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ANTIBACTERIAL ACTIVITY AND GC-MS ANALYSIS OF *TRICHAPTUM BIFORME* EXTRACT

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Key words : *Trichaptum biforme, Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922 Extract, Soxhlet, GC-MS.*

Abstract – In our study, mushrooms called *Trichaptum biforme* are harvested in west forest of Algeria in order to research their antibacterial activity. Description of mushrooms is carried out by morphological and microscopic methods. Extract is obteined by Soxhlet from the dried sporophores of *Trichaptum biforme*. This extract is tested *in vitro,* in Petri dishes, on the growth of a Gram + and Gram- bacteria: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. The results obtained showed a remarkable inhibitory effect of the *Trichaptum biforme* extract on the growth of the two pathogenic bacterial strains tested. The antibacterial activity of the extract towards the bacterial strains tested by the disc diffusion method has shown that this extract is biologically bacteriostatic. Analysis of the extract by GC-MS allowed the identification of 30 components. Octodrine, caryophyllene oxide, phenolic compounds, fatty acids and fatty acids esters were noted as key compounds in the *Trichaptum biforme* extract, which were perhaps causing the antibacterial activity. *Trichaptum biforme* are are therefore interesting candidates for obtaining antibacterial bioactives substances with therapeutic interest.

INTRODUCTION

Trichaptum biforme is a genus of mushrooms belonging to basidiomycetes, without stipes and with pores on the underside of the cap Gevry *et al.,* 2009.

Bioactive molecules have been reported in some basidiomycetes species. These molecules are antimicrobial, antioxidant, anti-tumor, cytostatic, antiallergic, hypoglycemic, anti-inflammatory and hepatoprotective (Wasser and Weis, 1999; Lindequist *et al.,* 2005).

Basidiomycetes are a poorly exploited source of antimicrobial substances. Indeed, it has been reported in the literature that they contain molecules with antiviral, antibacterial and antifungal activities (Wasser and Weis, 1999; Lindequist *et al.,* 2005; Colombo and Bosisio, 1996; Iwu *et al.,* 1999; Yamac and Bilgili, 2006; Alves *et al.,* 2012; Orion, 2013).

Our study focuses on the research of the antibaterial activity of the extract of *Trichaptum biforme, a* basidiomycete harvested in a west forest of Algeria (M'sila forest in Oran).

MATERIALS AND METHODS

Study Site

Trichaptum biforme is harvested in the forest of M'Sila, at 30 km west of Oran in Algeria (Fig. 1). This forest has poor soils, with a clay-siliceous texture, or sandy-clay-silty soils depending on the area (Aimé, 1991). Most of this forest is covered with cork oak trees (Benazza-Bouregba *et al.,* 2017). The prospection is carried out on January with the presence of foresters, after 3 days of rain, during the winter period.

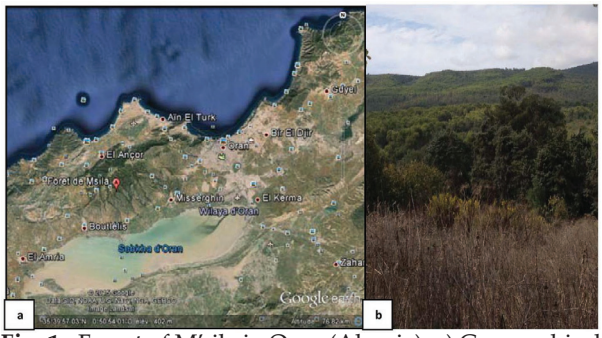


Fig. 1. Forest of M'sila in Oran (Algeria): a) Geographical position (google earth, 2019) and b) general view.

Mushrooms are harvested in a random manner, without particular statistical methods. They are carefully cleaned and placed in paper envelopes.

The mushroom *Trichaptum biforme*

The mushroom presents several characteristics (Fig.2). Cap: 10 × 5 cm, concentric grooves felted pale gray, often tinged with green, spread fan, margin corrugated and lobed. Flesh: Pale brown or purplish, hard. Hymenium (underside of the cap) angular, purple to reddish-brown pore. Basidiospores (8-10 x 2.5-3 µm), are cylindrical.

After drying in the sun, in an aerated place, mushrooms are crushed using a mechanical grinder; the powder thus obtained is used for extraction by the soxhlet.

10 g of dried fragments of *Trichaptum biforme* are used for extraction with the soxhlet. Fragments are introduced into a 500 mL round bottomed flask containing 300 mL of solvent (methanol, ethanol 96° or ethyl acetate). The body of the extractor (containing the fragments) is surmounted by a refrigerant. The solvent is vaporized and condensed while remaining in contact with the fragments.

The extraction is carried out at 77 °C for 1h30min. It is complete when the solvent becomes very colored. The solvent is then removed with Retavapor Buchi R200 (at 50°C) until brownish residue (concentrated extract) which is recovered using a dropper and then weighed.

Bacterial strains

Two bacterial strains are tested: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. These strains belong to the laboratory's collection (LBMB).

Preparation of bacterial culture

3 to 5 well isolated colonies are taken from a preculture of 24h and emulsified in a tube containing 10 mLof nutrient broth. After 24h of incubation at 37 °C, the tube of the bacterial suspension is compared with the Mc Farland standard 0.5 tube (10^8 CFU/mL) to adjust the turbidity of the bacterial suspension. Evaluation of antibacterial activity was determined using the disc diffusion method (Perez *et al.*, 1990).

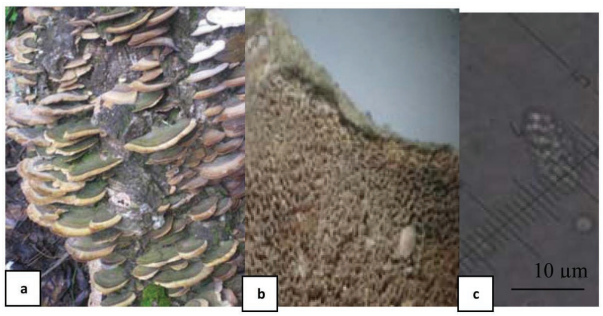


Fig. 2. The mushroom *Trichaptum biforme* : a) Sporophores of the mushroom on the trunk of cork oak, b) pores on the underside of the cap of the mushroom, c) basidiospores (G×1000).

In Vitro evaluation of the antibacterial activity

20 mL of Mueller-Hinton medium is poured in Petri dishes (90mm in diameter). 1mL of standardized bacterial inoculum (10^8UFC/ mL) is aseptically spread on the surface of the medium using a swab.

One disc of filter paper (6mm in diameter) is impregnated with mushroom extract and deposited on the surface of the medium. The control disc is impregnated with the extraction solvent (negative control) and deposited on the surface of the medium. The positive controls are standard antibiotics: chloramphenicol and streptomycine at 30µg/disc. Three repetitions are carried out for each bacterial strain tested.

The Petri dishes are left for a quarter of an hour at room temperature so that the contents of the discs diffuse into the medium. They are then sealed with tape and incubated at 37 °C for 24h.

The sensitivity of the bacterial strains tested is determined by measuring the diameters of the inhibition zones in the two perpendicular directions around the discs, in Petri dishes.

Statistical Analysis

The results were expressed as means followed by the standard error (means ± SD). Excel (Microsoft Corporation, USA) and Statistica (version 7.1) were used for statistical analysis.

Gas Chromatography- Mass Spectrometry (GC-MS) analysis

The changeable volatile compounds profiling of methanol extract, ethanol extract and ethyl acetate extract, from the mushroom *Trichaptum biforme*, was measured using Gas chromatography-Mass spectrometry (GC-MS). A quantity in the order of milligrams of the dried crude extract was dissolved in methanol. The method was described by (Hossain and Rahman, 2011).

GC-MS analysis is performed on a Perkin Elmer Clarus 500 equipped with an automatic injector. The column used is a Restek Rxi 5ms (5% diphenyl / 95% dimethylpolysiloxane) with a length of 60 meters in

diameter 0.25 mm.

The analysis parameters are:

-Injector temperature: 220 °C.

-volume injection 1 µL

-the carrier gas is helium with a flow rate of 1 mL / min.

-ramp 1, 10 °/ min to 150 °C.

-ramp 2, 3 °/ min up to 220 °C.

-Duration of the analysis 33 min.

-M / Z mass detector40-500 daltons.

-Ionization at 70 eV.

-temperature of the transfer line 250 °C.

-temperature of the source 220 °C.

In mass spectrometry, the ionization process used is electron impact ionization; it is the most used method for compounds that can go into the gaseous state. This is particularly the case of organic molecules. It consists in causing collisions between the initial no-charge molecules and the electrons obtained by thermo-ionic effect. In a shock, one of the least retained electrons of the molecule is torn off, which leads to an ion carrying a positive elementary charge.

The identification of the separated substances was done by comparing their mass spectra obtained by GC-MS with those provided by the libraries: wiley 9, Nist, AAFS0603, AAFSDrugs, NMStox$_1$, pflegerand mainlib.

RESULTS AND DISCUSSION

In vitro evaluation of the antibacterial activity

The extract of *Trichaptum biforme* had antibacterial activity against both bacterial strains tested (Fig.3, Table 1). These results are similar with those of many authors who found strong antimicrobial activity of several species of mushroom against bacteria (Rosa *et al.*, 2003).

Some authors reported that extracts of several basidiomycetes (*Cantharellus cibarius, Pleurotus ostreatus*) inhibit the growth of bacteria regardless of their Gram (*Bacillus subtillis* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27453) (Kalyoncu *et al.*, 2010). This therefore joins our results.

The extract strongly inhibits the growth of *Escherichia coli* ATCC 25922 (28mm inhibition zone) and *Staphylococcus aureus* ATCC 25923 (13mm inhibition zone).

The Gram + bacterial strain tested (*Staphylococcus aureus* ATCC 25923) is less sensitive than the Gram- bacterial strain (*Escherichia coli* ATCC 25922). It is well known that Gram- bacteria have a periplasmic space and a thinner peptidoglycan layer that are absent in Gram + bacteria and make them more sensitive (Basile *et al.*, 1998). In contrast, according to (Balakumar *et al.*, 2011), Gram +bacteria (*Staphylococcus aureus* and others) are more sensitive to extracts of mushroom species than Gram- bacteria.

Analysis of the *Trichaptum biforme* extracts using Gas Chromatography- Mass Spectrometry (GC-MS)

The analyzes by GC-MS provided the chromatograms illustrated respectively by Figs. 4, 5 and 6. These analyzes reveal the large number of products contained in the extracts. This is due to the extraction method, nature and selectivity of the organic solvents used. Soxhlet has been shown to be effective in driving a large number of volatile constituents when solvents such as methanol, ethyl acetate and ethanol are used.

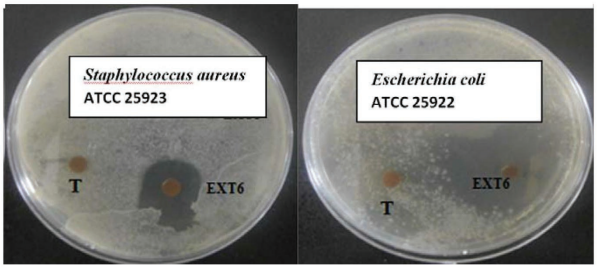


Fig. 3. Activity of the extract of *Trichaptum biforme* (EXT6) on the growth of *Staphylococcus aureus* ATCC 25923 (left) and *Escherichia coli* ATCC 25922 (right), after 24h of incubation at 37°C on Mueller-Hinton medium. Note the absence of an inhibition zone around the control disc (T).

Table 1. Diameters of inhibition zones of bacterial strains tested (values, including diameter of the filter paper disc (6.0 mm), are means±SD of three replicates).

Bacterial strains tested	Mushroom extract	Solvent (negative control : T)	Positive reference standards (30µg/disc)
Staphylococcus aureus ATCC 25923	13±2.08	6±0	27±1.5 (Chloramphenicol)
Escherichia coli ATCC 25922	28±2.51	6±0	19±1 (Streptomycine)

Identifications of the eluted compounds are grouped in Table 2. Retention times and spectral data are reported and compared with those of the literature.

GC-MS analysis of *Trichaptum biforme* extract showed a list of chemical compounds that includes a very large number of substances with antibacterial activity. According to Martchenko and Kim (2014), Octodrine ($C_8H_{19}N$) inhibits the proliferation of microbial growth, including growth of the genus *Candida* and both Gram negative and Gram positive bacteria. Many studies revealed that the chemical analysis of plant extracts and essential oils with antimicrobial activity show a remarkable presence of an Sesquiterpene, caryophyllene oxide ($C_{15}H_{24}O$)(Alsultan *et al.*, 2016). Caryophyllene oxide showed activity against *Staphylococcus sp.*, *Macrococcus caseolyticus*, *Enterococcus faecium* and *Enterococcus faecalis* (Xiong *et al.*, 2013)

Also, it is noted the presence of phenolic compounds with aromatic nucleus ($C_{14}H_{22}O$, $C_{13}H_{16}O_2$, $C_8H_6O_4$, $C_6H_2Cl_2O_2$). These compounds

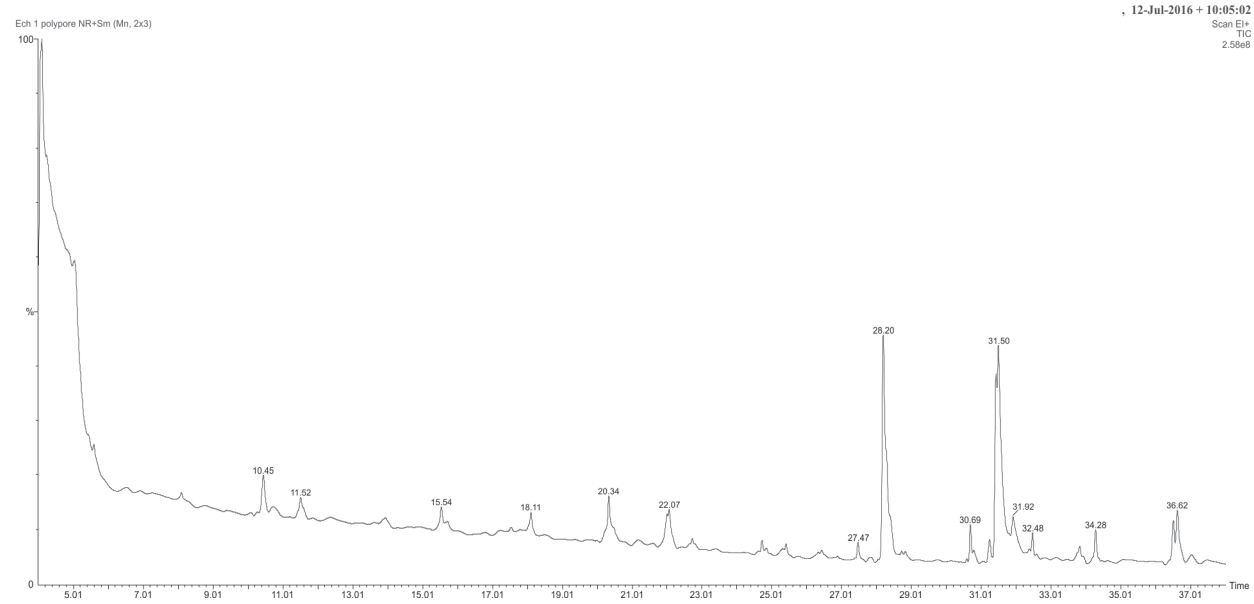


Fig. 4. Chromatogram obtained by GC-MS for the methanol extract

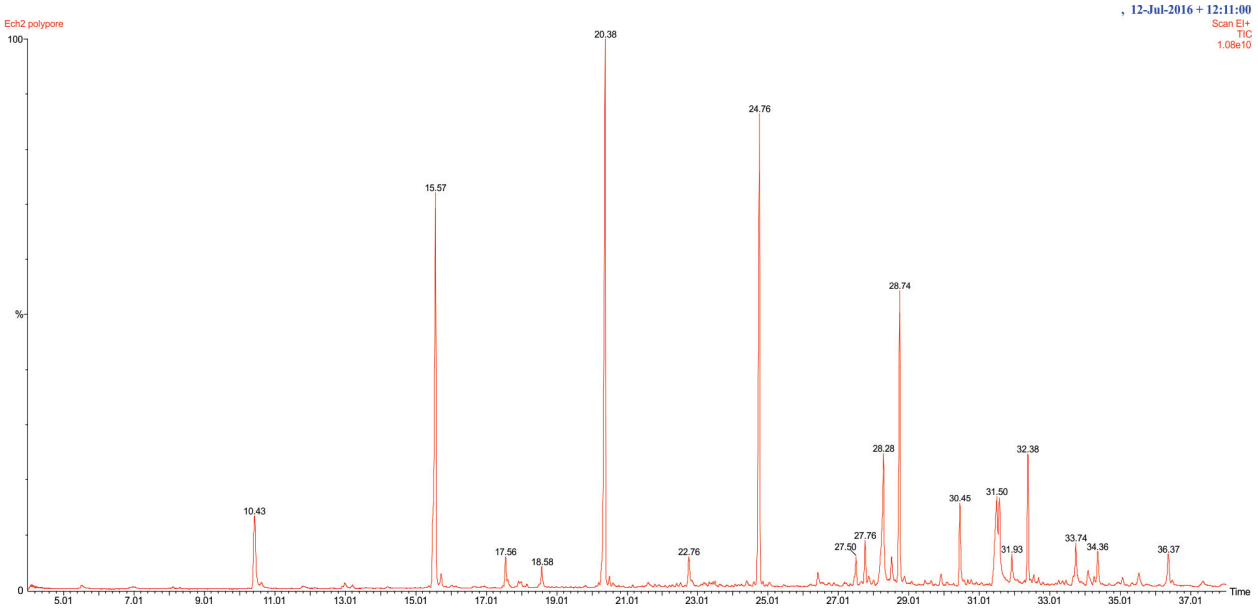


Fig. 5. Chromatogram obtained by GC-MS for the ethyl acetate extract

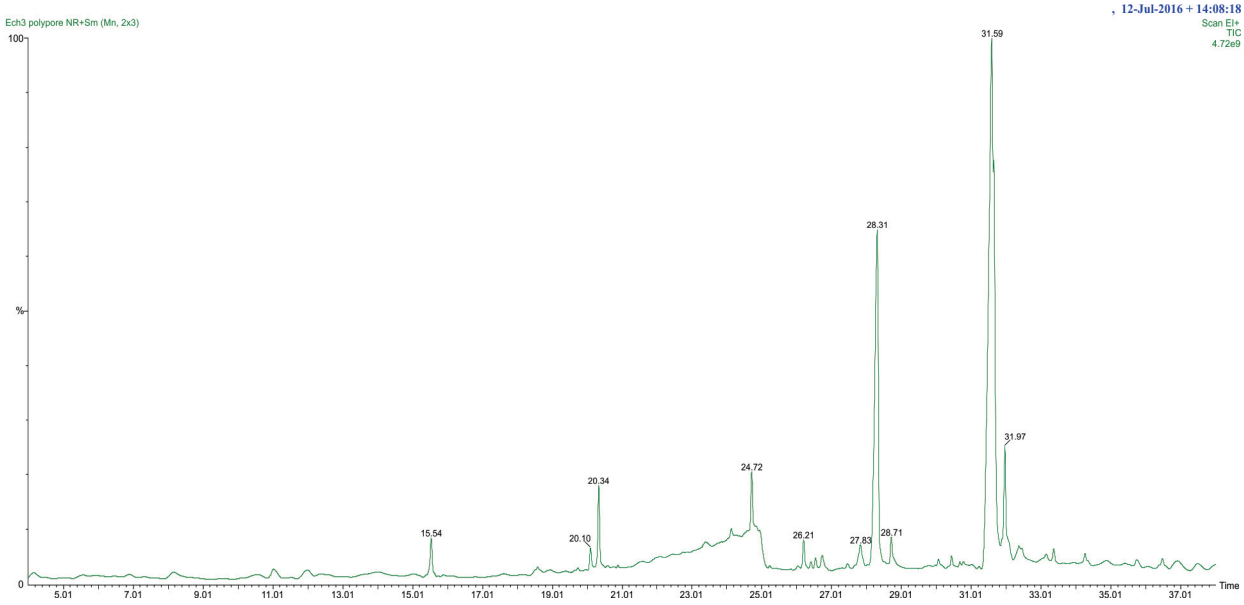


Fig. 6. Chromatogram obtained by GC-MS for the ethanol 96°extract

Table 2. Main components present in the extracts of *Trichaptum biforme* identified on the basis of GC-MS analysis.

Compound name	Molecular formula	Chemical 2D structure (PubChem : http:// pubchem. ncbi.nlm.nih.gov)	Retention time (min)	Extraction solvent
4,7-Benzofuradione, 3-acetyl-3a,7a-dihydro-2-methyl-3a,5,6,7a-tetrakis [(trimethylsilyl)oxy]	$C_{23}H_{42}O_8Si_4$		18.105	Methanol
Docosanoic acid, 1,2,3-propanetriyl ester	$C_{69}H_{134}O_6$		20.341	Methanol
Heneicosanoic acid	$C_{21}H_{42}O_2$		27.473	Methanol
Octodrine	$C_8H_{19}N$		27.849	Methanol
Hexadecanoic acid, dodecyl ester	$C_{28}H_{56}O_2$		28.199	Methanol
Pentanoic acid, 10-undecenyl ester	$C_{16}H_{30}O_2$		31.495	Methanol
Oleic acid	$C_{18}H_{34}O_2$		36.915	Methanol
Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$		36.622	Methanol
1-tetradecene	$C_{14}H_{28}$		15.569	Ethyl acetate
2,5-cyclohexadiene-1,4-dione, 2,6-dichloro	$C_6H_2Cl_2O_2$		17.560	Ethylacetate

Table 2. *Continued ...*

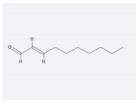
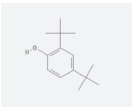
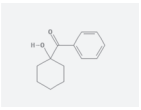
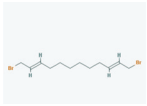
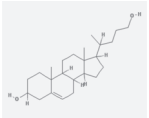
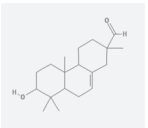
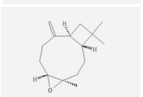
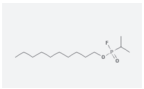
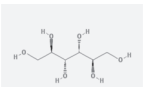
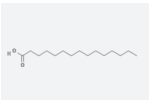
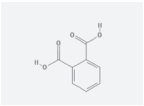
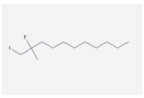
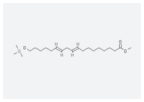
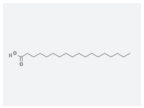
Compound name	Molecular formula	Chemical 2D structure (PubChem : http:// pubchem. ncbi.nlm.nih.gov)	Retention time (min)	Extraction solvent
2-Decenal, (E)-	$C_{10}H_{18}O$		17.805	Ethyl Acetate
Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$		18.585	Ethyl Acetate
Cetene	$C_{16}H_{32}$		20.381	Ethyl Acetate
Methanone, (1-hydroxycyclohexyl)phenyl-	$C_{13}H_{16}O_2$		22.762	Ethyl Acetate
(E,E)-1,12-dibromo-2,10-dodecadiene	$C_{12}H_{20}Br2$		27.764	Ethyl Acetate
Hexadecanoic acid, dodecyl ester	$C_{28}H_{56}O_2$		28.284	Ethyl acetate
n-Heptadecanol- 1	$C_{17}H_{36}O$		28.739	Ethyl Acetate
2-Fluoro-1-iodo-2-methylundecane	$C_{12}H_{24}FI$		30.450	Ethyl Acetate
Octadecanoic acid	$C_{18}H_{36}O_2$		31.925	Ethyl Acetate
5-cholene, 3,24-dihydroxy	$C_{24}H_{40}O_2$		32.545	Ethyl Acetate
2-phenanthrenecarboxaldehyde, 1,2,3,4,4a, 4b,5,6,7,8,8a,9-dodecahydro-7-hydroxy-2,4b,8,8-tetramethyl	$C_{19}H_{30}O_2$		33.741	Ethyl acetate
Octadecanol	$C_{18}H_{38}O$		34,366	Ethyl acetate
Caryophyllene oxide	$C_{15}H_{24}O$		35.532	Ethyl acetate

Table 2. *Continued ...*

Compound name	Molecular formula	Chemical 2D structure (PubChem : http:// pubchem. ncbi.nlm.nih.gov)	Retention time (min)	Extraction solvent
1-tetradecene	$C_{14}H_{28}$		15.539	Ethanol 96°
Isopropylphosphonic acid, fluoroanhydryde-, decyl ester	$C_{13}H_{28}FO_2P$		20.101	Ethanol 96°
D- Mannitol	$C_6H_{14}O_6$		24.132	Ethanol 96°
Cetene	$C_{16}H_{32}$		24.717	Ethanol 96°
Pentadecanoic acid	$C_{15}H_{30}O_2$		26.208	Ethanol 96°
1,2-benzendicarboxylic acid	$C_8H_6O_4$		26.413	Ethanol 96°
n-hexadecanoic acid	$C_{16}H_{32}O_2$		28.314	Ethanol 96°
2-Fluoro-1-iodo-2-methylundecane	$C_{12}H_{24}FI$		28.714	Ethanol 96°
9,12-octadecadienoic acid, 18-(trimethylsiloxy)-, methyl ester	$C_{22}H_{42}O_3Si$		31.590	Ethanol 96°
Octadecanoic acid	$C_{18}H_{36}O_2$		31.965	Ethanol 96°
Triacontane,11,20-didecyl-	$C_{50}H_{102}$		33.366	Ethanol 96°

reflect a strong antibacterial potency (Rapior *et al.*, 1996; Rapior *et al.*, 1997; Alsultan *et al.*, 2016; Khadhri *et al.*, 2017).

CONCLUSION

The extract of *Trichaptum biforme* showed an antibacterial activity against both bacterial strains tested *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. This mushroom extract exhibited bacteriostatic and bactericidal effect against these pathogenic bacteria. GC-MS analyses showed the presence of octodrine, caryophyllene oxide, phenolic compounds, fatty acids (palmitic

acid, phthalic acide) and fatty acids esters and other components that have been proposed to play an important role in the antimicrobial actions. This study revealed that *Trichaptum biforme* comprised constituents with antibacterial effect, which is considered important for application in the pharmacological field.

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