Review Article

Antimicrobial Activity of Secondary Metabolites Isolated from Endophytic Fungi Associated with Rubiaceae Species

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Abstract

The effectiveness of antibiotics has declined significantly due to development of drug resistance. Consequently, it results in millions of deaths due to infectious diseases. Many studies have suggested that bioactive compounds produced by endophytes could be an alternative to discover new antimicrobial compounds. Endophytes are microorganisms that reside within the tissues of living plants that cause no apparent harm to the host. Fungal endophytes may biosynthesize the same or similar compounds as their host plant and other diverse bioactive compounds, which provide various pharmacological activities. Therefore, this study aimed to conduct literature search on the antimicrobial properties of the isolated compounds produced by fungal endophytes associated with Rubiaceae. Literature was conducted on secondary metabolites of endophytic fungi via four databases; EBSCOhost, Google Scholar, PubMed, and Scopus. A search filter was performed to include only research articles from 2007 to 2021. The search was restricted to publications in English only. Overall, 29 publications were selected for full-text evaluation and were included in the study. Results showed that sixteen antimicrobial metabolites were isolated from six fungal endophytes of ten different plant species were identified. These compounds were classified as alkaloid, phenol, coumarin, steroid, diterpene, and meroterpene.

Keywords: Antimicrobial, secondary metabolites, endophytic fungi, fungal endophyte, Rubiaceae

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1.0 Introduction

Antimicrobial activity refers to the action of killing or suppressing the growth of microorganisms including bacteria, viruses, fungi, helminth, and protozoa. An antimicrobial is used to prevent and treat infections in humans, animals, and plants. However, the effectiveness of antibiotics declines significantly as pathogens are prone to develop drug resistance. Consequently, it leads to difficulty in treating infection and death. Antimicrobial resistance has been ranked as the top ten global public health threat by the World Health Organization (WHO) (1-5).

Recent studies have shown that bioactive compounds produced by endophytes could be an alternative source for the discovery of new antimicrobial compounds. Studies conducted by Schulz *et al.* (6) found that 51% of bioactive compounds produced by fungal endophytes have new chemical structures. Endophytes are microorganisms that reside within the tissues of living plants and cause no apparent harm to the host (7). The most common microbes that present as endophytes are fungi and bacteria (8). Endophytes, especially endophytic fungi are able to biosynthesize the same or similar compounds as their host plant and as well as diverse bioactive compounds that act as the protection to the host's microenvironment. These natural products from endophytes are said to have important biological activity such as antimicrobials, anticarcinogens, immune-suppressants or antioxidants (9-10).

The Rubiaceae belongs to a family of flowering plants and it is one of the largest angiosperm families (11). This family consists of 650 genera and 13,000 species of herbs, trees, and shrubs which are found mainly in tropical and subtropical regions [12]. Various species of Rubiaceae have been widely found to produce bioactive metabolites with a diverse and great pharmacological potential (13). This includes anti-inflammatory, analgesic, mutagenic, antiviral, antibacterial, and antioxidant. Besides, previous studies have shown that the secondary metabolites synthesized by endophytic fungi isolated from Rubiaceae also exhibited various biological activity including antimicrobial effect (14).

Therefore, the present study is aimed to conduct a literature search on antimicrobial properties of compounds produced by fungal endophytes originating from Rubiaceae species.

2.0 Methods

In this review, information on the secondary metabolite of fungal endophytes associated with Rubiaceae was gathered via four search databases, including EBSCOhost, Google Scholar, PubMed, and Scopus. A search filter was performed to include only research articles from 2007 to 2021. The search was restricted to English publications only.

After the extensive search, the articles found were manually screened and selected based on their relevance of evidence and aims in line with the current study. Overall, 29 articles were selected for full-text evaluation and included in the study. Publications that did not fulfil the inclusion and exclusion criteria were excluded from this study. Any potential articles from the reference section of the studies found were also reviewed. For ease of the review, the article was tabulated based on the article's title, year of publication, and place of research conducted.

3.0 Results and discussion

3.1. *Chemical and biological aspects of Rubiaceae*

Rubiaceae is the fourth largest family of flowering plants that comprise 650 genera and 13,000 species of herbs, trees,

and shrubs (11,12). Rubiaceae is found predominantly in the tropical region (12). The distribution of this family is ubiquitous except in the Antarctic Continent. Rubiaceae consists of three subfamilies which are Cinchonoideae, Rubioideae, and Ixoroideae. This family has a significant diversity of natural products such as alkaloids, anthraquinones, flavonoids, indole, iridoids, terpenoids, and other phenolic derivatives. Many Rubiaceae species plants are widely used especially in traditional medicine and some exhibit anti-inflammatory, analgesic, mutagenic, antiviral, antibacterial, and antioxidant activity (13-16).

In the Cinchonoideae subfamily, *Uncaria* species are found to be alkaloid-rich plants (13). Previous studies showed that alkaloid extract from *Uncaria tomentosa*, popularly known as cat's claw displayed immunostimulant and antitumor activity (17, 18). In Brazil, traditionally this plant is used for the treatment of arthritis. *Uncaria gambier* (W. Hunter) Roxb is native to Southeast Asia, particularly Malaysia and Indonesia. Traditionally gambier is used for the treatment of burn wound healing, diarrhea, sore throat anti-cancer and immunomodulator and sometimes as a food additive (19, 20). The genus of *Cinchona*, native to the Andean forests of South America, is an important source of quinine. Quinine from Cinchona bark was the first chemical compound that was found to effectively treat malaria, and it has been used for over 350 years (21). Cinchona is commonly used in folk medicine to treat fever and stomach problems and to stimulate the appetite. The bark of Cinchona is also a source of quinidine which is a compound that is useful for cardiac depressants.

Guettarda speciosa is predominantly found in the tropical country from East Africa to South Asia. The extract of *Guettarda speciosa* leaves is used traditionally in treatments of cold, cough, headache, and sore throat. Other parts of the plants have also been used in some countries, such as bark in New Guinea and In Tonga, to treat dysentery and epilepsy, respectively (22). The inner part of the bark is used in India as folk medicine to treat wounds, inflammation, ulcer, and anticholinergic applications. In addition, the stem of *Guettarda speciosa* is used as folk medicine in Fiji to stimulate menstruation (22, 23). *Guettarda speciosa* reported to contain phenolic and steroidal compounds such as squalene, campesterol and stigmasterol (24).

The genus *Mitragyna* distributed in tropical and subtropical regions of Africa and Asia has been used in traditional medicine to treat cough, fever, diarrhea, inflammation, and muscle aches (25, 26). In Malaysia and Thailand, the leaves of *Mitragyna speciosa* (Korth.) or also known as "biak," "ketum," and "kratom," have been used for their opium-like and cocaine-like effects to relieve fatigue and enhance work productivity. In the colonial era, Malaya used the leaves to replace opium and is also used to wean addicts off in morphine addiction by the native of Thailand (27). However, due to its narcotic effects, some countries have banned the consumption of the *Mitragyna speciosa* (Korth.) plant. Mitragynine, an indole alkaloid, has been the main constituent of the *Mitragyna* genus and other bioactive metabolites, including triterpenoids and flavonoids (26, 28-30). Gong *et al.* (31) have reported various therapeutic effects of the *Mitragyna* genus, including anti-inflammatory, antinociceptive, anti-diarrheal, antioxidant, anticancer, antimicrobial, and antidiabetic. As antidiabetic, the crude alkaloidal extracts of *Mitragyna speciosa* leaves were found to enhance the glucose uptake rate and increase glucose transport activity as it increases the key enzyme activities (32).

In the Rubioideae subfamily, the *Psychotria* genus is the most abundant species that consist of 1600 species (33). Psychotria genus can produce natural products that affect the central nervous system. For instance, the leaves of *Psychotria Viridis* are used as a part of making a hallucinogenic drink. Some of this *Psychotria* genera plant is broadly used as folk medicines to treat bronchitis, cough, ulcer, and stomachache. The *Spermacoce verticillata* (L.), also known as "vassourinha de botão", is indigenous to South America. This plant mainly contains indole alkaloids which are borrevine and borreverine compounds (34). Traditionally, Brazilians used this medicinal plant in the treatment of inflammatory processes. *Paederia foetida*, also known as skunk-vine with antispasmodic, anthelmintic, antitussive, antidiarrheal, anti-inflammatory and antioxidant activity, is another important genus (35). The decoction of the whole part of this plant is commonly used in Ayurveda, an Indian medicine system. Besides, the extraction of *Paederia foetida* fruits is used in the treatment of toothache.

Morinda citrifolia is commonly called Noni, and other vernacular names include "Indian mulberry", "mengkudu", "nhau", and "cheese fruit", which depends on the country (36). *Morinda citrifolia* been used as a food in the tropical country since the early centuries (37). It is also used as traditional medicine in Hawaii and Polynesia. Besides, the use has been extended worldwide as a dietary supplement and natural health enhance. A variety of biological activity of *Morinda citrifolia* has been reported which includes analgesics, anti-inflammatory, anti-oxidant, antidiabetic, antimicrobial, hypotensive and immune enhancer (37). All the plant parts, such as roots, barks, leaves, fruits, and seeds, are used, and about 200 phytochemicals were found (38). However, the study and research on the total amount of phytochemical of *Morinda citrifolia* is yet to be reported the fruits contribute to a large amount of important bioactive metabolites. In Taiwan, the study of seedless fermented *Morinda citrifolia* juice has been proposed to have potential in the production of probiotics resulting from the interaction of *Morinda citrifolia* juice with lactic acid of bacteria (*Lactobacillus plantarum* and *Lactobacillus casei*) (39).

In the *Coffea* genus, the *Coffea arabica* is the most studied and popular species (40). This genus belongs to the Ixoroideae subfamily. The seed extract from this coffee plant contains a biologically active substance such as alkaloids or specifically known as caffeine. This compound is a central nervous system stimulant that also exerts its effect on kidneys and blood vessels (41). The *Mussaenda* species is distributed mainly in the Old-World tropics, including West Africa and Asia. Some vital species were also broadly found in India, China, Sri Lanka, Eastern and Central Nepal. The *Mussaenda* species produce important bioactive metabolites, which are iridoids, flavonoids, and triterpene, that provide various pharmacological functions (42). In China, *Mussaenda pubescens* is used in folk medicines for antipyretic, antichloristic and diuretics. Besides, the plant is also used as a detoxifying, contraceptive, and abortive agent in early pregnancy (43).

3.2. *Endophytic fungi*

Endophytes can be divided into two distinct subgroups, namely, facultative and obligate. Facultative endophytes can live outside the plant tissues, such as soil and artificial nutrients (44). In comparison, obligate endophytes reside inside the plant tissues and rely on the metabolism of their hosts (45). The most common microbes present as endophytes

are fungi and bacteria (8). Endophytes have a significant biological diversity that grows naturally in tropical, temperate, and boreal forests (46). According to Bills *et al*. (47), tropical endophytes produce higher active metabolites than endophytes in temperate areas.

Among all the types of endophytes, endophytic fungi are the most widely isolated (9). Fungal endophytes can be classified into two large groups; Balansiaceous and non-Balansiaceous (48). The balansiaceous or grass endophytes belong to clavicipitaceous genera, and their growth involves vertical transmission. Non-balansiaceous endophytes consist of a broad range of fungi, from ascomycetes to basidiomycetes. Fungal endophytes can provide beneficial effects to their host plants primarily promoting plant growth and increasing the plant resistance to biotic and abiotic stresses (46). Biotic stresses include pathogens and herbivores. Meanwhile abiotic stresses consist of drought, salinity, and heavy metals. Endophytes employed several mechanisms for adapting the plant to these stresses, such as antimicrobial and antioxidant responses (49). The population distribution and structure of fungal endophytes are influenced by their hosts' ecological environment and genetics (46).

3.3. *Collection and isolation of endophytic fungi from plants*

The detection of fungal endophytes are associated with plant species, location, habitat distributions, tissue types, age, plant-endophyte interactions, fungal colonization species, and culture environment (50). Yet another study suggested that nutrition, isolation temperature, and surroundings may also contribute to the high quantity of fungal endophytes (51).

Isolation of endophytes is a crucial step as it needs careful action to ensure optimal number recovery of colonized endophytes and accuracy in obtaining the desired type of endophytic microorganisms. Endophytic fungi can be isolated from various parts of plants, such as stems, roots, leaves, bark, and fruits (9). Agusta *et al*. (52) reported fifty-three filamentous fungi isolated from the stems, leaves, fruits, and roots of *Uncaria gambier*. The stems of *Uncaria gambier* have the most abundant isolated fungi when compared to other parts the plant. Plants should appear to be healthy and disease-free, restricting the isolation of localized pathogenic endophytes (53, 54). A deep understanding of the methods and rationale used can increase the potential of good endophyte isolation since one plant could employ numerous endophytes.

3.4. *Antimicrobial activity of secondary metabolites produced by endophytic fungi isolated from Rubiaceae*

The study on the secondary metabolite of fungal endophytes isolated from Rubiaceae species was first published in 1999. This study observed taxol, an anticancer compound produced by the fungus *Seimatoantlerium tepuiense* collected from *Maguireothamnus speciosus* (55). From then on, research on endophytes associated with the Rubiaceae species has been extended and reported to produce several bioactive compounds with diverse chemical structures and pharmacological activities. Rubiaceae has a significant diversity of natural products such as alkaloids, anthraquinones, flavonoids, indole, iridoids, terpenoids, and other phenolic derivatives.

Five antifungal compounds were produced by *Penicillium* sp. an endophyte isolated from *Alibertia macrophylla*. (Table 1) *Penicillium* sp. is a widely studied fungus since antibiotics penicillin

was discovered. It also has an abundance of pharmacochemical constituents such as alkaloids, terpenoids, polyketides and peptides (56). The known compounds, (R)-7-hydroxymellein (**1**), (3R,4R)-4,7-dihydroxymellein (**2**), 4-hydroxymellein (**3**) and orcinol (**4**) (Figure 1) display potent antifungal activity against *C. cladosporioides* and *C. sphaerospermum* with a detection limit of 5.00 and 10.00 µg, respectively (57, 58). Meanwhile, the newly isolated coumarin compound, 8-methoxymellein (**5**) showed moderate fungitoxicity towards yeast with a detection limit of 10.0 and 25.0 µg, respectively (57).

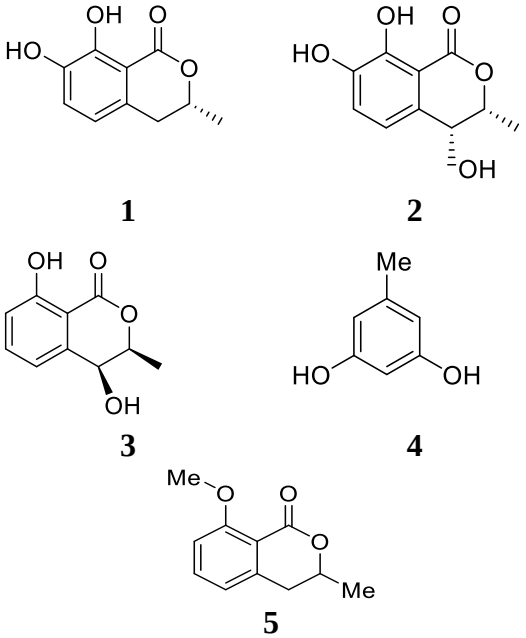


Figure 1: Compounds extracted from endophytic fungi of *Alibertia macrophylla*.

An antibacterial constituent, roquefortine C (**6**) and 11-bromoroquefortine C (**6** were produced by *Penicillium chrysogenum*, an endophyte isolated from green leaves of *Coffea arabica* (Figure 2) (15). The roquefortine C (**6**) and 11-bromoroquefortine C (**7**) inhibits *B. subtilis* at a concentration of 7.7 µmol L^{-1} and 15.4 µmol L^{-1}, respectively. Another study associated with roquefortine C (**7**) antimicrobial activity also has been reported (59). The bacteriostasis was observed at a concentration of 257 µmol L^{-1} meanwhile, mitigation occurred at 51.4 µmol L^{-1}.

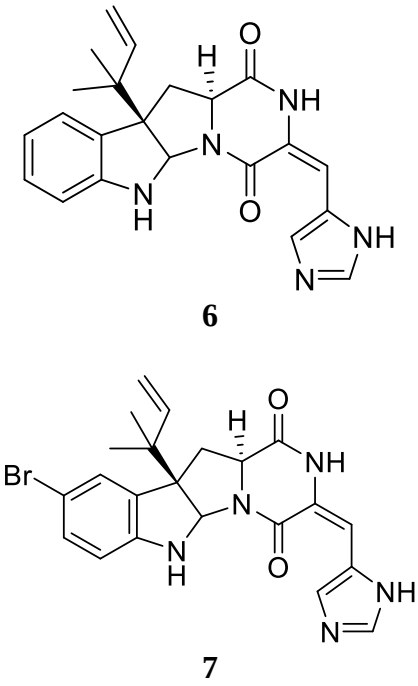


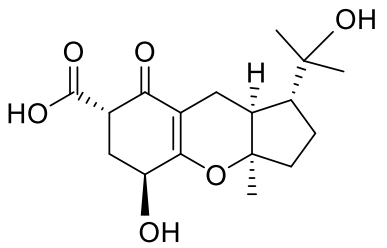
Figure 2: Compounds extracted from endophytic fungi of *Coffea arabica*.

Fungal endophytes from *Scyphiphora hydrophyllacea* were also found to display antimicrobial activity against *S. aureus*, MRSA-SK1, *C. albicans*, *C. neoformans* and *M. gypseum*. Fungus *Guignardia* sp. originating from the mangrove plant *Scyphiphora hydrophyllacea* was found to produce guignardone I (**8**) and B (**9**) (Figure 3) (60). At the concentration of 65 µmol L^{-1}, guignardone I (**8**) displayed moderate antibacterial activity meanwhile guignardone B (9) showed a weak effect against methicillin-resistant Staphylococcus aureus (MRSA) with diameters of inhibition zone 11.0 mm and 8.0 mm, respectively.

Table 1: Antimicrobial activity of compounds produced by endophytic fungi isolated from various genera of Rubiaceae.

Host plant	Compound	Endophyte	Microorganisms tested			Activity	Ref.
			Gram positive	Gram negative	Fungi		
Alibertia macrophylla	(R)-7-hydroxymellein (**1**) (3R,4R)-4,7-dihydroxymellein (**2**) 4-hydroxymellein (**3**) 8-methoxymellein (**5**) Orcinol (**4**)	*Penicillium* sp.	-	-	*C. cladosporioides, C. sphaerospermum*	Antifungal	57, 58
Coffea arabica	11-bromoroquefortine (**6**) Roquefortine C (**7**)	*Penicillium chrysogenum*	*B. subtilis*	-	-	Antibacterial	15
Scyphiphora hydrophyllacea	Guignardone I (**8**) Guignardone B (**9**)	*Guignardia* sp.	methicillin-resistant *S. aureus*, *S. aureus*	-	-	Antibacterial	60
Morinda citrifolia	Koninginols A (**10**) Koninginols B (**11**)	*Trichoderma koningiopsis*	*B. subtilis, S. aureus*	*E. coli*	-	Antibacterial	62
Mussaenda luteola	Resorcinol (**12**)	*Chaetomium cupreum*	*S. aureus*	*E. coli*	-	Antibacterial	51
			M. tuberculosis				

Host plant	Compound	Endophyte	Microorganisms tested			Activity	Ref.
			Gram positive	Gram negative	Fungi		
Mussaenda luteola	6- (heptacosa-18ˈZenyl)-2-(-18ˈˈ hydroxyl-1ˈˈenyl-19ˈˈoxy)-3-hydroxybenzoquinone (**13**) (3β–5α– Dihydroxy –6β–phenyl acetyloxy–ergosta –7, 22 – diene) (**14**)	*Chaetomium cupreum*	*M. tuberculosis*		-	Antimycobacterial	68
Uncaria gambier	(+)-1,1'-bislunatin (**15**) (+)-2,2'-epicytoskyrin A (**16**)	*Diaporthe* sp.	-	-	*A.flavus, A. niger, H. burtonii, W. anomalus, F. oxysporum, R. toruloides, R. minuta, Candida spp.,*	Antifungal	72



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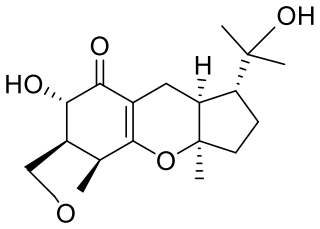

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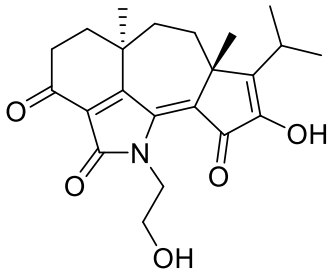
Figure 3: Compounds extracted from endophytic fungi of *Scyphiphora hydrophyllacea*.

Secondary metabolites of ten endophytic fungi obtained from twig *Morinda citrifolia* exhibited a broad-spectrum inhibitory activity against gram-positive, gram-negative bacteria and yeast. The tested microbes were B. subtilis, E. coli, S. aureus, S. typhimurium, and C. albicans. In addition, the antimicrobial activity of bacteria endophytes, as studied by Mai *et al.* (61), reported crude extract of *Actinomycetes* spp. collected from the leaf of *Morinda citrifolia* produced 118% of total inhibition. Meanwhile, gentamicin that acts as positive control showed 104% of total inhibition. Koninginols A (**10**) and koninginols B (**11**) (Figure 4) isolated from *Trichoderma koningiopsis* were positive against *B. subtilis* with the MIC values of 10μg/mL and 2μg/mL, respectively (62).



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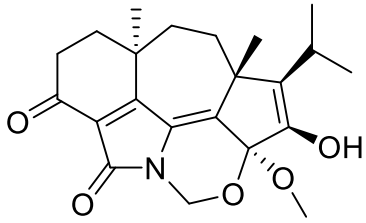

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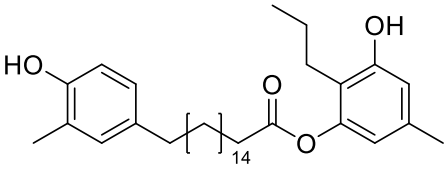

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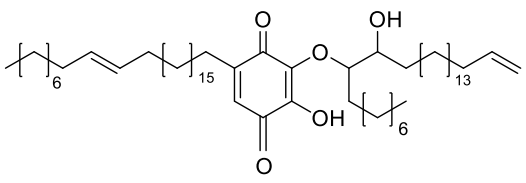
Figure 4: Compounds extracted from endophytic fungi of *Morinda citrifolia* and *Mussaenda luteola*, respectively.

Four fungal endophytes were isolated from several parts of *the Mussaenda luteola* plant (63). Crude extract of *Alternaria* sp. was positive against all microorganisms tested (*B. subtilis, S. aureus, E. coli* and *P. aeruginosa*. The highest inhibition zone of *Alternaria* sp. was 11.5 mm against *E. coli*. Besides, *Aspergillus* sp. and *Penicillium* sp. extracts displayed significant inhibition against *E. coli* and *P. aeruginosa*. Shylaja *et al.* (51) reported the isolation of a known compound, resorcinol (**13**) (Figure 4) type lipid synthesized by endophytic fungus *C. cupreum,* from leaves of *Mussaenda luteola*. *Chaetomium* sp. is one of the promising biological fungal endophytes discovered to produce diverse pharmacological metabolites such as anthraquinones, chaetoglobosins, depsidones, terpenoids, xanthones and steroids [64-66]. At a concentration of 40 μg/mL, resorcinol

(**12**) type lipid showed potent antibacterial activity against *S. aureus* and *E. coli* with 14 mm of inhibition zone. Resorcinol (**12**) inhibited the growth of *M. tuberculosis* with the MIC value of 6.3 µg/mL, equivalent to the streptomycin. Nagy *et al.* (67) discovered that the chemical structure of resorcinol lipids, 1, 3-dihydroxybenzene core with saturated chains at 5-position of the aromatic ring were associated with diverse biological function.

The continued search for endophytes associated with *Mussaenda luteola* led to two new compounds: 6-(heptacosa-180Zenyl)-2-(18" hydroxyl-1" enyl-19" oxy)-3-hydroxybenzoquinone (**13**) and (3β–5α–dihydroxy–6β–phenyl acetyloxy–ergosta–7, 22–diene) **(14)** (Figure 5) (68). These compounds produced by endophytic fungus *C. cupreum* exhibited significant antimycobacterial activity against *M. tuberculosis* with MIC values of 25µg/mL and 6.25µg/mL, respectively. The MIC value of this steroid compound **(14)** was equivalent to the streptomycin. Rugutt *et al.* (69) reported that optimum antimycobacterial was observed in C20 aliphatic compound (Phytol) with the MIC value of 2 µg/mL. Meanwhile, the structure of com-pound **(14)** with benzoquinone moiety attached to two linear alky chains more than C20 postulate to cause steric hindrance, which may reduce the antimycobacterial activity (Figure 5). However, the MIC obtained (25µg/mL) shows significant inhibition.



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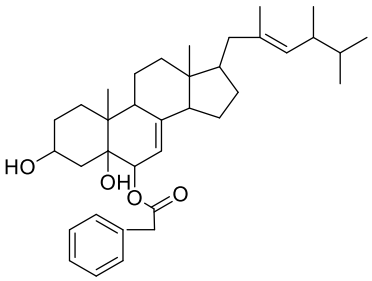

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Figure 5: Compounds extracted from endophytic fungi of *Mussaenda luteola*.

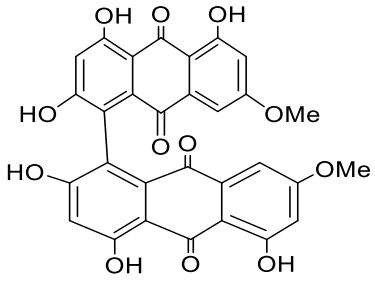
Thirawatthana *et al.* (70) reported fifty-five endophytic fungi were isolated from *Mitrajyna javanica* Koord and Val cultivated in Thailand. The extracts exhibited antimicrobial activity against at least one of the tested microorganisms, *B. subtilis, E. coli, S. aureus, P. aeruginosa, S. cerevisiae,* and *C. albicans*. In addition, *Cladosporium* sp., *Mycelia sterilia, Phomopsis* sp. and *Nodulisporium* sp. showed a broad-spectrum antimicrobial with the inhibition zone ranging from 8 to 40 mm. Among all fungal endophytes, *Nodulisporium* sp. showed a potent inhibition activity against all tested microorganisms. A total of forty-four fungal endophytes were isolated from the base and top leaves of Brazilian *Spermacoce verticillata* (71). In this study, the antibacterial test was performed using agar plug and disk diffusion assay. *Mycelia sterilia, Penicillium griseofulvum,* and *Penicilium aurantiogri-seum* exhibited inhibitory activity against *B. subtilis* and *S.aureus. Penicillium griseofulvum* and *Penicilium aurantiogriseum* produced inhibition zone more than 15 mm in both liquid and solid assays.

Ilyas *et al.* (52) explored endophytic fungi living in plants of *Uncaria gambier* Roxb. from West Sumatra. Fifty-three filamentous fungi were isolated from stems, leaves, fruits, and roots of *Uncaria gambier* var. nasi and var. udang, which mostly belong to Coelomycetes. The fungus *Diaporthe* sp. isolated from *Uncaria*

gambier produce two rare bisanthraquinones, (+)-1,1'-bislunatin (**15**) and (+)-2,2'-epicytoskyrin A (**16**) [72,] . (+)-2,2'-epicytoskyrin A (**16**) is analogues to rugulosin and cytoskyrin A (Figure 6) (72). (+)-2,2'-epicytoskyrin A (**1**) possess a similar anthraquinone backbone with rugulosin and has methoxy as a substitution group. Cytoskyrin A is an epimer of (+)-2,2'-epicytoskyrin A (**16**) with hydrogen at C-2 and C-2'. Cytoskyrin A was found to have a potent antibacterial activity towards gram-positive with MIC values ranged from 0.03 to 0.25 µg/mL (73). Therefore, the hydroxyl optical position (+)-2,2'-epicytoskyrin (**16**) proposed to play a role in the activity. The (+)-2,2'-epicytoskyrin A (**16**) exhibit a low antifungal activity with the MICs ranged from 16 to 128 ug/mL (72). The MIC values against *A. flavus* and *A.niger* were four times larger than MIC values of nystatin. The exposure of (+)-2,2'-epicytoskyrin A (**16**) displayed shrinkage of *C. tropicalis* and exhibited a similar trend of cytoplasmic material leakage as nystatin. This suggested that the action of (+)-2,2'-epicytoskyrin A (**16**) is through membrane disruption however, the specific mechanisms should be studied further. (+)-1,1'-bislunatin (**15**) isolated from *Diaporthe* sp. derived from *Uncaria gambier* displayed moderate antibiotic activity against several bacteria with MIC values ranging from 32 to 64 µg/mL (72). Exposure to (+)-1,1'-bislunatin (**15**) caused leakage of nucleic acids, protein, K^+ and Ca^{2+} in *B. subtilis* and *E. coli* cells. Compound **15** was found to interfere with the permeability of bacterial cell and caused changes in cell morphology.

4.0 Conclusion

Over all four known compounds, produced by *Penicillium* sp. an endophte isolated from *Alibertia macrophylla*. (R)-7-hydroxymellein (**1**), (3R,4R)-4,7-dihydroxymellein (**2**), 4-hydroxymellein (**3**) and orcinol (**4**) displayed potent antifungal activity against *C. cladosporioides* and *C. sphaerospermum.* This review revealed a significant interest in fungal endophytes isolated from Rubiaceae and its potential as a promising source of new novel metabolites to tackle the expanding drug resistance problems.



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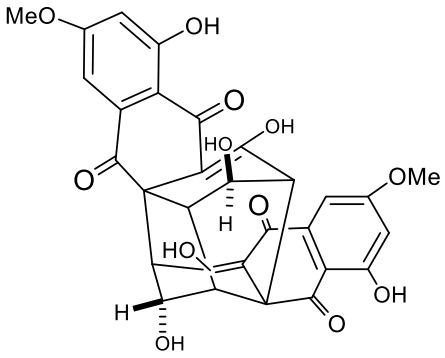

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Figure 6: Cytoskyrin A, rugulosin and compounds extracted from endophytic fungi of *Uncaria gambier*.

This review further highlights the phytochemical studies of secondary metabolites produced by endophytic fungi associated with Rubiaceae species. The antimicrobial metabolites described have been isolated from fungal endophytes of ten different plant species, and sixteen compounds were identified. These compounds can be classified as alkaloid, phenol, coumarin, steroid, diterpene, and meroterpene.

Conflicts of interests

The authors declare no conflict of interest.

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