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Antimicrobial Activity of Metabolites of an Endophytic Fungus Isolated from the Leaves of *Citrus jambhiri* (Rutaceae)

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ABSTRACT

Some few studies on the endophytic fungal populations of Nigerian medicinal plants have confirmed the enormous potentials which abound in these organisms as sources of novel bioactive molecules. These studies highlight the need to further explore Nigeria's plant biodiversity for endophytes producing biologically important molecules. In our study, an endophytic fungus was isolated from the leaves of *Citrus jambhiri* growing in South-East Nigeria. The fungus was subjected to solid state fermentation on rice medium and the metabolites were extracted using ethyl acetate. The fungal extract was screened for antimicrobial activity and some of the bioactive compounds of the extract were detected using high-performance liquid chromatography (HPLC) analysis. The antimicrobial assay was carried out using the agar diffusion method against several bacterial and fungal strains. The fungal extract, at a concentration of 1 mg/mL, showed antibacterial activity only against *Staphylococcus aureus* with an inhibition zone diameter (IZD) of 3 mm. No activity against the test fungi was recorded. The HPLC analysis of the extract revealed the presence of three bioactive compounds: protocathechuic acid, indole-3-acetic acid, and acropyrone. Results of this study suggest that endophytic fungi associated with *C. jambhiri* could be a promising source of novel compounds with pharmacological importance.

Keywords: *Citrus jambhiri*, endophytes, HPLC analysis, secondary metabolites.

Introduction

Citrus species are one of the most important fruit trees grown in Nigeria, as well as, globally due to their high nutritional value. The citrus industry is considered to be a major industry for the production of fruits and fruit products.[1,2]

Citrus fruits (Rutaceae) possess high amounts of bioactive compounds which can influence human health, these include: vitamin C, carotenoids (*β*-carotene), flavonoids, limonoids, essential oils, coumarins, acridone alkaloids, high quality soluble fibre, minerals, vitamin-B complex and related nutrients such as thiamine, riboflavin, nicotinic acid/niacin, pantothenic acid, pyridoxine, folic acid, biotin, choline, and inositol.[3] Health promoting effects of citrus include antioxidant, cardioprotective, anticarcinogenic, anti-allergic, antiplatelet, antiviral, antibacterial and antifungal activities.[4-6]

Nigeria is rich in plant biodiversity. These plants, which are hosts to millions of endophytic microbial communities, present the opportunity to discover a plethora of biologically important compounds and offer a renewable source of natural products. Recent studies on the endophytic fungal populations of Nigerian medicinal plants have confirmed the enormous potentials which abound in these organisms as sources of novel bioactive molecules.[7-15] Only a few studies on the presence of endophytes in citrus plants have been performed and little is known about the microbial endophytic community of the citrus plants.[16-19] Several endophytic bacterial species have however been isolated from *Citrus jambhiri*.[18,20,21]

Our study, therefore, seeks to further explore Nigeria's plant biodiversity for biologically important molecules by isolating an endophytic fungus from the leaves of *C. jambhiri* and identifying some of its bioactive metabolites.

Materials and Methods

Isolation of endophytic fungus, fermentation and extraction of metabolites

The isolation of the endophytic fungus, fermentation and extraction of metabolites were carried out as described by Abba *et al*.[12] and Akpotu *et al*.[14] Fresh healthy leaves of *C. jambhiri* were collected in June 2014 from Eziama-Uli, Anambra state, Nigeria. The plant leaves were washed thoroughly in running tap water and then cut into small fragments (about 1 cm^2). The leaf fragments were surface-sterilized by immersion in 2% sodium hypochlorite solution for 2 min, 70% ethanol for nearly 2 min, before a final rinse in sterile water for 5 min. The leaf fragments were put into Petri dishes containing malt extract agar (MEA) supplemented with chloramphenicol. The Petri dishes were then incubated at a temperature of 28ºC and fungal growths from the leaf fragments were monitored. Hyphal tips from distinct colonies emerging from leaf segments were sub-cultured onto fresh MEA plates to obtain pure colonies. Solid state fermentation of the endophytic fungus was carried out in 1L Erlenmeyer flask containing autoclaved rice medium (100 g of rice and 100 mL of distilled water). The flask was inoculated with 3 mm diameter agar blocks containing the fungi and incubated at 28°C for 21 days. At the completion of fermentation, the secondary metabolites (contained in the fermentation medium) were

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extracted with ethyl acetate and then concentrated under vacuum at 40ºC using a rotary evaporator.

Antimicrobial assay

Antibacterial and antifungal screening of the fungal extract was carried out using the agar well diffusion method described by Abba *et al*.[12]A concentration of 1 mg/mL of the extract was tested against laboratory strains of *Staphylococcus aureus, Salmonella typhi, Bacillus subtilis, Escherichia coli, Candida albicans and Aspergillus fumigatus.* Gentamicin (10 µg/mL) and ketoconazole (50 µg/mL) were used as positive controls in the antibacterial and antifungal tests respectively, while DMSO was used as the negative control in both tests. The inhibition zone diameters (IZDs) produced against the test isolates were measured and recorded.

High Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was carried on the crude fungal extract with a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S, Dionex Softron GmbH, Germering, Germany). The fungal extract (2 mg) was reconstituted with 2 mL of HPLC grade methanol. The mixture was sonicated for 10 min and thereafter centrifuged at 3000 rpm for 5 mins. Then, 100 µL of the dissolved sample was transferred into a vial containing 500 µL of HPLC grade methanol. The vial was then put in the HPLC machine for analysis. Detection was at 235 nm. The separation column (125 × 4 mm; length × internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany) and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent. The absorption peaks of the fungal extract were analyzed by comparing with those in the HPLC-UV/Vis database, which contains over 1600 registered compounds.

Results and Discussion

An endophytic fungus CJ-MR2 was isolated from the leaves of *C. jambhiri*. The result of the antimicrobial assay of the fungal extract revealed that at 1 mg/mL, the extract showed antibacterial activity only against *S. aureus* with an IZD of 3 mm (Table 1). The extract showed no antifungal activity against the test fungi *C. albicans* and *A. fumigatus*.

The extract of the endophytic fungus from *C. jambhiri* represents a dependable source of bioactive compounds, evidenced by the wide range of compounds with diverse biological properties present in these extracts. The HPLC analysis of the extract revealed the presence of protocathechuic acid, indole-3-acetic acid, and acropyrone**.** The HPLC chromatogram of the fungal extract, as well as the UV-spectra and chemical structures of detected compounds are presented in Figures 1 and 2.

Fungi are well known for producing many novel biologically active chemicals, and are among the most important groups of eukaryotic organisms that are being explored for therapeutic molecules. The compounds detected in the extract of the endophytic fungus isolated from *C. jambhiri* possess biological activities that are either antimicrobial, cytotoxic, anti-inflammatory or antioxidant.

The fungal extract showed mild antibacterial activity against *S. aureus* and this activity may be attributed to antimicrobial compounds present in the extract. Protocathechuic acid and acropyrone are known to exhibit antimicrobial activity,[22,23] and these compounds may have contributed greatly to the antimicrobial activity shown by the endophytic fungal extract.

Protocatechuic acid is a type of widely distributed naturally occurring phenolic acid and is widely distributed and present in most edible and medicinal plants.[24-26] Protocathechuic acid has been reported to show antioxidant,[27] antibacterial,[22] anticancer,[28] anti-ulcer,[29] antidiabetic,[30] anti-ageing,[31] antifibrotic,[32] antiviral,[33] anti-inflammatory,[34] analgesic activity,[34] anti-atherosclerotic,[35] cardiac,[36] hepatoprotective,[37] neurological,[38] and nephroprotective[39] activities.

Indole-3-acetic acid is a known plant compound, and the most common plant hormone of the auxin class which regulates various aspects of plant growth and development.[40-42] Many fungal species have been reported to be able to produce indole-3-acetic acid.[40] The compound has been reported to possess cytotoxic/anticancer, antioxidant, anti-inflammatory activities.[43-45] In this study, it was observed that indole-3-acetic acid was the most abundant compound in the fungal extract, as it showed the most prominent peak (B) in the HPLC chromatogram of the extract (Figure 1).

Acropyrone is an α-pyrone compound with cytotoxic[46,47] and antibacterial[23] activities. It has been previously isolated from *Acremonium strictum*[23] and *Acronychia pedunculata.*[46,47]

Indole-3-acetic acid and acropyrone have also been previously reported to be present in extracts of some endophytic fungi associated with Nigerian plants.[13-15] These endophytes can serve as a ready source for large-scale production of these bioactive compounds for pharmaceutical or industrial applications.

Nigeria's rich plant biodiversity presents an enormous platform for researchers to explore in the area of bioprospecting without the destructive harvesting of plants, but by exploring their associated endophytic organisms for pharmaceutically and industrially important molecules. The rapid depletion of rainforests, which hold a potential for endophytes and their promising products, is one of the major problems facing the future of natural product discovery. It is understood that when a plant species disappear, so does its associated endophytes. It is, therefore, necessary that steps be taken now to secure and conserve this plant biodiversity before they are completely lost.

Table 1: Results of the antimicrobial evaluation of the fungal extract showing the inhibition zone diameters (IZD) (mm) produced against test organisms.

Test Organisms	CJ-MR2 (1 mg/mL)	Positive control	Negative control
		Gentamicin (10 µg/mL)	DMSO
S. aureus	3	17	0
S. typhi	0	21	0
B. subtilis	0	22	0
E. coli	0	16	0
		Ketoconazole (50 µg/mL)	DMSO
C. albicans	0	17	0
A. fumigatus	0	4	0

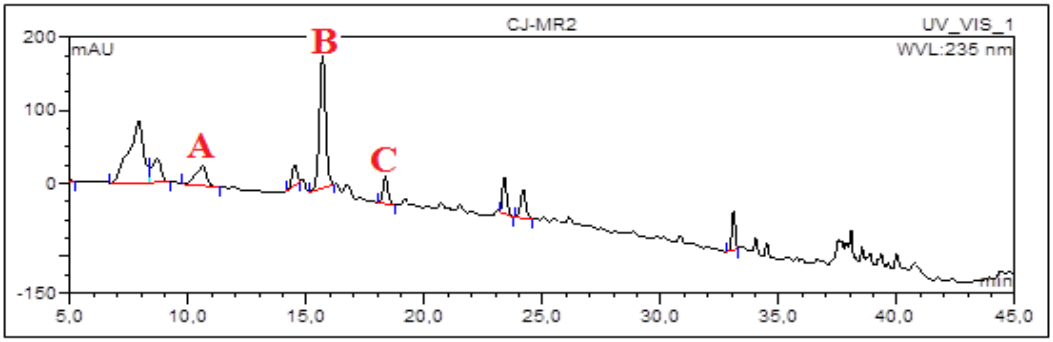

Figure 1: HPLC chromatogram of the endophytic fungal extract showing the detected compounds - (A) Protocathechuic acid, (B) Indole-3-acetic acid and (C) Acropyrone.

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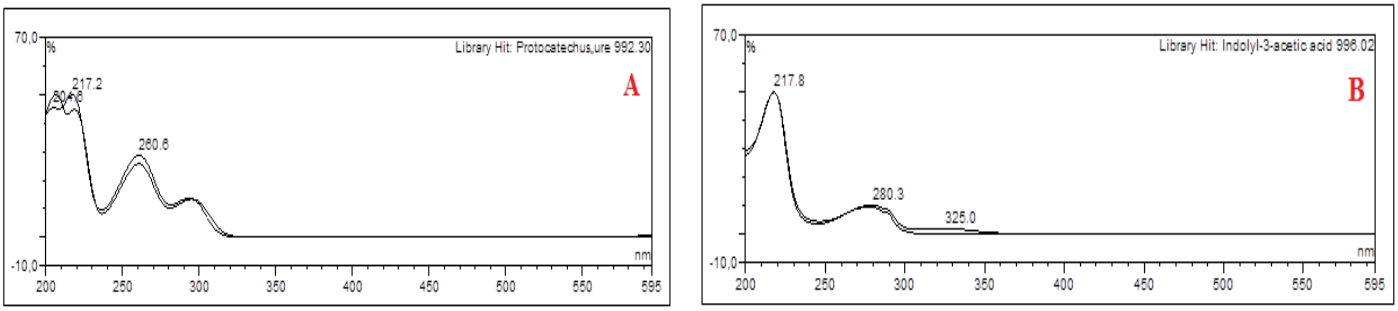
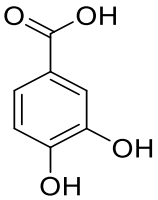
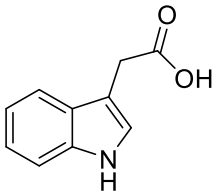
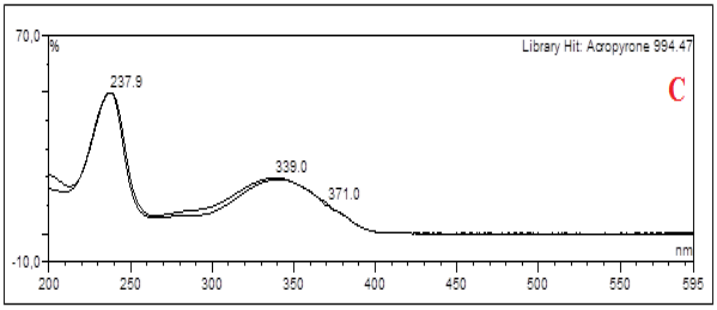


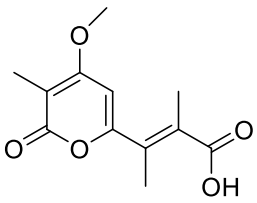


(A) Protocathechuic acid $C_7H_6O_4$, 154.12 g.mol^{-1}



(B) Indole-3-acetic acid $C_{10}H_9NO_2$, 175.19 g·mol^{-1}





(C) Acropyrone $C_{11}H_{12}O_5$, 224 g.mol^{-1}

Figure 2: UV Spectra and chemical structures of detected bioactive compounds: (A) Protocathechuic acid, (B) Indole-3-acetic acid and (C) Acropyrone**.**

According to Akpotu *et al.*,[14,15] the HPLC analysis has limitations as only compounds whose UV-spectra are already in the spectral library can be detected. Consequently, in the endophytic fungal extract, the undetected compounds or compounds whose spectra had no library hit may represent important or novel bioactive compounds. It is therefore recommended that further studies be carried out employing other more sensitive analytical tools such as mass spectrometry and/or NMR to validate the findings of this research.

Conclusion

The results of this study suggest that endophytic fungi associated with *C. jambhiri* could be a potential source of novel compounds for pharmaceutical and industrial applications.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Isenahume OP, Okonokhua BO. Comparative effects of seed storage methods on the development of established rough lemon (*Citrus jambhiri* lush) rootstock seedlings in the nursery. Nig J Agric Food Environ. 2014; 10(4):123-127.

2. Ali S, Mirza B. Micropropagation of rough lemon (*Citrus jambhiri* Lush.): Effect of explant type and hormone concentration. Acta Bot Croat. 2006; 65(2):137–146.
3. Ladaniya MS. Citrus fruit: Biology, technology and evaluation. USA: Academic Press/Elsevier 2008.
4. Manners GD, Hasegawa S. A new normal phase liquid chromatographic method for the analysis of limonoids in citrus. Phytochem Anal. 1999; 10:76–81.
5. Miller EG, Porter JL, Binnie WH, Guo IY, Hasegawa S. Further studies on the anticancer activity of citrus limonoids. J Agric Food Chem. 2004; 52:4908–4912.
6. Tripoli E, Guardia ML, Giammanco S, Majo DD, Giammanco M. Citrus flavonoids: Molecular structure, biological activity and nutritional properties. Food Chem. 2007; 104:466–479.
7. Okoye FBC, Nworu CS, Akah PA, Esimone CO, Debbab A, Proksch P. Inhibition of inflammatory mediators and reactive oxygen and nitrogen species by some depsidones and diaryl ether derivatives isolated from *Corynespora cassiicola*, an endophytic fungus of *Gongronema latifolium* leaves. Immunopharmacol Immunotoxicol. 2013a; 35(6):662-668.
8. Okoye FBC, Lu S, Nworu CS, Esimone CO, Proksch P, Chaldi A, Debbab A. Depsidone and Diaryl Ether Derivatives from the Fungus *Corynespora cassiicola*, an Endophyte of *Gongronema latifolium*. Tetrahedron Lett. 2013b; 54:4210–4214.
9. Okoye FBC, Nworu CS, Debbab A, Esimone CO, Proksch P. Two new Cytochalasins from an endophytic fungus, KL-1.1 isolated from *Psidium guajava* leaves. Phytochem Lett. 2015; 14:51-55.
10. Chen H, Daletous G, Okoye FBC, Lai D, Dai H, Proksch P. A new cytotoxic cytochalasin from the endophytic fungus *Trichodama harzianum*. Nat Prod Commun. 2015; 10(4):585-587.
11. Ebada SS, Eze P, Okoye FBC, Esimone CO, Proksch P. The Fungal Endophyte *Nigrospora oryzae* Produces Quercetin Monoglycosides Previously Known Only from Plants. Chem Sel. 2016; 1(11):2767-2771.
12. Abba CC, Nduka I, Eze PM, Ujam TN, Abonyi DO, Okoye FBC. Antimicrobial Activity of Secondary Metabolites of Endophytic *Aspergillus Species* Isolated from *Loranthus micranthus*. Afr. J. Pharma. Res. Dev. 2016; 8(2): 136-140.
13. Okezie UM, Eze PM, Okoye FBC, Ikegbunam MN, Ugwu MC, Esimone CO. Biologically Active Metabolites of an Endophytic Fungus Isolated from *Vernonia Amygdalina*. Afr J Pharm Res. Dev 2017; 9(1):24-29.
14. Akpotu MO, Eze PM, Abba CC, Umeokoli BO, Nwachukwu CU, Okoye FBC, Esimone CO. Antimicrobial activities of secondary metabolites of endophytic fungi isolated from *Catharanthus roseus*. J Health Sci. 2017; 7(1):15-22.
15. Akpotu MO, Eze PM, Abba CC, Nwachukwu CU, Okoye FB, Esimone CO. Metabolites of endophytic fungi isolated from *Euphorbia hirta* growing in Southern Nigeria. Chem Sci Rev Lett. 2017; 6(21):12-19.
16. Douanla-Meli C, Langer E, Mouafo FT. Fungal endophyte diversity and community patterns in healthy and yellowing leaves of *Citrus limon*. Fungal Ecol. 2013; 6:212-222.
17. Araujo WL, Maccheroni W Jr, Aguilar-Vildoso CI, Barroso PAV, Saridakis HO, Azevedo JL. Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. Can. J Microbiol 2001; 47:229–236.
18. Araujo WL, Marcon J, Maccheroni W Jr, van Elsas JD, van Vuurde JWL, Azevedo JL. Diversity of endophytic bacterial populations and their interactions with *Xylella fastidiosa* in Citrus plants. Appl. Environ. Microbiol. 2002; 10:4906–49.
19. Glienke-Blanco C, Aguilar-Vildoso CI, Vieira MLC, Barroso PAV, Azevedo JL. Genetic variability in the endophytic fungus *Guignardia citricarpa* isolated from citrus plants. Genet Mol Biol. 2002; 25(2):251-255.
20. Gardner JM, Chandler JA, Feldman AW. Growth response and vascular plugging of citrus inoculated with rhizobacteria and xylemresident bacteria. Plant Soil 1985; 86:996–1000.
21. Gardner JM, Feldman AW, Zablotowicz M. Identity and behavior of xylem-residing bacteria in rough lemon roots of Florida citrus trees. Appl Environ Microbiol. 1982; 43:1335–1342.
22. Chao CY, Yin MC. Antibacterial effects of roselle calyx extracts and protocatechuic acid in ground beef and apple juice. Foodborne Pathog Dis. 2009; 6(2):201–206.
23. Hammerschmidt L, Debbab A, Ngoc TD, Wray V, Hemphil CP, Lin W, Broetz-Oesterhelt H, Kassack MU, Proksch P, Aly AH. Polyketides from the mangrove-derived endophytic fungus *Acremonium strictum.* Tetrahedron Lett. 2014; 55(24):3463-3468.
24. Hudson EA, Dinh PA, Kokubun T, Simmonds MS, Gescher AC. Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidemiol. Biomark. Prev. 2000; 9:1163–1170.
25. Masella R, Cantafora A, Modesti D, Cardilli A, Gennaro L, Bocca A, Coni E. Antioxidant activity of 3,4-DHPEA-EA and protocatecuic acid: a comparative assessment with other olive oil biophenols. Redox Rep. 1999; 4(3):113–121.
26. Kayano SI, Kikuzaki H, Fukutsuka N, Mitani T, Nakatani N. Antioxidant activity of prune (*Prunus domestica* L.) constituents and a new synergist. J Agric Food Chem. 2002; 50(13):3708–3712.
27. Li X, Wang X, Chen D, Chen S. Antioxidant activity and mechanism of protocatechuic acid *in vitro*. Funct Foods Health Dis. 2011; 7:232–244.
28. Tanaka T, Tanaka T, Tanaka M. Potential cancer Chemopreventive activity of protocatechuic acid. J Exp Clin. Med. 2011; 3(1):27–33.
29. Kore KJ, Bramhakule PP, Rachhadiya RM, Shete RV. Evaluation of anti-ulcer activity of protocatechuic acid ethyl ester in rats. Int J Pharm Life Sci. 2011; 2(7):909.
30. Scazzocchio B, Vari R, Filesi C, D'Archivio M, Santangelo C, Giovannini C, Lacovelli A, Silecchia G, Volti GL, Galvano F, Masella R. Cyanidin-3-O-β- glucoside and protocatechuic acid exert insulin-like effects by upregulating PPARγ activity in human omental adipocytes. Diabetes 2011; 60(9):2234–2244.
31. Shi GF, An LJ, Jiang B, Guan S, Bao YM. Alpinia protocatechuic acid protects against oxidative damage *in vitro* and reduces oxidative stress *in vivo*. Neurosci Lett. 2006; 403(3):206–210.
32. Li C, Jiang W, Zhu H, Hou J. Antifibrotic effects of protocatechuic aldehyde on experimental liver fibrosis. Pharm Biol. 2012; 50(4):413–419.
33. Zhou Z, Zhang Y, Ding XR Chen SH, Yang J, Wang XJ, Jia GL, Chen HS, Bo XC, Wang SQ. Protocatechuic aldehyde inhibits hepatitis B virus replication both *in vitro* and *in vivo*. Antivir Res. 2007; 74(1):59–64.
34. Jaijoy K, Soonthornchareonnon N, Panthong A, Sireeratawong S. Anti-inflammatory and analgesic activities of the water extract from the fruit of *Phyllanthus emblica* Linn. Int J Appl Res Nat Prod. 2010; 3(2):28–35.
35. Borate AR, Suralkar AA, Birje SS, Malusare PV, Bangale PA. Antihyperlipidemic effect of protocatechuic acid in fructose induced hyperlipidemia in rats. Int J Pharm Bio Sci. 2011; 2(4):456.
36. Ciftci O, Disli OM, Timurkaan N. Protective effects of protocatechuic acid on TCDD-induced oxidative and histopathological damage in the heart tissue of rats. Toxicol Indus Health 2013; 29(9):806–811.
37. Liu CL, Wang JM, Chu CY, Cheng MT, Tseng TH. *In vivo* protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced rat hepatotoxicity. Food Chem Toxicol. 2002; 40(5):635–641.
38. Guan SG, Bao YM, Jiang BJ, An LJ. Protective effect of protocatechuic acid from *Alpinia oxyphylla* on hydrogen peroxide-induced oxidative PC12 cell death. Eur J Pharmacol. 2006; 538(1–3):73–79.
39. Lee JH, Lee HJ, Lee HJ, Choi WC, Yoon SW, Ko SG, Ahn KS, Choi SH, Ahn KS, Lieske JC, Kim SH. *Rhus verniciflua* Stokes prevents cisplatin-induced cytotoxicity and reactive oxygen species production in MDCK-I renal cells and intact mice. Phytomed. 2009; 16(2):188–197.
40. Fu S, Wei J, Chen H, Liu Y, Lu H, Chou J. Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Signal Behav. 2015; 10(8):e1048052.

41. Teale WD, Paponov IA, Palme K. Auxin in action: signalling, transport and the control of plant growth and development. Nat Rev Mol Cell Biol. 2006; 7:847-859.
42. Spaepen S, Vanderleyden J, Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev. 2007; 31(4):425-448.
43. Wardman P. Indole-3-acetic acids and horseradish peroxidase: a new prodrug/enzyme combination for targeted cancer therapy. Curr Pharm Des. 2002; 8(15):1363-1374.
44. Jeong YM, Oh MH, Kim SY, Li H, Yun HY, Baek KJ, Kwon NS, Kim WY, Kim DS. Indole-3 acetic acid/horseradish peroxidase induces apoptosis in TCCSUP human urinary bladder carcinoma cells. Pharmazie 2010; 65(2):122-126.
45. Jones LH, Abdalla DSP, Freitas JC. Effects of indole-3-acetic acid on croton oil and arachidonic acid-induced mouse ear edema. Inflamm Res. 1995; 44(9):372-375.
46. Ito C, Matsui T, Ban Y, Wu TS, Itoigawa M. Acetophenones Isolated from *Acronychia pedunculata* and their Antiproliferative Activities. Nat Prod Commun. 2016; 11(1):83-86.
47. Kouloura E, Halabalaki M, Lallemand M, Nam S, Jove R, Litaudon M, Awang K, Hadi HA, Skaltsounis A. Cytotoxic Prenylated Acetophenone Dimers from *Acronychia pedunculata*. J Nat Prod. 2012; 75:1270-1276.