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Sarcodon Mushrooms: Biologically Active Metabolites

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1. Introduction

Thelephorales Corn ex. Oberw. belong to the Agaricomycetes (Basidiomycota, Fungi) and comprise more than 177 species (Kirk *et al.*, 2008) that are classified into two families: the *Bankeraceae* Donk (mostly with colourless basidiospores) and the *Thelephoraceae* Chevall. (mostly with brown to yellowish basidiospores) (Yorou & Agerer, 2007). Bankeraceae are characterized by a typical fenugreek odour, with pileate and stipitate fruit bodies, hydnoid to spinose hymenophores with brown and lobed as well as colourless and evenly ornamented basidiospores. Bankeraceae comprise 5 distinct genera: *Bankera* Coker & Beers ex Pouzar, *Hydnellum* P. Karsten, *Phellodon* P. Karsten, *Sarcodon* Quél. ex P. Karst and *Boletopsis* Fayod.

The name *Sarcodon* was proposed by Quélet in 1878, but as any binomial was formed, the name was established as a genus only later by Karsten (Banker, 1913).

The name, derived from the ancient greek stems *sarco=* flesh and *odon=*tooth, is due to the presence of the spines that look like teeth on the hymenophore. For this reason *Sarcodon* species are colloquial called "tooth fungi" (Fig. 1).

The genus comprises more than 72 species, most of which are not edible, due to the bitter taste. Among these species only few have been phytochemically and biologically studied: *S. aspratus* (syn *S. imbricatus*), *S. cyrneus*, *S. glaucopus*, *S. leucopus*, *S. laevigetum* (syn. *S. laevigatus*) and *S. scabrosus*.

Phytochemical studies were aimed to study either the composition of different extracts or to isolate new metabolites and to evaluate biological activity of extracts and isolated compounds.

2. Phytochemical studies on Sarcodon spp

2.1 Sarcodon aspratus syn S. imbricatus

Sarcodon imbricatus (L.) P. Karst. is the current name used for *S. aspratus* (Berk.) even though several synonyms are used for this mushroom (see Index Fungorum: www.indexfungorum.org). It is commonly known as the *shingled hedgehog* or *scaly hedgehog*. The mushroom has a large, brownish cap with large brown scales and may reach 30 cm in diameter. On the underside it sports greyish brittle teeth instead of gills, and has white flesh.

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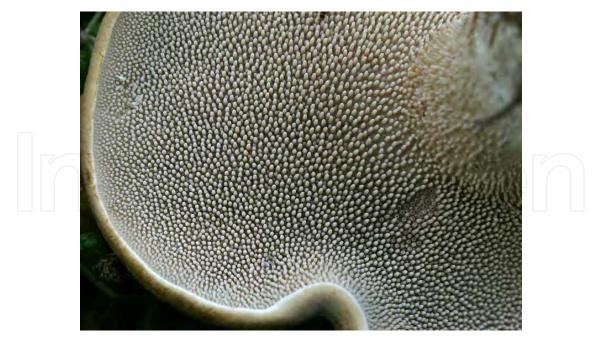


Fig. 1. Sarcodon imbricatus (L.: Fr.) Karsten hymenophore

The fungus can be bitter, although this is less apparent in younger specimens. Submerging the mushrooms in boiling water will remove this. It can be pickled or dried and used as flavouring. In Bulgaria it is collected, dried and finely ground to be used as an aromatic mushroom flour. It is reported as edible, but of poor quality in the United States by some sources but as deliciously edible by others. In China *Sarcodon aspratus*, also known as *"black tiger's paw"*, is a popular natural edible mushroom. It is used for lowering cholesterol level, muscles relaxation and blood circulation. Its special musky aroma enhances the taste in meat's dishes, especially hams (S.K. Kim, 2006), and particularly brings out the sweet flavour when making a clear soup out of it.

Several papers have been published regarding the use of *S. aspratus* for manufacturing foods and beverages (*e.g.* Hwang & Na, 2009a, 2009b; B.C. Kim *et al.*, 2008, 2009; Jang, 2008; Wang & Wang, 2008; J.K. Kim, 2007).

2.1.1 Phytochemistry of Sarcodon imbricatus

In view of its use as a food, the phytochemical composition of *S. imbricatus* has been studied.

Proximate constituents (moisture, fat, crude protein, ash and carbohydrates) of this mushroom have been reported (Barros *et al.*, 2007a). On the basis of the proximate analysis, it can be calculated that a portion of 100 g of this mushroom provides, on average, 24 kcal (100 kJ). The analysis of fatty acid composition allowed to determine 15 fatty acids.

Unsaturated fatty acids (MUFA, monounsaturated fatty acids, 46%, PUFA, polyunsaturated fatty acids, 36%) were predominant over SFA (saturated fatty acids, 18%). Methanolic extract of *S. imbricatus* showed the presence (0.5%) of total polyphenols, expressed as g of gallic acid /100 g dry weight that justify the radical scavenging activity (DPPH) (Marcotullio *et al.*, 2008). On the other hand the amounts of ascorbic acid, β -carotene and lycopene found in the mushroom were very low (Barros et al., 2007b). Sterol fraction was

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Fig. 2. Sarcodon imbricatus (L.: Fr.) Karsten

studied by several research groups. In addition to ergosterol, several other ergostane and cholestane compounds have been isolated (Huang et al., 2002; Ueno et al., 1999; Marcotullio et al., 2008). Particularly interesting is the presence in the methanolic extract of *S. imbricatus* of ergosterol peroxide (or $3-\beta$ -hydroxy- 5α , 8α -epidioxyergosta-6,22-diene) that shows a plethora of biological activities, such as antileukemic and anticancer (Kobori et al., 2006), apoptotic-inducing (Takei et al., 2005), and anti-inflammatory (Kobori et al., 2007). It is interesting to note that processing and cooking practices determine a lowering of nutrients concentrations and of antioxidant activity (Barros et al., 2007c).

Other interesting metabolites isolated and identified from *S. aspratus* are polysaccharides. Han and coworkers isolated a water soluble polysaccharide (HCP) with molecular mass of 6.7 x 105 Da (Han *et al.*, 2011). HCP resulted to be a linear glucan with a backbone structure of $(1\rightarrow 6)$ -linked- α -glupyranosyl residues. Another polysaccharide (HBP) with a molecular weight of 4.3 x 105 Da was isolated by the same author in 2010. Even in this case the backbone structure was constituted by $(1\rightarrow 6)$ -linked- α -glupyranosyl residues, which occasionally branched at O-3 position (Han *et al.*, 2010).

Other constituents isolated from S. aspratus were ceramide compounds (Yaoita et al., 2002).

2.2 Sarcodon cyrneus Maas Gest

Sarcodon cyrneus, according Myco Bank (www.mycobank.org), has a "pileus up to 65 mm across, planoconvex to somewhat depressed in centre, finely tomentose at margin, becoming felted farther back, or tomentum collapsed to form smooth and shiny pellicle. Stipe 15-30 x 6-15 mm, broader when fused, equal or somewhat enlarged below, with abruptly pointed base, straight to curved, tomentose, in places glabrescent, pale grey-brown, darkening with age and becoming more or less concolorous with, pileus, at extreme base with yellowish grey mycelium. Spines up to 3 mm long, 0.1-0.3 mm broad, long decurrent, often almost reaching base of stipe, crowded, subulate, first whitish, becoming purplish brown. Context pallid (whitish suffused with pinkish when fresh, according to the collector), not greenish in base of stipe" (Fig. 3). The taste is bitter and the mushroom is not edible.



Fig. 3. Sarcodon cyrneus Maas Geest.

2.2.1 Phytochemistry of Sarcodon cyrneus

In the literature only two papers regarding the secondary metabolites isolated from *S. cyrneus* have been reported (Marcotullio *et al.*, 2006b; Marcotullio *et al.*, 2007) and they deals with the structural identification of five cyathane diterpenes. These compounds were named cyrneine A-D (**1**-**4**) (Fig. 4) and glaucopine C (**7**) (Fig. 6).

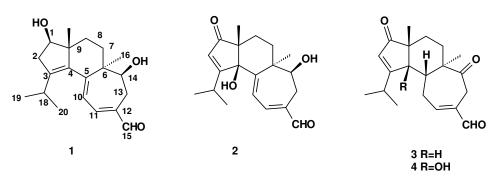


Fig. 4. Structures of cyrneines

2.3 Sarcodon glaucopus Maas Geest. & Nannf.

According MycoBank (www.mycobank.org) the mushroom has a "pileus up to 110 mm across, plano-convex to somewhat depressed; at first tomentose, then matted, forming a cuticle which breaks up into scales near margin, into areoles in centre; scales adhering to appressed, yellow-brown with vinaceous shade or pale to dark purplish brown, occasionally locally violet-grey (giving a peculiar leaden grey impression), at times very dark brown in centre, contrasting with dingy yellowish ground colour, not infrequently covered with minute yellowish dots of excreted matter when dried. Stipe 27-75 x 10-40 mm, cylindrical, tapering below or somewhat broadened below, tomentose, fibrillose, covered with adnate fibrillose squamules or partly matted, dingy whitish, soon pinkish brown to purplish brown above, grey-green below, with pointed, whitish base. Spines up to 5 mm long, 0.1-0.2 mm broad, whitish, finally purplish brown" (Fig. 5). The taste is bitter and the mushroom is not edible.

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Fig. 5. Sarcodon glaucopus Maas Geest. & Nannf.

2.3.1 Phytochemistry of Sarcodon galucopus

No phytochemical studies regarding proximate composition have been reported for *S. glaucopus*. In the literature there are only two studies on the secondary metabolites of *S. glaucopus*, and they deal with the isolation and structure elucidation of three cyathane diterpenes named glaucopine A (5), glaucopine B (6) (Curini *et al.*, 2005) and glaucopine C (7) (Marcotullio *et al.*, 2006a) (Fig. 6).

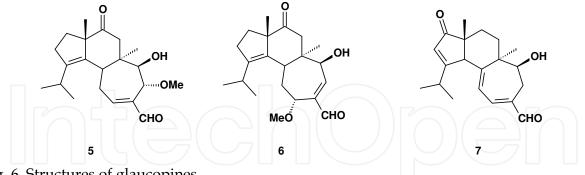


Fig. 6. Structures of glaucopines

2.4 Sarcodon laevigatus (Sw.) P. Karst.

Index Fungorum (www.indexfungorum.org) reports for *S. laevigatus* (syn. *S. leucopus*, often the term *S. laevigatum* is used instead of *S. laevigatus*). A description of *Sarcodon leucopus* (Pers.) Maas Geest. & Nannf. has been reported in MycoBank: "Pileus up to ca. 200 mm across, planoconvex or slightly depressed, without concentric or radiate markings, at first finely felted; felt collapsed to form smooth, more or less shiny, innate-scaly cuticle, the latter subsequently radiately rimose near margin, breaking up into areoles in centre, here with scales somewhat more pronounced and tips sometimes slightly raised; pale purplish brown

on yellowish drab ground colour or a rich purplish brown to dark brown. Stipe $40-80 \times 20-60$ mm, cylindrical to ventricose, finely tomentose, later with smooth or innate-scaly cuticle, concolorous with pileus or paler, whitish below, after some time with green spots (always?). Spines up to c. 15 x 1 mm, whitish, finally purplish brown. Context up to 40 mm thick near centre of pileus, whitish, suffused with purplish brown to violet tints, after some time pale greenish. Odour commonly experienced as disagreeable. Taste bitter after some time".

2.4.1 Phytochemistry of Sarcodon laevigatus or/and S. leucopus

In the literature no references to *S. laevigatus* have been reported, while it is possible to find two papers referring to *S. laevigatum* and to *S. laevigetum*. The first one reports the isolation and the structure identification of three p-terphenyls (8-10), of which 8 (named sarcodan) was a new compound (Ma *et al.*, 2006). The second report is a Patent and deals with the preparation of compound 9 (named B1-V) by extraction from *S. laevigetum* (Ma, 2009).

On the other hand, Tringali and coworkers (Tringali *et al.*, 1987) reported the isolation and structure identification of several p-terphenyls from *Sarcodon leucopus*.

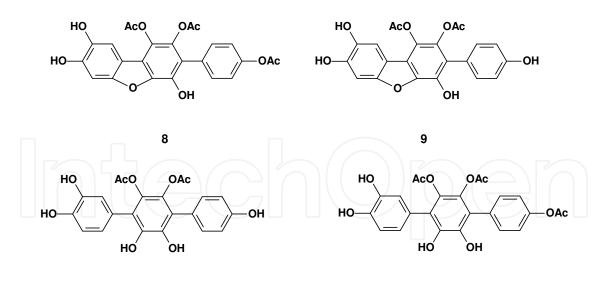
The first two isolated compounds were **10** and **11**. (Fig. 8)



Fig. 7. Sarcodon leucopus (Pers.) Maas Geest. & Nannf.

It is evident from these reports how the correct identification of the species is important for preventing mistakes in the identification of new secondary metabolites.

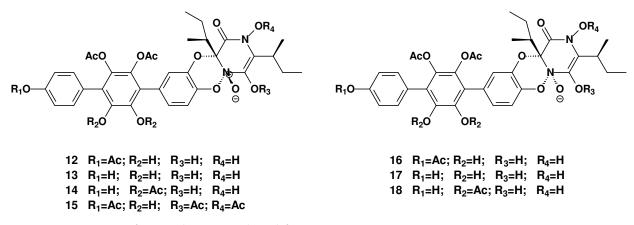
Later, the same research group isolated other nitrogen-containing p-terphenyl derivatives that they named **sarcodonins**. The first to be isolated was **12** in 2000 (Geraci *et al.*, 2000) and other six related compounds (**13-18**) were isolated in 2004 (Cali *et al.*, 2004) (Fig. 9).

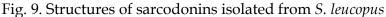


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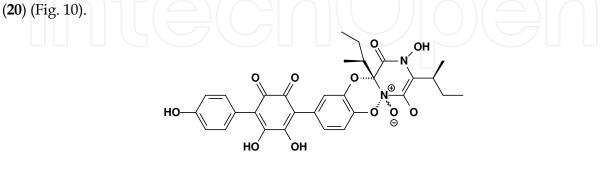
Fig. 8. Structures of *p*-terphenyls isolated from *S. laevigatum*

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Compounds **13-15** were named sarcodonin α , β , and γ , respectively, while compounds **16-18** *epi*-sarcodonin, *epi*-sarcodonin α and β , respectively. Cali *et al.*, (2004) from the same mushroom isolated oxidated sarcodonins and named them sarcoviolin (**19**) and epi-sarcoviolin



19 β -N-oxide 20 α -N-Oxide

Fig. 10. Structures of sarcoviolins

Recently the structures of sarcodonins and sarcoviolins have been revised by synthesis, and the new structure for sarcodonin **12** is reported in Fig. 11 (Lin *et al.*, 2011).

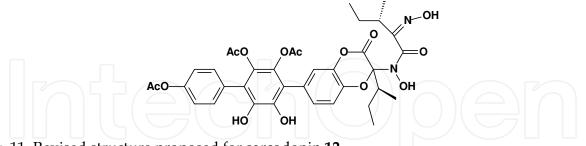


Fig. 11. Revised structure proposed for sarcodonin 12

It is very interesting to notice that screening the literature with the term "sarcodonin" different structures with the same name have been reported, as we will see checking the compounds isolated from *S. scabrosus*.

2.5 Sarcodon scabrosus (Fr.) P. Karst.

Myco Bank describes *S. scabrosus* "with pileus up to 120 mm across (fresh), plano-convex, more or less deeply depressed in the centre, coarsely scaly, the scales erect in the centre, decumbent farther outwards, adnate and woolly near the margin, brown in various shades (brick-colour, fulvous, ferruginous, bay) on a fairly pale yellow-brown ground, in some specimens passing into a delicate lilac at the margin, with age becoming very dark purplish brown, and the scale-tips even blackish, somewhat shiny when dried. Stipe 20-120 x 10-60 mm (fresh), tapering downwards, usually with pointed base, felted to subfibrillose, pinkish brown to brick-colour, becoming concolorous with the pileus, the lower part or the base grey-green, when young covered with white mycelium. Spines up to 5 mm long (dry), slender (up to 0.3 mm), decurrent, crowded, subulate, long remaining yellowish brown, finally purplish brown. Context dingy whitish in the pileus and the top of the stipe, vinescent, brownish-marbled with age, grey-green in the base of the stipe. Greenish mycelium from the base of the stipe staining dingy pinkish brown to reddish brown in KOH solution. Odour of water-melon (*Citrullus vulgaris*) when cut fresh" (Fig. 12).

Due to its bitter taste it is not edible.

2.5.1 Phytochemistry of Sarcodon scabrosus

An analysis of the literature revealed that *Sarcodon scabrosus* (syn *Hydnum scabrosus*), together with *S. imbricatus*, is one of the most studied species among *Sarcodon*. Two classes of secondary metabolites have been isolated from this species, and both are represented by cythane diterpenes: scabronines and *sarcodonins*. The use of the same name for different classes of secondary metabolites clearly shows how the use of common names can give rise to mistakes.

The first report about the isolation of cyathane diterpenes from *S. scabrosus* was by Shibata *et al.* (1989) and it is about sarcodonin A (**21**) and G (**22**) (Fig. 13).

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Fig. 12. Sarcodon scabrosus (Fr.) P. Karst.

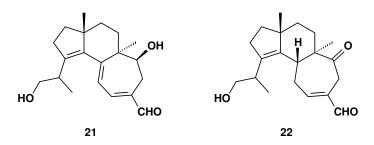


Fig. 13. Sarcodonins A and G

Generally sarcodonins and neosarcodonins isolated from *S. scabrosus* are characterized by the presence of a hydroxyl-methyl group in C-19 (Kryczkowski *et al.*, 2008).

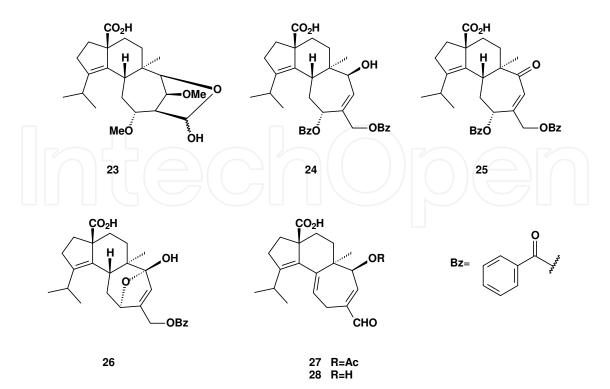


Fig. 14. Isolated scabronines

Scabronines are characterized by the oxidation of C-17 to carboxylic group. The first scabronine that was isolated was scabronine A (**23**) (Ohta *et al.*, 1998a) and later scabronines B-F (**24-28**) were isolated (Kita *et al.*, 1998) (Fig. 14).

Among different cyathane diterpenes, the most interesting for its biological properties is scabronine G (**29**) (Fig. 15), that has been isolated in 1998 (Ohta *et al.*, 1998b). In 2004, Ma and coworkers reported the isolation of another *scabronine* G (**30**), but this compound is structurally related to sarcodonins (Ma *et al.*, 2004).

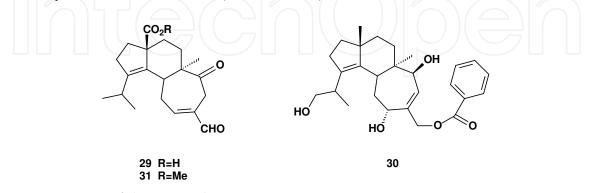


Fig. 15. Structures of the two scabronines G

Ma *et al.* isolated other scabronines (H-J), but they still have sarcodonins structures (Ma *et al.*, 2004; Ma *et al.*, 2008).

From *S. scabrosus* in addition to the already mentioned sarcodonins A (**21**) and G (**22**) in 2002 other three cyathane diterpenoids have been isolated (Hirota *et al.*, 2002). Three of them were named neosarcodonins A-C (**31-33**). The same group in 2004 isolated neosarcodonin O (**34**) and three acyl derivatives of sarcodonin A (**35-37**) (Kamo *et al.*, 2004) (Fig. 16).

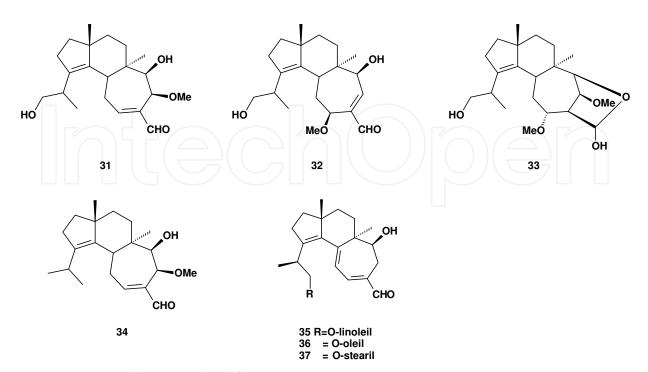


Fig. 16. Neosarcodonins isolated from S. scabrosus

3. Biological activities of Sarcodon metabolites

3.1 Biological activities of p-terphenyl derivatives

Terphenyls are aromatic hydrocarbons consisting of a chain of three benzene rings. From a structural point of view three different isomers are possible, in which the terminal rings are *ortho-, meta-,* or *para-substituents* of the central ring. Most of the natural terphenyls are *p*-terphenyl derivatives. In recent years, some terphenyls have been reported to exhibit significant biological activity, *e.g.,* potent immunosuppressant, neuroprotective, antithrombotic, anticoagulant, specific 5-lipoxygenase inhibitory, and cytotoxic activities (Liu, 2007).

3.1.1 Antibiotic properties

Compounds **10** and **11** (Tringali *et al.,* 1987) were tested against several Gram-positive and Gram-negative bacteria in an agar dilution test and the results showed that among Grampositive the most susceptible microorganism was *Proteus mirabilis* and among Gramnegative *Staphylococcus aureus* (MIC 75 and 50 µg/ml respectively).

3.1.2 Antitumoral activity

Sarcodonin (**12**) was tested against two different tumor cell lines and it resulted moderately active toward KB (ED_{50} 10.0 µg/ml) and P-388 (ED_{50} 27.0 µg/ml) (Geraci *et al.*, 2000). The higher cytotoxic activity of **10** with respect to **12**, coupled with mild antibacterial activity already described (Tringali *et al.*, 1987) suggest that **10** could play a role in the chemical defense of the mushroom.

Compounds **12**, **13** (sarcodonin α), **15** (sarcodonin γ), and **17** (episarcodonin) and the mixture of **19** and **20** (sarcoviolins) were tested in the three-cell line panel High Throughput PreScreen (one-dose primary anticancer assay) carried out at National Cancer Institute (Bethesda, USA). Fully aromatic terphenyls proved to be cytotoxic at a concentration of 5 x10⁻⁵ M against NCI-H460 (Lung), MCF7 (Breast), and SF-268 (CNS) cell lines. In particular, **12**, **15**, and **16** show the highest cytotoxicity towards SF-268 cells, with 96, 93, and 95% of cells killed, respectively. Sarcoviolins (**19** and **20**) significantly reduced the growth of all cell lines at 10⁻⁴ M (MCF7 totally blocked).

3.2 Biological activities of cyathane diterpenoids

In 1965 Brodie discovered a new bird's nest fungus of the genus *Cyathus* that was named *C. helenae* (Brodie, 1966) and he showed that the metabolites of the mushroom (cyathine complex) possessed antibiotic activity (Allbutt *et al.*, 1971). From this first report, in these 40 years, a great number of cyathane diterpenoids have been isolated, structurally identified and tested for their biological activities.

3.2.1 Antibiotic activity

In 1998 Shibata (Shibata *et al.*, 1998) and coworkers isolated from *S. scabrosus* sarcodonins L and M and sarcodonin A (**21**) and G (**22**). Sarcodonin L and M resulted to be identical to scabronines C (**25**) and B (**24**), respectively (Kita *etal.*, 1998). All these compounds were tested against *B. subtilis* and *S. aureus* and the results are reported in Table 1.

Compound	B. subtilis			S. aureus		
	1.0%*	0.2%	0.05%	1.0%	0.2%	0.05%
21	6.5**			7.0		
22	6.5			7.5	6.5	
24	14.0	11.0	8.0	22.0	15.0	9.0
25	9.5	7.5	7.0	18.0	12.0	7.5

*Disc were soaked in 35 μ l of each test sample (w/v%); **The diameter of the inhibitory zone was measured in mm.

Table 1. Antibiotic activity of sarcodonins A, G, L and M

3.2.2 Anti-inflammatory activity

Anti-inflammatory activity has been tested for sarcodonin A (21), sarcodonin G (22), neosarcodonin A-C (31-33) (Hirota *et al.*, 2002), neosarcodonin O (34), acyl derivatives of sarcodonin A (35-37) (Kamo *et al.*, 2004) and glaucopines A-C (5-7) (Curini *et al.*, 2005; Marcotullio *et al.*, 2006a). The results are reported in Table 2. The topical anti-inflammatory activity of compounds was evaluated observing the reduction of oedema induced by TPA, for sarcodonins and neosarcodonins, and Croton oil, for glaucopines, in mouse ear. Indomethacin was used as positive control in all tests.

Compound	Dose	Animals	% Oedema Reduction
5	1.0ª	10	62
6	1.0ª	10	55
7	1.0ª	6	39
Indomethacin	0.3ª	10	66
21	0.63 ^b	5	75
22	0.63 ^b	5	84
31	0.57 ^b	5	49
32	0.57 ^b	5	64
33	0.53 ^b	5	87
34	0.63b	5	36
35	0.63b	5	61
36	0.63b	5	-46
37	0.63b	5	72
Indomethacin	0.56 ^b	5	16

^a Dose= μMoles/cm²; ^bDose= μmol

Table 2. Anti-inflammatory activity of sarcodonins, neosarcodonins and glaucopines

3.2.3 Antiproliferative and antitumoral activities

The first report about the antitumor activity of a cyathane diterpene was about sarcodonin G isolated from the dichloromethane extract of *Sarcolon scabrosus* Karst, on HeLa cells in vitro. Sarcodonin G (**22**), isolated from the mushroom *Sarcodon scabrosus* and already reported to have anti-inflammatory activity, inhibited proliferation of HeLa cells (Dong *et al.*, 2009).

3.2.4 Stimulation of NGF (Nerve Growth Factor)

Sarcodon scabrosus and *S. cyrneus* metabolites have been studied for their unique activity to act as neuroprotective agents being able to stimulate the production of neurotrophic factors *in vitro*. Scabronines A-G (**23-29**) have been tested for their activity to induce NGF secretion from 1321N1 human astrocytoma cells and induce neuritogenesis in PC12 cells (rat pheochromocytoma cells) (Ohta *et al.*, 1998a, 1998b; Kita *et al.*, 1998; Oshima, Y. *et al.*, 1999). Scabronines increased the expression of mRNA for NGF, and the secretion of NGF from 1321N1 cells in a concentration-dependent mechanism (Obara *et al.*, 1999). Among different scabronines, scabronine G methyl ester (SG-ME) (**31**) resulted to be the most active and the mechanism of action was deeply studied. SG-ME activates PKC-ζ, induces the translocation NF-κB to nucleus and enhances its transcriptional activity (Fig. 17) (Obara *et al.*, 2001).

Cyrneine A-D (**1-4**) and glaucopine C (**7**) were tested to evaluate their activity to induce NGF production from 1321N1 cells, but they resulted much less active than scabronine G (Fig. 18) (Marcotullio *et al.*, 2007). Neverthless, cyrneine A and B showed an interesting activity, being able to induce directly differentiation on PC-12 cells (Marcotullio *et al.*, 2006b).

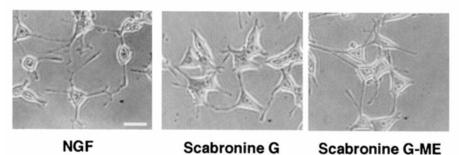
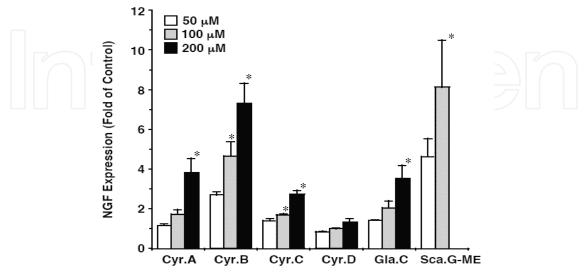
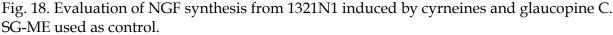


Fig. 17. Morphological changes of PC-12 cells by the 1321N1 cell culture medium conditioned by scabronines. NGF used as control.





As a transcriptional regulation is required for neurite extension, and the activity of three major transcription factors (activator protein-1 (AP-1), nuclear factor- κ B, and CREB) was determined. Cyrneines A and B enhanced activation of AP-1 and NF- κ B. Moreover, treatment with cyrneine A led to actin translocation and subsequently, to accumulation of F-actin at the tip of neurites. Rac1 activity was increased by cyrneine A and expression of a dominant-negative Rac1 mutant significantly inhibited the cyrneine A-induced extension of neurites. These results suggest that cyrneine A induces neurite outgrowth in a Rac1-dependent mechanism (Obara *et al.*, 2007).

4. Conclusions

The analysis of the literature about *Sarcodon* genus showed that these mushrooms produces interesting biologically active secondary metabolites such as cyathane e triphenyl derivatives. It is interesting to note that the use of trivial names instead of systematic ones generated some confusion (e.g. the term sarcodonins has been used to name both terphenyls and cyathanes). Furthermore, it was evidenced that sometimes there are problems in the use of different synonyms for the same species. Neverthless, the interesting biological properties of *Sarcodon* metabolites and the nutritional value of some spp make this genus worthy of further investigations.

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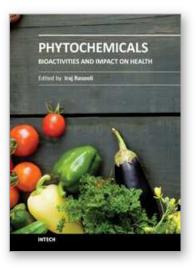
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Among the thousands of naturally occurring constituents so far identified in plants and exhibiting a long history of safe use, there are none that pose - or reasonably might be expected to pose - a significant risk to human health at current low levels of intake when used as flavoring substances. Due to their natural origin, environmental and genetic factors will influence the chemical composition of the plant essential oils. Factors such as species and subspecies, geographical location, harvest time, plant part used and method of isolation all affect chemical composition of the crude material separated from the plant. The screening of plant extracts and natural products for antioxidative and antimicrobial activity has revealed the potential of higher plants as a source of new agents, to serve the processing of natural products.

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