



Discovering the Combined *In-vitro* Anti-inflammatory Effects of *Smilax glabra* Standardized Rhizome Extract and *Berberis aristata* Standardized Root Extract

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ABSTRACT

No combined anti-inflammatory perspectives of *Smilax glabra* rhizome extract and *Berberis aristata* root extract have been described in an *in vitro* approach, according to a search of standard worldwide scientific and pharmaceutical databases (human red blood cells, HRBC). *S. glabra* standardized rhizome extract (SGSRE) and *B. aristata* standardized fruit extract (BASFE) were tested combined to see whether they had any synergistic action. At a concentration of 250 µg/mL, SGSRE provided 28.63 percent membrane stabilization/protection in HRBCs, whereas BASFE provided 38.11 percent. When the extracts SGSRE and BASFE were combined (at a concentration of 250 µg/mL each), their anti-inflammatory effect was increased to 58.14 percent. The anti-inflammatory impact was boosted by 20 percent to 30 percent when both extracts were given at the same time. Saponin, tannin, terpenoid, flavonoid, alkaloid, steroid, phenolics, and other phytoconstituents found in plants mediate anti-inflammatory effects by inhibiting various inflammatory enzymes and helping to stabilize the HRBC membrane. This study will open the door for further research into developing new herbal formulations to treat acute and chronic inflammation.

Key Words: *Smilax glabra*, *Berberis aristata*, Extract, HRBC Method, In-vitro anti-inflammatory, Phytochemicals

INTRODUCTION

Inflammation is the production of white blood cells and a range of critical chemical mediators in order to defend us against harmful organisms such as germs. While the mechanism is required for survival, when it is exacerbated, it produces excruciating pain and misery. ^[1] Inflammatory disorders, such as inflammation, make people feel far more than they should and attack their own tissue. As a result, anti-inflammatory drugs are seldom required to relieve pain and the associated symptoms. ^[2] Enzymes including cyclooxygenase-1/2 (COX-1/2), lipoxygenase (LOX), prostaglandin dehydrogenase (PGDH), and others catalyze several biochemical steps in the inflammatory process, culminating in the generation of leukotrienes, which are important in acute inflammation. ^[3] Anti-inflammatory drugs, both steroidal and non-steroidal, that have capabilities of blocking these various mediators have piqued pharmacotherapeutic interest, but they haven't been extensively utilized for a number of reasons, including poor pharmacokinetics, unanticipated side effects, and so on. ^[4] Attempts are undertaken on a daily basis to address

these anti-inflammatory-related issues utilizing a variety of pragmatic approaches in order to develop an optimal drug. ^[5]

Smilax glabra Roxb., a rhizome of the Liliaceae plant that belongs to the Smilacaceae family and is known as Tu fuling in Chinese, has long been utilized as a food and traditional medicine in many nations. The genus *Smilax* has over 300 species, the majority of which are found mostly in tropical climates. *S. glabra* Roxb., which is endemic to various Southeast Asian countries, including China, India, Vietnam, and Thailand, prefers warm, moist shade, woodlands, shrubs, and river valleys below 1800 m. *S. glabra* Roxb. is found across Gansu Province and the Yangtze River Basin, from Taiwan to Hainan Island in China. Modern pharmacological studies show that the active compounds in *S. glabra* Roxb., such as flavonoids and flavonoid glycosides, organic acids and phenolic acids, steroids and steroid glycosides, phenylpropanoids and phenylpropanoid glycosides, phenolic and phenolic glycosides, other glycosides, and volatile oil, have a variety of pharmacological actions, including anti-inflammatory, cytotoxicity, anti-oxidant, immune-modulatory,

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antiviral, hepatoprotective, cardioprotective, and antibacterial. [6]

B. aristata is traditionally known for its properties such as Lekhaniya, which reduces toxicity and unnecessary fats, Arshoghna, which is anti-haemorrhoidal, Stanyasodhana, which is lactode purant, Ropana, which is a wound healer, Svedala, which promotes sweating, Rasayana, which is rejuvenative, Kandughna, which is anti-pruritic, Because the qualities of *B. aristata*, also known as Daruharidra, are similar to those of Turmeric, also known as Haridra, both plants have been referred to as Haridra dvaya, or two Haridrads, namely Haridra and Daruharidra. It's a common ingredient in Indian traditional medicine, where it's used to treat allergies, metabolic abnormalities, ophthalmic and other eye illnesses, and as a laxative. It is one of the 73 plants that have historically been used to cure skin disorders in Nepal and the neighboring communities. In certain rural areas of India, a multi-herbal combination incorporating *B. aristata* is used to cure bleeding piles. When ovariectomized (OVX) rats were tested for the aqueous methanol extract of *B. aristata*, the traditional anti-osteoporosis action was verified. These data show that ethnic usage in the treatment of osteoporosis, joint pain, and menopause may continue. Rasaut, a decoction of *B. aristata* leaves, is often used to cure skin illnesses, menorrhagia, diarrhea, cholera, jaundice, eye and ear infections, and urinary tract infections, according to ethnobotanical research. Bhotiya people in India's Himalayan regions employ root decoctions to cure eye ailments. In addition, it is used by Malani tribal people in Himachal Pradesh, India, to treat skin problems, jaundice, piles, and malaria. Its fruits are used as both a laxative and an anti-scorbutic. *B. aristata* is a psychomedicine used in the Garhwal Himalaya to cure exorcism in children. Jaundice may be treated using the root of the plant. The fruit and leaf juice of the plant are used to cure diarrhea and dysentery in Nepal, while its bark and root decoctions are used to treat jaundice and fever. The plant extract is used by certain Himalayan tribes in India's Sikkim and Darjeeling as anti-diabetic and anti-hepatopathy. [7]

No combined anti-inflammatory perspectives of *S. glabra* rhizome extract and *B. aristata* root extract have been reported in an *in vitro* approach, according to a search of standard worldwide scientific and pharmaceutical databases (human red blood cells method). *S. glabra* standardized rhizome extract (SGSRE) and *B. aristata* standardized fruit extract (BASFE) were examined together to see whether they have a synergistic action.

MATERIALS AND METHODS

Chemicals

Bangalore-based Sigma Aldrich® Ltd. provided the standard diclofenac sodium. The reagents, consumables,

and chemicals used in this study were purchased via a local distributor from HiMedia® India Pvt. Ltd. in Mumbai. In the experiment, a Borosil® (India) double-distilled water apparatus was employed.

Instruments

A Shimadzu® Ultraviolet-Visible Spectrophotometer (Model UV-1800, Japan) with a spectral bandwidth of 1 nm and wavelength precision of 0.3 nm, as well as a pair of 10 mm path duration aligned quartz cells, was used for spectroscopic analysis. An Accro Tech® electronic balance was used to measure the chemicals (Model AT-266-1, India).

Plant materials

Standardized *S. glabra* rhizome extract (10% - 20% Saponins) and standardized *B. aristata* root extract were available from S. A. Herbal Bioactive in Mumbai, Maharashtra (5 percent - 97 percent Berberine).

In-vitro anti-inflammatory activity

Based on the notion that the release of lysosomal enzymes during inflammation produces some disarray, the anti-inflammatory effects of EJSFE and PNSFE were studied using an *in vitro* approach. Because of their extracellular activity, acute inflammation is the most common disease among them. The ability of experimental medications can be measured by inhibiting these chemical mediators or stabilizing the lysosomal membrane. Because the membranes of human red blood cells and lysosomal membrane components are similar, the prevention of hypotonicity-induced membrane lysis in human red blood cells (HRBC) was used to examine anti-inflammatory benefits. Blood was taken from a healthy person who had not used any anti-inflammatory medication in the previous 15 days for this study. Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, 0.42% sodium chloride) was diluted and centrifuged at 3000 rpm in the same amount. Plasma was carefully separated and stored. The sealed blood corpuscles are cleansed in a 0.9% saline solution before being suspended in a 10% solution. Aliquots of plant extract were made with distilled water at 250 µg/mL concentrations. 1 mL phosphate buffer, 2 mL hyposaline (hyposaline), and 0.5 mL HRBC solution were added to each concentration. The contents were centrifuged for 20 mins at 3000 rpm after 30 mins of incubation at 37°C±1°C. Spectrophotometry at 560 nm was used to determine the hemoglobin content in the supernatant solution, using diclofenac sodium as a reference standard. In addition to the extract, a control was created. The percentage hemolysis was determined assuming 100% hemolysis in the test group. [8] The formula was used to compute the percentage of HRBC

membrane stabilization by plant extract:

$$\% \text{ Protection} = 100 - \frac{\text{OD of Drug treated sample}}{\text{OD of Control}} \times 100$$

Statistical analysis

The experiment was conducted three times in total. The information was presented as a mean standard deviation (MSD). Minitab® version 17 was used to complete the mathematical calculations. The difference between the monitoring and study groups was evaluated using the unpaired Student t-test (two-tailed) for pharmacological treatments.

RESULTS

When compared to the usual medicine diclofenac sodium, *in vitro* anti-inflammatory effectiveness showed that all extracts, both alone and in combination, had a substantial effect (74.24 percent). At a concentration of 250 µg/mL, SGSRE provided 28.63 percent membrane stabilization/protection in HRBCs, whereas BASFE provided 38.11 percent. When the extracts SGSRE and BASFE were combined (at a concentration of 250 µg/mL each), their anti-inflammatory effect was increased to 58.14 percent (Table 1). The anti-inflammatory impact was boosted by

20 percent to 30 percent when both extracts were given at the same time.

DISCUSSION

Hypotonicity suppression and heat-induced red blood cell membrane lysis were used to determine the anti-inflammatory impact. These findings might be explained by the presence of phytochemicals such as saponin, tannin, terpenoid, flavonoid, alkaloid, steroid, phenolics, and others, all of which have high anti-oxidant capabilities. When lysosomal components are lyzed during inflammation, enzymes are generated, which may exacerbate a variety of diseases. Anti-inflammatory drugs work by preventing the release of lysosomal enzymes or by stabilizing the membranes that surround lysosomes. Because human red blood cell membranes and lysosomal membranes are so similar, the ability of thorn extract to prevent hypotonicity-induced HRBC membrane lysis was used to test anti-inflammatory potential. The extract's high phenolic and flavonoid content combined in a synergistic manner to stabilize the HRBC membrane. Flavonoids, for example, are thought to reduce inflammation by blocking important inflammatory mediators. [8]

Table 1: *In vitro* anti-inflammatory potential of *Smilax glabra* standardized rhizome extract and *Berberis aristata* standardized fruit extract combination.

Treatment	Concentration (µg/mL)	Absorbance (560 nm)	% Protection [#]
Control	-	0.559 ± 0.002	-
<i>Smilax glabra</i> standardized rhizome extract (SGSRE)	250	0.399 ± 0.003 ^{***a}	28.63
<i>Berberis aristata</i> standardized fruit extract (BASFE)	250	0.346 ± 0.001 ^{***a}	38.11
SGSRE + BASFE	250 + 250	0.234 ± 0.002 ^{***a}	58.14
Diclofenac sodium	100	0.144 ± 0.004 ^{***a}	74.24

All values represent mean ± SD of n = 3; ***p < 0.001 with respect to the control group. ^aDetermined as compared with the control group (solution of 0.9% sodium chloride) using the above formula. [#]% protection offered by the extract or standard refers to the prevention of hypotonicity-induced HRBC membrane lysis.

CONCLUSION

At a concentration of 250 µg/mL, a blend of standardized *Smilax glabra* rhizome extract and standardized *Berberis aristata* root extract demonstrated a considerable anti-inflammatory effect *in vitro*. Phytochemicals found in plant products such as saponin, tannin, terpenoid, flavonoid, alkaloid, steroid, phenolics, and others play a key role in mediating anti-inflammatory action by blocking numerous inflammatory enzymes. This research will open the way for further research into developing new herbal components for the treatment of acute inflammation as well as chronic inflammation.

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CONFLICT OF INTEREST

The authors declare no Conflict of Interest regarding the publication of the article.

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