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Review Article

Unearthing the Potential Secondary Metabolites Isolated from the Fungus *Aspergillus flocculosus:* A Comprehensive Review

Ashraqat Elsayed Ismail[a], Ahmed Mohamed Elissawy[a,b*], Haidy Abdel Moniem Gad[a], Abdel Nasser Badawy Singab[a,b]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt*
[b]*Center for Drug Discovery Research and Development, Faculty of Pharmacy, Ain Shams University, Cairo, 11566, Egypt*

ABSTRACT

Aspergillus flocculosus represents a relatively recent fungal source of interesting secondary metabolites. *A. flocculosus*, as a fungal endophyte, had been isolated from different sources including plants such as *Markhamia platycaly*, sponges, for example, *Phakellia fusca*, algae for example *Padina sp.* as well as sea sediment. This review presents a literature survey for the reported secondary metabolites isolated from *A. flocculosus,* demonstrating the potential biological activities of the reported metabolites. This review presents a comprehensive literature survey of the secondary metabolites isolated from the fungus *A. flocculosus* reporting eighty secondary metabolites belonging to different chemical classes including steroids, meroterpenoids and diketopiperazine alkaloids, drimane sesquiterpenoids as well as their nitro benzoyl derivatives as well as cerebrosides. Secondary metabolites reported from *A. flocculosus* possess numerous biological activities including anticancer, anti-inflammatory, anti-trypanosomal, antimicrobial, and neuroprotective activities. Despite the relatively limited reports for secondary metabolites represented in this review, the chemical diversity and the potential biological activities of the reported metabolites reported herein encourage further in-depth research for novel bioactive secondary metabolites from *A. flocculosus* especially those derived as endophytes from marine resources.

Keywords: *Aspergillus flocculosus; Fungal endophytes; Fungal sources; Secondary metabolites, terpenoids, alkaloids, polyketides.*

***Correspondence** | Ahmed Mohamed Elissawy; Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Organization of African Union, St. Abbassia, Cairo 11566, Egypt. Email: aelissawy@pharma.asu.edu.eg

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1. Introduction

Nature provides a huge reservoir of different sources of biologically active metabolites, many of which produce unique structural skeletons that can be utilized as lead structures for the development of novel drugs **[1, 2]**. Plants represent the major sources of medicinally active natural products with about 25% of prescribed drugs originating from plant sources. Furthermore, according to the World Health Organization (WHO) about 11% of 252 essential drugs are derived from plant origin. There are many synthetic drugs derived from plants for example, vinblastine and vincristine from *Catharanthus roseus*, digoxin from *Digitalis spp*, and codeine and morphine from *Papaver somniferum* **[3]**. As a result, extensive research work has been performed on medicinal plants throughout the last century, this drives the essential need to explore other interesting sources

of natural products; endophytes, especially fungal endophytes, represent one of the major recent sources of potential secondary metabolites **[4, 5]**.

Plant endophytes represent a relatively new source of interesting natural products **[6]**. Endophytes are a general term involving all microorganisms like; bacteria, and fungi that live inside plant tissues in a symbiotic relation where both sides share mutual benefits **[7, 8]**. Endophytic fungi have been attracting attention as a hopeful supply for structurally rare and biologically active natural products **[9-13]**. Endophytic fungi mainly be included in filamentous Ascomycetes that contain different genera like *Aspergillus spp.*, *Neurospora spp.*, *Monascus spp.*, *and Fusarium spp.* **[14, 15]**. Fungi belonging to the genus *Aspergillus* are famous for their potent biosynthetic capacities to produce variable secondary metabolites displaying a wide diversity of biological activities and important roles in pharmaceutical industries. **[16-22]**. *A. flocculosus* is known for its ability to produce a wide variety of secondary metabolites belonging to different chemical classes including steroids **[23, 24]**, meroterpenoids and diketopiperazine alkaloids **[25]**, drimane sesquiterpenoids as well as their nitro benzoyl derivatives **[26]** and cerebrosides **[27]**. Secondary metabolites isolated from *A. flocculosus* possess potential biological activities including anti-inflammatory, anti-trypanosomal, anti-cancer, antimicrobial, and neuroprotective activities **[25, 27-29]**.

This review aims to reveal the reported secondary metabolites isolated from the fungal endophyte *A. flocculosus* revealing their potential bioactivities. Different databases were surveyed including Egyptian Knowledge Bank (EKB), Marin Lit databases, Scifinder, and Reaxys.

2. Secondary metabolites isolated from *A. flocculosus.*

2.1. Alkaloids

Investigation of the Ethyl Acetate Extract of the halotolerant fungus *A. flocculosus* PT05-1 isolated from sea sediment collected from Putian, Fujian Province, China, led to the isolation of three acidic alkaloids (**1-3**), namely; neoaspergillic acid **(1),** hydroxyneoaspergillic acid **(2)** and 4-(1H-pyrrol-2-yl)-1-isoquinolone-3-carboxylic acid **(3).** Hydroxyneoaspergillic acid **(2)** is distinct from neoaspergillic acid **(1)** by oxygenated methine instead of methylene signals and desheilded H-5, C-6, and C-20 **[27]**.

Investigation of the sponge-derived fungal endophyte *A. flocculosus* isolated from the inner tissues of the marine sponge *Styllissa sp.* collected from Vietnam led to the isolation of two diketopiperazine alkaloids, identified as mactanamide **(4)** and cycloechoinulin **(5) [25]**. Mactanamide (**4**) was reisolated from a sediment specimen of *A.flocculosus* in Nha Trang Bay, South China Sea by Yurchenko et al. **[29]**.

Nine pyrrolidine alkaloids, pressing C−I **(6−12)** in addition to (11*R*)/(11*S*)-preussins J and (11*R*)/(11*S*)-preussins K **(13 and 14)**, were obtained from the marine-derived fungal endophyte *A.flocculosus* 16D-1 isolated from the marine sponge *Phakellia fusca* **[30]**. Structurally, preussins could be differentiated through C-5 side chains showing different degrees of oxidation. **Fig. 1.** illustrates the different Alkaloids isolated from *A. flocculosus***.**

2.2. Cerebrosides

Three different cerebrosides were obtained from the halotolerant *A. flocculosus* PT05-1 derived from sea sediment collected from Putian, Fujian Province, China and grown under hypersaline condition (20% saline) Cerebroside C **(15)**, (2R)-2-hydroxy-N-[(2S,3R,4E,8E)-1-b-D-glucopyranosyloxy-3-hydroxy-9-methylnonadec-4,8-dien-2-yl] hepta decanamide **(16)** and (2R,3E)-2-hydroxy-N-[(2S,3R,4E,8E)1-b-D-

glucopyranosyloxy-3-hydroxy-9-methylnonadec-4,8dien-2-yl] 44ietname-3-enamide **(17) [27].**

Fig. 2. illustrates the different cerebrosides isolated from *A. flocculosus***.**

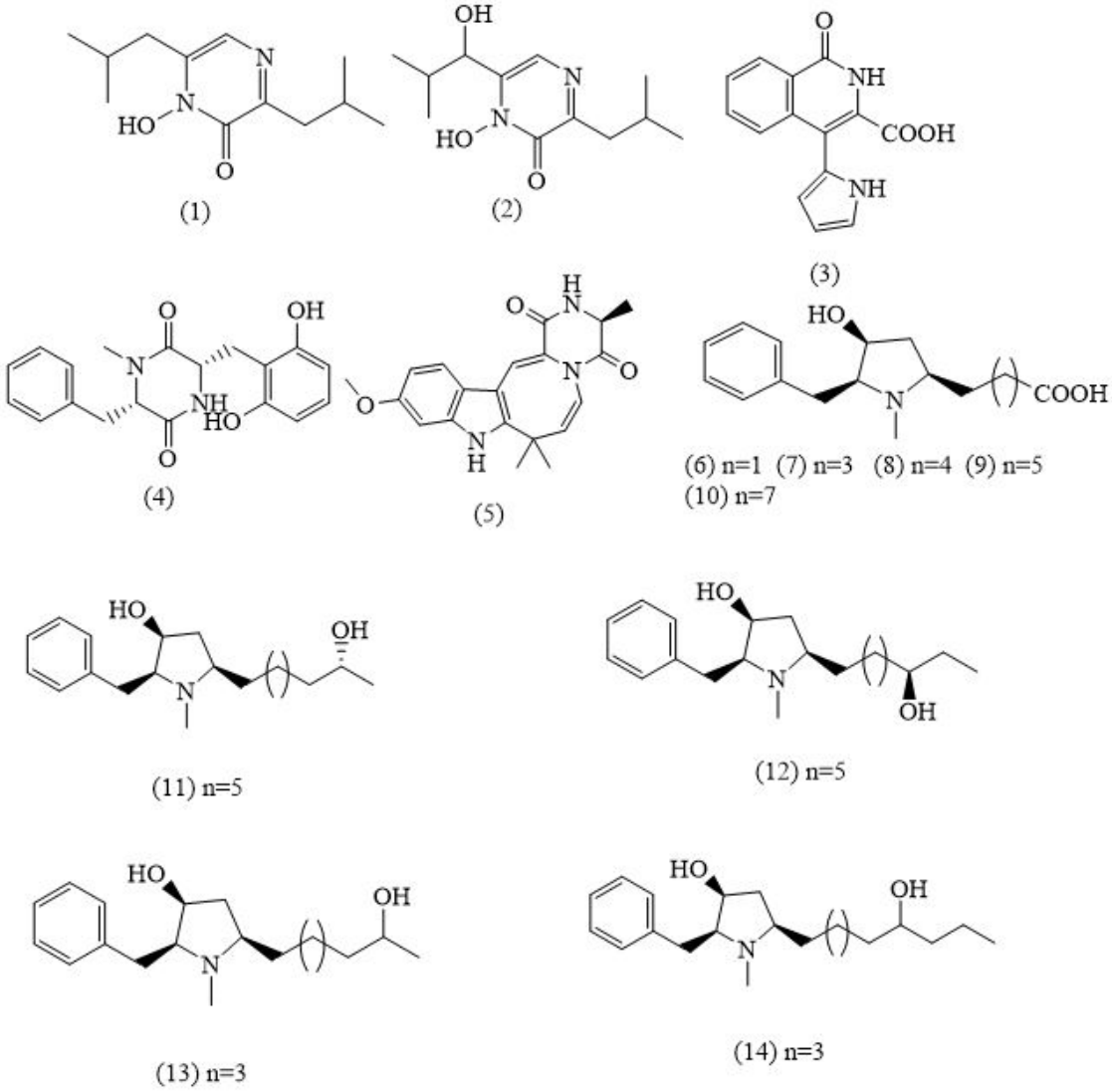

Fig. 1. Alkaloids reported from *A. flocculosus*

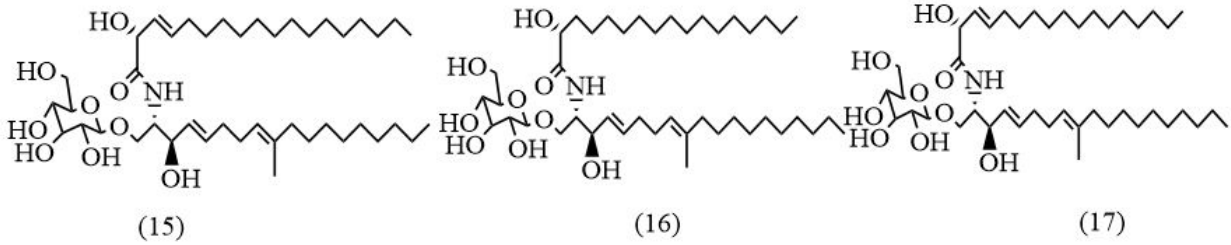

Fig. 2. Cerebrosides reported from *A. flocculosus*

2.3. Isocoumarins

Tawfike et al. used modern metabolomics technologies to separate isocoumarins from the fermentation medium of the endophyte *A. flocculosus* derived from the stem of *Markhamia platycalyx*. Fungus identification was confirmed by using phylogenetics **[28]**. During methanol fraction purification seven isocoumarins were isolated **(18-24)**. The isolated compounds were identified as botryoisocoumarin A **(18)** and mellein **(19)**, cis-4-hydroxymellein **(20)**, trans-4-hydroxymellein **(21)**, 3-hydroxymellein **(22)**, 5-hydroxymellein **(23)** and 4, 5-dihydroxymellien **(24) [28]**.

Additionally, a food-adulterating mycotoxin ochratoxin A **(25)** was obtained from *A.flocculosus* derived from the marine sponge *phakellia fusca* by Gu et al. **[23]**. Saccharonol A **(26)** was isolated from a liquid growth medium *A. flocculosus* derived from a marine source **[31]**. **Fig. 3.** illustrates the different isocoumarins isolated from *A. flocculosus***.**

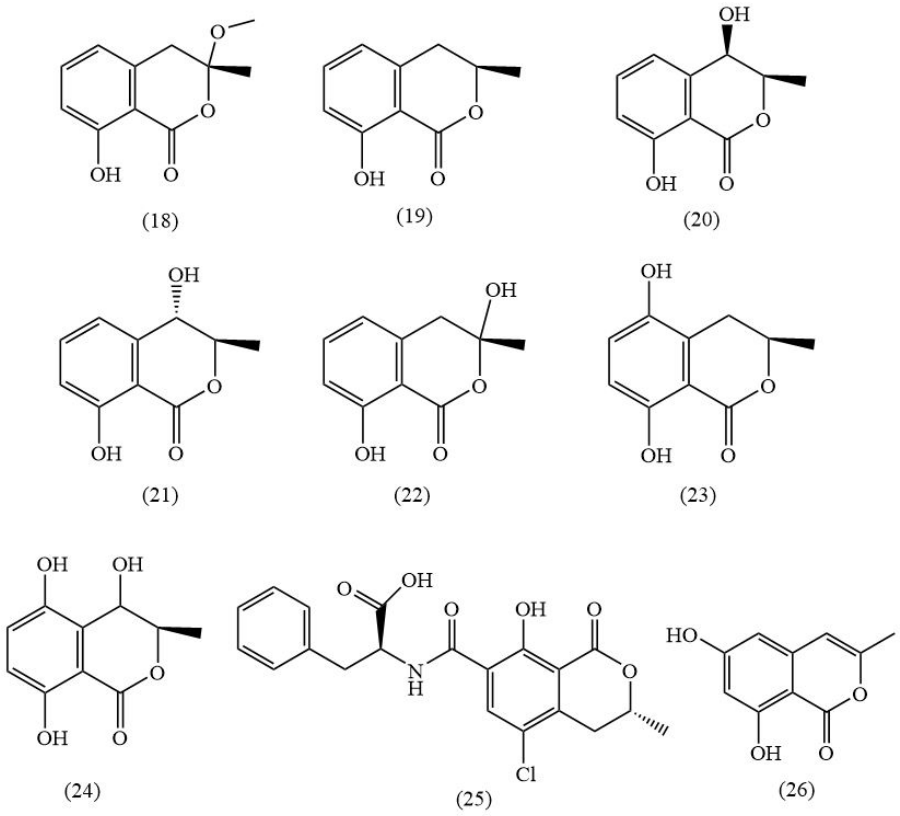

Fig. 3. iso coumarins reported from *A. flocculosus*

2.4. Polyketides

Six acidic polyketides (**27-32**) had been isolated from the fungal endophyte *A. flocculosus* isolated from the fresh stems of Markhamia *platycalyx*. The isolated metabolites were identified as 7-O-acetylkojicacid **(27),** methyl 2-(4-hydroxyphenyl) acetate **(28)**, dihydro penicillin acid **(29)**, p-hydroxy benzaldehyde **(30)**, 2-hydroxyphenyl acetic acid **(31)**, 4-hydroxyphenyl acetic acid **(32) [28]**.

Van Anh et al. identified 12-hydroxyhomovalencic acid **(33)** and homovalencic acid **(34)** from the marine-derived *A. flocculosus* isolated from *Stylissa* sp. Sponge

[32].

In fungi as pyrone related polyketides are common metabolites and are usually classified into three structure groups: γ-lactones (iso-aspinonene, aspilactonols), δ lactones (aspyrone), and linear (aspinonene) **[33]**.

Anton N. Yurchenko et al. isolated From *A. flocculosus* extracted from marine sediment in Vietnam, an aspyrone-related polyketide aspilactonol G **(35)** together with aspilactonols F **(36)** and determined stereoconfigurations of Aspilactonols G and F by a modified Mosher's method **[26]**.

Assessment of the ethyl acetate extract of *A. flocculosus* obtained from newly harvested stems of *Markhamia platycalyx* yielded a polyketide **(37),** (5,9-dihydroxy-2,4,6,8,10-pentamethyldodeca-2,6,10-trienal). Compound **(37)** was obtained from nature for the first time by Tawfike et al. and related in structure to polyketide TMC-151s **[28, 34]**. **Fig. 4**. illustrates the different polyketides isolated from *A. flocculosus*.

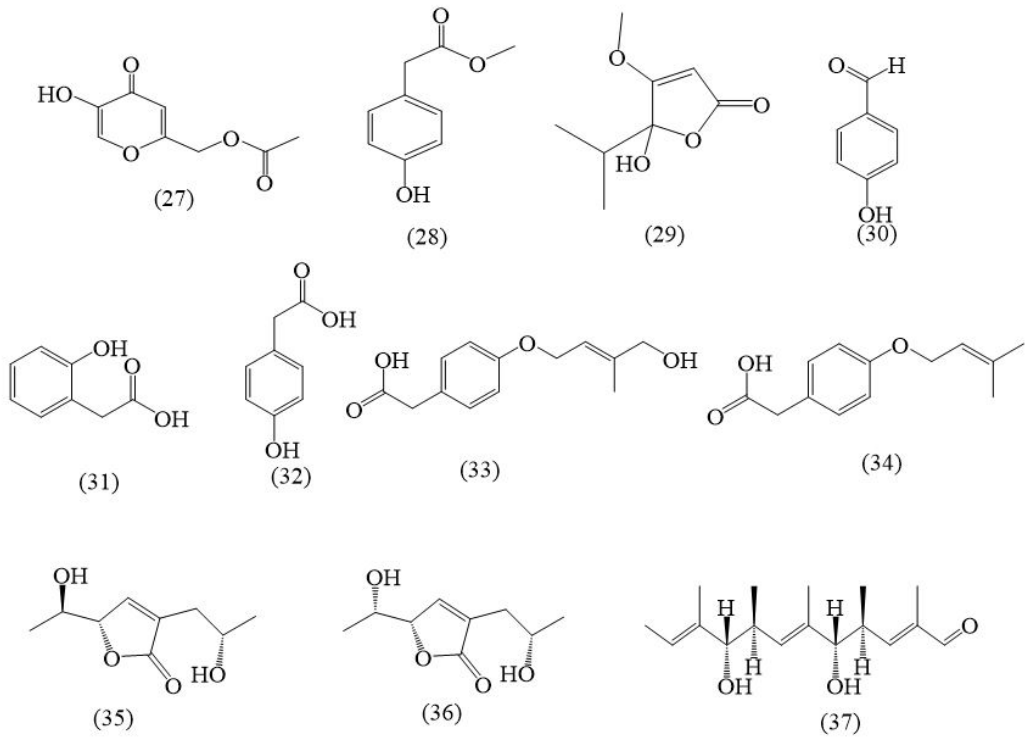

Fig. 4. Polyketides reported from *A. flocculosus*

2.5. Steroids

A heterodimer ochratoxin-ergosteroid Ochrasperfloroid **(38)** was obtained from endophytic *A. flocculosus* derived from the inner tissues of *Phakellia fusca* sponge **[23]**. Moreover, three compounds **(39-41)** were isolated from same sponge, Two of them were 11(9 → 10)-abeo-5,10-secosteroids having an unusual dioxatetraheterocyclic ring system, aspersecosteroids A **(39)** and aspersecosteroids B **(40),** third compound asperflosterol **(41)** was an ergosteroid that has similarity in C-22 side chain and rings D and E of **(39)** and **(40) [24]**.

Additionally, four compounds **(42-45)** were isolated from *A. flocculosus* PT05-1 grown in 20% saline medium collected from superficial

sediment collected from Putian, Fujian Province, China. The isolated compounds were identified as the ergosteroid, (22R,23S)-epoxy-3b,11a,14b,16b-tetrahydroxyergosta-5,7-dien-12-one **(42)**, in addition to 7-nor-ergosterolide **(43)**, 3b-hydroxyergosta-8,24(28)-dien-7-one **(44)** and cerevisterol **(45) [27]**.

The hexane fraction of the fungal endophyte *A. flocculosus* derived from the stem of *Markhamia platycalyx* yielded the known steroids ergosterol **(46)**, ergosterol peroxide **(47),** and campesterol **(48) [28]**.

Investigation of the dichloromethane – methanol fraction from (EtOAc) extract of the marine-derived fungal endophyte *A. flocculosus* 16D-1 isolated from the sponge *Phakellia fusca* collected from China led to the identification of Compounds **(49-52) [35]**. Two ergostanes Asperflotone **(49)** and asperfloroid **(50) [35]** together with two secosteroids asperfloketals A **(51)** and B **(52)** showing an unprecedented trioxahexaheterocyclic ring system **[36]**. **Fig. 5.** illustrates the different steroids isolated from *A. flocculosus*.

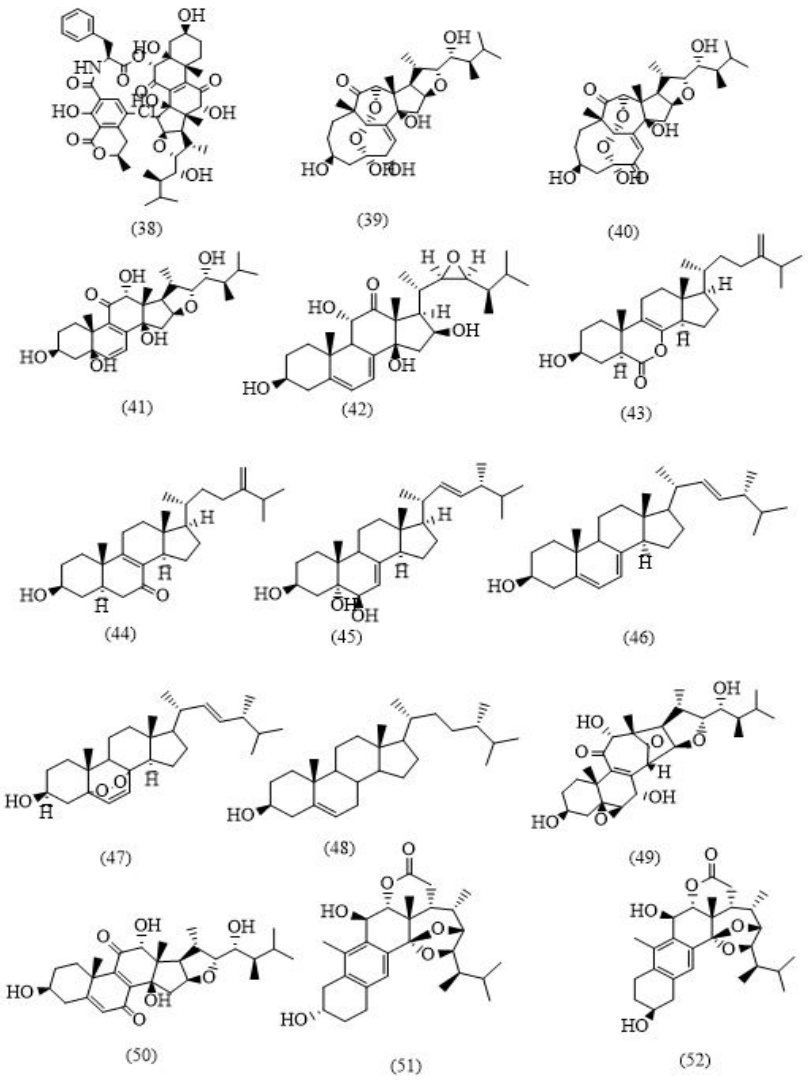

Fig. 5. Steroids reported from *A. flocculosus*

2.6. Terpenes

Nitro benzyl drimane sesquiterpenoids derivatives were first isolated from *A. insulicola* species. Currently, related fungi produce nitrobenzyl drimane derivatives too **[37]**. These compounds are distinguished with two isomeric skeletons confertifolin- and cinnamolide-based besides acyl groups in various locations. Usually, p-nitrobenzoic acid is substituted on -OH found at positions 9 or 14 **[38]**. Anton N. Yurchenko et al. isolated drimane-sesquiterpenoids and nitro benzoyl derivatives **(53-56)** from *A. flocculosus* derived from Vietnamese marine sediment, the isolated compounds had been identified as insulicolide A **(53)**, 9α-14-dihydroxy-6β-p-nitrobenzoylcinnamolide, 6β,9α,14-trihydroxycinnamolide **(54),** 6β,7β,14-trihydroxyconfertifolin **(55)** and 7α,14-dihydroxy-6β-p-nitrobenzoylconfertifolin **(56) [26]**. In addition to three aspertetranones meroterpenoids were yielded from same source **(57-59),** aspertetranone A **(57)**, aspertetranone D **(58)** and 12-epi-aspertetranone D **(59) [25, 26]**.

An angular tetracyclic α-pyrone merosesquiterpenoid, ochraceopone F **(60)**, was obtained from the marine fungus *A. flocculosus* 01NT.1.1.5 derived from the inner tissues of the sponge *Stylissa sp.* collected from Vietnam **[25]**.

Nine sesterterpenes **(61-69)** namely; 14,15-dehydro-6-epi-ophiobolin K **(61)**, 14,15-dehydroophiobolin K **(62)**, 14,15-dehydro-6-epi-ophiobolin G **(63),** 14,15-dehydro-ophiobolin G **(64)** and 14,15-dehydro-(Z)-14-ophiobolin G **(65),** 6-epi-ophiobolin C **(66)**, ophiobolin C **(67)**, 6-epi-ophiobolin N **(68)** and ophiobolin N **(69)** were identified from the endophyte *A. flocculosus* collected from the algae *Padina sp.*, The structures of ophiobolins **(66-69)** were determined by spectroscopic analysis **[39]**. **Fig. 6.** illustrates the different terpenes isolated from *A. flocculosus*.

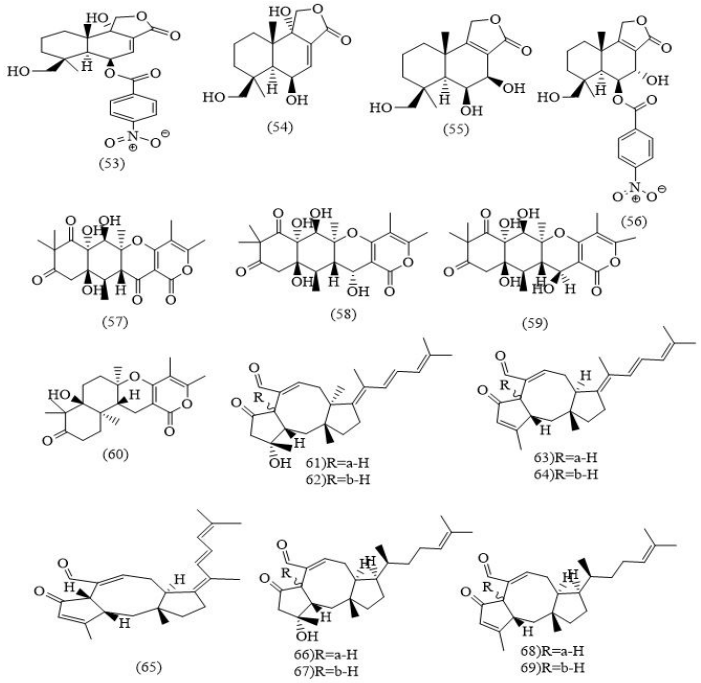

Fig. 6. Terpenes reported from *A. flocculosus*

2.7. Miscellaneous compounds

A red pyrrole pigment **(70),** 6-(1H-pyrrol-2-yl) hexa-1,3,5-trienyl-4-methoxy-2H-pyran-2-one (found in the form of a pair of epimers 1E,3E,5E and 1E,3Z,5E separately), was separated from the halotolerant *A. flocculosus* PT05-1 collected from marine sediment in China. Furthermore, three compounds were obtained from *A.flocculosus* from the newly harvested stems of *Markhamia platycalyx*, phomaligol A **(71)**, phomaligol A1 epimer **(72)**, and diorcinol **(73) [28, 31]**.

Wasabidienone E **(74)** was isolated from the marine-derived *A. flocculosus* isolated from the marine sponge *Stylissa sp* gathered from Vietnam **[25]**.

Dihydroaspyrone **(75)** was isolated from *A.flocculosus* derived from two different sources; newly harvested stems of *Markhamia platycalyx* and a sediment sample of the South China Sea in Vietnam **[26, 28, 40]**.

Two phomaligols, deketo-phomaligol A **(76)**, a membered ring phomaligol derivative and phomaligol E **(77)**, in addition to six compounds **(78–80)** were derived from a liquid growth medium of *A. flocculosus* collected from marine source (algae *Padina* sp.), the compounds were identified as sydowione A **(78)**, 2,6-dimethyl-3-O-methyl-4-(2-methyl butyryl) phloroglucinol **(79)**, phomaligol D **(80) [31]**, phomaligol A and phomaligol A1 **[28, 31]**. **Fig. 7.** illustrates Miscellaneous compounds isolated from *A. flocculosus*.

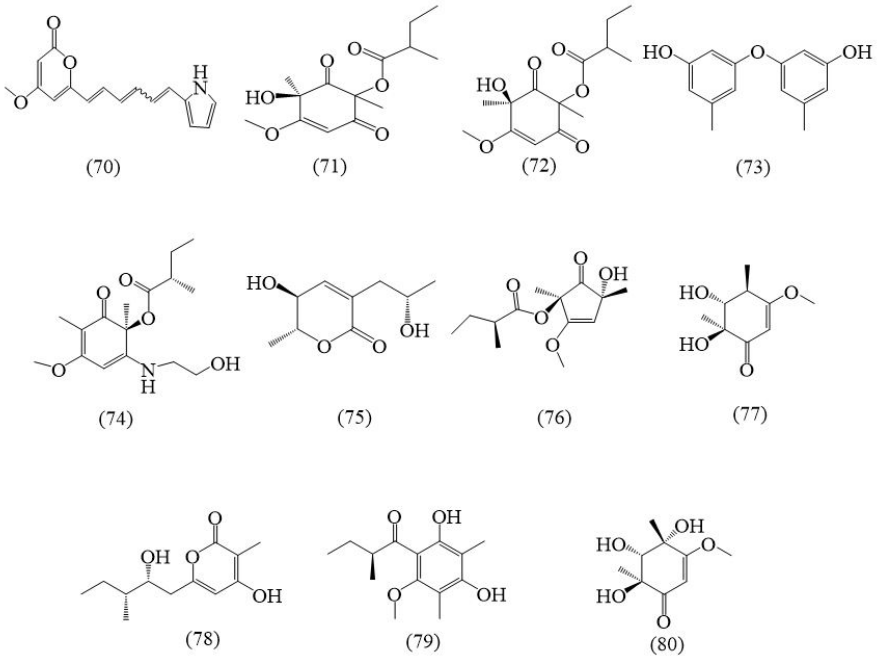

Fig. 7. Miscellaneous reported from *A. flocculosus*

3. Biological investigation

3.1. Cytotoxicity

Using sulforhodamine B (SRB) assay, 12-hydroxyhomovalencic acid **(33)** and homovalencic acid **(34)** did not show any cytotoxicity toward colon (HCT-15), stomach (NUGC-3), renal (ACHN), breast (MDA-MB-231) lung (NCI-H23) and prostate (PC-3) cancer cell lines **[32]**. In addition to mactanamide **(4)**, cycloechoinulin **(5),** aspertetranone D **(58),** ochraceopone F **(60)** and wasabidienone E **(74)**

were evaluated against five cell lines, HCT15, NUGC-3, MDA-MB-23,1 NCI-H23, PC-3 and ACHN and did not display any cytotoxicity up to 30 μg/mL**[25]**. further studies on mactanamide **(4)** displayed that it did not have any cytotoxic effect on Neuro2a cells up to 100 μM **[29]**.

Gu et al. evaluated preussins C−I **(6− 12)** in addition to (11*R*)/(11*S*)-preussins J and (11*R*)/(11*S*)-preussins K **(13 and 14)** against HepG2, THP-1, and A54 cell lines and did not show any cytotoxicity (IC50 > 20 μM) **[30]**.

The compounds, Ochratoxin A **(25)** and Ochrasperfloroid **(38)** presented cytotoxicity from weak to moderate toward HepG2 and A549 cell lines. **(25)** and **(38)** displayed IC_{50} values of 50.3 and 55 mM for A549 cells and 22.7 and 23.6 mM for HepG2 cells, respectively **[23]**.

Four isocoumarins, Botryoisocoumarin A **(18)**, mellein **(19)**, cis-4-hydroxymellein **(20),** and 5-hydroxymellein **(23)** demonstrated inhibitory activity against the K562 cell line at 30μM **[28]**.

In SRB assay and positive control Adriamycin, 14,15-dehydro-6-epi-ophiobolin K **(61)**, 14,15-dehydroophiobolin K **(62)**, 14,15-dehydro-6-epi-ophiobolin G **(63),** 14,15-dihydro-ophiobolin G **(64)**, 14,15-dihydro-(Z)-14-ophiobolin G **(65),** 6-epi-ophiobolin C **(66)**, ophiobolin C **(67)**, 6-epi-ophiobolin N **(68)** and ophiobolin N **(69)** were investigated toward NCI-H23, HCT-15, PC-3, ACHN, NUGC-3 and MDA-MB-231. Compounds **(61-69)** demonstrated strong activity with a range from 0.14 to 2.01 μM of GI_{50} values. 14,15-dehydro-6-epi-ophiobolin K **(61)** displayed 0.14,0.19 and 0.21 μM GI_{50} values toward MDA-MB-231, NUGC-3 and HCT-15 cell lines, respectively. Moreover, compounds **(61-65)** that have three double bonds were slightly less active than compounds **(66-69)** that have only one double bond. 14,15-dihydro-(Z)-14-ophiobolin G **(65)** displayed the least cytotoxicity range from 1.53 to.01 μM GI_{50} values **[39]**.

The compound Insulicolide A **(53)** demonstrated cytotoxicity against murine neuroblastoma (Neuro-2a) and human prostate cancer (22Rv1) cell lines with IC_{50}values 4.9, 3 μM, respectively, while its analogue 7α,14-dihydroxy-6β-p-nitrobenzoylconfertifolin **(56)** did not show any cytotoxicity up to 100 μM. In addition to weak cytotoxicity with IC_{50} of 59.6 μM toward MCF-7 a higher cytotoxicity was reported with IC_{50} equal 6.08 μM. The compound 6β,9α,14-trihydroxycinnamolide **(54)** displayed cytotoxicity with IC_{50} value 24.1μM against Neuro-2a, human prostate cancer 22Rv1 (IC_{50} value 31.5 μM) and no cytotoxicity up to100μM toward MCF-7 breast cancer cell line while 6β,7β,14-trihydroxyconfertifolin **(55)** displayed no cytotoxicity up to 100 μM against Neuro-2a cells **[26, 40, 41]**.

Ten variable compounds, aspilactonol G **(35),** aspilactonols F **(36),** insulicolide A **(53)**, 6β,9α,14-trihydroxycinnamolide **(54),** 6β,7β,14-trihydroxyconfertifolin **(55)**, 7α,14-dihydroxy-6β-p-nitrobenzoylconfertifolin **(56)**, aspertetranone A **(57)**, aspertetranone D **(58),** 12-epi-aspertetranone D **(59)** and Dihydroaspyrone **(75)** were investigated on the ability of formation colony and viability of human drug-resistant prostate cancer 22Rv1 cells. Compounds **(35)**, **(36)**, **(55), (56)**, **(57), (58), (59),** and **(75)** did not exhibit cytotoxic effects toward 22Rv1 up to 100 μM concentration **[26]**.

The compounds (22R,23S)-epoxy-3b,11a,14b,16b-tetrahydroxyergosta-5,7-dien-12-one **(42)**, together with 7-nor-ergosterolide **(43)**, 3b-hydroxyergosta-8,24(28)-dien-7-one **(44)** displayed cytotoxicity toward BEL-7402 and HL-60 cells with IC_{50} values of 12–18μM **[27]**. Moreover, asperfloketals A **(51)** and asperfloketals B **(52)** presented no cytotoxic effect toward SW480, HepG2, and HeLa cell

lines (IC50 > 80μM) **[36]**.

Cytotoxic activities for aspersecosteroids A **(39)**, aspersecosteroids B **(40)**, asperflosterol **(41)**, asperflotone **(49),** and asperfloroid **(50)** were evaluated against tumor cell lines A549 and HepG2 and showed no activity up to 80 μM **[24, 35]**.

3.2. Antioxidant activity

The compound **(4)**, mactanamide, showed an antiradical effect at 10μM and demonstrated scavenger activity for 15% DPPH radicals at a concentration of 100μM in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays **[29]**. In addition, mactanamide **(4)** inhibited 30% formation of ROS in the 6-Hydroxydopamin (6-OHDA)-treated neuronal cells but increased cell viability at 10 μM by 42%.

Neurotoxin was added for the PD model induced by 6-OHDA after adding mactanamide **(4)** for 1 hr., its neuroprotective effect was preserved although its concentration was reduced tenfold.

In the paraquat (PQ)-treated cells, mactanamide **(4)** reduced the formation of ROS by 32% and 37%, at 1 and 10 μM concentrations, respectively. However, it did not display any activity on the PQ-treated cell's viability **[29]**.

The compound 12-hydroxyhomovalencic acid **(33)** was screened for DPPH radical scavenging assay and did not display activity **[32]**.

Furthermore, dihydroaspyrone **(75)** raised statistically the PQ-treated cell's viability and decreased the level of ROS in neurotoxin-treated Neuro-2a. Additionally, hydroxylation of the compound **(75)** may perform an important role in its anti-ROS activity in neurotoxin-induced Parkinson's disease (PD) cell models **[40]**.

3.3. Anti-inflammatory and immune modulation activity

By Chen et al. method and using Corylifol A as positive control **[42]**, ochratoxin A **(25)** and ochrasperfloroid **(38)** were assessed for inhibiting Interleukin-6 (IL-6) production in lipopolysaccharide (LPS)-induced THP-1 cells and with using Diphenyleneiodonium chloride as positive control to determine the production of nitric oxide (NO) in LPS-activated RAW264.7 macrophages. **(25)** did not inhibit NO and IL-6 productions (IC_{50} > 25 mM) while **(38)** exhibited potent inhibitory activities with an IC_{50} value of 1.11 on NO production and with an IC_{50} value of 2.02 on IL-6 production **[23]**.

A CCK-8 assay described by Tang et al. **[43]** was used to find out whether the suppressive effects of compounds **(25)** and **(38)** were related to cell viability. They did not display any cytotoxic activity toward RAW264.7 and THP-1 cells up to IC_{50} values > 20 mM), but inhibited IL-6 and NO productions **[23]**.

The compounds preussins C–I **(6– 12)** and (11*R*)/(11*S*)-preussins J and (11*R*)/(11*S*)-preussins K **(13 and 14)** were examined for inhibiting IL-6 production in LPS-activated THP-1 cells. Compounds **(10)**, **(12),** and **(13)** displayed potent inhibitory effects on the production of IL-6 with IC_{50} values of 0.11, 0.19, and 2.3 μM, respectively. Furthermore, **(6–9)**, **(11)**, and **(14)** showed moderate inhibitory effects, with IC_{50} values from 8.2 to 22 μM **[30]**.

In vitro immune suppression effects were evaluated for aspersecosteroids A **(39)**, aspersecosteroids B **(40),** and asperflosterol **(41)** and exhibited IC_{50} values 21, 26, and 24 μM against Il-6 in LPS-stimulated THP-1 cells and 28, 31, and 28 μM against TNF-α **[24]**.

The compounds Asperflotone **(49)** and Asperfloroid **(50)** displayed inhibitory activity with IC_{50} values of 22 μM toward IL-6 secretion in the LPS-induced THP-1 cell line **[35]**.

Using $CuSO_4$ induced acute inflammatory

response and stimulated the infiltration of macrophages in mechanosensory and lateral line neuromast cells in zebrafish. Then, *in vivo* anti-inflammatory activity was evaluated for Asperfloketals A **(51)** and Asperfloketals B **(52)** with a positive control, ibuprofen. **(51)** and **(52)** exhibited stronger anti-inflammatory activity than control in the $CuSO_4$-induced zebrafish through the reduction of macrophage number in the neuromast of zebrafish **[36]**.

At a concentration of 100 μM phomaligol A **(71)**, phomaligol A1 **(72)**, deketo-phomaligol A **(76)**, sydowione A **(78)**, 2,6-dimethyl-3-O-methyl-4-(2-methylbutyryl) phloroglucinol **(79)** and phomaligol D **(80)** were investigated for inhibiting neuroinflammation, cytotoxicity and NO production in LPS-induced Murine microglial (BV-2) cells. compound **(79)** exhibited moderate activity without cytotoxicity with an IC_{50} value of 56.6 μM against inflammation through suppressing pro-inflammatory mediators' production in activated microglial cells. further study was conducted to determine NO production and expression levels of iNOS and COX-2 proteins, **(79)** displayed a reduction in NO production and reduced iNOS and COX-2 proteins expression in a dose-dependent manner **[31]**.

3.4. Antimicrobial activity

Activity of preussins C−I **(6− 12)** and (11*R*)/(11*S*)-preussins J and (11*R*)/(11*S*)-preussins K **(13 and 14)** were assessed for antifungal effects toward *Trichophyton rubrum* ATCC4438, *Monilia albican* ATCC10231 and *Trichophyton mentagrophytes* ATCC4439 and did not exhibit any activity (MIC > 50 μg/mL) **[30]**.

In a recent study investigating the antimicrobial potential of secondary metabolites isolated from *A. flocculosus* using the agar dilution method **[44]**, (22R,23S)-epoxy-3b,11a,14b,16b-tetrahydroxyergosta-5,7-dien-12-one **(42)**, 7-nor-ergosterolide **(43)** and 3b-hydroxyergosta-8,24(28)-dien-7-one **(44)** and 6-(1H-pyrrol-2-yl)hexa-1,3,5-trienyl-4-methoxy-2H-pyran-2-one **(70)** were investigated against *Candida albicans*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Staphylococcus aureus* for antimicrobial activity. Compound **(42)** exhibited medium activities toward *E. aerogenes, C. albicans*, *P. aeruginosa* and with MIC values of 1.6/3.3/3.3 μM, while **(43)** showed moderate antimicrobial activities with MIC values of 7.5/7.5/1.9 μM. toward *P. aeruginosa*, *E. aerogenes*, and *C. albicans*, respectively. Moreover, **(44)** displayed activity toward *E. aerogenes* only with a MIC value of 15μM. Also, **(70)** produced medium activity toward *E.aerogenes* only with a MIC value of 3.7μM **[27]**.

3.5. Antitrypanosomal

Anti-trypanosomal activity was investigated for 3-hydroxymellein **(22)**, dihydropenicillic acid **(29)**, Ergosterol **(46)**, Ergosterol peroxide **(47)**, Phomaligol A1 **(72)** and Diorcinol (73) toward *T. brucei*. 3-hydroxymellein **(22)** Displayed 56% inhibitory action toward *T. brucei*. The activity of dihydropenicillic acid **(29)** was medium with MIC of 25 μg/mL (145.3 μM) while Ergosterol **(46)** recorded toward *T. brucei* MIC of 31.6μM (12.5 μg/mL). Additionally, Ergosterol peroxide **(47)** showed strong activity with MIC of 7.3μM (3.12 μg/mL). Furthermore, Phomaligol A1 **(72)** had moderate activity with MIC of 88μM (M25 μg/mL) and Diorcinol **(73)** showed a 97% inhibitory action toward *T. brucei*. and displayed activity with an MIC of 25 μg/mL **[28]**.

3.6. Osteoclastogenesis inhibition

In a study using tartrate-resistant acid phosphatase (TRAP), a diketopiperazine alkaloid Mactanamide **(4)** was evaluated for its inhibitory effects on receptor activator of nuclear factor-κB

ligand RANKL-induced osteoclast differentiation of bone marrow macrophages (BMMs). **(4)** showed an inhibitory effect against osteoclastogenesis by suppressed RANKL-induced differentiation and did not exhibit cytotoxic effects on BMMs **[25]**. aspertetranone D **(58)** displays suppressive activity on RANKL-induced differentiation of BMMs into osteoclasts.

Table 1. Summarizes the different biological activities of the secondary metabolites isolated from *A. flocculosus.*

Table 1. Biological activities of the secondary metabolites isolated from *A. flocculosus*

Compound Name (Number)	Chemical Class	Biological Activities	References
Mactanamide (4)		Inhibit osteoclastogenesis. Not cytotoxic against neuroblastoma Neuro2a cells up to 100μM Neuroprotective effect through antiradical effect	**[25, 29]**
Preussins C−I (6-12) and (11R)/(11S)-Preussins J and (11R)/(11S)-Preussins K (13 and 14)	**Alkaloids**	Inhibitory activity on the production of IL-6	**[30]**
Botryoisocoumarin A (18), **Mellein (19),** ***Cis*-4-hydroxymellein (20),** **And** **5-hydroxymellein (23)**	**Iso coumarin**	Cytotoxic against K562 cancer cell line	**[28]**
3-hydroxymellein (22)		Anti-trypanosomal activity	
Ochratoxin A (25)		Cytotoxic against HepG2 and A549 tumor cell lines.	**[23]**
Dihydropenicillic acid (29)	**Polyketide**	Antitrypanosomal	**[28]**
Ochrasperfloroid (38)		Inhibit IL-6 and NO production. Cytotoxicity against A549 and HepG2 tumor cell lines.	**[45]**
Aspersecosteroids A (39), **Aspersecosteroids B (40)** **and Asperflosterol (41)**		Inhibitory effects on the production of IL-6 and TNF-α in LPS-stimulated THP-1	**[24]**
(22R,23S)-epoxy-3b,11a,14b,16b-tetrahydroxyergosta-5,7-dien-12-one (42)	**Steroids**	Antimicrobial activities Cytotoxicity against HL-60 and BEL-7402 cells.	**[27]**
7-nor-ergosterolide (43)		Antimicrobial activities Cytotoxic toward BEL-7402 and HL-60 cells.	
3b-hydroxyergosta-8,24(28)-dien-7-one (44)		Antibacterial activity against E. aerogenes Cytotoxic toward BEL-7402 and HL-60 cells.	

Ergosterol (46)		Anti-trypanosomal activity	**[28]**
Ergosterol peroxide (47)		Anti-trypanosomal activity	
Asperflotone (49)		Inhibit the secretion of IL-6	**[35]**
Asperfloroid (50)			
Asperfloketals A (51)		Anti-inflammatory activity	**[36]**
Asperfloketals B (52)		No cytotoxicity toward SW480, HeLa, and HepG2 cell lines.	
Insulicolide A (53)		Cytotoxic against Neuro-2a, MCF-7, and 22Rv1 cells.	**[26, 41]**
6β,9α,14-trihydroxycinnamolide (54)		Cytotoxic toward MCF-7, 22Rv1, and Neuro-2a cells.	
6β,7β,14-trihydroxyconfertifolin (55)		Non-cytotoxic toward Neuro-2a cells.	
7α,14-dihydroxy-6β-p-nitrobenzoylconfertifolin (56)		Cytotoxic 22Rv1cells. Non-cytotoxic 22Rv1 and MCF-7 cells	
Aspertetranone A (57)		Non-cytotoxic against 22Rv1 Neuro-2a, cells.	**[26]**
Aspertetranone D (58)	**Terpenes**	Non-cytotoxic against 22Rv1 and Neuro-2a cells. Inhibit osteoclastogenesis.	**[25, 26]**
12-epi-aspertetranone D (59)		Non-cytotoxic toward Neuro-2a cell Cytotoxic against 22Rv1 cells.	**[26]**
Nine ophiobolin derivatives (61-69)		Cytotoxic against HCT15, NCI-H23, NUGC-3, ACHN, MDA-MB-231 and PC-3 cancer cell lines.	**[39]**
6-(1H-pyrrol-2-yl) hexa-1,3,5-trienyl-4-methoxy-2H-pyran-2-one (70)		Antibacterial activity.	**[27]**
Phomaligol A1 (72)		Antitrypanosomal	**[28]**
Diorcinol (73)		Anti-trypanosomal Cytotoxic effect against K562 cell line	
Wasabidienone E (74)	**Miscellaneous**	Inhibit osteoclastogenesis.	**[25]**
2,6-dimethyl-3-O-methyl-4-(2-methylbutyryl) phloroglucinol (79)		Inhibited the production of NO and expression levels of iNOS and COX-2 proteins	**[31]**

Conclusion

Isolation of *Aspergillus flocculosus* was reported from dissimilar sources, plants, sponges, algae, and sea sediment. Eighty compounds were reported from *Aspergillus flocculosus*. Those compounds are divided into fourteen alkaloids, three cerebrosides, nine isocoumarins, eleven polyketides, fifteen steroids, seventeen terpenes, and eleven miscellaneous compounds. Secondary metabolites reported from *Aspergillus flocculosus* possess various biological activities such as anti-inflammatory, anti-trypanosomal, anti-cancer, antimicrobial, and neuroprotective activities.

The current review provides updated

information about the different sources of *A. flocculosus* and its ability to biosynthesize a wide variety of potential secondary metabolites, additionally, throughout this review, we could conclude that the endophyte *A. flocculosus* is a relatively recent source for secondary metabolites, and further investigation of different strains isolated from different hosts and grown under variable conditions is highly recommended.

Declarations

Consent to publish

All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article in the main manuscript.

Conflict of Interest

The authors assert that there are no conflicts of interest.

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Authors Contribution

Conceptualization was performed by Abdel Nasser Singab, data preparation and collection of the draft was performed by Ashraqat Ismail and Ahmed Elissawy, and revision of the first draft was performed by Ahmed Elissawy, Haidy Gad and Abdel Nasser Singab. All authors have read and approved the final manuscript.

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