ABIE TANE QUINONE DITERPENES WITH ANTITUBERCULOSIS ACTIVITY ISOLATED FROM THE GENUS SALVIA

Abstract. The name Salvia, the largest genus of Lamiaceae, derives from the Latin "salvere" (= to feel good, healthy). In the Mediterranean basin, S. officinalis has been used since the time of the ancient Egyptians for medicinal purposes. Pliny the Elder was the first known author to describe a plant called "Salvia" by the Romans. Recent scientific research has confirmed the antituberculosis properties of numerous species present in the traditional ethnobotany.

Keywords: Salvia, abietane diterpenes, structure, anti-tuberculosis, toxicity, Multiorthoquinone, Horminone, Tanshinones.

The genus Salvia is present spontaneously in all continents except Australia, with diffusion in the temperate and tropical regions of the two hemispheres.

The species of this genus, about 900, prefer forest or mountainous habitats, but also ruderal environments. Six geographical areas constitute the centers of biodiversity of this kind: Central-southern states of the U.S.A., Central America where the Mexican area is the area with the greatest presence of taxa, South America, Southern Africa, Eastern and Western Asia. This vast geographical and habitat distribution makes the genus Salvia the most numerous and complex in the family Lamiaceae. The area including the Mediterranean Basin has about 250 species [4].

Abietane quinone diterpenes are widespread in the genus Salvia L. [2, 11, 12]. Many of them show interesting biological activities when tested both "in vitro" and "in vivo" [5, 6, 10]. Species of the genus Salvia have been studied as a potential resource of quinone diterpenes with antimicrobial and antituberculosis activity [3, 17].

Based on the literature 175 diterpene quinones isolated from 130 species of the
genus Salvia were examined: about 54 compounds show different biological activity, while many have not yet been tested or are inactive; out of 175 molecules, 151 have the structure of abietane and 24 have a structure different from abietic acid [4, 15].

Diterpene quinones are classified into 4 main groups (Fig. 1).

The first two groups include the compounds endowed with the abietanic skeleton with the quinone group in the –para or –ortho position; the third group includes compounds in which a ring of the abietanic skeleton is open or rearranged; the fourth group includes all compounds with a molecular structure other than abietic acid.

![Fig. 1. Classification of diterpene quinones [4, 15, 18]](image)

Several studies evidenced that the most typical activity of all diterpene quinones is the anticancer and antimicrobial activity (including antituberculosis), which was demonstrated by 38% and 30% of the molecules tested, respectively [4].

Diterpenoids are often found both in various organs of the Salvia species and in the volatile fraction. Quinone diterpenes constitute one of the largest and most
diverse classes of plant compounds with more than 10,000 different structures. Diterpenoids isolated from Lamiaceae have more than 50 different skeletons [15].

Diterpenoids can be isolated from all plant organs, but most of them are found in the roots (Fig. 2).

![Diagram showing distribution of diterpene quinones in plant organs of the 130 species reported in the literature.](image)

**Fig. 2. Distribution of diterpene quinones in plant organs of the 130 species reported in the literature.**

Some diterpene quinones are very common in several species of the genus *Salvia* (mostly “Tashinones”) (Fig. 3). In that way, around 100 compounds are extremely uncommon and each has been found in only one species. All this can be explained by the fact that many species are still poorly studied or that some diterpene quinones are exclusive to certain species [4].

Several *Salvia* species are the most important sources of diterpene quinones with antimicrobial and anti-mycobacterial activity; about 30 chemical compounds were isolated from 57 species.

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). In 2019, an estimated 10 million people fell ill with tuberculosis (TB) worldwide (a total of 1.4 million people died) [1, 20].
Study of antimicrobial and anti-tuberculosis properties of diterpene quinones are fairly recent and have increased with interest in the last decade as reported in a recent review of Bisio et al. (2019) [15, 19].

According to recent studies, many tanshinonoi have been shown to be active in the treatment of tuberculosis. Tanshinonoi from *Salvia miltiorrhiza* (Fig. 4) inhibit *Mycobacterium tuberculosis* via disruption of the cell envelope surface and oxidative stress. In this work, Sieniawska E. et al. successfully applied the combination of genes expression analysis and metabolomics to explore the
antimycobacterial potential of tanshinones. Gene expression results suggested that tanshinones caused surface and oxidative stress. Then, metabolomic analysis revealed the disruption of cell envelope surface manifested by uncontrolled leakage of the cell envelope constituents and by strong rearrangement of molecules placed in outer compartment of cell envelope. The following metabolic pathways analysis confirmed the activation of detoxification mechanisms and biosynthetic processes related to lipids formation and nucleic acids repair, which aimed to overcome the tanshinones influence [17].

![Fig.4. The major constituents of Salvia miltiorrhiza extract used in the metabolomic experiment by Sieniawska E. et al](image)

Some quinone diterpenes have much more significant MIC against *Mycobacterium tuberculosis* than the positive control. Diterpene quinones with tuberculous activity have a wide range of activity. Some of them are significantly more active than anti-TB drugs on the market [4, 15].

In particular, 12-Methyl-5,6-dehydroacethylhorminone (Fig. 5), from *S. multicaulis* (root), showed much higher MIC values against *Mycobacterium tuberculosis* than Streptomycin, suggesting its potential use in anti-TB therapy: MIC 0.89 $\mu$g/ml MIC 2.0 $\mu$g/ml Streptomycin [6].

![Fig.5. 2-Methyl-5,6-dehydroacetylhorminone](image)
12-Methyl-5-dehydrohorminone (Fig. 6), from *S. multicaulis* (root) *S. recognita* Fisch. & C.A. Mey., *S. verbenaca*, showed much higher MIC values against *Mycobacterium tuberculosis* than Streptomycin, suggesting its potential use in anti-TB therapy: MIC 1.2 μg/ml MIC 2.0 μg/ml Streptomycin [6].

![Fig.6. 12-Methyl-5-dehydrohorminone](image)

12-Demethylmultiorthoquinone (Fig. 7), from *S. multicaulis* (root), showed much higher MIC values against *Mycobacterium tuberculosis* than Kanamycin, suggesting its potential use in anti-TB therapy: MIC 1.2 μg/ml MIC 5.0 μg/ml Kanamycin [6].

![Fig.7. 12-Demethylmultiorthoquinone](image)

Multiorthoquinone (Fig. 8), from *S. multicaulis* (root), showed much higher MIC values against *Mycobacterium tuberculosis* than Kanamycin, suggesting its potential use in anti-TB therapy: MIC 2.0 μg/ml MIC 5.0 μg/ml Kanamycin [6].

![Fig.8. Multiorthoquinone](image)
Hypargenin F (Fig. 9), *S. hypargeia* Fisch. & C.A. Mey., *S. montbretii* Benth. also appeared to be active against *Mycobacterium tuberculosis*, but much weaker than the above compounds (MIC 250 μg/ml) [14].

![Fig.9. Hypargenin F](image)

Many authors hypothesize that the presence of the quinone group in abietane quinone diterpenes is responsible for their antibacterial and antituberculosis activity [7, 8, 9].

This compounds have a typical structure of abietic acid, but at the same time exhibit different strength of antituberculosis activity (MIC from 0.89 to 250 μg/ml) suggesting that the presence of substituents in the abietane skeleton (hydroxyl, methyl, acetyl groups, etc.) affects the anti-tuberculosis activity of the above molecules.

Despite centuries-old use of species of the genus *Salvia*, very few studies have been conducted on the toxicity of both the species themselves and their diterpene quinones today. However, according to the information available, diterpene quinones are considered safe and not toxic.

A prolonged toxicity study in rats demonstrated that administration of *S. miltiorrhiza* extract of 8000 mg / kg for 3 months was not associated with toxic effects, as was a single oral dose of 15 g / kg in mice [13].

The LD of the aqueous and methanolic extract of *S. deserta* administered to mice is 30-40 g / kg. The LD₅₀ of the ethanol extract of *S. Przewalski*, administered to mice orally, intraperitoneally and intramuscularly, is 2547.7, 780.8, and 901.3 mg / kg, respectively [15].

In conclusion, it seems that toxic effects are not particularly relevant for *Salvia* species containing quinine diterpenes [4, 15].
Despite the fact that abietane diterpene quinones long been used in national
traditional medicine around the world, especially in Chinese, only one monograph
devoted to Tanshinone IIA (a typical representative of the class of diterpene
quinones of the abietane type) was included in the European Pharmacopoeia 10th
[16].

From the point of view of MIC (Minimum Inhibitory Concentration) values,
the selected compounds are promising for further deeper study of their anti-
tuberculosis activity and determination of toxicity.

Based on the above, it is possible to conclude that both the Salvia genus as a
whole and its abietane diterpene quinones certainly deserve attention and require
more careful and in-depth research from the scientific community.

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References:
2. Esquivel B, Sanchez A. (2005) Rearranged icetexane diterpenoids from the roots of Salvia
   thymoides (Labiatae). Nat Prod Res 19:413–417
   (2018) Sessein and isosessein with anti-inflammatory, antibacterial and antioxidant activity
   isolated from Salvia sessei Benth. J Ethnopharmacol 217:212–219
   botanici, chimici ed attività biologica” Tesi, Allegato I-III
   Georgiev V, Pavlov A (eds) Salvia biotechnology. Springer, Cham, pp 31–132
   of diterpenoids from hairy roots of Salvia sclarea L.: salvipisone as a potential antibiofilm
   agent active against antibiotic resistant Staphylococci. Phytomedicine 14:31–35


20. World Health Organization https://www.who.int/news-room/fact-sheets/detail/tuberculosis