Siddiqui *et al. Chem Synth* 2023;3:25
DOI: 10.20517/cs.2023.02

Chemical Synthesis

Review

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Biotransformation studies on bioactive compounds: 25 years of interesting research at the ICCBS

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How to cite this article: Siddiqui M, AtW, Choudhary MI, AuR. Biotransformation studies on bioactive compounds: 25 years of interesting research at the ICCBS. *Chem Synth* 2023;3:25. https://dx.doi.org/10.20517/cs.2023.02

Received: 23 Jan 2023 **First Decision:** 28 Feb 2023 **Revised:** 30 Mar 2023 **Accepted:** 10 Apr 2023 **Published:** 24 May 2023

Academic Editor: Bao-Lian Su **Copy Editor:** Yanbing Bai **Production Editor:** Yanbing Bai

Abstract

Biotransformation of natural, synthetic, and semi-synthetic compounds has emerged as a frontier branch of chemical sciences that is progressively being applied in numerous fields. In the present review, we have summarized our biotransformation studies on bioactive compounds from 1997 to 2022. Various microbial and plant cell cultures were used for biocatalytic structural transformations. We present here an overview of biotransformation of 53 compounds belonging to various classes of natural, synthetic, and semi-synthetic compounds, published in several leading journals. The structures of the resulting metabolites have been elucidated by detailed spectroscopic studies. Oxidation, reduction, dehydrogenation, chlorination, aromatization, methylation, demethylation, rearrangements, etc. were the main reactions that occurred during the biotransformation processes. Many of the biotransformed products exhibited interesting biological activities. Structural transformations in some cases have also led to improved pharmacokinetic profiles. This review is aimed to provide a focused account of extensive work carried out in our laboratories in this field, as well as the immense potential of biocatalytic transformations in organic chemistry.

Keywords: Biotransformation, whole-cell catalysis, terpenes, steroids, steroidal alkaloids, biological activity evaluation

BIOTRANSFORMATION- AN EFFICIENT GREEN CHEMISTRY APPROACH

Biotransformation techniques are among the most efficient approaches for structural transformation of various classes of organic compounds. These techniques involve the use of low-cost, eco-friendly, and non-toxic biocatalysts. They are normally conducted under ambient reaction conditions and involve oxidation, dehydrogenation, chlorination, reduction, aromatization, methylation, demethylation, rearrangements, etc. Biotransformation procedures can also reduce the total number of reaction steps towards the desired products. They do not require the use of toxic chemicals and harsh conditions that may otherwise be necessary for the functional group activation, protection, and deprotection steps, resulting in less production of wastes as compared to the desired products. These techniques have effectively been employed in drug discovery and development, affording libraries of compounds around core structures. These libraries are then evaluated for various biological activities in the drug discovery phase. The most significant aspect of biocatalytic transformations is the conservation of the original carbon skeleton of the starting material after transformation[1-10].

Biotransformation techniques are catalyzed by various biological systems, such as actinomycetes, algae, fungi, bacteria, yeasts, and plants and animals cell cultures, as well as by pure enzymes, affording compounds with high stereo-, regio-, and chemo-selectivity. They may be applied on potentially important scaffolds for the design, discovery, and development of new bioactive multi-functional compounds, including pharmaceuticals. Fungi have been widely used in whole-cell biocatalysis of organic compounds due to the presence of cytochrome P450 systems. In addition, fungi have higher metabolic and multiplication rates, thus serving as an excellent source for whole-cell biocatalysis. Pure enzyme-catalyzed biotransformation reactions often produce single and specific metabolites. Biotransformation reactions with whole colonies of microorganisms or plant/animal cell cultures may, however, produce more than one metabolite due to the involvement of a range of enzymes. Whole-cell biocatalysts are cost-effective, easy to handle, and usually stable in the long term. Moreover, biotransformation reactions by whole-cell colonies do not require co-factors[11-20].

BIOTRANSFORMATION STUDIES ON BIOACTIVE COMPOUNDS

Bioactive compounds are extra-nutritional constituents that usually occur in small quantities in plants/foods. They have been extensively evaluated for their effects on human health. These bioactive compounds have diverse structures and distributions in nature. At present, several bioactive compounds have been derivatized through bio-catalysis with applications in the field of medicine. Bio-catalytic transformation of bioactive compounds has emerged as a frontier field of chemical sciences that is being extensively employed in numerous other fields. Several natural products, e.g., monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, anabolic, contraceptive, and anti-cancer steroids, steroidal alkaloids, and flavonoids, as well as other bioactive compounds of synthetic origin, have been structurally transformed using biocatalytic approaches in our laboratories since 1997. In the present review, we have compiled the results of these biotransformation studies.

For the biotransformation studies, media was prepared by mixing specific media ingredients, transferred into flasks, cotton plugged, autoclaved, inoculated with microbial/plant cell cultures under sterilized conditions, and placed on a rotary shaker (2-4 days). After the maximum growth of microbial/plant cell cultures, substrates/drugs were dissolved in water-miscible solvents, such as methanol, DMSO, and acetone, and fed in flasks containing microbial/plant cell cultures. The material was placed again on a rotary shaker for 2 to 15 days. Oxidation, reduction, dehydrogenation, chlorination, aromatization, methylation, demethylation, and rearrangements were the main reactions observed during the biotransformation studies. The reaction was terminated by adding water-immiscible solvents, such as dichloromethane (DCM) or ethyl

acetate (EtOAc), filtered to separate biomass, extracted thrice with DCM/EtOAc, and evaporated using a rotary evaporator.

The crude materials were initially fractionated by column chromatography by using hexanes-ethyl acetate or hexanes-acetone solvent systems. The fractions were finally purified by reverse phase or/and normal phase HPLC, followed by thin layer chromatography (TLC). The structures of purified derivatives were established by using 1D-, and 2D-NMR (Nuclear Magnetic Resonance), HREI-MS (High-Resolution Electron Ionization Mass Spectrometry), HRESI-MS (High-Resolution Electrospray Ionization Mass Spectrometry), HRFAB-MS (High-Resolution Fast Atom Bombardment Mass Spectrometry), IR (Infrared), and UV (Ultraviolet–visible) spectral data, and single-crystal X-ray diffraction analyses. Fully purified compounds were evaluated for different biological activities.

In certain cases, solid phase fermentation was also employed to increase the yields and diversity of metabolites.

Spectroscopy is the investigation and measurement of spectral data produced by the interaction of samples with electromagnetic radiation. NMR spectroscopy plays a major role in the structure determination of organic molecules, and other biological macromolecules. Chemical shifts are accurately measured by NMR parameters as sensitive probes of molecular structures. HSQC spectroscopy determines the correlations between two different types of nuclei (^{1}H with ^{13}C or ^{1}H with ^{15}N), which are separated by one bond. HMBC spectroscopy correlates ^{1}H and ^{13}C nuclei through two, three, or sometimes four bonds. COSY (^{1}H-^{1}H Correlation) Spectroscopy shows the correlation between hydrogens that are coupled to each other in the ^{1}H-NMR spectrum. NOESY is frequently used to determine the spatial structure of organic molecules. The CD (Circular dichroism) is the difference in absorption of left and right circularly polarized light. Only chiral molecules display CD, and enantiomers have CD of equal magnitude but opposite sign.

KEY OBJECTIVES

The main objectives of our work on biotransformation studies were as follows: 1: To synthesize libraries of new and novel analogues of natural, synthetic, and semisynthetic compounds through eco-friendly and cost-effective biotransformation techniques with the aim of improving their pharmacodynamic profiles; 2: To produce potentially interesting regio-, stereo-, enantio-selective compounds without the use of toxic chemicals and harsh conditions; 3: To evaluate the resultant transformed products for different biological activities, e.g., enzyme inhibition, anti-inflammatory, anti-cancer, anti-bacterial, etc.

BIOTRANSFORMATION OF MONOTERPENES

Biotransformation of (-)-α-(1), and β-pinene (6)

α-(1), and *β*-pinene (**6**) are well-known monoterpenes having a range of pharmacological activities, such as anti-microbial, anti-inflammatory, anti-oxidant, anti-malarial, and anti-leishmanial. They are the major constituents of many aromatic plants. Biotransformation of (-)-α-pinene (**1**) with the fungal culture of *Botrytis cinerea,* afforded three new hydroxylated metabolites, 3-hydroxy-(-)-*β*-pinene (**2**) (16.5%), 9-hydroxy-(-)-*α*-pinene (**3**) (13.5%), and 4-hydroxy-(-)-α-pinene-6-one (**4**) (20%), along with a known metabolite verbenone (**5**) (28%)[21] [Figure 1].

Similarly, biotransformation of (-)-*β*-pinene (6), a structural isomer of (-)-α-pinene, with the fungal culture of *Botrytis cinerea* yielded four new hydroxylated metabolites, (-)-6α-hydroxy-*β*-pinene (**7**) (22%), (-)-4*β*,5*β*-dihydroxy-*β*-pinene (**8**) (10%), (-)-2*β*,3*β*-dihydroxypinane (**9**) (12%), and (-)-4*β*-hydroxy-*β*-pinene-6-one (**10**) (9%)[22] [Figure 2].

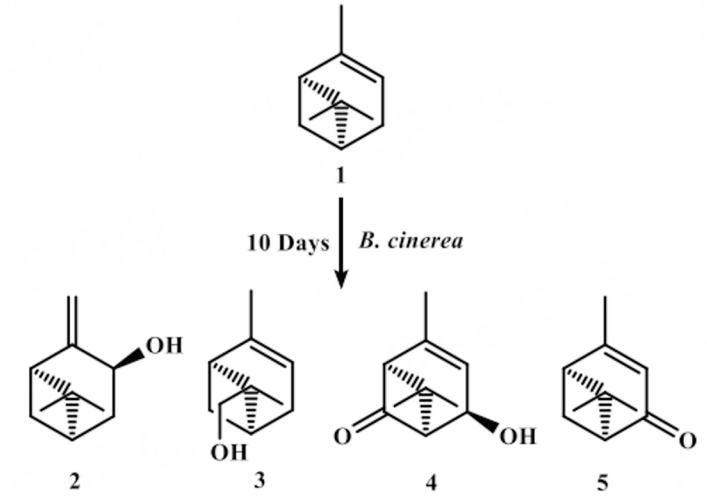

Figure 1. Biotransformation of (-)-α-pinene (**1**) with *Botrytis cinerea*.

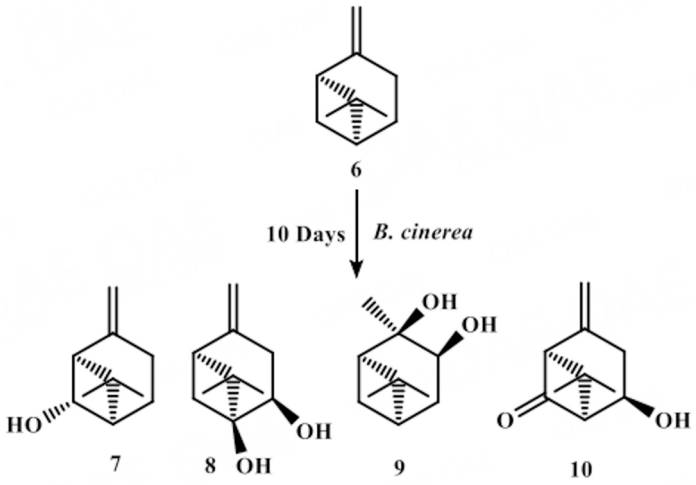

Figure 2. Biotransformation of (-)-*β*-pinene (**6**) with *Botrytis cinerea*.

Biotransformation of terpinolene (11)

Two new metabolites, 2,3-dihydro-3*β*,6*β*-dihydroxy-terpinolene (**12**) (39%), and 2,3-dihydro-1*α*,3*α*-dihydroxy-terpinolene (**13**) (20%) of the anti-bacterial and anti-fungal monoterpene terpinolene (**11**) were synthesized through biotransformation of compound 11 with the fungal culture of *Botrytis cinerea*. These derivatives 12 and 13 showed no activity against the plant pathogenic fungus *Cladosporium herbarum*, as compared to the substrate 11[23] [Figure 3].

Biotransformation of (-)-menthol (14), and (+)-menthol (21)

Menthol, a commercially used constituent of the mint plant, is used to treat minor pains in the muscles and joints. Biotransformation of (-)-menthol (**14**) by the fungal culture of *Cephalosporium aphidicola* yielded four new metabolites, 10-acetoxymenthol (**15**) (3%), 4*α*-hydroxymenthol (**16**) (7.5%), 3*α*-hydroxymenthol (**17**) (2.3%), and 10-hydroxymenthol (**18**) (21.5%), along with the two known metabolites, 7-hydroxymenthol (**19**) (4.8%), and 9-hydroxymenthol (**20**) (14%)[24] [Figure 4].

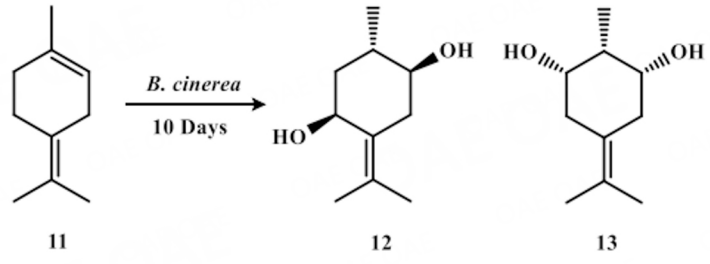

Figure 3. Biotransformation of terpinolene (**11**) with *Botrytis cinerea.*

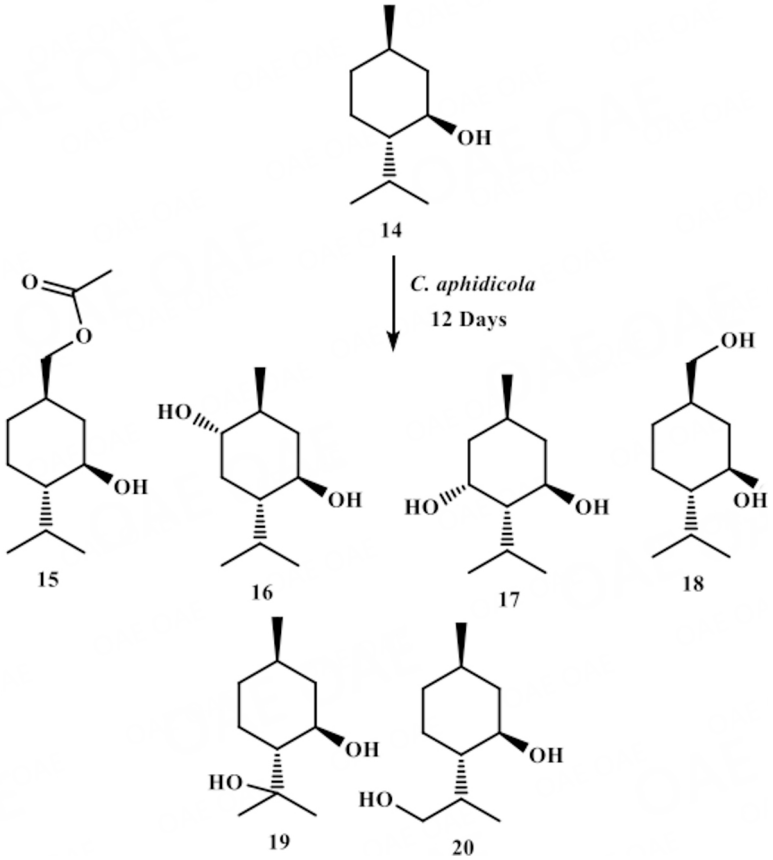

Figure 4. Biotransformation of (-)-menthol (**14**) with *Cephalosporium aphidicola.*

Similarly, biotransformation of (+)-menthol (**21**), with the fungal culture of *Macrophomina phaseolina* afforded five new hydroxylated metabolites, 2β, 8-dihydroxymenthol (**22**) (1%), 8, 9-dihydroxymenthol (**23**) (0.8%), 6β, 8-dihydroxymenthol (**24**) (1.3%), 1β, 8-dihydroxymenthol (**25**) (1.1%), and 7, 8-dihydroxymenthol (**26**) (1.5%), along with four known metabolites, 8-hydroxymenthol (**27**) (7.4%), 6β-hydroxymenthol (**28**) (2.5%), 1β-hydroxymenthol (**29**) (5.5%), and 9-hydroxymenthol (**30**) (2.2%)[25] [Figure 5].

Biotransformation of thymoquinone (31)

Biotransformation of a major constituent of black seeds, *Nigella sativa*, thymoquinone (**31**) with the plant pathogenic fungus *Aspergillus niter* afforded a new seven-membered lactone derivative, 5-isopropyl-2-

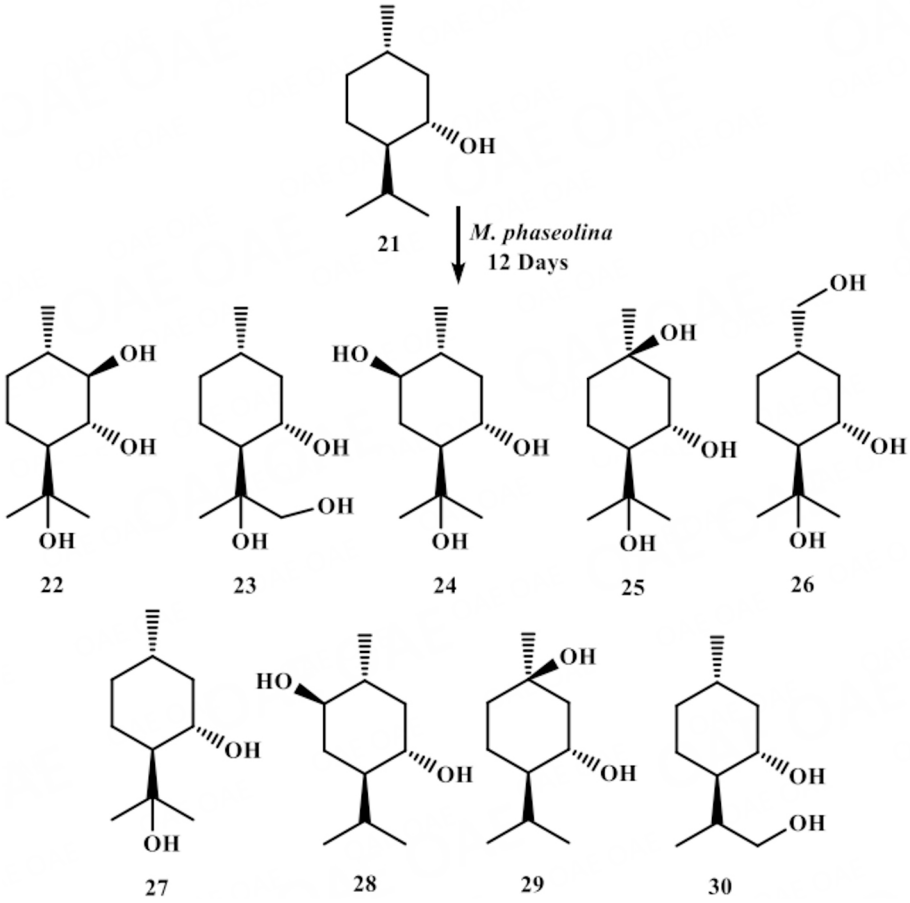

Figure 5. Biotransformation of (+)-menthol (**21**) with *Macrophomina phaseolina.*

methyloxepin-1-one (**32**) (2%) [Proposed Supplementary Figure 1], along with two known metabolites, 3-hydroxy-5-isopropyl-2-methylcyclohexa-2,5-diene-1,4-dione (**33**) (8%), and 5-isopropyl-2-methylbenzene-1,4-diol (**34**) (28%)[26] [Figure 6] [Proposed Supplementary Figure 2].

BIOTRANSFORMATION OF SESQUITERPENES

Biotransformation of (+)-isolongifolen-4-one (35)

Five new oxidative derivatives, 12-hydroxyisolongifolen-4-one (**36**), 13-hydroxyisolongi-folen-4-one (**37**), 11-hydroxyisolongifolen-4-one (**38**), 10-hydroxyisolongifolen-4-one (**39**), and 9-hydroxyisolongifolen-4-one (**40**) were synthesized through the microbial transformation of (+)-isolongifolen-4-one (**35**)[27] [Figure 7]. Compound **35** is used in the perfumery industry. Derivatives **37** (IC_{50} = 9.64 ± 0.0008 μM) and **40** (IC_{50} = 6.68 ± 0.0096 μM) were identified as potent inhibitors of tyrosinase, an important enzyme for melanin biosynthesis, as compared to substrate 35 (IC_{50} = 51.91 ± 0.0245 μM). Metabolite 36 showed moderate inhibitory activity (IC_{50} = 101.01 ± 0.1978 μM).

Biotransformation of (-)-isolongifolol (41)

Biotransformation of (-)-isolongifolol (**41**) with *Fusarium lini* resulted in three new polar oxygenated derivatives, 10-oxoisolongifolol (**42**) (1.2%), 10α-hydroxyisolongifolol (**43**) (16%), and 9α-hydroxyisolongifolol (**44**) (22%). The presence of an α-OH at C-10 in the metabolite **42** increased its inhibitory potential against butyrylcholinesterase, an enzyme whose inhibition reduces memory deficiency in Alzheimer's patients, with the IC_{50} value of 13.6 μM, while the presence of an α-OH at C-11 in the

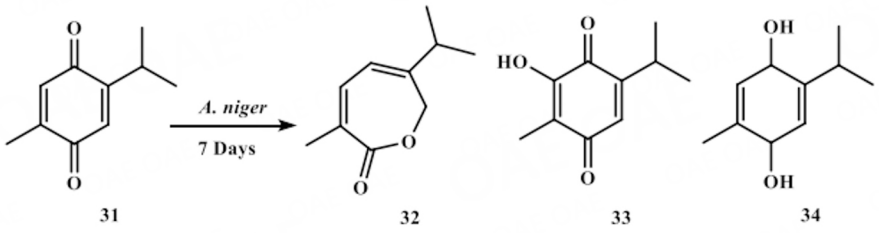

Figure 6. Biotransformation of thymoquinone (**31**) with *Aspergillus niger*.

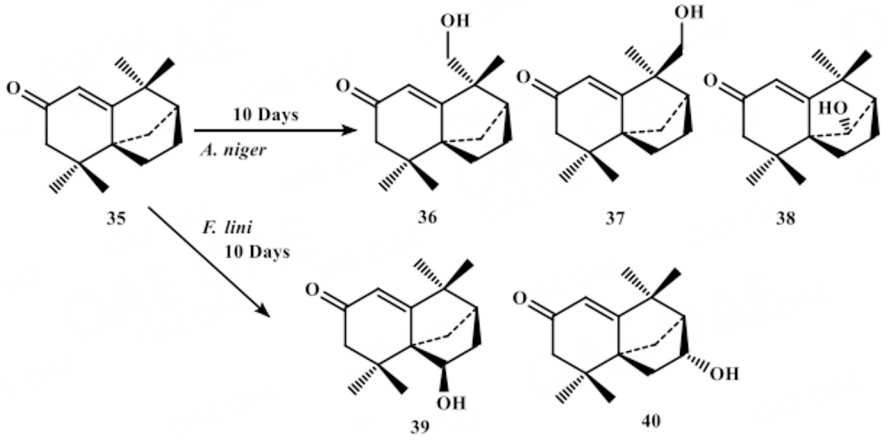

Figure 7. Microbial transformation of (+)-isolongifolen-4-one (**35**) with *Aspergillus niger* and *Fusarium lini*.

metabolite **43** decreased its activity (IC_{50} = 299.5 μM)[28] [Figure 8].

Biotransformation of 5α-hydroxycaryophylla-4(12), 8(13)-diene (45)

Macrophomina phaseolina-mediated structural transformation of the naturally occurring sesquiterpene, 5α-hydroxycaryophylla-4(12), 8(13)-diene (**45**) resulted in three new metabolites, 4β-methoxycaryophyllene-5α, 14-diol (**46**) (3.5%), 4β-methoxycaryophyllene-5α, 15-diol (**47**) (7.6%), and caryophyllene-5α, 15-diol (**48**) (2.7%) [Figure 9]. The transformed products **46** (IC_{50} = 3.09 ± 2.61 μg/mL), **47** (IC_{50} = 0.72 ± 0.17 μg/mL), and **48** (IC_{50} = 1.35 ± 0.43 μg/mL) showed significant anti-malarial activity, in comparison to the standard, chloroquine diphosphate (IC_{50} = 0.025 ± 0.01 μg/mL) by using parasite lactate dehydrogenase assay *in vitro*[29].

Biotransformation of (-)-caryophyllene oxide (49)

Biotransformation of another sesquiterpene (-)-caryophyllene oxide (**49**), a common constituent of essential oils, with the cell suspension culture of medicinal plant *Catharanthus roseus* afforded two new derivatives, 2β-hydroxycaryophyllene oxide (**50**) (7.5%), and 2-hydroxy-4, 5- epoxycaryophyllan-13-ol (**51**) (4.6%), along with two known derivatives, 15-hydroxycaryophyllene oxide (**52**) (2%), and 4β, 5α-dihydroxycaryophyll-8 (**13**) -ene (**53**) (6%)[30] [Figure 10].

In addition, biotransformation of (-)-caryophyllene oxide (**49**) with the fungal cell culture *Cephalosporium aphidicola* yielded two known metabolites, 4β, 5α-dihydroxycaryophyll-8 (**13**) -ene (**53**) (2.7%) and clovane-5,9-diol (**54**) (1.3%) [Figure 11] [Proposed Supplementary Figure 3]. Similarly, fungal transformation of 49 with *Macrophomina phaseolina* also yielded two known transformed products, 15-hydroxycaryophyllene

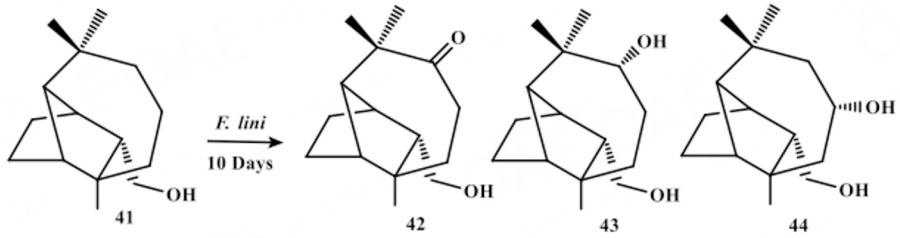

Figure 8. Biotransformation of (-)-isolongifolol (**41**) with *Fusarium lini.*

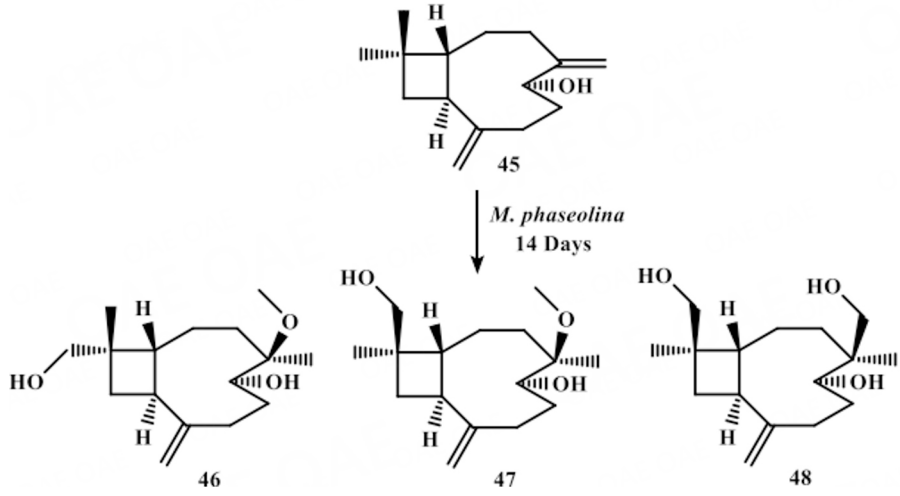

Figure 9. Biotransformation of 5α-hydroxycaryophylla-4(12), 8(13)-diene (**45**) with *Macrophomina phaseolina.*

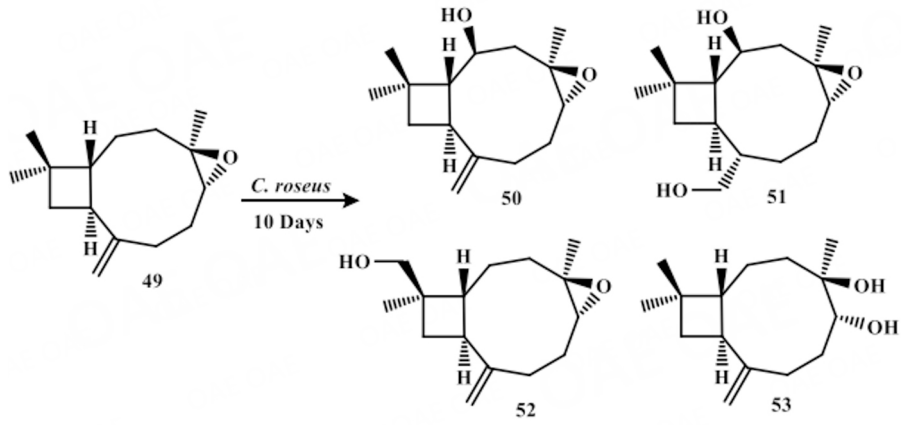

Figure 10. Biotransformation of (-)-caryophyllene oxide (**49**)with *Catharanthus roseus*

oxide (**52**) (3.2%), and 4, 5-epoxycaryophyllan-8 (**13**) -en-14-ol (**55**) (4.6%)[31] [Figure 11]. Moreover, four new derivatives, 4, 5-epoxy-13-norcaryophyllan-8-one (**56**) (2.5%), caryolane-5, 8, 13-triol (**57**) (2.3%), clovane-5, 9, 12-triol (**58**) (2.1%), and 4, 5-epoxycaryophyllan-3, 13-diol (**59**) (2.1%), were also synthesized by our research group through the biotransformation of (-)-caryophyllene oxide (**49**) with *Rhizopus stolonifer*, *Aspergillus niger*, *Gibberella fujikuroi*, and *Fusarium lini*, respectively[31] [Figure 12].

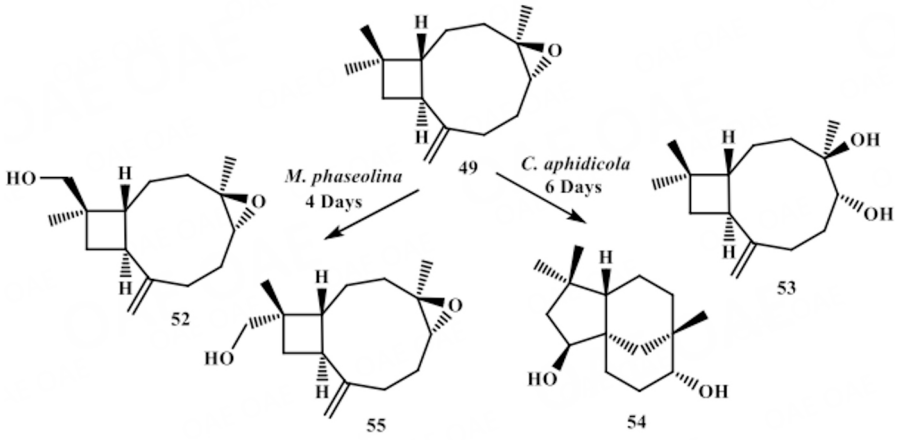

Figure 11. Microbial transformation of (-)-caryophyllene oxide (**49**) with *Cephalosporium aphidicola*, and *Macrophomina phaseolina*.

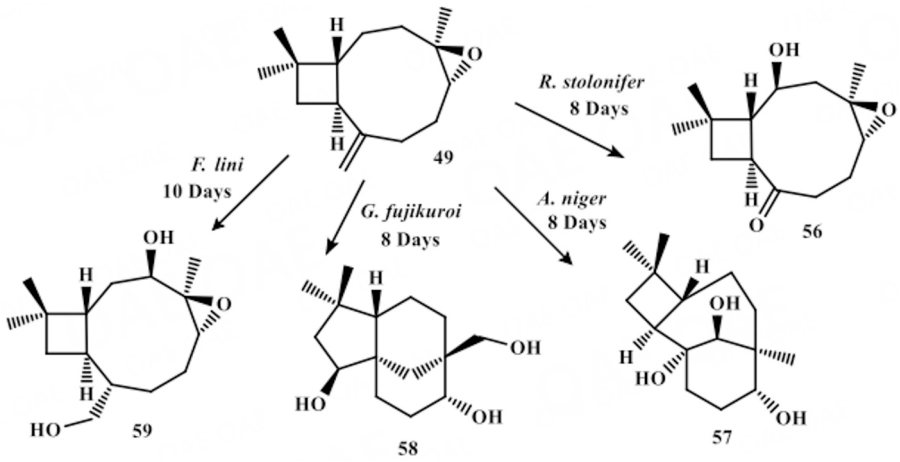

Figure 12. Microbial transformation of (-)-caryophyllene oxide (**49**) with *Rhizopus stolonifer*,*Aspergillus niger, Gibberella fujikuroi*, and *Fusarium lini*.

Derivatives **52** (IC_{50} = 44.0 ± 0.2 μM), **53** (IC_{50} = 455.8 ± 0.1 μM), **54** (IC_{50} = 189.5 ± 0.2 μM), **55** (IC_{50} = 10.9 ± 0.2 μM), **56** (IC_{50} = 458.7 ± 0.5 μM), **57** (IC_{50} = 23.6 ± 0.1 μM), **58** (IC_{50} = 43.6 ± 0.3 μM), and **59** (IC_{50} = 154.6 ± 0.3 μM) showed a moderate to significant inhibitory potential against butyrylcholinesterase, as compared to the substrate **49** (IC_{50} = 208.4 ± 0.8 μM).

Biotransformation of (+)-cycloisolongifol-5β-ol (60)

Cunninghamella elegans-mediated transformation of a cyclic sesquiterpene (+)-cycloisolongifol-5*β*-ol ((**60**) afforded three new metabolites, cycloisolongifol-3*β*,5*β*-diol (**61**) (3.7%), cycloisolongifol-5*β*-ol-11-one (**62**) (4%), and cycloisolongifol-3*β*, 5*β*, 11*α*-triol (**63**) (3%)[32] [Figure 13].

Biotransformation of (-)-ambrox (64)

Fungal transformation of another perfumery sesquiterpene, (-)-ambrox (**64**) with *Fusarium lini* yielded four compounds, ambrox-1*α*-ol (**65**) (2.7%), ambrox-1*α*,11*α*-diol (**66**) (1.3%), ambrox-1*α*,6*α*-diol (**67**) (3.2%), and ambrox-1*α*,6*α*,11*α*-triol (**68**) (**4.6%**) [Figure 14]. Similarly, four more derivatives, ambrox-3-one (**69**) (1.3%), ambrox-3*β*-ol (**70**) (1%), ambrox-3*β*,6*β*-diol (**71**) (1.9%), and tetranorlabdane-3, 8, 12-triol (**72**) (4.7%) of substrate **64** were also synthesized through its biotransformation with *Rhizopus stolonifer*[33] [Figure 15].

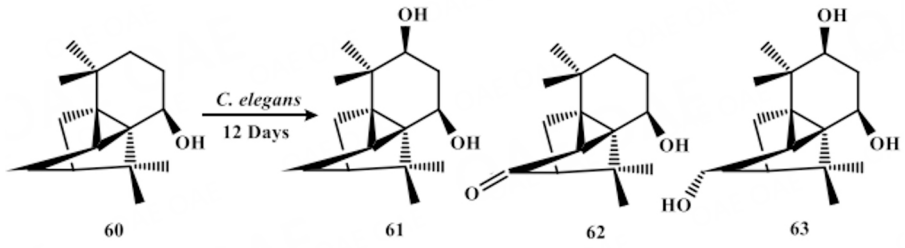

Figure 13. Biotransformation of (+)-cycloisolongifol-5β-ol (**53**) with *Cunninghamella elegans*.

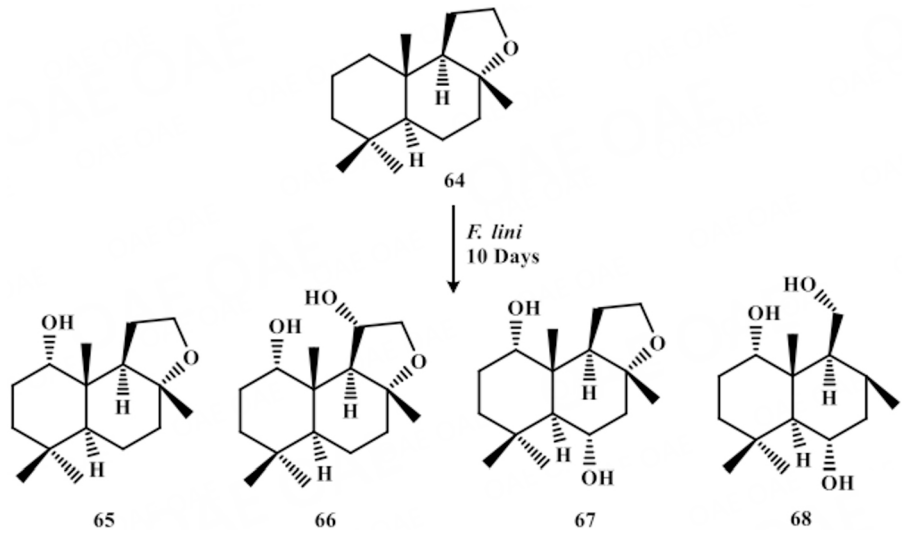

Figure 14. Biotransformation of (-)-ambrox (**64**) with *Fusarium lini*.

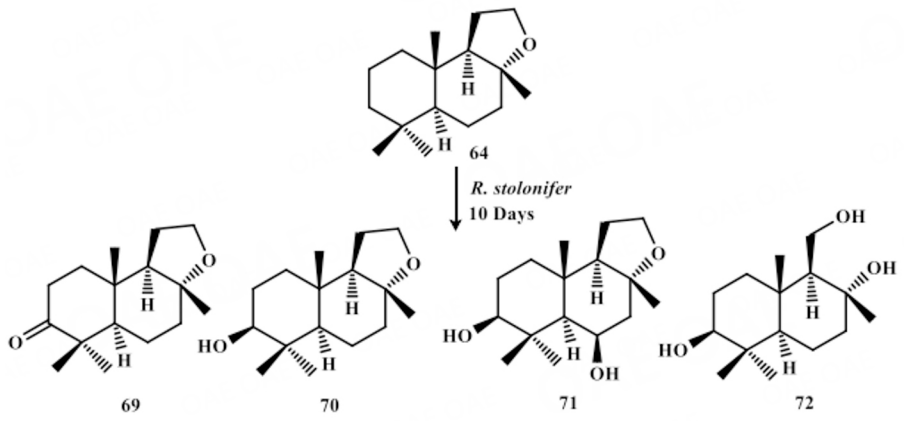

Figure 15. Biotransformation of (-)-ambrox **(**64**)** with *Rhizopus stolonifer*.

Derivatives **65**, **67**-**69**, ambrox-1α, 3β-diol (**73**) (10.1%), and ambrox-1α, 6β-diol (**74**) (15.7%) were also obtained *via* incubation of (-)-ambrox (**64**) with *Actinidia deliciosa* (**Kiwi fruit**)[34] [Figure 16].

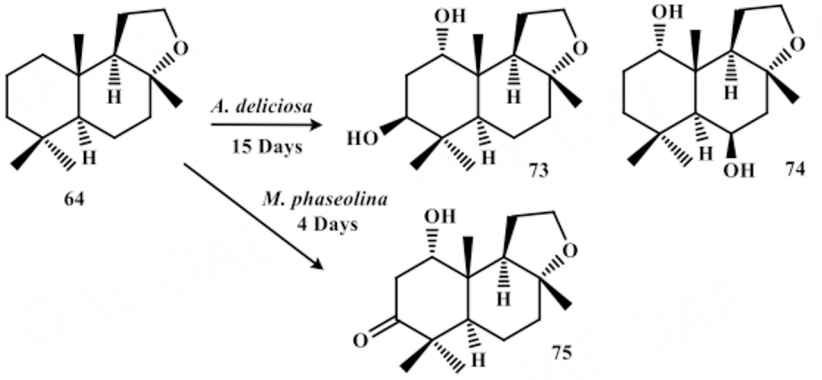

Figure 16. Microbial transformation of (-)-ambrox (**64**) with *Actinidia deliciosa,* and *Macrophomina phaseolina*.

Moreover, biotransformation of (-)-ambrox (**64**) with *Macrophomina phaseolina* afforded a new compound, 1α-hydroxy-3-oxoambrox (**75**) (3.5%), along with four known compounds **70**, and 72-74[35] [Figure 16]. Three more derivatives, **69**, ambrox-2α-ol (**76**) (2.5%), and ambrox-2α,3β-diol (**77**) (3%), were also synthesized through biotransformation of (-)-ambrox (**64**) by using cell suspension culture of plant *Peganum harmala*[35] [Figure 17]. Some of the resulting metabolites exhibited exotic aroma, different from the substrate **64**.

Biotransformation of artemether (78)

Biotransformation of an anti-malarial sesquiterpenoid drug artemether (**78**) with plant cell suspension culture of *Azadirachta indica* afforded two derivatives, 9α-acetoxy, 10β-methoxyartemethin (**79**) (1.2%), and 3α-hydroxy, 12β-methoxyartemethin (**80**) (2.5%) [Figure 18]. Three derivatives **79**, **80**, and 3α-12β-dihydroxyartemethin (**81**) (3.5%) were obtained from *Macrophomina-phaseolina*-mediated transformation of 78 [Figure 18] [Supplementary Figure 4]. In addition, two new derivatives, peroxy-linkage, 9α-hydroxyartemethin (**82**) (2.8%), and 10β-hydroxyartemethin (**83**) (4.6%), were obtained *via* the biotransformation of artemether (**78**) with *Fusarium lini*[36] [Figure 18] [Supplementary Figure 4]. Compounds 79-83 showed no anti-malarial activity (*Plasmodium falciparum*, 3D7 strain) *in vitro*.

Biotransformation of sclareolide (84)

Curvularia lunata-catalyzed transformation of a sesquiterpene lactone sclareolide (**84**) afforded a new derivative, 1α, 3β-dihydroxysclareolide (**85**) (16%), along with four derivatives, 3-ketosclareolide (**86**) (8.7%), 1β-hydroxysclareolide (**87**) (9.3%), 3β-hydroxysclareolide (**88**) (12.2%), and 1β,3β-dihydroxysclareolide (**89**) (7%). Biotransformation of **84** with *Aspergillus niger* also yielded metabolites **85**-**89**. Transformed products **85**-**88** were also obtained by the transformation of **84** with *Gibberella fujikuroii,* while fermentation of **84** with *Fusarium lini* produced metabolites **87** and **88**[37] [Figure 19]. Similarly, biotransformation of sclareolide (**84**) with *Cunninghamella elegans* afforded six derivatives **86**, **88**, **89**, 2α-hydroxysclareolide (**90**) (5.3%), 2α, 3β-dihydroxysclareolide (**91**) (23.6%), 1α, 3β-dihydroxysclareolide (**92**) (2.7%), and 3β-hydroxy-8-episclareolide (**93**) (1.3%)[33] [Figure 20]. Structural transformations in derivatives **86** (100%), **88** (87.5%), **89** (72.3%), **90** (82.7%), and **92** (75%) have increased their phytotoxicity against *Lemna minor* L., in comparison to the substrate **84** (62.5%), while derivative **91** (50%) showed weak phytotoxicity at 100 µg/mL.

Biotransformation of (-)-guaiol (94)

Biotransformation of a sesquiterpene (-)-guaiol (**94**) with *Rhizopus stolonifer* afforded a new compound, 1-guaiene-9α,11-diol (**95**) (1.6%) [Figure 21]. Similarly, *Cunninghamella elegans*-assisted transformation of **94** yielded compounds, 1-guaiene-3α,11-diol (**96**) (1.8%), and 1(5)-guaiene-3α,9,11-triol (**97**) (1.8%)

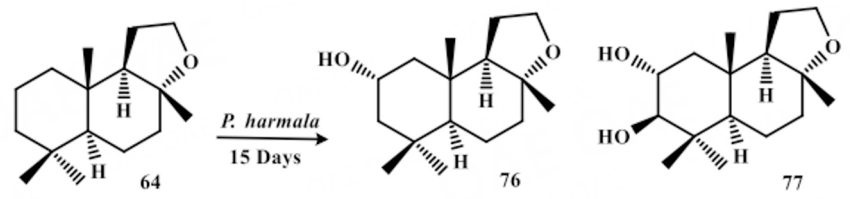

Figure 17. Biotransformation of (-)-ambrox (64) with *Peganum harmala.*

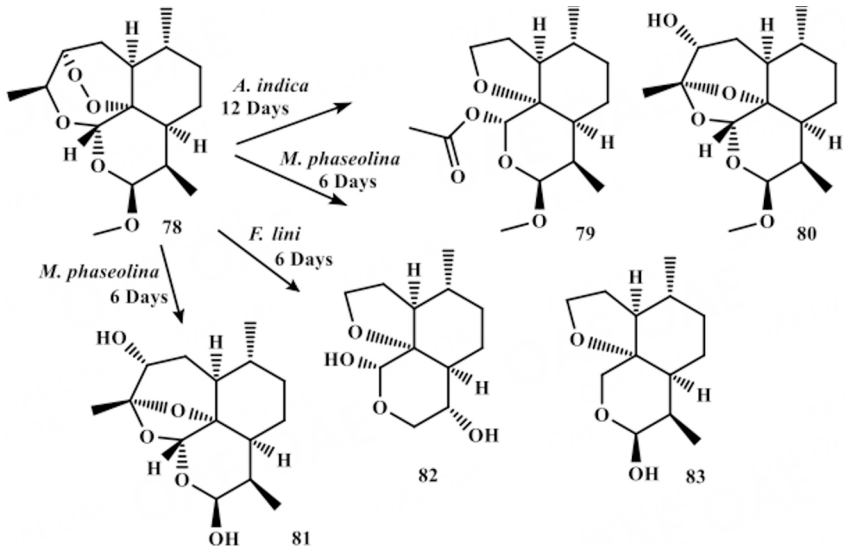

Figure 18. Biotransformation of artemether (**78**) with *Azadirachta indica*, *F. lini*, and *M. phaseolina*

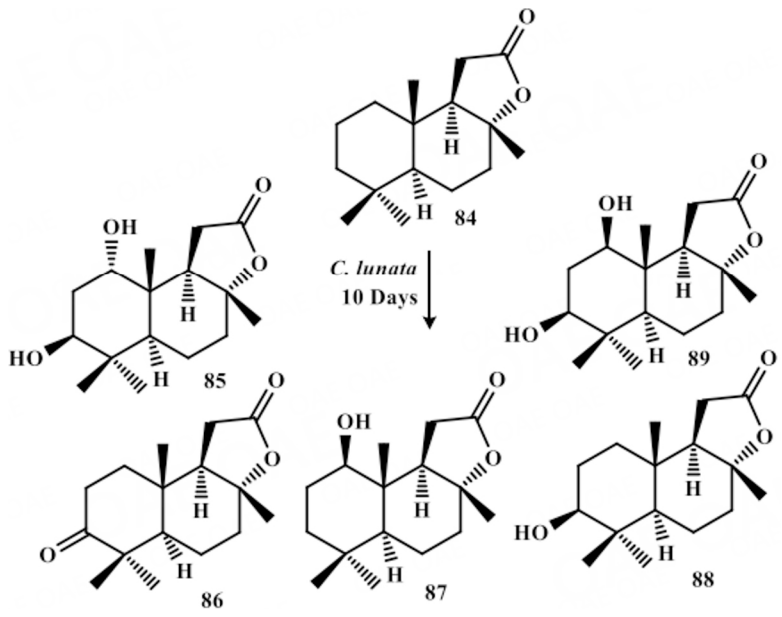

Figure 19. Biotransformation of sclareolide (**84**) with *Curvularia lunata.*

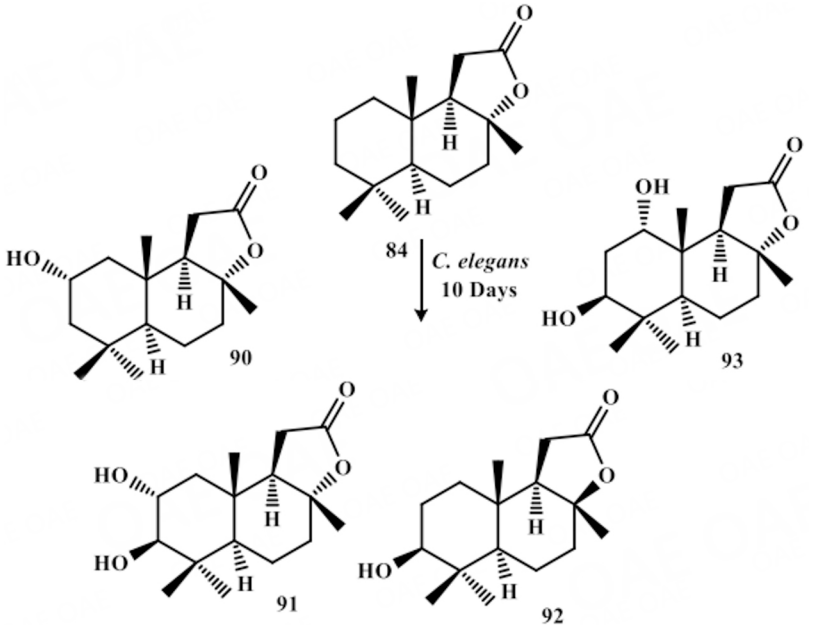

Figure 20. Biotransformation of sclareolide (**84**) with *Cunninghamella elegans.*

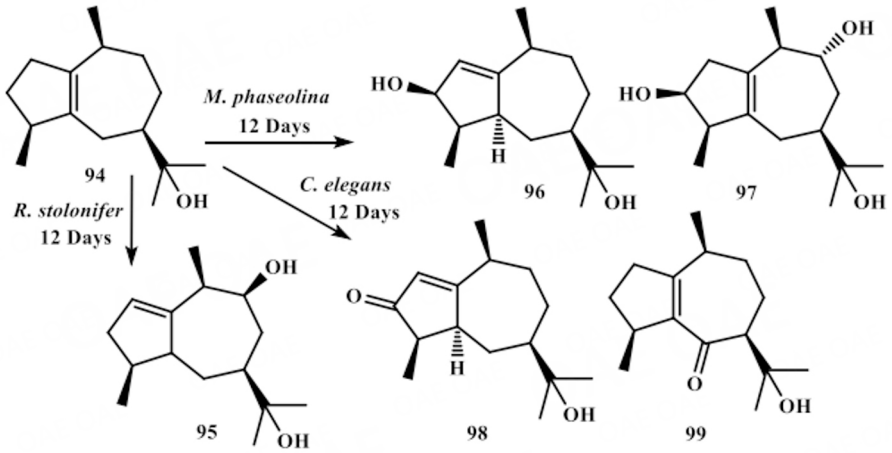

Figure 21. Biotransformation of (-)-guaiol (**94**) with *Macrophomina phaseolina, Rhizopus stolonifer, and Cunninghamella elegans.*

[Figure 21], while *Macrophomina phaseolina*-catalyzed biotransformation of **94** resulted in two derivatives, 1(5)-guaien-11-ol-6-one (**98**) (2.1%), and 1-guaien-11-ol-3-one (**99**) (2%)[38] [Figure 21].

BIOTRANSFORMATION OF DITERPENES

Biotransformation of sclareol (100)

Two new derivatives, 1β-hydroxysclareol (**101**) (1.3%) and 12-hydroxysclareol (**102**) (1%), were synthesized through the biotransformation of the diterpene, sclareol (**100**) with *Fusarium lini* [Figure 22]. Similarly, two new derivatives, 3β-hydroxysclareol (**103**) (2.7%) and 18-hydroxysclareol (**104**) (1.3%), and the two known metabolites, 6α,18-dihydroxysclareol (**105**) (3.2%) and 11,18-dihydroxysclareol (**106**) (4.6%), were also synthesized through the biotransformation of sclareol (**100**) with *Rhizopus stolonifer*[39] [Figure 23].

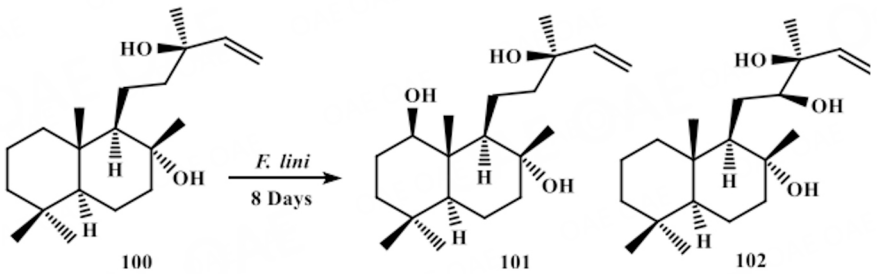

Figure 22. Biotransformation of sclareol (**100**) with *Fusarium lini*.

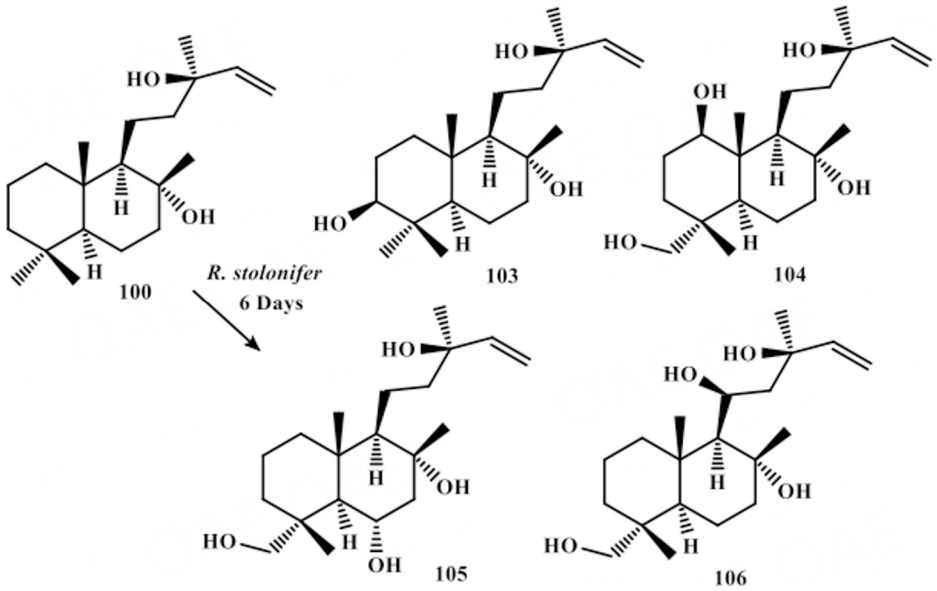

Figure 23. Biotransformation of sclareol (**100**) with *Rhizopus stolonifer*.

Biotransformation of andrographolide (107)

Biotransformation of andrographolide (**107**) with *Cephalosporium aphidicola* and *Cunninghamella elegans* yielded two derivatives, andropanolide (**108**) (**1.7%**) and 14-deoxy-11, 12-didehydroandrographolide (**109**) (1.6%) [Figure 24], respectively[40].

Biotransformation of dehydroabietic acid (110)

Three new derivatives, 1β-hydroxydehydroabietic acid (**111**) (0.8%), 15-hydroxy dehydroabietic acid (**112**) (1.1%), and 16-hydroxy dehydroabietic acid (**113**) (1.4%), were obtained *via* microbial transformation of the diterpene dehydroabietic acid (110)[41] [Figure 25]. Compounds **110** (IC_{50} = 11 ± 01 μM), **111** (IC_{50} = 130 ± 15 μM), **112** (IC_{50} = 99 ± 43 μM), and **113** (IC_{50} = 81 ± 90 μM) showed a potent α-glucosidase inhibitory activity, as compared to the standard acarbose (IC_{50} = 780 ± 20 μM). α-Glucosidase inhibitors delay the digestion of carbohydrates, resulting in a reduction of post-prandial sugar levels in diabetic patients.

BIOTRANSFORMATION OF SESTERTERPENE

Biotransformation of leucosceptrine (114)

Microbial transformation of the sesterterpene leucosceptrine (**114**) with the fungus *Rhizopus stolonifer* by our research group synthesized two polar derivatives, 1α-hydroxyleucosceptrine (**115**) (6.5%) and 8α-

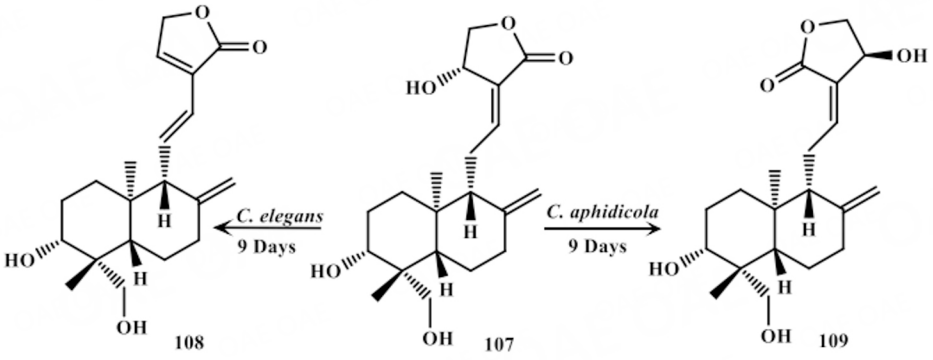

Figure 24. Microbial transformation of andrographolide (**107**) with *Cephalosporium aphidicola*.

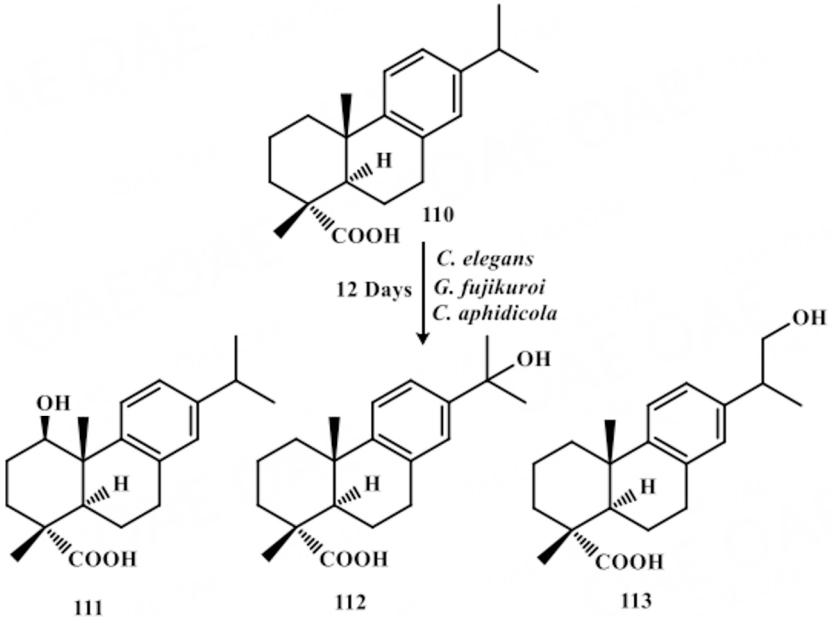

Figure 25. Microbial transformation of dehydroabietic acid (**110**) with *Cunninghamella elegans Gibberella fujikuroi*, and *Cephalosporium aphidicola*.

hydroxyleucosceptrine (**116**) (2.6%) [Figure 26][42].

BIOTRANSFORMATION OF TRITERPENE

Biotransformation of oleanolic acid (117)

Biotransformation of a pentacyclic triterpene, oleanolic acid (**117**) with *Fusarium lini* yielded a new compound, 2α, 3β, 11β-trihydroxyolean-12-en-28-oic acid (**118**) (4%), and the known metabolite, 2α,3β-dihydroxyolean-12-en-28-oic acid (**119**) (3%)[43] [Figure 27]. Compounds **117** (IC_{50} = 12.8 ± 0.00 μM), **118** (IC_{50} = 444.0 ± 8.0 μM), and **119** (IC_{50} = 666.0 ± 20.0 μM) showed a significant α-glucosidase inhibitory activity, as compared to the standard, drug acarbose (IC_{50} = 780.0 ± 0.28 μM).

Biotransformation of 18-glycyrrhetinic acid (120)

Biotransformation of 18-glycyrrhetinic acid (**120**) with *Cunninghamella elegans* yielded the metabolite, 3, 7-dihydroxy-11-oxo-olean-12-en-30-oic acid (**121**) (4%), while fermentation of **120** with *Fusarium lini*

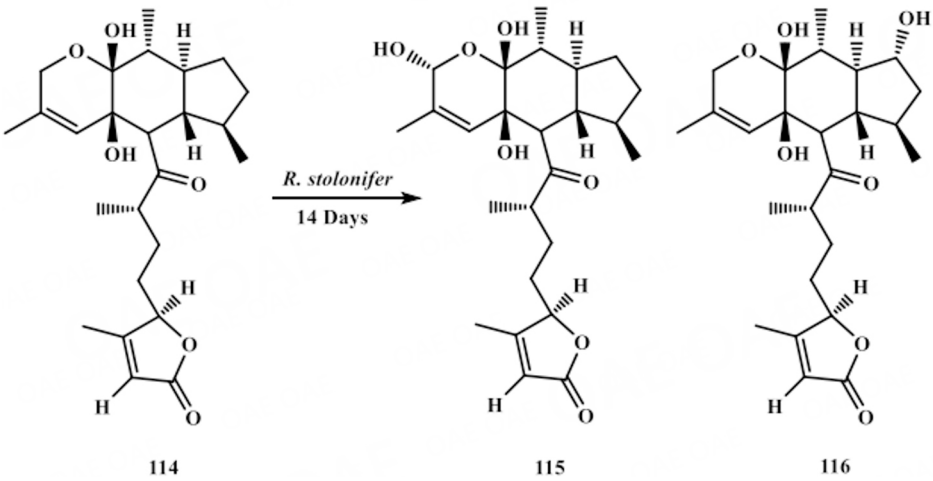

Figure 26. Biotransformation of leucosceptrine (**114**) with *Rhizopus stolonifer.*

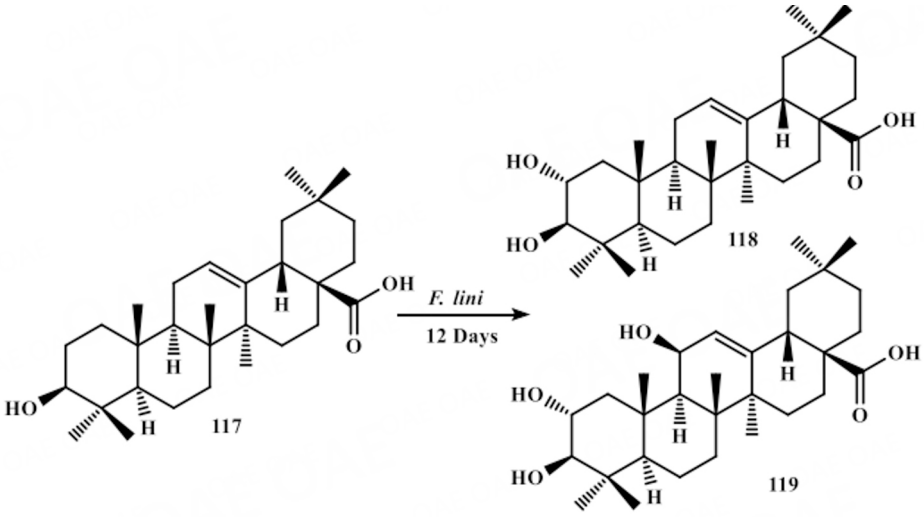

Figure 27. Biotransformation of oleanolic acid (**117**) with *Fusarium lini.*

afforded the metabolite, 3, 11-dioxoolean-12-en-30-oic acid (**122**) (2.5%)[44] [Figure 28]. Compounds **120** (IC_{50} = 225.1 ± 0.5 μM), **121** (IC_{50} ≥ 300 μM), and **122** (IC_{50} = 144.2 ± 0.2 μM) exhibited good lipoxygenase (LOX) inhibitory activity, as compared to the standard drug, baicalein (IC_{50} = 22.4 ± 0.5 μM). LOX inhibitors are used for the treatment of various diseases, i.e., asthma, inflammation, cancer, and some autoimmune diseases.

BIOTRANSFORMATION OF ANABOLIC-ANDROGENIC STEROIDS

Biotransformation of testosterone (123)

Biotransformation of the male steroidal hormone testosterone (**123**) with *Curvularia lunata* yielded 17-dehydrotestosterone (**124**) (2.1%), while *Pleurotus ostreatus*-assisted transformation of 123 afforded 15α-hydroxytestosterone (**125**) (2.9%)[45] [Figure 29]. Four derivatives, testolactone (**126**) (4.6%) (anti-cancer drug), 11α-hydoxytestolactone (**127**) (0.5%), 11α-hydroxyandrost-4-en-3, 17-dione (**128**) (0.3%), and 17β-hydroxy-androst-4-en-3, 6-dione (**129**) (1%) were synthesized through the biotransformation of **123** with *Rhizopus stolonifer*[46] [Figure 29]. Similarly, biotransformation of **123** with *Fusarium lini* afforded four

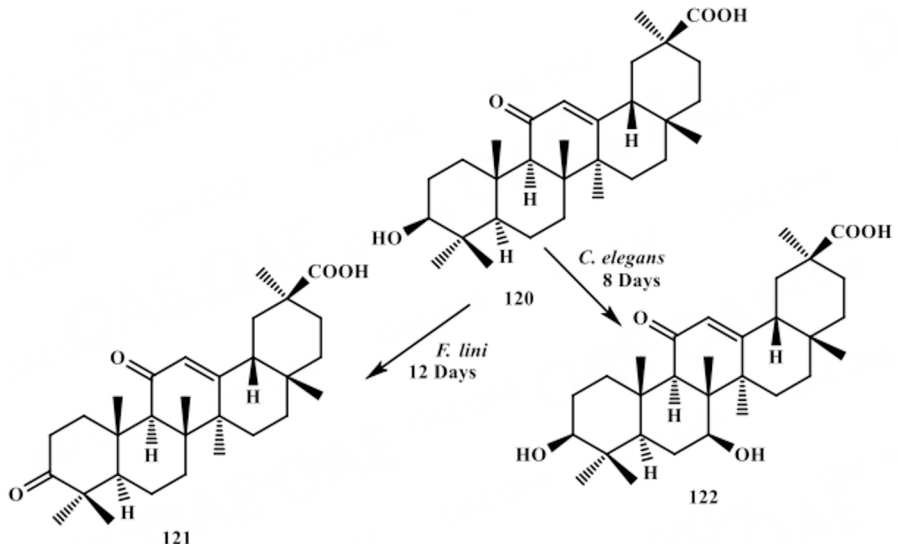

Figure 28. Microbial transformation of 18-glycyrrhetinic acid (**120**) *with Cunninghamella elegans, and Fusarium lini.*

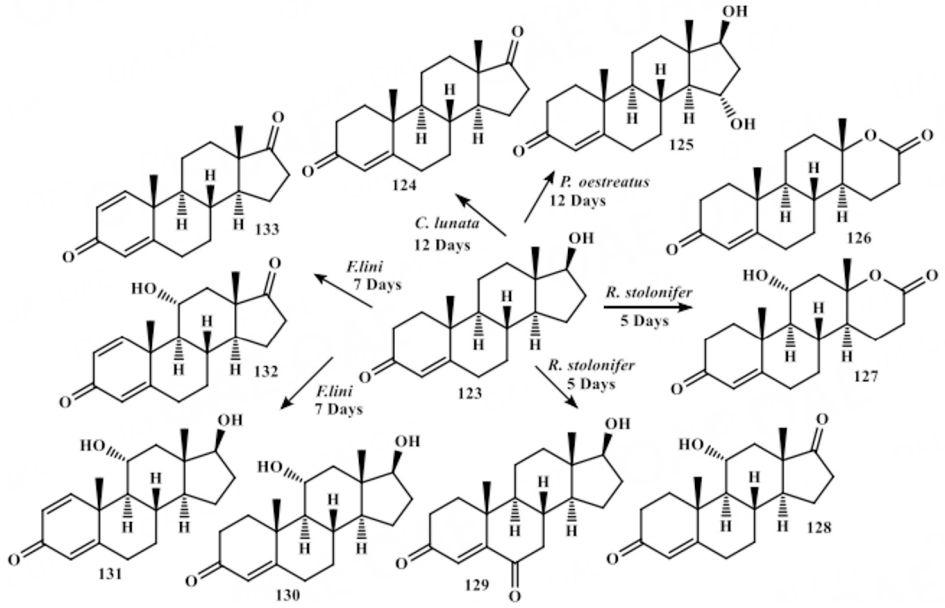

Figure 29. Microbial transformation of testosterone (**123**) with *Curvularia lunata, Pleurotus oestreatus, Rhizopus stolonifer,* and *Fusarium lini.*

derivatives, 17β,11α-dihydroxy-androst-4-en-3-one (**130**) (0.6%), 17β,11α-dihydroxy-androst-1, 4-dien-3-one (**131**) (1.2%), 11α-hydroxy-androst-1, 4-dien-3,17-dione (**132**) (3.1%), and androst-1, 4-dien-3, 17-dione (**133**) (2.5%)[46] [Figure 29] [Proposed Supplementary Figure 6].

Biotransformation of dihydrotestosterone (134)

Four derivatives, 1α-hydroxy-androst-1, 4-dien-3, 17-dione (**132**) (2.2%), and androst-1, 4-dien-3, 17-dione (**133**) (2.4%), 17β-hydroxyandrosta-1, 4-dien-3-one (**135**) (1.9%) [Supplementary Figure 5], and 7α, 17β-dihydroxyandrosta-1, 4-dien-3-one (**136**) (1.6%), were synthesized *via Fusarium oxysporum*-mediated transformation of the male steroidal hormone dihydrotestosterone (**134**)[47] [Figure 30] [Proposed

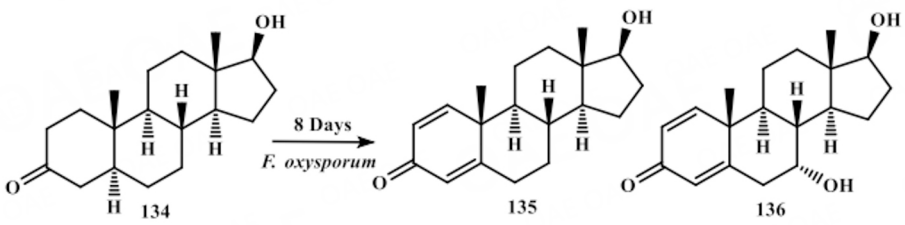

Figure 30. Biotransformation of dihydrotestosterone (**134**) with *Fusarium oxysporum.*

Supplementary Figure 6].

Biotransformation of androsterone (137)

Trichothecium roseum-catalyzed biotransformation of another steroidal hormone androsterone (**137**) afforded 3*α*, 17*β*-dihydroxyandrostane (**138**) (21.5%)[48] [Figure 31] [Supplementary Figure 6].

Biotransformation of trans-androsterone (139)

Transformed product 3*β*, 7*β*-dihydroxy-5*α*-androstan17-one (**140**) (5.1%) was obtained from the fermentation of steroidal hormone *trans*-androsterone (**139**) with the *Rhizopus stolonifer* fungal culture, while two derivatives 6*β*-hydroxy-5*α*-androstan-3, 17-dione (**141**) (0.5%) and 3*β*, 6*β*-dihydroxy-5*α*-androstan-17-one (**142**) (0.5%) were isolated from the transformation of substrate **139** with the fungal culture of *Fusarium lini*[49] [Figure 32].

Biotransformation of dehydroepiandrosterone (143)

Fermentation of dehydroepiandrosterone (DHEA) (**143**) with the fungal cell culture of *Cephalosporium aphidicola* resulted in two derivatives, 3*β*-hydroxy androst-4-en-17-one (**144**) (14%), and 3*β*, 4*β*-dihydroxyandrost-5-en-17-one (**145**) (17.3%)[48] [Figure 33]. Similarly, biotransformation of DHEA (**143**) with another fungal cell culture *Rhizopus stolonifer* yielded seven metabolites, 3*β*, 17*β*-dihydroxyandrost-5-ene (**146**) (20%), 3*β*, 17*β*-dihydroxyandrost-4-ene (**147**) (12%), 17*β*-hydroxyandrost-4-ene-3-one (**148**) (34%), 3*β*, 11*β*-dihydroxyandrost-4-ene-17-one (**149**) (15%), 3*β*, 7*α*-dihydroandrost-5-ene-17-one (**150**) (12%), 3*β*, 7*α*, 17*β*-trihydroxyandrost-5-ene (**151**) (20%), and 11*β*-hydroxyandrost-4, 6-diene-3, 17-dione (**152**) (15%)[50] [Figure 33]. The metabolites, 5*β*-androstane-3, 17-dione (**153**), 5*α*-androstane-3, 17-dione (**154**), androst-4-ene-3, 17-dione (**155**), and 17*β*-hydroxyandrost-4-en-3-one (**156**) were isolated through the biotransformation of 143 with plant cell culture *Codiaeum variegatum*[51] [Figure 34].

In addition, *M. phaseolina*-catalyzed transformation of DHEA (**143**) resulted in the synthesis of eight metabolites **146**, **150**, **154-156**, 17*β*-hydroxy-androst-4,6-diene-3-one (**157**), 3*β*-hydroxy-androst-4-en-6, 17-dione (**158**), and 3*β*, 7*β*, 17*β*-trihydroxy-androst-4-en (**159**)[52] [Figure 34]. Compounds **143** (IC_{50} = 77.9 ± 1.95 μM), **150** (IC_{50} = 373.5 ± 9.57 μM), **152** (IC_{50} = 430 ± 7.13 μM), **154** (IC_{50} = 221.6 ± 12.5 μM), and **155** (IC_{50} = 191.4 ± 1.17 μM) showed significant activity against *β*-glucuronidase enzyme, while metabolites **146**, **150**, **155**, and **157** were found to be inactive. *β*-Glucuronidase is an enzyme that catalyzes the hydrolysis of glucuronides, and generates free toxins. As a result, endogenous exposure of the organs to carcinogens increases. Its inhibition, therefore, has therapeutic significance against diseases such as colorectal carcinoma, GI tract infections, etc.

Biotransformation of boldione (160)

Biotransformation of the steroidal anabolic compound boldione (**160**) with the fungus *Cephalosporium aphidicola* afforded six metabolites, androst-4-ene-3, 17-dione (**161**) (2.0%), 17*β*-hydroxyandrosta-1, 4-

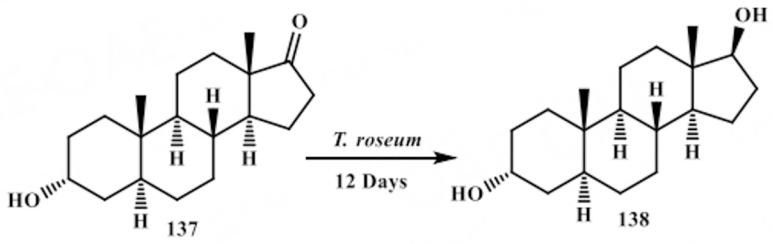

Figure 31. Biotransformation of androsterone (**137**) *Trichothecium roseum.*

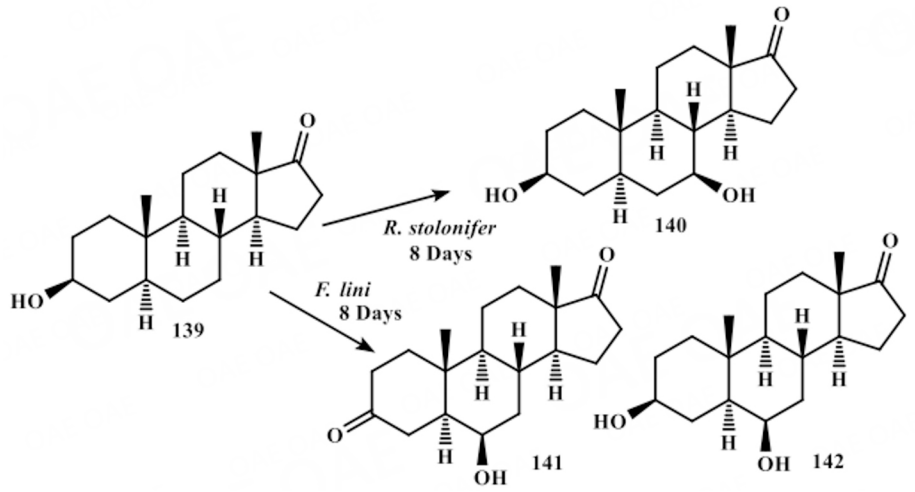

Figure 32. Microbial transformation of trans-androsterone (**139**) with *Rhizopus stolonifer*, and *Fusarium lini*.

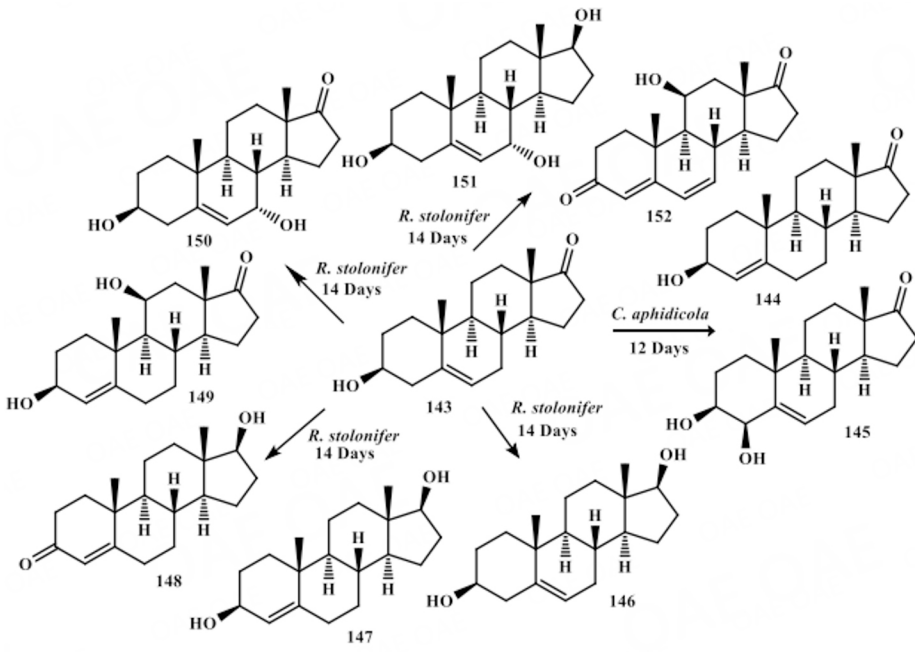

Figure 33. Microbial transformation of DHEA (**143**) with *Rhizopus stolonifer*, and *Cephalosporium aphidicola*. DHEA: dehydroepiandrosterone.

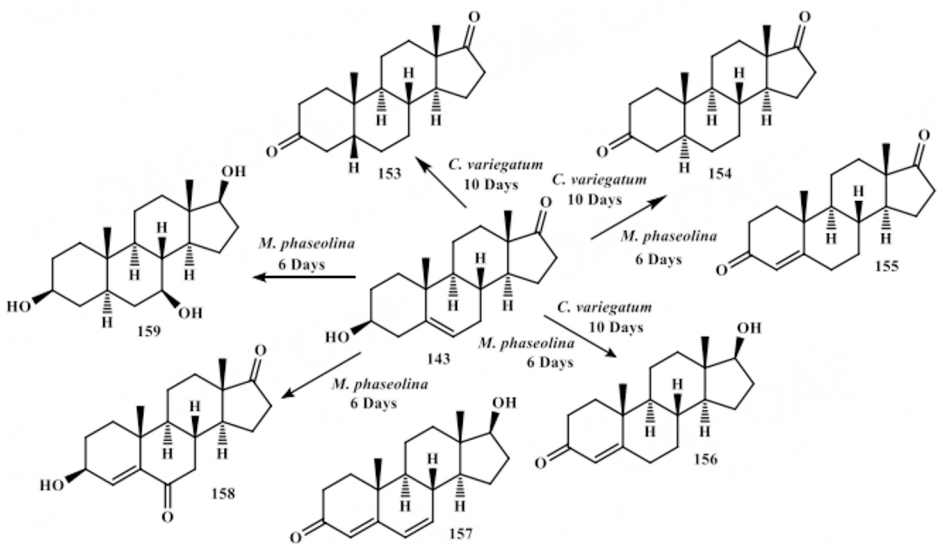

Figure 34. Biotransformation of DHEA (**143**) with *Cephalosporium aphidicola, Rhizopus stolonifer,* and *Codiaeum variegatum.* DHEA: dehydroepiandrosterone.

diene-3-one (**162**) (4.1%), 11α-hydroxyandrosta-1, 4-diene-3, 17-dione (**163**) (3.1%), 11α-hydroxyandrost-4-ene-3, 17-dione (**164**) (2.5%), 11α, 17β-dihydroxyandrost-4-ene-3-one (**165**) (5.3%), and 11α, 17β-dihydroxyandrosta-1, 4-diene-3-one (**166**) (2.5%) [Figure 35]. Metabolites **161**–**166** were also obtained *via* the fermentation of **160** with *Fusarium lini*[53].

Biotransformation of adrenosterone (167)

Microbial transformation of adrenosterone (**167**) with *Cephalosporium aphidicola* yielded three metabolites, androsta-1, 4-diene-3, 11, 17-trione (**168**) (11.2%), 17β-hydroxyandrost-4-ene-3, 11-dione (**169**) (8.1%), and 17β-hydroxyandrosta-1, 4-diene-3, 11-dione (**170**) (36.8%)[54] [Figure 36]. In addition, three new compounds, 9α-hydroxy-androsta-1-ene-3, 11, 17-trione (**171**) (3.3%), 9α, 17β-dihydroxy-androsta-1-ene-3, 11-dione (**172**) (12.4%), and 6β, 17β-dihydroxy-androsta-1-ene-3, 11-dione (**173**) (13.7%), along with the known compound 6β-hydroxy-androsta-1-ene-3, 11, 17-trione (**174**) (4.2%) were also synthesized *via* the biotransformation of **167** with *Cunninghamella elegans*[55] [Figure 36].

Biotransformation of nandrolone (175)

Rhizopus stolonifer-assisted transformation of nandrolone (**175**) yielded the new compound 6α, 17β-dihydroxy-19-norandrost-1, 4-dien-3-one (**176**) (20%), along with the known compound, 19-norandrost-4-en-3, 17-dione (**177**) (34%)[56] [Figure 37].

Three new derivatives, 10β, 12β, 17β-trihydroxy-19-nor-4-androsten-3-one (**178**) (0.2%), 10β, 16α, 17β-trihydroxy-19-nor-4-androsten-3-one (**179**) (1.5%), and 6β, 10β, 17β-trihydroxy-19-nor-4-androsten-3-one (**180**) (0.5%), along with four known metabolites, 10β, 17β-dihydroxy-19-nor-4-androsten-3-one (**181**) (0.25%), 10β-hydroxy-19-nor-4-androsten-3, 17-dione (**182**) (0.91%), and 16β, 17β-dihydroxy-19-nor-4-androsten-3-one (**183**) (0.83%)[57] [Figure 37]. Compounds 175 (IC_{50} = 32.0 ± 0.5 µM), **178** (IC_{50} ≥ 100 µM), **179** (IC_{50} = 77.39 ± 5.52 µM), **180** (IC_{50} = 70.90 ± 1.16 µM), **181** (IC_{50} = 54.94 ± 1.01 µM), **182** (IC_{50} = 80.23 ± 3.39 µM), and **183** (IC_{50} = 61.12 ± 1.39 µM) exhibited significant anti-leishmanial activity *in vitro* against *Leishmania major*. Leishmaniasis is a parasitic neglected tropical disease (NTD) affecting millions of people in over 80 countries in the global south. It causes self-healing lesions to be single and large skin ulcers.

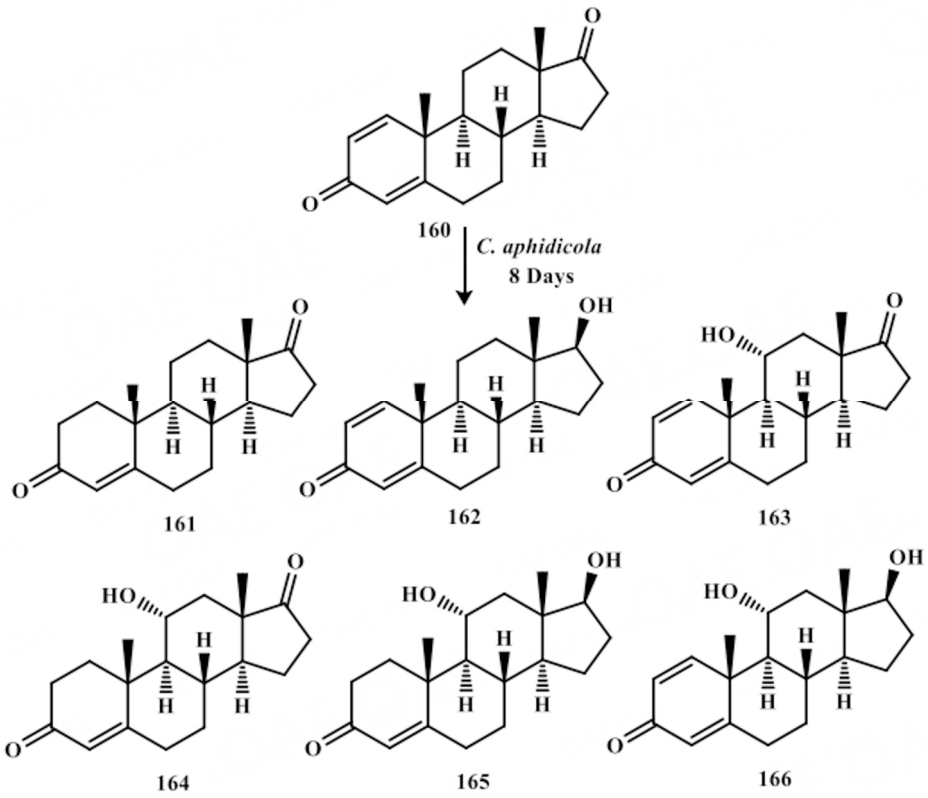

Figure 35. Biotransformation of boldione (**160**) with *Cephalosporium aphidicola.*

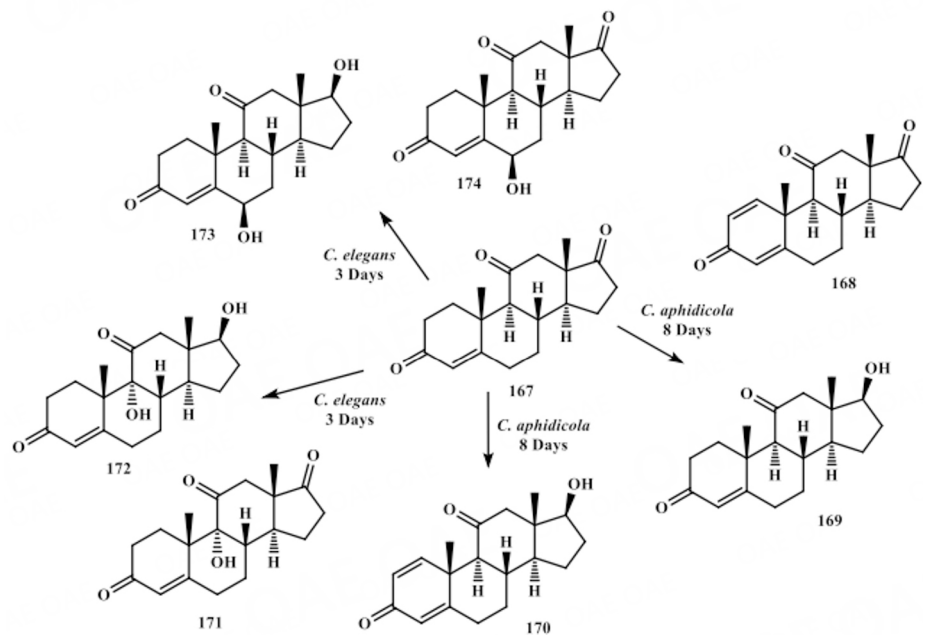

Figure 36. Microbial transformation of adrenosterone (**167**) with *Cephalosporium aphidicola*, and *Cunninghamella elegans.*

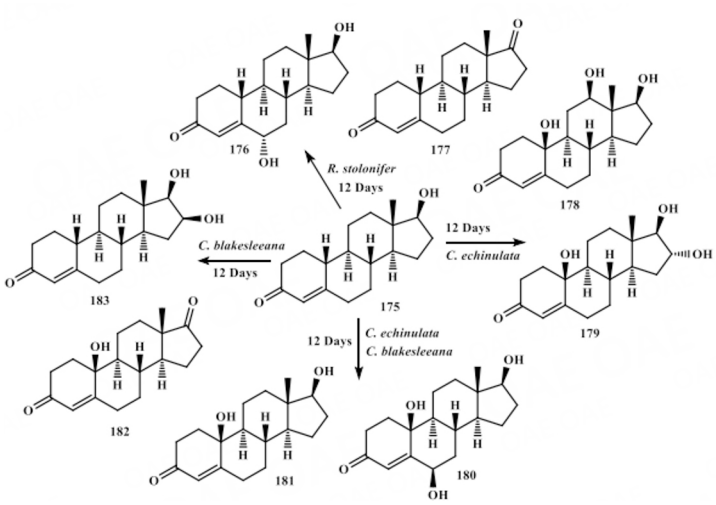

Figure 37. Microbial transformation of nandrolone (**175**) with *Cunninghamella blakesleeana*, *Cunninghamella echinulata*, and *Rhizopus stolonifer*.

Biotransformation of oxandrolone (184)

Rhizopus stolonifer-assisted transformation of the steroidal lactone, oxandrolone (**184**) yielded three new metabolites, 11*α*,17*β*-dihydroxy-2-oxa-androstan-3-one (**185**) (25%), 6*α*, 17*β*-dihydroxy-2-oxa-androstan-3-one (**186**) (5.0%), and 9*α*, 17*β*-dihydroxy-2-oxa-androstan-3-one (**187**) (8.0%) [Figure 38]. Compounds **185** (IC_{50} = 190.3 ± 1.18 μM) and **187** (IC_{50} = 482.66 ± 6.86 μM) showed weak inhibition of *β*-glucuronidase, as compared to the standard inhibitor, D-saccharic acid 1, 4 lactone (IC_{50} = 48.4 ± 1.25 μM)[58].

A new metabolite, 12*β*,17*β*-dihydroxy-17*α*-methyl-2-oxa-5*α*-androstan-3-one (**188**) (3.4%), was synthesized *via* the transformation of substrate **184** with *Cunninghamella blakesleeana*[59] [Figure 38]. Similarly, structural transformation of oxandrolone (**184**) with *Macrophomina phaseolina* afforded four new metabolites, 11*β*, 17*β*-dihydroxy-17*α*-(hydroxymethyl)-2-oxa-5*α*-androstan-3-one (**189**) (2.5%), 5*α*, 11*β*, 17*β*-trihydroxy-17*α*-methyl-2-oxa-androstan-3-one (**190**) (1.0%), 17*β*-hydroxy-17*α*-methyl-2-oxa-5*α*-androstan-3, 11-dione (**191**) (1.5%), and 11*β*, 17*β*-dihydroxy-17*α*-methyl-2-oxa-5*α*-androstan-3-one (**192**) (3.0%)[59] [Figure 38]. *Glomerella fusarioides*-mediated transformation of oxandrolone (**184**) also yielded a new compound, 17*β*, 11*α*-dihydroxy-17*α*-methylandrosta-2-oxa-4-ene-3-one (**193**) (3.8%)[60] [Figure 38]. The new metabolite 193 showed a remarkable aromatase inhibitory activity with an IC_{50} = 0.6 ± 0.005 μM, as compared to the substrate **184** (IC_{50} = 0.808 ± 0.07 μM), and standard anti-breast cancer drug exemestane (IC_{50} = 0.232 ± 0.031 μM)[60]. Aromatase is an enzyme that catalyzes the production of estrogen through the aromatization of steroidal ring-A, and thus helps in the proliferation of breast cancer cells. Its inhibition is the standard treatment of ER+ breast cancers.

Biotransformation of mesterolone (194)

Mesterolone (**194**) is a steroidal anabolic-androgenic drug used for the treatment of disorders in men where their bodies cannot produce enough natural androgens. Eight metabolites, 1α-methylandrostane-3, 17-dione (**195**) (1.2%), 1-methylandrost-1-en-3, 17-dione (**196**) (0.25%), 6*α*, 17*β*-dihydroxy-1*α*-methylandrosta-3-one (**197**) (0.36%), 7*α*, 17*β*-dihydroxy-1*α*-methylandrosta-3-one (**198**) (0.60%), 11*α*, 17*β*-dihydroxy-1*α*-methylandrosta-3-one (**199**) (0.55%), 15*α*-hydroxy-1*α*-methylandrosta-3, 17-dione (**200**) (1.05%), 15*α*, 17*β*-dihydroxy-1*α*-methylandrosta-3-one (**201**) (0.86%), 15*α*, 17*β*-dihydroxy-1-methylandrosta-1en-3-one (**202**) (0.37%), and 3*β*, 17*β*-dihydroxy-1*α*-methylandrostane (**203**) (0.65%) of mesterolone (**194**) were synthesized by using *Cephalosporium aphidicola*, *Fusarium lini*, and *Rhizopus stolonifer* fungal cell cultures. Metabolites

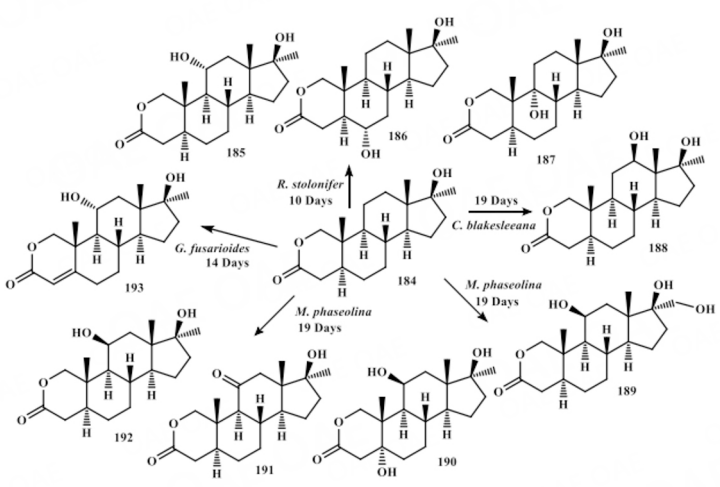

Figure 38. Microbial transformation of oxandrolone (**184**) with *Cunninghamella blakesleeana*, *M. phaseolina*, *Glomerella fusarioides*, and *Rhizopus stolonifer*.

197-**202** were found to be new[61] [Figure 39]. Compounds **194**, **196**, **199**, **200**, **201**, and **202** showed significant anti-inflammatory activity with the IC_{50} values of 202, 117, 250, 183, 295, 50, and 127 μM, respectively, as compared to the standard drug prednisolone (IC_{50} = 83 μM). Metabolite 195 was found to be inactive[61].

Similarly, *Cunninghamella blakesleeana*-mediated fermentation of mesterolone (**194**) (0.60%) yielded seven new compounds, 1*α*-methyl-11*β*, 14*α*, 17*β*-trihydroxy-5*α*-androstan-3-one (**204**), 1*α*-methyl-7*β*, 17*β*-dihydroxy-5*α*-androstan-3-one (**205**) (0.70%), 1*α*-methyl-17*β*-hydroxy-5*α*-androstan-3, 7-dione (**206**) (1.0%) [62] [Figure 40], 1*α*-methyl-1*β*, 11*α*, 17*β*-trihydroxy-5*α*-androstan-3-one (**207**) (0.90%), 1*α*-methyl-7*α*, 11*β*,17*β*-trihydroxy-5*α*-androstan-3-one (**208**) (0.40%), 1*α*-methyl-1*β*, 6*α*, 17*β*-trihydroxy-5*α*-androstan-3-one (**209**) (0.40%), and 1*α*-methyl-1*β*, 11*β*, 17*β*-trihydroxy-5*α*-androstan-3-one (**210**) (0.50%), along with three known metabolites, **197**, **198**, and **199**[63] [Figure 40]. Fermentation of mesterolone (**194**) with *Macrophomina phaseolina* afforded the new compound, 1*α*-methyl, 17*β*-hydroxy-5*α*-androstan-3, 6-dione (**211**) (0.40%)[63] [Figure 40].

Biotransformation of mibolerone (212)

Mibolerone (**212**) is a potent synthetic anabolic steroid, marketed by Upjohn Company (USA) under the brand name Cheque Drops, for the treatment of estrous (heat) in female dogs. Biotransformation of mibolerone (**212**) was carried out at room temperature using *Cunninghamella echinulata*, and *Macrophomina phaseolina*. This afforded six new metabolites, 10*β*, 17*β*-dihydroxy-7*α*, 17*α*-dimethylestr-4-en-3-one (**213**) (**4.0%**), **6□, 17□-dihydroxy-7□, 17□-dimethylestr-4-en-3-one (214) (2.0%), 6□, 10□, 17□-trihydroxy-7□, 17□-dimethylestr-4-en-3-one** (**215**) (30.0%), 11*β*, 17*β*, 20-trihydroxy-7*α*, 17*α*-dimethylestr-4-en-3-one (**216**) (0.4%), 1*α*, 17*β*-dihydroxy-7*α*, 17*α*-dimethylestr-4-en-3-one (**217**) (0.24%), 1*α*, 11*β*, 17*β*-trihydroxy-7*α*, 17*α*-dimethylestr-4-en-3-one (**218**) (0.34%), and a known metabolite, 11*β*, 17*β*-dihydroxy-7*α*, 17*α*-dimethylestr-4-ene-3-one (**219**) (3.0%)[64] [Figure 41].

Biotransformation of metenolone acetate (220)

Metenolone acetate (**220**) is another synthetic steroidal anabolic drug, sold under the brand names Nibal and Primobolan Depot, for the treatment of anemia. Drug **220** is also used by athletes, and for sports animals, to enhance their muscular strength and physical performances. Microbial transformation of

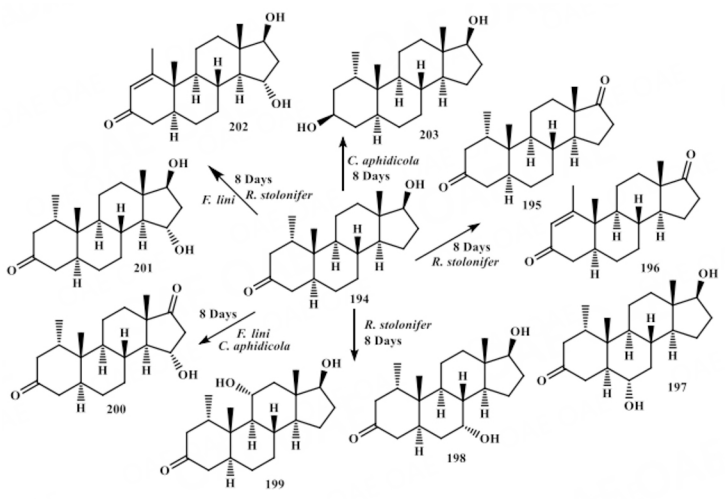

Figure 39. Microbial transformation of mesterolone (**194**) with *Cephalosporium aphidicola*, *Rhizopus stolonifer*, and *Fusarium lini*.

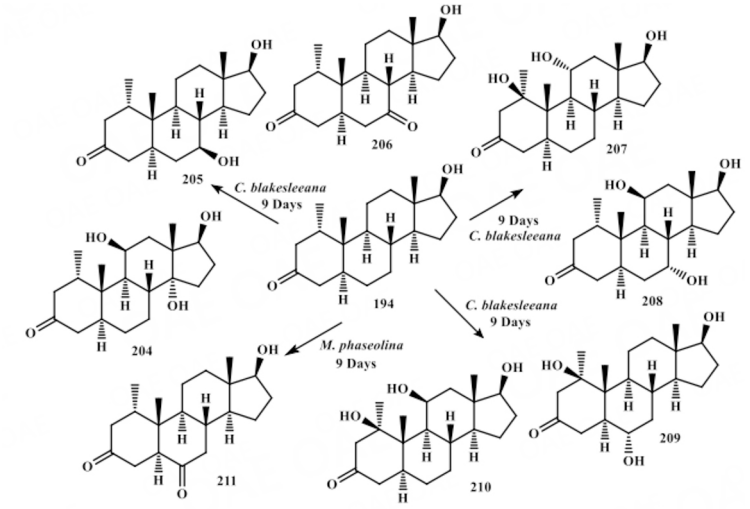

Figure 40. Microbial transformation of mesterolone (**194**) with *Cunninghamella blakesleeana*, and *Macrophomina phaseolina*.

metenolone acetate (**220**) was carried out under ambient reaction conditions using *Rhizopus stolonifer*, *Fusarium lini*, *Cunninghamella elegans*, and *Aspergillus alliaceous*. This afforded fourteen transformed products, 6α-hydroxy-1-methyl-3-oxo-5α-androst-1-en-17-yl acetate (**221**) (1.1%), 6α, 17β-dihydroxy-1-methyl-3-oxo-5α-androst-1-en (**222**) (1.0%), 7β-hydroxy-1-methyl-3-oxo-5α-androst-1-en-17-yl acetate (**223**) (0.50%), 15β, 20-dihydroxy-1-methyl-3-oxo-5α-androst-1-en-17-yl acetate (**224**) (2.6%), 15β-hydroxy-1-methyl-3-oxo-5α-androst-1-en-17-yl acetate (**225**) (1.0%), 17β-hydroxy-1-methyl-3-oxoandrosta-1,4-dien (**226**) (0.40%) [Figure 42], 17β-hydroxy-1-methyl-3-oxo-5α-androst-1-en (**227**) (7.1%)[65], 17β-hydroxy-1-methyl-3-oxo-5β-androst-1-en (**228**) (0.84%), 1-methyl-5β-androst-1-en-3,17-dione (**229**) (0.30%), 12β, 17β-dihydroxy-1-methyl-3-oxoandrosta-1, 4-dien (**230**) (0.70%), 1-methyl-androsta-1, 4-dien-3, 17-dione (**231**) (24.4%), 17β-hydroxy-1α-methyl-5α-androstan-3-one (**232**) (0.45%), 17β, 15α-dihydroxy-1α-methyl-5α-androstan-3-one (**233**) (1.0%), and 7β, 15β, 17β-trihydroxy-1-methyl-3-oxo-5α-androst-1-en (**234**) (0.54%). Among them, compounds **221**-**225**, **230**, and **234** were identified as new[65] [Figure 43], Compounds **220** (62.5% ± 4.4%), **221** (73.4% ± 0.6 %), **224** (81.0% ± 2.5 %), **227** (69.7% ± 1.4%), **229** (73.2% ± 0.3%), **232** (60.1% ±

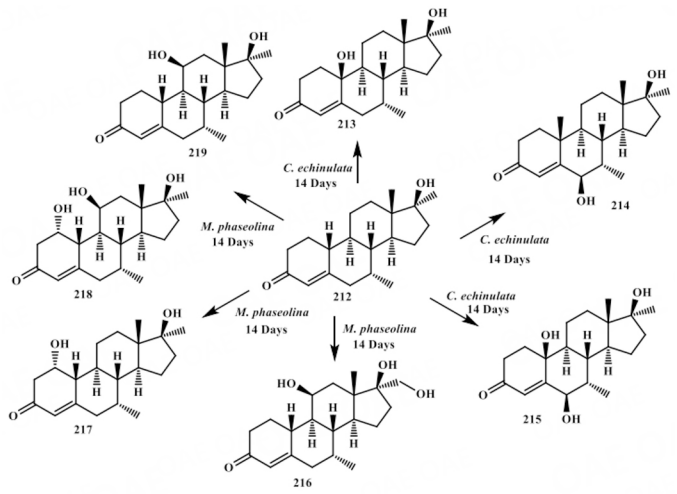

Figure 41. Microbial transformation of mibolerone (**212**) with *Cunninghamella echinulata,* and *Macrophomina phaseolina.*

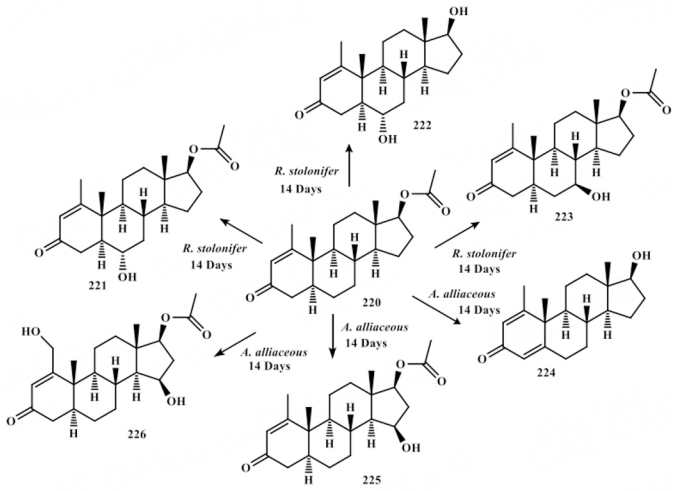

Figure 42. Microbial transformation of metenolone acetate (**220**) with *Aspergillus alliaceous,* and *Rhizopus stolonifer.*

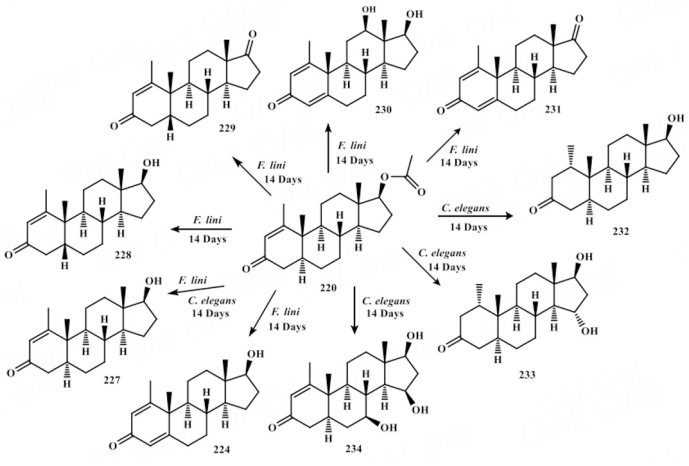

Figure 43. Microbial transformation of metenolone acetate (**220**) with *Cunninghamella elegans,* and *Fusarium lini.*

3.3%), and **233** (71.0% ± 7.2%) showed good inhibition of cytokine (TNF-α) production. Compounds 223 (53.7% ± 1.4 %), 226 (46.6% ± 5.2 %), and **234** (52.9% ± 2.4%) showed moderate activity, compounds **222** (33.5% ± 6.6%), and **225** (37.8% ± 1.1%) showed a weak activity, while metabolites **228**, **234**, and **235** were found inactive. Compounds **222** (IC_{50} = 4.4 ± 0.01 µg/mL), and **224** (IC_{50} = 10.2 ± 0.01 µg/mL) showed significant activity against T-cells proliferation, in contrast to the standard drug, prednisolone (IC_{50} = 3.51 ± 0.03 µg/mL) *in vitro*[65]. TNF- α, and T-cells are essential components of innate inflammatory cascade, and their inhibition is used for the treatment of chronic inflammations.

Biotransformation of metenolone enanthate (235)

Four new derivatives of steroidal anabolic drug, metenolone enanthate (**235**), namely 17β-hydroxy-1-methyl-5α-androst-1-ene-3, 16-dione (**236**), 15β, 17β -dihydroxy-1-methyl-5α-androstan-1-ene-3-one (**237**), 12β, 17β -dihydroxy-1-methyl-5α-androstan-1-ene-3-one (**238**), and 16β, 17β-dihydroxy-1-methyl-5α-androstan-1-ene-3-one (**239**), were obtained through biotransformation of drug **235** with *Aspergillus niger*[66] [Figure 44]. The metabolites **236** and **237** showed potent inhibition of ROS production by whole blood with the IC_{50} values of 8.60 ± 1.0 and 7.05 ± 1.3 µg/mL, respectively, while drug **235** was found to be inactive. Compounds **236** and **237** also showed potent activity against isolated polymorphonuclear leukocytes (PMNs) with the IC_{50} values of 14.0 ± 1.7 and 4.70 ± 0.5 µg/mL, respectively.

Biotransformation of danazol (240)

Cunninghamella blakesleeana-catalyzed transformation of the anabolic steroidal drug, danazol (**240**), afforded three new metabolites, 15β, 17β-dihydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**241**) (1.0%), 1α, 17β-dihydroxy-17α-pregna-2, 4-dien-20-yno-[2, 3-d]-isoxazole (**242**) (1.2%), 6β, 17β-dihydroxy-17α-pregna-2, 4-dien-20-yno-[2,3-d]-isoxazole (**243**) (0.8%), along with the known metabolite, 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-1, 4-dien-20-yn-3-one (**244**) (1.2%)[67] [Figure 45]. Compound **241** showed potent cytotoxicity against HeLa (cervical) cancer cell line with the IC_{50} = 0.283 ± 0.013 µM, as compared to the standard anti-cancer drug, doxorubicin (IC_{50} = 0.506 ± 0.015 µM), where compound **242** was identified as significantly active (IC_{50} = 13.42 ± 0.819 µM)[67].

Biotransformation of dianabol (245)

Biotransformation of another anabolic steroidal drug dianabol (**245**) with *Cunninghamella elegans* afforded five new metabolites, 6β, 17β-dihydroxy-17α-methylandrost-1, 4-dien-3-one (**246**) (3.7%), 15α, 17β-dihydroxy-17α-methylandrost-1, 4-dien-3-one (**247**) (18.6%), 11α, 17β-dihydroxy-17α-methylandrost-1, 4-dien-3-one (**248**) (13.0%), 6β, 12β, 17β-trihydroxy-17α-methylandrost-1, 4-dien-3-one (**249**) (4.0%), 6β, 15α, 17β-trihydroxy-17α-methylandrost-1, 4-dien-3-one (**250**) (3.2%)[68] [Figure 46]. Three new metabolites, 17β-hydroxy-17α-methylandrost-1,4-dien-3,6-dione (**251**) (1.2%), 7β, 17β-dihydroxy-17α-methylandrost-1, 4-dien-3-one (**252**) (11.0%), and 15β, 17β-dihydroxy-17α-methylandrost-1, 4-dien-3-one (**253**) (3.0%), along with the known metabolite, 11β, 17β-dihydroxy-17α-methylandrost-1, 4-dien-3-one (**254**) (1.7%) were synthesized by *Macrophomina phaseolina*-assisted biotransformation of drug 245. The metabolite 247 showed a remarkable β-glucuronidase inhibitory activity (IC_{50} = 60.7 µM), as compared to the standard, D-saccharic acid-1, 4-lactone (IC_{50} = 48.4 µM)[68].

Biotransformation of methasterone (255)

Five new derivatives, 7α, 17β-dihydroxy-2α, 17α-dimethyl-5α-androstane-3-one (**256**) (2.0%), 7α, 16β, 17β-triihydroxy-2α, 17α-dimethyl-5α-androstane-3-one (**257**) (0.7%), 5α, 12β, 17β-trihydroxy-2α, 17α-dimethyl-5 α-androstane-3-one (**258**) (1.0%), 7α, 12β, 17β-triihydroxy-2α, 17α-dimethyl-5α-androstane-3-one (**259**) (1.5%), and 7α, 9α, 17β-triihydroxy-2α, 17α-dimethyl-5α-androstane-3-one (**260**) (0.5%) were isolated through biotransformation of steroidal anabolic drug, methasterone (**255**) with *Cunninghamella blakesleeana.* Likewise, six new derivatives, 6β, 17β-dihydroxy-2, 17α-dimethylandrosta-1, 4, 14-triene-3-one

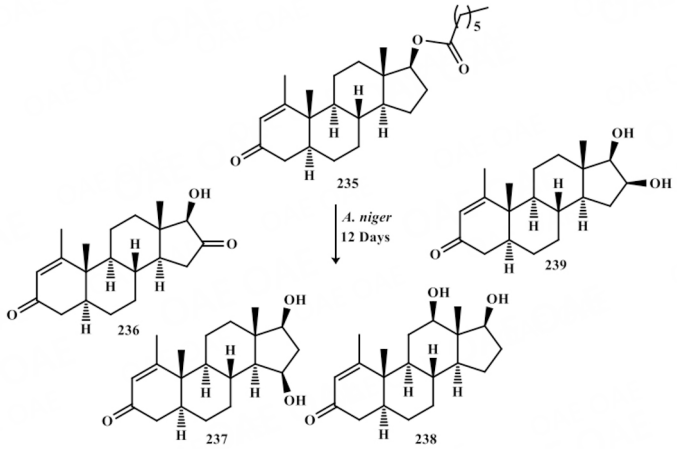

Figure 44. Biotransformation of metenolone enanthate (**235**) with *Aspergillus niger* (**235**).

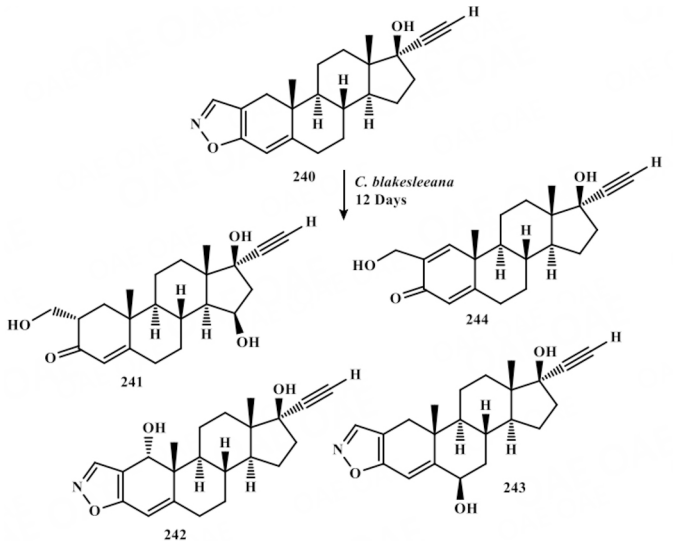

Figure 45. Biotransformation of danazol (**240**) with *Cunninghamella blakesleeana* (**240**).

(**261**) (1.0%), 15*α*, 17*β*-dihydroxy-2, 17*α*-dimethylandrosta-1, 4-diene-3-one (**262**) (0.6%), 6*β*,17*β*-dihydroxy-2, 17*α*-dimethylandrosta-1, 4-diene-3-one (**263**) (0.4%), 14*α*, 15*α*-dihydroxy-2, 17-dimethylandrosta-1, 4, 16-triene-3-one (**264**) (0.3%), 17*β*-hydroxy-2, 17*α*-dimethylandrosta-1, 4-diene-3, 6-dione (**265**) (0.3%), 17*β*-hydroxy-2, 17*α*-dimethylandrosta-1, 4-diene-3-one (**266**) (1.0%) were synthesized by *Fusarium lini* transformation of drug **255**[69] [Figure 47]. The metabolite **259** showed a potent inhibition against TNF-*α* production with the IC_{50} value of 8.1 ± 0.9 μg/mL, as compared to the standard drug, pentoxifylline (IC_{50} = 94.8 ± 2.1 μg/mL). Derivatives **259** (86.7% ± 2.3%) and **266** (62.5% ± 1.5%) also showed an excellent inhibition of NO proliferation, as compared to the standard N^G-monomethyl-l-arginine acetate (65.6% ± 1.1%)[69].

Further, a new compound, 6*β*, 9*α*, 17*β*-trihydroxy-2*α*, 17α-dimethyl-5α-androstane-3-one (**267**), was obtained through the biotransformation of compound **255** with *Macrophomina phaseolina*[69]. While four new compounds, 6*β*, 7*β*, 17*β*-trihydroxy-2*α*, 17*α*-dimethyl-5*α*-androstane-3-one (**268**) (0.54%), 6*β*, 7*α*, 17*β*-trihydroxy-2*α*, 17*α*-dimethyl-5*α*-androstane-3-one (**269**) (0.53%), 6*α*, 17*β*-dihydroxy-2*α*, 17*α*-dimethyl-5*α*-

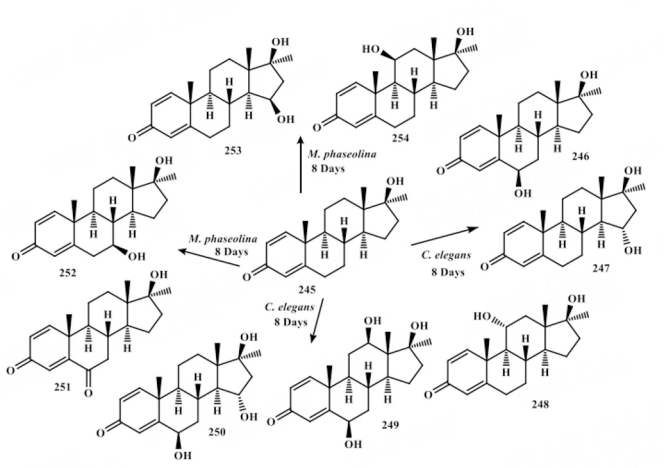

Figure 46. Biotransformation of dianabol (**245**) with *Macrophomina phaseolina* and *Cunninghamella elegans.*

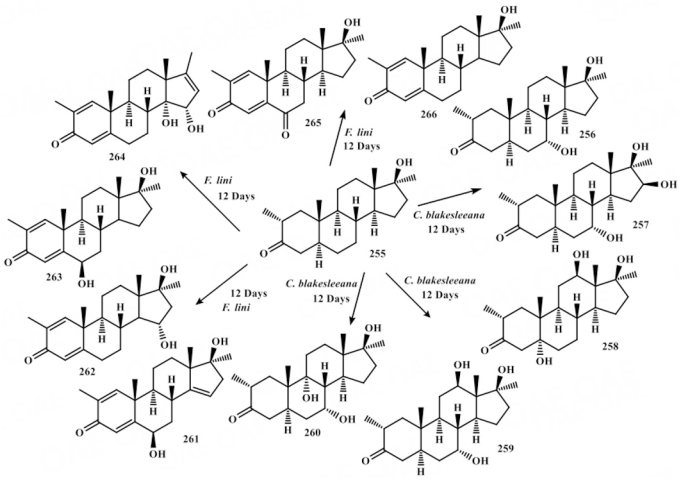

Figure 47. Biotransformation of methasterone (**255**) with *Cunninghamella blakesleeana* and *Fusarium lini.*

androstane-3, 7-dione (**270**) (0.51%), and 3*β*, 6*β*, 17*β*-trihydroxy-2*α*, 17*α*-dimethyl-5*α*-androstane-7-one (**271**) (0.53%) were synthesized by biotransformation of drug **255** with *Cunninghamella blakesleeana*[70] [Figure 48]. Metabolites **267** and **268** showed moderate inhibition of NO production with the IC_{50} values of 40.2 ± 3.3, and 38.1 ± 0.5 μg·mL^{-1}, respectively.

BIOTRANSFORMATION OF CONTRACEPTIVE STEROIDS

Biotransformation of desogestrel (272)

Whole-cell bio-catalytic conversion of steroidal contraceptive drug desogestrel (**272**) by *Cunninghamella blakesleeana* yielded three new metabolites, 13-ethyl-11-methylene-18, 19-dinor-17*α*-pregn-4-en-20-yn-6*β*, 15*β*, 17*β*-triol (**273**) (2.5%), 13-ethyl-11-methylene-18, 19-dinor-17*α*-pregn-4-en-20-yn-3*β*, 6*β*, 17*β*-triol (**274**) (15.2%), and 13-ethyl-11-methylene-18, 19-dinor-17*α*-pregn-20-yn-3*α*, 5*α*, 6*β*, 17*β*-tetraol (**275**) (1.9%), along with the known metabolite, 13-ethyl-11-methylene-18, 19-dinor-17*α*-pregn-4-en-20-yn-6*β*, 17*β*-dihydroxy-3-one (**276**) (9.2%)[71] [Figure 49]. Compounds **272** and **273** showed potent activity against *Staphylococcus aureus* EMRSA-17, *Staphylococcus aureus* NCTC 13277 (MRSA-252), and *Staphylococcus aureus* NCTC 13143, and clinically isolated Pakistani strain of *Staphylococcus aureus* in an in-vitro MABA

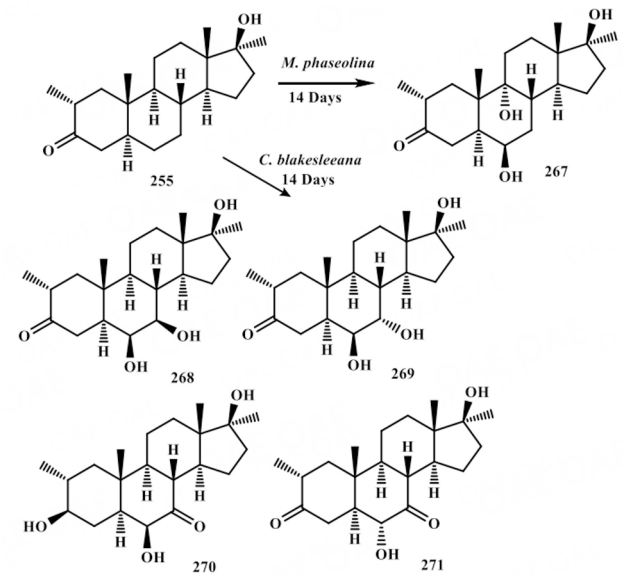

Figure 48. Biotransformation of methasterone (**255**) with *Macrophomina phaseolina* and *Cunninghamella blakesleeana*.

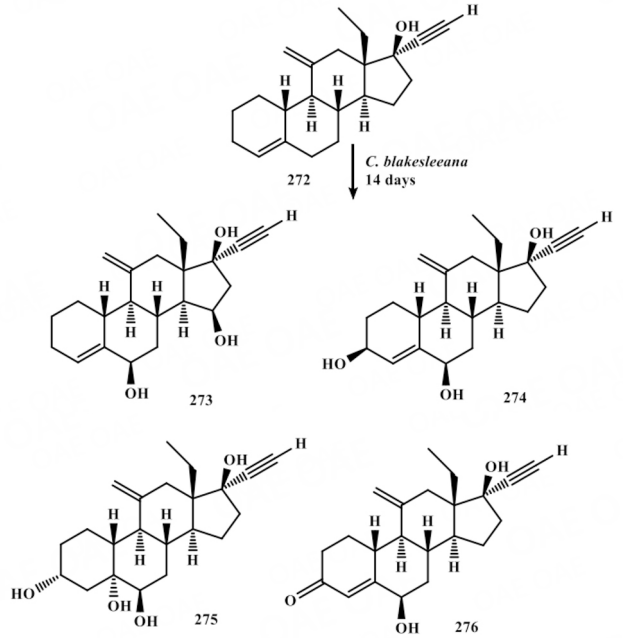

Figure 49. Biotransformation of desogestrel (**272**) with *Cunninghamella blakesleeana.*

(Microplate Alamar Blue) assay.

Biotransformation of ethisterone (277)

Cunninghamella elegans-assisted transformation of another steroidal contraceptive compound, ethisterone (**277**), afforded a new derivative, 17α-ethynyl-11α, 17β-dihydroxyandrost-4-en-3-one (**278**) (5.5%)[72] [Figure 50]. Biotransformation of the drug **277** with *Cunninghamella blakesleeana* afforded two new metabolites, 17α-ethynyl-6β, 15β, 17β-trihydroxyandrost-4-en-3-one (**279**) (0.80%) and 17α-ethynyl-7β, 15β, 17β-trihydroxyandrost-4-en-3-one (**280**) (0.30%), while fermentation of ethisterone (**277**) with *Aspergillus*

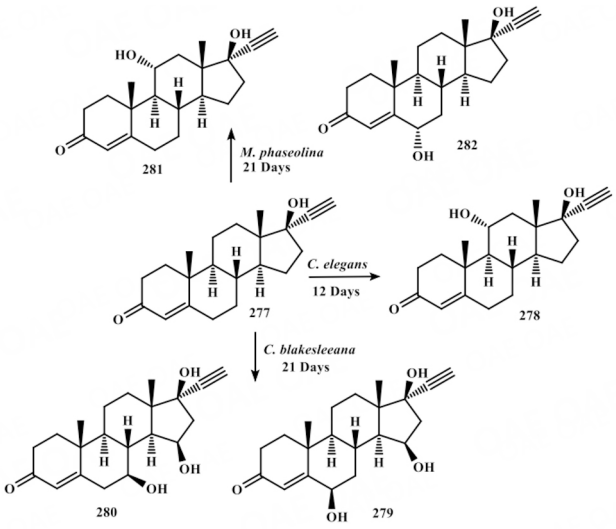

Figure 50. Microbial transformation of ethisterone (**277**) *Cunninghamella blakesleeana,Cunninghamella elegans* and *Macrophomina phaseolina*.

niger yielded a new metabolite, 17α-ethynyl-6α, 17β-dihydroxyandrost-4-en-3-one (**281**) (0.60%), along with the known metabolite, 17α-ethynyl-11α, 17β-dihydroxyandrost-4-en-3-one (**282**) (0.30%)[73] [Figure 50].

Two new derivatives, 17α-ethyl-11α, 17β-dihydroxyandrost-4-en-3-one (**284**) (0.80%), and 17α-ethyl-6α, 17β -dihydroxy-5α-androstan-3-one (**285**) (60.0%) were also synthesized through the biotransformation of 17α-ethyl-17β-hydroxyandrost-4-en-3-one (**283**) with *C. elegans*[72] [Figure 51]. Derivatives **278** (IC_{50} = 5.95 ± 0.00078 µM), **284** (IC_{50} = 3.46 ± 0.01046 µM), and **285** (IC_{50} = 1.72 ± 0.00089 µM) showed remarkable tyrosinase inhibitory activity, as compared to the substrates **277** (IC_{50} = 2.61 ± 0.037328 µM), **283** (IC_{50} = 1.53 ± 0.001088 µM)[72]. Tyrosinase is an enzyme involved in the melanin biosynthesis; therefore, its inhibitors are used in the prevention of excessive production dermal melanin.

Biotransformation of mestranol (286)

Biotransformation of an oral steroidal contraceptive drug, mestranol (**286**), with the fungal culture of *Cunninghamella elegans* afforded a new compound, 6β, 12β-dihydroxymestranol (**287**) (3.6%), and the known derivative, 6β-hydroxymestranol (**288**) (2.7%)[74] [Figure 52].

Biotransformation of methyloestrenolone (289)

Six transformed products, 17α-methyl-6β,17β-dihydroxyestr-4-en-3-one (**290**) (1.8%), 17α-methyl-11β, 17β, 20-trihydroxyestr-4-en-3-one (**291**) (0.8%), 17α-methyl-2α, 11β, 17β-trihydroxyestr-4-en-3-one (**292**) (0.6%), 17α-methyl-1β, 17β-dihydroxyestr-4-en-3-one (**293**) (4.5%), 17α-methyl-11α, 17β-dihydroxyestr-4-en-3-one (**294**) (0.45%), and 17α-methyl-11β, 17β-dihydroxyestr-4-en-3-one (**295**) (1.4%) were synthesized via biotransformation of a steroidal contraceptive drug, methyloestrenolone (**289**) with *Macrophomina phaseolina*. Compounds **290**-**295** were found to be new compounds[75] [Figure 53]. Two known derivatives, 17α-methyl-10β, 17β-dihydroxyestr-4-en-3-one (**296**) (0.8%), and 17α-methyl-17β, 20-dihydroxyestr-4-en-3-one (**297**) (1.2%) were obtained through biocatalysis of **289** with *Aspergillus niger*[75] [Figure 54].

Biotransformation of drospirenone (298)

Drospirenone (**298**) is an orally active contraceptive drug, marketed under the brands Sylnd and Yasmin. *Cunninghamella elegans*-catalyzed transformation of drospirenone (**298**) afforded four new metabolites, 6β,

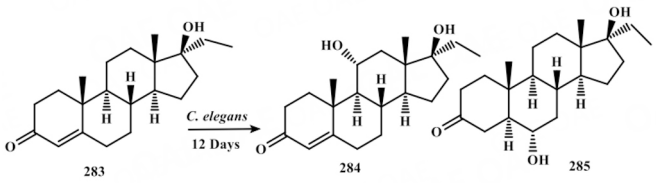

Figure 51. Biotransformation of drug **283** with *Cunninghamella elegans*.

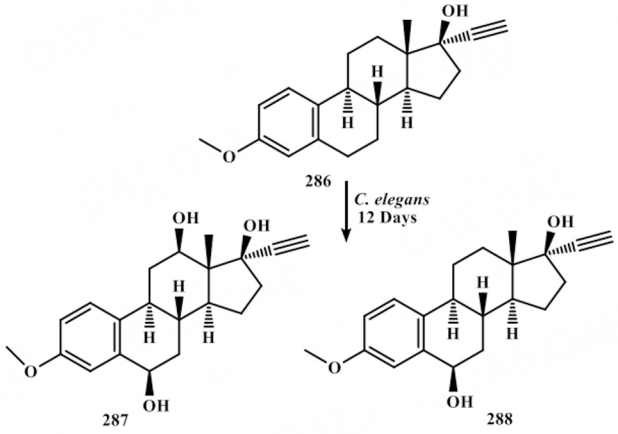

Figure 52. Biotransformation of mestranol (**286**) with *Cunninghamella elegans*.

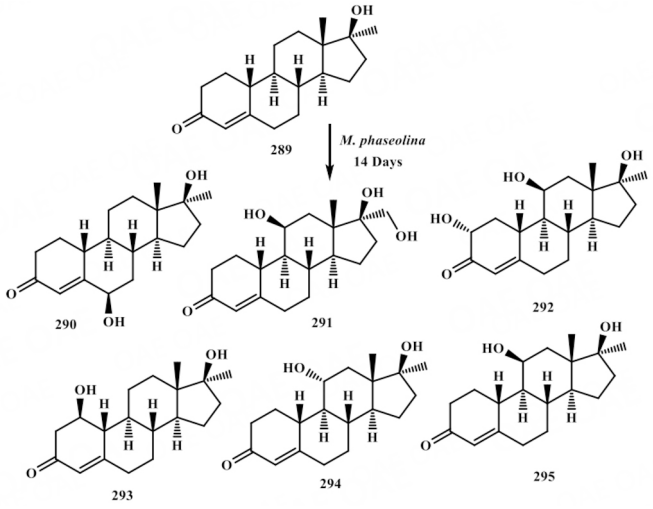

Figure 53. Biotransformation of methyloestrenolone (**289**) with *Macrophomina phaseolina*.

7*β*, 15*β*, 16*β*-dimethylene-3-oxo-14*α*-hydroxy-17*α*-pregn-4-ene-21, 17-carbolactone (**299**) (3.1%), 6*β*,7*β*,15*β*,16*β*-dimethylene-3, 11-dioxo-17*α*-pregn-4-ene-21, 17-carbolactone (**300**) (4.2%), 6*β*, 7*β*, 15*β*, 16*β*-dimethylene-3, 12-dioxo-17*α*-pregn-4-ene-21, 17-carbolactone (**301**) (2.9%), and 6*β*, 7*β*, 15*β*, 16*β*-dimethylene-3-oxo-11*β*, 14*α*-dihydroxy-17*α*-pregn-4-ene-21, 17-carbolactone (**302**) (6.2%), along with the known metabolite, 6*β*, 7*β*, 15*β*, 16*β*-dimethylene-3-oxo-11*α*-dihydroxy-17*α*-pregn-4-ene-21, 17-carbolactone (**303**)[76] [Figure 55].

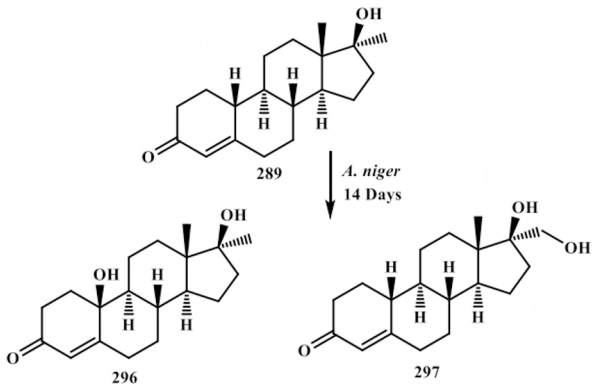

Figure 54. Biotransformation of methyloestrenolone (**289**) with *Aspergillus niger*.

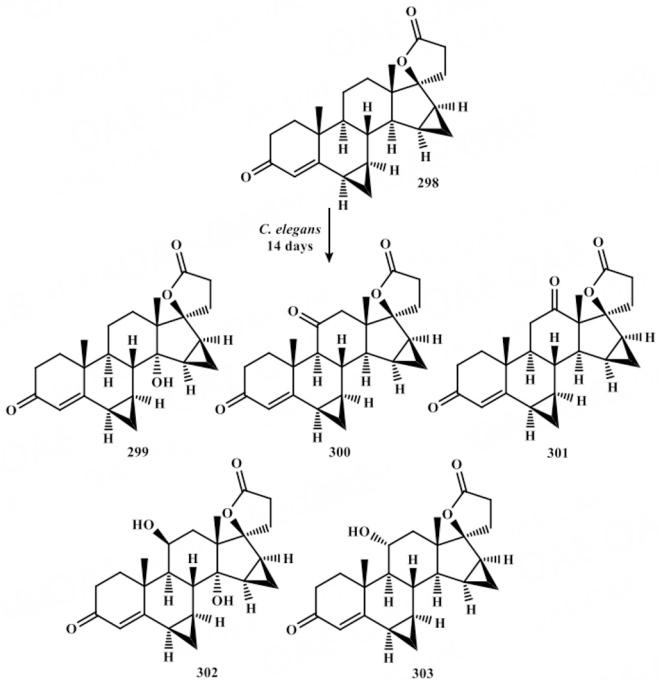

Figure 55. Biotransformation of drospirenone (**298**) with *Cunninghamella elegans*.

Biotransformation of etonogestrel (304)

Biotransformation of another contraceptive drug (brands: Nexplanon and Implanon) etonogestrel (**304**) with *Cunninghamella blakesleeana* afforded three new metabolites, 6β-hydroxy-11, 22-epoxy-etonogestrel (**305**) (3.2%), 11, 22-epoxy-etonogestrel (**306**) (1.5%), 10β-hydroxy-etonogestrel (**307**) (4.6%), and two known compounds, 6β-hydroxy-etonogestrel (**308**) (3.4%), and 14α-hydroxy-etonogestrel (**309**) (2.7%). Compounds **305**, **307**, and **309** were also obtained from the fermentation of **304** with *Cunninghamella echinulata*[77] [Figure 56]. Derivative **308** was found to be significantly active against β-glucuronidase enzyme with the IC_{50} value of 13.97 ± 0.12 μM, as compared to the standard drug, D-saccharic acid 1,4-lactone (IC_{50} = 45.75 ± 2.16 μM)[77].

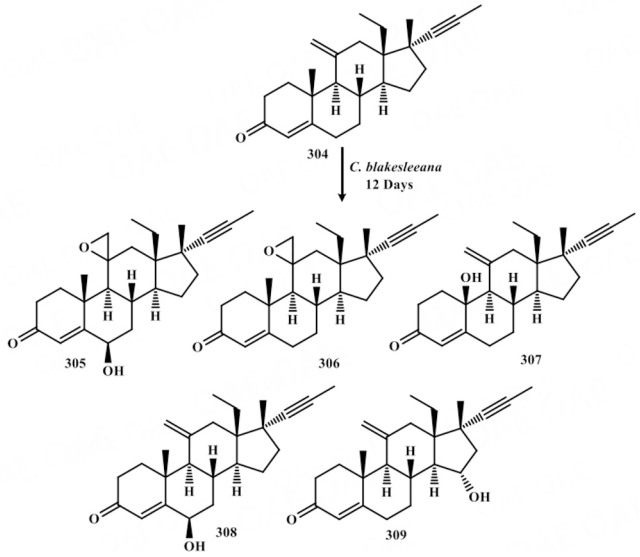

Figure 56. Biotransformation of etonogestrel (**304**) with *Cunninghamella blakesleeana.*

Biotransformation of ethynodiol diacetate (310)

Biotransformation of another synthetic steroidal contraceptive drug, ethynodiol diacetate (**310**), with the fungus *Cunninghamella elegans* yielded three new derivatives, 17*α*-ethynylestr-4-en-3*β*, 17*β*-diacetoxy-6α-ol (**311**) (0.5%), 17*α*-ethynylestr-4-en-3*β*, 17*β*-diacetoxy-6*β*-ol (**312**) (1.0%), and 17*α*-ethynylestr-4-en-3*β*, 17*β*-diacetoxy-10*β*-ol (**313**) (0.5%), and the known metabolite, 17*α*-ethynyl-17*β*-acetoxyestr-4-en-3-one (**314**) (1.4%). In addition, four known metabolites, 314, 17α-ethynyl-17*β*-hydroxyestr-4-en-3-one (**315**) (3.3%), 17*α* -ethynyl-3*β*-hydroxy-17*β*-acetoxyestr-4-ene (**316**) (0.58%), and 17α-ethynyl-5*α*, 17*β*-dihydroxyestr-3-ene (**317**) (0.45%) were also obtained by the biotransformation of drug **310** with the plant cell culture of *Ocimum basilicum*[78] [Figure 57].

BIOTRANSFORMATION OF ANTI-CANCER STEROIDS

Biotransformation of exemestane (318)

Exemestane (**318**) is a steroidal-based aromatase inhibitor for the treatment of estrogen-dependent (ER+) breast cancers. Drug **318** is marketed under the brand name Aromasin. Microbial transformation of exemestane (**318**) afforded three new derivatives, 11*α*-hydroxy-6-methylene-androsta-1,4-diene-3,17-dione (**319**) (0.4%), 16*β*,17*β*-dihydroxy-6-methylene-androsta-1,4-diene-3-one (**320**) (1.0%), and 17*β*-hydroxy-6-methylene-androsta-1,4-diene-3, 16-dione (**321**) (0.5%), and the known metabolite, 17*β*-hydroxy-6-methylene-androsta-1, 4-diene-3-one (**322**) (0.6%)[79] [Figure 58]. The metabolite **319** showed moderate cytotoxicity against PC-3 (prostrate) (IC_{50}= 16.83 ± 0.96 μM), and HeLa (cervical) (IC_{50} = 24.87 ± 0.72 μM) cancer cell lines.

Six metabolites, 6-methylene-5*α*-androstane-3*β*, 16*β*, 17*β*-triol (**323**), 17*β*-hydroxy-6-methyleneandrosta-4-ene-3-one (**324**), 6α-spiroxirandrost-4-ene-3, 17-dione (**325**), 6-methyleneandrosta-4-ene-3, 17-dione (**326**), 6*β*,17*β*-dihydroxyandrost-4-en-3-one (**327**), and 17*β*-hydroxy-6α-spiroxirandrost-1, 4-diene-3-one (**328**), were obtained from the *Cunninghamella blakesleeana*-catalyzed transformation of drug **318**[79] [Figure 59]. Two derivatives, 17*β*-hydroxy-6*α*-hydroxymethylandrosta-1, 4-dien-3-one (**329**) and 6*α*-hydroxymethylandrosta-1, 4-diene-3,17-dione (**330**), were synthesized *via* the fermentation of 318 with *Curvularia lunata*[80] [Figure 60]. 17*β*-Hydroxy-6-methyleneandrosta-1, 4-diene-3, 16-dione (**331**) was obtained by the biotransformation of **318** with *Aspergillus niger*[80] [Figure 60]. Fermentation with *Gibberella*

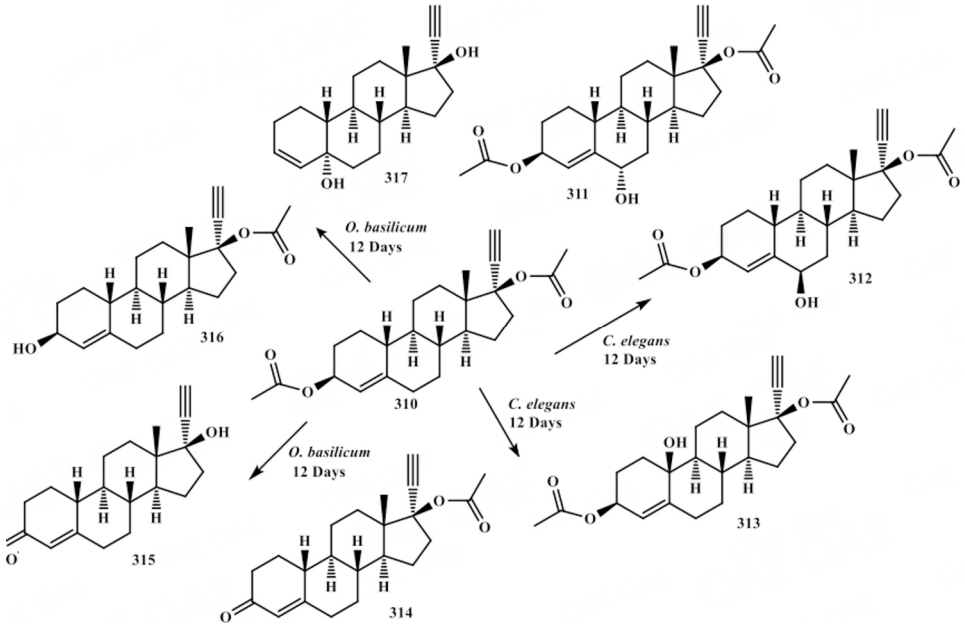

Figure 57. Biotransformation of ethynodiol diacetate (**310**) with *Cunninghamella elegans*, and *Ocimum basilicum*.

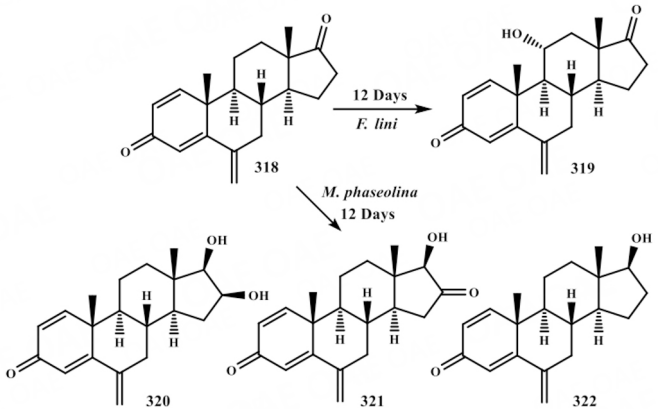

Figure 58. Microbial transformation of exemestane (**318**) with *Macrophomina phaseolina*, and *Fusarium lini*.

fujikuroi afforded two derivatives, 6α-hydroxy-4-androstene-3, 17-dione (**332**) and 6α-hydroxymethylandrost-4-ene-3, 17-dione (**333**). Metabolites **324** and **330** were found to be new compounds[80] [Figure 60]. The derivative **325** showed moderate cytotoxicity against MCF-7 breast cancer cell line with an IC_{50} of 33.43 ± 4.01 µM, in comparison to the standard anti-cancer drug, doxorubicin (IC_{50} = 0.92 ± 0.1 µM)[80].

Biotransformation of drostanolone enanthate (334)

Five new transformed products, 2α-methyl-3α,14α,17β-trihydroxy-5α-androstane (**335**) (0.8%), 2-methylandrosta-11α-hydroxy-1, 4-diene-3,17-dione (**336**) (0.9%), 2-methylandrosta-14α-hydroxy-1, 4-diene-3, 17-dione (**337**) (1.2%), 2α-methyl-7α-hydroxy-5α-androstan-3, 17-dione (**340**) (1.1%), and 2-methyl-5α-androsta-7α-hydroxy-1-ene-3, 17-dione (**341**) (0.75%), along with three known metabolites, 2α-methyl-3α, 17β-dihydroxy-5α-androstane (**338**) (3.5%), 2-methylandrosta-1, 4-diene-3, 17-dione (**339**)

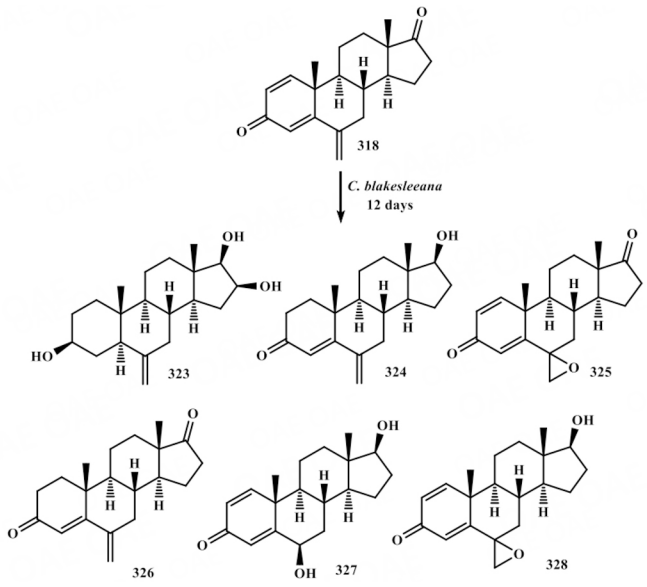

Figure 59. Microbial transformation of exemestane (**318**) with *Cunninghamella blakesleeana.*

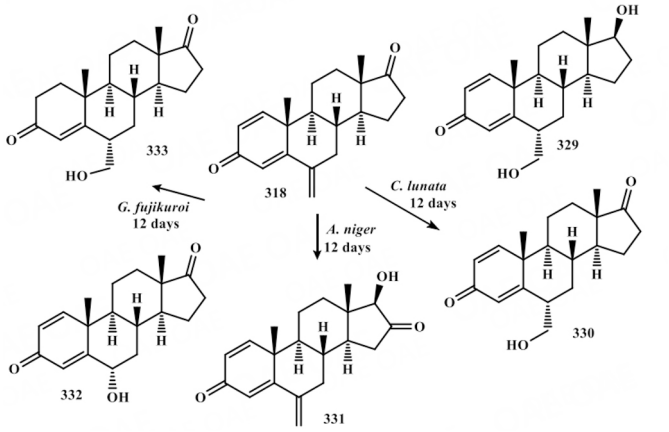

Figure 60. Microbial transformation of exemestane (**318**) with *Curvularia lunata*, *Gibberella fujikuroi*, and *Aspergillus niger*.

(4.2%), and 2α-methyl-5α-androsta-17*β*-hydroxy-3-one (**342**) (0.5%) were produced by the biotransformation of the anti-cancer drug, drostanolone enanthate (**334**), with *Cephalosporium aphidicola* and *Fusarium lini*[81] [Figure 61].

Metabolite **341** (IC_{50} = 19.6 ± 1.4 μM) exhibited potent activity against HeLa (cervical) cancer cells, in contrast to the parent drug **334** (IC_{50} = 54.7 ± 1.6 μM), and the standard drug, cisplatin (IC_{50} = 40.1 ± 2.0 μM). Compounds **335** (IC_{50} = 64.3 ± 3.0 μM), **336** (IC_{50} = 40.7 ± 0.9 μM), **337** (IC_{50} = 40.7 ± 0.9 μM), **338** (IC_{50} = 49.5 ± 2.2 μM), **339** (IC_{50} = 39.8 ± 1.5 μM), and **342** (IC_{50} = 30.1 ± 1.0 μM) also displayed remarkable activity against HeLa cell line. Metabolites **335** (IC_{50} = 58.4 ± 1.6 μM), **336** (IC_{50} = 59.1 ± 2.6 μM), **337** (IC_{50} = 60.4 ± 0.9 μM), **338** (IC_{50} = 51.8 ± 3.4 μM), **339** (IC_{50} = 68.1 ± 1.2 μM), and **340** (IC_{50} = 39.1 ± 2.0 μM) showed a significant anti-cancer activity against PC-3 (prostate) cells, compared to compounds **342** (IC_{50} = 96.2 ± 3.0 μM), **335** (IC_{50} = 84.6 ± 6.4 μM), **339** (IC_{50} = 84.0 ± 3.1 μM), and standard cisplatin (IC_{50} = 76.5 ± 1.2 μM). Compounds **334** (IC_{50} = 5.0 ± 1.2 μM), **338** (IC_{50} = 12.4 ± 2.3 μM), **340** (IC_{50} = 16.7 ± 2.6 μM), and

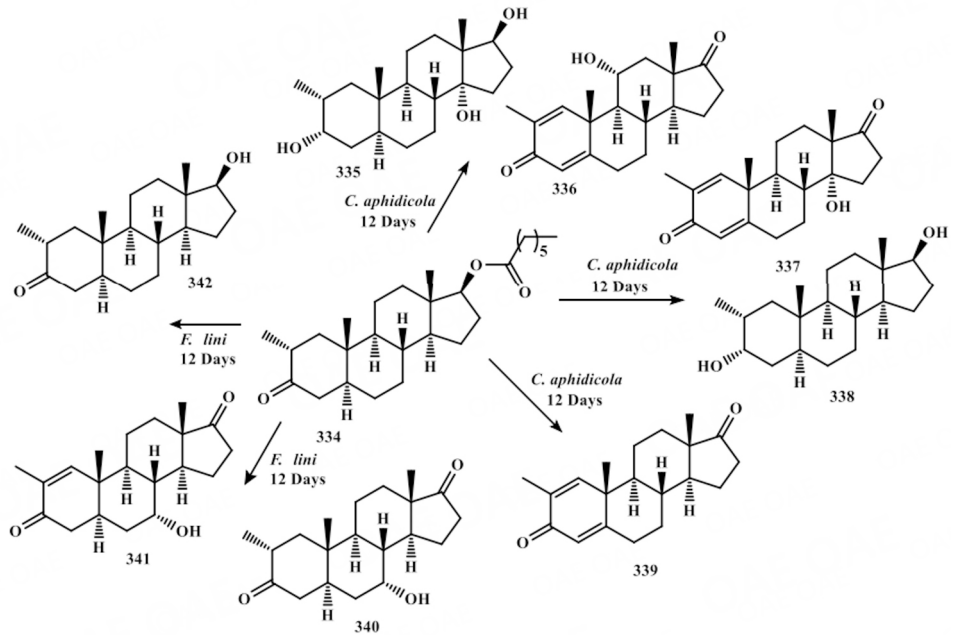

Figure 61. Microbial transformation of drostanolone enanthate (**334**) with *Cephalosporium aphidicola*, and *Fusarium lini.*

90 (IC_{50} = 14.7 ± 2.6 μM) showed potent activity against H460 (lung) cells, as compared to the cisplatin (IC_{50} = 22.2 ± 2.1 μM). Compounds **335** (IC_{50} = 44.4 ± 2.0 μM), **336** (IC_{50} = 33.2 ± 1.0 μM), **337** (IC_{50} = 38.5 ± 2.8 μM), **339** (IC_{50} = 31.9 ± 1.8 μM), and **340** (IC_{50} = 26.4 ± 0.9 μM) also presented good anti-cancer activity against H460 cells. Compound **334** (IC_{50} = 3.1 ± 3.2 μM) showed potent anti-cancer activity against HCT116 (colon) cells, in contrast to the standard cisplatin (IC_{50} = 11.2 ± 3.0 μM). While compounds **335** (IC_{50} = 39.4 ± 2.0 μM), **336** (IC_{50} = 45.9 ± 4.2 μM), **337** (IC_{50} = 46.6 ± 3.0 μM), **339** (IC_{50} = 30.4 ± 1.6 μM), **340** (IC_{50} = 55.0 ± 1.9 μM), **341** (IC_{50} = 42.8 ± 1.2 μM), and **342** (IC_{50} = 25.4 ± 1.6 μM) showed a weak anti-cancer activity against H460 cells. Interestingly, compounds **334**-**335**, **340**, and **342** were identified as non-cytotoxic against mouse fibroblast (3T3 normal) cell line, while compounds **341** (IC_{50} = 74.6 ± 3.7 μM) and **342** (IC_{50} = 47.6 ± 3.7 μM) were found to be cytotoxic.

In addition, nine more derivatives, 2α-methyl-7α, 11*β*, 17*β*-trihydroxy-5*α*-androstan-3-one (**343**), 2α-methyl-7*β*, 15*α*, 17*β*-trihydroxy-5α-androstan-3-one (**344**), 2*α*-hydroxymethyl-11*β*, 17*β*-dihydroxy-5*α*-androstan-3-one (**345**), 2α-methyl-7*α*, 11*α*, 17*β*-trihydroxy-5α-androstan-3-one (**346**), 2*α*-methyl-11*α*, 15*β*, 17*β*-trihydroxy-5*α*-androstan-3-one (**347**), 2*α*-methyl-3*β*, 5*α*, 17*β*-trihydroxy-5*α*-androstane (**348**), 2*α*-methyl-3*β*, 14*α*, 17*β*-trihydroxy-5*α*-androstane (**349**), 2*α*-methyl-17*β*-hydroxy-5*α*-androstan-3-one (**350**), and 2*α*-methyl-3*β*, 17*β*-dihydroxy-5*α*-androstane (**351**) were also synthesized through the biotransformation of anti-cancer drug **334** with *Beauveria bassiana,* and *Macrophomina phaseolina*[82] [Figure 62].

BIOTRANSFORMATION OF GLUCOCORTICOIDS

Biotransformation of melengestrol acetate (352)

Biotransformation of the progestin medication, melengestrol acetate (**352**) (Brand names, Heifermax and MGA) with *Glomerella fusarioides* and *Rhizopus stolonifer* afforded four new oxidative products, 17*α*-acetoxy-11*α*-hydroxy-6-methyl-16-methylenepregna-4, 6-diene-3, 20-dione (**353**), 17*α*-acetoxy-11*α*-hydroxy-6-methyl-16-methylenepregna-1, 4, 6-triene-3,20-dione (**354**), 17*α*-acetoxy-6, 7*α*-epoxy-6*β*-methyl-16-methylenepregna-4, 6-diene-3, 20-dione (**355**), and 17*α*-acetoxy-11*β*, 15*β*-dihydroxy-6-methyl-16-

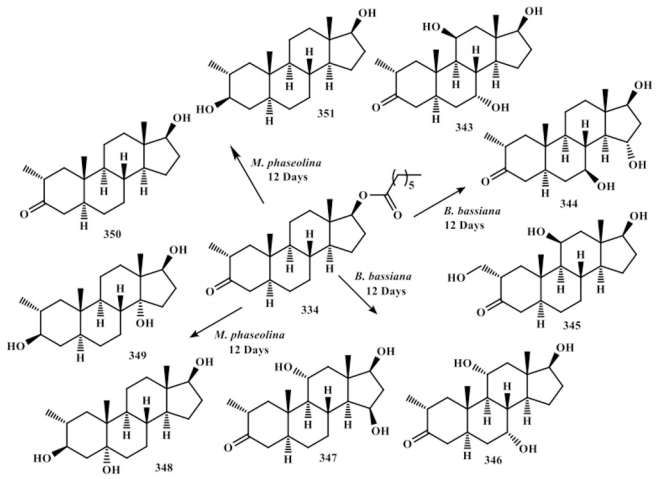

Figure 62. Microbial transformation of drostanolone enanthate (**334**) with *Macrophomina phaseolina*, and *Beauveria bassiana.*

methylenepregna-4, 6-diene-3,20-dione (**356**)[83] [Figure 63]. Drug **352** (IC_{50} = 2.77 ± 0.08 μM), and its derivatives **353** (IC_{50} = 2.78 ± 0.07 μM), **355** (IC_{50} = 2.74± 0.1 μM), and **356** (IC_{50} ≤ 2 μM) showed potent T-cell proliferation inhibitory activities *in vitro*. While derivative **253** (IC_{50} = 29.9 ± 0.09 μM) showed moderate activity, as compared to the standard anti-inflammatory drug, prednisolone (IC_{50} = 9.73 ± 0.08 μM)[83].

Biotransformation of medrysone (357)

Medrysone is a synthetic glucocorticoid, which is marketed under the brand names HMS and Medrocort for the treatment of inflammatory diseases. Seven new metabolites, 14α-hydroxy-6α-methylpregn-4-ene-3, 11, 20-trione (**358**) (1.88%), 6β-hydroxy-6α-methylpregn-4-ene-3, 11, 20-trione (**359**) (1.55%), 15β-hydroxy-6α-methylpregn-4-ene-3, 11, 20-trione (**360**) (1.11%), 6β, 17α-dihydroxy-6α-methylpregn-4-ene-3, 11, 20-trione (**361**) (1.66%), 6β, 20(S)-dihydroxy-6α-methylpregn-4-ene-3, 11-dione (**362**) (0.64%), 11β, 16β-dihydroxy-6α-methylpregn-4-ene-3-one (**363**) (0.66%), and 15β, 20(R)-dihydroxy-6α-methylpregn-4-ene-3, 11-dione (**364**) (1.77%) of medrysone (**357**) were synthesized through the microbial transformation of drug **357** with the fungal cultures of *Cunninghamella blakesleeana*, *Neurospora crassa*, and *Rhizopus stolonifer*[84] [Figure 64]. Compounds **357** (IC_{50} = 2.0 ± 0.04 µg/mL), 358 (IC_{50} = 20.0 ± 0.9 µg/mL), **359** (IC_{50} = 14.6 ± 2.1 µg/mL), **360** (IC_{50} = 9.2 ± 0.7 µg/mL), **361** (IC_{50} = 1.2 ± 0.02 µg/mL), **362** (IC_{50} = 15.2 ± 2.4 µg/mL), **363** (IC_{50} ≤ 0.2 µg/mL), and **364** (IC_{50} = 10.4 ± 0.42 µg/mL) showed potent anti-inflammatory activities through T-cell proliferation inhibition, as compared to the standard drug, prednisolone (IC_{50} ≤ 3.1 µg/mL)[84].

BIOTRANSFORMATION OF PHYTOSTEROIDS

Biotransformation of (E)-guggulsterone (365)

Guggulsterone (**365**) is a naturally occurring bioactive plant sterol isolated from the gum resin of guggul (*Commiphora wightii*). This compound dramatically reverses multi-drug resistance in a number of human cancer cell lines, extending the efficacy of existing chemotherapy. The new metabolites, 12α-hydroxypregn-4-ene-3,16-dione (**366**) (1.5%), (17*Z*)-11α-hydroxypregna-4, 17-diene-3, 16-dione (**367**) (2.5%), (17*E*)-11α-hydroxypregna-4, 17-diene-3, 16-dione (**368**) (2.66%), (17*Z*)-6β, 11α-dihydroxypregna-4, 17-diene-3, 16-dione (**369**) (0.34%)[85] [Figure 65], (17*E*)-6β, 11α-dihydroxypregna-4, 17-diene-3, 16-dione (**370**) (1.52%), 6 α, 11 α -dihydroxypregn-4-ene-3, 16-dione (**371**) (1.82%), and 11α, 16β-dihydroxypregn-4-en-3-one (**372**) (1.92%) were synthesized through the microbial transformation of **365** with *Rhizopus stolonifer and Gibberella fujikuroi*[85,86] [Figure 66].

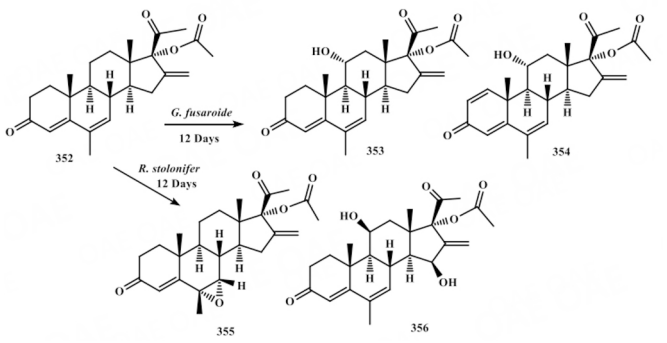

Figure 63. Microbial transformation of melengestrol acetate (**352**) with *Glomerella fusarioides*,and *Rhizopus stolonifer*.

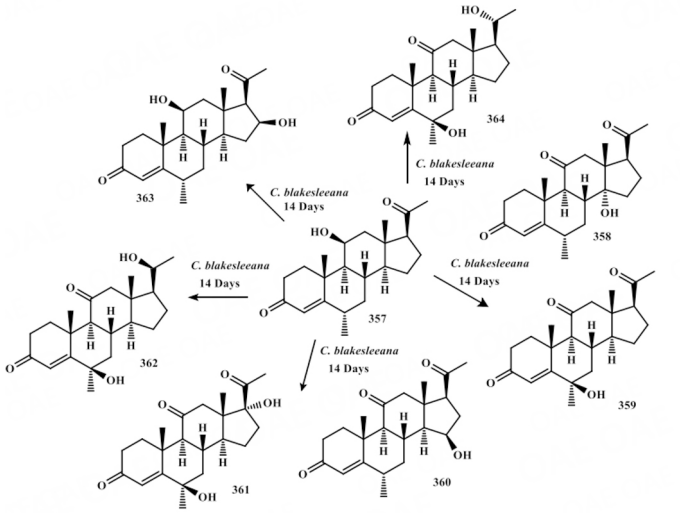

Figure 64. Microbial transformation of medrysone (**357**) with *Cunninghamella blakesleeana.*

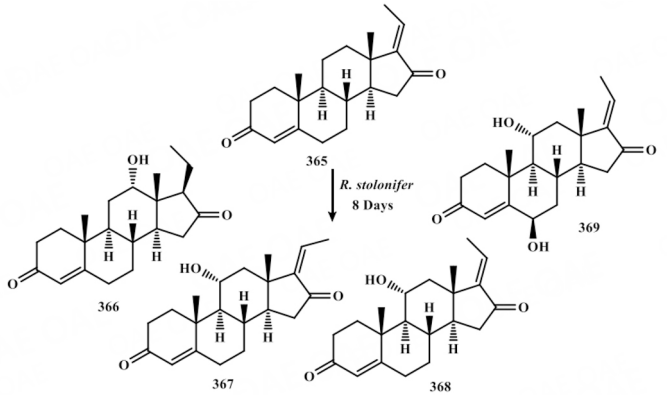

Figure 65. Biotransformation of (*E*)-guggulsterone (**365**) with *Rhizopus stolonifer.*

Biotransformation of physalin H (373)

Rhizopus stolonifer-catalyzed the transformation of physalin H (**373**) and yielded the new derivative, 6, 7-dehydrophysalin H (**374**) (2.10%)[87] [Figure 67], while structural transformation with *Cunninghamella elegans* afforded a new compound, 6-deoxyphysalin H (**375**) (4.10%), along with the known metabolite,

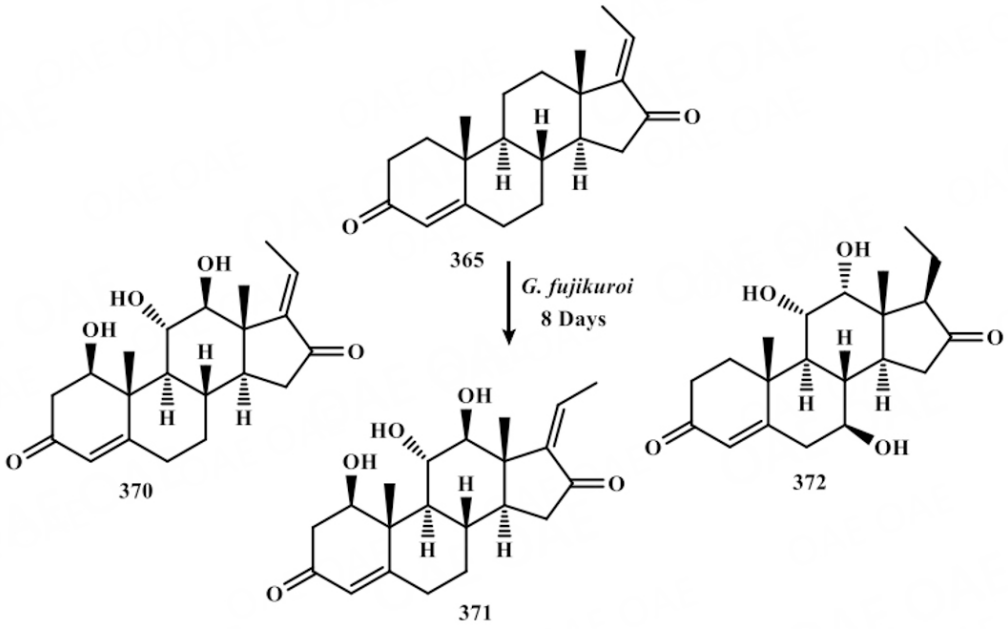

Figure 66. Biotransformation of (*E*)-guggulsterone (**365**) with *Gibberella fujikuroi.*

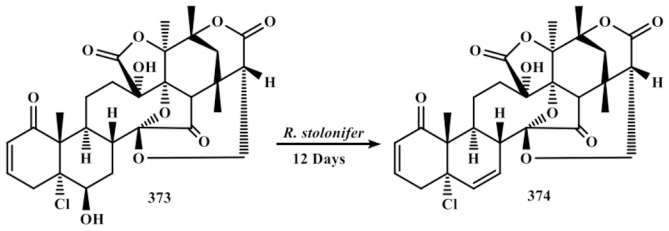

Figure 67. Biotransformation of physalin H (**373**) with *Rhizopus stolonifer.*

isophysalin B (**376**) (5.25%)[87] [Figure 68]. Compounds **373** (IC_{50} = 6.03 ± 0.005 μM), **374** (IC_{50} = 7.74 ± 0.015 μM), **375** (IC_{50} = 6.34 ± 0.03 μM), and 376 (IC_{50} = 13.8 ± 0.05 μM) showed potent anti-leishmanial activity *in vitro*, compared with the standard drug, amphotericin B (IC_{50} = 0.129 ± 0.105 μM) against promastigotes of *Leishmania major* (DESTO).

BIOTRANSFORMATION OF NEUROSTEROIDS

Biotransformation of pregnenolone (377)

Pregnenolone (**377**) is a naturally produced hormone by the adrenal glands in the human body. It is the starting material in the synthesis of steroidal hormones, including cortisol, testosterone, progesterone, estrogen, etc. Biotransformation of pregnenolone (**377**) with the fungus *Cunninghamella elegans* yielded two new metabolites, 3*β*, 6*α*, 11*α*, 12*β*-tetrahydroxypreg-5-en-20-one (**378**) (4.05%), and 3*β*, 6*β*, 11*α*-trihydroxypreg-5-en-20-one (**379**) (2.29%), along with the known metabolite, 3*β*, 7*β*, 11*α*-trihydroxypreg-5-en-20-one (**380**) (28.1%)[88] [Figure 69]. Two more derivatives, 3*β*, 7*β*-dihydroxypreg-5-en-20-one (**381**) (3.03%), and 3*β*, 6*β*, 7*β*-trihydroxypreg-5-en-20-one (**382**) (2.41%) were also synthesized through the biotransformation of drug **377** with *Gibberella fujikuroi*[88] [Figure 70]. Similarly, microbial transformation of pregnenolone acetate (**383**) with the fungus *Cunninghamella elegans* afforded four known metabolites,

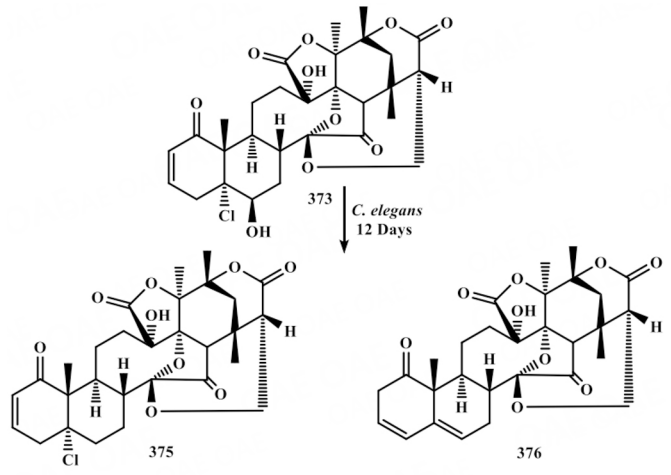

Figure 68. Biotransformation of physalin H (**373**) with *Cunninghamella elegans.*

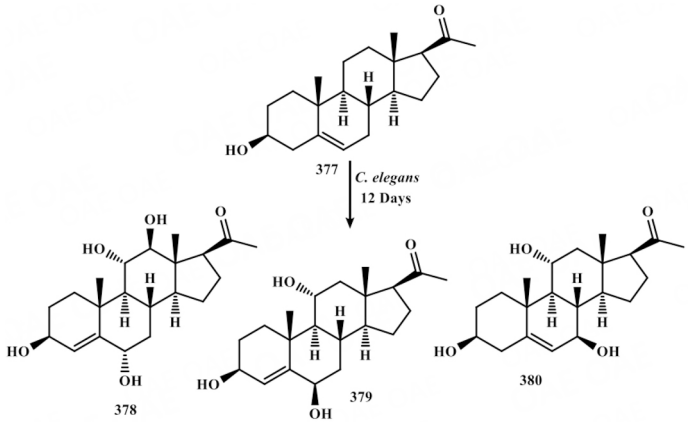

Figure 69. Biotransformation of pregnenolone (**377**) with *Cunninghamella elegans.*

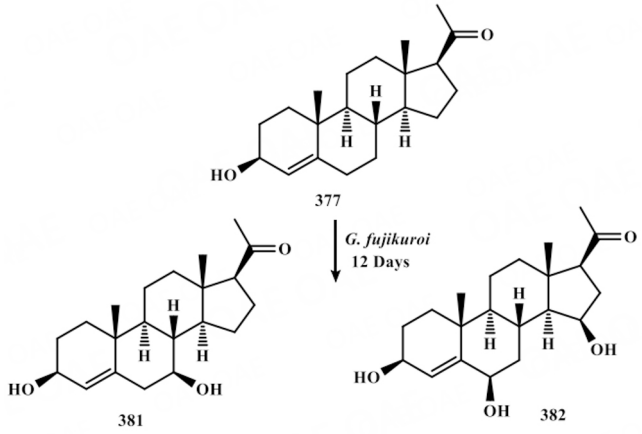

Figure 70. Biotransformation of pregnenolone (**377**) with *Gibberella fujikuroi.*

pregnenolone (**384**) (4.0%), androsta-1, 4-diene-3, 17-dione (**385**) (2.04%), 6β,15β-dihydroxyandrost-4-ene-

3,17-dione (**386**) (2.04%), and 11α, 15β-dihydroxypreg-4-ene-3, 20-dione (**387**) (2.30%)[88] [Figure 71].

Biotransformation 6-dehydroprogesterone (388)

6-Dehydroprogesterone (**388**) is a synthetic derivative of progesterone hormone. Biotransformation of 6-dehydroprogesterone (**388**) with the fungal cell culture of *Aspergillus niger* afforded three new metabolites, 6β-chloro-7α, 11α-dihydroxypregna-4-ene-3, 20-dione (**389**) (1.0%), 7α-chloro-6β, 11α-dihydroxypregna-4-ene-3,20-dione (**390**) (1.33%), and 6α, 7α-epoxy-11α-hydroxypregna-4-ene-3,20-dione (**391**) (1.33%), along with the two known metabolites, 6α, 7α-epoxypregna-4-ene-3,20-dione (**392**) (2.0%), and 11α-hydroxypregna-4, 6-diene-3,20-dione (**393**) (2.33%)[89] [Figure 72]. Whereas, *Gibberella fujikuroi*-catalyzed transformation of substrate 388 yielded the known compound, 11α, 17β-dihydroxyandrosta-4, 6-dien-3-one (**394**) (15.4%)[89] [Figure 73].

Biotransformation 20-hydroxymethylpregna-1,4-dien-3-one (395)

(20S)-20-Hydroxymethylpregna-1, 4-dien-3-one (**395**) is another neurochemical, which is obtained by microbial-catalyzed degradation of sterols. Compound **395** is also used as an intermediate in the synthesis of many steroid-based drugs. Six new metabolites, 11α-hydroxy-20- acetoxymethylpregna-1,4-dien-3-one (**396**) (0.40%), 17α-hydroxy-20-hydroxymethylpregna-1, 4-dien-3-one (**397**) (0.46%), 6β, 11α-dihydroxy-20-hydroxymethyl pregna-1, 4-dien-3-one (**398**) (0.60%), 11α, 15β-dihydroxy-20-hydroxymethylpregna-1, 4-dien-3-one (**399**) (2.0%), 11α, 17α-dihydroxy-20-hydroxymethylpregna-1, 4-dien-3-one (**400**) (0.40%), 14α, 15β, 17α-trihydroxy-20-hydroxymethylpregna-1, 4-dien-3-one (**401**) (0.40%), along with the known metabolite, 11α-hydroxy-20-hydroxymethylpregna-1, 4-dien-3-one (**402**) (8.66%), were synthesized through the biotransformation of 20-hydroxymethylpregna-1, 4-dien-3-one (**395**) with *Cunninghamella elegans*[90] [Figure 74]. Three new derivatives, 15β-hydroxy-20-hydroxymethylpregna-1, 4-dien-3-one (**403**) (1.12%), 7β-hydroxy-20-hydroxymethylpregna-1, 4-dien-3-one (**404**) (0.62%), and 7β, 15β-dihydroxy-20-hydroxymethylpregna-1, 4-dien-3-one (**405**) (8.70%), were also synthesized by *Macrophomina phaseolina*-catalyzed transformation of compound **395**[90] [Figure 75].

Biotransformation ganaxolone (407)

Ganaxolone (**407**) is a synthetic steroidal-based anti-epileptic drug (CCD-1042) under development by Marinus Pharmaceuticals. Compound 407 is a 3β-methylated derivative of allopregnanolone (neurosteroid). The new metabolite, 14α-hydroxy-5α-pregnan-1-ene-3, 20-dione (**408**) (1.30%), along with the four known metabolites, 11α-hydroxy-pregn-1, 4-diene-3,20-dione (**409**) (2.87%), 6β, 11α-dihydroxy-pregn-4-ene-3, 20-dione (**410**) (4.66%), 5α-pregnan-3, 20-dione (**411**) (4.56%), and 5α-pregnan-4-ene-3, 20-dione (**412**) (5.46%) [60] [Figure 76]. Ganaxolone (**407**) showed good aromatase inhibitory activity (IC_{50} = 13.76 ± 2.00 μM), while its derivatives were found to be inactive.

BIOTRANSFORMATION OF STEROIDAL ALKALOID

Biotransformation of dictyophlebine (413)

The new compound, 3β-(propyloxycarbonylamino)-dictyophlebin-16-ene (**414**) (0.55%), along with the two known derivatives, 5, 6-dihydrosarconidine (**415**) (0.52%) and iso-*N*-formylchonemorphine (**416**) (0.54%), were synthesized through the biotransformation of the steroidal alkaloid, dictyophlebine (413)[91] [Figure 77]. The new derivative **414** was found to be the potent inhibitor of acetyl-, and butyrylcholinesterase enzymes with the IC_{50} values of 2.2 and 1.2 μM, respectively, as compared to the standard drug, galanthamine (IC_{50} 0.5 and 8.2 μM for AChE and BChE, respectively).

BIOLOGICAL ACTIVITY EVALUATION OF TRANSFORMED PRODUCTS

Fully characterized derivatives were evaluated through various cell-based and biochemical assays. Bio-catalytic structural changes in natural/synthetic compounds of various classes have affected their biological

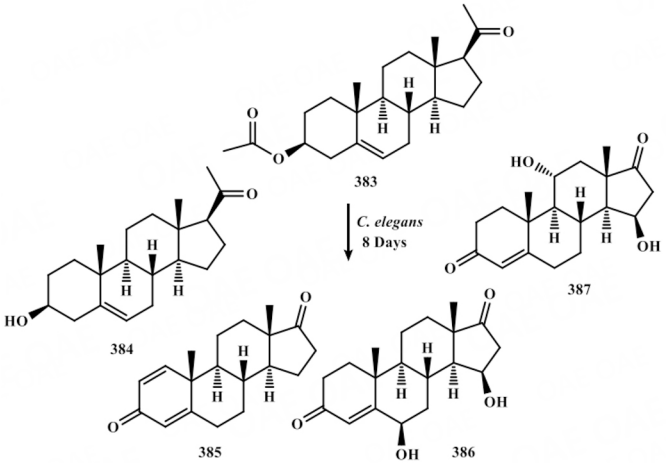

Figure 71. Biotransformation of pregnenolone acetate (**383**) with *Cunninghamella elegans.*

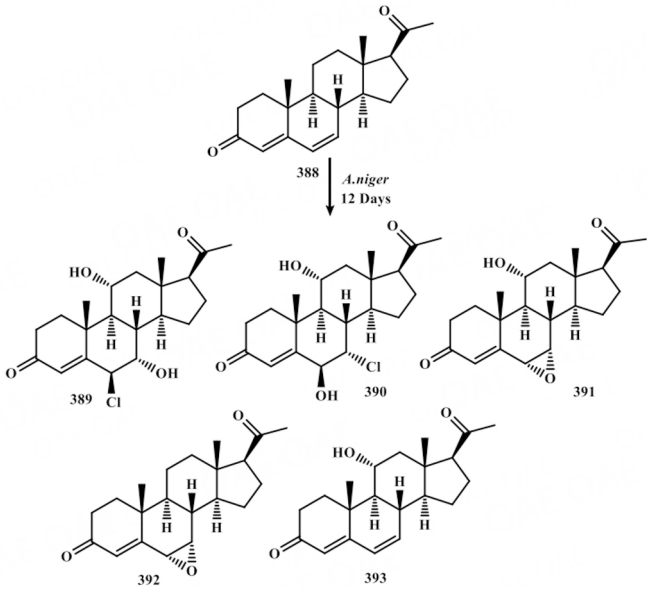

Figure 72. Biotransformation of 6-dehydroprogesterone (**388**) with *Aspergillus niger.*

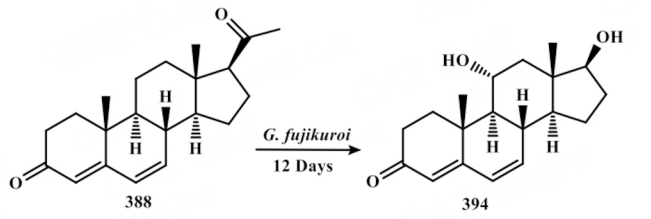

Figure 73. Biotransformation of 6-dehydroprogesterone (**388**) with *Gibberella fujikuroi.*

activities.

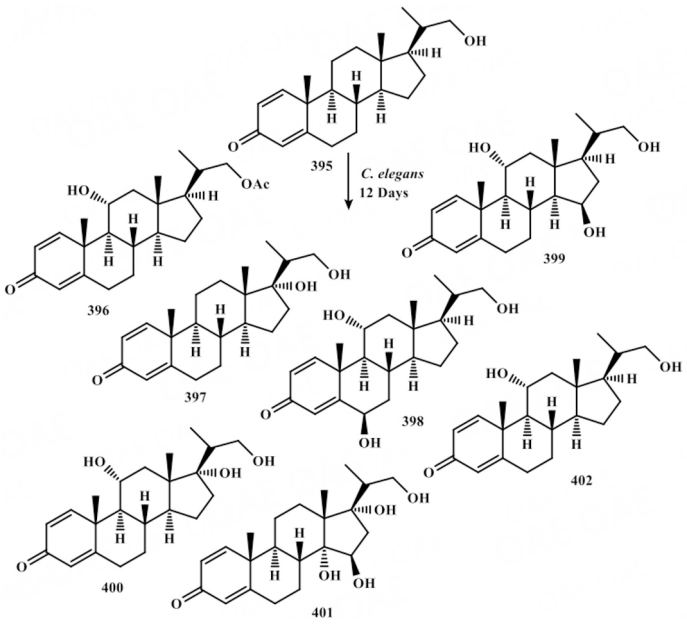

Figure 74. Biotransformation of 20-hydroxymethylpregna-1,4-dien-3-one (**395**) with *Cunninghamella elegans*.

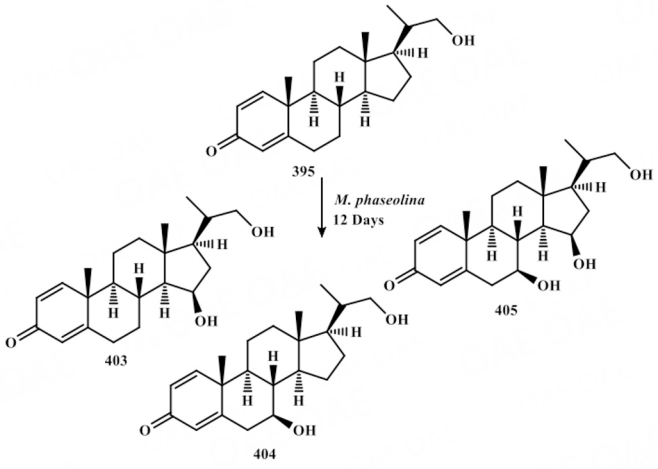

Figure 75. Biotransformation of 6-dehydroprogesterone (**395**) with *Macrophomina phaseolina*.

Hydroxylation in derivatives, 13-hydroxyisolongi-folen-4-one (**37**) (IC_{50} = 9.64 ± 0.0008 μM) and 9-hydroxyisolongifolen-4-one (**40**) (IC_{50} = 6.68 ± 0.0096 μM), has increased their activities against tyrosinase, as compared to substrate, (+)-isolongifolen-4-one (**35**) (IC_{50} = 51.91 ± 0.0245 μM). Similarly, hydroxylation in derivatives, 17*α*-ethynyl-11*α*, 17*β*-dihydroxyandrost-4-en-3-one (**278**) (IC_{50} = 5.95 ± 0.00078 μM), 17*α*-ethyl-11*α*, 17*β*-dihydroxyandrost-4-en-3-one (**284**) (IC_{50} = 3.46 ± 0.01046 μM), and 17*α*-ethyl-6*α*, 17*β*-dihydroxy-5*α*-androstan-3-one (**285**) (IC_{50} = 1.72 ± 0.00089 μM) also increased their tyrosinase inhibitory activities, as compared to substrate, ethisterone (**277**) (IC_{50} = 2.61 ± 0.037328 μM).

Structural changes in metabolites, 4*β*-methoxycaryophyllene-5α, 14-diol (**46**) (IC_{50} = 3.09 ± 2.61 μg/mL), 4*β*-methoxycaryophyllene-5α, 15-diol (**47**) (IC_{50} = 0.72 ± 0.17 μg/mL), and caryophyllene-5α,15-diol (**48**) (IC_{50} = 1.35 ± 0.43 μg/mL), have significantly increased their anti-malarial activity *in vitro*, in comparison to the

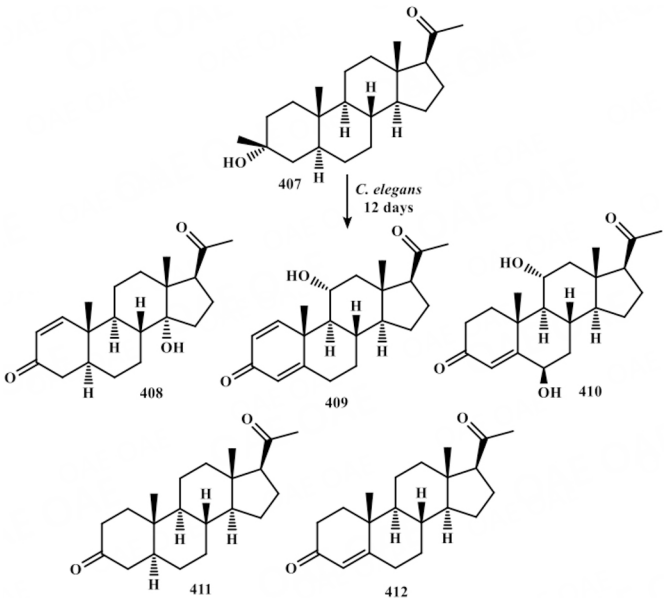

Figure 76. Biotransformation of ganaxolone (**407**) with *Cunninghamella elegans*.

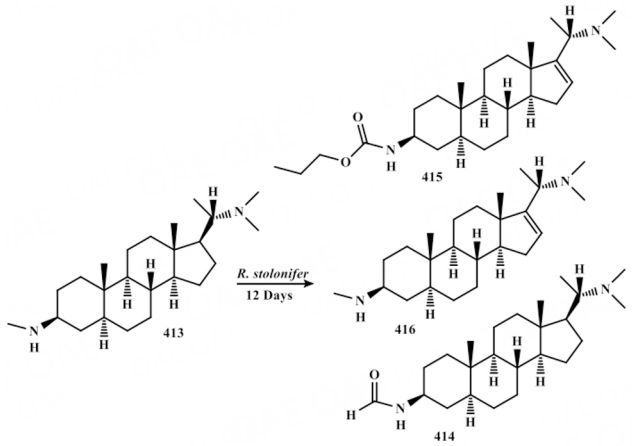

Figure 77. Biotransformation of 6- dictyophlebine (**413**) with *Rhizopus stolonifer*.

standard drug, chloroquine diphosphate (IC_{50} = 0.025 ± 0.01 µg/mL). Derivatives, 15-hydroxycaryophyllene oxide (**52**) (IC_{50} = 44.0 ± 0.2 µM), 4*β*, 5α-dihydroxycaryophyll-8(13)-ene (**53**)(IC_{50} = 455.8 ± 0.1 µM), clovane-5, 9-diol (**54**) (IC_{50} = 189.5 ± 0.2 µM), 4, 5-epoxycaryophyllan-8(13)-en-14-ol (**55**) (IC_{50} = 10.9 ± 0.2 µM), 4, 5-epoxy-13-norcaryophyllan-8-one (**56**) (IC_{50} = 458.7 ± 0.5 µM), caryolane-5, 8, 13-triol (**57**) (IC_{50} = 23.6 ± 0.1 µM), clovane-5, 9, 12-triol (**58**) (IC_{50} = 43.6 ± 0.3 µM), and 4, 5-epoxycaryophyllan-3, 13-diol (**59**) (IC_{50} = 154.6 ± 0.3 µM), showed a moderate to significant inhibitory potential against butyrylcholinesterase enzyme, as compared to their substrate, (–)-caryophyllene oxide (**49**) (IC_{50} = 208.4 ± 0.8 µM).

Structural transformations in derivatives, 3-ketosclareolide (**86**) (100%), 3*β*-hydroxysclareolide (**88**) (87.5%), 1*β*, 3*β*-dihydroxysclareolide (**89**) (72.3%), 2*α*-hydroxysclareolide (**90**) (82.7%), and 1*α*, 3*β*-dihydroxysclareolide (**92**) (75%), have increased their phytotoxicity against *Lemna minor* L., in comparison to sclareolide (**84**) at 100 µg/mL. Compounds, dehydroabietic acid (**110**) (IC_{50} = 11 ± 01 µM), 1*β*-hydroxydehydroabietic acid (**111**), (IC_{50} = 130 ± 15 µM), 15-hydroxy dehydroabietic acid (**112**) (IC_{50} = 99 ±

43 μM), and 16-hydroxy dehydroabietic acid (**113**) (IC_{50} = 81 ± 90 μM), showed potent α-glucosidase inhibitory activity. In addition, Compounds, oleanolic acid (**117**) (IC50 = 12.8 ± 0.00 μM), 2*α*, 3*β*, 11*β*-trihydroxyolean-12-en-28-oic acid (**118**) (IC50 = 444.0 ± 8.0 μM), and 2*α*, 3*β*-dihydroxyolean-12-en-28-oic acid (**119**) (IC50 = 666.0 ± 20.0 μM), showed significant *α*-glucosidase inhibitory activity, as compared to the standard drug, acarbose (IC50 = 780.0 ± 0.28 μM). Compounds, 18-glycyrrhetinic acid (**120**) (IC_{50} = 225.1 ± 0.5 μM), 3,7-dihydroxy-11-oxo-olean-12-en-30-oic acid (**121**) (IC_{50} ≥ 300 μM), and 3, 11-dioxo-olean-12-en-30-oic acid (**122**) (IC_{50} = 144.2 ± 0.2 μM), exhibited a good lipoxygenase (LOX) inhibitory activity, as compared to the standard drug, baicalein (IC_{50} = 22.4 ± 0.5 μM).

Compounds, DHEA (**143**) (IC_{50} = 77.9 ± 1.95 μM), 3*β*,7*α*-dihydroandrost-5-ene-17-one (**150**) (IC_{50} = 373.5 ± 9.57 μM), 11*β*-hydroxyandrost-4, 6-diene-3, 17-dione (**152**) (IC_{50} = 430 ± 7.13 μM), 5*α*-androstane-3, 17-dione (**154**)(IC_{50} = 221.6 ± 12.5 μM), and androst-4-ene-3, 17-dione (**155**) (IC_{50} = 191.4 ± 1.17 μM), showed significant activity against *β*-glucuronidase enzymes. Compounds **185** (IC_{50} = 190.3 ± 1.18 μM) and 187 (IC_{50} = 482.66 ± 6.86 μM) showed weak inhibition of *β*-glucuronidase. Derivative, 15*α*, 17*β*-dihydroxy-17*α*-methylandrost-1, 4-dien-3-one (**247**) showed a remarkable *β*-glucuronidase inhibitory activity (IC_{50} = 60.7 μM). Interestingly, derivative, 6*β*-hydroxy-etonogestrel (**308**), was found to be significantly active against *β*-glucuronidase enzyme with the IC_{50} value of 13.97 ± 0.12 μM, compared to the standard inhibitor, D-saccharic acid 1, 4 lactone (IC_{50} = 48.4 ± 1.25 μM).

Nandrolone (**175**) (IC_{50} = 32.0 ± 0.5 μM), and its derivatives, 10*β*, 12*β*, 17*β*-trihydroxy-19-nor-4-androsten-3-one (**178**) (IC_{50} ≥ 100 μM), 10*β*, 16*α*, 17*β*-trihydroxy-19-nor-4-androsten-3-one (**179**) (IC_{50} = 77.39 ± 5.52 μM), 6*β*, 10*β*, 17*β*-trihydroxy-19-nor-4-androsten-3-one (**180**) (IC_{50} = 70.90 ± 1.16 μM), 10*β*, 17*β*-dihydroxy-19-nor-4-androsten-3-one (**181**) (IC_{50} = 54.94 ± 1.01 μM), 6*β*, 17*β*-dihydroxy-19-nor-4-androsten-3-one (**182**) (IC_{50} = 80.23 ± 3.39 μM), 10*β*-hydroxy-19-nor-4-androsten-3, 17-dione (**183**) (IC_{50} = 61.12 ± 1.39 μM), and 16*β*, 17*β*-dihydroxy-19-nor-4-androsten-3-one (**184**) (IC_{50} = 29.55 ± 1.14 μM) exhibited significant anti-leishmanial activity against *Leishmania major in vitro*.

Compounds, metenolone acetate (**220**) (62.5% ± 4.4 %), 6*α*-hydroxy-1-methyl-3-oxo-5*α*-androst-1-en-17-yl acetate (**221**) (73.4% ± 0.6 %), 15*β*, 20-dihydroxy-1-methyl-3-oxo-5*α*-androst-1-en-17-yl acetate (**224**) (81.0% ± 2.5 %), 17*β*-hydroxy-1-methyl-3-oxo-5*α*-androst-1-en (**227**) (69.7% ± 1.4 %), 1-methyl-5*β*-androst-1-en-3, 17-dione (**229**) (73.2% ± 0.3%), 17*β*-hydroxy-1*α*-methyl-5*α*-androstan-3-one (**232**) (60.1% ± 3.3 %), and 17*β*, 15*α*-dihydroxy-1*α*-methyl-5*α*-androstan-3-one (**233**) (71.0% ± 7.2 %), showed good inhibition of cytokine (TNF-*α*) production. Melengestrol acetate (**352**) (IC_{50} = 2.77 ± 0.08 μM), and its derivatives, 17*α*-acetoxy-11*α*-hydroxy-6-methyl-16-methylenepregna-4,6-diene-3,20-dione (**353**) (IC_{50} = 2.78 ± 0.07 μM), 17*α*-acetoxy-6, 7*α*-epoxy-6*β*-methyl-16-methylenepregna-4, 6-diene-3,20-dione (**355**) (IC_{50} = 2.74± 0.1 μM), and 17*α*-acetoxy-11*β*, 15*β*-dihydroxy-6-methyl-16-methylenepregna-4, 6-diene-3, 20-dione (**356**) (IC_{50} ≤ 2 μM), showed potent T- cell proliferation inhibitory activities. Similarly, medrysone (**357**) (IC_{50} = 2.0 ± 0.04 μg/mL), and its derivatives, 14*α*-hydroxy-6*α*-methylpregn-4-ene-3, 11, 20-trione (**358**) (IC_{50} = 20.0 ± 0.9 μg/mL), 6*β*-hydroxy-6*α*-methylpregn-4-ene-3, 11, 20-trione (**359**) (IC_{50} = 14.6 ± 2.1 μg/mL), 15*β*-hydroxy-6*α*-methylpregn-4-ene-3, 11, 20-trione (**360**) (IC_{50} = 9.2 ± 0.7 μg/mL), 6*β*, 17*α*-dihydroxy-6α-methylpregn-4-ene-3, 11, 20-trione (**361**) (IC_{50} = 1.2 ± 0.02 μg/mL), 6*β*, 20(S)-dihydroxy-6*α*-methylpregn-4-ene-3, 11-dione (**362**) (IC_{50} = 15.2 ± 2.4 μg/mL), 11*β*, 16*β*-dihydroxy-6*α*-methylpregn-4-ene-3-one (**363**) (IC_{50} ≤ 0.2 μg/mL), and 15*β*, 20(R)-dihydroxy-6*α*-methylpregn-4-ene-3, 11-dione (**364**) (IC_{50} = 10.4 ± 0.42 μg/mL), also showed potent activities against T-cell proliferation *in vitro*, as compared to the standard drug, prednisolone (IC_{50} ≤ 3.1 μg/mL).

Biotransformed products, 17*β*-hydroxy-1-methyl-5*α*-androst-1-ene-3, 16-dione (**236**), and 15*β*, 17*β* -dihydroxy-1-methyl-5α-androstan-1-ene-3-one (**237**), also showed potent activity against isolated polymorphonuclear leukocytes (PMNs) with the IC_{50} values of 14.0 ± 1.7, and 4.70 ± 0.5 μg/mL, respectively.

Derivative, 14*β*, 17*β*-dihydroxy-2-(hydroxymethyl)-17*α*-pregn-4-en-20-yn-3-one (**241**) showed potent cytotoxicity against HeLa cancer cell line with the IC_{50} = 0.283 ± 0.013 μM, as compared to the standard drug, doxorubicin (IC_{50} = 0.506 ± 0.015 μM). Metabolite, 11*α*-hydroxy-6-methylene-androsta-1, 4-diene-3, 17-dione (**319**) showed moderate cytotoxicity against PC-3 (IC_{50}= 16.83 ± 0.96 μM) and cancer HeLa (IC_{50}= 24.87 ± 0.72 μM). Metabolite 2α-methyl-5α-androsta-17*β*-hydroxy-3-one (**342**) (IC_{50} = 19.6 ± 1.4 μM) exhibited potent activity against HeLa cells, in contrast to drostanolone enanthate (**334**) (IC_{50} = 54.7 ± 1.6 μM), and standard cisplatin (IC_{50} = 40.1 ± 2.0 μM). Derivatives, 2*α*-methyl-3*α*, 14*α*, 17*β*-trihydroxy-5*α*-androstane (**335**) (IC_{50} = 64.3 ± 3.0 μM), 2-methylandrosta-11α-hydroxy-1, 4-diene-3, 17-dione (**336**) (IC_{50} = 40.7 ± 0.9 μM), 2-methylandrosta-14α-hydroxy-1, 4-diene-3, 17-dione (**337**) (IC_{50} = 40.7 ± 0.9 μM), 2α-methyl-3α,17*β*-dihydroxy-5α-androstane (**338**) (IC_{50} = 49.5 ± 2.2 μM), 2-methylandrosta-1, 4-diene-3, 17-dione (**339**) (IC_{50} = 39.8 ± 1.5 μM), 2-methyl-5α-androsta-7α-hydroxy-1-ene-3, 17-dione (**341**) (IC_{50} = 58.0 ± 1.0 μM), and 2α-methyl-5α-androsta-17*β*-hydroxy-3-one (**342**) (IC_{50} = 30.1 ± 1.0 μM) also displayed a remarkable activity against HeLa cell line. Metabolites 335 (IC_{50} = 58.4 ± 1.6 μM), 336 (IC_{50} = 59.1 ± 2.6 μM), 337 (IC_{50} = 60.4 ± 0.9 μM), 338 (IC_{50} = 51.8 ± 3.4 μM), 339 (IC_{50} = 68.1 ± 1.2 μM), and 340 (IC_{50} = 39.1 ± 2.0 μM) showed significant anti-cancer activity against PC-3 cells, compared to compounds 342 (IC_{50} = 96.2 ± 3.0 μM), 335 (IC_{50} = 84.6 ± 6.4 μM), 339 (IC_{50} = 84.0 ± 3.1 μM), and standard cisplatin (IC_{50} = 76.5 ± 1.2 μM). Compounds 334 (IC_{50} = 5.0 ± 1.2 μM), 338 (IC_{50} = 12.4 ± 2.3 μM), 340 (IC_{50} = 16.7 ± 2.6 μM), and 90 (IC_{50} = 14.7 ± 2.6 μM) showed potent activity against H460 cells, as compared to cisplatin (IC_{50} = 22.2 ± 2.1 μM). Compounds 335 (IC_{50} = 44.4 ± 2.0 μM), 336 (IC_{50} = 33.2 ± 1.0 μM), 337 (IC_{50} = 38.5 ± 2.8 μM), 339 (IC_{50} = 31.9 ± 1.8 μM), and 340 (IC_{50} = 26.4 ± 0.9 μM) also presented good anti-cancer activity against H460 cells. Compound 334 (IC_{50} = 3.1 ± 3.2 μM) showed potent anti-cancer activity against HCT116 cells, in contrast to standard cisplatin (IC_{50} = 11.2 ± 3.0 μM).

Physalin H (**373**) (IC_{50} = 6.03 ± 0.005 μM), and its structural analogues, 6, 7-dehydrophysalin H (**374**) (IC_{50} = 7.74 ± 0.015 μM), 6-deoxyphysalin H (**375**) (IC_{50} = 6.34 ± 0.03 μM), and isophysalin B (**376**) (IC_{50} = 13.8 ± 0.05 μM), showed potent anti-leishmanial activity, compared to the standard drug, amphotericin B (IC_{50} = 0.129 ± 0.105 μM), against promastigotes of *Leishmania major* (DESTO).

CONCLUSION AND FUTURE PERSPECTIVES

The scope of present biotransformation studies conducted in our laboratories was to synthesize new analogues of monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, and steroidal-based anabolic, contraceptive, anti-cancer, and anti-epileptic drugs by using the cost-effective, and eco-friendly bio-catalytic approach. In the present review, over 350 new and known metabolites are presented through biotransformation of natural/synthetic/semisynthetic compounds. Aromatization, hydroxylation, epoxidation, hydrogenation, and dehydrogenation were the main reactions that occurred during the whole-cell bio-catalyzed transformation reactions. The technique of biotransformation was found to be a robust method to produce compounds having structural similarities with their parent molecules. Newly synthesized derivatives were evaluated for various biological activities. Variations in the structures of the transformed products often led to changes in their biological activities in comparison to their parent drugs.

Several bio-transformed products exhibit biological activities and have been explored for different applications, mainly in the food and pharmaceutical industries. These structurally altered compounds are extensively being studied for their effects on human health. In the search for biologically active compounds,

derivatization of natural products and existing drugs has emerged as an important research area. In the future, further biotransformation studies will include the production of biologically important transformed products in bulk quantities for their *in vitro*, *ex vivo*, and *in vivo* studies. After successful evaluation, these compounds may serve as new therapeutic agents against various ailments. The mode of actions of potent derivatives through NMR spectroscopy and molecular docking studies will also be performed. Moreover, the biologically important metabolites need to be studied at enzymatic levels using modern molecular and structural biology techniques in order to understand the Supplementary Figures involvedin the biotransformation reactions.

DECLARATIONS

Acknowledgments

We would like to acknowledge present and past students and research officers of the biotransformation laboratories whose names are cited in the references. Financial support from national and international funding agencies is also appreciated here.

Authors' contributions

Designed the topic and provided ideas: Choudhary M, Rahman A
Collected the data and drafted the review: Siddiqui M, Wahab A
Checked and finalized this review article: Choudhary M, Rahman A

Availability of data and materials

Not applicable.

Financial support and sponsorship

Not applicable.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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