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Research Article

The secondary metabolites profiling of the phytopathogenic fungus *Sclerotinia Sclerotiorum*

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Abstract

Sclerotinia sclerotiorum is a necrotrophic plant pathogen causing more than 60 different disease symptoms in approximately 400 plants globally. Hence, due to this distinctive characteristic, *S. sclerotiorum* has been the subject of various research to comprehend its pathogenicity mechanism, including virulent genes, proteins, and metabolites. Likewise, the genomic annotation of *S. sclerotiorum* uncovered its remarkable potential for producing secondary metabolites, of which genome mining has additionally prompted the disclosure of these uncharacterized metabolic pathways, which might aid the pathogenicity process. To comprehend the secondary metabolites secreted by *S. sclerotiorum* that might be involved in its pathogenicity, a secondary metabolite-level investigation of this plant pathogen was performed. Profiling and characterizing these secondary metabolites produced during *in vitro* germination would increase the current knowledge of this pathogen.

In this study, *S. sclerotiorum* secondary metabolites profile examination was conducted, utilizing the Ultra-High Resolution Qq-Time-Of-Flight mass spectrometer (UHR-QqTOF). Proficient data analysis and verification with the genomic pathways of *S. sclerotiorum* gave an unequivocal metabolome profile of this pathogen. Two hundred and thirty secondary metabolites were identified in all three biological replicates, and their bodily functions were identified.

More Information

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Introduction

Sclerotinia sclerotiorum is a necrotrophic plant pathogen that is the causative agent of approximately 60 symptomatic diseases, including the notorious Sclerotinia stem rot, drop, crown rot, blossom blight, and white mould (which is the most prevalent) [1,2].

Sclerotinia sclerotiorum is a highly destructive phytopathogenic fungus that poses a significant threat to numerous plant species [3]. This fungus is responsible for causing Sclerotinia stem rot or white mould, a disease that results in considerable yield losses and economic damage in agriculture. The pathogenic mechanisms employed by *S. sclerotiorum* involve the production of enzymes and toxins [4]. It secretes various cell wall-degrading enzymes, such as polygalacturonases and cellulases, which break down plant cell walls, facilitating fungal penetration and colonization [5]. Oxalic acid, another virulence factor the fungus produces, contributes to tissue maceration and cell death [6]. These mechanisms collectively lead to symptoms including wilting, stem cankers, water-soaked lesions, and the formation of characteristic white cottony mycelium and sclerotia on infected tissues [6,7].

To combat *S. sclerotiorum*, a range of strategies for prevention and control are currently employed. Cultural practices play a vital role, including crop rotation with non-host plants to disrupt the pathogen's life cycle and reduce inoculum levels in the soil. Proper spacing between plants improves air circulation and reduces humidity, creating an unfavorable environment for fungal growth. Timely and appropriate irrigation practices minimize plant wetness and limit disease development [4]. Chemical control utilizing fungicides, such as boscalid, iprodione, and thiophanate-methyl, has demonstrated efficacy in managing the disease [8]. However, adopting integrated pest management practices and considering potential environmental impacts is essential [4,9].

Biological control agents, including certain species of Trichoderma and Bacillus, show promise in suppressing *S. sclerotiorum* [10]. These beneficial microorganisms compete with the pathogen for resources, produce antimicrobial compounds, and induce plant defense mechanisms. Furthermore, breeding programs for resistant cultivars offer a long-term and sustainable solution. Breeders aim to reduce crop susceptibility to the disease by incorporating resistance traits into commercial varieties. However, the complex nature of host-pathogen interactions presents challenges in achieving broad-spectrum resistance [11].

Hence, a comprehensive approach combining cultural practices, chemical control, biological agents, and host resistance is essential for effectively managing *S. sclerotiorum*.

This epidemic has opened *S. sclerotiorum* to broad research, including its biology, genomic analysis, and proteome-level studies [6,7,12]. All these are proposed for systematic searches for its molecular characteristics and the bases of its pathogenicity [13,14]. More needs to be done to investigate the metabolites secreted by this necrotrophic pathogen. Consequently, the collection of metabolites involved in the necrotrophic lifestyle of this pathogen remains vague, thereby creating a gap in the available knowledge on this pathogen.

Given these perceptions, it could be hypothesized that *S. sclerotiorum* produces numerous metabolites that could be by-products of the proteins. Henceforth, this investigation aims to profile secondary metabolites produced during *in vitro* germination of *S. sclerotiorum*, characterize them based on their functions, and discover the pathways in which they are implicated. The discovery of such metabolites would fill the knowledge vacuum of *S. sclerotiorum* metabolomics, creating opportunities for novel metabolite disclosures.

Materials and methods

Metabolites were extracted from the actively growing mycelia of *S. sclerotiorum* as described by [15]. They were profiled utilizing the automated Ultra-High Resolution Qq-Time-Of-Flight mass spectrometer that generated chromatograms from detected chemical compounds available in the extract. This step was then preceded by data analysis using the Magma web tool to interpret and identify the secondary metabolites from the chromatogram.

Finally, the identified metabolites were classified based on their chemical composition and biological functions.

Fungi sample preparation and metabolite extraction

The virulent *S. sclerotiorum* wild-type strain 1980 UF-70 acquired from the Agricultural Research Council – Plant Protection Research, Tshwane, was utilized for this investigation. Mycelia of the fungus were harvested from a 5-day-old culture growing on potato dextrose agar at temperatures ranging from 4 °C to room temperature. Harvested mycelia were ground in liquid nitrogen using a mortar and pestle, followed by a metabolite extraction technique.

Extraction of metabolites secreted by *S. sclerotiorum* was performed according to [16], with slight modification. Fifty milligrams of the powdered *S. sclerotiorum* mycelia samples were weighed, and then 1.5 mL methanol: water (75%:25%, v/v) was added to the samples, following ultrasonic blending for 5 minutes. The mixtures were centrifuged at 12,000 rpm for 15 minutes at 4 °C [17] and the supernatants were dispensed into 1.5 mL centrifuge tubes for subsequent mass spectrometry investigation.

Analysis of metabolites extracted from *Sclerotinia sclerotiorum*

One microliter of *S. sclerotiorum* metabolites extracts was separated using an RP C18 column (50 x 2 mm, 1.7 µm particle size) on UHR-QqTOF (Bruker Daltonics). The system was connected to a networked series printer for recording chromatograms, Chromeleon Data System (Thermoscientific). The following gradient was utilized for the separation; the flow rate was 400 µL/min using (A) water + 0.1% HCOOH (B) Acetonitrile + 0.1% HCOOH as the mobile phase. The gradient was at 0 minutes 1% B; 1 minute 1% B; 10 minutes 99% B; 12 minutes 99% B; 12.5 minutes 1% B; 14 minutes 1% B ESI-MS measurements were performed using positive ionization on the maXis UHR-QqTOF MS m/z range: 100- 1200 m/z, acquisition rate: 3, 5, 10, 20 Hz [18].

Data analysis was done utilizing the MAGMa web tool https://www.emetabolomics.org/ according to [19]. Chromatogram generated by the UHR-QqTOF was queried against the KEGG compound database and the PubChem database, respectively, excluding peaks corresponding to contaminants, solvents, or media used.

Lastly, the functional characterization of the identified secondary metabolites was done utilizing the MetaboAnalyst and KEGG BRITE resources [20].

The workflow for characterizing the *S. sclerotiorum* metabolome is shown in Figure 1.

Pathway analysis of identified secondary metabolites produced by *Sclerotinia sclerotiorum*

All the secondary metabolites identified above were subjected to the pathway analysis module of the MetaboAnalyst web tool. MetaboAnalyst pathway analysis module utilizes the results from pathway enrichment (using hypergeometric test) and pathway topology analysis to detect the most significant pathways in the present investigation [21].

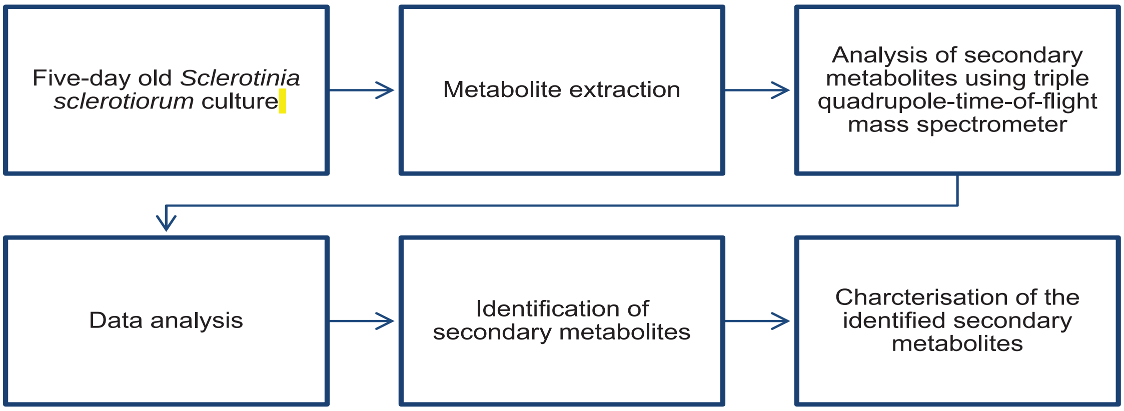

Figure 1: A schematic workflow for analyzing secondary metabolites produced by *Sclerotinia sclerotiorum.*

Results

In the present investigation, 230 secondary metabolites were discovered in the mycelia of *S. sclerotiorum* during its *in vitro* germination. These metabolites were grouped based on their chemical composition and biological functions. Pathway analysis revealed all the enriched pathways which are discussed.

Identification and characterisation of secondary metabolites extracted from *Sclerotinia sclerotiorum*

Table 1 shows the chemical formula, chemical name, KEGG identification code, function, group, and subgroup of individual secondary metabolites detected in *S. sclerotiorum* in the current study.

The distribution of functional classification of secondary metabolites produced by *S. sclerotiorum* is represented in Figure 2. The secondary metabolites profile shows that *S. sclerotiorum* has; phytochemical compounds (100), lipids (89), carcinogens (7), pesticides (5), major components of natural products (5), target-based classification of chemical compounds (4), chemical compounds with biological roles (3), pharmaceutical additives in Japan (2), risk category of Japanese otc drugs (2), natural toxins (1), animal drugs in Japan (1), classification of Japanese otc drugs (1), endocrine disrupting compounds (1) and 94 unclassified secondary metabolites.

Pathway analysis of secondary metabolites produced by *Sclerotinia sclerotiorum*

The pathway analysis results of all the secondary metabolites detected in this investigation were represented graphically in Figure 3 and Table 2 to simplify the biological implication of the enriched pathways connected with the identified secondary metabolites.

Figure 3 distinctively identified the phenylalanine metabolism pathway as the most significant pathway with an impact value of 0.7, followed by glycine, serine, and threonine metabolism pathways having 0.2 as an impact value; however, other identified pathways have negligible impact values.

Discussion

Although significant advancement has been made in understanding the molecular characteristics of the necrotrophic plant pathogen- *S. sclerotiorum*, several aspects of its lifecycle and infection processes remain vague [22].

This research is a progression from these fungi's genomic, transcriptomic, and proteomic analysis. While transcriptomics studies generated essential data relating to *S. sclerotiorum* gene expression during in vitro growth stage [23], proteomic results justified the transcriptomic results with the list of the corresponding protein [7] and the secondary metabolites profile gave a clear picture of the outcome of the cellular processes that occur within *S. sclerotiorum*. This research profiled the secondary metabolites produced by *S. sclerotiorum* during in vitro germination, revealing this fungi's richness.

S. sclerotiorum, like many other phytopathogenic fungi, is a biosynthetically endowed organism that produces a massive range of chemically diverse and biologically significant molecules known as metabolites. Nonetheless, the *S. sclerotiorum* metabolome profiling conducted in the current study revealed a catalog of secondary metabolites produced by *S. sclerotiorum* during *in vitro* germination is discussed below.

Sclerotinia sclerotiorum produces a plethora of diverse and bioactive secondary metabolites

Secondary metabolites produced by *S. sclerotiorum*, identified in this study, were classified as phytochemicals, lipids, and natural toxins based on their chemical constituents and known functions (Table 1).

Phytochemical compounds: In the current study, 100 phytochemical compounds were identified as part of *S. sclerotiorum* secondary metabolites. These phytochemicals were classified into sub-groups: alkaloids, amino acid-associated compounds, flavonoids, fatty acids-related compounds, phenylpropanoids, polyketides, skate/acetate-malonate pathway derivative compounds, and terpenoids.

Table 1: Secondary metabolites produced by *Sclerotinia sclerotiorum*.

Formula	Metabolite name	Kegg Number	Function	Group	Subgroup	Chemical function
$C_4H_9NO_3$	L-homoserine (12647)	C00263	Biological role	Peptide	Amino acids	Amino acid
$C_4H_9NO_3$	L-threonine (6288)	C00188	Biological role	Peptide	Amino acids	Amino acid
$C_4H_8N_2O_3$	Methylazoxymethanol acetate (5363199)	C19258	Carcinogens	Group 2A carcinogenic compounds		Methyl ester, Azoxy compound
C_8H_8O	Styrene oxide (7276)	C02083	Carcinogens	Group 2A carcinogenic compounds		Epoxide
$C_4H_8N_2O_3$	N-nitroso-n-methylurethane (12001)	C19301	Carcinogens	Group 2A carcinogenic compounds		Nitroso compound
$C_{21}H_{40}O_3$	Glycidyl stearate (62642)	C19427	Carcinogens	Group 3: Not carcinogenic to humans		Ester
$C_3H_6N_2O$	N-nitrosomethylvinylamine (20678)	C19282	Carcinogens	Group 2A is probably carcinogenic to human compounds		Nitroso compound
$C_{29}H_{48}O_2$	C11509 (443238)	C11509	Lipids	Sterol lipids	Cholesterol and derivatives	Unknown
$C_{29}H_{50}O_2$	C04814 (440493)	C04814	Lipids	Sterol lipids	Cholesterol and derivatives	Unknown
$C_{31}H_{48}O_3$	3-hydroxy-vitamin k (5280540)	C02785	Lipids	Quinones and hydroquinones	Vitamin K	Hydroxylated vitamin K
$C_5H_{11}NO_2$	5-aminovaleric acid (138)	C00431	Lipids	Fatty acyls	Amino fatty acids	Amino acid derivative
$C_{18}H_{28}O_3$	Alpha-licanic acid (5281118)	C08319	Lipids	Fatty acyls	Oxo fatty acid	Carboxylic acid
$C_5H_{11}NO_2$	L-norvaline (65098)	C01826	Lipids	Fatty acyls	Amino fatty acids	Amino acid
$C_{15}H_{11}O_{7+}$	Delphinidin (128853)	C05908	Lipids	Flavonoids	Anthocyanidins	Flavonoid
$C_{24}H_{38}O_4$	C11637 (443323)	C11637	Lipids	Sterol lipids	Bile acid, alcohols and derivatives	unknown compound
$C_{40}H_{54}O$	Echinenone (5281236)	C08592	Lipids	Prenol lipids	Isoprenoids	Carotenoid
$C_{13}H_{18}O_2$	Plastoquinol-1 (24892729)	C02185	Lipids	Quinones and hydroquinones	Ubiquinones	Quinone derivative
$C_{18}H_{28}O_3$	12,13(s)-eotre (20843328)	C04672	Lipids	Fatty acyls	Epoxy fatty acid	unknown
$C_{40}H_{56}O_2$	Deoxymyxol (16061292)	C15933	Lipids	Prenol lipids	Isoprenoids	unknown
$C_{40}H_{54}O$	Hydroxychlorobactene (10099075)	C15911	Lipids	Prenol lipids	Isoprenoids	unknown
$C_8H_8O_2$	3-vinylcatechol (441226)	C07085	Lipids	Octadecanoids	12-oxophytodienoic acid metabolites	Vinyl-substituted catechol
$C_{16}H_{30}O$	Hexadecenal (5280541)	C06123	Lipids	Fatty acyls	Fatty aldehydes	Aldehyde
$C_{18}H_{28}O_2$	Stearidonic acid (5312508)	C16300	Lipids	Fatty acyls	Polyunsaturated fatty acids	Fatty acid
$C_{18}H_{28}O_3$	9,10-eotre (23724711)	C16324	Lipids	Fatty acyls	Other octadecanoids	Unknown
$C_{31}H_{48}O_3$	2-hydroxy-vitamin k (11953813)	C02793	Lipids	Quinones and hydroquinones	Vitamine K	Hydroxylated vitamin K
$C_{13}H_2O_3$	Methyl jasmonate (5281929)	C11512	Lipids	Fatty acyls	Jasmonic acid	Methyl ester, Jasmonate
$C_{18}H_{28}O_3$	10-opda (23724712)	C16325	Lipids	Fatty acyls	Other octadecanoids	Unknown
$C_{15}H_{10}O7$	2'-hydroxypseudobaptigenin (5280616)	C03662	Lipids	Flavonoids	Isoflavonoids	Hydroxylated flavonoid
$C_{29}H_{48}O_2$	(24r,28r)-fucosterol epoxide (440161)	C03910	Lipids	sterol lipids	Stigmasterols	Epoxide
$C_{17}H_{14}O_6$	Aflatoxin b2 (2724360)	C16753	Lipids, Mycotoxins	Polyketides, Aflatoxins	Aflatoxin and related substances	Mycotoxin
$C_{29}H_{50}O_2$	Alpha-tocopherol (14985)	C02477	Lipids, Pharmaceutical additives in Japan, Japanese, OTC drugs risk category of Japanese OTC drugs	Quinones and hydroquinones, 3rd class OTC drugs, Nourishing tonics and health supplements	Vitamine E, Stabilizing agent	Vitamin E
$C_{27}H_{30}O_{16}$	Multinoside a (5319943)	C17563	A major component of natural products	Crude drug		Glycoside
$C_4H_8N_2O_3$	Asparagine (236)	C16438	A major component of natural products	Crude drug		Amino acid
$C_5H_{11}NO_2$	Betaine (247)	C00719	A major component of natural products	Crude drug		Quaternary ammonium compound
$C_{18}H_{28}O_2$	Neoprene (6434236)	C19042	Pesticides	Insect growth regulator	Juvenile hormone mimics	Insect growth regulator
$C_{18}H_{26}O_2$	Cinmethylin (91745)	C10903	Pesticides	Herbicides		Herbicide
$C_9H_{11}NO_2$	Metolcarb (14322)	C18747	Pesticides, Target based compound	Insecticides, Enzyme	Inhibitor	Carbamate insecticide
$C_{18}H_{26}O_2$	Empenthrin (6434488)	C18524	Pesticides, Target based compounds, Japanese Animal drugs	Insecticides, Ion channels, not therapeutic	Modulator	Insecticide

$C_5H_{14}NO^+$	Choline (305)	C00114	Pesticides, Risk category of Japanese OTC drugs	Plant growth regulator, 3rd class OTC drugs	Inorganic and organic chemicals	Quaternary ammonium compound
$C_4H_8N_2O_3$	Glycylglycine (11163)	C02037	Pharmaceutical additives in Japan	Buffering agent		Dipeptide
$C_{15}H_{10}O_4$	Chrysin (5281607)	C10028	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{15}H_{12}O_5$	Naringenin (439246)	C00509	Phytochemicals	Flavonoids	Flavanones	Flavonoid
$C_{15}H_{12}O_5$	Butein (5281222)	C08578	Phytochemicals	Flavonoids	Chalcones	Flavonoid
$C_{16}H_{12}O_5$	Wogonin (5281703)	C10197	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{17}H_{14}O_6$	Pinobanksin 3-o-acetate (148556)	C16418	Phytochemicals	Flavonoids	Dihyroflavonols	Acetylated flavonoid
$C_{21}H_2O_{12}$	Bracteatin 6-o-glucoside (23724746)	C16410	Phytochemicals	Flavonoids	Aurones	Glucosylated flavonoid
$C_{16}H_{12}O_5$	3-methylgalangin (5281946)	C11577	Phytochemicals	Flavonoids	Flavonols	Methylated flavonoid
$C_{27}H_{30}O_{16}$	Lucenin-2 (442615)	C10102	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{15}H_{10}O_5$	5-deoxykaempferol (5281611)	C10037	Phytochemicals	Flavonoids	Flavonols	Flavonoid
$C_{16}H_{12}O_5$	2'-hydroxyformononetin (5280551)	C02920	Phytochemicals	Isoflavonoids	Isoflavones	Hydroxylated isoflavone
$C_{15}H_{10}O_5$	Morindone (442756)	C10376	Phytochemicals	Polyketides	Anthraquinone	Unknown
$C_{21}H_2O_{12}$	Isoquercitrin (5280804)	C05623	Phytochemicals	Flavonoids	Flavonols	Flavonoid, Glycoside
$C_{15}H_{10}O_4$	4',6-dihydroxyflavone (182362)	C14344	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{16}H_{12}O_5$	Acacetin (5280442)	C01470	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{15}H_{12}O_5$	Pinobanksin (73202)	C09826	Phytochemicals	Flavonoids	Dihyroflavonols	Flavonoid
$C_{15}H_{10}O_7$	Isoetin (5281649)	C10079	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{15}H_{10}O_5$	Baicalein (5281605)	C10023	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_8H_8O_2$	3,4-dihydroxystyrene (151398)	C06224	Phytochemicals	Phenylpropanoids	Caffeate derivatives	Hydroxylated phenyl compound
$C_{16}H_{16}O_4$	Vestitol (177149)	C10540	Phytochemicals	Isoflavonoids	Isoflavanes	Isoflavone
$C_{15}H_{12}O_4$	Aloe-emodin anthrone (122840)	C16760	Phytochemicals	Polyketides	Anthrone	Anthraquinone derivative
$C_{15}H_{10}O_7$	Robinetin (5281692)	C10177	Phytochemicals	Flavonoids	Flavonols	Flavonoid
$C_{21}H_2O_{12}$	Quercimeritrin (5282160)	C12639	Phytochemicals	Flavonoids	Flavonols	Flavonoid, Glycoside
$C_{15}H_{10}O_4$	7,4'-dihydroxyflavone (5282073)	C12123	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{16}H_{16}O_4$	Perforatin a (441968)	C09014	Phytochemicals	Pyrones	Chromoes	Unknown
$C_{15}H_{10}O_7$	8-hydroxykaempferol (5280544)	C02806	Phytochemicals	Flavonoids	Flavonols	Flavonoid
$C_{29}H_{48}O_3$	Messagenin (46173776)	C08631	Phytochemicals	Triterpenoids	Dammarenes	Anthraquinone derivative
$C_{15}H_{11}O_5$	Luteolinidin (441701)	C08652	Phytochemicals	Flavonoids	Anthocyanidins and anthocyanins	Flavonoid
$C_{15}H_{10}O_4$	1,4-dihydroxy-2-methyl anthraquinone (99300)	C10329	Phytochemicals	Polyketides	Anthraquinone	
$C_{15}H_{10}O_5$	3',4',7-trihydroxy isoflavone (5284648)	C14313	Phytochemicals	Isoflavonoids	Isoflavones	
$C_{16}H_{12}O_5$	Lucidin omega-methyl ether (149782)	C10370	Phytochemicals	Polyketides	Anthraquinone	Methylated anthraquinone
$C_{15}H_{12}O_5$	Rubrofusarin (72537)	C09047	Phytochemicals	Pyrones	Naphthopyrones	Unknown
$C_{15}H_{12}O_4$	Liquiritigenin (114829)	C09762	Phytochemicals	Flavonoids	Flavanones	Flavonoid
$C_{15}H_{10}O_7$	Hypolaetin (5281648)	C10078	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{15}H_{10}O_7$	Bracteatin (5281221)	C08577	Phytochemicals	Flavonoids	Aurones	Flavonoid
$C_{15}H_{10}O_5$	Purpurin 1-methyl ether (442766)	C10397	Phytochemicals	Polyketides	Anthraquinone	Methylated anthraquinone
$C_{15}H_{10}O_7$	Tricetin (5281701)	C10192	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{15}H_{10}O_4$	Alizarin 2-methyl ether (80103)	C10291	Phytochemicals	Polyketides	Anthraquinone	Flavonoid
$C_{15}H_{10}O_5$	Apigenin (5280443)	C01477	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{15}H_{10}O_5$	Norwogonin (5281674)	C10113	Phytochemicals	Flavonoids	Flavones	Flavonoid
$C_{16}H_{12}O_5$	Genkwanin (5281617)	C10046	Phytochemicals	Flavonoids	Flavones	Unknown
$C_{15}H_{10}O_4$	Primetin (11055)	C10121	Phytochemicals	Flavonoids	Flavones	Glycoside
$C_{15}H_{10}O_4$	Digiferrugineol (32209)	C10327	Phytochemicals	Polyketides	Anthraquinone	Unknown
$C_{27}H_{30}O_{16}$	Rutin (5280805)	C05625	Phytochemicals	Flavonoids	Flavonols	Anthraquinone derivative
$C_{16}H_{12}O_5$	Question (160717)	C01448	Phytochemicals	Polyketides	Anthraquinone	Flavonoid
$C_{15}H_{10}O_5$	Emodin (3220)	C10343	Phytochemicals	Polyketides	Anthraquinone	Flavonoid
$C_{15}H_{12}O_5$	2,7,4'-trihydroxyisoflavanone (11954208)	C15567	Phytochemicals	Isoflavonoids	Isoflavones	
$C_{15}H_{10}O_7$	Morin (5281670)	C10105	Phytochemicals	Flavonoids	Flavonols	
$C_{15}H_{10}O_5$	Norobtusifolin (442759)	C10379	Phytochemicals	Polyketides	Anthraquinone	
$C_{15}H_{10}O_4$	Rubiadin (124062)	C10402	Phytochemicals	Polyketides	Anthraquinone	
$C_{21}H_{20}O_{12}$	Myricitrin (5281673)	C10108	Phytochemicals	Flavonoids	Flavonols	
$C_{15}H_{12}O_5$	Chalconaringenin (5280960)	C06561	Phytochemicals	Flavonoids	Chalcones	

$C_{15}H_{11}O_4$	Apigeninidin (441647)	C08574	Phytochemicals	Flavonoids	Anthocyanidins and anthocyanins	
$C_{15}H_{12}O_4$	Pinocembrin (68071)	C09827	Phytochemicals	Flavonoids	Flavanones	
$C_{27}H_{30}O_{16}$	Sophoraflavonoloside (5282155)	C12634	Phytochemicals	Flavonoids	Flavonols	
$C_{15}H_{12}O_4$	Pinocembrin chalcone (6474295)	C16404	Phytochemicals	Flavonoids	Chalcones	
$C_{16}H_{12}O_5$	Physcion (10639)	C17045	Phytochemicals	Polyketides	Anthraquinone	
$C_{15}H_{10}O_7$	6-hydroxykaempferol (5281638)	C10068	Phytochemicals	Flavonoids	Flavonols	
$C_{16}H_{16}O_4$	2',6'-dihydroxy-4'-methoxydihydrochalcone (169676)	C09644	Phytochemicals	Flavonoids	Dihydrochalcones	
$C_{15}H_{12}O_4$	3,9-dihydroxypterocarpan (162933)	C04271	Phytochemicals	Isoflavonoids	Pterocarpans	
$C_{17}H_{14}O_6$	Ventinone a (442767)	C10407	Phytochemicals	Polyketides	Anthraquinone	
$C_{16}H_{12}O_5$	Melanin (442808)	C10504	Phytochemicals	others	Neoflavonoids	
$C_{15}H_{12}O_5$	Garbanzol (442410)	C09751	Phytochemicals	Flavonoids	Dihyroflavonols	
$C_{15}H_{12}O_4$	Hydrangenol (119199)	C10262	Phytochemicals	Skimate / acetate-malonate pathway-derived compounds	Miscellaneous stilbenoids	
$C_{27}H_{30}O_{17}$	Baimaside (5282166)	C12667	Phytochemicals	Flavonoids	Flavonols	
$C_{15}H_{10}O_7$	6-hydroxyluteolin (5281642)	C10072	Phytochemicals	Flavonoids	Flavones	
$C_{21}H_{20}O_{12}$	Gossypetin 8-rhamnoside (5281620)	C10050	Phytochemicals	Flavonoids	Flavonols	
$C_{15}H_{10}O_7$	Quercetin (5280343)	C00389	Phytochemicals, Carcinogens, transporter	Flavonoids, Group 3-not carcinogenic to humans, Solute carrier	Flavonols, Inhibitor	
$C_{24}H_{38}O_4$	Dioctyl phthalate (8343)	C03690	Phytochemicals, Carcinogens, Endocrine disrupting compound	Polyketides, Group 2B, possibly carcinogenic to humans compounds, Plasticizers, and plastics	Anthraquinone, Phthalates	
$C_{17}H_{15}O_6$	Rosinidin (441777)	C08729	Phytochemicals, lipids	Flavonoids	Anthocyanidins and anthocyanins	
$C_{15}H_{11}O_5$	Pelargonidin (440832)	C05904	Phytochemicals, lipids	Flavonoids	Anthocyanidins and anthocyanins	
$C_{15}H_{10}O_4$	Daidzein (5281708)	C10208	Phytochemicals, lipids	Flavonoids	Isoflavones	
$C_{40}H_{56}O_2$	Zeaxanthin (5280899)	C06098	Phytochemicals, lipids	Carotenoids, apocarotenoids, and prenol lipids	Carotenoids, isoprenoids	
$C_{15}H_{12}O_5$	2'-hydroxydihydrodaidzein (440047)	C03567	Phytochemicals, lipids	Flavonoids	Flavones	
$C_{17}H_{14}O_6$	Pisatin (101689)	C10516	Phytochemicals, lipids	Isoflavonoids	Pterocarpans	
$C_{16}H_{12}O_5$	Inermin (91510)	C10502	Phytochemicals, lipids	Isoflavonoids	Pterocarpans	
$C_{27}H_{31}O_{16}$	Cyanidin 3-o-sophoroside (11169452)	C16306	Phytochemicals, lipids	Flavonoids	Anthocyanidins and anthocyanins	
$C_{15}H_{12}O_4$	Isoliquiritigenin (638278)	C08650	Phytochemicals, lipids	Flavonoids	Chalcones	
$C_{16}H_{12}O_5$	Prunetin (5281804)	C10521	Phytochemicals, lipids	Isoflavonoids	Isoflavones	
$C_{15}H_{10}O_4$	His idol (5281254)	C08644	Phytochemicals, lipids	Flavonoids	Aurones	
$C_{21}H_{21}O_{12}$	Mirtillin (443650)	C12138	Phytochemicals, lipids	Flavonoids	Anthocyanidins and anthocyanins	
$C_{40}H_{56}O_2$	Lactucaxanthin (5281242)	C08599	Phytochemicals, lipids	Carotenoids, apocarotenoids, and prenol lipids	Carotenoids, isoprenoids	
$C_{15}H_{10}O_4$	Anhydroglycinol (442667)	C10200	Phytochemicals, lipids	Isoflavonoids	Pterocarpans	
$C_{26}H_{28}O_{16}$	C12637 (5487635)	C12637	Phytochemicals, lipids	Isoflavonoids	Pterocarpans	
$C_{16}H_{12}O_5$	Biochanin a (5280373)	C00814	Phytochemicals, lipids	Isoflavonoids	Isoflavones	
$C_{16}H_{12}O_5$	Calycosin (5280448)	C01562	Phytochemicals, lipids	Isoflavonoids, a crude drug	Isoflavones	
$C_{16}H_{12}O_5$	Texasin (5281812)	C10536	Phytochemicals, lipids	Isoflavonoids	Isoflavones	
$C_{17}H_{14}O_6$	Irisolidone (5281781)	C10471	Phytochemicals, lipids	Flavonoids	Isoflavones	
$C_{27}H_{31}O_{17}$	Delphin (10100906)	C16312	Phytochemicals, lipids	Flavonoids	Anthocyanidins and anthocyanins	

$C_{15}H_{10}O_5$	2'-hydroxydaidzein (5280520)	C02495	Phytochemicals, lipids	Isoflavonoids	Isoflavones	
$C_{15}H_{10}O_5$	Genistein (5280961)	C06563	Phytochemicals, lipids	Isoflavonoids	Isoflavones	
$C_{16}H_{12}O_5$	Glycitein (5317750)	C14536	Phytochemicals, lipids	Isoflavonoids	Isoflavones	
$C_{15}H_{10}O_5$	Aloe-emodin (10207)	C10294	Phytochemicals, lipids	Polyketides	Anthraquinone	
$C_{27}H_{31}O_{17+}$	Delphinidin 3-o-sophoroside (23724705)	C16307	Phytochemicals, lipids	Flavonoids	Anthocyanidins and anthocyanins	
$C_{27}H_{31}O_{16+}$	Tulipanin (5492231)	C16315	Phytochemicals, lipids	Flavonoids	Anthocyanidins and anthocyanins	
$C_{15}H_{10}O_4$	Chrysophanol (10208)	C10315	Phytochemicals, a major component of natural products	Polyketides, crude drug	Anthraquinone	
$C_{16}H_{12}O_5$	Obtusifolin (3083575)	C17039	Phytochemicals, a major component of natural products	Polyketides	Anthraquinone	
$C_{37}H_{40}O_9$	Resiniferatoxin (442082)	C09179	Phytochemicals, target-based compounds	Terpenoids, voltage-dated cations channels	Daphnanes, Agonist	
$C_{40}H_{56}O_2$	Lutein (5281243)	C08601	Phytochemicals, lipids	Carotenoids, apocarotenoids, and prenol lipids	Carotenoids, isoprenoids	
$C_{27}H_{31}O_{16+}$	Cyanin (441688)	C08639	Phytochemicals, lipids	Flavonoids	Anthocyanidins and anthocyanins	
$C_{20}H_{23}N_5O_6S$	Azlocillin (6479523)	C06839	Unclassified			
$C_9H_{11}NO_2$	4-hydroxy-1-(3-pyridinyl)-1-butanone (107819)	C19565	Unclassified			
$C_8H_8O_2$	Benzylformate (7708)	C05613	Unclassified			
$C_{24}H_{38}O_4$	Diisooctyl phthalate (33934)	C14577	Unclassified			
$C_8H_{14}O_5S$	2-(3'-methylthio)propyl malate (24883455)	C17214	Unclassified			
$C_8H_8O_2$	2-methyl benzoic acid (8373)	C07215	Unclassified			
$C_9H_{11}NO_2$	Tricaine (11400)	C18090	Unclassified			
$C_{24}H_{38}O_4$	Di-n-octyl phthalate (8346)	C14227	Unclassified			
$C_9H_{11}NO_2$	Benzocaine (2337)	C07527	Unclassified			
$C_{17}H_{14}O_6$	4',6-dihydroxy-5,7-dimethoxyflavone (244386)	C15100	Unclassified			
$C_4H_9NO_3$	2-methylserine (439656)	C02115	Unclassified			
$C_{15}H_{12}O_5$	P-coumaroyltriacetic acid lactone (54704424)	C12087	Unclassified			
$C_{15}H_{10}O_5$	Sulfuretin (5281295)	C08730	Unclassified			
$C_{18}H_{28}O_3$	Etherolenic acid (23724709)	C16319	Unclassified			
$C_{35}H_{36}N_4O_5$	Pheophorbide a (5323510)	C18021	Unclassified			
$C_8H_8O_2$	Phenylacetate (31229)	C00548	Unclassified			
$C_{16}H_{16}O_4$	1,2-bis(4-hydroxy-3-methoxyphenyl)ethylene (5280698)	C04547	Unclassified			
$C_{16}H_{16}O_4$	9-methoxy-alpha-lapachone (442754)	C10372	Unclassified			
$C_{15}H_{12}O_5$	Licodione (439528)	C01592	Unclassified			
$C_{15}H_{10}O_5$	6-hydroxydaidzein (5284649)	C14314	Unclassified			
$C_{18}H_{28}O_3$	17beta-hydroxy-2-oxa-5alpha-androstane-3-one (252289)	C14911	Unclassified			
$C_{18}H_{26}O_2$	Prenortestosterone (235672)	C15257	Unclassified			
C_8H_8O	3-methylbenzaldehyde (12105)	C07209	Unclassified			
$C_{21}H_{20}O_{12}$	6-hydroxy luteolin 7-glucoside (185766)	C17763	Unclassified			
$C_8H_8O_2$	Methyl benzoate (7150)	C20645	Unclassified			
$C_{16}H_{16}O_4$	Eleutherin (10166)	C10340	Unclassified			
$C_{15}H_{12}O_5$	Toralactone (5321980)	C17673	Unclassified			
$C_{24}H_{38}O_4$	Apocholic acid (101818)	C15375	Unclassified			
$C_{27}H_{31}O_{16+}$	Cyanidin 3,7-di-o-beta-d-glucoside (5491675)	C20469	Unclassified			
$C_{47}H_{70}O_3$	2-octaprenyl-6-methoxy-1,4-benzoquinone (5280835)	C05813	Unclassified			

$C_8H_8O_2$	M-toluic acid (7418)	C07211	Unclassified			
$C_7H_{14}N_2O_6S$	Glutaurine (68759)	C05844	Unclassified			
$C_9H_{11}NO_2$	5-(3-pyridyl)-2-hydroxytetrahydrofuran (179630)	C19578	Unclassified			
$C_8H_{21}NO_6P+$	Glycerophosphocholine (439285)	C00670	Unclassified			
$C_{47}H_{70}O_3$	3-octa prenyl-4-hydroxybenzoate (5280831)	C05809	Unclassified			
$C_8H_8O_2$	4-hydroxyphenyl acetaldehyde (440113)	C03765	Unclassified			
$C_{15}H_{10}O_5$	Lucidin (10163)	C10369	Unclassified			
$C_5H_{11}NO_2$	Valine (1182)	C16436	Unclassified			
$C_{18}H_{26}O_2$	Nandrolone (9904)	C07254	Unclassified			
$C_8H_8O_2$	Phenylacetic acid (999)	C07086	Unclassified			
$C_{40}H_{56}O_2$	Rhodopinal (20055178)	C16270	Unclassified			
$C_{27}H_{31}O_{17+}$	Delphinidin 3,7-di-o-beta-d-glucoside (72734296)	C20496	Unclassified			
$C_{15}H_{10}O_5$	Islandicin (10151)	C16796	Unclassified			
C_8H_8O	P-tolu aldehyde (7725)	C06758	Unclassified			
$C_{13}H_{20}O_3$	(6s,9r)-vomifoliol (5280462)	C01760	Unclassified			
$C_{29}H_{48}O_3$	C04840 (440507)	C04840	Unclassified			
$C_{13}H_{18}O_2$	Ibuprofen (3672)	C01588	Unclassified			
$C_8H_{14}O_5S$	3-(3'-methylthio)propyl malate (44237293)	C17215	Unclassified			
C_8H_8O	Phenylacetaldehyde (998)	C00601	Unclassified			
$C_5H_9NO_2$	Proline (614)	C16435	Unclassified			
$C_{17}H_{14}O_6$	Cirsimaritin (188323)	C17785	Unclassified			
$C_8H_8O_2$	4'-hydroxyacetophenone (7469)	C10700	Unclassified			
$C_8H_8O_2$	P-anisaldehyde (31244)	C10761	Unclassified			
C_8H_8O	2-methylbenzaldehyde (10722)	C07214	Unclassified			
$C_{15}H_{11}O_{7+}$	6-hydroxycyanidin (441697)	C08646	Unclassified			
$C_{16}H_{12}O_5$	Geraldine (5281618)	C10047	Unclassified			
$C_4H_8N_2O_3$	N-carbamoylsarcosine (439375)	C01043	Unclassified			
$C_8H_8O_2$	3-methylsalicylaldehyde (522777)	C14087	Unclassified			
C_8H_8O	Acetophenone (7410)	C07113	Unclassified			
$C_9H_{11}NO_2$	Phenylalanine (994)	C02057	Unclassified			
$C_{15}H_{10}O_5$	Galangin (5281616)	C10044	Unclassified			
$C_5H_{11}NO_2$	4-methylaminobutyrate (70703)	C15987	Unclassified			
$C_{15}H_{12}O_5$	Dihydrogenistein (9838356)	C14458	Unclassified			
$C_4H_8N_2O_3$	3-ureidopropionate (111)	C02642	Unclassified			
$C_{15}H_{12}O_5$	Butin (92775)	C09614	Unclassified			
$C_{16}H_{12}O_5$	Cypripedium (174864)	C10323	Unclassified			
$C_{35}H_{52}O_4$	Hyperforin (441298)	C07608	Unclassified			
$C_5H_{11}NO_2$	2-amino-2-methyl butanoate (94744)	C03571	Unclassified			
$C_{15}H_{10}O_5$	3,6,4'-trihydroxy flavone (676308)	C15222	Unclassified			
$C_{15}H_{12}O_4$	3',5'-dihydroxy flavanone (11954216)	C15609	Unclassified			
$C_{17}H_{14}O_6$	4'-methylcapillarisin (5320438)	C17784	Unclassified			
$C_{16}H_{30}O$	Bombykol (445128)	C16873	Unclassified			
$C_{31}H_{48}O_3$	Dehydroeburicoic acid (15250826)	C16950	Unclassified			
$C_{17}H_{14}O_6$	Aflatoxicol (53297443)	C19584	Unclassified			
$C_8H_8O_2$	4-methyl benzoic acid (7470)	C01454	Unclassified			
$C_{15}H_{10}O_7$	Nortangeretin (96506)	C15031	Unclassified			
C_8H_8O	4-vinylphenol (62453)	C05627	Unclassified			
$C_{15}H_{12}O_5$	(-)-Glycinol (129648)	C01263	Unclassified			
$C_4H_9NO_3$	(-)-Erythro-(2r,3r)-dihydroxybutylamide (443073)	C11108	Unclassified			
$C_{18}H_{28}O_3$	Colnelenic acid (6441679)	C16320	Unclassified			
$C_5H_9NO_2$	3-acetamidopropanal (5460495)	C18170	Unclassified			
$C_{27}H_{30}O_{16}$	Quercetin 3-o-rhamnoside 7-o-glucoside (6325870)	C19796	Unclassified			
$C_{26}H_{29}O_{16+}$	Delphinidin 3-o-beta-d-sambubioside (10196837)	C20491	Unclassified			
$C_4H_9NO_3$	Gabob (2149)	C03678	Unclassified			
$C_5H_{11}NO_2$	Isoamyl nitrite (8053)	C07457	Unclassified			

$C_8H_8O_2$	2-hydroxyacetophenone (68490)	C07189	Unclassified			
$C_{16}H_{16}O_4$	Deoxyshikonin (98914)	C18133	Unclassified			
$C_{15}H_{12}O_5$	6,7,4'-trihydroxyflavanone (23724670)	C16232	Unclassified			
$C_{18}H_{28}O_3$	12-opda (5280411)	C01226	Unclassified			
$C_{15}H_{12}O_4$	Cis-3,4-phenanthrenedihydrodiol-4-carboxylate (49787035)	C18256	Unclassified			
$C_9H_{11}NO_2$	L-beta-phenylalanine (686703)	C20487	Unclassified			

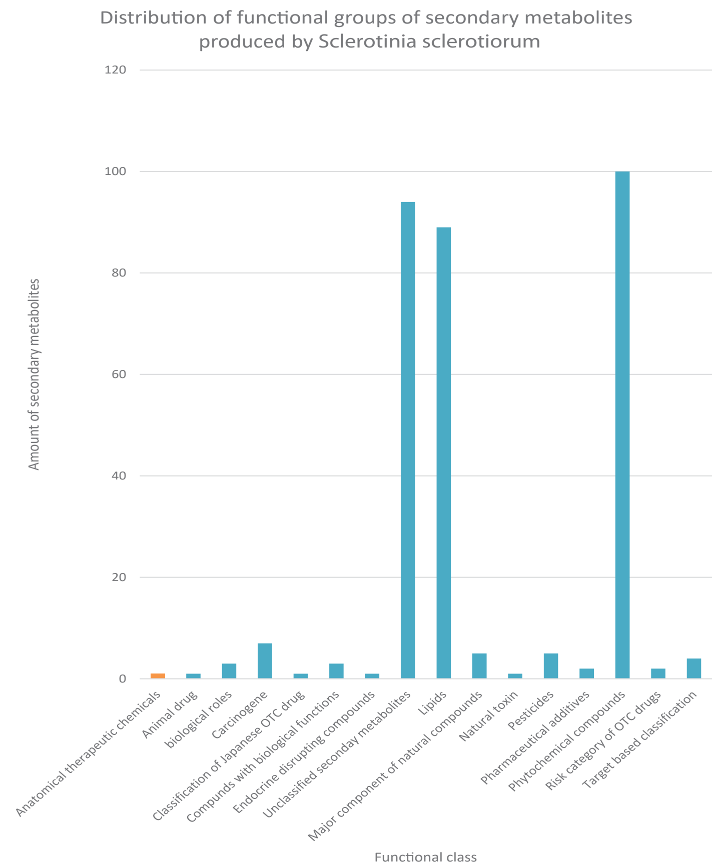

Figure 2: Functional classification of secondary metabolites produced by *Sclerotinia sclerotiorum*, based on the annotation from the KEGG database.

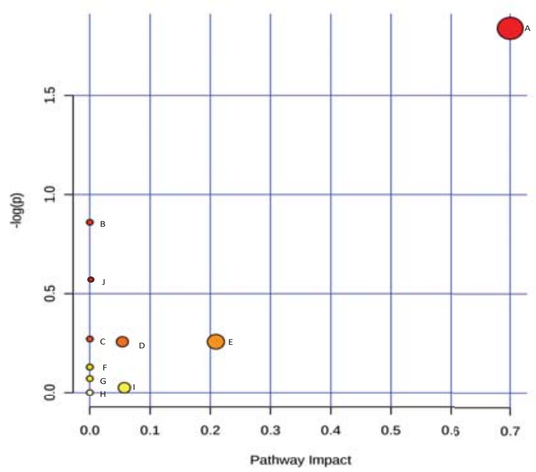

Figure 3: Graphical representation of impact value of enriched pathways associated with secondary metabolites produced by Sclerotinia sclerotiorum. –log p is the log value of the original p value calculated from the enrichment analysis; pathway impact is the impact value calculated from pathway topology analysis. A (Phenylalanine metabolism); B (Taurine and hypotaurine metabolism); C (Sulfur metabolism); D (Glycerophospholipid metabolism); E (Glycine, serine, and threonine metabolism); F (Lysine biosynthesis); G (Tyrosine metabolism); H (Valine, leucine and isoleucine biosynthesis); I (Cysteine and methionine metabolism); J (Aminoacyl-tRNA biosynthesis).

Table 2: Pathway Analysis of secondary metabolites produced by *Sclerotinia sclerotiorum.*

Pathway	Total	Expected	Hits	Raw p	-log(p)	Holm adjust P	FDR	Impact
Phenylalanine metabolism	7	0.73	2	1.59E-01	1.84E+00	1.00E+00	1.00E+00	0.70
Taurine and hypotaurine metabolism	5	0.52	1	4.23E-01	8.60E-01	1.00E+00	1.00E+00	0.00
Sulfur metabolism	13	1.35	1	7.63E-01	2.71E-01	1.00E+00	1.00E+00	0.00
Glycerophospholipid metabolism	26	2.70	2	7.73E-01	2.57E-01	1.00E+00	1.00E+00	0.05
Glycine, serine, and threonine metabolism	26	2.70	2	7.73E-01	2.57E-01	1.00E+00	1.00E+00	0.21
Lysine biosynthesis	19	1.98	1	8.79E-01	1.29E-01	1.00E+00	1.00E+00	0.00
Tyrosine metabolism	19	1.98	1	8.79E-01	1.29E-01	1.00E+00	1.00E+00	0.00
Valine, leucine, and isoleucine biosynthesis	24	2.50	1	9.31E-01	7.15E-02	1.00E+00	1.00E+00	0.00
Cysteine and methionine metabolism	33	3.43	1	9.75E-01	2.51E-02	1.00E+00	1.00E+00	0.06
Aminoacyl-tRNA biosynthesis	67	6.97	1	1.00E+00	4.65E-04	1.00E+00	1.00E+00	0.00

In particular, the Total is the total number of metabolites in the pathway; the Hits is the matched number of metabolites from the current study; Raw p is the *p* - value calculated from the enrichment analysis; the Holm p is the *p* - value adjusted by the Holm-Bonferroni method; the FDR p is the *p* - value adjusted using False Discovery Rate; Impact is the pathway impact value calculated from pathway topology analysis.

Moreover, several identified secondary metabolites belong to the flavonoid group, which could indicate the antimicrobial properties inert within *S. sclerotiorum* [24]. As shown in Table 1, the results show that *S. sclerotiorum* produces more flavonoids as secondary metabolites, although the function of these flavonoids in *S. sclerotiorum* is still largely unknown. However, according to [25], a similar *ALT1* ligand was identified as a methylated flavonoid produced by *Alternaria* spp associated with Asthma in humans.

Lipids: In the metabolome of *S. sclerotiorum*, 89 lipid compounds were identified as part of its secondary metabolites. These lipids belong to eight classes, including fatty acyls, glycerolipids, glycerophospholipid, sphingolipids, sterol lipids, prenol lipids, saccharolipids, polyketides, exhibiting varying functions, including energy storing and acting as structural components of cell membranes [26]. For instance, 18 polyketides (molindone, aloe emodin anthrone, and 1,4-Dihydroxy-2-methylanthraquinone) were secreted by *S. sclerotiorum,* yet their mechanism of action is still elusive. However, studies have demonstrated that many polyketides, whose backbones are often frequently changed by glycosylation or oxidation, e.g., erythromycins, tetracyclines, and avermectins, are commonly utilized antimicrobial, anti-parasitic, and anti-cancer and antitumor compounds [27,28]. Five of the lipids identified in *S. sclerotiorum* were reported to belong to the alpha-linolenic acid metabolism pathway, and eight others were implicated in the biosynthesis of the secondary metabolites pathway.

Natural toxins: Natural toxins include fungal toxins (mycotoxin), phytotoxins, cyanotoxins, marine biotoxins, and venoms. Aflatoxin B_2 and resiniferatoxin (identified in the current study) are toxins produced by *S. sclerotiorum* as this collaborated with the report of [29], wherein they identified *S. sclerotiorum* P450 enzymes that are associated with aflatoxin biosynthetic pathway.

Impact of phenylalanine metabolism in the biochemical pathways associated with *Sclerotinia sclerotiorum:* Pathway analysis results identified 2-phenylacetamide, phenylacetic acid, phenylacetaldehyde, phenyl pyruvic acid, and L-phenylalanine secondary metabolites involved in the phenylalanine metabolism pathway that was enriched compared to other detected pathways (Figure 4). Although the significance of this pathway in *S. sclerotiorum* is still vague, it was reported that reprogramming of the phenylalanine cycle is responsible for soybean resistance against *S. sclerotiorum* attack [30]. Phenylpyruvic acid is a pyruvate dehydrogenase inhibitor essential for the metabolism of glucose, fatty acids, and cholesterol [31]. Likewise, phenylacetaldehyde is responsible for polyesters synthesis, managing additive activities during the polymerization process [32], while phenylacetic acid is a nitrogen and ammonium binding agent [33]. Based on the known functions of these individual secondary metabolites implicated in the phenylalanine pathway, it could be proposed that; Phenylalanine metabolism pathway is responsible for inhibiting the host plant phenylalanine defense mechanism [34,35].

Limitations of the study: The study on the secondary metabolites profiling of the phytopathogenic fungus *Sclerotinia sclerotiorum* has several limitations. Firstly, the research may have only focused on a specific strain or isolates of *S. sclerotiorum*, which may limit the generalizability of the findings to other strains or species [36]. The study might have included a partial analysis of all possible secondary metabolites produced by the fungus, as detecting and identifying secondary metabolites can be challenging and dependent on the analytical

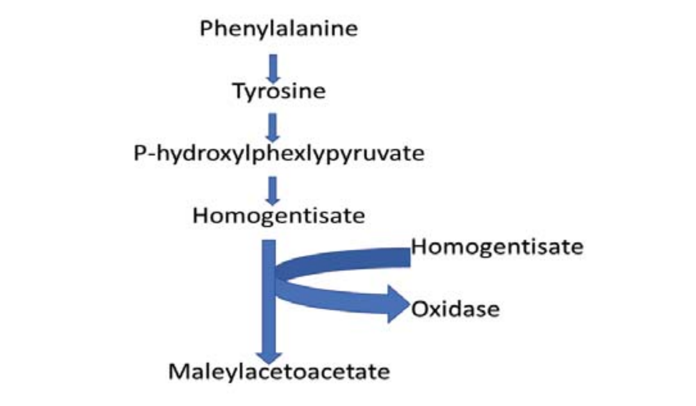

Figure 4: Phenylalanine metabolism pathway, the most significant pathway implicated with the secondary metabolism of *Sclerotinia sclerotiorum*.

techniques employed [37]. Moreover, the study may have been conducted under specific laboratory conditions, which may not fully represent the natural environment in which the fungus interacts with plants [38]. Additionally, the functional characterization of the identified secondary metabolites and their role in pathogenicity may require further investigation [39]. These limitations should be taken into consideration when interpreting and extrapolating the results of the study.

Conclusion

In conclusion, the study on the secondary metabolites profiling of the phytopathogenic fungus *Sclerotinia sclerotiorum* revealed significant insights into its chemical composition. The research successfully identified and characterized several secondary metabolites produced by *S. sclerotiorum*, providing valuable information about its bioactive compounds and their potential role in pathogenicity. Two hundred and forty metabolites were found to vary in abundance between biological replicates. The metabolites included essential groups of compounds such as phytochemicals, lipids, and toxins, amongst others. Many of these metabolites were involved in critical pathways associated with resistance, nitrogen remobilization, cell signaling, and secondary metabolic defenses [40]. Metabolites discovered in this research are potentially primarily related to the production of secondary metabolites, indicating the level of all housekeeping metabolites since the pathogen was grown *in vitro*, excluding the metabolites expressed during the pathogenicity of host plants.

In summary, these data support that both secondary metabolites are involved in multiple interconnecting pathways that contribute immensely to the pathogenicity of *S. sclerotiorum.*

These findings contribute to our understanding of *S. sclerotiorum's* chemical arsenal and offer potential targets for disease management strategies. However, further investigations are needed to fully comprehend the functional significance of these secondary metabolites and their interactions with host plants. Future studies could focus on elucidating the mechanisms underlying the fungus-host interactions and exploring the potential application of these metabolites in agricultural practices. Such research holds promise for developing innovative approaches to combating plant diseases caused by *S. sclerotiorum* [41].

Acknowledgment

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