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PHARMACOLOGICAL ASSESSMENT OF CONSTITUENTS OF SPECIES *ALLIUM SAXATILE* AND *ALLIUM PONTICUM* GROWING IN GEORGIA

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საქართველოში გავრცელებული *Allium saxatile* და *Allium ponticum* სახეობების მეორადი მეტაბოლიტების ფარმაკოლოგიური შეფასება

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რეზიუმე

წინამდებარე კვლევა მიზნად ისახავდა საქართველოში მოზარდი Allium saxatile და Allium ponticum-ის მეორადი მეტაბოლიტების ფარმაკოლოგიური მოქმედების შეფასებას. მცენარიდან მიღებული ჯამური ექსტრაქტების და გამდიდრებული ფრაქციების ტკივილგამაყუჩებელი, ანთების საწინააღმდეგო და გასტროპროტექტორული აქტივობა შეფასდა in vivo ექსპერიმენტში აღიარებული მოდელების გამოყენებით: ცხელი ფირფიტა, კარაგინანით გამოწვეული შეშუპება და ეთანოლით გამოწვეული კუჭის წყლული. ექსპერიმენტების შედეგებმა გამოავლინა საკვლევი მცენარეებიდან მიღებული ჯამური ექსტრაქტების და გამდიდრებული ფრაქციების ღირებული ეფექტები. კერძოდ, A. saxatile-ის ჯამურმა ექსტრაქტმა აჩვენა ზომიერად აღმავალი პროლონგირებული ტკივილგამაყუჩებელი ეფექტი (70.3%), ხოლო A. ponticum-ის ჯამურმა ექსტრაქტმა - სწრაფი, ძლიერი, მაგრამ ხანმოკლე ეფექტი (105%). იგივე ტენდენცია ნარჩუნდება მცენარის გამდიდრებული ფრაქციების შესწავლისას. კარაგენანით გამოწვეული თათების შეშუპების მოდელში, A. saxatile-ის ჯამური ექსტრაქტი და გამდიდრებული ფრაქციები უფრო ეფექტური აღმოჩნდა (63.5%), ვიდრე A. ponticum-დან (32.7%) მიღებული საკვლევი ობიექტები. ეთანოლით გამოწვეული კუჭის წყლულის მოდელში მხოლოდ A. saxatile-ის ჯამურმა ექსტრაქტმა შეამცირა (44%-ით) წყლულის ინდექსი (UI), ხოლო A. ponticum-ის ექსტრაქტმა, პირიქით, გაზარდა წყლულის ინდექსი (UI). მიღებული შედეგების საფუძველზე შეიძლება დავასკვნათ, რომ კვლევებმა გამოავლინა პერსპექტიული მცენარეული ობიექტები, როგორც ბიოლოგიურად აქტიური ნაერთების წყარო ახალი წამლის ფორმების შემუშავებისთვის.

Introduction. Compounds that originate from natural sources, including plants, have been used for medicinal purposes for thousands of years and still remain extremely important raw material for drug development [13].

The first records from Mesopotamia are dated by 2600 BC: amongst the approximately 1000 plant-derived substances were oils from *Cedrus* species (cedar) and *Cupressus sempevirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh), *Papaver somniferum* (poppy juice), etc., all of which are still in use for treatment of ailments ranging from coughs and colds to parasitic infections and inflammation [19]. Allium species have been traditionally used for its remedial characteristics in the management of various ailments since the early ages. Allium cultivation traces back over 4000 years to ancient Egypt [25]. In Anatolia *Allium cepa* and *A. sativum* have been used as a protection from infections, sunstroke, hypertension, rheumatism, antihelmintic, for headache and as an analgesic [14]. Iranian folk

medicine used Allium species for treatment of atherosclerosis, rheumatism, tuberculosis, gastric and lung diseases [12]. Allium species are well known for Georgian traditional medicine as an antifungal, antiseptic and antibacterial remedy [1,2].

Historically, natural products have played a key role in drug discovery, especially for cancer and infectious diseases, but also in other therapeutic areas, including cardiovascular diseases (for example, statins) and multiple sclerosis (for example, fingolimod) [6,7]. Up to 35 percent of the 1.1 trillion US$ worth annual global market of medicines is occupied by remedies that directly or indirectly originate from natural sources including: plants (25%), microorganisms (13%) and animals (about 3%) [8].

Newman and Cragg (2019) reviewed the role of natural products in the drugs approved by FDA between 1981 and 2019. They found that during this period the FDA approved 1881 drugs, amongst them 71 (3.8%) were unaltered natural products, 14 (0.8%) - botanical drugs (mixtures), 356 (18.9%) - natural product derivatives and 65 (3.2%) - synthetic drugs that contain natural products pharmacophore [18].

The most important secondary metabolites (terpenoids, phenolics, flavonoids, alkaloids and glycosides) exhibit diverse pharmacological activity and represent a valuable source for nutraceuticals and modern medicines [27].

Allium species have been shown to have anti-inflammatory and analgesic properties. According to Ogbole et al., *A. cepa* is a plant that has traditionally been used to treat inflammatory diseases [20]. *A. ascalonicum* (Shallot) was reported as having an effective anti-inflammatory property [16].

Inflammation associated pain is one of the major indicators of inflammation. In many cases, when inflammation is controlled, the associated pain is automatically relieved [22]. Oyewusi et al. investigated the anti-inflammatory and analgesic effects of methanol extract of red cultivar *A. cepa* bulbs (MERCACB) on rats using a hot-plate test and a carrageenan-induced paw oedema assay. Indomethacin (10mg/kg) significantly ($p<0.05$) increased the percentage inhibition of oedema formation at 90- and 120-min post induction (pi) by 80.77 and 82.46 percent, respectively, when compared to the control. Similarly, all the doses of methanol extract of red cultivar *A. cepa* bulb significantly ($p<0.05$) increased the percentage inhibition of oedema formation with 200 mg/kg inhibiting up to 62.50% 76.92% at 30- and 60-min pi, respectively [22]. The results of the mice response to thermal pain induced by hot plate indicated that MERCACB exhibited analgesic effect by significantly ($p<0.05$) increased pain reaction time compared with the mice treated with indomethacin and control groups. This response was more pronounced at 60 min post induction of pain but diminished steadily at 90- and 120-min post induction except for the group treated with 800 mg/kg [22].

Methanolic extract of *A. paradoxum* has also significant analgesic activity. The latter was evaluated by Hot plate and acetic acid induced writhing test in male Balb/C mice. In both models, the extracts demonstrated significant analgesic activity. In the writhing test, the extract demonstrated significant analgesic activity in all doses tested when compared to the control group, as well as reduced writhing behaviors ($p<0.001$). In the Hot plate test, the extract increased the pain threshold compared to the control, particularly in the 30th minute of the test ($p<0.001$) [15].

The anti-inflammatory activity of *A. subhirsutum* aqueous extract was assessed using the carrageenan-induced oedema method. In the inflamed groups, the size of the oedema and the percentage of inflammation increased rapidly, peaking around the third hour after carrageenan injection. Rats given *A. subhirsutum* for one week prior to oedema induction had a significant reduction in paw thickness after carrageenan injection. When compared to the reference drug (indomethacin), treatment with *A. subhirsutum* had the greatest effect on reducing paw oedema [28]. Nonsteroidal anti-inflammatory drugs (NSAIDs) have become one of the most used groups of pharmaceuticals due to the need to effectively treat inflammation, pain, and/or fever.

Unfortunately, the use of these agents is frequently accompanied by adverse side effects, the most common of which are those affecting the gastrointestinal (GI) tract [24]. Human intestinal endothelial cells exposed to NSAIDs show mitochondrial dysfunction and an increase in reactive oxygen species (ROS) formation [21,26]. Considering this, molecules and plant extracts with antioxidant properties have been thoroughly researched.

Gastroprotective effect of garlic was studied using indomethacin induced gastric ulcer in rats. The ulcerated indomethacin group showed ulcer score of 29 ± 4.8. Rat groups treated with garlic extract or

omeprazole before indomethacin showed a significant decrease of ulcer score, which was 4.8 ± 1.4 and 1.6 ± 0.51, respectively. Omeprazole showed a greater gastroprotective effect with preventive index 94.5%, while garlic extract showed a preventive index of 83.4% [10].

Adao et al. have investigated antiulcerogenic activity of steroidal saponin isolated from *A. ampeloprasum* by measuring acute gastric lesions induced by acidified ethanol. Studied compound has shown significant reduction in gastric hyperemia and severity of lesions [3].

Previous studies on the extracts and fractions of plants *A. saxatile* and *A. ponticum* have shown that, these plants have very good antioxidant properties [5]. These results and bibliographical data about the plants genus Allium, encouraged us to evaluate the analgesic, anti-inflammatory and gastroprotective activity of extracts and fractions from *A. saxatile* and *A. ponticum* using accepted *in vivo* models.

The present study aimed to estimate pharmacological potency of products from some Allium species growing in Georgia.

Materials and methods

Plant materials. The objects of the research were plants *A. saxatile* and *A. ponticum* growing in Georgia. The whole plants of *Allium saxatile* and *Allium ponticum* were collected, respectively, in Racha and Javakheti, regions of Georgia. Plants were collected and identified by Prof. Ts. Ghviniashvili (Ilia State University Botanical Institute). Both plants were collected in July 2018.

Extraction and fractionation. 500 g of each plant were dried and powdered to 1 mm particles. Powdered plants were extracted with 80% EtOH, using an ultrasonic water bath at 50 ˚C. Extracts were concentrated with a rotary evaporator to yield a total extract of *A. saxatile* (35.7 g) and *A. ponticum* (30.5 g). 15 g of dried extracts of each plant were subjected to Diaion HP-20 column chromatography. The mobile phase was H_2O-MeOH in gradient condition (100:0; 50:50; 0:100 v/v) and finally EtOAc to give 4 enriched fractions of each plant (**A.s.F1**_8.2g; **A.s.F2**_1.7g; **A.s.F3**_3.5g; **A.s.F4**_0.02g; **A.p.F1**_6.7g; **A.p.F2**_3.9g; **A.p.F3**_2.3; **A.p.F4**_0.2g).

In this following research were used total extracts of both plants, **A.s.tot** and **A.p.tot** and fractions: **A.sF2**; **A.s.F3**; **A.p.F2** and **A.p.F3.**

Animals. Outbred white mice weighing 24-28 g were obtained from the animal house of Tbilisi State Medical University I. Kutateladze Institute of Pharmacochemistry and quarantined for 1 week in the Department of Preclinical Pharmacological Research of above Institute. Animals were kept under standard conditions (temperature 20 ± 2°C, humidity 55-65%, 12/12-hour light/darkness cycle, granulated food - 4 g/animal/day, water ad libitum). All experiments were carried out in accordance with the requirements of the EU Directive 2010/63 [9]. Research protocol was authorized by the Tbilisi State Medical University Ethics Committee on Animal Research (approval #AP-56-2022).

Analgesic activity (Hot plate assay). The animals were individually placed in an open cylindrical space consisting of a metal floor heated to a temperature of 52 ± 1°C and transparent vertical walls. The time between placing the animal on the floor and the first nociceptive reaction (hind paw licking or jumping) was recorded as the hot-plate latency. The measurements were taken before and after the intraperitoneal administration of 50 mg/kg **A.s.tot** and **A.p.tot** extracts and fractions: **A.s.F2**, **A.s.F3**, **A.p.F2**, **A.p.F3** with 25 mg/kg and 50 mg/kg concentrations (baseline latency), as well as 30 minutes and one hour. The analgesic effect was calculated by the formula: $E\% = ((T_0-T_n)/T_0) \times 100$, where T_0 is the reaction time prior to the **A.s.tot** and **A.p.tot** extracts and fractions injection, and T_n - after the corresponding period (30 or 60 min) after injection, respectively [23].

Gastroprotective activity (Ethanol induced ulcer model). The experiment was carried out in accordance with the method described by Adinortey et al. [4]. In brief, 18 outbred mice were randomly distributed in three groups of animals, each consisting of six mice. 24 hours prior to the experiment the access to food was restricted, and animals were relocated in cages with raised floors of wide wire mesh to prevent coprophagy. During the fasting period, all mice received a nutritive solution of 8% sucrose in 0.2% NaCl to avoid excessive dehydration. On day 2 absolute ethanol was given orally (1 ml/100 g) to all animals. **A.s.tot** extract in a dose of 50 mg/kg, i.p. (Group III) and **A.p.tot** extract - 50 mg/kg, i.p. (Group II) was given 1 hour prior the ethanol administration. Mice of control group (Group I) got 0.2 ml of saline. Animals were euthanized by CO2 inhalation 1 hour after the ethanol administration. The stomachs were

immediately removed, opened along the great curvature, rinsed consequently with water and 10% formalin solution which contains about 4% formaldehyde w/v, fixed on white EPS foam board, and digitally photographed. Ulcer index (UI) was calculated for each stomach according to the following scale, by three independent observers: 1 - no lesions; 2 - single petechial lesions; 2.5 - multiple petechial or short linear hemorrhagic lesions; 3 - long linear hemorrhagic lesions; 4 - continuous linear hemorrhagic lesions along the entire length of the glandular part of stomach. The efficacy of **A.s.tot** and **A.p.tot** extracts expressed as percentage of ulcer inhibition (% I) was estimated based on the UI and calculated using the formula:

$$\% I = \frac{UI_C - UI_T}{UI_T} \times 100$$

Where UI_C and UI_T are macroscopic ulcer indexes in control and test groups, respectively.

Anti-inflammatory activity (Carrageenan induced paw oedema assay). 50µl of 1% carrageenan solution in saline was injected in the aponeurosis of the right hind paw of the animal. One hour prior the onset of oedema, 0.5 ml of saline and 0.5 ml of **A.s.tot** and **A.p.tot** extracts at a dose of 50 mg/kg are administered intraperitoneally to control and experimental animals, respectively. The thickness of the paw was measured with a digital micrometer before the carrageenan injection (baseline) and after 2 hours. Anti-inflammatory efficacy was calculated by the following formula: $E\% = (1-(\Delta T_{exp} / \Delta T_{con})) \times 100$, where ΔT_{con} and ΔT_{exp} are the mean differences in paw thickness before and 2 hours after carrageenan administration in control and experimental group animals, respectively [17].

Statistics. All data were processed statistically using one-way ANOVA Tukey test [11].

Results and discussion

Hot plate assay. The hot-plate latency in the experimental group was 11.5 seconds before the intraperitoneal administration of 50 mg/kg **A.s.tot** and **A.p.tot** extracts and 25 mg/kg and 50 mg/kg fractions: **A.s.F2**, **A.s.F3**, **A.p.F2**, **A.p.F3**. Analgesic activity of the *A. saxatile* total extract ascends with time and reaches its maximum at 60 min after the administration. Differently, *A. ponticum* total extract revealed faster onset of action but shorter duration of action. Hot-plate latency for the **A.s.tot** extract was 17.9 and 20 seconds on the 30th and 60th minutes after the injection, in case of **A.p.tot** extract, latency time was 23.7 and 23.3 seconds, respectively, yielding the analgesic effect 55.2 % after 30 minutes and 70.3% after 60 minutes for *A. saxatile* total extract, for *A. ponticum* the effect was 105.6 % and 81.3 % respectively.

Similar tendency was observed when studying the efficacy of fractions obtained from total extracts. Moreover, fractions obtained from both *A. saxatile* and *A. ponticum* showed dose-dependent activity (Fig.1, Table.1). Analysis of the obtained data, especially that **A.s.F3** fraction has even higher activity than total extract, allows to conclude that this fraction contains compound(s) responsible for analgesic effect.

Table 1. Analgesic effects of *A. saxatile* and *A. ponticum* total extracts and fractions.

	Baseline latency #	30 min	60 min
Control		11.6±2.6	11.8±2.4
A.s. tot (50 mg/kg ip)		17.9±5.6*	20.1±7.3**
A.s. F2 (25 mg/kg ip)		13.5±5.3	15.1±5.6
A.s. F2 (50 mg/kg ip)		16.6±2.3*	18.6±2.5**
A.s. F3 (25 mg/kg ip)		17.4±5.4	19.5±7.2*
A.s. F3 (50 mg/kg ip)	11.5±3.5	19.0±5.4*	21.2±6.7***
A.p. tot (50 mg/kg ip)		23.7±6.7**	21.3±6.4*
A.p. F2 (25 mg/kg ip)		14.6±2.9	13.2±2.6
A.p. F2 (50 mg/kg ip)		18.0±4.5*	16.2±4.4
A.p. F3 (25 mg/kg ip)		14.3±3.5	12.9±3.1
A.p. F3 (50 mg/kg ip)		18.3±2.5***	16.5±2.2*

Data in sec are represented as mean (n=6) ±S.E.M. #Baseline latency calculated as mean±S.E.M. from all groups; * - $p<0.05$; ** - $p<0.01$; * - $p<0.001$;

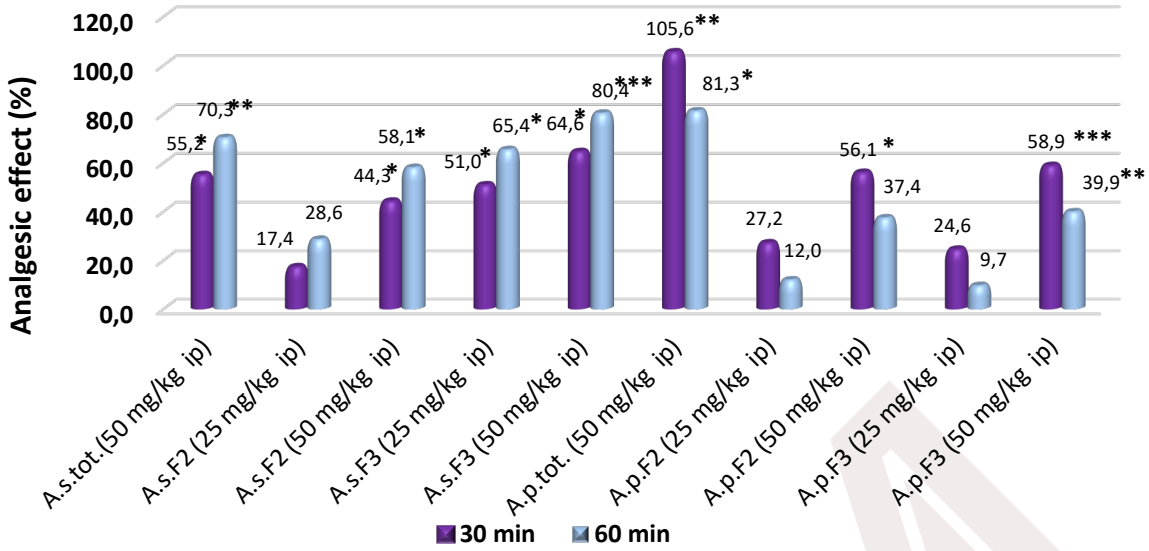


Figure 1. Analgesic effects of *A. saxatile* and *A. ponticum* total extracts and fractions.

Ethanol induced ulcer model. In untreated animals, administration of absolute ethanol induced marked gross mucosal lesions, including full length hemorrhagic streaks along the longitudinal axis of the glandular part of stomach and petechial lesions. (Fig.2, A I). In mice given 50 mg/kg i.p. **A.s.tot** and **A.p.tot** extract only single petechial lesions were present (Fig. 1 A III), whereas in animals pretreated with 50 mg/kg **A.p.tot** extract mostly continuous linear hemorrhagic lesions along the entire length of the glandular part of stomach, were observed (Fig. 2 A II). Correspondingly, the UI was significantly reduced in animals pretreated with **A.s.tot** extract (UI=1.44; %I=44, $p<0.05$) when compared with untreated mice (UI=2.57) and **A.p.tot** extract (UI=2.67; %I=-3.9), which, apparently, increased hemorrhagic lesions.

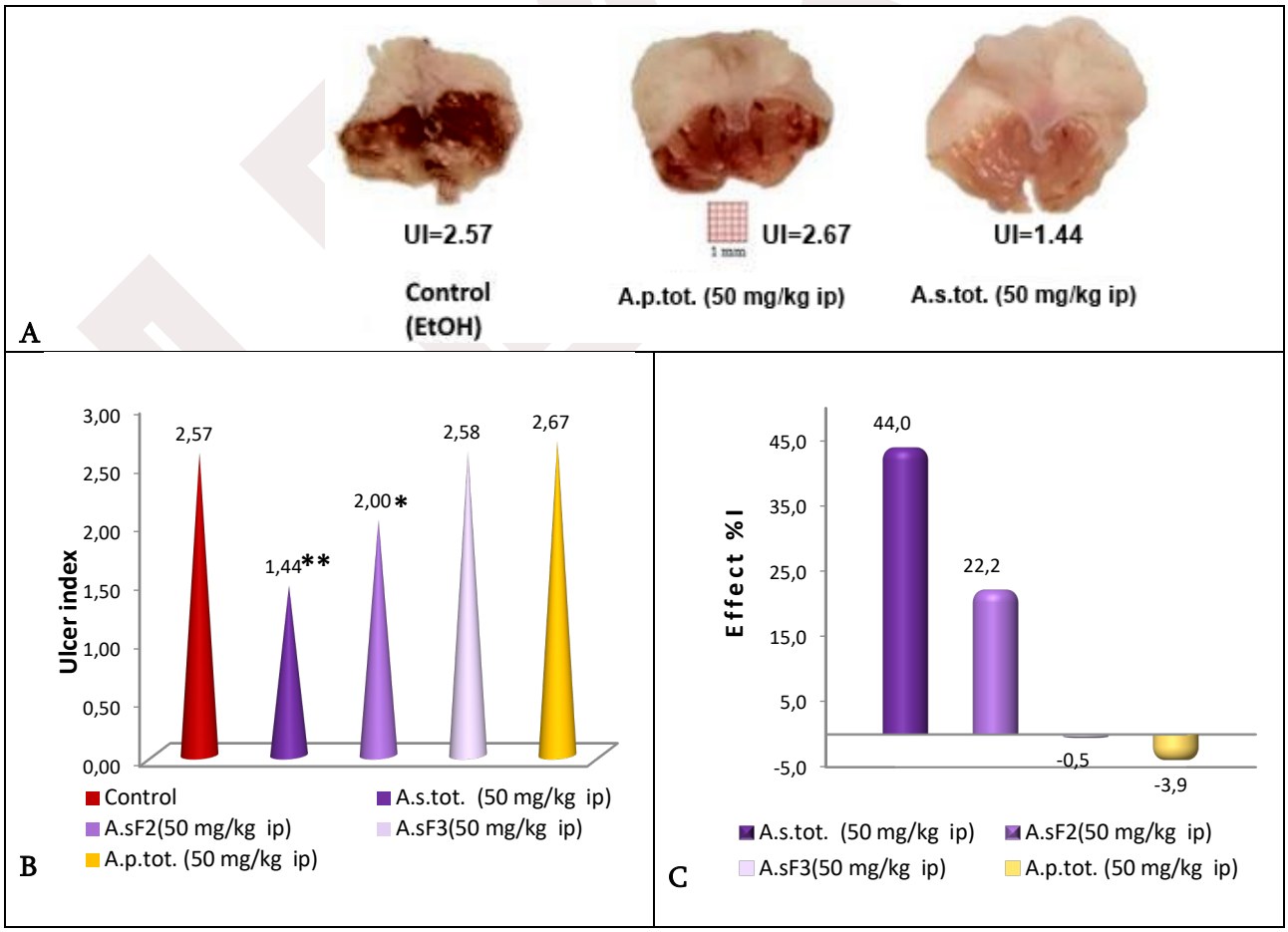


Figure 2. Gastroprotective effect of A.s. and A.p. total extracts. **A** – Ethanol induced ulcer lesions in control (I) and A.s. and A.p. total extracts treated (II and III) mice; **B** – Ulcer index (UI); **C** - Efficacy of extracts (%I). Data represented as mean (n=6). * - $p<0.05$; **- $p<0.01$

Taking above into consideration we proceeded only with fractions obtained from *A. saxatile* - **A.s.F2** and **A.s.F3** in a dose 50 mg/kg ip. The results showed that fraction A.s. F2 has a moderate gastroprotective effect - 22.2 % - whereas fraction **A.s.F3** appeared to be inactive. (Fig. 2. B, C).

Carrageenan oedema assay. In experimental animals, an increase in paw thickness 1 hour after the carrageenan administration, in groups that received **A.s.tot** extract and **A.p.tot** extract was 39.4 μm and 72.7 μm, respectively, which was less than in control group animals (80.2 μm). The efficacy of **A.s.tot** and **A.p.tot** extracts amounted to 63.5% and 32.7%, respectively, evidencing a notable anti-inflammatory activity of *A. saxatile* (Fig. 4. A, B). As *A. saxatile* extract was significantly active than *A. ponticum*, further study was continued only on the *A. saxatile* fractions **A.s.F2** and **A.s.F3**. and revealed their efficacy equal to 59.5% and 21.4% correspondingly. Thus, it is likelihood that fraction **A.s.F3** contains compound(s) responsible for the observed anti-inflammatory activity (Fig.4. A, B).

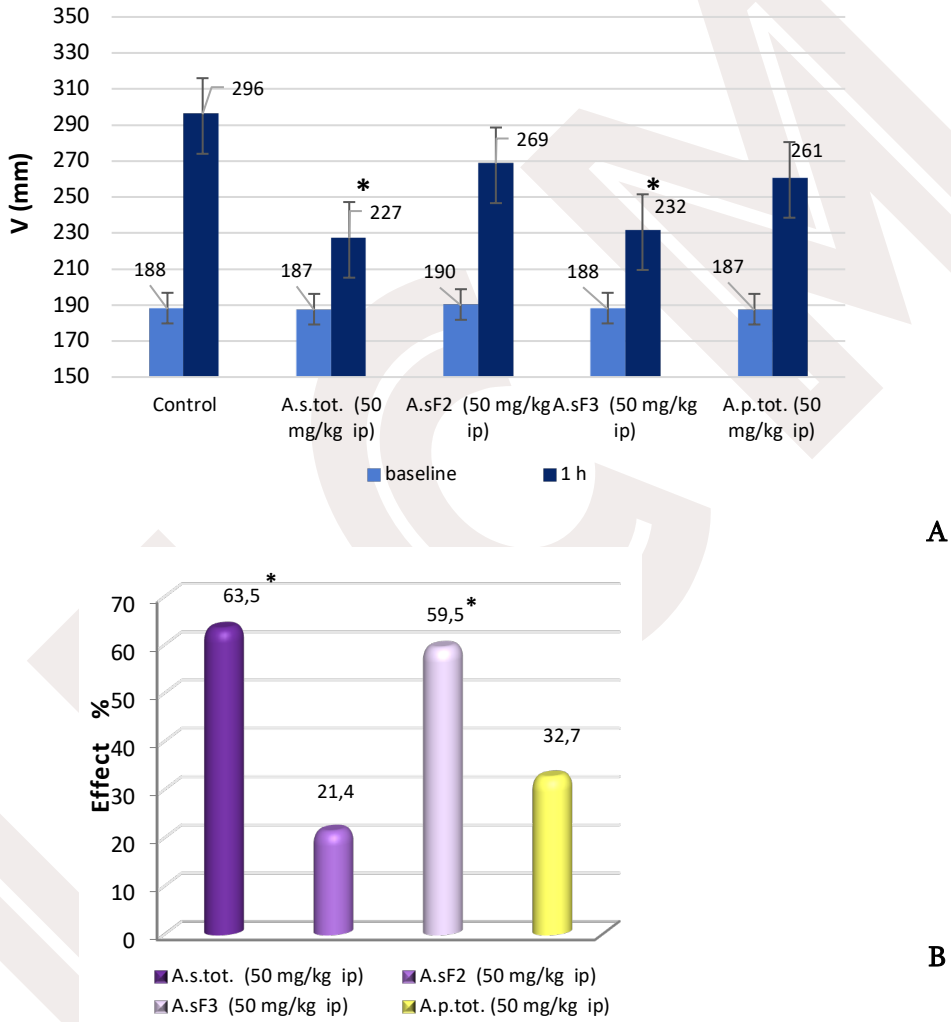
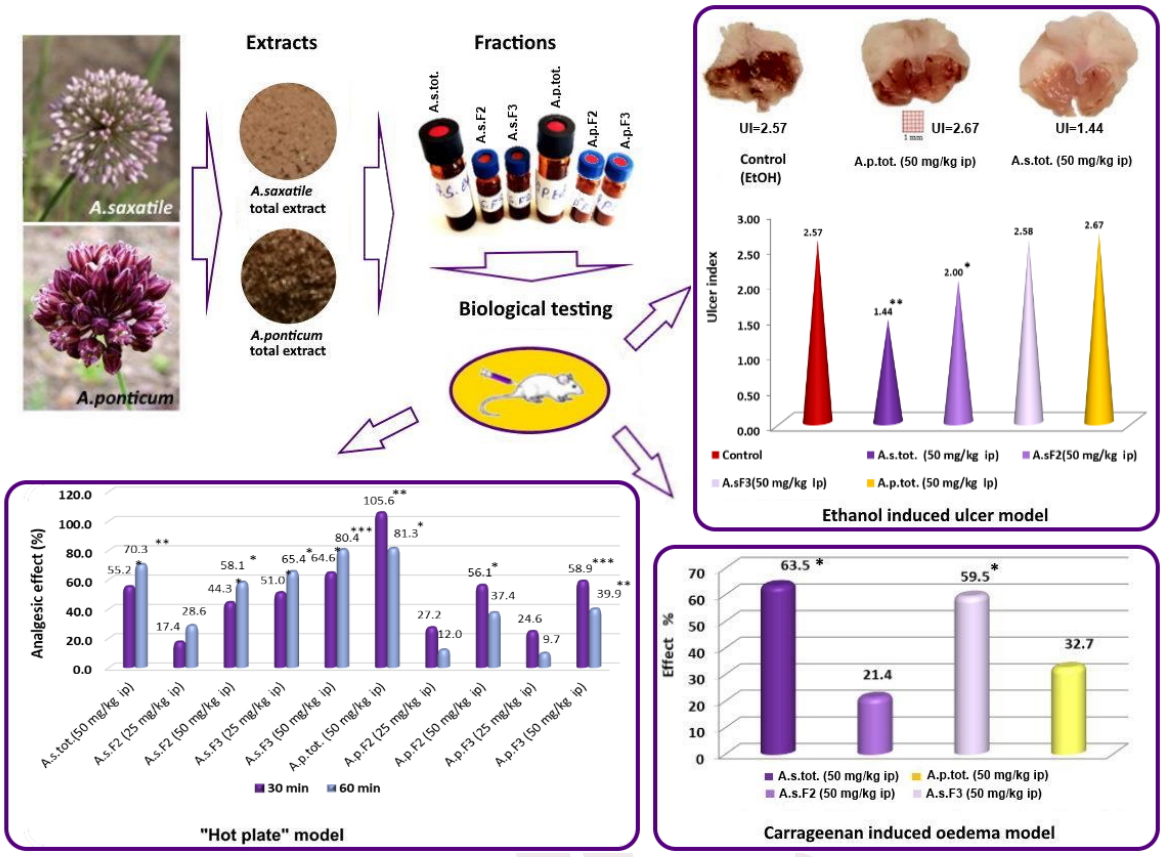


Figure 3. Anti-inflammatory effect of A.s. and A.p. total extracts and fractions. **A** - thickness of the paw, of control and experimental groups. **B** - Efficacy of the extracts and fractions. Data represented as mean (n=6). * - $p<0.01$ vs control group.

Conclusion

Comparative study of *A. ponticum* and *A. saxatile* pharmacological efficacy revealed the advantage of the latter, and the same tendency was noticed when separate fractions were studied. In particular, total extract of *A. saxatile* is characterized with marked analgesic and anti-inflammatory activity presumably because of A.S-F3 content, that well correlates with the worsening of gastric mucosa condition, suggesting having mechanism of action similar to the one of NSAIDs [29]. At the same time pronounced analgesic activity of *A. ponticum* should be taken into consideration, as well, but its mechanism of action remains unclear and thus needs further justification. Summarizing the results, it can be concluded that the study revealed *A. saxatile* and *A. ponticum* as a promising raw of bioactive compounds for drug development.

Graphical Abstract



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ФАРМАКОЛОГИЧЕСКАЯ ОЦЕНКА КОМПОНЕНТОВ ВИДОВ *ALLIUM SAXATILE* И *ALLIUM PONTICUM* ПРОИЗРАСТАЮЩИХ В ГРУЗИИ

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РЕЗЮМЕ

Задачей настоящего исследования была оценка фармакологической активности соединений из некоторых видов лука – *Allium saxatile* и *Allium ponticum*, произрастающих в Грузии. Анальгетическую, противовоспалительную и гастропротекторную активность общих экстрактов и фракций из них изучали на общепринятых *in vivo* моделях: горячая пластинка (hot plate), каррагинан-индуцированный отек и этанол-индуцированная язва желудка соответственно. Результаты экспериментов выявили ценные эффекты суммарных экстрактов и фракций, полученных из *A. saxatile* и *A. ponticum*. В частности, общий экстракт *A. saxatile* проявлял умеренно нарастающую пролонгированную анальгетическую активность (70,3%), тогда как общий экстракт *A. ponticum* оказал быстрый, высокий (105%), но кратковременный эффект. Эта тенденция сохранилась и при изучении фракций. Точно так же общий экстракт *A. saxatile* и его фракции более эффективно (63,5%), чем экстракт, полученный из *A. ponticum* (32,7%), уменьшали воспаление вызванное каррагинаном. В модели язвы желудка, вызванной этанолом, только общий экстракт *A. saxatile* и его фракции эффективно снижали язвенный индекс (UI) на 44%, тогда, когда экстракт *A. ponticum*, напротив, увеличивал язвенный индекс (UI). Обобщая результаты исследования, можно сделать вывод, что *A. saxatile* и *A. ponticum* представляют собой перспективное сырье для получения биоактивных соединений, используемых для разработки лекарственных средств.

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PHARMACOLOGICAL ASSESSMENT OF CONSTITUENTS OF SPECIES *ALLIUM SAXATILE* AND *ALLIUM PONTICUM* GROWING IN GEORGIA

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SUMMARY

The present study aimed to estimate pharmacological potency of products from some Allium species - *Allium saxatile* and *Allium ponticum*, growing in Georgia. Analgesic, anti-inflammatory and gastroprotective activity of total extracts and fractions from was evaluated using accepted in vivo models: hot-plate, carrageenan induced oedema and ethanol induced gastric ulcer, respectively. The results of the experiments have revealed valuable effects of the total extracts and fractions obtained from *A. saxatile* and *A. ponticum*. In particular, *A. saxatile* total extract demonstrated moderately ascending prolonged analgesic activity (70.3%), whereas total extract of *A.ponticum* exhibited rapid, high, but shortlasting effect (105%). Same tendency was noticed when fractions were studied. Similarly, *A. saxatile* total extract and its fractions appeared more effective (63.5%), than one obtained from *A.ponticum* (32.7%) in carrageenan induced paw oedema model. In ethanol induced gastric ulcer model only *A. saxatile* appeared to effectively reduce ulcer index (UI) by 44%, when *A. ponticum* extract, oppositely, increased ulcer index

(UI). Summarizing the results, it can be concluded that the study revealed *A.saxatile* and *A.ponticum* as a promising raw of bioactive compounds for drug development.

Keywords: *Allium saxatile*; *Allium ponticum*; Analgesic activity, Anti-inflammatory activity; Gastroprotective activity;