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Phytochemistry of the genus *Selaginella* (Selaginellaceae)

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The Selaginellaceae family includes the single genus *Selaginella*, which is found worldwide and comprises approximately 700 to 750 species. A number of species have been traditionally used for medicinal purposes in the whole world, and the phytochemistry of some has been investigated. For this review, the search was carried out using Web of Sciences, Chemical Abstracts and the data bank NAPRALERT (acronym for NAtural PRoducts ALERT), updated to October 2012. The references found in the search were then studied in detail. This review refers to 32 species and 130 compounds isolated from plants of the genus *Selaginella*, which are classified in appropriate chemical groups. The compounds isolated have been identified belonging to the classes of alkaloids, benzenoids, carbohydrates, chromones, coumarins, flavonoids, lignans, oxygen heterocycle, phenylpropanoids, pigments, quinoids and steroids. Some aspects of bioactivity of the secondary metabolites produced are discussed. For this purpose 75 references were consulted.

Key words: Selaginellaceae, *Selaginella*, phytochemistry, review.

INTRODUCTION

The family Selaginellaceae Willk. is a distinctive family including the single genus *Selaginella*. *Selaginella* is a nearly worldwide genus of about 700 species (Tryon and Tryon, 1982) or 750 species (Judd et al., 1999), with about 270 of them in America. This genus is widely distributed in America, Africa and Europe, east to the Bering Straits, to Kamchatka, Japan, and to New Guinea and Australia also in the Pacific East to the Hawaiian Islands, the Marquesas, Tahiti and Rapa. In America, *Selaginella* occurs from Northern Alaska East to Greenland, and South to Mendoza and Buenos Aires in Argentina. It is certainly better represented in the Amazon basin, for 31 species are known from that region (Tryon and Tryon, 1982).

Members of the Selaginellaceae occurs mostly terrestrial, herbaceous and perennial plants under 2 cm tall. Roots dichotomously branching; rhizophores usually produced from the stem, dichotomously branching. Stems are erect or creeping. Leaves about 0.5 to 1 cm long, spirally arranged and often 4-ranked on the secondary and ultimate branches. Distribution of these plants is mainly in tropical regions, with a few species

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extending into arctic regions of both hemispheres, occupying a wide range of habitats. The family has a nearly cosmopolitan distribution, but is not significant economically (Judd et al., 1999). Plants of *Selaginella* vary greatly in size. Some small species have stems of about 3 cm long, while larger ones have stems 50 cm to *ca.* 1 m long (Tryon and Tryon, 1982).

Several *Selaginella* species are used in traditional medicine in various countries to treat a variety of diseases such as cancer, cardiovascular problems (Lin et al., 1994), diabetes (Darias et al., 1989), gastritis (Han et al., 1984), hepatitis (Lin and Kan, 1990), skin diseases (MacFoy and Sama, 1983) and urinary tract infections (Winkelman, 1986). Extracts from some *Selaginella* spp. have shown activity as antinociceptive (Sá et al., 2012), anti-inflammatory (Han et al., 1972), antimutagenic (Meng et al., 1990), antispasmodic (Itokawa et al., 1983), cytotoxic, imunnostimulant and RNA reverse transcriptase inhibitory agents (Ono et al., 1989).

Previous studies involving some species of *Selaginella* revealed that this genus is a rich source of steroids, biflavonoids, alkaloids, secolignans, neo-lignans and caffeoyl derivatives. Other compounds, such as alkaloidal glycosides, phenylpropanones and lignans, were also reported in some *Selaginella* spp. (Sá et al., 2012). However, only a few studies on the bioactive components of species in this genus have been performed. In a recent work, Setyawan (2011) studied the diversity of natural products from *Selaginella*, especially biflavonoid and trehalose compounds; and biological activity of *Selaginella*'s biflavonoid in modern medication.

This study is a compilation of recent literature reports on phytochemistry and pharmacological importance of plants of the Selaginellaceae family.

MATERIALS AND METHODS

With the objective of contributing to these studies, a literature search on the natural products from the genus *Selaginella* was carried out. The keywords used for the literature search for this review were *Selaginella*, Selaginellaceae, natural products, phytochemistry and medicinal plants. The search was carried out using Web of Sciences, Chemical Abstracts, and the data bank of the University of Illinois in Chicago NAPRALERT (acronym for NAtural PRoducts ALERT), updated to October 2012. The references found in the search were then studied in detail.

RESULTS AND DISCUSSION

Consultation of various literature sources resulted in the elaboration of a list of natural products that have been grouped into appropriate chemical classes, as well as a list of species where these compounds were isolated (Table 1). It should be noted that most of the references cited are not first-hand observations, but compilations copied from other sources. For details, the original references should be consulted.

This review reported the study of 32 species of the genus *Selaginella*. 130 chemically defined natural products reported in the literature from species of this genus were found. The compounds isolated have been identified belonging to the classes of alkaloids (14), benzenoids (4), carbohydrates (3), chromones (3), coumarins (3), flavonoids (60), lignans (13), oxygen heterocycle (1), phenylpropanoids (8), pigments (9), quinoids (4) and steroids (8).

Phytochemistry of the genus *Selaginella*

The extensive use of species in this genus to treat a variety of diseases in traditional medicine in several countries, coupled with the fact that only limited literature information on the pharmacological activity of its constituents was available, had suggested the phytochemical investigation of species of this family (Silva et al., 1995).

Bioactivity of metabolites

The biological activities of the metabolites from species of the Selaginellaceae are considered here. The intention is to compare these properties attributed to the medicinal plants to determine the degree of correspondence and, possibly, provide leads for biological testing on one hand, and to individuate species whose phytochemistry could be investigated further, on the other hand. Chemical structures of some bioactive compounds are shown in Figure 1.

Chromones

Uncinoside A (1) and B (2) showed potent antiviral activities against respiratory syncytial virus (RSV) with IC_{50} value of 6.9 and 1.3 µg/ml, moderate antiviral activities against parainfluenza type 3 virus (PIV 3) with value of 13.8 and 20.8 µg/ml, respectively (Ma et al., 2003).

Lignans

A previous investigation on *Selaginella doederleinii* demonstrated that its cytotoxic activity against L929 murine carcinoma cells was correlated to its lignan constituents (Lin et al., 1994), although several biflavones also isolated from this species were found to be inactive in this same assay. Thus, the folkloric use of many of these species to treat cancer may not be entirely explained by the presence of lignans, since such compounds have been isolated thus far from only the above-mentioned *Selaginella* spp.

Table 1. Chemical constituents isolated from species of the genus *Selaginella*.

Chemical compound	Class	Species	References
Hordenine	Alkaloid	*S. doederleinii*	Chao et al. (1987)
Hordenine-[6-*O*-(4-hydroxy-cinnamoyl)-β-*D*-glucosyl]-(1,3)-α-*L*-rhamnoside	Alkaloid	*S. doederleinii*	Chao et al. (1990)
Hordenine-*O*-(6"-*O*-*trans*-cinnamoyl)-4'-*O*-β-*D*-glucopyranosyl-α-*L*-rhamnopyranoside	Alkaloid	*S. doederleinii*	Chao et al. (1987)
Hordenine-*O*-α-*L*-rhamnopyranoside	Alkaloid	*S. doederleinii*	Chao et al. (1987)
N-methyltyramine-*O*-α-*L*-rhamnoside	Alkaloid	*S. doederleinii*	Chao et al. (1987)
Selaginellic acid	Alkaloid	*S. moellendorfii*	Wang et al. (2009)
5-Hydroxyselaginellic acid	Alkaloid	*S. moellendorfii*	Wang et al. (2009)
5-Hydroxy-N_8,N_8-dimethylpseudophrynaminol	Alkaloid	*S. moellendorfii*	Wang et al. (2009)
N-Selaginelloyl-*L*-phenylalanine	Alkaloid	*S. moellendorfii*	Wang et al. (2009)
N-(5-Hydroxyselaginelloyl)-*L*-phenylalanine	Alkaloid	*S. moellendorfii*	Wang et al. (2009)
Neoselaginellic acid	Alkaloid	*S. moellendorfii*	Wang et al. (2009)
N-(5-Hydroxyneoselaginelloyl)-*L*-phenylalanine	Alkaloid	*S. moellendorfii*	Wang et al. (2009)
Adenosine	Alkaloid	*S. tamariscina*	Zheng et al. (2004b)
Guanosine	Alkaloid	*S. tamariscina*	Zheng et al. (2004b)
Arbutin	Benzenoid	*S. tamariscina*	Zheng et al. (2004)
4-Hydroxy-benzoic acid	Benzenoid	*S. pulvinata*	Zheng et al. (2001)
Vanillic acid	Benzenoid	*S. tamariscina*	Bi et al. (2004)
Syringic acid	Benzenoid	*S. tamariscina*	Bi et al. (2004)
Selaginose	Carbohydrate	*S. adunca*	Fischer and Kandler (1975)
		S. asperula	
		S. epirrhizos	
		S. galeotti	
		S. geniculata	
		S. kraussiana	
		S. marginata	
		S. parkeri	
		S. plumosa	
		S. sanguinolenta	
		S. stellata	
		S. sulcata	
2-Carboxy-arabinitol	Carbohydrate	*S. mertensii*	Moore et al. (1993)
Mycose	Carbohydrate	*S. pulvinata*	Zheng et al. (2001)
8-Methyl-eugenitol	Chromone	*S. uncinata*	Ma et al. (2002, 2003)
Uncinoside A	Chromone	*S. uncinata*	Ma et al. (2002, 2003)

Table 1. Contd.

Uncinoside B	Chromone	*S. uncinata*	Ma et al. (2002, 2003)
Isopimpinellin	Coumarin	*S. doederleinii*	Chen et al. (1995)
		S. moellendorfii	Che and Yu (1986)
Umbelliferone	Coumarin	*S. tamariscina*	Bi et al. (2004)
3-(4-Hydroxyphenyl)-6,7-dihydroxy coumarin	Coumarin	*S. tamariscina*	Liu et al. (2010)
Amentoflavone	Flavonoid	*S. braunii*	Ma et al. (2001)
		S. davidii	Ma et al. (2001)
		S. delicatula	Lin et al. (2000)
		S. denticulata	Lopez-Saez et al. (1994a)
		S. kraussiana	Qasim et al. (1985)
		S. moellendorfii	Sun et al. (1997)
		S. pulvinata	Ma et al. (2001)
		S. rupestris	Chanravarthy et al. (1981)
		S. sanguinolenta	Huneck and Khaidav (1985)
		S. selaginoides	Lopez-Saez et al. (1994b)
		S. sinensis	Ma et al. (2001)
		S. stauntoniana	Ma et al. (2001)
		S. tamariscina	Lee et al. (1992)
		S. uncinata	Ma et al. (2003)
		S. willdenowii	Silva et al. (1995)
2,3-Dihydroamentoflavone	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
2”,3”-Dihydroamentoflavone	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
Tetrahydro-amentoflavone	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
Amentoflavone-7,4,7,4-tetramethylether	Flavonoid	*S. moellendorfii*	Cao et al. (2010a)
4’,7”-Di-*O*-methyl-amentoflavone	Flavonoid	*S. sinensis*	Ma et al. (2001)
		S. willdenowii	Silva et al. (1995)
7,7”-Di-*O*-methyl-amentoflavone	Flavonoid	*S. doederleinii*	Lin et al. (1994)
4’,4”’,7,7”-Tetra-*O*-methyl-amentoflavone	Flavonoid	*S. doederleinii*	Lin et al. (1994)
		S. moellendorfiii	Sun et al. (1995)
7,4’,7”,4”-tetramethylether-amentoflavone	Flavonoid	*S. moellendorfii*	Sun et al. (1997)
Apigenin	Flavonoid	*S. doederleinii*	Chen et al. (1995)
Apigenin-7-*O*-β-neohesperidoside	Flavonoid	*S. moellendorfii*	Feng et al. (2011)
Apigenin-8-*C*- β-*D*-glucopyranoside	Flavonoid	*S. moellendorfii*	Feng et al. (2011)
6,8-Di-*C*-β-*D*-glucopyranosyl-apigenin	Flavonoid	*S. moellendorfii*	Zhu et al. (2008)
6-*C*-β-*D*-Glucopyranosyl-8-*C*-β-*D*-xylopyranosyl-apigenin	Flavonoid	*S. moellendorfii*	Zhu et al. (2008)

Table 1. Contd.

6-*C*-β-*D*-Xylopyranosyl-8-*C*-β-*D*-glucopyranosyl-apigenin	Flavonoid	*S. moellendorfii*	Zhu et al. (2008)
6-(2-Hydroxy-5-acetylphenyl)-apigenin	Flavonoid	*S. tamariscina*	Liu et al. (2009, 2010)
6-(5-Carboxyl-2-methoxyphenyl)-apigenin	Flavonoid	*S. uncinata*	Zheng et al. (2008)
2',8"-Biapigenin	Flavonoid	*S. tamariscina*	Lee et al. (2008)
2",3"-Dihydro-3',3"'-biapigenin	Flavonoid	*S. labordei*	Xu et al. (2009)
Bilobetin	Flavonoid	*S. willdenowii*	Silva et al. (1995)
Chamaecyparin	Flavonoid	*S. difusa*	Meurer-Grimes et al. (1999)
		S. jungermannioides	Meurer-Grimes et al. (1999)
		S. stellata	Meurer-Grimes et al. (1999)
Chrysoeriol	Flavonoid	*S. moellendorfii*	Cao et al. (2010a)
Cryptomerin B	Flavonoid	*S. denticulata*	Lopez-Saez et al. (1994[a])
		S. tamariscina	Shin and Kim (1991)
Delicaflavone	Flavonoid	*S. delicatula*	Lin and Chou (2000)
Genistin	Flavonoid	*S. sinensis*	Dai et al. (2001)
Ginkgetin	Flavonoid	*S. moellendorfii*	Sun et al. (1997)
		S. sinensis	Dai et al. (2001)
Heveaflavone	Flavonoid	*S. doederleinii*	Lin et al. (1994)
		S. lepidophylla	Qasim et al. (1985)
Hinokiflavone	Flavonoid	*S. denticulata*	Lopez-Saez et al. (1994a, 1995)
		S. kraussiana	Qasim et al. (1985)
		S. selaginoides	Lopez-Saez et al. (1994b)
		S. uncinata	Ma et al. (2003)
2,3-Dihydrohinokiflavone	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
2",3"-Dihydrohinokiflavone	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
Tetrahydro-hinokiflavone	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
Tetra-*O*-methyl-hinokiflavone	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
Isocryptomerin	Flavonoid	*S. denticulata*	Lopez-Saez et al. (1994a)
		S. tamariscina	Shin and Kim (1991)
		S. willdenowii	Silva et al. (1995)
2,3-Dihydro-isocryptomerin	Flavonoid	*S. delicatula*	Lin and Chou (2000)
2",3"-Dihydro-isocryptomerin	Flavonoid	*S. willdenowii*	Silva et al. (1995)
Kayaflavone	Flavonoid	*S. moellendorfii*	Sun et al. (1997)
			Sun et al. (1995)
Lanaroflavone	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
Podocarpusflavone A	Flavonoid	*S. moellendorfii*	Sun et al. (1995)

Table 1. Contd.

			Sun et al. (1997)
Robustaflavone	Flavonoid	*S. delicatula*	Lin et al. (2000)
		S. denticulata	Lopez-Saez et al. (1994a)
		S. lepidophylla	Qasim et al. (1985)
		S. selaginoides	Lopez-Saez et al. (1994b)
		S. sinensis	Ma et al. (2001)
		S. willdenowii	Silva et al. (1995)
2,3-Dihydro-robustaflavone	Flavonoid	*S. lepidophylla*	Aguilar et al. (2008)
2,3-Dihydro-5-methylether-robustaflavone	Flavonoid	*S. lepidophylla*	Aguilar et al. (2008)
2",3"-Dihydro-4',7,7"-trimethylether-robustaflavone	Flavonoid	*S. delicatula*	Lin et al. (2000)
2,3-Dihydro-4',7,7"-trimethylether-robustaflavone	Flavonoid	*S. delicatula*	Lin and Chou (2000)
2",3"-Dihydro-4',7,-dimethylether-robustaflavone	Flavonoid	*S. delicatula*	Lin et al. (2000)
4',7-Dimethylether-robustaflavone	Flavonoid	*S. delicatula*	Lin et al. (2000)
4'-Methylether-robustaflavone	Flavonoid	*S. delicatula*	Lin et al. (2000)
		S. doederleinii	Lee et al. (2008)
7''-*O*-Methyl-robustaflavone	Flavonoid	*S. sinensis*	Ma et al. (2001)
		S. willdenowii	Silva et al. (1995)
2,2'',3,3''-Tetrahydro-4',7,7''-trimethylether-robustaflavone	Flavonoid	*S. doederleinii*	Lee et al. (2008)
4,7,7''-Trimethylether-robustaflavone	Flavonoid	*S. doederleinii*	Lee et al. (2008)
Sciadopitysin	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
Sequoiaflavone	Flavonoid	*S. bryopteris*	Kunert et al. (2008)
Sotetsuflavone	Flavonoid	*S. denticulata*	Lopez-Saez et al. (1994[a])
Sumaflavone	Flavonoid	*S. tamariscina*	Yang et al. (2006)
2,3-Dihydro-5,5",7,7",4'-pentahydroxy-6,6"-dimethyl-[3'-*O*-4"']-biflavone	Flavonoid	*S. labordei*	Xu et al. (2009)
2",3"-Dihydroochnaflavone	Flavonoid	*S. labordei*	Xu et al. (2009)
5-Carboxymethyl-4'-hydroxyflavone-7-*O*-β-*D*-glucopyranoside	Flavonoid	*S. moellendorfii*	Zhu et al. (2008)
Taiwaniaflavone	Flavonoid	*S. tamariscina*	Lee et al. (2008)
5-Carboxymethyl-4',7-dihydroxyflavone	Flavonoid	*S. moellendorfii*	Cao et al. (2010a)
[7-Hydroxy-2-(4-hydroxy-phenyl)-4-oxo-4H-chromen-5-yl]-acetic acid ethyl ester	Flavonoid	*S. moellendorfii*	Cao et al. (2010a)
[7-Hydroxy-2-(4-hydroxy-phenyl)-4-oxo-4H-chromen-5-yl]-acetic acid butyl ester	Flavonoid	*S. moellendorfii*	Cao et al. (2010a)
5-Acethyl-dihydro-2-(3',5'-dimethoxy-4'-hydroxy-phenyl)-7-methoxybenzofuran	Lignan	*S. tamariscina*	Zheng et al. (2004b)
(-)-Lirioresinol A	Lignan	*S. doederleinii*	Lin et al. (1994)
(-)-Lirioresinol B	Lignan	*S. doederleinii*	Lin et al. (1994)
(+)-Matairesinol	Lignan	*S. doederleinii*	Lin et al. (1994)
Moellenoside A	Lignan	*S. moellendorfii*	Zheng et al. (2008)

Table 1. Contd.

Moellenoside B	Lignan	*S. moellendorfii*	Feng et al. (2011)
(-)-Nortracheloside	Lignan	*S. doederleinii*	Lin et al. (1994)
Pinoresinol diglucoside	Lignan	*S. sinensis*	Dai et al. (2001)
Sinesiol A	Lignan	*S. sinensis*	Wang et al. (2007)
Syringaresinol	Lignan	*S. tamariscina*	Bi et al. (2004)
Tamariscinoside B	Lignan	*S. tamariscina*	Zheng et al. (2004b)
Tamariscinoside C	Lignan	*S. tamariscina*	Zheng et al. (2004b)
(+)-Wilkstromol	Lignan	*S. doederleinii*	Lin et al. (1994)
2,4-Dihydroxy-3-methylenehydroxy-5-methoxy-tetrahydrofuran	Oxygen heterocycle	*S. lepidophylla*	Perez et al. (1994)
Caffeic acid	Phenylpropanoid	*S. tamariscina*	Bi et al. (2004)
Ferulic acid	Phenylpropanoid	*S. tamariscina*	Bi et al. (2004)
Isochlorogenic acid A	Phenylpropanoid	*S. delicatula*	Lin et al. (2000)
Isochlorogenic acid B	Phenylpropanoid	*S. delicatula*	Lin et al. (2000)
Isochlorogenic acid C	Phenylpropanoid	*S. delicatula*	Lin et al. (2000)
3-Hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-propan-1-one	Phenylpropanoid	*S. doederleinii*	Lin et al. (1994)
3-Hydroxy-1-(3-methoxy-4-hydroxyphenyl)-propan-1-one	Phenylpropanoid	*S. doederleinii*	Lin et al. (1994)
Tamariscine ester A	Phenylpropanoid	*S. tamariscina*	Bi et al. (2004)
Selaginellin	Pigments	*S. sinensis*	Zhang et al. (2007)
Selaginellin A	Pigments	*S. tamariscina*	Cheng et al. (2008)
Selaginellin B	Pigments	*S. tamariscina*	Cheng et al. (2008)
Selaginellin C	Pigments	*S. pulvinata*	Tan et al. (2009)
Selaginellin D	Pigments	*S. pulvinata*	Cao et al. (2010b)
Selaginellin E	Pigments	*S. pulvinata*	Cao et al. (2010b)
Selaginellin F	Pigments	*S. pulvinata*	Cao et al. (2010b)
Selaginellin G	Pigments	*S. pulvinata*	Cao et al. (2010c)
Selaginellin H	Pigments	*S. pulvinata*	Cao et al. (2010c)
Chrysophanic acid	Quinoid	*S. stauntoniana*	Liu et al. (2004)
Emodin	Quinoid	*S. stauntoniana*	Liu et al. (2004)
1-Methoxy-3-methylanthraquinone	Quinoid	*S. tamariscina*	Liu et al. (2010)
Physcion	Quinoid	*S. stauntoniana*	Liu et al. (2004)
Cholesterol	Steroid	*S. delicatula*; *S. doederleinii*	Chiu et al. (1988)
22-Dehydrocampesterol	Steroid	*S. delicatula*; *S. doederleinii*	Chiu et al. (1988)
3β-16α-Dihydroxy-(5α)-cholestan-21-oic acid	Steroid	*S. pulvinata*	Zheng et al. (2007)
24α-Ethyl-cholest-5-en-3β-ol	Steroid	*S. delicatula*; *S. doederleinii*	Chiu et al. (1988)
24α-Methyl-cholest-5-en-3β-ol	Steroid	*S. delicatula*; *S. doederleinii*	Chiu et al. (1988)

Table 1. Contd.

24β-Methyl-cholest-5-en-3β-ol	Steroid	*S. delicatula*; *S. doederleinii*	Chiu et al. (1988)
24α-Ethyl-cholesta-5,22-dien-3β-ol	Steroid	*S. delicatula*; *S. doederleinii*	Chiu et al. (1988)
β-Sitosterol	Steroid	*S. doederleinii*	Chen et al. (1995)
		S. moellendorfii	Che and Yu (1986)
		S. pulvinata	Zheng et al. (2001)

Flavonoids

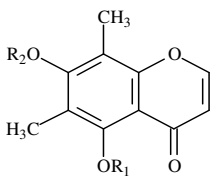
Selaginellaceae is a family rich in biflavonoids, these compounds shows potent activity such as antimalarial, anti-inflammatory, antibacterial, anti-oxidant and antiviral. The biflavonoids isolated from *Selaginella delicatula* were tested against a panel of human cancer cell lines according to established protocols. With the exception of compounds 4'-methylether-robustaflavone (3) and 2",3"-dihydro-4',7-dimethylether-robustaflavone (4), there was no inhibitory activity on tumor cells whose IC_{50} values are higher than 100 µM. Both compounds significantly inhibited Raji and Calu-1 cell growth in a concentration-dependent manner. By contrast, the compounds had no supressory activity on K562, HeLa, Vero and Wish tumor cell lines. These results show that *S. delicatula* biflavonoids possess mainly the C-3'-C-6" interflavonoid linkage instead of the C-3'-C-8" interflavonoid linkage, which is common for most of the biflavonoids in the genus of *Selaginella*. *S. delicatula*, similar to other *Selaginella* spp., contained biflavonoid substances that exhibited the cytotoxic activities against cancer cells in cultures (Sun et al., 1997; Silva et al., 1995).

Among the active and inactive biflavonoids obtained from *Selaginella willdenowii,* are novel compound 2",3"-dihydro-isocryptomerin (5), the cytotoxic biflavones 4',7"-di-*O*-methyl-amentoflavone (6) isocryptomerin (7), bilobetin (8), 7"-*O*-methyl-robustaflavone (9), amentoflavone (10), and robustaflavone (11). All of the isolated biflavonoids were tested against a panel of human cancer cell lines according to established protocols. Although, the biflavonoid 2",3"-dihydro-isocryptomerin was inactive, the parent molecule, isocryptomerin (7), displayed significant activity against the HT-1080 and Lu1 cell lines. Among the remaining biflavone isolates, amentoflavone was found to be inactive, consistent with previous assay data, and bilobetin displayed only marginal activity against the HT-1080 cell line (Silva et al., 1995). However, the presence of two methoxyl groups, as in 4',7"-di-*O*-methylamentoflavone was observed to enhance the cytotoxicity of the compound, as in the case of Col2 and U373 cell lines. Although, there was a tendency to increase the activity with increasing numbers of methoxyl groups in the molecule, 7,7"-di-*O*-methylamentoflavone (12) and 7,4',7",4'"-tetra-*O*-methyl-amentoflavone (13) have been found by others to be inactive against L929 murine cells (Lin et al., 1994). Only two compounds of the robustaflavone series were isolated and tested. Consistent with earlier literature (Lin et al., 1989), robustaflavone showed a lack significant cytotoxicity, but its monomethyl derivative, 7"-*O*-methylrobustaflavone was found to be a potent cytotoxic agent against the HT-8080, Lu1 and U373 cell lines. Its *in vitro* potency is comparable to that of calycopterone, a new type of biflavonoid isolated by Wall et al. (1994).

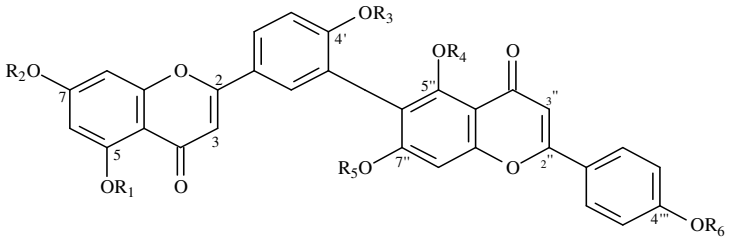
Amentoflavone, a dimmer of apigenin, has several known pharmacological activities which includes anti-inflammatory (Kim et al., 1994) and antioxidative effects (Huguet et al., 1990). Amentoflavone also inhibits phospholipase C1 (Lee et al., 1996) and cAMP-dependent phosphodiesterase (Saponara and Bosisio, 1998), which could be associated with its diverse physiological activities. Amentoflavone inhibited the production of nitric oxide in a concentration-dependent manner and also blocked the lipopolysaccharide (LPS)-induced expression of inducible nitric oxide synthase (iNOS). These findings suggest that the inhibition of LPS-induced NO formation by amentoflavone is due to its inhibition of NF-κB by blocking I-κBα degradation, which may be the mechanistic basis of the anti-inflammatory effects of amentoflavone (Woo et al., 2005).

Bioassay-directed fractionation of an ethanolic extract of *Selaginella moellendorfii* has led to the isolation of a known biflavone, ginkgetin (14). A dose-dependent inhibition was observed on the growth of OVCAR-3 (human ovarian adeno-carcinoma) cells with 50% inhibition occurring at 1.8 µg/ml. Non bioactive fractions yielded four additional known biflavones, 7,4',7",4'"-tetramethylether-amentoflavone, kayaflavone, podocarpusflavone A and amentoflavone (Sun et al., 1997).

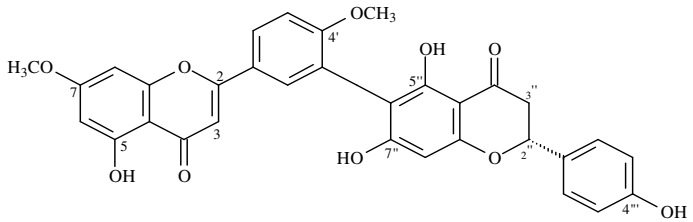
An ethyl acetate-soluble extract of *Selaginella tamariscina* was found to exhibit distinctive vasorelaxant activity. Further purifications of the



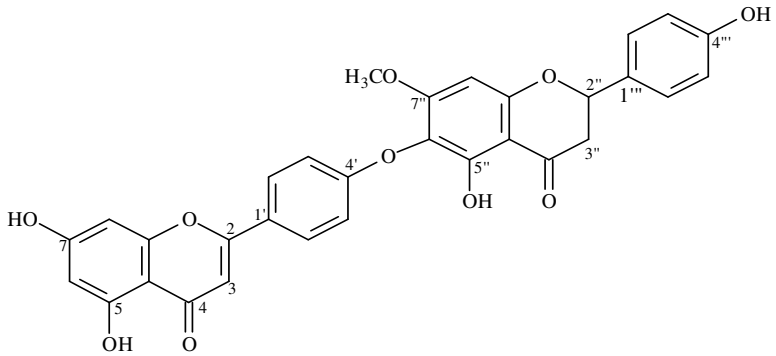
(**1**) R_1= H; R_2= Glc
(**2**) R_1= Ac; R_2=Glc



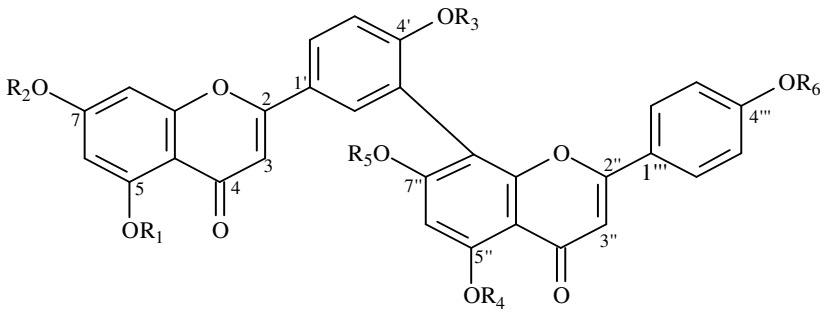
(**3**) R_1= H; R_2= H; R_3= CH_3; R_4= H; R_5= H; R_6= H
(**9**) R_1= H; R_2= H; R_3= H; R_4= H; R_5= CH_3; R_6= H
(**11**) R_1= H; R_2= H; R_3= H; R_4= H; R_5= H; R_6= H



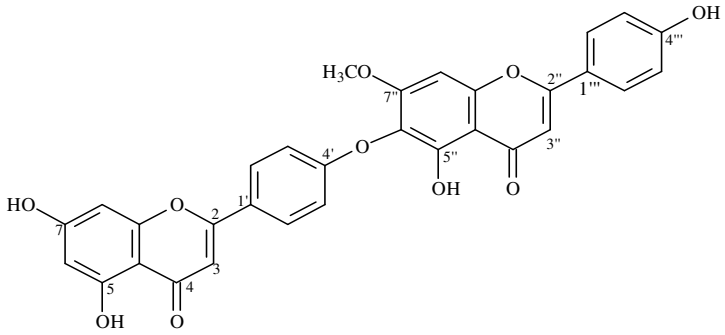
(**4**)



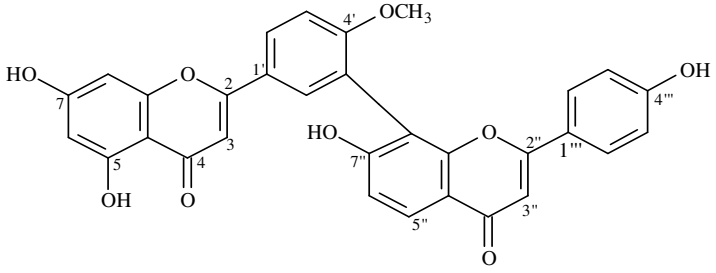
(**5**)



(**6**) R_1= H; R_2= H; R_3= CH_3; R_4= H; R_5= CH_3; R_6= H
(**10**) R_1= H; R_2= H; R_3= H; R_4= H; R_5= H; R_6= H
(**12**) R_1= H; R_2= CH_3; R_3= H; R_4= H; R_5= CH_3; R_6= H
(**13**) R_1= H; R_2= CH_3; R_3= CH_3; R_4= H; R_5= CH_3; R_6= CH_3
(**14**) R_1= H; R_2= CH_3; R_3= CH_3; R_4= H; R_5= H; R_6= H



(7)



(8)

Figure 1. Chemical structures of some bioactive compounds in *Selaginella*.

extract as guided by *in vitro* vasorelaxant assay afforded an active biflavonoid, amentoflavone. Amentoflavone induced concentration-dependent relaxation of the phenylephrine-precontracted aorta, which disappeared by removal of functional endothelium. Amentoflavone-induced relaxations were also markedly attenuated by addition of tetraethylammonium (TEA) or verapamil. However, the relaxant effect of amentoflavone was not blocked by pretreatment with indomethacin, glibenclamide, atropine, or propranolol. Incubation of endothelium-intact aortic rings with amentoflavone increased the production of cGMP, but this effect was blocked by endothelium-denudation or pretreatment with L-NAME or ODQ. These results suggest that amentoflavone relaxes vascular smooth muscle via endothelium-dependent nitric oxide-cGMP signaling, with possible involvement of non-specific K^+ and Ca^{2+} channels (Kang et al., 2004).

Conclusion

This work shows that species of the genus *Selaginella* are a rich source of bioactive compounds. The flavonoids are the major compounds isolated from these species. In particular, the chemical constituents belonging to class of biflavonoids, such as amentoflavone, ginkgetin, isocryptomerin and robustaflavone presented several biological activities in experimental models. More research is needed to further explore the actions of these compounds. *Selaginella* research exhaustively needs to be conducted to explore all natural products constituents and their bioactivities (Setyavan, 2011).

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