Advances in Biochemistry
2022; 10(1): 11-17
http://www.sciencepublishinggroup.com/j/ab
doi: 10.11648/j.ab.20221001.12
ISSN: 2329-0870 (Print); ISSN: 2329-0862 (Online)

Evaluation of the Antioxidant Activity and Mycochemical Screening of Two *Penicillium oxalicum* Isolated from Soil and Leaves of *Solanum lycopersicum* Respectively

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To cite this article:
Delchinor Arioste Doh Gneho, Dodehe Yeo, Alex Gilles Pakora, David Jean N'Guessan. Evaluation of the Antioxidant Activity and Mycochemical Screening of Two *Penicillium oxalicum* Isolated from Soil and Leaves of *Solanum lycopersicum* Respectively. *Advances in Biochemistry*. Vol. 10, No. 1, 2022, pp. 11-17. doi: 10.11648/j.ab.20221001.12

Received: December 22, 2021; **Accepted**: January 10, 2022; **Published**: January 15, 2022

Abstract: The purpose of the work was to compare the mycochemical composition and antioxidant properties of acetate extracts of *Penicillium oxalicum* from two different sources, soil and leaves of *Solanum lycopersicum* (tomato). After extraction of the metabolites on rice medium, qualitative analyses of the extracts were carried out by colorimetric tests and thin layer chromatography (TLC). The contents of total polyphenols and flavonoids were determined respectively by the Folin-Ciocalteu method and the $AlCl_3$ method. For antioxidant capacity, the 1,1-diphenyl-2picrylhydrazyl free radical trapping method and the FRAP method were used. *Penicillium oxalicum* from the soil was characterised by molecular biology. Mycochemical analysis revealed the existence of polyphenols, flavonoids, alkaloids, polyterpenes and sterols and saponosides in the extract of *Penicillium oxalicum* from soil. Except for saponosides, the other secondary metabolites were present in the endophyte extract but in low quantities. In addition, the contents of total flavonoids and phenols in soil *Penicillium oxalicum,* which are respectively 11.67 ± 1.03 mg QE/ g extract and 58.36 ± 2.779 mg GAE/ g extract, were higher than those of the endophyte 6.03 ± 0.95 mg QE/ g extract and 36.66 ± 2.01 mg GAE/ g extract. Concerning the antioxidant activity, the IC_{50} of *Penicillium oxalicum* extracts were 67.73 ± 0.58 µg/mL for the soil extract and 2.74 ± 0.33 mg/mL for the endophyte extract. The reducing power of *Penicillium oxalicum* from the soil is higher than that of the endophyte. It appears that *Penicillium oxalicum* from soil is rich in secondary metabolites. Also, it showed a better antioxidant activity. This work shows that soil *Penicillium oxalicum* is the best candidate for the production of antioxidant compounds.

Keywords: Soil *Penicillium oxalicum*, Endophytic *Penicillium oxalicum*, Antioxidants, DPPH, FRAP

1. Introduction

Oxidative stress is an etiological factor implicated in the cause of various human diseases, such as cancer, diabetes *mellitus*, cardiovascular and neurodegenerative diseases, inflammation, ageing, skin damage and arthritis [1-3]. Indeed, oxidative damage is achieved by free radical attack on various biomolecules, in particular proteins, lipids and DNA, ultimately resulting in cell degradation and death [4, 5]. To protect itself against this damage, the body has defence systems [6, 7]. However, these are not always effective. Therefore, antioxidants are used to form a cooperative defence system. Antioxidants are defined as compounds that possess the ability to protect biological systems from the potentially harmful effects of processes or reactions that may cause excessive oxidation [8]. Many synthetic antioxidants such as butylated hydroxyanisole (BHA), tert-butylhydroxyquinone (TBHQ) and butylated hydroxytoluene (BHT) exist, but they are suspected of causing liver damage and carcinogenesis [9-11].

As a result of the above, the search for new bioactive molecules in plants, as well as in fungi such as *Penicillium oxalicum*, becomes imperative.

Fungi live in both terrestrial and aquatic environments [12]. Thus, they readily synthesise a wide range of metabolites such as alkaloids, benzoquinones, flavanoids, phenols, steroids, terpenoids, tetralones and xanthones [13].

These metabolites possess several bioactive properties including antioxidant properties [14].

Among the fungi, *Penicillium oxalicum* drew our attention. *Penicillium oxalicum* is a ubiquitous fungus in soil, seas and plants [15].

Studies conducted on *Penicillium oxalicum* isolated from medicinal plants have shown its antioxidant activity [16-20]. However, the origin (soil and plant), environmental cues and harvesting period of the fungi, influence their bioactive properties [21-23]. *Solanum lycopersicum* (tomato), an herbaceous plant belonging to the Solanaceae family, is one of the most widely grown vegetable crops in the world [24]. The antioxidant activity of tomato leaves was reported by [25]. The aim of the present work was to compare the antioxidant activities of two *Penicillium oxalicum* extracts from different sources and their mycochemical screening.

2. Material and Method

2.1. Material

Two *Penicillium oxalicum* from different sources were used as fungal material in this study. One was isolated from the leaves of *Solanum lycopersicum* (tomato) (NR121232.1) under high parasitic pressure and the other from the soil in Songon located 1 Km from the Abidjan-Dabou road axis in the South of Côte d'Ivoire, whose coordinates are (5°18′ 5 ″ North, 4°14′ 56″ West) with an altitude of 10 m. The endophyte was provided by the Laboratory of Bacteriology and Virology of the UFR of Medical Sciences (Felix Houphouët Boigny University).

2.2. Methods

2.2.1. Isolation

The soil sample was collected from the same *Solanum lycopersicum* plot in Songon according to the method described by [26]. Briefly, on the plot, using a sterile spatula, 100 g of soil was carefully taken at random and stored in a sterile packaging bag (L x W: 200 x 145 mm). This soil sample was placed in a cooler and immediately transported to the laboratory where it was stored at 4°C.

Penicillium oxalicum was isolated by the suspension-dilution method [27] on Potato Dextrose Agar (PDA) medium (Conda SA, Spain). The stock suspension was prepared by mixing 1 g of soil in 9 mL of sterile distilled water. After vertexing the mixture for 5 min, a series of decimal dilutions from 10^0 to 10^{-5} was performed. Suspensions of 10^{-3} to 10^{-5} were plated on PDA medium supplemented with Chloramphenicol. Three trials were performed. The plates were incubated at 28°C for 7 days. The colonies obtained were subcultured several times on PDA medium until pure cultures were obtained.

2.2.2. Molecular Identification

(i). DNA Extraction

The extraction of genomic DNA from *Penicillium oxalicum* was performed as described by [28] with some modifications. To extract the DNA, the fungal isolate was grown on PDA agar and incubated at 28°C for 7 days for mycelial growth. Next, liquid culture of the fungal isolate was performed by adding 100 µL of the fungal suspension to 3 mL of Potato Dextrose Broth (PDB) medium, which was incubated at 28°C for 3 days. Then, 1.5 mL of the mixture was placed in a cryotube and centrifuged at 10,000 g for three (3) minutes. After removal of the supernatant, the pellet was homogenised in 400 µL of TBS buffer (0.4 M NaCl, 10 mM Tris-HCl pH 8 and 2 mM EDTA pH 8) and vortexed for 1 min. Then, 40 µL of 20% SDS and 8 µL of Proteinase K (20 mg/mL) were added. The mixture was incubated at 60°C for 3 hours. After incubation, 300 µL of 6 M NaCl was added to the tube which was also vortexed for 30 seconds and then centrifuged at 10,000 g for 30 min at 4°C. The supernatant was transferred to a 2 mL tube to which an equal volume of 100% Isopropanol was added. This tube was incubated at -20°C for one hour and then centrifuged at 10,000g for 20 min at 4°C. The pellet was washed with 70% ethanol and then centrifuged again for 10 min at 10,000 g. The pellet from the latter centrifugation was recovered and dried at 37°C for 5 min. The resulting DNA was re-suspended in 300 µL of sterile Tris-EDTA (TE) buffer and stored at -20°C for amplification.

(ii). PCR Amplification of the Internal Transcribed Spacer (ITS)

The fungal isolate was identified by sequencing the ITS (Internal Transcribed Spacer) regions of the rDNA (Eurofins Genomics ITS4 and ITS5). For this purpose, four (4) µL of 5x Master Mix buffer (Slis biodyne FIREPOL), 0.75 µL of each fungal primer at 10µM, 5 µL of fungal DNA were combined in a final volume of 25 µL. PCR amplifications were performed with primers ITS4 (TCCTCCGGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) according to the following program: 4 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C and 2 min at 72°C, with a final extension of 15 min at 72°C.

(iii). Sequencing and Sequence Comparison with the Database

The resulting amplificate was packaged and sent to Eurofin Genomic for Sanger sequencing according to the recommendations of the service provider. Analysis of the sequence obtained after PCR was performed with the BLAST local base alignment tool from GenBank (http://www.ncbi.nlm.nih.gov).

2.2.3. Extraction of Metabolites from Penicillium oxalicum

For the extraction of metabolites, each *Penicillium oxalicum* was pre-picked on PDA medium and incubated at 28°C for 7 days. From the obtained young fungal cultures, a suspension of spores was made in sterile distilled water. One millilitre (1mL) of suspension was transferred to rice medium. In total, the fungal suspension was spread on ten plates of rice medium and incubated at 28°C for 21 days for

each of the fungi. At the end of the incubation, the cultures were collected in 1L Erlenmeyer flasks to which 500 mL of ethyl acetate was added and macerated for 24 h under agitation at room temperature (about 28°C). The solutions obtained after maceration were filtered three times on cotton wool and once on wattman paper. The filtrates recovered were concentrated using a rotary evaporator. The extracts obtained were respectively the acetate extract of *Penicillium oxalicum* from soil and that of *Solanum lycopersicum* [29].

2.2.4. Qualitative Screening of Secondary Metabolites

The mycochemical screening consisted in characterising some chemical groups present in the ethyl acetate extracts of *Penicillium oxalicum*. Thus, chemical groups such as alkaloids, polyphenols, flavonoids, tannins, saponosides, quinones, polyterpenes and sterols were searched for in the extracts by the methods described by [30]. Table 1 summarises the reagents for the secondary metabolites of interest and the characteristic reaction results.

Table 1. *Reagents corresponding to the secondary metabolites investigated and characteristic reaction results.*

Secondary metabolites	Reagents	Reaction indicating the positivity of the test
Alkaloids	Dragendorff	Orange precipitate.
	Bouchardât	Reddish brown precipitate.
Flavonoids	Cyanidin	Purplish coloration.
Polyphenols	Ferric chloride	More or less dark blue-black or green colouring.
Quinones	Bornstraegen	Red or yellow colouring violet.
Saponosides	Foam test	Persistent moss, larger than 1 cm.
Sterols and Polyterpenes	Liebermann	The appearance at the interface of a purplish purple ring, which then turns blue and then green.
Tannins	Stiasny	Catechic: precipitate in large flakes. Gallic: intense blue-black colouring.

2.2.5. Thin Layer Chromatography (TLC)

The support used in this study is a 20 × 20 cm silica gel aluminium plate (silica gel 60 F_{254}). Ethyl acetate/methanol (9:1) was the only migration system used for performing TLC of alkaloid, phenolic and terpene compounds. The visualisation of the spots was done by UV lamp at wavelengths of 254 and 365 nm. The reagents used were Draggendorf reagent for alkaloid compounds and FastBlue reagent for phenolic compounds. For terpenic compounds and sterols, the TLC plates were heated to 100°C after immersion in sulphuric vanillin.

2.2.6. Determination of Total Flavonoids

To a volume of 5 mL (0.1 mg/mL) of extract, 1.5 mL of methanol, 0.1 mL of 10% aluminium trichloride, 0.1 mL of potassium acetate (1M) and 2.5 mL of distilled water are added successively. After incubation at room temperature for 30 min, the optical densities are read with a spectrophotometer at 415 nm.

The flavonoid concentration was deduced from the range of the calibration curve established with Quercetin (0-100 µg/mL) used as standard. The result was expressed as milligram quercetin equivalent per gram of extract (mg EQ/g extract) [31].

2.2.7. Determination of Total Phenols

To 100 µL of extract dissolved in methanol (1 mg/mL), 500 µL of Folin-Ciocalteau reagent (10-fold dilution) was added. After 2 minutes, 1.5 mL of 20% Na_2CO_3 was added to the mixture. The final volume was made up to 5 mL by adding distilled water. The mixture was incubated in the dark at room temperature for 30 minutes and the absorbance was read at 765 nm. Quantification was done according to the standard curve for gallic acid. The result was expressed as milligram of gallic acid equivalent (GAE) per gram of extract (mg GAE/g extract) [32].

2.2.8. Assessment of Antioxidant Activity

(i). Measurement of Antiradical Activity by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Test

The antiradical activity of the fungal extract was measured by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) test according to the method of [33].

A range of concentrations (0-200 µg/mL) of fungal extract or ascorbic acid (reference antioxidant) is prepared in methanol. An equal volume of DPPH solution (0.5 mmol/mL) was added to the test sample or control and incubated for 30 minutes at room temperature in the dark. After 30 min of incubation, the absorbance of the sample was measured at 517 nm using a spectrophotometer (Jenway 7305). The inhibition of the DPPH radical by a given extract is expressed as a percentage (%) and determined by the following formula:

$$I(\%) = [(Abs\ control - Abs\ test) / Abs\ control] \times 100$$

I(%): percentage of free radical inhibition;

Abs control: absorbance of the medium containing all reagents except the extract.

Abs test: absorbance of the reaction medium containing the DPPH and the test extract.

(ii). FRAP Method (Reducing Power of Iron)

The FRAP test was carried out according to the method of [34] with some modifications. The FRAP working solution is prepared by mixing in a volume ratio (10 - 1 - 1) the following three solutions: a 300 mM sodium acetate buffer solution (pH= 3.6), a 10 mM TPTZ solution (40 mM HCl as solvent) and a 20 mM FeCl solution respectively. The FRAP working solution is kept in a water bath at 37°C. The test consists of reacting 100 µL of each extract with 3 mL of the FRAP working solution in glass haemolysis tubes. The absorbance of the reaction mixture was measured at 593 nm

after 4 min incubation at room temperature. This was done in triplicate.

2.2.9. Statistical Analysis

Analyses of variance (ANOVA) were carried out to highlight significant differences between the contents of flavonoid, polyphenol, IC_{50} and the reducing power of the fungal crude extract, using GraphPad Prism 5.0 software.

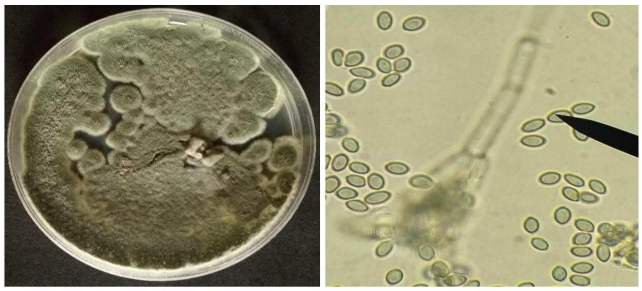
3. Results and Discussion

3.1. Identification of the Isolate

The main objective of our study was to compare the antioxidant activity of *Penicillium oxalicum* in the soil and in the plant.

After isolation of the fungal strain from the soil, identification was made on the basis of morphological and molecular characters. Colonies were fast growing reaching a diameter of 3.2 cm on PDA medium after 3 - 4 days at 28°C and were dark green, powdery showing abundant sporulation without reverse pigmentation (Figure 1A). The hyphae were septate ending in conidiophores passing through phialides (Figure 1B).

Following sequencing, a sequence length of 554 bp and a percentage of 98.91% of the sample was obtained. A BLASTn with the Megablast program was used to process the sequence. The database used was RNA typestrains / ITS_RefSeq Fungi. Thus, the isolate was identified as *P. oxalicum on the* basis of morphological and molecular characteristics [35]. Table 2 shows all the information collected.



A B

Figure 1. *(A) Macroscopic observation of the morphology of Penicillium oxalicum colonies on PDA medium (1440×720); (B): Microscopic observation of the shape of the mycelium and spores (G×100).*

3.2. Mycochemical Screening

The mycochemical screening showed the presence of different chemical groups in the acetate extract of *Penicillium oxalicum* from the soil and that of the endophyte of *Solanum lycopersicum*. Thus, from the intensity of the staining obtained for each of the characterisation tests, it appears that:

The acetate extract of *Penicillium oxalicum* from the soil contained a high amount of polyphenols, flavonoids and alkaloids, whereas the extract of *Penicillium oxalicum* endophytic contained an average amount of these three secondary metabolites. Sterols and polyterpenes were in average quantity in both extracts. Regarding quinones, they were present only in the soil extract of *Penicillium oxalicum*. However, an absence of saponoside and tannins was observed in both extracts (Table 3). Thin layer chromatography confirmed the presence of the different chemical groups sought Table 4.

Table 2. *Identification of the fungus isolated from the soil.*

Source	DNA sequence of the isolate (FASTA file)	Name of the fungus
Soil	5'-GGAAGGACATTACCGAGTGAGGGCCCTCTGGGTCCACCT CCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCC GCCTCACGGCCGCCGGGGGGCATCCGCCCCCGGGCCCGC GCCCGCCGAAGACACACAAACGAACTCTTGTCTGAAGAT TGCAGTCTGAGTACTTGACTAAATCAGTTAAAACTTTCA ACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCA GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG AATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTA TTCCGGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCT CAAGCACGGCTTGTGTGTTGGGCTCTCGCCCCCCGTTTCC GGGGGGCGGGCCCGAAAGGCAGCGGCGGCACCGCATCC GGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAG GCCCGGCCGGCGCCCGCCGGAGAACACCATCAATCTTAA CCAGGTTGACCTCGGATCATGTAGGGATACCCGCTGAAC TTACGCG-3'	*Penicillium oxalicum*

Table 3. *Secondary metabolite composition of ethyl acetate fungal extracts.*

Reclaimed mycocomposites	*Penicillium oxalicum* from soil	*Penicillium oxalicum* endophytic
Sterols and polyterpenes	1	1
Polyphenols	2	1
Flavonoids	2	1
Tannins	0	0
Quinones	1	0
Alkaloids	2	1
Saponosides	0	0

(2): Abundance; (1): Presence; (0): Absence.

Table 4. *Characterisation of compounds by thin layer chromatography.*

Mycochemicals	Revealer	*Penicillium oxalicum* from soil	*Penicillium oxalicum* endophytic
Alkaloids	Dragerndorff	x	X
Polyphenols	Fast blue B	x	X
Sterols and terpenes	Vanillin-sulfuric acid	x	X

(x): presence.

The secondary metabolites detected in the extracts are known to have beneficial use in the pharmaceutical industry. It has been reported that polyphenols exhibit antimicrobial, anti-inflammatory, antimutagenic, antitumour and antioxidant activities [36]. Concerning, flavonoids, they have antibacterial, antiviral, antidiabetic, antioxidant activities [37]. For terpenes, activities such as antibacterial, anti-inflammatory, antioxidant have been reported [38]. As for alkaloids, they have anticholinergic, antitumor, antidiuretic, antiviral, antihypertensive, antidepressant, antimicrobial, antioxidant and anti-inflammatory activities [39]. Quinones exhibit anticancer and antioxidant activities [40].

This study showed that *P. oxalicum* from the soil is richer in secondary metabolites compared to that of the endophyte.

3.3. Determination of Total Flavonoids and Polyphenols

Polyphenols are the main antioxidant components derived from fungi, as are flavonoids, which are considered the most important class of polyphenols. Thus, the determination of antioxidant capacity in various studies has focused mainly on these compounds [12, 41]. Therefore, the total content of flavonoids and polyphenols in the extracts of the tested fungi were determined quantitatively. The total phenol content of the extracts was determined graphically by linear regression of the ascorbic acid standard curve ($y=10.122x + 0.0778$; $R^2= 0.9939$) and expressed as ascorbic acid equivalent. The results show that the soil extract of *P. oxalicum* had a content of 58.36 ± 2.779 mg EAG/ g extract and that of the endophyte 36.66 ± 2.01 mg EAG/ g extract. The total flavonoid content of the extracts was determined graphically by linear regression of the quercetin standard curve ($y=13.7009x$; $R^2= 0.9976$) and expressed as Quercetin equivalent. The results reveal that the total flavonoid content of the *P. oxalicum* soil extract was 11.67 ± 1.03 mg EQ/g extract and for that of the endophyte, 6.03 ± 0.95 mg EQ/g extract.

It is inferred that the *P. oxalicum* extract from the soil had high levels compared to that of the endophyte.

The total polyphenol content of *P. oxalicum* in the soil is much higher than that obtained by Arora and Chandra [42] in the extract of *Aspergillus fumigatus* (5.68 mg GAE/g), the same for the work of Abo-Elmagd [43] in the acetate extract of *Chaetomium madrasense* (17.43 mg GAE/g), the same is true for Arora and Chandra [44] in the freeze-dried extracellular extract of *Penicillium citrimum* (28 mg GAE/g), and finally for Arora and Chandra [45] in the extract of *Aspergillus PR78* (17.32 mg GAE/g). The composition of the phenolic content of fungi generally depends on genetic, environmental and other factors [46].

3.4. Assessment of Antioxidant Activity

To determine the *in vitro* antioxidant activity of acetate extracts of *P. oxalicum* from soil and endophyte, DPPH and FRAP assays were performed. Antioxidants scavenge radicals through hydrogen donation to form the stable DPPH molecule. The antioxidant activity of an extract is related to its low inhibitory concentration. Indeed, a low IC_{50} value indicates a better antioxidant activity [47]. Thus, the best free radical scavenging activity was obtained with vitamin C, the reference molecule (IC_{50} 8.64 ± 0.13µg/mL), followed by the acetate extract of *P. oxalicum* from soil (IC_{50} 67.73 ± 0.58 µg/mL), and finally the acetate extract of endophytic *P. oxalicum* (IC_{50} 2.74 ± 0.33 mg/mL).

The best antiradical activity observed with the *P. oxalicum* extract from the soil, would be due to the phenolic compounds [48]. Indeed, similar results have been reported by other authors who have shown that there is a relationship between the content of phenolic compounds and the antiradical activity of fungi extracts [49, 50]. The antiradical effect of polyphenols, especially flavonoids, is attributed to their low redox potential which makes them thermodynamically capable of reducing free radicals by transferring hydrogen atoms from hydroxyl groups [51, 52].

For the FRAP test, recall that the method is based on the ability of the extract to reduce ferric Fe^{3+} to ferrous Fe^{2+}[47]. The antioxidants reduce the Fe^{3+} ferricyanide complex to the ferrous form by donating an electron. The colour of the solution to be examined then changes from yellow to various shades of green and blue [53].

The FRAP values of soil *P. oxalicum* and endophyte *P. oxalicum* were 260.9 ± 24.68 and 143.80 ± 1.43 µmol Eq Trolox/ g EXS, respectively. The FRAP value of soil *P. oxalicum* was significantly higher than that of the endophyte.

This ability to reduce Fe (III) is attributed to phenolic compounds that can donate hydrogen atoms [54]. Generally, the reducing properties are associated with breaking the radical chain by donating a hydrogen atom [55]. Extracts from fungi that have shown the presence of the compounds and total flavonoids would act in the same way.

4. Conclusion

From this study, it was found that *Penicillium oxalicum* from soil, in addition to quinones, is richer in phenolic, alkaloid and terpene compounds than that isolated from tomato leaves.

Also, *Penicillium oxalicum* from soil showed a higher antioxidant potential than that of the endophyte, as indicated by its DPPH free radical scavenging capacity and its iron

reducing capacity. The antioxidant activity of *Penicillium oxalicum* from soil was attributed to phenolic compounds. Such compounds found in soil *Penicillium oxalicum* with antioxidant activity, makes this fungus an interesting natural source for its industrial and medical use.

References

[1] Ahmad, W., Ijaz B., Shabbiri, K., Ahmed, F. and Rehman, S. (2017) Oxidative toxicity in diabetes and Alzheimer's disease: mechanisms behind ROS/ RNS generation. *Journal of Biomedical Science*, 24, 76.

[2] Sarr, S. O., Fall, A. D., Gueye, R., Diop, A., Diatta, K., Diop, N., Ndiaye, B. and Diop, Y. M. (2015) Study of the antioxidant activity of Vitex doniana (Verbenaceae) leaf extracts. *International Journal of Biological and Chemical Sciences*, 9 (3): 1263-1269.

[3] Harrison, I. P. and Selemidis, S. (2014) Understanding the biology of reactive oxygen species and their link to cancer: NADPH oxidases as novel pharmacological targets. *Clinical and Experimental Pharmacology*, 41, 533-542.

[4] Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. and Mazur, M. (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, 160: 1-40.

[5] Moon, J. K. and Shibamoto T. (2009) Antioxidant assays for plant and food components. *J Agr Food Chem*, 57: 1655–66.

[6] Sharma, S., Shrivastav, B. R. and Shrivastav, A. (2013) Free Radicals, Antioxidants and Oxidative Stress. *International Journal of Advanced Research*, 1 (9), 252-258.

[7] Chandra, P., Sharma, K. R. and Arora, S. D. (2019) Antioxidant compounds from microbial sources: a review. *Food Research International*, 1-74.

[8] Krinsky, I. N. (1989) Antioxydant functions of carotenoids. *Free Radical Biology and Medicine*, 7 (6): 617-635.

[9] Qi, H. M., Zhao, T. T., Zhang, Q. B., Li Z. E., Zhao, Z. Q. and Xing, R. (2005) Antioxidant activity of different sulfated polysaccharides of different molecular weight from Ulva pertusa Kjellm (Chlorophyta). *Journal of Applied Phycology*, 527-534.

[10] Djeridane, A., Yousfi, M. and Brunel, J M. (2010) Isolation and characterization of a new steroid derivative as a powerful antioxidant from Cleome arabica in screening the in vitro antioxidant capacity of 18 Algerian medicinal plants. *Food ChemToxicol*, 48: 2599–606.

[11] Edziri, H., Mastouri, M. and Aouni M. (2012) Polyphenols content, antioxidant and antiviral activities of leaf extracts of Marrubium deserti growing in Tunisia. *South Afr J Botany*, 80: 104-9.

[12] Jakovljevic, D. V., Nikolic N. J., Matic, L. S., Stanic M. S. and Vrvic, M. M. (2018) "Mycochemical screening, antioxidant and DNA protecting activity of *Penicillium cyclopium* and *Penicillium brevicompactum*," *FARMACIA*; 66 (3) 494–501.

[13] Archer, D. B. (2000) "Filamentous fungi as microbial cell factories for food use," *Current Opinion in Biotechnology*, 11 (5) 478–483.

[14] Subha, K., Baskar, V., Kanimozhi, K. and Panneerselvam, A. (2015) *In vitro* investigation on antioxidant activity of terrestrial and marine *Penicillium chrysogenum* strain. *Int J Res Pure Applied Microbiology*, 5: 1-5.

[15] Bian, X., Bai, J. B., Hu, X., Wu X., Xue, C., Han, A., Su G., Hua, H. and Pei, Y. (2015) Penioxalicin, a novel 3-nor-2,3-seco-labdane type diterpene from the fungus Penicillium oxalicum TW01-1. *Tetrahedron Letters*, 56, 5013–5016.

[16] Bisht, R., Bhatt, A. and Agarwal, P. K. (2016) Antimicrobial, Antioxidant Activity and Phytochemical Analysis Of An Endophytic Fungi Penicillium Oxalicum Isolated from a Gymnosperm Tree Cupressus Torulosa D. Don. Of Garhwal Region *International Journal of Science, Engineering and Management*; 1: 90-96.

[17] Bhattacharjee, S., Debnath, G., Das, R. A., Saha, K. A. and Das, P. (2017) Characterization of silver nanoparticles synthesized using an endophytic fungus, *Penicillium oxalicum* having potential antimicrobial activity. Advances in Natural Sciences: *Nanoscience and Nanotechnology*, 8, 6p.

[18] Kaur, R., Kaur, J., Kaur, M., Kalotra, V., Chadha, P., Kaur, A. and Kaur, A. (2019) An endophytic Penicillium oxalicum isolated from Citrus limon possesses antioxidant and genoprotective potential. *Journal of Applied Microbiology*, 128: 1400-1413.

[19] Yen, Gow-Chin, Lee, Che-An. (1996) Antioxidant Activity of Extracts from Molds. *Journal of Food Protection*, 59 (12) 1327-1330.

[20] Fadhillah, F., Elfita, E., Muharni, M., Yohandini, H. and Widjajanti, H. (2021) Antioxidant Compound of Endophytic Fungi *Penecillium oxalicum* Isolated from *Swietenia mahagoni* L. Jacq. *Tropical Journal of Natural Product Research*, 5 (3): 543-548.

[21] Aly, A. H., Debbab, A. and Proksch, P. (2011) Fungal endophytes: unique plant inhabitants with great promises. *Appl J Microbiol Biotechnol*, 90: 1829–1845.

[22] Hoffmeister, D. and Keller, N. P. (2007) Natural products of filamentous fungi: enzymes, genes, and their regulation. *Nat Prod Rep*, 24: 393-416.

[23] Elkhateeb, A. W. and Daba, M. G. (2018) Where to Find? A Report for Some Terrestrial Fungal Isolates, and Selected Applications Using Fungal Secondary Metabolites. *Biomed J Sci & Tech Res*, 4, 4: 1-4.

[24] Sinno, M., Ranesi, M., Gioia, L., d'Errico, G. and Woo, L. S. (2020) Endophytic Fungi of Tomato and Their Potential Applications for Crop Improvement. *Agriculture*, 10, 587.

[25] Piao, X-M., Jang, E-K., Chung, J-W., Lee, G-A., Lee, H-S., Sung, J-S., Jeon, Y-A., Lee, J-R., Kim, Y-G. and Lee, S-Y. (2013) Variation in Antioxidant Activity and Polyphenol Content in Tomato Stems and Leaves. *Plant Breed. Biotech*, 1 (4): 366-373.

[26] Barillot, C. D. C., Sarde, C. O., Bert, V., Tarnaud, E. and Nelly, C. (2012) A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. *Ann Microbiol*. 9p.

[27] Rapilly F. (1968) Les techniques de mycologie en Pathologie végétale, *Ann. Epiphyt*., 19, 97p.

[28] White, T. J., Bruns, T., Lee, S. and Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). *Academic Press, New York, USA*: 315–322.

[29] Gogoi, D. K., Deka, H. P., Saikia, B. R. and Bora, T. C. (2007) Optimization of process parameters for improved production of bioactive metabolite by a novel endophytic fungus Fusarium sp. DF2 isolated from Taxus wallichiana of North East India. *World J. Microbiol Biotechnol*, 1-9.

[30] Koulaï, D., Doumbia, I., Yeo, S. O., M'boh, G. M., Kipré, G. R. and Djaman, A. J. (2021) Triphytochemistry and effects of aqueous and hydro-ethanolic extracts of Spathodea campanulata P. beauv. (Bignoniaceae) on blood-sugar level and markers of pancreatitis in type 2 diabetic rats. *International Journal of Innovation and Applied*, 33, 2, 426-434.

[31] Yi, Z-B., Yu, Y., Liang, Y-Z. and Zeng, B. (2007) In vitro antioxidant and antimicrobial activities of the extract of *Pericarpium Citri* Reticulatae of a new Citrus cultivar and its main flavonoids, *LWT-Food Science and Technology*, 4: 1000-1016.

[32] Yadav, M., Yadav, A. and Yadav, J. P. (2014) In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from Eugenia jambolana Lam. *Asian Pacific Journal of Tropical Medicine*, 7 (1): S256-S261.

[33] Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J. and Codina, C. (2002) Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. *Journal of Agricultural and Food Chemistry*, *50* (23), 6882-6890.

[34] Benzie, F. F. I., Strain, J. J. (1996) The Ferric Reducing Ability of Plasma (FRAP) as a Measure of ''Antioxidant Power'': The FRAP Assay. *Analytical Biochemistry*, 239, 70–76.

[35] Zhao, R-B., Bao, H-Y. and Liu, Y-X. (2010) Isolation and Characterization of *Penicillium oxalicum* ZHJ6 for Biodegradation of Methamidophos. *Agricultural Sciences in China*, 9 (5): 695-703.

[36] Xia, E-Q., Deng, G-F., Guo, Y-J. and Li H-B. (2010) Biological Activities of Polyphenols from Grapes. *Int. J. Mol. Sci.*, 11, 622-646.

[37] Karak, P. (2019) biological activities of flavonoids: an overview. *IJPSR,* 10 (4): 1567-1574.

[38] Elissawy, M. A., El-Shazly, M., Ebada, S. S., Singab, B. A. and Proksch, P. (2015) Bioactive Terpenes from Marine-Derived Fungi. *Mar. Drugs*, *13*, 1966-1992.

[39] Souto, A. L., Tavares, J. F., da Silva M. S., Diniz, M. F. F. M., de Athayde-Filho P. F. and Filho, J. M. B. (2011) Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. *Molecules*, 16: 8515-8534.

[40] El-Najjar, N., Gali-Muhtasib, H., Ketola, A. R., Vuorela, P., Urtti, A. and Vuorela, H. (2011) The chemical and biological activities of quinones: overview and implications in analytical detection. *Phytochem Rev*, 19p.

[41] Nsimba, Y. R., Kikuzaki, H. and Konishi, Y. (2008)"Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus* spp. seeds," *Food Chemistry*, 106 (2) 760–766.

[42] Arora, S. D. and Chandra, P. (2011) Antioxidant Activity of Aspergillus fumigatus. *ISRN Pharmacology*, 11p.

[43] Abo-Elmagd, I. H. (2014) Evaluation and optimization of antioxidant potentiality of Chaetomium madrasense AUMC 9376. *Journal of Genetic Engineering and Biotechnology*, 1-6 p.

[44] Arora, S. D. and Chandra, P. (2011) In Vitro Antioxidant Potential of Some Soil Fungi: Screening of Functional Compounds and their Purification from Penicillium citrinum. *Appl Biochem Biotechnol*, 165: 639–651.

[45] Chandra, P. and Arora, S. D. (2009) Antioxidant activity of fungi isolated from soil of different areas of Punjab, India. *Journal of Applied and Natural Science*, 1 (2): 123-128.

[46] Heleno, S. A., Barros, L., Sousa, M. J., Martins, A. and Ferreira, I. C. (2010) Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. Food Chemistry 119, 1443–1450.

[47] Nandhakumar, E. and Indumathi, P. (2013) In vitro antioxidant activities of methanol and aqueous extract of *Annona squamosa* (L.) fruit pulp. *Journal of Acupuncture and Meridian Studies*, 6 (3): 142-148.

[48] Sikandar, A., Zhang, M., Wang, Y., Zhu, X., Liu, X., Fan, H., Xuan, Y., Chen, L. and Duan, Y. (2020) Mycochemical Screening and Analysis, Antioxidant Activity, and Biochemical Composition of Fermentation Strain Snef1216 (*Penicillium chrysogenum*). *Journal of Analytical Methods in Chemistry*, 8 p.

[49] Ramesha, A. and Srinivas, C. (2014) "Antimicrobial activity and phytochemical analysis of crude extracts of endophytic fungi isolated from *Plumeria acuminata* L. and *Plumeria obtusifolia* L." *European Journal of Experimental Biology*, 4, 35–43.

[50] Sajeesh, T., Arunachalam K and Parimelazhagan, T. (2011) "Antioxidant and antipyretic studies on *Pothos scandens* L," *Asian Pacific Journal of Tropical Medicine*, 4 (11), 889–899,

[51] Siddhuraju, P. and Becker, K. (2007) The antioxidant and free radical scavenging activities of processed cowpea *Vigna unguiculata* L. Walp seed extracts. *Food Chemistry*.; 101: 10-19.

[52] Amarowicz, R., Pegg, R. B., Moghaddam, P. R., Barl, B. and Weil, J. A. (2004) *Food Chemistry*, 84 551 -562.

[53] Amaeze, O. U., Ayoola, G. A., Sofidiya, M. O., Adepoju-Bello, A. A., Adegoke, A. O. and Coker, H. A. B. (2011) Evaluation of antioxidant activity of *Tetracarpidium conophorum* (Müll. Arg) Hutch & Dalziel leaves. *Oxidative Medicine and Cellular Longevity*, 7p.

[54] Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992) Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agriculture and Food Chemistry*, 40: 945- 948.

[55] Othman, A. Ismail, A., Ghani, N. A. and Adenan, I. (2007) Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry*, 100, 1523-1530.