PharmacologyOnLine

Archives • 2021 • vol.2 • 105-112

# PHARMACOLOGICAL STUDY OF THE POLYPHENOL-CONTAINING PHYTOSUBSTANCE OBTAINED FROM THE ANISE HYSSOP HERB

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**Abstract**

Phytochemical and pharmacological studies of non-officinal species among essential oil-bearing plants are promising areas of modern Pharmacognosy. The aim of the research was the experimental study the pharmacological activities and safety of a dry extract obtained from the *Lophanthus anisatus* herb (LAE) and evaluation its polyphenols using high performance liquid chromatography (HPLC) method. *In vivo* studies found the dose-dependent anti-inflammatory and antipyretic activity of the LAE. Acute administration of the developed LAE in doses up to 5.0 g/kg did not induce any toxic reactions in experimental animals. The HPLC analysis of polyphenols in the LAE revealed the high amounts of rosmarinic acid and flavonoid apigenin-7-*O*-glucoside possessing significant therapeutic properties. Overall, the tested LAE is highly promising substance for further development of safe and effective herbal medicinal products for the treatment of the inflammatory diseases.

**Keywords**: *Lophanthus anisatus*, dry extract, HPLC analysis, toxicity, anti-inflammatory effect, antipyretic effect

## Introduction

Herbal medicinal products based on plant raw materials are a great source of pharmaceutics [1]. Phytochemical and pharmacological studies of non-officinal species among essential oil-bearing plants to create new phytopreparations are promising areas of the modern Pharmacognosy [2-4]. Phytosubstances are mostly multi-target drugs because of containing many of biologically active components [5, 6].

The representatives of the Mint (*Lamiaceae* Martinov) family are beneficial sources of phytoconstituents including terpenoids and polyphenols with valuable healing properties [7–9]. Such genus of this family as *Lophanhus* Adans. (synonym *Agastache* Clayton ex Gronov.) comprises 22 species of herbal plants which are common in North America and Southeast Asia [10]. These species are used in folk medicine for treatment mainly the problems of digestive and respiratory tract caused by inflammation, as melliferous or ornamental plants [11].

Anise Hyssop (*Lophanthus anisatus* (Nutt.) Benth.) has the North American origin and nowadays it is gradually spreading in Europe. Despite on availability of some experimental data regarding chemical composition and medicinal properties of this species [12–15], it is not included to the Europoean Pharmacopoeia [16]. The essential oil of Anise Hyssop was studied in the most details comparatively to the other biologically active substances of this plant [13, 14, 17]. In particular, American researchers [17] found that essential oil of *Agastache foeniculum* can accumulate 88–93% of estragol. Estragol (93.45%) was also the main component of Bolgarian *Agastache foeniculum* essential oil possessing significant antibacterial and antioxidant effects [13]. However, the valuable pharmacological effects of polyphenols and other metabolites of the *Lamiaceae* plants which are related to the antioxidant effect could be useful in the healing of inflammation, pain, cancer, etc. [4, 8].

Many chronic diseases lead to releasing reactive oxygen species, causing cells and tissues injury [18]. As a result, an inflammatory process develops, body temperature rises and pain occurs due to sensitizing of nociceptors [19]. In this regard, it is quite relevant to develop new herbal medicinal products with anti-inflammatory and antipyretic effects which could be used for the treatment of diseases of the oral cavity, upper respiratory tract, gastrointestinal tract, etc. The representatives of the *Lamiaceae* family are very promising for this purpose [20, 21].

The aim of the research was to study the pharmacological activities and safety of a dry extract obtained from the *Lophanthus anisatus* herb (LAE) and to evaluate its polyphenols chromatographically.

## Methods

*Plant material*

The aerial part of *Lophanthus anisatus* was harvested from the experimental plots in Ternopil region (Ukraine) during the flowering period, then dried and ground.

*Preparation of the LAE*

The LAE was obtained from the *Lophanthus anisatus* herb in two stages [22]. 100.0 g of the plant raw material was ground and passing through a sieve with a diameter of 1 mm. Then 50% ethanol was poured into the extraction flask (the ratio of raw material to extractant was 1: 2) and infused for 1 h at room temperature. After swelling of the raw material, it was extracted for 40 min with the same solvent (total ratio of raw material to extractant was 1:10) at the boiling point of the extractant. The obtained extract was placed for 24 h in the refrigerator for sedimentation of the ballast. 1.5 L of purified water was poured to the waste and it was extracted using a boiling water heater for 60 min (the raw material-extractant ratio was 1:15). The obtained extract was maintained for 24 h in the refrigerator. After decantation and filtration, each of the extracts were evaporated in a vacuum rotary evaporator to 1/10 of their primary volume. Then they were combined and dried to a dry extract (LAE). The developed dry extract was a hygroscopic brown powder with a specific pleasant 'anise' smell and a bitter-spicy taste. The yield of the finished product was 22.53 %.

*Chemicals*

The tablets of "Diclophenac" (produced by pharmaceutical factory "Chervona zirka", Ukraine) and "Acetylsalicylic acid" (produced by pharmaceutical factory "Darnitsa", Ukraine) were purchased from the pharmacies in Ukraine. Carrageenan and Tween-80 were purchased from

Sigma-Aldrich (Poland). All the reagents and solvents were of analytical grade. trifluoroacetic acid, acetonitrile and standards for high performance liquid chromatography (HPLC) were purchased from Merck (Germany).

*Chromatographic analysis*

The HPLC analysis of phenolic compounds was performed using Shimadzu HPLC-DAD system and Phenomenex Luna C18 column (250 x 4.6 mm x 5 µm) at 35 °C [4]. The gradient elution was riched by mixing the mobile phases I (0.1% trifluoroacetic acid in water) and II (0.1% trifluoroacetic acid in acetonitrile).

*Acute toxicity and pharmacological activities*

All the *in vivo* experiments were conducted according to [23]. Such animals as mice (20–25 g) and albino rats (180–220 g) were used for the experiments. The animals were kept in plastic cages; they were fed *ad libitum* and had free access to water. The acute toxicity and anti-inflammatory (AIA) studies of the LAE were conducted according to methods described by Shanaida et al. [4].

The antipyretic activity (APA) of the LAE was studied using male rats of both sexes in a model of aseptic inflammation named "milk fever" [24]. The increase in body temperature of the animals was provoked by subcutaneous administration of skim milk at a dose of 5 mL/kg (previously boiled and cooled to 37 °C). The investigated doses of the extract (50 mg/kg, 100 mg/kg and 200 mg/kg) shaken with 1% solution of Tween-80 in purified water were administered intragastrically in a prophylactic mode (in 1 h before pyrogen injection). Animals in the reference drug group were pretreated with the reference drug "Acetylsalicylic acid" (AcA) in dose 50 mg/kg. Animals in the control group were given the purified water. The rectal temperature was measured with an electronic thermometer in the dynamics of 1, 2, 3, 4, 5 and 6 h after administration of the tetsed LAE.

The APA (%) of the studied doses of the LAE and the reference drug was determined by the ability to reduce the body temperature of the animals in comparison with the control group. The calculation was performed according to the formula:

$$APA = \frac{(T_{pic} - T_{nc}) - (T_{teh} - T_{ten})}{T_{pic} - T_{nc}}$$

where: $T_{pic}$ – body temperature in the control group after the induction of inflammation (for a certain hour), °C;

$T_{nc}$ – initial (normal) body temperature in the control group, °C;

$T_{teh}$ – body temperature of the group of animals to which the extract or reference drug was administered (for a certain hour), °C;

$T_{ten}$ – initial (normal) body temperature of the animals to which the extract or reference drug was administered, °C.

Statistical analyzes were performed using the Statistica software, version 13.1 (StatSoft).

## Results and Discussion

The acute toxicity research of the LAE was recorded for 2-weeks post treatment of the experimental animals. It was found that LAE in tested doses of 0.5 g/kg, 1.5 g/kg and 5.0 g/kg did not provoke death or any side effects of rats. Thus, up to 5 g/kg, the LAE could be considered as safe.

Since fever and pain are the key signs of the inflammatory process in cause of traumas, infectious diseases, etc., searcing for alternative anti-inflammatory, analgesic and antipyretic medicines of natural origin which do not have the side effects of synthetic drugs is a very prespective [1, 18]. It is known that the water-ethanol mixtures and purified water are very effective for extracting complexes of polyphenolic compounds from the plant raw materials of the *Lamiaceae* representatives [2, 4, 13].

The results of the conducted study of the anti-inflammatory activity of LAE in the model of carrageenan edema are presented in Table 1. The AIA was manifested by LAE in a dose-dependent manner. The maximum antiexudative effect was shown by LAE at a dose of 200 mg/kg. Its highest antiexudative effect (41.38%) was observed for 3 h of the experiment, at the peak of inflammation. During this period it was close to reference-drug "Diclofenac" (48.28%). This indicates the possibility of cyclooxygenase conversion of arachidonic acid under the influence of biologiccally active substances of the tested extract.

It should be noted that different *Lamiaceae* representatives demonstrate promising antioxidant and anti-iflammatory properties. The significant antioxidant activity was found for the ethyl acetate extract of *Ocimum gratissimum* herb [7]. Pudziuvelyte et al. [8] revealed that extracts from the different parts of *Elsholtzia ciliata* possessed the antioxidant and anti-inflammatory activities. The ether extract of *Thymus serphyllum* herb produced maximum effect 34% inhibition against carrageenan paw edema in experimental animals at the end of 3 h [25]. *Thymus algeriensis* and *T. fontanesii* were also the interesting candidates for the healing such oxidative stress-related disorders like inflammation [9]. Machado et al. [20] established that extract of *Hyptis suaveolens* could protects colon from TNBS-induced inflammation via antioxidant, immunomodulatory, and anti-proliferative mechanisms. Czech reseachers [21] found that rosmarinic acid as the main component of *Prunella vulgaris* extract can suppress lipopolysaccharide-induced alteration in gingival fibroblasts of humans. The dried leaf powder of *Agastache foeniculum* aerial part increased the stress resistance and lifespan in *Drosophila melanogaster* by inhibiting free radical processes [15]. Presence of the Sage (*Salvia officinalis*) extract in the herbal-based toothpastes caused their antibacterial and anti-inflammatory properties [26].

The results of the antipyretic activity study of LAE in the model of milk fever are presented in Table 2. As can be seen from the results of Table 2, an increase in body temperature of animals in the control group was observed during all hours of the experiment; the peak of fever was recorded 5 h after the injection of pyrogen. The APA was manifested by tested LAE in a dose-dependent manner. The highest of the studied doses of LAE (200 mg/kg) showed the maximum antipyretic activity (66.26%) atr 6 h of the experiment.

It should be mentioned that species of the *Lamiaceae* family possess the significant antinociceptive and antipyretic properties. Such effects of the aqueous extract of *Mentha longifolia* leaf were found by Amabeoku et al. [27]. Hussain et al. [28] revealed the promising anti-inflammatory, analgesic and antipyretic activity of the 70% methanol extract of *Salvia moorcroftiana* leaves. The lyophilized aqueous extract (100 mg/kg) of *Thymus serphyllum* herb demonstrated the highest antipyretic activity when compared to ethanolic and ether ones [25].

To interpret the obtained data it was carried out the chromatographic analysis of phenolic compounds in the LAE. The component analysis of polyphenols in the LAE was performed using HPLC method (Table 3). The amounts of a lot of hydroxycinnamic acids and flavonoids were evaluated.

Polyphenols revealed in the tested LAE possess the significant antioxidant, anti-inflammatory, antipyretic, analgesic, and immunomodulatory potential and are playing a beneficial role in the prevention of chronic illnesses such as neurodegeneration, cancers, diabetes, obesity, and cardiovascular diseases [19, 29]. Rosmarinic acid was regarded as the major polyphenol of many *Lamiaceae* representatives [3, 4, 13, 21]. Rosmarinic acid (50.6 mg/g extract) and flavonoids luteolin (0.9 mg/g), and apigenin (0.6 mg/g) were detected as predominant components of *Agastache foeniculum* aqueous extract [13]. Rosmarinic acid (91.23 mg/g extract) and luteolin-O-7-glucoside (76.30 mg/g) prevailed in the dry extract obtained from *Monarda fistulosa* L. hydrodistilled residue by-product [4]. Sobeh et al. [9] concluded that the methanol extracts of the *Thymus fontanesii* and *Thymus algeriensis* leaves contained the rosmarinic acid as a major compound could demonstrate the significant anti-inflammatory and antipyretic effects. It can be speculated that LAE rich in rosmarinic acid and other polyphenols shows anti-inflammatory effect via the inhibition of cytokines [18, 19, 30].

Flavones as the predominant components of the developed LAE can inhibit the inflammatory response provoked by lipopolysaccharide [31]. Park and Song [31] found the anti-inflammatory properties of luteolin and its derivative luteolin-7-O-glucoside. It could be supposed that biologically active triterpenoids extracted from the *Lamiaceae* representatives using water-ethanol solutions possess the significant antioxidant, anti-inflammatory, antispasmodic, hepatoprotective, etc. properties and may synergistically affect the overall therapeutic influences of the phytosubstances containing polyphenols [32–34].


*ISSN: 1827-8620*

## Conclusions

*In vivo* studies shown the dose-dependent anti-inflammatory and antipyretic activity of LAE. Acute administration of the extract in doses 0.5-5.0 g/kg did not induce any toxic reactions in the experimental animals. The HPLC analysis of polyphenols in the obtained LAE revealed the high amounts of such polyphenols as hydroxycinnamic rosmarinic acid and flavonoid apigenin-7-*O*-glucoside with valuable therapeutic properties. The developed LAE has the healthful potential to be safely used for inflammation and fever. The findings of this study also suggest that the revealed anti-inflammatory and antipyretic properties provide a scientific evidence of the application of *Lophanthus anisatus* in ethnopharmacology.

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ISSN: 1827-8620

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**Table 1.** The anti-inflammatory effect of LAE on the model of carrageenan edema

| Treatment | Dose (mg/kg) | Increase of paw oedema | | | | | |
|---|---|---|---|---|---|---|---|
| | | After 1 h | | After 3 h | | After 6 h | |
| | | Df | % AIA | Df | % AIA | Df | % AIA |
| Control | - | 0.37±0.03 | - | 0.58±0.03 | - | 0.54±0.04 | - |
| LAE | 50 | 0.34±0.03[2] | 8.11 | 0.42±0.03[1,2] | 27.59 | 0.46±0.02[1] | 14.81 |
| | 100 | 0.31±0.01[1,2] | 16.22 | 0.36±0.02[1,2] | 37.93 | 0.43±0.02[1] | 20.38 |
| | 200 | 0.29±0.02[1,2] | 21.62 | 0.34±0.02[1,2] | 41.38 | 0.41±0.01[1] | 24.07 |
| Diclofenac | 8 | 0.2±0.01[1] | 45.94 | 0.3±0.02[1] | 48.28 | 0.43±0.03[1] | 20.37 |

Note: Df – difference in paw volume before and after injection of carrageenan; [1] – significantly difference compared to control group (p≤0.05); [2] – significantly difference compared to diclofenac group (p≤0.05).

**Table 2.** The antipyretic activity of LAE in a model of milk fever

| Treatment | Dose (mg/kg) | Temperature /APA | Indicators of body temperature by hours of the experiment | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Initial | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| Control | - | t, °C | 37.32±0.04 | 37.55±0.05[1] | 37,97±0.03[1] | 38.19±0.04[1] | 38.36±0.03[1] | 38.62±0.05[1] | 38.15±0.06[1] |
| LAE | 50 | t, °C | 37.22±0.03 | 37.45±0.03[1] | 37,86±0.04[1] | 38.06±0.05[1] | 38.13±0.02[1,2] | 38.25±0.04[1,2] | 37.84±0.02[1,2] |
| | | APA, % | - | 0 | 1.54 | 3.45 | 12.50 | 20.77 | 25.3 |
| | 100 | t, °C | 37.25±0.06 | 37.47±0.04[1,2] | 37,82±0.06[1,2] | 37.95±0.07[1,2] | 38.01±0.05[1,2] | 37.93±0.04[1,2] | 37.63±0.03[1,2] |
| | | APA, % | - | 4,35 | 12.31 | 19.54 | 26.92 | 47.69 | 54.22 |
| | 200 | t, °C | 37.16±0.02 | 37.38±0.05[1,2] | 37,71±0.04[1,2] | 37.85±0.06[1,2] | 37.82±0.06[1,2] | 37.69±0.03[1,2] | 37.42±0.04[1,2] |
| | | APA, % | - | 4.35 | 15.38 | 20.69 | 36.53 | 59.23 | 66,26 |
| AcA | 50 | t, °C | 37.15±0.06 | 37.36±0.04[1,2] | 37,55±0.06[1,2] | 37.63±0.03[1,2] | 37.49±0.04[1,2] | 37.38±0.08[1,2] | 37.26±0.05[1,2] |
| | | APA, % | - | 8.70 | 38.46 | 44.83 | 67.31 | 82.31 | 86.75 |

Note: [1] – the differences are statistically significant relatively to the initial temperature values (p≤0.05);
[2] – the differences are statistically significant for the values of the control pathology group (p≤0.05).

**Table 3.** Contents of phenolic compounds in LAE evaluated using HPLC analysis

| Compound | Retention time, min | Content, mg/g |
|---|---|---|
| Neochlorogenic acid | 14.8 | 2.40±0.05 |
| Catechin | 19.5 | 0.82±0.02 |
| Chlorogenic acid | 20.4 | 1.94±0.07 |
| Caffeic acid | 21.6 | 9.53±0.19 |
| Rutin | 30.9 | 1.40±0.02 |
| Hyperoside | 31.6 | 3.24±0.06 |
| Ferulic acid | 32.3 | 1.71±0.07 |
| Luteolin-7-*O*-glucoside | 33.1 | 13.73±0.19 |
| Apigenin-7-*O*-glucoside | 36.8 | 29.22±0.28 |
| Rosmarinic acid | 37.8 | 73.20±1.12 |
| Acacetin-7-*O*-glucoside | 45.8 | 0.51±0.02 |
| Quercetin | 46.6 | 2.33±0.08 |
| Luteolin | 47.0 | 3.92±0.14 |
| Apigenin | 52.4 | 8.10±0.22 |


*ISSN: 1827-8620*