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Antimicrobial activity of essential oil from the rhizomes of *Amomum rubidum* growing in Vietnam

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Abstract
Bearing in mind the lack of scientific studies focused on the biological activity of essential oil from *Amomum rubudum*, this work was designed to validate the antimicrobial property ascribed to this traditional species. Essential oil was extracted from the air-dried and pulverized rhizomes of *A. rubidum* by hydrodistillation in an all glass Clevenger-type apparatus. The antimicrobial activity was established by the method of microdilution broth susceptibility assay. *A. rubidum* essential oil displayed inhibitory action against *Escherichia coli* (ATCC 25922) and *Fusarium oxysporum* (ATCC 48112) with Minimum inhibitory concentration (MIC) values of 50 µg/mL. The antimicrobial activity of *A. rubidum* rhizome essential oil was being reported for the first time. Overall, the results herein presented sustain and strengthen the antimicrobial property traditionally ascribed to *A. rubidium*.

Keywords: Amomum rubudum, *essential oil, antimicrobial activity*

1. Introduction

Amomum rubidum Lamxay & N. S. Lý (Syn. *Conamomum rubidum* (Lamxay & N.S. Lý) is included in the list of threatened and endanger species [1]. The plant has been used in ethnomedicine for the treatment of inflammation related disorder, ulcer and fever. In addition, various parts of the plant were used to treat microbial infections [2]. Till moment no information could be seen on either the chemical constituents of volatile and non-volatile fractions or their biological activities. However, the essential oils isolated from other *Amomum* plants were known to have exhibited a number of biological activities of importance. The oil of *A. uliginosum* exhibited larvicdial action against *Aedes aegypti* [3]. The essential oils from *A. krervanh* were highly effective as oviposition deterrent, ovicidal and adulticidal activities against *Ae. Albopictus* [4] while the oil from *A. subulatum* was toxic to the insect pests such as *Anopheles subpictus*, *Ae. albopictus* and *Culex tritaeniorhynchus* [5]. The essential oil from various parts of *A. subulatum* showed good antimicrobial efficacy against *Bacillus pumilus, Staphylococcus epidermidis, Pseudomona aerugenosa, Aspergillus niger* and *Saccharomyces cerevisiae* [6-8]. The antimutagenic and cytotoxicity activities of the essential oils from *A. uliginosum* [9] have been reported. Also, the cytotoxicity and antioxidant potentials of essential oils of *A. tsao-ko* [10] had also been recently reported.

Previously, the compositions and biological activities of essential oils from various parts of *A. rubidium* were determined and reported. 1,8-Cineole (37.7%), δ-3-carene (19.5%) and limonene (16.3%) were the main compounds in the leaf oil, while δ-3-carene (21.9%), limonene (17.8%) and β-phellandrene (14.6%) occurred in higher quantity in the stem essential oil [11]. The leaf and stem essential oils of of *A. rubidium* displayed strong antimicrobial activity towards *P. aeruginosa* with minimum inhibitory concentration (MIC) of 25 µg/mL and 50 µg/mL respectively. The stem essential oil was also active against *Candida albicans* (MIC, 50 µg/mL) while both essential oils inhibited the growth of *Fusarium oxysporum* (MIC 50 µg/mL) [11]. The rhizome oil whose major constituents were β-phellandrene (16.1%), limonene (14.4%), δ-3-carene (13.9%) and eugenol (9.2%) was found to displayed larvicidal activity against *Ae. aegypti* with LC_{50} values of 22.85µg/mL (24 h) and 22.62 µg/mL (48 h) [12].

The aim of the present study was to examine the antimicrobial activity of essential oil from the rhizomes of *A. rubidum* grown in Vietnam for the first time and determine its potential uses.

2. Materials and methods

2.1 Plant collection and identification

The rhizomes of *A. rubidum* were harvested from mature plants growing in Bidoup Nui Ba National Park, Lam Dong Province on October 2018. Botanical identification was achieved by Dr. Dai, D.N. A voucher specimen DND 749 was deposited at the Botany Museum, NghệAn College of Economics, Vietnam.

2.2 Hydrodistillation of essential oil

For this experiment, 3 kg of the air-dried and pulverized rhizomes of *A. rubidum* was used at different times. Known weight of samples was carefully introduced into a 5 L flask and distilled water was added until it covers the sample completely. Essential oils were obtained hydrodistillation which was carried out in an all glass Clevenger-type distillation unit designed according to the Vietnamese Pharmacopoeia [13]. The distillation time was 3 h and conducted at normal pressure. The volatile oils distilled over water and were collected by running through the tap in the receiver arm of the apparatus into clean and previously weighed sample bottles. The oils were kept under refrigeration (4 ºC) until the moment of analyses as described previously [12].

2.1 Antimicrobial assay

Eight standardized ATCC strains from laboratory stock cultures were used in the evaluation of the antimicrobial activity of the oils of *A. rubidum*. The Gram-negative strains were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25923). The Gram-positive strains were *Bacillus subtilis* (ATCC 11774) and *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Aspergillus niger* (ATCC 9763) and *Fusarium oxysporum* (ATCC 48112). Two strains of yeast, *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 16404) were also used for the experiment. Testing media included Mueller-Hinton Agar (MHA) used for bacteria and Sabouraud Agar (SA) used for fungi. The Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay [14, 15]. For the assays, the essential oil was diluted with DMSO and loaded into the microtiter plate with each of the microbial strains. The plate was then incubated overnight at 37ºC. One hundred microlitre of microbial culture of an approximate inoculums size of 1.0 x 10^6 CFU/mL was added to all well and incubated at 37ºC for 24 h. The last row, containing only the serial dilutions of sample without microorganisms, was used as a negative control. Sterile distilled water and DMSO served as a positive control. All experiments were performed in triplicate. The MIC values were determined as the lowest concentration of the test sample that completely inhibits the growth of microorganisms.

2.2 Statistical analysis

Statistical analysis of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD) of three independent measurements using Microsoft excel program 2003.

3. Results & Discussion

The yield of essential oils was 0.13% (v/w), calculated on a dry weight basis. The essential oil was light yellow coloured. The rhizome essential oil of *A. rubidum* showed stronger inhibitory effect on both *A. niger* and *F. oxysporum* with MIC values of 50 µg/mL. As shown in Table 1, however, the essential displayed insignificant activity against other microorganisms used in this experiment. The observed antimicrobial activity of the rhizome oil against *F. oxysporum* was in agreement with data obtained for the leaf and stem essential oils [11]. However, while the rhizome oil in this study inhibited the growth of *A. niger*, the lea and stem oil displayed stronger inhibition of *P. aeruginosa* [11]. In addition, only the stem essential oil was active against *C. albicans* (MIC, 50 µg/mL) [11] contrary to the leaf and rhizome oil.

The observed result of *A. rubidium* oil was in agreement with information that *Amomum* oil samples posses' antimicrobial actions. The antimicrobial activities of essential oils of some *Amomum* species were reported previously. The essential oil from whole fruits of *A. subulatum* showed good antimicrobial results against *B. pumilus, S. epidermidis, P. aerugenosa* and *S. cerevisiae* with diameter of zone of inhibition (ZI) of 20 mm, 20 mm, 16 mm and 16 mm respectively [6]. The essential oil from leaf of *A. subulatum* inhibited the growth of *C. albicans* (ATCC 2019) and *S. cerevisiae* (11-161) with MIC values of 3.125 mg/mL and MIC 6.25 mg/mL respectively [7]. The seed oils of *A. subulatum* were also active against *S. aureus* (ATCC 6816), *E. coli* (ATCC 25922), *B. subtilis* (ATCC 6633) and *Salmonella typhinurium* (ATCC 14028) all with MIC of 0.0468mg/mL [7]. Moreover, essential oils from the rind of *A. subulatum* exhibited activity against the fungus *A. niger* with MIC of 19.5 µg/mL [8].

The antimicrobial activity of essential oil from the fruit of *A. uliginosum* were also determined and reported. The oil exhibited antibacterial activity against *E. coli* (O157:H7), *E. coli* (DMST 12743) and *S. aureus* (DMST 8013) with MIC values of 75 µL/mL [9]. The fruits essential oil of *A. cannicarpum* showed good antimicrobial activity against *Salmonella typhi*, *P. aeruginosa*, *Proteus vulgaris*, *C. albicans, C. glabrata* and *S. aurues* with diameter of zone of inhibitions determined to be 10 mm, 10.3 mm, 11 mm, 11.7 mm, 11.7 mm and 11 mm respectively [16]. However, the leaf oil displayed more potent and significant antimicrobial activity against some pathogens including *B. subtilis* (MTCC 441), *P. fluorescens* (MTCC 103) and *E. coli* (MTCC 443) with ZI of 20 mm, 13 mm and 12 mm respectively [17]. Essential oils obtained from the fruits of *A. kravanh* also possessed antibacterial activity depicted by inhibiting the growth of *S. albus* (ATCC 25923; MIC 5.0 mg/mL), *B. subtilis* (ATCC 6051; MIC 2.5 mg/mL), *Salmonella enterica* (ATCC 19430; MIC 2.5 mg/mL), *Shigella dysenteriae* (CMCC 51252; MIC 1.25 mg/mL) and *E. coli* (ATCC 25922; MIC, 2.5 mg/mL) [18]. The oil of *A. tsao-ko* exerted the strong bactericidal activity against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *B. subtilis* (ATCC 6051) and *Salmonella typhinurium* (ATCC 14028) with MIC of 3.13, 3.13, 6.25 and 6.25 mg/mL respectively [19]. The fruit oil of *A. tsao-ko* exerted antimicrobial activity against *A. oryzae, B. subtilis, S. albus,* and *Rhizopus sp.* with ZI of 23.1 mm, 20.5 mm, 17.2 mm and 16.2 mm respectively [20]. Moreover, the seed oil of *A. tsao-ko* also displayed antimicrobial potentials towards *S. aureus* (CCTCC AB91118), *B. subtilis* (CCTCC AB92068), *E. coli* (CCTCC AB90054), *P. vulgaris* (CCTCC AB91103), *S. typhinurium* (CCTCC AB94010), and *Candia* sp. (CCTCC AY910001) with MIC of 0.20, 3,13, 1.56, 6.25, 6.25 and 3.13 mg/mL respectively [21].

It could be deduced that the essential oil hydrodistilled from the rhizome of *A. rubidium* grown in Vietnam exhibited antimicrobial activity against targeted micro-organisms with MIC values favorably comparable with data obtained from other *Amomum* oil samples [6-12, 16-21] tested for their antimicrobial effects.

Essential oils are complex mixture of odoriferous substances consisting of diverse structural pattern. Majority of these substances were reported to displayed potent biological importance. The biological action exhibited by an essential oil normally depends on the major constituents or synergy between the major and some minor compounds. The observed antimicrobial activity of *A. rubidum* rhizome essential oil can be related to the major compounds or a synergy between the major and some minor compounds present therein [22]. The main constituents of the rhizome essential oil of *A. rubidum* [12] used for this study namely limonene [23, 24], eugenol [25], α-phellandrene [26] and δ-3-carene [22] were previously reported to inhibit significantly the growth and cell viability of potential infectious of broad spectrum microorganisms. Therefore, the observed antimicrobial action of the essential oil may be attributed to the compounds such as limonene, eugenol, α-phellandrene and δ-3-carene, previously identified in the oil sample.

Table 1: Antimicrobial activity of rhizome essential oil of *A. rubidium*

Microorganisms	Minimum inhibitory concentration (µg/mL)
E. coli (ATCC 25922)	-
P. aeruginosa (ATCC 25923)	-
B. subtilis (ATCC 11774)	-
S. aureus subsp. *aureus* (ATCC 11632)	-
A. niger (ATCC 9763)	50 ±0.50
F. oxysporum (ATCC 48112)	50 ±1.23
S. cerevisiae (ATCC 10231)	-
C. albicans (ATCC 16404)	-

- No significant activity recorded

4. Conclusions

The present work highlights, for the first time, the antimicrobial potentials of the volatile extract from the rhizomes of endemic species, *A. rubidum.* The oil exhibited antimicrobial activity against both *A. niger* and *F. oxysporum* with MIC values of 50 µg/mL. This result validates and reinforces the traditional uses ascribed to the plant in the treatment of microbial infections. The antimicrobial activity of the tested *A. rubidum* oil should make the oil to be potential for the use in health-care system.

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