Abstract

Scrophularia species have been used since ancient times as folk remedies for some medical treatments including scrofula, scabies, tumours and inflammatory affections. Some compounds isolated from these species, such as iridoids and phenylpropanoids, are considered responsible for these activities. This review summarizes mainly the biological activity associated with this genus.

Keywords: Biologically active substances, iridoids, phenylpropanoids, Scrophularia, Scrophulariaceae.

Introduction

The genus Scrophularia, consisting of about 300 species, is one of the most important genera belonging to the Scrophulariaceae. Many species belonging to this genus have been used since ancient times as folk remedies for some medical treatments (scrophulas, scabies, tumours, eczema, psoriasis, inflammatory affections, etc.) (Heather & Henderson, 1994; Paris & Moyse, 1976). One species, Scrophularia ningpoensis (officially indexed drug in the Chinese Pharmacopoeia), is even cultivated as a medicinal plant in China. It has been used for the treatment of fever, swelling, constipation, pharyngitis, neuritis, and laryngitis in traditional Chinese medicine (Miyazawa et al., 1998). Scrophularia grossheimi and S. nodosa have been used as diuretic plants in traditional medicine (Akhmedov et al., 1969; Schaunbenger & Paris, 1977) and S. oldhamii has been used as an antipyretic and for the treatment of inflammation (Won Sick Woo, 1963). Other species have been used as antiinflammatory, antihypertensive, and antihypercholesterolemic agents (Kajimoto et al., 1989).

According to the literature, many Scrophularia species have been investigated and found to contain many classes of secondary metabolites including iridoids, phenylpropanoids, phenolic acids, flavonoids and saponins. Some of these compounds were shown to have antiinflammatory, antibacterial, hepatoprotective, immuno-modulator, cardiovascular, diuretic, protozoocidal, fungicidal, molluscicidal, cytotoxic, cytostatic, antitumour activities (Ghisalberti, 1998; Bermejo Benito et al., 1998; Emam et al., 1997; Nishibe, 1994; Lacaille-Dubois & Wagner, 1996).

A survey of the presently available chemical and biological data suggests that the iridoid glycosides are the main classes of substances of interest to pharmacologists, and it was suggested that the therapeutic action of these plants depends on the presence of iridoids (Ghisalberti, 1998). A number of reports have been published which demonstrate that iridoids possess a number of biological properties, such as choleretic, vasoconstrictor, hepatoprotective, antiviral and antimicrobial activities, and these compounds could act synergically with other active substances (Tables 1 and 2).

Antiinflammation

Most species belonging to the Scrophularia genus have been used as antiinflammatory drugs by folk medicine. Iridoids and phenylpropanoids are considered to be the active principles of these drugs (García et al., 1996; Fernández et al., 1998).

The roots of Scrophularia ningpoensis have been used for the treatment of inflammation in Chinese traditional medicine (Kajimoto et al., 1989). This plant has also been prescribed as an antipyretic and antiinflammatory in diseases causing heat or fever, dry cough and pulmonary tuberculosis. This antiinflammatory effect has been demonstrated in animals (Qian et al., 1991). The hydrophilic extract of the roots from S. ningpoensis had intense antiinflammatory activity with the carrageenin-induced rat paw model for edema. From this species were isolated harpagide (1), harpagoside (2), aucuboside (3), 6-O-methylcatalpol (4), ningpogenin (5), ningpogoside A (6), ningpogoside B (7),
Table 1. Activities of some species from the *Scrophularia* genus.

<table>
<thead>
<tr>
<th>S. auriculata</th>
<th>S. frutescens</th>
<th>S. ningpoensis</th>
<th>S. scorodonina</th>
<th>S. nodosa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HEPATOPROTECTIVE</strong></td>
<td></td>
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<tr>
<td><strong>IMMUNOMODULATOR</strong></td>
<td></td>
<td></td>
<td>(Kajimoto et al., 1989; Ody, 1993)</td>
<td>(Karimova et al., 1966)</td>
</tr>
<tr>
<td><strong>CARDIOVASCULAR</strong></td>
<td></td>
<td></td>
<td>(Fernandez et al., 1993)</td>
<td>(Akhmedov et al., 1969; Schaubnenger &amp; Paris, 1977)</td>
</tr>
<tr>
<td><strong>DIURETIC</strong></td>
<td></td>
<td></td>
<td></td>
<td>(Martin et al., 1998; Emam et al., 1997)</td>
</tr>
<tr>
<td><strong>PROTOZOOCIDAL</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>FUNGICIDAL AND MOLLUSCICIDAL</strong></td>
<td>(Garcia et al., 1998)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ANTITUMOR CYTOTOXIC AND CYTOSTATIC</strong></td>
<td>(Miyazawa et al., 1998; Pinkas et al., 1994; Liu &amp; Wu, 1993)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NEUROPROTECTIVE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ANTIPRURITIC</strong></td>
<td></td>
<td></td>
<td>(Tohda et al., 2000)</td>
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<tr>
<td><strong>OTHERS</strong></td>
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Verbascoside (8), angoroside A (9) and angoroside C (10) (Kajimoto et al., 1989; Zhang et al., 1994; Qian et al., 1992). Pharmacological investigations are necessary for evaluating their potential as antiinflammatory agents.

*S. auriculata* is a medicinal plant used in traditional medicine against inflammatory skin diseases. Its aqueous alcohol extract showed a marked effect on both acute and chronic models of inflammation (Cuellar et al., 1998).

Two catalpol derivates \([6-O\alpha-L-(2\"-O-acetyl-3\",4\"-O-di-p-methoxycinnamoyl-rhamnopyranosyl)-catalpol (11)\) and \(6-O\alpha-L-(4\"-O-acetyl 2\",3\"-O-di-p-methoxycinnamoyl)-rhamnopyranosyl-catalpol (12)\), isolated from *S. auriculata* L., exert high antiinflammatory activity on mouse ear edema induced by tetradecanoylphorbol acetate (TPA). When these products were assayed at the same dose as indomethacin (0.5 mg/ear), they exhibited nearly the same effect as this reference drug with an edema percentage inhibition of 73.4% (Giner et al., 1991).

Two saponins, verbascosaponin A (13) and verbascosaponin (14), and two iridoids, scropolioside A (15) and
Biologically active substances from *Scrophularia*

<table>
<thead>
<tr>
<th>S. canina</th>
<th>S. sambucifolia</th>
<th>S. koelzii</th>
<th>S. grosheimi</th>
<th>S. buergeriana</th>
<th>S. oldhamii</th>
<th>S. scopolii</th>
</tr>
</thead>
</table>

Scrovalentinoside (16), isolated from *S. auriculata* ssp. *pseudoauriculata*, assayed with various acute and chronic experimental models, showed antiinflammatory activity against all the inducers with the exception of arachidonic acid (Giner et al., 2000). Both saponins significantly inhibited mouse paw edema induced by single and multiple doses of 12-O-tetradecanoylphorbol 13-acetate (TPA). Verbascosaponin A (13) showed a potency twice as high as that of indomethacin in the acute TPA model. Verbascosaponin A (13) and scropolioside A (15) were active after a long latency period against ethyl phenylpropiolate edema, as are glucocorticoids. When the putative corticoid-like mechanism of the two compounds was studied, verbascosaponin A (13) activity was notably reduced by the mRNA synthesis inhibitor, actinomycin D, while the effect of scropolioside A (15) was partially blocked by the anti-glucocorticoid drugs used. Both iridoids were active on the delayed-type hypersensitivity reaction. They significantly reduced the inflammatory lesion and suppressed cellular infiltration.

The aqueous extract and harpagoside (2) isolated from *S. frutescens* L. were tested for antiinflammatory activity on rat paw edema. The results obtained showed that the aqueous
Table 2. Bioactive compounds, parts or fractions and activities derived from *Scrophularia* species.

<table>
<thead>
<tr>
<th>Plant sources</th>
<th>Bioactive compound, parts or fraction and activities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(11–16) Iridoids and saponins (antiinflammatory), plant, hidroalcoholic extract</td>
<td>(Giner et al., 1991, 2000; Cuellar et al., 1998)</td>
</tr>
<tr>
<td><em>S. buergeriana</em></td>
<td>Chloroform methanol extract from roots, phenylpropanoids (neuroprotective)</td>
<td>(Kim &amp; Kim, 2000)</td>
</tr>
<tr>
<td><em>S. frutescens</em></td>
<td>Aqueous extract, phenolic acids, iridoid (antiinflammatory)</td>
<td>(Garcia et al., 1996; Fernandez et al., 1996, 1998)</td>
</tr>
<tr>
<td></td>
<td>Aqueous extract of the aerial parts (ashes), flavonoids and saponins (diuretic)</td>
<td>(Fernandez Arche et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>Phenolic acids (antitumor, cytotoxic and cytostatic)</td>
<td>(Garcia et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>Aerial part, phenolic acids (antibacterial)</td>
<td>(Fernandez et al., 1996)</td>
</tr>
<tr>
<td><em>S. grossheimi</em></td>
<td>Plant (diuretic)</td>
<td>(Akhmedov et al., 1969; Schaumbenger &amp; Paris, 1977)</td>
</tr>
<tr>
<td></td>
<td>Extract, flavonoids (cardiovascular)</td>
<td>(Akhmedov et al., 1969)</td>
</tr>
<tr>
<td></td>
<td>Flavonoid fraction (hepatoprotective)</td>
<td>(Akhmedov et al., 1969)</td>
</tr>
<tr>
<td><em>S. koelzii</em></td>
<td>Alcoholic extract, chloroform fraction from alcoholic extract of the aerial parts, iridoids (hepatoprotective and immunomodulator)</td>
<td>(Garg et al., 1994)</td>
</tr>
<tr>
<td></td>
<td>Alcoholic extract of the whole plant, iridoid (CNS depressant)</td>
<td>(Bhandari et al., 1992)</td>
</tr>
<tr>
<td></td>
<td>Phenolic acid (hepatoprotective)</td>
<td>(Swiatek, 1970)</td>
</tr>
<tr>
<td></td>
<td>Infusion, saponin (cardiovascular)</td>
<td>(Karimova et al., 1966)</td>
</tr>
<tr>
<td></td>
<td>Infusion (inhibited of motor activity)</td>
<td>(Karimova et al., 1966)</td>
</tr>
<tr>
<td></td>
<td>Plant (diuretic)</td>
<td>(Akhmedov et al., 1969; Schaumbenger &amp; Paris, 1977)</td>
</tr>
<tr>
<td><em>S. ningpoensis</em></td>
<td>(1–10) Roots, hydrophilic extract, iridoids and phenolic acids (antiinflammatory)</td>
<td>(Kajimoto et al., 1989; Quian et al., 1991, 1992; Zhang et al., 1194)</td>
</tr>
<tr>
<td></td>
<td>Methanol extract from roots (antipruritic)</td>
<td>(Tohda et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>Aqueous extract (cardiovascular)</td>
<td>(Kajimoto et al., 1989; Ody, 1993)</td>
</tr>
<tr>
<td></td>
<td>(33, 38–40) Plant, methanol extract from roots and phenolic acids (antitumoral, cytotoxic and cytostatic)</td>
<td>(Miyazawa et al., 1998; Pinkas et al., 1994; Liu &amp; Wu, 1993)</td>
</tr>
<tr>
<td><em>S. oldhamii</em></td>
<td>(33) Ethanol extract from roots, phenolic acid (antibacterial)</td>
<td>(Won Sick Woo, 1963)</td>
</tr>
<tr>
<td></td>
<td>Plant (antiinflammatory)</td>
<td>(Won Sick Woo, 1963)</td>
</tr>
<tr>
<td><em>S. sambucifolia</em></td>
<td>(17–23, 31, 32) Aerial part, phenolic acids (antibacterial)</td>
<td>(Fernandez et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>Aqueous extract of the aerial parts (ashes), flavonoids and saponins (diuretic)</td>
<td>(Fernandez Arche et al., 1993)</td>
</tr>
<tr>
<td><em>S. scorpolii</em></td>
<td>(9) Phenylpropanoid glycoside (antitumor, cytotoxic and cytostatic)</td>
<td>(Saracoglu et al., 1997)</td>
</tr>
<tr>
<td><em>S. scorodonia</em></td>
<td>(24) Methanol extract from flowers, Buddlejasaponin I (protozoocidal, fungicidal and molluscicidal)</td>
<td>(Martin et al., 1998; Emam et al., 1997)</td>
</tr>
</tbody>
</table>
Biologically active substances from *Scrophularia*

Extract from this species can be considered as a potential mild anti-inflammatory agent on an acute inflammation process, although harpagoside is not considered the principal responsible of the anti-inflammatory effect (García et al., 1996). Probably, other bioactive substances are involved, such as phenolics acids, previously isolated from the aqueous extract of this species (Fernández et al., 1996), since it has been reported already that some of these compounds have anti-inflammatory action (Fernández et al., 1998). These authors showed that *p*-coumaric (17), caffeic (18), ferulic (19), gentisic (20), protocatechuic (21), syringic (22) and isovanillic (23) acids isolated from *S. frutescens* are moderate systemic anti-inflammatory agents but have a strong anti-inflammatory effect when applied locally at the site of

![Figure 1. Structures and sources of compounds from *Scrophulania* ssp.](image-url)
Figure 2. Structures and sources of compounds from *Scrophulania* ssp.
Biologically active substances from *Scrophularia*

Figure 3. Structures and sources of compounds from *Scrophulania* ssp.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>R</th>
<th>SPECIES</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>(11) 6-O-α-L-(2″-O-acetyl-3″, 4″-O-di-p-methoxy-cinnamoyl-rhamnopyranosyl)-catalpol</td>
<td>R¹ = CH₃-CO-&lt;br&gt;R² = R³ = p-CH₃O-C₆H₄-CH = CH-CO-</td>
<td><em>S. auriculata</em></td>
<td>(Giner et al., 1991)</td>
</tr>
<tr>
<td>(12) 6-O-α-L-(4″-O-acetyl 2″, 3″-di-p-methoxycinnamoyl)-rhamnopyranosyl-catalpol</td>
<td>R¹ = R² = p-CH₃O-C₆H₄-CH = CH-CO-&lt;br&gt;R³ = CH₃-CO-</td>
<td><em>S. auriculata</em></td>
<td>(Giner et al., 1991)</td>
</tr>
<tr>
<td>(27) 6-O-α-L-(3″-O-acetyl-2″-trans-O-cinnamoyl)-rhamnopyranosyl-catalpol (Scorodioside)</td>
<td>R¹ = C₆H₅-CH = CH-CO-&lt;br&gt;R² = CH₃-CO-&lt;br&gt;R³ = H</td>
<td><em>S. scorodonia</em></td>
<td>(Bermejo Benito et al., 2000)</td>
</tr>
<tr>
<td>(28) 6-O-α-L-(2″-O-acetyl-3″, 4″-di-O-trans-cinnamoyl)-rhamnopyranosyl-catalpol (Scropolioside B)</td>
<td>R¹ = CH₃-CO-&lt;br&gt;R² = R³ = C₆H₅-CH = CH-CO-</td>
<td><em>S. scorodonia</em></td>
<td>(Bermejo Benito et al., 2000)</td>
</tr>
<tr>
<td>(15) 6-O-α-L-(2″, 4″-di-O-trans-cinnamoyl)-rhamnopyranosyl-catalpol (Scrovalentinoside)</td>
<td>R¹ = R² = CH₃-CO-&lt;br&gt;R³ = CH₃O-C₆H₄-CH = CH-CO-</td>
<td><em>S. auriculata</em></td>
<td>(Giner et al., 2000)</td>
</tr>
<tr>
<td>(34) 6-O-α-L-(4″-O-acetyl-2″, 3″-di-O-cinnamoyl)-rhamnopyranosyl-catalpol (Koelzioside)</td>
<td>R¹ = R² = C₆H₅-CH = CH-CO-&lt;br&gt;R³ = H</td>
<td><em>S. koelzii</em></td>
<td>(Garg et al., 1994)</td>
</tr>
<tr>
<td>(35) 6-O-α-L-(3″-O-p-methoxycinnamoyl)-rhamnopyranosyl-catalpol</td>
<td>R¹ = R² = CH₃-CO-&lt;br&gt;R³ = p-CH₃O-C₆H₄-CH = CH-CO-</td>
<td><em>S. koelzii</em></td>
<td>(Garg et al., 1991)</td>
</tr>
</tbody>
</table>

Inflammation. In the topical model, the best antiinflammatory activity might be afforded by compounds related to benzoic acid derivatives, compounds with methoxy group substitution at C-3 or C-5, or both. After topical administration, the compounds, especially syringic (22), protocatechuic (21) and ferulic (19) acids, showed good antiinflammatory activity. Their effect on leukocyte migration to the inflamed site might be an important aspect of their mechanism of action. It seems that the phenolic acids that are the active principles of some orally administered medicinal plants might merely
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>R</th>
<th>SPECIES</th>
<th>REFERENCES</th>
</tr>
</thead>
</table>
| (17) p-coumaric | R¹ = CH = CHCOOH  
R² = R³ = H  
R³ = OH | S. sambucifolia  
S. frutescens | (Fernandez et al., 1996)  
(Fernandez et al., 1996)  
(Fernandez et al., 1998)  
(Garcia et al., 1996)  
(Garcia et al., 1998)  
(Kim & Kim, 2000) |
| (18) Caffeic   | R¹ = CH = CHCOOH  
R² = R³ = OH  
R³ = H | S. sambucifolia  
S. frutescens  
S. buergeriana | (Fernandez et al., 1996)  
(Fernandez et al., 1996)  
(Fernandez et al., 1998)  
(Garcia et al., 1996)  
(Garcia et al., 1998)  
(Kim & Kim, 2000) |
| (19) Ferulic    | R¹ = CH = CHCOOH  
R² = OCH₃  
R³ = OH  
R⁴ = H | S. sambucifolia  
S. frutescens  
S. nodosa  
S. buergeriana | (Fernandez et al., 1996)  
(Fernandez et al., 1996)  
(Fernandez et al., 1998)  
(Garcia et al., 1996)  
(Garcia et al., 1998)  
(Paris & Moyse, 1976)  
(Schaunbenger & Paris, 1977)  
(Vigneau, 1985)  
(Swiatek, 1970)  
(Rombouts & Links, 1956)  
(Kim & Kim, 2000) |
| (22) Syringic  | R¹ = CH = CHCOOH  
R² = R³ = OCH₃  
R³ = O-glucose | S. buergeriana  
S. sambucifolia  
S. frutescens  
S. buergeriana | (Fernandez et al., 1996)  
(Fernandez et al., 1996)  
(Fernandez et al., 1998)  
(Garcia et al., 1996)  
(Garcia et al., 1998)  
(Kim & Kim, 2000)  
(Kim & Kim, 2000) |
| (33) Methoxycinnamic | R¹ = CH = CHCOOH  
R² = R³ = H  
R³ = OCH₃ | S. ningpoensis  
S. oldhamii  
S. buergeriana | (Miyazawa et al., 1998)  
(Won Sick Woo, 1963)  
(Kim & Kim, 2000) |
| (38) Trans-cinnamic | R¹ = CH = CHCOOH  
R² = R³ = H  
R⁴ = H | S. ningpoensis  
S. buergeriana | (Miyazawa et al., 1998)  
(Kim & Kim, 2000) |
| (39) 3,4-dimethoxycinnamic | R¹ = CH = CHCOOH  
R² = R³ = OCH₃  
R⁴ = H | S. ningpoensis | (Miyazawa et al., 1998) |
| (40) 4-hydroxy-3-methoxycinnamic | R¹ = CH = CHCOOH  
R² = OCH₃  
R³ = OH  
R⁴ = H | S. ningpoensis | (Miyazawa et al., 1998) |
| (45) p-methoxycinnamic methylester | R¹ = CH = CHCOOCH₃  
R² = R³ = H  
R⁴ = OCH₃ | S. buergeriana | (Kim & Kim, 2000) |

Figure 4. Structures and sources of compounds from Scrophulania ssp.
Biologically active substances from *Scrophularia*

![Chemical structure](image)

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>R</th>
<th>SPECIES</th>
<th>REFERENCES</th>
</tr>
</thead>
</table>
| (20) Gentisic | $R_1 = R_4 = OH$  
$R_2 = R_3 = H$ | *S. frutescens*  
*S. sambucifolia* | (Fernandez et al., 1996)  
(Fernandez et al., 1998)  
(Fernandez et al., 1996) |
| (21) Protocatechuic | $R_1 = R_3 = H$  
$R_2 = R_4 = OH$ | *S. frutescens*  
*S. sambucifolia* | (Fernandez et al., 1996)  
(Fernandez et al., 1998)  
(Garcia et al., 1996)  
(Garcia et al., 1998) |
| (23) Isovanillic | $R_1 = R_4 = H$  
$R_2 = OCH_3$  
$R_3 = OH$ | *S. frutescens*  
*S. sambucifolia* | (Fernandez et al., 1996)  
(Fernandez et al., 1998)  
(Garcia et al., 1996)  
(Garcia et al., 1998) |
| (31) $p$-Hydroxybenzoic | $R_1 = R_2 = R_4 = H$  
$R_3 = OH$ | *S. frutescens*  
*S. sambucifolia* | (Fernandez et al., 1996)  
(Fernandez et al., 1996) |
| (32) Vanillic | $R_1 = R_4 = H$  
$R_2 = OCH_3$  
$R_3 = OH$ | *S. frutescens*  
*S. sambucifolia* | (Fernandez et al., 1996)  
(Fernandez et al., 1996) |

Figure 5. Structures and sources of compounds from *Scrophularia* ssp.

...and synergistically with other active substances, for example, harpagoside (2), isolated from *S. frutescens*, might be another bioactive compound involved in its action (Fernández et al., 1998).

Buddlejasaponin I (24), a biologically active compound from *S. scorodonia* L., exerts potent *in vivo* antiinflammatory effects on mouse ear edema induced by phorbol myristate acetate (PMA). The screening for *in vitro* effects of this saikosaponin on cellular systems generating cyclooxygenase (COX) and lipoxygenase (LOX) metabolites showed a significant effect. These data support the inhibition of arachidonic acid metabolism as one of the biochemical mechanisms that might be the rationale for the putative antiphlogistic activity of this saikosaponin (Bermejo Benito et al., 1998).

Seven iridoid glycosides isolated from different extracts of *S. scorodonia* L., namely bartsioside (25), aucuboside (3), harpagide (1), harpagoside (2), 8-0-acetyl-harpagide (26), scorodioside (27) and scropolioside B (28), have been evaluated for their *in vitro* antiinflammatory activity in cellular systems generating cyclooxygenase (COX) and lipoxygenase (LOX) metabolites (Bermejo Benito et al., 2000). Iridoids did not show a cytotoxic effect even at the higher concentration of 100μM. Most compounds assayed did not exhibit any significant effect on prostaglandin E$_2$ (PGE$_2$) and leukotriene C$_4$ (LTC$_4$) released from calcium ionophore-stimulated mouse peritoneal macrophages. In the PGE$_2$-release assay, only harpagoside (2) and 8-0-acetyl-harpagide (26) showed an inhibition rate of 30–40%. In the LTC$_4$ assay, only aucuboside (3) showed a significant effect, with a IC$_{50}$ value of 72μM. Harpagoside (2) and harpagide (1) also inhibited release of LTC$_4$, but it was not a very significant effect. However, most iridoids assayed showed a significant effect on thromboxane B$_2$ (TXB$_2$) release from calcium ionophore-stimulated human platelets, with inhibition percentages slightly lower than the reference drug ibuprofen. Only harpagide (1), scorodioside (27) and scropolioside B (28) had no significant effect on TXB$_2$-release. These results indicated that selective inhibition of thromboxane synthase enzyme may be the primary target of action of most of these iridoids, and one of the mechanisms through which they exert their antiinflammatory effects. This result does not contradict information in the literature, where some authors found negative results with *in vivo* models after oral administration (Lanhers et al., 1992) and other authors obtained positive results in topical processes (Recio et al., 1994). Further conclusions about iridoid structure-activity relationships are that substitutions with an additional moiety at C-8...
is a positive chemical feature for thromboxane-synthase activity \([8-O\text{-acetylharpagide (26)}\) and harpagoside (2) versus harpagide (1)]. The presence of a foreign moiety at C-6 is unfavourable, as is apparent when the activity of bart-sioside (25) (iridoid with no hydroxyl substituents in the body of the molecule, and only active on thromboxane synthase) is compared with scorodioside (27) and scropolioside B (28), which are inactive.

\( S. nodosa \) has also been considered to possess antiinflammatory properties. From this species have been isolated aucuboside (3), catalpol (29), catalposide (30), ferulic acid (19) and ester derivatives of harpagide. These compounds could be responsible of the activity, together with other substances (Paris & Moyse, 1976; Schaunbenger & Paris, 1977; Vigneau, 1985). \( S. oldhamii \) has been used traditionally in medicine for the treatment of inflammation (Won Sick Woo, 1963).

\begin{tabular}{|c|c|c|c|}
\hline
\textbf{COMPOUND} & \textbf{R} & \textbf{SPECIES} & \textbf{REFERENCE} \\
\hline
(13) Verbascosaponin A & \( R_1 = \text{Rha}\ (1\rightarrow4) \text{Glc}(1\rightarrow3)(\text{Glc}(1\rightarrow2))\text{Fuc} \) & \textit{S. auriculata} & (Giner et al., 2000) \\
\hline
\end{tabular}

\begin{tabular}{|c|c|c|c|}
\hline
\textbf{COMPOUND} & \textbf{R} & \textbf{SPECIES} & \textbf{REFERENCES} \\
\hline
(14) Verbascosaponin & \( R = \text{H} \) & \textit{S. auriculata} & (Giner et al., 2000) \\
(24) Buddlejasaponin I & \( R = \text{OH} \) & \textit{S. scorodonia} & (Emam et al., 1997) \\
& & & (Bermejo Benito et al., 1998) \\
\hline
\end{tabular}

\begin{tabular}{|c|c|c|}
\hline
\textbf{COMPOUND} & \textbf{SPECIES} & \textbf{REFERENCE} \\
\hline
(36) 5,7,3’-trihydroxy-4’-flavone (tetrahydroxy-m-hydroxyflavone) & \textit{S. grossheimi} & (Akhmedov et al., 1969) \\
\hline
\end{tabular}
Biologically active substances from Scrophularia

Antibacterial

*S. nodosa*, *S. auriculata* and *S. canina* have been used since the Middle Ages as a remedy for scrophulas and several dermatoses (scabies, tumours) (Paris & Moyse, 1976). Also, different reports have suggested that the antiseptic properties of these species could be attributed to the presence of phenolic acids (Swiatek, 1970, 1973; Van Hellemont, 1986). At present, *S. nodosa* is considered by several authors to possess bacteriostatic properties (Schaunbenger & Paris, 1977; Swiatek, 1970). The following glycosides have been isolated from this species: aucuboside (3), catalpol (29), catalposide (30), ferulic acid (19) and ester derivates of harpagide (1).

The therapeutic properties of the Figwort, *S. nodosa*, probably depend of these constituents. For example, aucuboside (3) and ferulic acid (19) possess antibacterial properties (Rombouts & Links, 1956; Davini et al., 1986; Fernández et al., 1998).

The phenolic fractions of the aerial parts of *S. frutescens* and *S. sambucifolia* showed potent antibacterial activity (Fernández et al., 1996). However, *S. frutescens*, the species most rich in phenolic acids, demonstrated a more pronounced activity than *S. sambucifolia*. The phenolic fractions of both species showed more activity against Gram-positive bacteria, specifically against *Bacillus* sp. The higher concentration of the phenolic compounds detected in *S. frutescens* could explain the more potent activity of this species. These preliminary results suggest that the antibacterial activity of these species can be attributed to the presence of phenolic acids [ferulic (19), isovanillic (23), p-hydroxybenzoic (31), syringic (22), caffeic (18), gentisic (20), protocatechuic (21), p-coumaric (17) and vanillic (32) acids]. Therefore, these species could be considered as potentially antiseptic agents on bacteriologic infections, especially in processes where Gram-positive bacteria are involved (Fernández et al., 1996). Methoxycinnamnic acid (33), isolated by EtOH extract from roots of *S. oldhamii*, showed good antipyretic action. Both *S. frutescens*, the species most rich in phenolic acids, demonstrated a more pronounced activity than *S. sambucifolia*. The phenolic fractions of both species showed more activity against Gram-positive bacteria, specifically against *Bacillus* sp. The higher concentration of the phenolic compounds detected in *S. frutescens* could explain the more potent activity of this species. Therefore, these species could be considered as potentially antiseptic agents on bacteriologic infections, especially in processes where Gram-positive bacteria are involved (Fernández et al., 1996). Methoxycinnamnic acid (33), isolated by EtOH extract from roots of *S. oldhamii*, showed good antipyretic action. Both *S. frutescens* and the EtOH extract from *S. oldhamii* exhibited good antipyretic activity when tested on typhoid-vaccinated rabbits (Won Sick Woo, 1963).

Hepatoprotective

The alcoholic extract of the aerial parts of *S. koelzii* significantly protected against thioacetamide-induced hepatic damage (Garg et al., 1994). This extract was fractionated with different solvents (hexane, chloroform, butanol and water). Hepatoprotective activity was localized in the chloroform fraction from which four iridoid glycosides, namely, scropolioside A (15), koelzioside (34), harpagoside (2) and 6-O-α-L-(3”-O-p-methoxyinnamoyl)-rhamnopyranosyl-catalpol (35), were isolated. Among these, scropolioside A (15) showed the greatest hepatoprotective activity. The activity of this compound was comparable to that of silymarin, a clinically used hepatoprotective drug. The important observation, however, is that the greatest protection was achieved with the alcoholic extract only. Hence, it is likely that the hepatoprotective activity of *S. koelzii* may not be due to any single constituent. Rather, there may be

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>R</th>
<th>SPECIES</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>(41) Buergeris A</td>
<td>R1 = CH3-CO-</td>
<td>S. buergeriana</td>
<td>(Kim &amp; Kim, 2000)</td>
</tr>
<tr>
<td></td>
<td>R2 = (E)p-CH3O-C6H4-CH = CH-CO-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R3 = (E)p-CH3O-C6H4-CH = CH-CO-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(42) Buergeris B</td>
<td>R1 = CH3-CO-</td>
<td>S. buergeriana</td>
<td>(Kim &amp; Kim, 2000)</td>
</tr>
<tr>
<td></td>
<td>R2 = (E)p-CH3O-C6H4-CH = CH-CO-</td>
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<tr>
<td></td>
<td>R3 = OH-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(43) Buergeris C</td>
<td>R1 = CH3-CO-</td>
<td>S. buergeriana</td>
<td>(Kim &amp; Kim, 2000)</td>
</tr>
<tr>
<td></td>
<td>R2 = (Z)p-CH3O-C6H4-CH = CH-CO-</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>R3 = OH-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(44) Buergeris D</td>
<td>R1 = OH-</td>
<td>S. buergeriana</td>
<td>(Kim &amp; Kim, 2000)</td>
</tr>
<tr>
<td></td>
<td>R2 = OH-</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>R3 = (E)p-CH3O-C6H4-CH = CH-CO-</td>
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</table>

Figure 7. Structures and sources of compounds from *Scrophulania* ssp.
certain other constituents acting synergistically for a better activity profile.

Ferulic acid (19), isolated from S. nodosa, has been shown to possess cholalogue properties (Swiatek, 1970) and the flavonoid fractions of S. grossheimi showed weak but positive cholalogue activity (Akmedov et al., 1969).

**Immunomodulating**

Scroplioside A (15), koelzioside (34), harpagoside (2) and 6-O-α-L-(3″-O-p-methoxy-cinnamoyl)-rhamnopyranosyl-catalpol (35), were isolated from the chloroform soluble fraction of an alcoholic extract of the aerial parts of S. koelzii. This fraction was shown to have immunomodulating activity (Garg et al., 1994). Therefore, an immunostimulant response was observed with all the four iridoids. Maximum induction of immune response with respect to all the parameters studied (macrophage migration index, haemagglutinating antibody titre and plaque forming cell) was observed with harpagoside (2) and 6-O-α-L-(3″-O-p-methoxy-cinnamoyl)-rhamnopyranosyl-catalpol (34) when administered intraperitoneally. The immunostimulant activity observed with pure glycosides was of nearly equal intensity and suggests that the catalpol nucleus of the iridoid is responsible for this activity. The hepatoprotective activity and immunostimulant activity of these four iridoids seem to be complimentary.

Some of the catalpol glycosides are reported to show immunostimulant activity (Pandey & Das, 1988) and these compounds are present in a number of species from the Scrophulariaceae genus (Bhandari et al., 1992, 1997; Calis et al., 1993; De Santos et al., 1998; Giner et al., 1998; Zhang et al., 1992).

**Cardiovascular**

In sedated rabbits, cats and dogs, an infusion of S. nodosa considerably reduced arterial pressure, stimulated respiration, caused bradycardia, lengthened the PQ segment (interval between auricle and ventricle contraction) and changed the configuration of the T wave (representing repolarization of the ventricles) of the electrocardiogram. S. nodosa increased the amplitude and slowed down the frequency of contractions of an isolated frog heart. This activity of S. nodosa apparently could be due to the saponins present in the plant extract (Karimova et al., 1966). However, the dependence of the cardiovascular activity on these compounds is still obscure.

Small doses of an extract from S. grossheimi showed hypotensive activity and increased capillary tonicity. Different flavonoid aglycones, mainly 5,7,3’-trihydroxy-4’-flavone (36) and its derivates (tetrahydroxy-m-hydroxyflavones), have been isolated from this species (Akmedov et al., 1969).

Some authors (Kajimoto et al., 1989; Ody, 1993) have attributed hypotensive activity to the aqueous extract of S. ningpoensis. However, pharmacological investigations are necessary for assessing the potential as a hypotensive agent.

**Diuretic**

S. nodosa and S. grossheimi have been used in traditional medicine as diuretic agents (Akmedov et al., 1969; Schaumbenger & Paris, 1977). The aqueous extract and the ash from the aerial parts of S. frutescens and S. sambucifolia subsp. sambucifolia have also shown pronounced diuretic activity. The increase in urine volume was more significant with S. frutescens than with S. sambucifolia. Flavonoids and saponins, which are present in the extract tested, were suggested as being responsible for the activity (Fernández Arche et al., 1993).

**Protozoocidal, fungidal and molluscicidal**

The methanol extract from flowers of S. scorodonia revealed protozoocidal activity against Trichomonas vaginalis (LC$_{100}$ = 100μg/ml) and Leishmania infantum (LC$_{100}$ = 250μg/ml) (Martin et al., 1998). From this extract, a saikosaponin, buddejasaponin I (24), has been isolated. The biological activity of this saikosaponin was evaluated in vitro as a protozoocidal agent against Trichomonas vaginalis and Leishmania infantum, as a molluscicidal agent against Biomphalaria alexandrina snails, and as a fungicidal agent against nine yeast strains. The results showed that the saponin killed 100% of the snails at 10μg/ml within 24 h, whereas the LC$_{100}$ values against Trichomonas, Leishmania and the nine yeast strains were 20, 40, and 100μg/ml, respectively (Emam et al., 1997).

**Antitumour, cytotoxic and cytostatic**

Several phenylpropanoid glycosides were found to show antitumour activity. Angoroside A (9) isolated from S. scopolii showed cytotoxic and cytostatic activities, but the methylated derivates [angoroside B (37) and C (10)] isolated from the same species did not show any cytotoxic activity at 1–200μg/ml concentrations against cancer cell lines. Angoroside A (9) (1–50μg/ml) exhibited cytostatic activity against HeLa cells (human epithelial carcinoma) and also showed slight cytotoxic activity at higher concentrations (>50μg/ml) against HeLa cells. Angoroside A (9) exhibited cytostatic activity against S-180 cells (sarcoma) at 1–40μg/ml concentrations. This compound, at a concentration of 12.7 μg/ml, showed cytostatic activity against P-388/D1 cells (mouse lymphoid neoplasma). By contrast, when angoroside A (9) was used at 119μg/ml, it showed cytotoxic activity (biphasic effect). Phenylpropanoid glycosides did not show any cytotoxic effects against primary-cultures of rat hepatocytes. Against dRLh-84 cells (rat hepatoma), angoroside A (9) exhibited significant cytotoxic activity. As a result, the cytotoxic and cytostatic activities of phenylpropanoid glycosides were found to be mainly dependent on the ortho-dihydroxy aromatic systems in their structures. Methylation of at least one of the phenolic hydroxyl groups abolished the activity which may explain why the methylether derivatives,
angorosides B (37) and C (10), completely lost their activities (Saracoglu et al., 1997). The mechanism by which phenylpropanoid glycosides exhibit these activities is under investigation.

Seven phenolic acids: p-coumaric (17), caffeic (18), ferulic (19), gentisic (20), protocatechuic (21), syringic (22), and isovanillic (23) acids, isolated from S. frutescens, have been tested on two cell lines: Hep-2 and McCoy (derived from the synovial fluid in the knee joint of a patient suffering from degenerative arthritis) (Garcia et al., 1998). All the compounds tested showed higher activity against Hep-2 and McCoy cells. Since the phenolic acids of the cinnamic compounds tested showed higher activity against Hep-2 cells (considered as expectorant) is included in this formula. Clinical research work should be necessary to support the justification of traditional medicine theories, and is promising for the therapy of malignant skin inflammatory affections. Related to this application, these authors demonstrated previously the antiseptic and antiinflammatory effects of the phenolic acids isolated from S. frutescens (Garcia et al., 1996; Fernández et al., 1998).

However, the data obtained with the phenolic acid of the benzoic group showed the ID₅₀ for these samples were higher than those recommended by the National Cancer Institute, except for syringic acid (22) (ID₅₀ = 3.87 ± 0.34) and isovanillic acid (23) (ID₅₀ = 5.25 ± 0.70) against McCoy cells. The results are in accord with the popular uses of different species of the Scrophularia genus, traditionally used in scrophulas, and indicate that some of the phenolic acids assayed may be promising for the therapy of malignant skin inflammatory affections. Related to this application, these authors demonstrated previously the antiseptic and antiinflammatory effects of the phenolic acids isolated from S. frutescens (Garcia et al., 1996; Fernández et al., 1998).

On the other hand, the methanol extract from S. ningpoensis showed antimutagenic activity. Trans-cinnamic acid (38), p-methoxycinnamic acid (33), 3,4-dimethoxycinnamic acid (39) and 4-hydroxy-3-methoxycinnamic acid (40), isolated from this extract, exhibited the same activity, especially trans-cinnamic acid (38). Methyl esters of these compounds showed greater suppressive potency against all mutagens assayed, especially the methyl ester of p-methoxycinnamic acid (33) (Miyazawa et al., 1998).

S. ningpoensis is present in a formula officially used for bronchopulmonary cancers. No mention for any anticancer activity in vitro or in vivo has been found until now for this species. According to Chinese medicine, bronchopulmonary cancer could be due to a fluid or mucous concentration in lungs. This could be the reason why S. ningpoensis (considered as expectorant) is included in this formula. Clinical research work should be necessary to support the justification of traditional medicine theories, the possible complementary contribution of which could be very useful for modern occidental therapy in this difficult field (Pinkas et al., 1994). S. ningpoensis has also been shown to alleviate the adverse effects of high-dose methotrexate plus vincristine which is utilized in chemotherapy of postoperative osteogenic sarcoma (Liu & Wu, 1993). S. nodosa has been used as a folk medicine for cancer (Pauli et al., 1995).

Neuroprotective

A chloroform-methanol extract from the roots of S. buergeriana Miq. exhibited significant neuroprotective activity against glutamate-induced neurotoxicity (Kim & Kim, 2000). Ten phenylpropanoids [buergerisides A₁ (41), B₁ (42), B₂ (43), C₁ (44) and cinnamic (38), p-methoxycinnamic (33), p-methoxycinnamic methyl ester (45), p-coumaric (17), caffeic (18) and ferulic acids (19)] isolated from this species may exert significant protective effects against glutamate-induced neurodegeneration in primary cultures of cortical neurons. At concentrations of 0.1–10.0µM, compounds 41–44 blocked the release of lactate dehydrogenase (LDH) from glutamate-insulted primary cultures of rat cortica cells significantly and also preserved the cell survival rate. At higher concentrations (above 10µM), these compounds showed no improvement in the cell survival rate due to inherent cytotoxicity. Compounds 17–19, 33, 38, 45 also reduced the release of LDH and showed some improvement in the cell survival rate in a dose-dependent manner. On the basis of these results, phenylpropanoids bearing a cinamoyl moiety exerted significant neuroprotective effects on primary cultures of rat cortical cells injured by glutamate.

Antipruritic

In a search for new antipruritic drugs, some authors (Tobita et al., 2000) screened a methanol extract from the roots of S. ningpoensis using substance P (SP) as a pruritogen in mice. This extract inhibited SP-induced scratching response in mice and showed clear dose-dependent inhibition of the scratching. The methanol extract did not inhibit locomotor activity at 200 mg/kg, a dose which was effective against SP-induced scratching. Therefore, the scratch-inhibition action of this extract may be due to inhibition of the itching sensation and/or scratching reflex, rather than to sedation or depression of general functions of the central nervous system.

Miscellaneous

The chloroform fraction of the alcoholic extract of the whole plant of S. koelzii showed significant CNS depressant activity. From this active fraction, koelzioside (34) (triacylrhamnopyranosyl catalpol derivate) has been isolated (Bhandari et al., 1992). However, investigations are needed for the pharmaceutical potential of this product.

Infusions of S. nodosa injected in mice inhibited locomotor activity and prolonged sleep caused by hexenal. This infusion dilated capillary of an isolated rabbit ear and decreased tone of an isolated rabbit or cat intestine (Karimova et al., 1966).

Discussion

Undoubtedly, the plant Kingdom still holds many species of plants containing substances of medicinal value which have
yet to be discovered; large numbers of plants are constantly being screened for their possible pharmacological value. Some of these plants belong to the Scrophularia genus. This genus may prove to be a richer source of compounds with possible pharmacological values, but more pharmacological investigations are necessary.

However, most of the reported biological studies of Scrophularia constituents and extracts were carried out in vitro, although bioactive iridoids found in Scrophularia can often lead to a multiplicity of compounds after in vivo administration. For this reason more biological and chemical attention is needed.

Acknowledgements

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References


