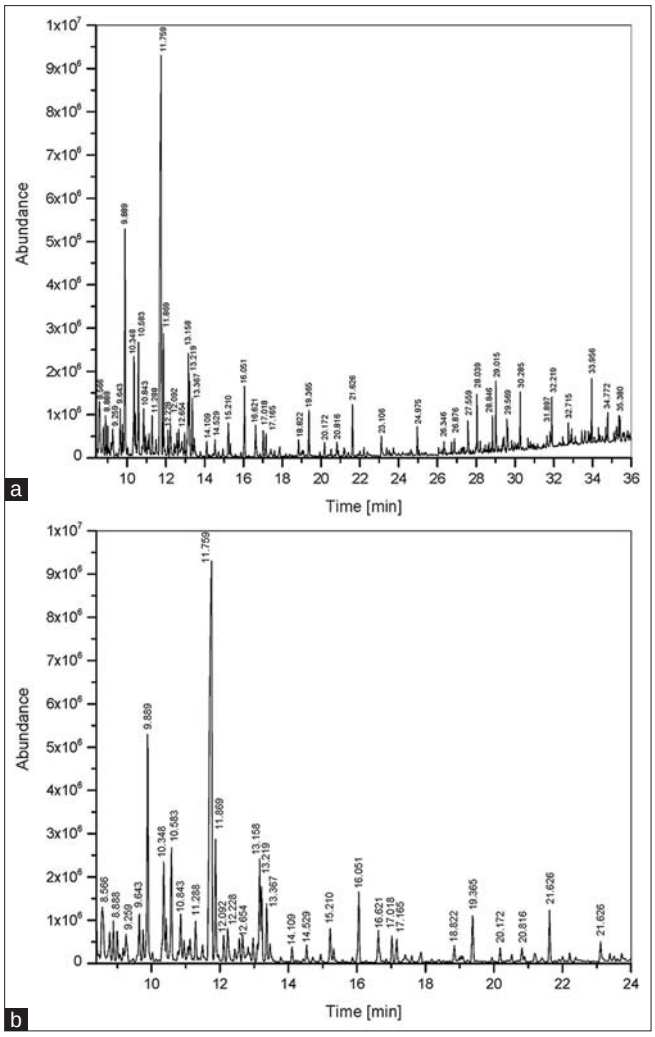

Figure 2: Gas chromatography-mass spectrometry chromatogram of *xGraptoveria* butanol extract include the identified components: (a) Retention time (R_t) = 8.6-36.0 min; (b) R_t = 8.6-24.5 min

The GC/MS analysis allowed for identification of most of the fresh juice components. However, searching the literature did not reveal any anti-conjunctivitis activity for the identified single compounds. Instead we came across some data showing interesting biological functions for these constituents that may be in relation with the observed effect.

Conjunctiva, which is the place where conjunctivitis occurs, provides a major source of immune components in the cornea. It produces the antigen immunoglobulin A that plays a critical role in mucosal immunity and also contains macrophages, neutrophilic granulocytes, mast cells, lymphocytes, and other aspects of the general mucosal immune system [15]. The macrophages play a part in modulating the T-cell immune response and mediating both the innate and acquired immune responses. Interestingly, in this relation we observed that the fresh juice of *xGraptoveria* leaves is rich of alkylamines, mostly *n*-butylamine and some ethylamine [Table 2]. Alkylamines are known immune activators. For example, *sec*-butylamine and *iso*-butylamine can activate Vγ9Vδ2 T cells in humans as a consequence of inhibition of farnesyl diphosphate synthase and the intracellular accumulation of isopentenyl pyrophosphate [16-18]. Structure analysis of several antigenic and non-antigenic alkylamines indicates that a straight or branched alkyl chain of two to five carbons with a single primary amine group as the only substituent is active, while alkylamines with one carbon or more than five carbons, or any substituent other than the primary amino group have no effect [16,19].

Presence of tryptophan in the fresh juice may be useful for influence upon the inflammatory process. Furthermore, a number of recent studies have shown a clear association between tryptophan catabolism and inflammatory reactions in a vast array of disease states [20]. The remainder of the organic acids identified in the juice possess antimicrobial activity predominantly or a combination of several biological activities. Malic acid manifests antioxidant, anti-inflammatory and antibacterial activities [21-23]. Oleic acid is active against several Gram-positive bacteria [24]. Interesting synergistic relationships were observed between some of the acids. Palmitic, pentadecanoic and oleic acids gave a mixture which was much more potent as antimicrobial agent than the single acids against 11 microorganisms [25-27]. Another example concerns oleic and linoleic acids, which were more active together against *Staphylococcus aureus* and *Micrococcus kristinae* [24,28].

After all, we decided to speculate that the healing effect of *xGraptoveria* fresh leaf juice might be due to a synergistic effect of its constituents, part of which affect the immune response while the rest act against the invading microorganisms.

CONCLUSION

This is a preliminary study on the chemical composition and anti-conjunctivitis effect of *xGraptoveria* leaf fresh juice. It is hypothesized that the effect is due to the synergistic action of the bioactive constituents – mainly alkylamines, hydroxycarboxylic, aliphatic and aromatic carboxylic acids.

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