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Review Article

THE PHARMACOLOGY OF *ANCHUSA ITALICA* AND *ANCHUSA STRIGOSA*. A REVIEW

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ABSTRACT

Anchusa italica and *Anchusa strigosa* belong to the Boraginaceae family were distributed in the temperate, especially in Mediterranean and tropical regions**.** Chemical studies showed that Anchusa italica contained alkaloids, tannins, oil, triterpenes and polyphenols, while, *Anchusa strigosa* contained aliphatic hydrocarbons, oil, proteins, pyrrolizidine alkaloids and polyphenols. Anchusa italica possessed many pharmacological effects; these included anticancer, antioxidant, antiviral, central nervous, endocrine and many other effects. On the other hand, gastric protective effect, antimicrobial, hypotensive and antidiabetic effects were recorded for Anchusa strigosa. The present review will highlight the pharmacological and therapeutic effects of *Anchusa italica* and *Anchusa strigosa*.

Keywords: *Anchusa italica*, *Anchusa strigosa,* Pharmacology

INTRODUCTION

Anchusa italica **(**cow's tongue plant, bugloss) and *Anchusa strigosa* **(**bugloss, alkanet**)** belong to the Boraginaceae family were distributed in the temperate, especially in Mediterranean and tropical regions [1-2]. *Anchusa italica* was used traditionally as stimulant, tonic, demulcent, in bilious complaints, fever, cough, and asthma and as diuretic in bladder and kidney stones [3-5]. It was also used as diaphoretic, narcotic, hypnotic, antiarthritis, anirheumatic and cathartic [6]. The leaves of the plant were used as decoction in cold, sore throat, and chest pain [7]. *Anchusa strigosa* was used as antiulcer, for wound healing, as a tonic and tranquilizer, as a diuretic and for abdominal pain. It also used as diaphoretic antipyretic, narcotic, antipyretic, antirheumatic, cathartic, hypnotic and antiarthritis [6, 8-11]. Anchusa italica contained alkaloids, tannins, oil, triterpenes and polyphenols, while *Anchusa strigosa* contained aliphatic hydrocarbons, oil, proteins, pyrrolizidine alkaloids and polyphenols. Anchusa italica possessed many pharmacological effects including anticancer, antioxidant, antiviral, central nervous, endocrine and many other effects. On the other hand, gastric protective effect, antimicrobial, hypotensive and antidiabetic effects were recorded for Anchusa strigosa. The aim of this review is to highlight the chemical constituents and the pharmacological and therapeutic effects of *Anchusa italica* and *Anchusa strigosa*.

I- Anchusa italica

Synonym: *Anchusa azurea* Mill.

Parts used medicinally: aerial part or leaves [6, 12].

Chemical constituents

The plant show positive tests for alkaloids and tannins, and it contained oil rich in vitamin E. The flowers yield anthocyanins and the leaf, stem yield bornesitol [3]. The seeds of Anchusa italica contained 21 %(v/w) oil.The gamma-linolenic acid represented 13% (v/v) of the oil and 2.7% (v/w) of the seeds [13].

The total lipid content of Anchusa italica leaves was 0.93 g / 100 g. It contained 16.59% saturated fatty acids, 3.15% monounsaturated fatty acids and 4.85% poly unsaturated fatty acids. Oil contained the following compounds : capric acid (0.07%), undecanoic acid (0.01%), lauric acid (0.07%), tridecanoic acid (0.01%), myristic acid (0.35%), myristoleic acid (0.16%), pentadecanoic acid (0.12%), palmitic acid (10.45%), palmitoleic acid (0.14%), heptadecanoic acid (0.22%), stearic acid (1.67%), oleic acid (2.20%), linoleic acid (12.16%), γ-linolenic acid (1.46%), α-linolenic acid (64.74%), arachidic acid (1.64%), eicosenoic acid (0.17%), cis-8.11.14-eicostrienoic acid (1.69%), cis-11.14.17-eicosatrienoic acid, heneicosanoic acid (0.21%), behenic acid (1.25%), eruicic acid (0.07 %), tricosanoic acid (0.02%), lignoceric acid (0.72%), and nervonic acid (0.40%)[14]. However, Conforti et al showed that Anchusa italica yield linoleic acid 2.57 % and linolenic acid 5.02 %. It also contained hydroalcoholic extracts 23.8 %, and the total phenolics content (using Folin– Ciocalteau method) was 85.5 chlorogenic acid equivalents (mg/g)[15]. *Anchusa italica* also contained triterpenes. Kuruüzüm isolated the old seven and four new triterpene glycosides, named oleanazuroside 1, oleanazuroside 2, ursolazuroside 1, and ursolazuroside 2 from the methanolic extract of the aerial parts of *Anchusa azurea* MILLER var. *azurea* [16]. Polysaccharides, poly[3-(3,4-dihydroxyphenyl) glyceric acid, alkaloids and saponins were also isolated from Anchusa italica[17-19]. The total phenolic contents of *Anchusa italica* aqueous extract was 12.3 and in methanolic extract was 16.2 (Gallic acid equivalents per g dry weight) [20].

Pharmacological effects

Anticancer and antioxidant effects

The cytotoxic activity of *Anchusa italic*a against MCF-7, HepG2, WEHI and MDBK cell lines was evaluated. IC_{50} was more than 100 µg/ml against all evaluated cell lines[12]. Anchusa italica is one of the thirteen plants contained in the Abnormal Savda Munziq of Traditional Uighur formula (ASMq), which used for the treatment and prevention of cancers. The effects of ethanol extract of ASMq on cultured human hepatoma cells (HepG2) was carried out to explore the mechanism of its putative anticancer properties by using many experimental methods including the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) bromide, neutral red and lactate dehydrogenase (LDH) leakage, the incorporation of ^{3}[H]-leucine and ^{3}[H]-nucleosides into protein, DNA and RNA, and quantifying the formation of malondialdehyde-thiobarbituric acid (MDA). ASMq ethanol extract significantly inhibited the growth of HepG2 and cell viability, increased the leakage of LDH after 48 hours or 72 hours treatment in a concentration and time dependent manner ($P < .05$). Cellular protein, DNA and RNA synthesis were inhibited in a concentration and time dependent manner ($P < .05$). No significant MDA release in culture medium and no lipid peroxidation in cells were observed. According to the results, the cytotoxic effects of ASMq ethanol extract might be related to inhibition of cancer cell growth, alteration of cell membrane integrity and inhibition of cellular protein, DNA and RNA synthesis[21].

The IC50 values of inhibition of nitric oxide (NO) production and cytotoxicity of *Anchusa italica* were 123 ug/ml and >1000 respectively. The IC50 value of free radical scavenging activity on DPPH of *Anchusa italica* was 84 ug/ml[15].

The butanol extract of *Anchusa italica* and two of the triterpenes compounds isolated by Kuruüzüm-Uz *et al*, produced strong free radical-scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH)[16]. The antioxidant activity of *Anchusa italica* aqueous extract was 83.3 and methanolic extract was 88.2 (Trolox equivalents per g dry weight) [20].

Central nervous and endocrine effects

Oral administration of Abnormal Savda Munsiq (ASMq) which contained *Anchusa italica*, also found to exert a memory-enhancing effect in the chronic stressed mice induced by electric foot-shock. The memory improvement of the stressed mice was shown by an increase of the latency time in the step-through test and the decrease of the latency time in the Y-maze test. Treatment with ASMq induced significant decrease the serum levels of adrenocorticotropic hormone, corticosterone and *β*-endorphin as well as the brain and serum level of norepinephrine. Furthermore, ASMq was able to significantly reverse the chronic stress by decreasing the brain and serum levels of the monoamine neurotransmitters dopamine, 5-hydroxytryptamine and 3, 4-dihydroxyphenylalanine [22].

Antiviral effects:

The antiinfluenza virus activity of aqueous and alcoholic extract of *Anchusa italica* plant (2.5-80 µg/ml) was investigated on the viral infected Madin-Darbey -Canine Kidney cell monolayer. *Anchusa italica* extracts possessed higher antiviral properties when used one hour before infection compared to their usage after infection. However, the antiviral effect of alcoholic extract was more pronounced than that of the aqueous preparation. The antiviral activity of *Anchusa italica* was likely due to interference with viral replication and transcription; accordingly *Anchusa italica* can be use such as amantadin for the treatment of influenza [1].

Other pharmacological effects:

The anti-inflammatory activity of different extracts from the aerial parts and the roots of *Anchusa italica* were investigated in rats using carrageenan-induced acute inflammation. The methanolic extract from the aerial parts, its *n*-butanol fraction, and rosmarinic acid, which was isolated from the *n*-butanol fraction of the methanol extract, showed significant dose-dependent anti-inflammatory activity. During the acute phase of inflammation, the anti-inflammatory activity of rosmarinic acid was comparable to that of ibuprofen [23].

The methanolic extract of Anchusa italica (200 µg/ml) was evaluated *in vitro* for their hormone sensitive lipase (HSL) inhibitory potential. It produced 57.41% inhibition, and their inhibition profile was dose-dependent. Further evaluation by estimating the IC50 values showed that IC_{50} of Anchusa italica methanolic extract was 132.8 µg/ml [24]. *Anchusa italica* boiling water extract (5%) inhibited the mean height of rabbit jejunum smooth muscle contractions to 35% in comparison with normal contractions [25]. The antiulcer activity of different extracts from the aerial parts and the roots of *Anchusa italica* were investigated. No antigastric ulcer activity was recorded in indomethacin-induced gastric damage in rats[23].

II- *Anchusa strigosa*

Parts used medicinally: roots and leaves [26]

Chemical constituents:

Phytochemical investigation of the flowers of *Anchusa strigosa* showed the presence of four aliphatic hydrocarbons in chloroform extract. Various carbohydrates were detected in the methanolic extract. The aqueous extract was rich in the free amino acids and proteins. The anthocyanidins malvidin and pelargonidin, present in the methanol-HCL extract, were responsible for the pink-violet color of the followers. The aqueous extract had pH 7 and ash content of 3.5 per cent of the dried material [27]. Four triterpenes were isolated from *A. strigosa* roots, these identified as oleanolic acid, beta-amyrin, crataegolic acid and beta-sitosteryl glucoside [2].

Pyrrolizidine alkaloids was isolated from *A. strigosa*, the highest total concentration was detected in the leaves (23.63 mg/g of dried part), followed by the flowers (19.77 mg/g), and finally by the roots (1.80 mg/g). These pyrrolizidine alkaloids included 7, 7′-bis-(4-hydroxy-3, 5-dimethoxyphenyl)-8, 8′-dihydroxymethyltetrahydrofuran 4′-*O*-β-*D*-glucopyranoside, rosmarinic acid, caffeic acid, tormentic acid 28-*O*-β-*D*-glucopyranoside, euscaphic acid 28-*O*-β-*D*-glucopyranoside, euscaphic acid, and allantoin. However Braca *et al* isolated new six pyrrolizidine alkaloids included a new carboxylic acid, a new phenolic and a new oleanane glycoside[28-29].

The total lipid of dry flowers of *Anchusa strigosa* was 4.4% (26.1% volatile oils and 52.8% fixed oils). It contained two phospholipids types, phosphatidy ethanol amine and one triglyceride compound (tripalmetin). The fats composition included (µg /100g dry weight), tetradecanoic 0.6424, pentadecanoic 0.7495, hexadecanoic 3.6404, heptadecanoic 1.2849, octadecanoic 4.6040, eicosanoic 0.7495, heneicosapentanoic 0.6424 and docosanoic 2.1414[30-31]. The total phenolic contents of *Anchusa strigosa* aqueous extract was 12.3 and methanolic extract was 16.2 (Gallic acid equivalents / g dry weight) [20].

Gastric protective effects:

Anti- ulcer activity of different root extracts of *Anchusa strigosa* was studied in ethanol-induced ulcer model in rats. Petroleum ether-soluble fraction was the most effective in reducing ulcer index and gave 91% protection. Chloroform soluble fraction gave 86% protection while butanol-soluble fraction was less effective (65% protection). On the other hand, water-soluble fraction was not effective in protecting the stomach from the ulcerative agent [2].

The ulcer index values expressed as a percentage of total stomach surface area affected by the ulcer was lowered when *Anchusa strigosa* root extracts was administration at a dose of 0.080 g prior to ethanol induction of stomach ulcer in rats. Healing of the induced ulcer in guinea pigs was achieved by oral administration of *Anchusa strigosa* root extracts at the therapeutic dose of extract of 0.286 g/kg body weight/day for 24 days [8].

A pepsin inhibitor (undetermined chemical composition) was isolated from the aqueous extracts of the roots of *Anchusa strigosa*. The extract of 1 g dry roots inhibited 9380±390 µg of pepsin [32].

Antimicrobial effects

The antibacterial activity of the extracted lipid of *Anchusa strigosa* against different bacteria strains has been investigated. This antibacterial effect was significant at different concentrations of the extracted lipids (0.01-10mg/ml). It appeared that *Anchusa strigosa* lipids were more effective against Gram positive microorganisms in comparison with Gram negative. The antibacterial activity against Gram positive as follow : *Streptococcus faecalis > Staphylococcus aureus >Bacillus sp*., while the effect against Gram negative was in the flowing sequent : *Pseudomonas aeruoginosa > Proteus sp. > E. coli > Enterobacter sp. > Klebsiella sp*.[30]

The volatile oil of *Anchusa strigosa* exhibited potent antibacterial activity against both Gram positive and Gram negative bacteria, especially in a high concentrations (200 and 500µgm /ml). On the other hand, the fixed oil showed good activity against *Klebsiella sp., Proteus sp*. and *Pseudomonos aeruginosa* especially at higher concentration (500µg /ml). However, the volatile oil showed greater inhibitory activity when compared to fixed oil [31]. The antibacterial activity of aqueous extracts of *Anchusa strigosa* was also studied on the fish bacterial pathogens including *Aeromonas hydrophila, Photobacterium damselae subspecies piscicida, Streptococcus iniae,* and *Vibrio alginolyticus*. A high inhibitory effect (14-19.5 mm) was produced by *Anchusa strigosa* aqueous extracts[33].

The aqueous extract of *Anchusa strigosa* ((15 mg ml^{-1} medium)) also exerted antifungal activity, the means of percentage of mycelial

inhibition against *M. canis, T. mentagrophytes* and *T. violaceum* were l50.1±9.84, 36.7±3.80, and 71.7±1.91 respectively[26].

Hypotensive effects

The aqueous extract of the plant (100 to 200 mg/kg, iv) in anaesthetized dogs produced hypotensive effect for more than half an hour after an initial transient rise. The hypotensive effect was not blocked by atropine or mepyramine maleate. The extract failed to modify the effect of acetylcholine, histamine or epinephrine. The extract has no significant effect on isolated frog heart. However, the extract was found to have slight inhibitory effect on the auricular contraction in bilaterally vagotomised dog but there was no effect on ventricular contraction in this animal. These results indicate that the site of action is probably blood vessel [34].

Antidiabetic effects

The antidiabetic activity of aqueous extract of flowers of *A. strigosa* was examined in streptozotocin induced diabetic rats. The aqueous extract of *A. strigosa* flowers in a dose of 250 mg/kg and 500 mg/kg orally for 30 days caused a dose-dependent fall in blood glucose and an improvement in serum insulin levels. Cholesterol and triglyceride levels showed significant reduction in comparison with diabetic control group. The extract also caused significant increase in hepatic glycogen levels [35].

Other pharmacological effects

The antioxidant activity was 66.7 *for* aqueous extract of *Anchusa strigosa* and 43.6 for methanolic extract (Trolox equivalents per g dry weight) [20]. The aqueous and ethanolic extracts of *Anchusa strigosa* were studied to inhibit aryl hydrocarbon hydroxylase activity (AHH) and ^{3}H-benzo [a] pyrene (^{3}H-BP) binding to rat liver microsomal protein. The aqueous extracts showed no inhibitory effect while the ethanolic extracts exhibited strong inhibitory effect on both AHH and ^{3}H-BP binding to the microsomal protein [36].

Contraindication and adverse effects

The intraperitoneal LD_{50} of *Anchusa strigosa* root extracts in mice was 0.080 g extract/kg body weight. Replacing of water intake by *Anchusa strigosa* root extracts of variable concentrations (2.865, 3.57 and 4.284 g/l) per animal per day for 90 days showed no histopathological changes in all organs of the rat [8]. Acute toxicity study revealed the non-toxic nature of the aqueous extract of *A. strigosa.* No mortality was observed in the extract treated rats, and observations showed the normal behavior of the treated rats. There was no lethality or any toxic reactions found with the selected dose (of 250 mg/kg and 500 mg/kg orally in rats for 30 days) [35]. The lethal dose in dog was found to be 4g/kg ip and in mice 2g/kg iv, which indicates a good safety margin [34].

CONCLUSION

The paper reviewed *Anchusa italica* and *Anchusa strigosa* as promising natural medicinal plants with wide range of pharmacological activities which could be utilized in several medical applications because of their effectiveness and safety.

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