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# Bioprospecting Certain Freshwater-derived Fungi for Phenolic Compounds with Special Emphasis on Antimicrobial and Larvicidal Activity of Methyl Gallate and *p*-coumaric Acid

**Marwa T. A. Abdel-Wareth[1*], Mosad A. Ghareeb[2]**
[1]*Environmental Research and Medical Malacology Department, Theodor Bilharz Research Institute, Giza, Egypt.*
[2]*Medicinal Chemistry, Biochemistry and Molecular Biology Department, Theodor Bilharz Research Institute, Giza, Egypt.*

FUNGI naturally produce secondary metabolites which have been investigated as biologically active compounds. This work aimed at exploring phenolic compounds profile of three freshwater derived fungi, and investigating the antimicrobial and larvicidal activity of methyl gallate and *p*-coumaric acid as two major compounds detected. Reverse phase high performance liquid chromatography coupled with diode array detector (RP-HPLC-DAD) revealed the presence of 22 phenolic compounds in the filtrate extracts of *Penicillium implicatum*, *Aspergillus niveus* and *Aspergillus petrakii*, although they varied in their concentrations from one species to another, methyl gallate was one of the major compounds in *Penicillium implicatum* and *Aspergillus niveus* filtrates, and the compound of the highest concentration in *Aspergillus petrakii* filtrate. While *p*-coumaric acid was the major compound in *Aspergillus niveus* filtrate. Methyl gallate was effective on *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* sub sp. *aureus* (MRSA**)**, while *p*-coumaric acid was only effective on *Bacillus cereus*. Regarding larvicidal activity, *p*-coumaric acid was more effective on *Schistosoma mansoni* cercariae than methyl gallate. The investigated fungi comprise a rich source of phenolic compounds, and methyl gallate and *p*-coumaric acid represent promising antibacterial and larvicidal agents.

**Keywords:** *Penicillium, Aspergillus,* RP-HPLC, methyl gallate, *p*- coumaric acid, antimicrobial effect, Larvicidal activity

## Introduction

Naturally occurring secondary metabolites are considered leading compounds that can be used for new drug discovery [1]. At present, the search for new producers of biologically active compounds is diligently underway among fungi from unusual or specialized ecological niches, because the synthesis of new secondary metabolites is crucial for these fungi to survive and adapt to their environments [2-4]. Additionally, freshwater-derived fungi are being accepted as a potentially important source of novel compounds that might prove suitable for specific medicinal or agrochemical applications [5]. Many novel bioactive compounds have been reported from freshwater-derived fungi; *Stachybotrys* sp. [6]*, Kirschsteiniothelia* sp. [7], *Dendrospora tenella* [8], *Massarina tunicata* [9], *Annulatascus triseptatus* [10], *Ophioceras venezuelense* [11], *Decaisnella hyridioides* [12] and *Helicodendron giganteum* [13]. On the other hand, the increasing occurrence of highly resistant pathogenic microorganisms against antibiotics is a great challenge. Thus, there is a crucial demand for the isolation and identification of new therapeutic agents to overcome infectious diseases [14]. Schistosomes larval stages; miracidia and cercariae are key organisms in incidence of schistosomiasis, so searching for compounds from biological origin to kill these larvae is encouraged.

The reversed phase-high performance liquid chromatography coupled with diode array detector (RP-HPLC-DAD) is a unique technique widely used for the separation and quantification of polyphenolic compounds either in plants, marine organisms or fungal extracts [15]. Therefore, the aims of the current study were to identify

*Corresponding author e-mail: marwatamim2001@hotmail.com;
Tel: 02001008578166, Fax: 020 002-02-34508125
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the phenolic compounds profile of filtrates from three fungal species; *Penicillium implicatum*, *Aspergillus niveus* and *Aspergillus petrakii* using RP-HPLC-DAD technique, and to determine the presence of biologically active compounds by evaluating their *in vitro* antimicrobial and larvicidal activity.

## Materials and Methods

*Chemicals*

Sabouraud dextrose agar media, Mueller-Hinton agar medium, glucose, acetic acid, acetonitrile, toluene, ethyl acetate, formic acid and DMSO were analytical grade. Standard gallic acid, pyrogallol, 4-amino-benzoic acid, protocatechuic acid, catechin, chlorogenic acid, methyl gallate, epicatechin, caffeic acid, vanillic acid, *p*-coumaric acid, ferulic acid, iso-ferulic acid, reversetrol, ellagic acid, *e*-vanillic acid, *α*-coumaric acid, benzoic acid, 3,4,5- methoxy cinnamic acid, coumarin, salicylic acid and cinnamic acid were purchased from Sigma-Aldrich Co., polymyxin, ampicillin, kanamycin and nystatin antibiotics were provided by faculty of Agriculture, Cairo University.

*Preparation of fungal filtrates*

Three fungal species; namely *Penicillium implicatum*, *Aspergillus niveus* and *Aspergillus petrakii* were previously isolated from watercourses at Ismailia, Giza and Gharbeya governorates, respectively [16]. They were identified microscopically using universal manuals [17,18], maintained by continuous sub culturing on Sabouraud dextrose agar at constant intervals, and the slants then kept refrigerated. Fungal cultures were prepared by inoculating conical flasks (250 ml capacity) containing 50 ml of potato dextrose broth medium with fungal discs (5 mm diameter) which were cut from 7 days old cultures [19] . The inoculated flasks were incubated on a rotary shaker (150 rpm) at 28°C for 10 days. The mycelia then were separated by filtration, using a membrane filter. The resultant filtrates were used for analysis.

*Separation and quantification of phenolic compounds*

Separation and determination of phenolic compounds were performed by reverse phase HPLC (RP-HPLC)/diode array detection (DAD) (Hewlett Packard 1050) using a column Alltima C18, 5mm (150 mm_4.6 mm id) with a guard column Alltima C18, 5mm (Alltech). The solvent system used was a gradient of A ($CH_3COOH$ 2.5% v/v), B ($CH_3COOH$ 8% v/v) and C (acetonitrile). The best separation was obtained with the following gradient: at 0 min, 5% B; at 20 min, 10% B; at 50 min, 30% B; at 55 min, 50% B; at 60 min, 100% B; at 100 min, 50% B and 50% C; at 110 min, 100% C until 120 min. The solvent flow rate was 1ml/min, and the separation was performed at 35°C. The volume injected was 10 ml. Phenolic compounds were assayed by external standard calibration at 280 nm and expressed in µg/100ml. All values were the mean of two injections [20].

*Chromatographic isolation of methyl gallate and p-coumaric acid from fungal filtrates extracts*

As the largest peak areas of methyl gallate and *p*-coumaric acid were noticed in the filtrates of *Penicillium implicatum* and *Aspergillus niveus*, respectively, preparative thin layer chromatography (PTLC) was used to separate methyl gallate and *p*-coumaric acid from these species.TLC plates (20 x 20 cm Merck aluminum sheet, silica gel 60, layer thickness 0.2 mm) were used. Each fungal filtrate extract was spotted at the start of the silica gel plates, and allowed to dry, then eluted using toluene: ethyl acetate: 90% formic acid (TEF) (5:4:1, v/v/v) in a solvent saturated atmosphere, then allowed to air dry. TLC plates were examined under UV light (365 nm) to determine the spots which had the same $R_f$ values of authentic methyl gallate and *p*-coumaric acid [21, 22]. Then, the *in vitro* antimicrobial and larvicidal activity of the purified compounds were evaluated.

*Antimicrobial activity*

The antimicrobial activity was determined using disc diffusion assay [23]. *In vitro* antibacterial activity was carried out against *Escherichia coli* and *Pseudomonas aeruginosa* (Gram negative bacteria), *Bacillus cereus* and *Staphylococcus aureus* sub sp. *aureus* (MRSA) (Gram positive bacteria), using Mueller-Hinton agar medium. Antifungal activity was carried out against *Aspergillus niger* and *Candida albicans* using Sabouraud dextrose agar medium. Kanamycin (30µg), Ampicillin (10 µg), Polymyxin (130 units) and Nystatin (100 units) were used as positive reference standards for Gram positive, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activity, respectively. DMSO was used as solvent and negative control. Briefly, 20 ml of sterilized media was poured onto the sterilized Petri-dishes and allowed to

solidify. 0.5 ml of each microbial suspension was poured on the surface of the solidified media, and evenly distributed using a sterile swab. Discs saturated with 15µl of the purified compound (with a concentration equals 10% v/v) and antibiotics solutions were placed in triplicates on the surface of the media. The plates were incubated at 30°C for *Bacillus cereus*, 37°C for other bacterial species, and 25°C for tested fungi; all plates were incubated and investigated after 24-48 hrs. Zones of inhibition were measured in mm scale.

*Larvicidal effect*

To investigate miracidicidal effect, *Schistosoma mansoni* ova were allowed to hatch in dechlorinated tap water. 250 µm of water containing about 50-100 freshly hatched miracidia was mixed with 250 µm of each concentration tested (25mg/ml, 50 mg/ml and 100 mg/ml) in a divided Petri dish. 250 µm of dechlorinated water containing about 50-100 miracidia was kept as control. After 15, 30, 45 and 60 min, the miracidia were observed under a dissecting microscope for alterations in their motility. Motionless miracidia were considered dead and the mortality rates were recorded. For cercariae, 250 µm of water containing about 50-100 freshly shed *S. mansoni* cercariae were mixed with 250 µm of the tested concentrations as in the miracidicidal test. Motionless cercariae were considered dead and their mortality was recorded [24]. *Schistosoma mansoni* ova and cercariae were kindly provided by Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI).

## Results and Discussion

RP-HPLC/DAD analysis of *Penicillium implicatum* filtrate extract showed the presence of two major compounds namely; *e*-vanillic acid (2.14%) and methyl gallate (1.12%), and other minor compounds like 3,4,5-methoxy cinnamic acid (0.78%) and *p*-coumaric acid (0.75%) (Table 1 and Fig. 1). Regarding *Aspergillus niveus* filtrate extract, the analysis revealed the presence of one major compound, namely; *p*-coumaric acid (1.35%), beside methyl gallate (0.98%), protocatechuic acid (0.84%), 4-amino-benzoic acid (0.66%) and chlorogenic acid (0.63%) (Table 2 and Fig. 2). While, for *Aspergillus petrakii* extract, this analysis showed the presence of methyl gallate (0.73%), vanillic acid (0.70%), gallic acid (0.57%) and *e*-vanillic acid (0.50%) (Table 3 and Fig. 3). From the obtained results, we can conclude that there are mutual compounds in the three tested species but in different concentrations, and this indicated the chemical similarity of these fungal species. To the best of our knowledge, there are very limited data concerning the RP-HPLC-DAD analysis of the three investigated fungal species. Reviewing the literature revealed that some bioactive phenolic secondary metabolites were isolated and identified in different *Penicillium* species; *viz.*, (3,1' -didehydro-3[z2"(3"',3'''-dimethyl-prop-2-enyl)-3"indolyl methylene]-6-methyl pipera-zine-2,5-dione) [25], 7-methoxy-2,2-dimethyl-4-octa-4′,6′-dienyl-2*H*-napthalene-1-one [26], citreorosein, emodin, janthinone, citrinin, citrinin H1 and dicitrinol [27]. On the other hand, sesquiterpenes, alkaloids and quinines have been previously reported in some *Aspergillus* species [28,29].

Methyl gallate is a derivative of gallic acid. It was previously isolated from plants showing medicinal properties, e.g. *Toona sureni* [30] and *Spondias pinnata* [31]. Also, it was identified and isolated from the filtrate of the fungus *Penicillium janthinellum* [16]. Many studies showed its multiple biological activities as antioxidant, anti proliferative and anticancer agent [31-33]. The current study showed that methyl gallate was effective on both tested Gram negative bacteria; *Pseudomonas aeruginosa* and *Escherichia coli* as the diameters of inhibition zones were 9 and 13 mm as compared to 10 and 15 mm for polymyxin and ampicillin, respectively. Additionally, it was effective against *Staphylococcus aureus* sub sp. *aureus* (MRSA) as a Gram positive bacterium with an 8 mm inhibition zone (Table 4 and Fig. 4). On the other hand, methyl gallate did not show antimicrobial effect on each of *Bacillius cereus*, *Aspergillus niger* and *Candida albicans*. These results agree with the findings reported by Choi et al., they mentioned that methyl gallate, as a major component of *Galla rhois*, exhibited strong antimicrobial activity against *E. coli* [34]. Moreover, Ekaprasada *et al*. showed that 10 mg/l of methyl gallate isolated from the leaves of *Toona sureni* showed antibacterial activities against each of *Bacillus subtilis*, *Staphylococcus aureus* and *E. coli* with inhibition zones equal 8, 10 and 8 mm, respectively [30]. Phenolic compounds are thought to inhibit microbial enzymes possibly through reaction with sulfhydryl groups (the oxidized phenols) or through nonspecific interactions with the proteins [35].

**TABLE 1. The phenolic compounds identified in *Penicillium implicatum* filtrate by reverse phase HPLC with diode-array detection.**

| Peak No. | R.T. (min) | Area % | Identified compound | µg/100ml |
|---|---|---|---|---|
| **1** | 7.26 | 0.32 | Gallic acid | 53.74 |
| **2** | 7.37 | 0.12 | Pyrogallol | 833.58 |
| **3** | 7.93 | 0.30 | 4-Amino-benzoic acid | 18.66 |
| **4** | 8.65 | 0.18 | Protocatechuic acid | 80.70 |
| **5** | 8.69 | 0.10 | Catechin | 56.69 |
| **6** | 9.21 | 0.23 | Chlorogenic acid | 57.86 |
| **7** | 9.53 | 1.12 | Methyl gallate | 285.00 |
| **8** | 9.69 | 0.10 | Epicatechin | 23.83 |
| **9** | 10.24 | 0.25 | Caffeic acid | 19.19 |
| **10** | 10.35 | 0.17 | Vanillic acid | 63.48 |
| **11** | 11.75 | 0.75 | *p*-Coumaric acid | 89.24 |
| **12** | 11.86 | 0.13 | Ferulic acid | 19.90 |
| **13** | 12.08 | 0.40 | Iso-ferulic acid | 68.51 |
| **14** | 12.63 | 0.14 | Reversetrol | 7.65 |
| **15** | 13.03 | 0.35 | Ellagic acid | 932.51 |
| **16** | 13.10 | 2.14 | *e*-vanillic acid | 8021.09 |
| **17** | 13.28 | 0.26 | *α*-Coumaric acid | 42.48 |
| **18** | 13.43 | 0.30 | Benzoic acid | 516.93 |
| **19** | 13.92 | 0.78 | 3,4,5- methoxy cinnamic acid | 11.73 |
| **20** | 14.04 | 0.19 | Coumarin | 23.41 |
| **21** | 14.39 | 0.19 | Salicylic acid | 131.51 |
| **22** | 15.15 | 0.20 | Cinnamic acid | 12.08 |
| | | **Total 8.72%** | | |

**TABLE 2. The phenolic compounds identified in *Aspergillus niveus* filtrate by reverse phase HPLC with diode-array detection.**

| Peak No. | R.T. (min) | Area % | Identified compound | µg/100ml |
|---|---|---|---|---|
| **1** | 7.29 | 0.13 | Gallic acid | 12.04 |
| **2** | 7.34 | 0.27 | Pyrogallol | 970.06 |
| **3** | 7.93 | 0.66 | 4-Amino-benzoic acid | 22.21 |
| **4** | 8.64 | 0.84 | Protocatechuic acid | 202.17 |
| **5** | 8.68 | 0.20 | Catechin | 56.79 |
| **6** | 9.24 | 0.63 | Chlorogenic acid | 86.84 |
| **7** | 9.55 | 0.98 | Methyl gallate | 135.72 |
| **8** | 9.72 | 0.28 | Epicatechin | 34.73 |
| **9** | 10.27 | 0.58 | Caffeic acid | 24.26 |
| **10** | 10.38 | 0.54 | Vanillic acid | 108.31 |
| **11** | 10.64 | 1.35 | *p*-Coumaric acid | 87.07 |
| **12** | 10.88 | 0.32 | Ferulic acid | 26.90 |
| **13** | 12.20 | 0.58 | Iso-ferulic acid | 54.33 |
| **14** | 12.61 | 0.11 | Reversetrol | 3.08 |
| **15** | 13.01 | 0.03 | Ellagic acid | 57.26 |
| **16** | 13.04 | 0.06 | *e*-vanillic acid | 124.44 |
| **17** | 13.28 | 0.22 | *α*-Coumaric acid | 20.38 |
| **18** | 13.52 | 0.61 | Benzoic acid | 573.71 |
| **19** | 13.90 | 0.04 | 3,4,5- methoxy cinnamic acid | 3.19 |
| **20** | 13.97 | 0.10 | Coumarin | 7.02 |
| **21** | 14.35 | 0.15 | Salicylic acid | 56.31 |
| **22** | 15.20 | 0.12 | Cinnamic acid | 3.89 |
| | | **Total 8.80%** | | |

**TABLE 3. The phenolic compounds identified in *Aspergillus petrakii* filtrate by reverse phase HPLC with diode-array detection.**

| Peak No. | R.T. (min) | Area % | Identified compound | µg/100ml |
|---|---|---|---|---|
| **1** | 7.23 | 0.57 | Gallic acid | 118.41 |
| **2** | 7.34 | 0.13 | Pyrogallol | 1098.17 |
| **3** | 7.92 | 0.19 | 4-Amino-benzoic acid | 14.62 |
| **4** | 8.65 | 0.11 | Protocatechuic acid | 65.20 |
| **5** | 8.69 | 0.07 | Catechin | 50.91 |
| **6** | 9.21 | 0.10 | Chlorogenic acid | 31.91 |
| **7** | 9.53 | 0.73 | Methyl gallate | 222.38 |
| **8** | 9.72 | 0.17 | Epicatechin | 48.15 |
| **9** | 10.26 | 0.01 | Caffeic acid | 1.77 |
| **10** | 10.41 | 0.70 | Vanillic acid | 339.55 |
| **11** | 11.70 | 0.12 | *p*-Coumaric acid | 17.84 |
| **12** | 11.74 | 0.29 | Ferulic acid | 55.42 |
| **13** | 12.15 | 0.06 | Iso-ferulic acid | 13.62 |
| **14** | 12.68 | 0.14 | Reversetrol | 9.11 |
| **15** | 12.98 | 0.13 | Ellagic acid | 455.32 |
| **16** | 13.10 | 0.50 | *e*-vanillic acid | 2344.51 |
| **17** | 13.31 | 0.11 | $\alpha$-Coumaric acid | 23.21 |
| **18** | 13.43 | 0.28 | Benzoic acid | 607.15 |
| **19** | 13.93 | 0.25 | 3,4,5- methoxy cinnamic acid | 40.90 |
| **20** | 14.04 | 0.32 | Coumarin | 48.51 |
| **21** | 14.32 | 0.17 | Salicylic acid | 147.31 |
| **22** | 15.20 | 0.22 | Cinnamic acid | 16.08 |
| | | **Total 5.37%** | | |

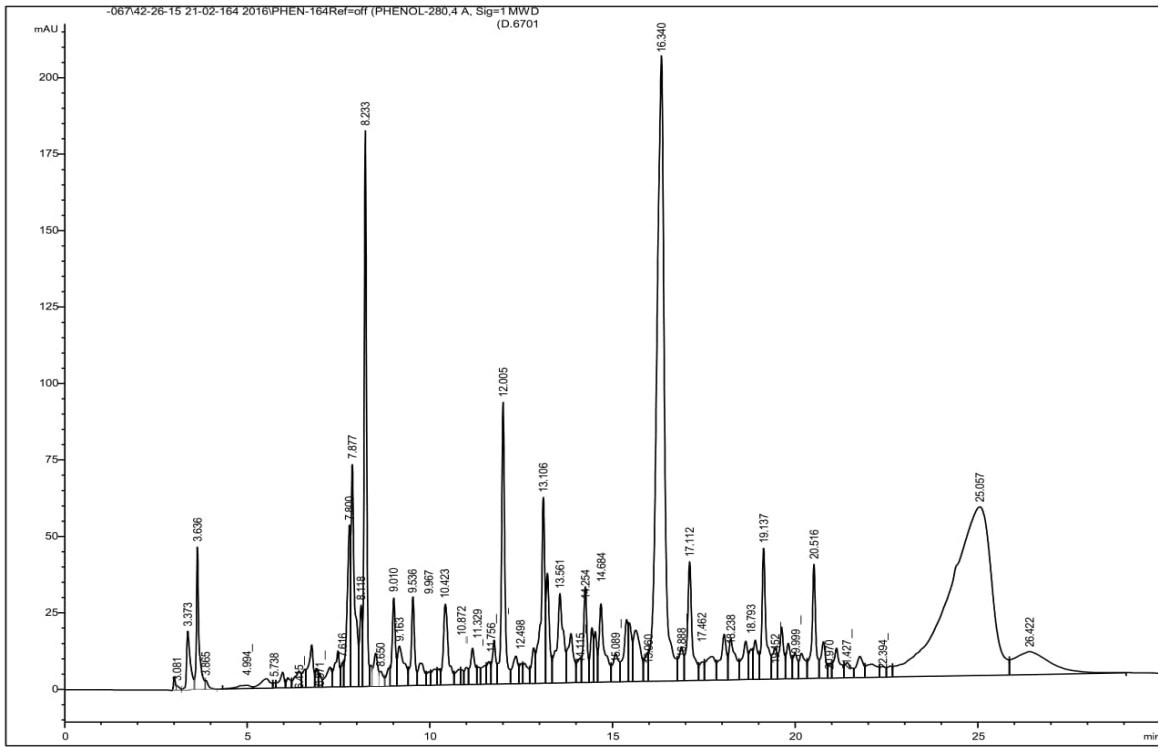


**Fig. 1. HPLC chromatogram of *Penicillium implicatum* filtrate extract.**

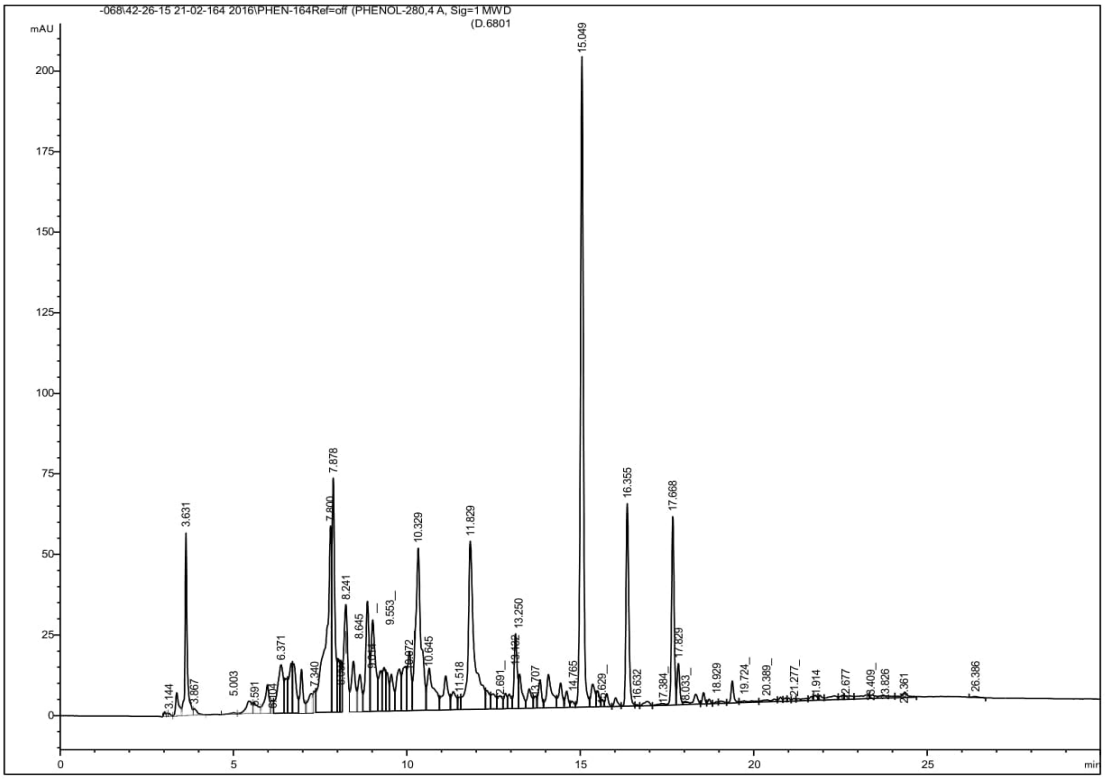


**Fig. 2. HPLC chromatogram of *Aspergillus niveus* filtrate extract.**

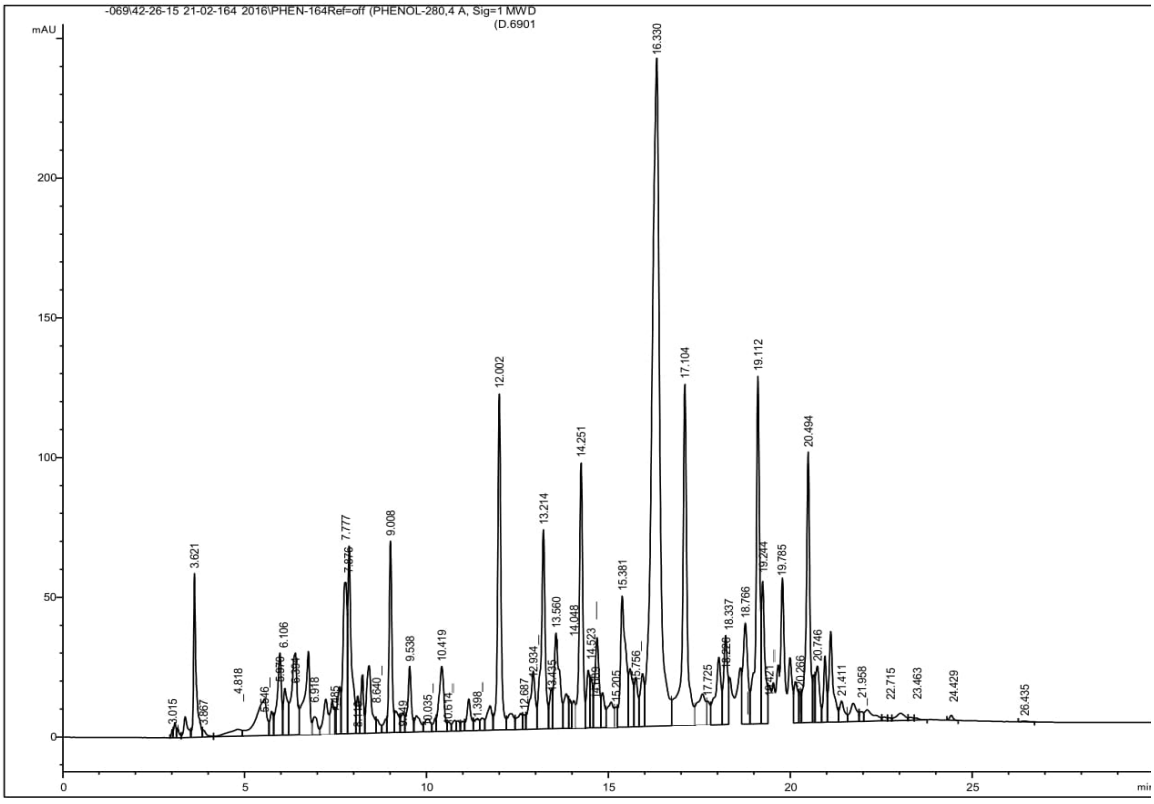


**Fig. 3. HPLC chromatogram of *Aspergillus petrakii* filtrate extract.**

**TABLE 4. Inhibition zones diameters (mm) of microorganisms exposed to methyl gallate, *p*-coumaric acid (10% v/v) and reference antibiotics.**

| Tested microorganisms \ Sample | Methyl gallate | *p*-coumaric acid | Positive reference standard |
|---|---|---|---|
| **Gram Negative bacteria** | | | **Polymyxin** |
| *Pseudomonas aeruginosa* (ATCC9027) | 9± 0.5 | NI | 10±0.0 |
| | | | **Ampicillin** |
| *Escherichia coli* (ATCC 35218) | 13±0.0 | NI | 15±0.0 |
| **Gram Positive bacteria** | | | **Kanamycin** |
| *Bacillus cereus* (ATCC33018) | NI | 9±0.6 | 12±0.0 |
| *Staphylococcus aureus* sub sp. *aureus* (MRSA) (ATCC43300) | 8±0.7 | NI | 10±0.0 |
| **Fungi** | | | **Nystatin** |
| *Aspergillus niger* (nrrl 326) | NI | NI | 15±0.0 |
| *Candida albicans* (ATCC 10231) | NI | NI | 13±0.0 |

NI: No inhibition

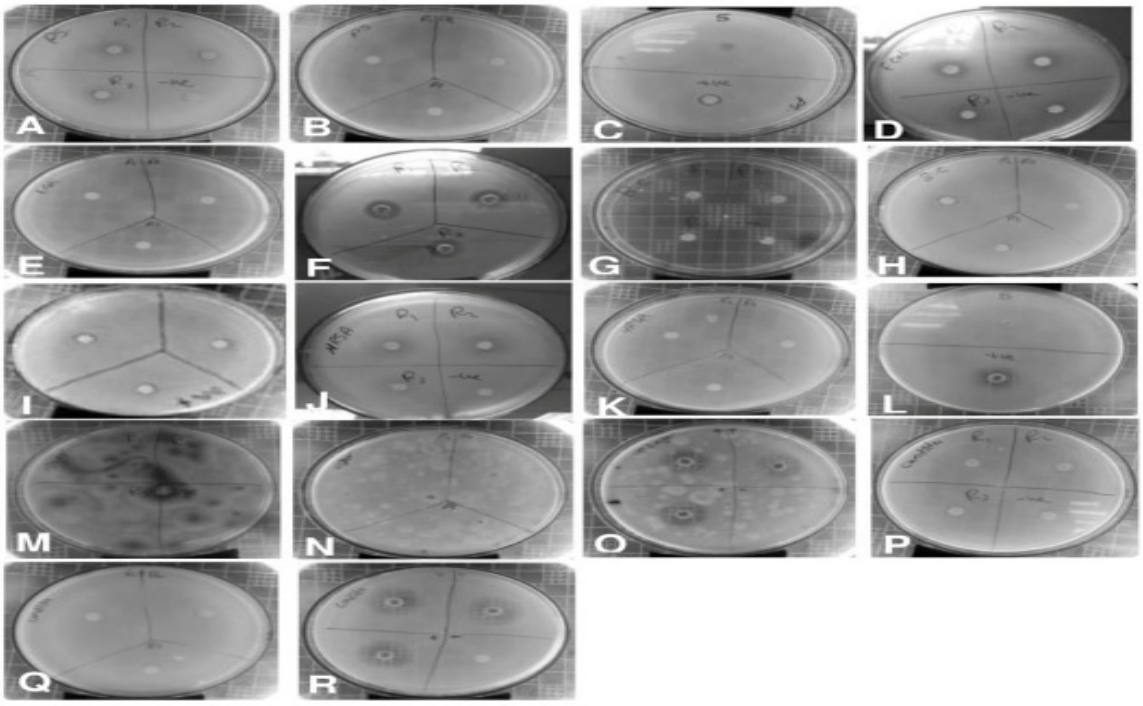


**Fig. 4. Photos of media inoculated with tested microorganisms and exposed to discs saturated with methyl gallate, *p*-coumaric acid and reference antibiotics; A: *P. aeruginosa* + methyl gallate, B: *P. aeruginosa* + *p*-coumaric acid, C: Polymyxin, D: *E. coli* + methyl gallate, E: *E. coli* + *p*-coumaric acid , F: Ampicillin, G: *B. cereus*+ methyl gallate, H: *B. cereus*+ *p*-coumaric, I: Kanamycin, J: *S. aureus* sub sp. aureus (MRSA)+ methyl gallate, K: (MRSA)+ *p*-coumaric acid, L: Kanamycin, M: *A.niger*+ methyl gallate, N: *A. niger*+ *p*-coumaric acid, O:Nystatin, P: *C. albicans*+ methyl gallate, Q: *C. albicans*+ *p*-coumaric acid, R: Nystatin. R1, R2 and R3 represent three replicates, -ve represents DMSO as negative control, +ve represents reference antibiotic as positive control.**

Generally, phenolic acids are divided into two groups as hydroxybenzoic acid and hydroxycinnamic acid derivatives, *p*-coumaric acid belongs to the second category [36,37]. It is indicated that *p*-coumaric acid, which is stated to have biological activities and many physiological functions, is one of the most important phenolic acids [38-40]. *p*-coumaric acid (4-hydroxy-cinnamic acid), classified as a neutraceutical and phytochemical, is identified at significant levels in many fruits and vegetables as well as cereals.

To the best of our knowledge, this is the first time to identify this compound in fungal metabolites, the present work indicated that *p*-coumaric acid was only effective against *Bacillus cereus* with inhibition zone of 9 mm as compared to 12 mm for Kanamycin (Table 4 and Fig. 4 ). Our results coincide with Acar *et al*., they declared that *p*-coumaric acid extracted from *Crocus baytopiorum* has a considerable antimicrobial effect against *Bacillus cereus* [41]. It is stated that *p*-coumaric acid exerts its effect through altering permeability of the cell membrane and disturbing cell functions [42]. Concerning the lack of antimicrobial activity of *p*-coumaric acid against certain bacteria in the current study, Krishna *et al*. noticed similar results especially at low concentrations, as the compound did not inhibit any of *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Bacillus subtilis* [43].

On the other hand, the results of the present study disagree with the findings of Sathish et al., they reported that *p*-coumaric acid showed high effect against *Staphylococcus aureus* and *Escherichia coli* and moderate effect on *Streptococcus pyogenes* and *Klebsiella pneumonia* while it had no effect on *Salmonella typhi* [44]. The observed different response of the same bacterial species to *p*-coumaric acid may be attributed to the difference in strains.

Regarding larvicidal activity, methyl gallate and *p*-coumaric acid were highly effective on miracidia as all the tested concentrations resulted in death of exposed miracidia after 15 min. For cercariae exposed to the three concentrations of methyl gallate, no mortality was observed after 15 min. As the time extended, the effect of these concentrations became more pronounced as 100% mortality was recorded after 45 min of cercarial exposure to 25 and 100 mg/ml, while 60 min were required to attain this percentage in case of 50 mg/ml (Fig. 5). Few studies demonstrated the adverse effect of methyl gallate on schistosomiasis worms and snails. Methyl gallate was previously isolated from the roots of *Elephantorrhiza goetzei* plant by Moyo et al. [45]. In Malawi and Zimbabwe, the root decoction of this plant is taken orally for the treatment of Bilharzia [46, 47]. Moreover, water extract of the plant roots showed lethal concentration of 0.5 mg/ml against *Schistosoma mansoni*. *E. goetzei* stem bark extract was also effective against schistosomules as its lethal concentration was 0.8 mg/ml [48]. Recently, methyl gallate was found to be responsible for the molluscicidal effect of *Penicillium janthinellum* against schistomiasis snails [16].

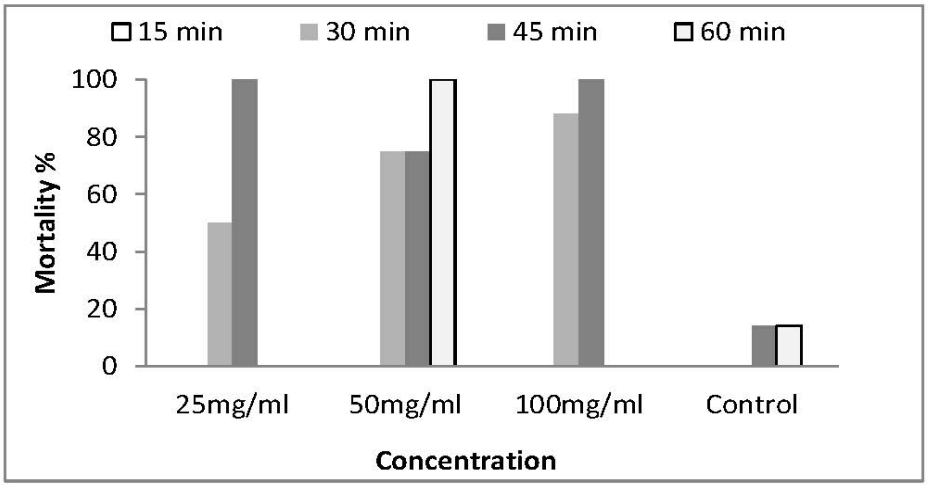


**Fig. 5. Effect of methyl gallate on mortality percentage of *S. mansoni* cercariae at certain time intervals.**

For cercariae exposed to 25 mg/ml *p*-coumaric acid in the current study, 40% mortality was observed after 15 min, as the time extended to 30 min, mortality percentage increased to 90%. All cercariae exposed to that concentration died after 45 min, while in case of cercariae treated with the two higher concentrations, 100% mortality was attained just after 15 min (Fig. 6). It was found that the extracts from plants belonging to family Lamineceae, which were effective against infectious diseases, contain *p*-coumaric acid among their constituents [49]. Moreover, Alvarenga *et al*. demonstrated that the ethyl acetate fraction of *Cuspidaria pulchra* plant which killed the adult schistosomes *in vitro* was shown to contain *p*-coumaric acid as one of the five major compounds identified [50].

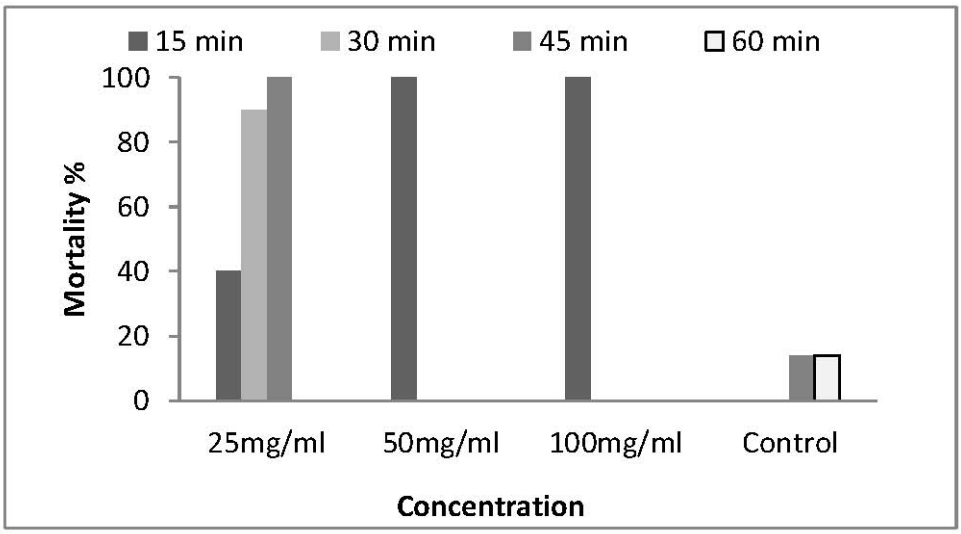


**Fig. 6. Effect of *p*-coumaric acid on mortality percentage of *S. mansoni* cercariae at certain time intervals.**

## Conclusion

*Penicillium implicatum*, *Aspergillus niveus* and *Aspergillus petrakii* are three promising freshwater-derived fungi as they produce a number of phenolic compounds, amongst them methyl gallate and *p*-coumaric acid. These two compounds were shown to have antibacterial effect, and miracicidal and cercaricidal activities.

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## التنقيب الحيوى عن المركبات الفينولية فى بعض فطريات المياه العذبة مع التركيز على النشاط المضاد للميكروبات والمميت لليرقات لكل من الميثيل جاللات و حمض الباراكوماريك

**مروة تميم أحمد عبد الوارث[١] و مسعد أحمد غريب[٢]**
[١]قسم بحوث البيئة و الرخويات الطبية – معهد تيودور بلهارس للأبحاث- الجيزة- مصر.
[٢]قسم الكيمياء الحيوية و البيولوجيا الجزيئية و الكيمياء العلاجية – معهد تيودور بلهارس للأبحاث- الجيزة- مصر.

تنتج الفطريات بشكل طبيعى مواد أيض ثانوية و التى تتم دراسة نشاطها الحيوى فى العديد من الأبحاث. تهدف هذه الدراسة إلى استكشاف المركبات الفينولية التى تنتجها ثلاثة أنواع من الفطريات المعزولة من بيئة المياه العذبة بالإضافة إلى دراسة النشاط المضاد للميكروبات و المميت للأطوار السابحة لديدان شيستوسوما مانسونى لكل من ميثيل جاللات و حمض الباراكوماريك كإثنين من المركبات الكبرى التى تنتجها الفطريات موضع الدراسة.

أوضح جهاز الفصل الكوماتوجرافى للسوائل ذو الأداء العالى المصحوب بالكاشف ذوالصمام الثنائى وجود ٢٢ مركب فينولى فى مستخلصات رشيح كل من فطر بنيسيليوم إمبليكاتم و أسبيرجيللس نيفياس و أسبيرجيللس بيتراكياى. بالرغم من الإختلاف فى تركيزات هذه المركبات من نوع فطرى لآخر، كان ميثيل جاللات واحد من المركبات الكبرى فى مستخلص رشيح كل من بنيسيليوم إمبليكاتم و أسبيرجيللس نيفياس، و كان هو المركب صاحب أعلى تركيز فى مستخلص رشيح أسبيرجيللس بيتراكياى. بينما كان حمض الباراكوماريك هو الأعلى تركيزا فى مستخلص رشيح أسبيرجيللس نيفياس. أوضحت الدراسة أن ميثيل جاللات كان فعالا ضد كل من سودوموناس إيوريجينوزا، إيشيريشيا كولاى، ستافيلوكوككس أوريوس تحت نوع أورياس، بينما كان حمض الباراكوماريك فعال فقط على باسيليس سيريوس. أما بخصوص الفعالية على الأطوار السابحة لدودة شيستوسوما مانسونى ، فلقد كان حمض الباراكوماريك أكثر فاعلية على السركاريا مقارنة بميثيل جاللات . تعتبر الأنواع الفطرية موضع الدراسة مصدرا غنيا للمركبات الفينولية ، و يمثل كل من ميثيل جاللات وحمض الباراكوماريك موادا مضادة للبكتريا و مميتة للأطوار اليرقية لديدان الشيستوسوما.