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Preliminary Investigation of the Fungal Endophytic Extract Isolated from *Tabernaemontana pandacaqui* Leaves and Evaluation of its Antioxidant and Cytotoxic Potentials

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ABSTRACT

Objectives: *Tabernaemontana pandacaqui* is used in Indonesian folklore as a cure for ulcers, inflammation, skin sores and stomach pain, it was recently grown in Egypt for ornamental purposes. It can host endophytes which produce variety of secondary metabolites of potential biological activities. Based on traditional use of this plant, it was essential to study endophytes living in this host plant and evaluate their phytochemical and biological values. **Methods:** *T. pandacaqui* leaves were used as the microbial host material for the isolated fungus which was mass cultured and extracted. Fungal ethyl acetate extract was analyzed with GC-MS. Quantitative and qualitative identification of the compounds was performed based on the comparison of their retention times and mass spectra with those of the NIST, WILLY library data of the GC-MS system. Its antioxidant activity was evaluated using DPPH and ABTS assays and expressed as Trolox Equivalent Antioxidant Capacity (TEAC) and the cytotoxic screening against four cell lines was carried out using SRB assay. **Results:** The isolated fungus (TMP16) was identified as *Aspergillus* species. GC-MS analysis revealed the presence of high percentage of nitrogenous compounds (75.38%) of which porphyrin derivatives dominate (42.85%). The fungal extract gave antioxidant scavenging power of 881.49 ± 44.6 and 866.86 ± 50.0 µM TE/ mg extract (TEAC) in both DPPH and ABTS assays respectively. While it showed no cytotoxic activity against A-549, HepG2, PC-3 and MCF-7 cell lines with the used concentrations. **Conclusion:** Ethyl acetate extract of (TMP16), isolated from *T. pandacaqui* leaves for the first time, exhibited a significant antioxidant activity which may be attributed to high percentage of porphyrin derivatives.

Keywords: *Antioxidant; Cytotoxic; GC-MS; Tabernaemontana pandacaqui; Endophyte.*

INTRODUCTION

Endophytes are endosymbionts that colonize healthy plant tissue causing no disease manifestation, rather competent of biosynthesizing active compounds, used by plants as guards against pathogens[1]. Hundreds of bioactive compounds have been recently reported from endophytes, mostly are known to have functions as antibiotics, immunosuppressants, anticancer agents and so forth[2]. For decades, fungi have been known as pathogens, causing health hazards, today they are considered as antimicrobials that act against bacteria saving millions of lives. Endophytic *Aspergillus* species have confirmed their capability to produce various bioactive secondary metabolites, such as butenolides, alkaloids, terpenoids, terphenyls, xanthones, sterols, diphenyl ether and anthraquinones derivatives, of great importance pharmaceutically and commercially[3]. The metabolites produced by different endophytic *Aspergillus* spp. showed various biological activities, such as anti-inflammatory, anticancer, antibacterial and antiviral[3].

Tabernaemontana pandacaqui Poir., belongs to family Apocynaceae, commonly known as banana bush and in Philippine known as pandakaking-puti and kampupot[4]. *T. pandacaqui* is widely distributed in Indonesia, New Guinea, northern Australia, Cuba and the Pacific, recently grown in Egypt as ornamental plant. Alkaloids of different groups have been isolated from this species[5,6]. It is used in traditional medicine as a remedy for ulcers, skin sores, inflammations and stomachache. The reported reviews demonstrated various pharmacological activities of *T. pandacaqui* such as analgesic, anti-inflammatory, antipyretic, antinociceptive, cytotoxic and free-radical scavenging activity[7-10]. Recently, gas chromatography-mass spectrometry (GC-MS) is extensively used for fingerprint analysis of secondary metabolites in different plant extracts, as it is fast and accurate technique to detect various compounds, including alkaloids, nitro compounds, organic acids, steroids, long chain hydrocarbons, esters and amino acids, and requires a small volume of plant extract[11]. Due to the medicinal value of *T. pandacaqui* and the capability of endophytes to synthesize bioactive compounds, so it was deemed of interest to analyze the phytochemical constituents of the endophytic extract of *T. pandacaquis'* leaves using GC-MS technique and evaluate some of its biological activities.

MATERIAL AND METHODS

Microbial host material

The microbial host *T. pandacaqui* leaves was identified by Dr. Trease Labib, former specialist of plant Taxonomy, El Orman Botanical Garden, Giza, Egypt. The reference specimen (000463tc/07-07-01-23) is available in the Herbarium of El Orman Botanical Garden, Giza, Egypt. Storage of the collected microbial host samples was carried out at 4°C in Microbial Chemistry laboratory, National Research Centre, Egypt until investigation of their symbiotic endophytes.

Isolation of fungal endophytes

The selected plant samples were rinsed thrice with sterilized water, then treated with 70% ethanol for 30 seconds followed by washing with sterile distilled water to remove any attached debris or organisms. After that, the plant leaves were aseptically cut into small fragments after being scratched with sterile scalpel to reach their inner tissue surface .

For the isolation of the endophytic fungi, the cleaved plant leaves were agitated in 100 mL conical flasks containing 50 mL sterile sea water using reciprocal water bath, at 30°C for 30 min. Subsequently, the resulted suspensions were serially diluted (10^{-1} to 10^{-6}) and aliquots of the serially diluted samples (0.1 mL) were used to inoculate potato dextrose agar (PDA) plates. The petri dishes were then incubated at 30°C for 6 weeks. The colonies with distinct morphological characteristics were selected and transferred onto slants (freshly prepared solid media) and refrigerated at 4°C for investigation[12].

Mass culture and preparation of fungal extract

Mass culture and preparation of ethyl acetate extract of the isolated fungus were carried out similarly as mentioned in our previous work[13].

Antioxidant assays

DPPH assay

Radical scavenging activity of the endophytic fungal extract was evaluated by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay. It was carried out according to the method of Boly et al., 2016.[14] The tested sample concentration was 0.04 mg/mL in methanol. All the assays were carried out in six replicates. The results were recorded at 540 nm using microplate reader FluoStar Omega-Germany, and the percentage of inhibition is calculated according to the following equation:

$$\% \text{ inhibition} = [(A0 - A1) / (A0)] \times 100$$

Where A0 = Average absorbance of blank and A1 = average absorbance of the sample

ABTS assay

ABTS (2, 2'- azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonuim salt) assay was carried out according to the method of Arno et al., 2001,[15] with minor modifications to be carried out in microplates, briefly; 192 mg of ABTS were dissolved in distilled water and transferred to 50 mL volumetric flask then the volume was completed with distilled water. One mL of this solution was added to 17 µL of 140 mM

potassium persulphate and the mixture was left in the dark for 24 h. After that, 1mL of the reaction mixture was completed to 50 mL with methanol to obtain the final ABTS dilution used in the assay. Freshly prepared ABTS reagent (190 µL) was mixed with 10 µL of the sample (0.2 mg/mL in methanol) in 96 wells plate (n=6), the reaction was incubated at room temperature for 30 min in dark. At the end of incubation time, the decrease in ABTS color intensity was measured at 734 nm using microplate reader FluoStar Omega, and the percentage of inhibition is calculated according to the same equation as in DPPH assay.

Trolox was used as positive control for both assays and results of the sample are presented as Trolox Equivalent Antioxidant Capacity (TEAC), Trolox equivalent µM TE/mg sample extract means ± SD, using the linear regression equation extracted from calibration curve (linear dose-inhibition curve of Trolox).

Linear dose-inhibition curve of Trolox

A stock solution of 1 mM concentration of Trolox was prepared in methanol from which 7 concentrations were prepared including 5, 10, 20, 40, 50, 60, and 80 µM for DPPH calibration curve and 5 concentrations 500, 250, 125, 62.5 and 31.25 µM for ABTS calibration curve. Each calibration curve was done separately for each assay, the average of the readings of six replicates was taken after subtraction from the control.

Cytotoxic assay

Prostate Cancer (PC-3), Lung Cancer (A-549), Breast Adenocarcinoma (MCF-7) and Hepatocellular carcinoma (HepG2) cell lines were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO_2 atmosphere at 37 °C.

Cell viability was assessed by SRB (sulphorhodamine B) assay[16,17]. The fungal extract that used was in 2 concentrations (10 and 100 µg/mL). The absorbance was measured at 540 nm using a BMG LABTECH®-FLUO star Omega microplate reader (Ortenberg, Germany). Wells without cells were used as blanks. All the assays were carried out in triplicates. The data were presented as cell viability % means ± standard deviation (SD).

Gas chromatography mass spectrometry (GC-MS) Analysis

The GC-MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used, helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 50°C (hold 2 min) to150°C at an increasing rate of 7°C /min. then to 270 at an increasing rate 5°C /min (hold 2min) then to 310 as a final temperature at an increasing rate of 3.5 °C /min (hold 10 min).

The quantification of all the identified components was carried out using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their retention times and mass spectra with those of the NIST, WILLY library data of the GC-MS system

RESULTS

Identification of the fungal endophyte

The fungal isolate was preliminary identified using cultural and morphological features such as colony growth pattern, and conidial morphology. The fungus culture was transferred to freshly prepared PDA plate and identified morphologically, based on macro- and microscopic characters. The fungus colonies were fast growing, attaining a diameter of 3-4 cm at 28 °C in 10 days on PDA plate, fungus growth is initially white, but they changed to black after a few days. Conidial heads are Globose to Loosely radiate. Conidiophores are 6 µm in diameter. With conidia, sub-spherical 3.5 µm. The Morphology results of the isolated fungus confirmed the isolated fungus as *Aspergillus* spp. as shown in Figure 1 and assigned as TMP16. The fungus was mass cultured and extracted as the technique mentioned above.

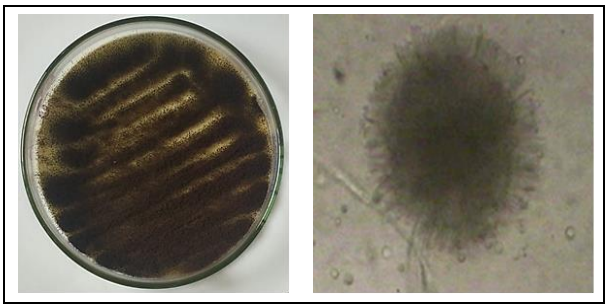

Figure 1. Morphological examination of TMP16

Evaluation of antioxidant activity of the fungal extract

DPPH and ABTS antioxidant assays were used in this study for the estimation of the radical scavenging activity, the DPPH assay is frequently used for estimating the antioxidant activity of different natural (plant or endophyte) extracts and natural or synthetic

pure compounds due to experimental feasibility, high stability, and low cost[18].

The inhibition power estimated as (TEAC) µM TE/mg sample using the linear regression equation extracted from the calibration curves (linear dose-inhibition curve of Trolox for DPPH and ABTS assays) (**Figure 2**).

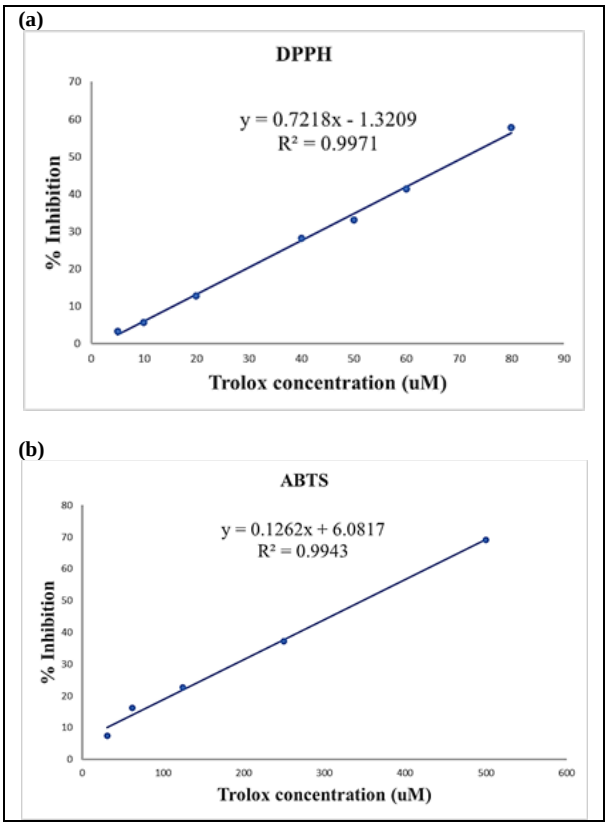

Figure 2. (a) Linear dose – inhibition curve of Trolox in DPPH assay, (b) Linear dose-inhibition curve of Trolox in ABTS assay

The results revealed that the ethyl acetate extract showed radical scavenging power of 881.49±44.6 µM TE/mg extract according to DPPH assay and 866.86±50.0 µM TE/mg extract according to ABTS assay which is considered as significant antioxidant activity (**Table 1**).

Cytotoxicity screening of the fungal extract

Cytotoxicity of the ethyl acetate extract was evaluated against different human cancer cell lines PC-3, A-549, MCF-7 and HepG2 was screened using the SRB assay. The Sulforhodamine B (SRB) is a bright pink amino xanthine dye with two sulfonic acid groups. They bind to basic amino acid residues under mild acidic conditions to provide a sensitive index of cellular protein content, which is directly proportional to cell viability which dissociate under alkaline conditions.[16,17] The extract is used in two concentrations 10 and 100 µg/mL and the % of cell viability was assessed (**Table 2**).

Analysis of bioactive Compounds in the fungal extract by GC-MS

GC-MS analysis indicated that the ethyl acetate extract of TMP16 gave about 50 bioactive compounds of which 39 were tentatively identified (92.93 %), only compounds with the highest probability were considered. (**Table 3, Figure 3**).

Nitrogenous compounds of different classes were highly observed, among these classes the porphyrin derivatives were represented in meso-tetraphenylporphineZinc, 5,15-Bis(3-methoxyphenyl)-10-phenyl-20-propylporphyrin, 2-nitro-5,10,15,20-tetraphenyl[2-(2)H1]prophyrinatonickel(II), 2-methoxy-3-nitro-5,10,15,20-etraphenyl-2,3-dihydroporphyrin, 5-[4(e)-(2-Methyl-1,4-naphthoquinon-3-yl)cyclo-hex(e)-yl]-10,15,20-tris (4-methylphenylene)prophyrin, 2-hydroxy-5,10,15,20-tetra phenylporphinatozinc(II), 5,10-bis-3-aminophenyl-15,20-diphenylporphyrin, 3-(E)-t-butoxtcarbonylpropenyl-2,7,12,18-tetramethyl-21H,23Hporphine-13,17-ipropyldimethylester, methyl sulfinato(5,10,15,20-tetra phenylporphyrinato) iron, 3-[7,12-bis-(2-hydroxy-ethyl) -18-(2-methoxycarbonyl-ethyl)-3,8,13 , 17-tetramethyl-porphyrin-2-yl]-propionic acid methyl ester, tetraphenylporphyrinatodichloro titanium(IV), Methyl-5-ethyl-2-(1-hydroxyethyl)-5-demethyl-.delta.-methyl-2-devinylpyro phaeophorbide A and homologues, ë-chloro-2,4-bis(2-chloroethyl)-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5-trimethyl porphyrin, 13,17-bis(2-methoxycarbonylethyl)-12,18 -bis-methoxycarbonylmethyl-2,2,8,8-tetramethyl isobacterioChlorin and 5,10,15,20-tetra phenyl[2-(2)H1]prophyrinatonickel (II). These compounds collectively sum up to total presence of 42.85 % in the extract.

Additionally, alkaloids were detected like diethyl-dehatridine (1.43%) and acrimarine A (3.63%), also, carbazoles (3 [9,9-Dihexyl-7-(pyridine-2-yl)fluoren-2-yl]-9-hexylcarbazole and 1,3,6,8-tetrabromo-9-(4'-iodophenyl) carbazole) are present in total concentration of 6.38 %.

N,N'-Dicyclohexyl-1,7-dipyrrolidinylperylene -3,4:9,10 -tetracarboxylic acid bisimide was noticed in concentration of 3.9 % fall under the category of perylene-diimide derivatives. Other classes detected from the nitrogenous compounds are indole derivatives (4,6-Dimethoxy-7-(4',6'-dimethoxyI-7'-(4",6"-dimethoxyindol-2"-yl)indol-2'-yl)-2,3-diphenylindole 1.47%), Benzo(c)cinnoline (3,8-Dimethoxy-4,7-di-(4-methoxy-3,5-di-t-butylphenyl)-benzo[c]cinnoline 1.76%) and selenadiazolopyridine (4,7-Diphenyl-6-hydroxymethyl-1,2,5-selenadiazolo[3,4-c] pyridine 2.15%)

Table 1. Antioxidant activity of ethyl acetate extract of TMP16 calculated as µM TE/mg extract in DPPH and ABTS assays

	TEAC (µM TE/mg extract) ± (SD)	
	DPPH Assay	**ABTS Assay**
Eth. Ac. Ext (TMP16)	881.49±44.6	866.86±50.0

Table 2. Results of cytotoxicity screening of the ethyl acetate extract

Eth. Ac. Ext (TMP16) conc.	Average cell viability % ± SD			
	PC-3	A-549	MCF-7	HepG2
10 µg/mL	100.028 ± 1.45	102.336 ± 2.42	95.901±0.80	97.323±0.61
100 µg/mL	85.916 ± 0.51	62.144± 2.43	85.207±2.93	72.464±2.71

DISCUSSION

Medicinal plants believed to be valuable source for endophytes producing associated plant natural products[19]. *T. pandacaqui*, an ethnic medicinal plant, has been used by Indonesian to treat various illnesses. Despite all the pharmacological studies, there is no reported information about bioactivity of endophytes associated with this plant.

Aspergillus spp. produce structurally diverse classes of secondary metabolites that exhibit a wide range of biological activities including antioxidant and cytotoxic[20,21]. In the present study, the biological efficacy of secondary metabolites (determined by GC-MS) as antioxidant and cytotoxic was investigated. The pharmacological activity of the selected fungal isolate (*Aspergillus* sp. TMP16) herein showed that the fungal ethyl acetate extract has antioxidant and mild to low cytotoxic activities with four different cell lines due to the presence of bioactive natural compounds.

It was apparent that the fungal extract exhibited significant antioxidant potential 881.49±44.6 µM TE/ mg with DPPH assay and 866.86±50.0 µM TE/ mg with ABTS assay (TEAC). The Trolox equivalent antioxidant capacity (TEAC) measures the antioxidant power of the sample using the DPPH and ABTS decolorization Assays[22]. The TEAC assay has been used for evaluating antioxidant capacity for many compounds and food samples due to its operational simplicity [23-25].

Cytotoxicity screening was conducted against four cancer cell lines to evaluate the effect of the bioactive compounds on cell proliferation. It was observed that the fungal extract displayed no cytotoxic activity against the used cell lines, but still affecting the cell viability at 100 µg/mL concentration. The % of cell viability against A-549 (lung cancer) and HepG2 (hepatocellular carcinoma) cell lines were 62.144 ± 2.43 and 72.464 ± 2.71 respectively, while against PC-3 (prostate cancer) and MCF-7 (breast adenocarcinoma) cell lines were 85.916±0.51 and 85.207±2.93 respectively.

According to GC-MS analysis of the fungal extract, it was abundant that high percentage of nitrogenous compounds of different classes were noticed (75.38 %), concluding they may be responsible for the biological activity of the extract.

Porphyrin-derivatives constitute the highest percentage of the nitrogenous compounds (42.85 %). Porphyrins are derivatives of porphin (Fig. 4a), which consists of four monopyrroles linked by methene bridges. The four nitrogen atoms of tetrapyrrole ring can coordinate with a central transition metal ion to form a metalloporphyrin (**Figure 4b**) as porphyrins tend to form chelates[26]. A porphyrin which has no metal in the cavity is called a free base.

Reported data confirmed that the lipophilic free bases of porphyrins and their complexes with metals can be accumulated in the lipid bilayer of cell membranes and are capable of inactivating free radicals. The unique porphyrin structure provides good opportunities for developing systems with electrochemical properties as their redox mechanism is attributed to their structure and stereochemistry[27-34]. Also, the macro-tetrapyrrole ring has conjugated π-system together with the significant variation of the properties of porphyrins, due to the presence of substituents of different nature, like aromatic (phenol) OH groups in different positions of the ring, and their distinctive electrochemical properties make them well-favored regarding their potential use as antioxidants[35-37]. In addition, the antioxidant activity of metalloporphyrins was reported to be higher compared to their free bases. The higher antioxidant activity of the coordinated porphyrins can be due to the presence of an additional scavenging metal center, which make the molecule more able to stabilize the radical[38].

Table 3. List of identified phytocompounds of ethyl acetate extract of endophytic fungus TMP16 by GC-MS analysis

Number	Retention time (min)	Area %	Compound name	Molecular formula	Molecular weight
1	5.08	3.22	5,5′-Bis(3,5-di-tertbutyl-4-oxo-2,5-cyclohexadien-1-ylidene)-5,5′-dihydro-2,2′-biselenophene	$C_{36}H_{44}O_2Se_2$	668
2	5.15	4.96	3-[9,9-Dihexyl-7-(pyridine-2-yl)-fluoren-2-yl]-9-hexylcarbazole	$C_{48}H_{56}N_2$	660
3	5.23	6.13	(5,10,15,20-tetraphenyl[2-(2) H1] prophyrinato) zinc (II) (meso-Tetraphenylporphine, Zinc)	$C_{44}H_{28}N_4Zn$	676
4	5.35	2.24	5,15-Bis(3-methoxyphenyl)-10-phenyl-20-propylporphyrin	$C_{43}H_{36}N_4O_2$	640
5	7.15	4.60	(2-Nitro-5,10,15,20-tetraphenyl [2-(2)H1] prophyrinato) nickel(II)	$C_{44}H_{27}N_5NiO_2$	715
6	8.14	1.59	2-Methoxy-3-nitro-5,10,15,20-tetraphenyl-2,3-dihydroporphyrin	$C_{45}H_{31}N_5O_3$	689
7	9.66	1.42	1,3,6,8-tetrabromo-9-(4'-iodophenyl) carbazole	$C_{18}H_8Br_4IN$	681
8	10.57	1.64	Pyrroline, 3-[2-(methoxycarbonyl)ethyl] -4-methyl-4-[(tert-butoxycarbonyl)methyl] -2-[(tert-butoxycarbonyl) methylidene]-5-[[3-[2-(methoxycarbonyl)ethyl]-5-cyano 4,5-dimethyl-4-[(tert-butoxycarbonyl)methyl]-1-pyrrolin-2-yl]methylidene]-.delta1-pyrroline	$C_{39}H_{59}N_3O_{10}$	729
9	11.32	2.40	N,N'-Dicyclohexyl-1,7-dipyrrolidinylperylene -3,4:9, 10 -tetracarboxylic acid Bisimide	$C_{44}H_{44}N_4O_4$	692
10	11.66	1.47	4,6-Dimethoxy-7-(4',6'-dimethoxyI-7'-(4",6"-dimethoxyindol-2"-yl)indol-2'-yl)-2,3-diphenylindole	$C_{42}H_{37}N_3O_6$	679
11	12.80	2.43	(E)-3-Benzyl-1,2,3,4,5,6-hexahydro-2-(2-benzyloxy-3-methyl-4,5-methylenedioxybenzylidene)-9-methoxy-8,11-imethyl-1,5-imino-3-benzocine-4-one	$C_{37}H_{36}N_2O_5$	588
12	13.61	1.65	Bis[2,2'-bis(N,N'-methoxyacetamido) diphenylether	$C_{36}H_{38}N_4O_{10}$	686
13	13.98	2.17	5-[4(e)-(2-Methyl-1,4-naphthoquinon-3-yl) cyclohex(e)-yl]-10,15,20-tris (4-methylphenylene) prophyrin	$C_{58}H_{48}N_4O_2$	832
14	14.14	2.15	4,7-Diphenyl-6-hydroxymethyl-1,2,5-selenadiazolo[3,4-c]pyridine	$C_{18}H_{13}N_3OSe$	367
15	14.60	1.79	3,5-Diphenyl-3,5-(9,10-phenanthylene) tricyclo [5.2.1.0]decane-4-one-8-exo-9-endodicarboxylic acid	$C_{44}H_{36}O_9$	708
16	14.76	6.78	(2-hydroxy-5,10,15,20-traphenylporphinato) zinc (II)	$C_{44}H_{28}N_4OZn$	692
17	15.13	1.76	(P)-N,N'-Dimethyl [1+1] cycloamide	$C_{46}H_{46}N_2O_2$	658
18	15.47	1.79	Dimethyl5"-(1,1-Dimethylethyl)-2,2',2",2"',2""-pentamethoxy[1,1':3',1":3",1"':3"'.1""-quinquephenyl] -3,3""-dicarboxylate	$C_{43}H_{44}O_9$	704
19	15.62	1.99	5,10-bis (3-aminophenyl)-15,20-diphenylporphyrin	$C_{44}H_{32}N_6$	644
20	15.73	1.55	tetra-tert-butyl-2,6-di(3-propenyl)-3,7-dimethoxybicyclo [3.3.0]octa3,7-diene-2,4,6,8-dicarboxylate	$C_{36}H_{54}O_{10}$	646
21	15.82	2.12	3-[(E)-t-Butoxtcarbonylpropenyl]-2,7,12,18-tetramethyl-21H,23H-porphine-13,17-dipropyl dimethylester	$C_{38}H_{56}N_4O_6$	664
22	16.05	1.55	Methylsulfinato(5,10,15,20-tetraphenylporphyrinato) iron	$C_{45}H_{31}FeN_4O_2S$	747
23	18.57	1.76	3,8-Dimethoxy-4,7-di-(4-methoxy-3,5-di-t-butylphenyl)-benzo[c]cinnoline	$C_{44}H_{56}N_2O_4$	676
24	20.62	1.51	7,8,17,18-Tetrahydro-35-methoxy-1,3,21,23-tetramethyl-16H,31H-5,9,15,19-dimethano-10,14-metheno-26,30-nitrilo-6H,25H-dibenzo(b,s)(1,21,4,8,14,18) dioxatetraazacyclooctacosine	$C_{38}H_{41}N_5O_5$	647
25	22.58	1.43	Diethyl-dehatridine	$C_{39}H_{40}N_2O_6$	632
26	23.34	3.76	3-[7,12-bis-(2-hydroxy-ethyl)-18-(2-methoxycarbonyl-ethyl)-3,8,13,17-tetramethyl-	$C_{36}H_{42}N_4O_6$	626

			porphyrin-2-yl]-propionic acid methylester		
27	24.92	1.43	tetraphenylporphyrinatodichlorotitanium(IV)	$C_{44}H_{28}Cl_2N_4Ti$	730
28	30.99	3.63	Acrimarine-A	$C_{31}H_{29}NO_8$	543
29	32.16	2.09	Methyl-5-ethyl-2-(1-hydroxyethyl)-5-demethyl-delta-methyl-2-devinylpyrophaeophorbide A and homologues	$C_{38}H_{46}N_4O_4$	622
30	32.80	1.47	3,3'",5,5'"-Tetrachloro-4,4",4'",6'- tetramethoxy-2',2"-dinitro -m-quaterphenyl	$C_{28}H_{20}Cl_4N_2O_8$	652
31	34.11	1.57	1,2-Cyclohexanedimethanol	$C_8H_{16}O_2$	144
32	34.27	1.54	Docosanedioic acid, dimethyl ester (CAS)	$C_{24}H_{46}O_4$	398
33	41.55	2.85	9-(Ethyloxy)-3-(2'-ethyloxyethenyl)-2-ethyl-1-propyl-3,7-diphenyl-2,3,3a,4,5,6-hexahydro-1H-azacyclopenta[d] indene	$C_{34}H_{43}NO_2$	497
34	42.63	1.98	ë-chloro-2,4-bis(2-chloroethyl)-6,7-bis[2-(methoxyc arbonyl) ethyl]-1,3,5-trimethylporphyrin	$C_{35}H_{37}Cl_3N_4O_4$	682
35	42.75	1.81	13,17-bis(2-methoxycarbonylethyl)-12,18-bis (methoxy carbonylmethyl)-2,2,8,8-tetramethylisobacterioChlorin	$C_{38}H_{46}N_4O_8$	686
36	43.49	1.46	NaMonB (monensin B sodium)	$C_{35}H_{59}NaO_{11}$	678
37	44.76	2.65	5-(Dibromomethyl)-1,3-bis (tribromomethyl) benzene	$C_9H_4Br_8$	744
38	44.93	1.98	1,2,3-Trichloro-4,9,10-tris (phenylethynyl) anthracene	$C_{38}H_{19}Cl_3$	580
39	48.07	2.61	(5,10,15,20-tetraphenyl[2-(2)H1] prophyrinato)nickel (II)	$C_{44}H_{27}N_4Ni$	670
Percent of identified compounds			92.93 %		
Percent of unidentified compounds			7.07 %		
Percent of nitrogenous compounds			75.38 %		

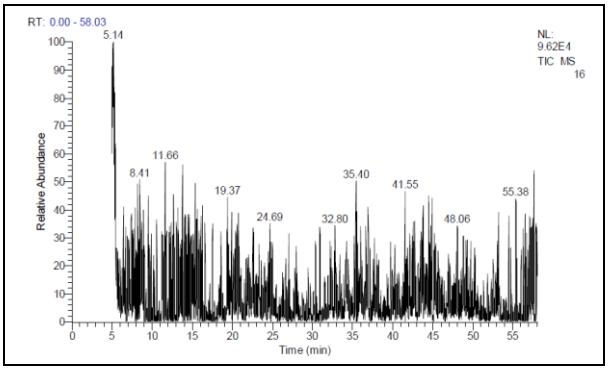

Figure 3. GC-MS Chromatogram of ethyl acetate extract of endophytic fungus TMP16

Porphyrin derivatives have been used as photosensitive drugs in cancer treatments using photodynamic therapy (PDT)[39,40]. Porphyrin derivatives can selectively localize in cancer tissues where positively charged porphyrin molecules showed a strong interaction with biological targets, which are negatively charged, such as DNA, RNA and specific proteins[41,42]. The different cytotoxic activity of the porphyrins towards various cell lines depends on the formation of intermolecular hydrogen bonds between the porphyrin derivatives and DNA bases and the functional group addition to any unsaturated moiety in DNA leading to its damage. Additionally, the negative charges on the cell wall attracts the positive charges on the porphyrins molecule[43]. So, it can be concluded that the porphyrin moiety with or without peripheral substituents have electronic properties that contribute to their antioxidant activity. Although the total extract displayed no cytotoxicity against the tested cell lines, but still affecting their cell viability, which may attributed to the porphyrins content.

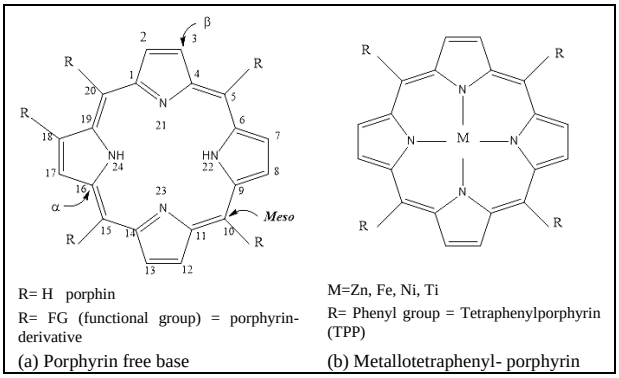

Figure 4. (a) Porphyrin free base show positions of substitutions, (b) Examples of metalloporphyrin.

CONCLUSION

The preliminary study of ethyl acetate extract of (TMP16), isolated from *T. pandacaqui* leaves for the first time, was carried out by GC-MS analysis for tentative identification of its bioactive metabolites. It showed prominent presence of porphyrin derivatives that can be responsible for its antioxidant activity. The results obtained from this study suggested that ethyl acetate extract of (TMP16) is a good candidate for further phytochemical and biological investigation.

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Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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