Diazole and triazole derivatives of castor oil extract: synthesis, hypoglycemic effect, antioxidant potential and antimicrobial activity

F. Taieb Brahimi, F. Belkhadem, B. Trari and A.A. Othman

Departement de classe preparatoire, Ecole Superieure en Genie Electrique et Energetique d'Oran, Bp. 64 CH2 Achaba Hanifi USTO 31003 Oran, Algerie.

Laboratoire de Synthese Organique Bioactive, Departement de Chimie Organique Industrielle, Faculte de Chimie, Universite des Sciences et de la Technologie d'Oran, Mohamed Boudiaf-USTO-MB, BP. 1505, El-M'naouer, 31003 Oran, Algerie.

Laboratoire scientifique et technique regional de police ORAN 31000, Algerie.

*Corresponding author: fawziatb@yahoo.fr

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SUMMARY: The ricinoleate triglyceride was extracted from castor-oil seeds grown in Algeria and isolated by catalytically methanolyse to methyl ricinoleate. Six diazole and triazole derivatives of ricinoleic acid were synthesized and characterized: 1,3,4-oxadiazole-5-thione (4); 1,3,4-thiadiazole-5-thione (5); 4-N-amino-1,2,4-triazole-5-thiol (7); 1,2,4-triazole-5-thione (9); 5-amino-1,3,4-oxadiazole (10) and 5-amino-1,3,4-thiadiazole (11). The antibacterial and antifungal screening data of synthesized compounds showed appreciable inhibition and among them, 5, 7 and 8 showed more inhibition on Gram positive Enterococcus faecalis than reference ampicilnine; while compounds 1, 7, 8, 10 and 11 showed competitive antifungal effects compared to reference amphotericin B. In addition, all synthesized compounds (1-11) showed competitive antioxidant properties, particularly compounds 7 at 125, 250, 500 and 1000 µg/mL and compounds 4, 5 and 9 at a concentration of 1000 µg/mL. The intermediate compounds 1, 2 and 8 showed anti-α-amylase activity at various concentrations in the range of IC50 = (120.25 ± 1.17 - 130.42 ± 2.48). Oxadiazole 4 showed the best α-amylase inhibition by 78.5% at a concentration of 1000 µg/mL.

KEYWORDS: Anti diabetic; Antimicrobial; Antioxidant; Castor oil; Extraction; Heterocycle

RESUMEN: Diazoles y triazoles derivados del extracto de aceite de ricino: síntesis, efecto hipoglucémico, potencial antioxidante y actividad antimicrobiana. Los triglicéridos de ricinoleico se extrajeron de semillas de aceite de ricino cultivadas en Argelia y se sintetizó catalíticamente con metanolisis el ricinoleato de metilo. Seis derivados de diazoles y triazoles de ácido ricinoleico se han sintetizado y caracterizado: 1,3,4-oxadiazol-5-tiona (4); 1,3,4-thiadiazol-5-tiona (5); 4-N-amino-1,2,4-triazol-5-tiol (7); 1,2,4-triazol-5-tiona (9); 5-amino-1,3,4-oxadiazol (10) y 5-amino-1,3,4-thiadiazol (11). Los datos de detección antibacteriana y antifúngica de los compuestos sintetizados mostraron una inhibición apreciable, entre ellos, los compuestos 5, 7 y 8 mostraron más inhibición en Enterococcus faecalis Gram positivo que la ampicilina de referencia. Mientras que los compuestos 1, 7, 8, 10 y 11 mostraron una influencia antifúngica competitiva en comparación con la anfotericina de referencia B. Como todos los compuestos sintetizados (1-11) mostraron propiedades antioxidantes competitivas, particularmente los compuestos 7, a 125, 250, 500 y 1000 µg/mL también compuestos 4, 5 y 9 a una concentración de 1000 µg/mL. Los compuestos intermedios 1, 2 y 8 mostraron actividad anti-α-amilasa a diversas concentraciones en el rango de IC50 = (120.25 ± 1.17 - 130.42 ± 2.48). El oxadiazol 4 mostró la mejor inhibición de la α-amilasa en un 78.5% a una concentración de 1000 µg/mL.

PALABRAS CLAVE: Aceite de ricino; Antidiabético; Antimicrobiano; Antioxidante; Extracción; Heterociclo

ORCID ID: Taieb Brahami F https://orcid.org/0000-0002-9460-8193, Belkhadem F https://orcid.org/0000-0002-7113-7798, Trari B https://orcid.org/0000-0001-7997-3638, Othman AA https://orcid.org/0000-0001-8116-4506

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1. INTRODUCTION

Castor oil is a natural production of the castor plant (Ricinus Communis) (Mubofu, 2016). Castor oil plants are widespread throughout the globe, particularly in tropical regions such as India, the southeastern Mediterranean Basin of North Africa (Trochain, 1930), Los Angeles, California (Witchard, 1997) and elsewhere. Castor oil has many uses; for example, it remains of commercial importance as a non-freezing, antimicrobial, pressure-resistant lubricant for special purposes, either for latex or metals, or as a lubricating component in fuels (Imankulov, 2012). Castor oil has long been used on the skin to prevent dryness. Whether pure or processed, it is still a component of many cosmetics (Bianchi et al., 2011; Rachapudi et al., 2017).

The high percentage of ricinoleic acid residues in castor oil and their derivatives inhibit viruses, bacteria or fungi (Ghosh et al., 2013). The literature has revealed several synthetic modifications and utilization of ricinoleic acid. These modifications vary between ethylene bond (Goddard et al., 2013) hydroxyl group (Thames et al., 2006) and carboxylic acid group (Dutta et al., 2011; Lavanya et al., 2012).

Heterocyclic-fatty acid hybrids such as oxadiazole, thiadiazole and triazole derivatives of vegetable oils are a new class of fatty acid derivatives with a wide range of biological activities and significance in the field of medicinal chemistry. They possess a broad spectrum of therapeutic uses such as analgesic, antimicrobial, anti HIV activity, antitumor, antimarial, anticancer, anticonvulsant, anti-diabetic, antioxidiant (Cao et al., 2014; Ahmad et al., 2017).

This work is mainly concerned with the extraction of castor oil from seeds of the plant and the isolation of ricinoleic acid. The latter was subjected to synthetic modifications focused on the carboxylic group to ultimately give six diazole derivatives. The synthetic intermediates and final products were studied to determine the biological evaluation of their α-amylase inhibitory, antimicrobial and antioxidiant activities.

2. MATERIALS AND METHODS

2.1. General

All reactions were monitored by TLC, silica gel F254, made by Merck, Germany. Melting points (°C) were measured in open glass capillaries using a Branstead 9001 Electrothermal melting point apparatus and were not corrected. The UV visible electron spectroscopy was recorded on an Optisan View 42 spectrometer. The IR spectra were recorded using KBr disks in a GENESIIS FTIR spectrophotometer, in μ units of cm^{-1}. The ¹H and ¹³C-NMR spectra (1D) were recorded on a Bruker AC 400 MHz spectrometer (University of Lyon 1, France) in DMSO-d₄ and referenced to TMS. Symbols δ were used for chemical shifts in ppm, s = singlet, d = doublet, dd = double doublet and m = multiplets. Mass spectra were obtained using a GC-MS CLARIS 500 (Laboratoire Régional de Police Scientifique d’Oran, Algeria). The microorganisms in this study were supplied and identified by the laboratory of microbiology by the university hospital of Oran1. The Mueller Hinton medium was supplied by (Difco).

2.2. Chemistry

2.2.1. Oil extraction

The castor oil (1) was extracted with a soxhlet extractor. Hexane (1L) and ground castor beans (200 g) were packed into a filter paper thimble. The extraction lasted 10 h. The crude oil was treated with hot water to remove gums, hydrates, phosphates and other impurities and then it was neutralized with 0.1N NaOH to remove free fatty acid and soap to give castor oil (1), (84 g, 40.2%) (Nakarimi et al., 2014). UV (λ max) nm: 205; 210. IR (CCl₄), ν cm⁻¹: 3452. 92(OH); 3008(CH saturated); 2925, 2855 (alkyl groups); 1742.37(C = O). ¹H NMR (400 MHZ, DMSO d₆) δ (ppm): 5.37-5.34 (m, 6H, CH₄₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋_-...
DMSO $d_6$ $\delta$ ppm: 175.0; 172.9; 130.8; 129.9; 127.8; 70.4; 60.0; 35.6; 34.2; 31.2; 29.6; 29.5; 28.6; 27.5; 26.8; 22.9; 14.2. MS: Molecular formula $C_{19}H_{36}O_3$: 311, $m/z$: 311 (M+, 100%).

2.2.3. Ricinoleic acid hydrazide

Methyl ricinoleate (2), (2.11 g; 0.01mol), ethanol (50 mL) and hydrazine hydrate 64% (12 mL) were refluxed for 10 h. Ethanol and hydrazine were evaporated under reduced pressure, white solid was obtained, recrystallized from acetone/cyclohexane to give ricinoleic hydrazide (3), (2.2g, 90%). M.p. (80–85 °C); UV ($\lambda$ max) nm: 2849.31 and 2918.73 (alkyl groups); 205; 252. IR (KBr), v cm$^{-1}$: 3317.23 (NH and NH); m/z: 364 m/z: 364 (M+, 100%).

2.2.4. 5-Ricinoleyl-1,3,4-oxadiazole-2-thione-thiol

Ricinoleic acid hydrazide (3), (1 g; 0.0032 mol) and KOH (1.5 g) in absolute ethanol (10 mL) were mixed together until the solution became clear. Cs$_2$ (25 mL) was added. The solution was stirred for 10 h at room temperature, and then diethyl ether (20 mL) was added to form a precipitate ricinoyl-potassium thiocarbazinic acid (6). The intermediate product was washed with (NH$_3$N$_2$H$_2$O) (4 mL). The solution was refluxed for 1.5 h until the color of the solution became clear green. After cooling to room temperature, ice water (10 mL) was added to the reaction mixture, which was then neutralized with 3N HCl to form a precipitate. The precipitate was isolated by filtration and purified by recrystallization from ethanol/water to afford the desired product in the form of yellow crystal: 5-Ricinoleyl-4-amino-1, 2, 4-triazole-3-thiol (7), 1.2g, 65%; $R_e$ 0.61 (cyclohexane /acetone: 6/4); $M_p$ (132°C) UV ($\lambda$ max) nm: 205; 240. IR (KBr), v cm$^{-1}$: 3124.17 (OH); 3177 (NH); 3068 (alkyl groups); 2700 (alkyl groups); 205; 262. (C = N); m/z: 312 (M+, 100%).

2.2.5. 5-Ricinoleyl-1,3,4-thiadiazole-2-thione

The ricinoleic acid hydrazide (3), (1 g, 0.0032 mole) was mixed with KOH (0.5 g) dissolved in absolute ethanol. A solution of Cs$_2$ (2.5 g) was added to the mixture with 15 mL of ethanol. The mixture was stirred for 1 h at room temperature and then refluxed for 24 hours. The final product was acidified with hydrochloric acid. The resulting solid residue was filtered and recrystallized from ethanol. The product was obtained in the form of yellowish crystals (5), (1.45g, 75%); $R_e$ 0.35 (cyclohexane /acetone 6/4); $M_p$ 97 °C; UV ($\lambda$ max) nm: 205; 285. IR (CCl$_4$), v cm$^{-1}$: 3308.29(OH,NH); 2980.45(C = C); 2921.63,2849.31 (alkyl groups); 1589.06(C = N); 1462.74(C = S). 1H NMR (400 MHz, DMSO d$_6$) $\delta$ (ppm): 5.42 (s, 1H, NH); 5.33 (m, 1H, H$_{10}$); 4.75 (m, 1H, H$_{10}$); 3.32 (s, 1H, OH); 3.24-1.16 (m, 32H, CH paraffinic); 0.84 (t, 3H, CH$_3$ terminal). 13C NMR (400 MHz, DMSO d$_6$) $\delta$ ppm: 173.2; 123.4; 69.7; 37.9; 37.6; 34.2; 31.2; 29.6; 29.4; 28.6; 25.6; 22.9; 21.9; 14.28. MS: Molecular formula $C_{19}H_{34}N_2O$: 312, $m/z$: 312 (M+, 100%).

2.2.6. 5-Ricinoleyl-4-amino-1, 2, 4-triazole-3-thiol

Ricinoleic acid hydrazide (3), (1g; 0.0032 mol) and KOH (1.5 g) in absolute ethanol (10 mL) were mixed together until the solution became clear. Cs$_2$ (25 mL) was added. The solution was stirred for 10 h at room temperature, and then diethyl ether (20 mL) was added to form a precipitate ricinoyl-potassium thiocarbazinic acid (6). The intermediate product was washed with (NH$_3$N$_2$H$_2$O) (4 mL). The solution was refluxed for 1.5 h until the color of the solution became clear green. After cooling to room temperature, ice water (10 mL) was added to the reaction mixture, which was then neutralized with 3N HCl to form a precipitate. The precipitate was isolated by filtration and purified by recrystallization from ethanol/water to afford the desired product in the form of yellow crystal: 5-Ricinoleyl-4-amino-1, 2, 4-triazole-3-thiol (7), (1.2g, 65%); $R_e$ 0.61 (cyclohexane /acetone: 6/4); $M_p$ (132°C) UV ($\lambda$ max) nm: 205; 240. IR (KBr), v cm$^{-1}$: 3124.17 (OH); 3177 (NH); 3068 (alkyl groups); 2700 (SH); 1617 (C = N). 1H NMR (400 MHz, DMSO d$_6$) $\sigma$ (ppm): 11.97 (s, 1H, SH); 4.22 (d, 2H, NH$_3$); 4.01 (d, 2H, NH$_3$); 5.75 (m, 1H, H$_{10}$); 5.39 (m, 1H, H$_{10}$); 3.34 (s, 1H, OH); 2.69-1.23 (m, 32H, CH paraffinic); 0.85 (t, 3H, CH$_3$ terminal). 13C NMR (400 MHz, DMSO d$_6$) $\delta$ ppm: 181.0; 174.3; 164.7; 128.2; 123.2; 70.0; 69.9; 60.3; 37.9; 34.3; 31.9; 25.7; 22.5; 14.5. MS: Molecular formula $C_{19}H_{34}N_2O$: 368; the pseudo-molecular peak [M + H$^+$] with $m / z$ 367.

2.2.7. N-Thiosemicarbazide ricinoleic

Ricinoleic acid hydrazide (3), (1g; 0.0032 mol) was dissolved in ethanol (20 mL) with stirring. Ammonium thiocyanate (0.58 g) and HCl (30%) were added, and the reaction mixture was refluxed for 6 h. Excess solvent was evaporated to almost dryness and recrystallized from methanol/petroleum ether to give N-Thiosemicarbazide ricinoleic (8), 0.97g, 65%; $R_e$: 0.45 (cyclohexane /acetone: 7/3); $M_p$ (97 °C); UV ($\lambda$ max) nm: 210; 265. IR (KBr) v cm$^{-1}$: 3373.85 (OH); 3271.64, 3172.33, 3106.76 (NH and NH$_2$); (2851.24 and 2919.70)

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2.2.8. 5-Ricinoleyl-4H-I, 2, 4-triazole-3-thiol

N-Thiosemicarbazide ricinoleic (8), (1.0 g; 0.0024 mol) in ethanol (15 mL) was added to an alcoholic solution of 10% NaOH (20 mL), and the reaction mixture was refluxed for 12 h. The mixture was cooled and acidified with dilute HCl to pH (5–6). The crude compound was recrystallized from ethanol to give white needle-like crystals: 5-Ricinoleyl-4H-I, 2, 4-triazole-3-thiol (9), (0.75 g, 75%); m.p (128 °C); Rf: 0.46 (cyclohexane /acetone: 6/4) ; UV (λmax) nm: 210; 275. IR (KBr) v cm⁻¹: 3433 (OH, NH); 3100(C = C); 2953 and 2868 (alkyl groups); 2722.05 (alkyl groups); 1654.31 (O-C = N); 1119 ( = C-O-C = ).

2.2.9. 5-Ricinoleyl-2-amino-1,3,4-oxadiazole

N-Thiosemicarbazide ricinoleic (8), (1.0 g, 0.0024 mol) was dissolved in an alcoholic solution of NaOH (5N) with the aid of stirring. The mixture was refluxed for 10 h in an oil bath. The precipitated solid was filtered, washed with water, dried and recrystallized with ethanol to give brown crystals (11, 0.40g, 40%); m.p. (110 °C); Rf: 0.43 (cyclohexane /acetone: 6/4) UV (λmax) nm: 210; 240. IR (KBr) v cm⁻¹: 3444.24 (OH, NH); 2955.38(C = C-CH); 2921.63 and 2850.27 (alkyl groups); 1636.3 (C = N). MS: Molecular formula C₁₉H₂₃N₂O₅S: 371, m/z 371 (M+, 100%).

2.2.10. 5-Ricinoleyl-2-amino-1,3,4-thiadiazole 11.

N-Thiosemicarbazide ricinoleic (8, 1.0 g; 0.0024 mol) was added gradually under stirring to the cooled concentrated H₂SO₄ (30 mL) for 1 h. The reaction mixture was refluxed for 10 h in an oil bath. The precipitated solid was filtered, washed with water, dried and recrystallized with ethanol to give brown crystals (11, 0.40g, 40%); m.p. (110 °C); Rf: 0.43 (cyclohexane /acetone: 6/4) UV (λmax) nm: 210; 240. IR (KBr) v cm⁻¹: 3444.24 (OH, NH); 2955.38(C = C-CH); 2921.63 and 2850.27 (alkyl groups); 1636.3 (C = N). MS: Molecular formula C₁₉H₂₃N₂O₅S: 371, m/z 371 (M+, 100%).
presence of the DPPH radicals gave a dark purple color to the solution and which was absorbed rapidly at 517 nm when reduced. The color became pale yellow. During the reaction, the layer of this radical became saturated on contact with an antioxidant, which explained the disappearance of its coloring. This discoloration highlighted the trapping power of the free radical by the tested product. The percentage of the anti-free radical activity was estimated according to the equation below (Vijayalaxmi et al., 2015):

\[
\text{PI} \% = \frac{(\text{Abs control} - \text{Abs product})}{\text{Abs control}} \times 100
\]

PI: Percentage inhibition
Abs control: Absorbance at the 517 nm wavelength of the negative control (DPPH + methanol).

2.3.3. α-Amylase inhibition activity

The α-amylase inhibitory activity of the synthetic compounds was determined using the chromogenic DNSA method with a few modifications (Adegboye et al., 2018). 300 μl of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) and 300 μl of sample at different concentrations (62.5; 125; 250; 500; 1000) μg/mL were incubated at 37 °C for 30 min. Afterwards, 300 μl of a 1% starch solution in 0.02 M sodium phosphate buffer were added to each tube at timed intervals. The reaction mixtures were then incubated at 37 °C for 15 min. The reaction was stopped with 0.5 mL of dinitrosalicylic acid (DNSA) color reagent. The reaction mixture was then diluted after adding 5 mL of distilled water, and absorbance was measured at 540 nm. The α-amylase inhibitory activity was calculated as follows:

\[
\text{Inhibition} \% = 1 - \frac{A_{\text{samp}}}{A_{\text{cont}}} \times 100\%.
\]

Where \(A_{\text{samp}}\) and \(A_{\text{cont}}\) were defined as absorbance of the sample and the control, respectively.

3. RESULTS AND DISCUSSION

3.1. Synthesis

The ricinoleate triglyceride (1) was catalytically transesterified with methanol to give methyl ricinoleate (2) in quantitative yield (Kumar et al., 2017) and converted to hydrazide (3) (Joshi et al., 2017), as summarized in Figure 1. Hydrazide (3) was subjected to synthetic modifications to reveal ricinoleic diazoles and triazole derivatives: 1, 3,4-oxadiazole-5-thione (4), 1,3,4-thiadiazole-5-thione (5), 4-N-amino-1,2,4-triazole-5-thiol (7), 1,2,4-triazole-5-thiol (9), 5-amino-1,3,4-oxadiazole (10), 5-amino-1,3,4-thiadiazole (11) and as summarized in
Figure 3. Characteristic signals in $^1$H NMR of heterocyclic derivatives: 5-Ricinoleyl-1,3,4-oxadiazole-2-thione-thiol (4), 5-Ricinoleyl-1,3,4-thiadiazole-2-thione (5), 5-Ricinoleyl-4-amino-1, 2, 4-triazole-3-thiol (7), 5-Ricinoleyl-4H-1,2,4-triazole-3-thiol (9), 5-Ricinoleyl-2-amino-1,3,4-oxadiazole (10), 5-Ricinoleyl-2-amino-1,3,4-thiadiazole (11).
The first set of heterocycle derivatives 4, 5 and 7 were achieved by refluxing hydrazide 3 with CS$_2$ and aqueous KOH to give 2-ricinoleyl-1,3,4-oxadiazole-5-thiol (4) (Taieb Brahimi et al., 2017). While using the excess CS$_2$ it yielded 2-ricinoleyl-1,3,4-thiadiazole-5-thione (5) (Gad El-Karim et al., 2013). However, heating hydrazide (3) with CS$_2$ in alcoholic KOH resulted in the crude ricinoleic potassium thiocarbazinic acid (6), which without separation and further analysis was refluxed with hydrazine hydrate to give 5-ricinoleyl-4-amino-1,2,4-triazole-3-thiol (7).

The second set of heterocycles (9-11) was prepared from N-thiosemicarbazide ricinoleic (8), which had already been prepared by treating hydrazide (3) with ammonium thiocyanide. 5-Ricinoleyl-4H-1,2,4-triazole-3-thiol (9) was obtained by the cyclization of 8 with KOH (Belkhadem et al., 2017); while 2-amino oxadiazole (10) was isolated.

| Compound | Gram-positive bacteria | Gram-negative bacteria |
|----------|------------------------|-----------------------|
|          | Zone of inhibition in mm and MIC (minimum inhibitory concentration) in µg/mL |                      |
| 1        | -                      | 7 (100) 7 (25) 7 (25) 8 (50) 10 (6.5) 10 (25) |
| 2        | -                      | 7 (50) 7 (25) 8 (25) 7 (50) 12 (25) 10 (25) |
| 3        | -                      | - 10 (25) 10 (25) 15 (25) - |
| 4        | 14 (6.25) 7 (50) 7 (100) 10 (12.5) 10 (25) 15 (12.5) 8 (25) |
| 5        | 10 (6.25) 12 (25) - 8 (25) 10 (25) 10 (6.25) 8 (25) |
| 7        | 7 (25) 9 (50) 7 (25) 7 (25) - 10 (25) 10 (25) - |
| 8        | 25 (100) 7 (100) 7 (50) 8 (25) 10 (6.25) 8 (50) 8 (6.25) 10 (100) |
| 9        | 7 (25) 7 (50) - 7 (50) 7 (25) 10 (25) 7 (50) 8 (25) |
| 10       | 7 (50) 7 (12.5) 7 (50) 7 (50) - 8 (25) 10 (25) - |
| 11       | 8 (50) 8 (50) 7 (100) 7 (100) - 10 (25) 10 (25) - |
| Amp      | 25                     | 8 32 15 20 32 18 22 |

The figures in the table show the zone of inhibition (mm) and the corresponding MIC (µg/mL) values in brackets.

S.a. (Staphylococcus aureus), E.f. (Enterococcus faecalis), B.c. (Bacillus cereus), P.a. (Pseudomonas aeruginosa), E.c. (Escherichia coli), K.P. (Klebsiella planticola), Sal. (Salmonella), P.v. (Proteus vulgarus).

| Compound | Fungi |
|----------|-------|
|          | Candida albicans | Trichosporon Sp | Aspergillus niger | Fusarium | Penicillium Sp | Altenaria |
| 1        | -      | -               | +++              | -        | -              | +++       |
| 2        | -      | -               | ++               | -        | -              | +++       |
| 3        | ++     | ++              | -                | +        | +++             | -         |
| 4        | ++     | ++              | -                | -        | -              | +         |
| 5        | ++     | ++              | -                | -        | -              | +         |
| 7        | +++    | -               | -                | -        | -              | +         |
| 8        | -      | +++             | -                | +++      | +++             | -         |
| 9        | -      | -               | -                | -        | -              | +         |
| 10       | ++     | +++             | -                | +++      | -              | +++       |
| 11       | -      | ++              | -                | +++      | -              | +++       |
| Ref      | +++    | ++              | +++              | +        | +++             | +++       |

Key to the inhibition zones activities: Highly active = (21-30 mm) +++; Moderately active = (16-20 mm) ++; Slightly active = (10-15 mm) +; Inactive = (< 10mm) -
Ref: amphotericin B (100 μg/disc.).
after treating 8 with NaOH in the presence of I$_2$/KI. 2-Amino thiadiazole (11) was collected after reacting (8) with H$_3$PO$_4$. The structural determination of all synthetic compounds 2-11 was confirmed spectroscopically by IR, UV, $^1$H- NMR, $^{13}$C-NMR and MS (see Figure 3). All compounds 1-11 became available, and a study of their antimicrobial, antioxidant and hypoglycemic activities was carried out.

3.2. Pharmacological screening

3.2.1. Antimicrobial activities

The results for antimicrobial activities are summarized in Tables 1 and 2.

The gram-positive bacteria under consideration showed zones of inhibition inferior to those observed by the Gram-negative bacteria. In general, the first
site of action of the products tested on bacterial cells was the plasma membrane. This was directly related to the amphiphilic nature of the tested products which facilitated their insertion between the membrane phospholipids and ensured their solubilization in the lipid bilayer (Soliman et al., 2015). The good activity was attributed to the presence of pharmacologically active groups NH-CS-NH, C = O and C = S attached to the heterocyclic nuclei (triazoles, oxadiazoles and thiadiazoles). The presence of amine functions (NH$_2$) provided the tested molecules a higher activity on mushrooms than those of other products.

### 3.2.2. Antioxidant activity

From a methodological point of view, the free radical test, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) is recommended for compounds containing the SH, NH and OH (Barbuceanu et al., 2014) groups and was carried out at ambient temperature. This made it possible to eliminate any risk of thermal degradation of labile molecules (Li et al., 2018). This test consisted of the reduction of an alcoholic solution of the radical species DPPH$^-$ in the presence of a hydrogen donor antioxidant (AH), which resulted in the formation of a non-radical form of DPPH-H. Based on the experimental results (see Figure 4), the starting ricinoleic triglyceride (1), ester (2) and diazoles (4, 5, 7, 9, 10 and 11) showed high activity at different concentrations. Indeed, the structure-activity relationship of the heterocycle showed that the scavenging activity of the radicals increased with the presence of the hydrogen donor groups (-NH$_2$, -NH, -SH, -OH). The conjugation between the free radicals of the hetero atoms (Nitrogen, Oxygen, Sulfur) and the π electrons of the aromatic ring represented an additional factor to increase the stability of the radical structure.

IC$_{50}$ (Inhibitory concentration 50), also referred to as EC$_{50}$ (Efficient Concentration 50), is the concentration of the test sample needed to reduce 50% of the DPPH radical. The IC$_{50}$ are calculated graphically by percent inhibition as a function of different concentrations of the tested product. From the value shown in Table 3, we noted a strong antiradical power for castor oil, which resulted in a fairly low IC$_{50}$, comparable to that of the standard compound ascorbic acid.

### 3.2.3. Hypoglycemic effect (α-amylase inhibition)

The digestive enzyme (α-amylase) was responsible for hydrolyzing dietary starch (maltose), which broke down into glucose prior to absorption. The inhibition of α-amylase led to a reduction in post prandial hyperglycemia under diabetic conditions (Yilmazer-Musa et al., 2012; Menteșe et al., 2014). α-Amylase activity can be measured in-vitro by the hydrolysis of starch in the presence of the α-amylases enzyme. The α-amylase inhibitory activity was determined by using dinitro salicylic acid (DNSA). Triglyceride (1), its corresponding ester (2), synthetic products 3-11

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### Table 3. IC$_{50}$ values for castor oil 1 and its synthesized derivatives (2-11) with references of DPPH scavenging and α amylase inhibitory activity.

| Compound | DPPH Scavenging Activity (IC50 (μg/mL)) | Antiradical Power (1/ IC50) | α-amylase inhibitory activity (IC50 (μg/mL)) |
|----------|----------------------------------------|-----------------------------|---------------------------------------------|
| 1        | 32.16 ± 0.58                           | 0.031                       | 130.42 ± 2.48                               |
| 2        | 44.45 ± 0.45                           | 0.022                       | 120.25 ± 1.17                               |
| 3        | ND                                     | ND                          | 400.64 ± 2.75                               |
| 4        | 56.74 ± 0.32                           | 0.019                       | 325.90 ± 2.75                               |
| 5        | 58.24 ± 0.30                           | 0.020                       | 552.80 ± 3.15                               |
| 7        | 50.15 ± 0.41                           | 0.018                       | 736.00 ± 5.66                               |
| 8        | ND                                     | ND                          | 125.00 ± 1.75                               |
| 9        | 57.49 ± 0.35                           | 0.017                       | 920.56 ± 8.15                               |
| 10       | 48.46 ± 0.28                           | 0.001                       | ND                                          |
| 11       | 52.48 ± 0.38                           | 0.001                       | ND                                          |
| Ascorbic acid † | 33.67 ± 0.45                           | 0.030                       | -                                           |
| Acarbose ‡ | -                                     | -                           | 85.65 ± 1.09                               |

Each point shows the average value of three replicates ± SD ND (Not detected). † (reference of antioxidant activity); ‡ (reference of anti diabetic activity). DPPH (2, 2-diphenyl-1-picrylhydrazyl radical); IC$_{50}$ (Inhibitory concentration 50).
(except 6) and acarbose, as control, were evaluated for in vitro α-amylase and showed a wide range of inhibitory activity (Figure 5). At a lower IC₅₀ concentration (µg/mL), the above mentioned compounds were grouped into three inhibitory groups: Group A, consisting of compounds (1), (2) and (8), which exhibited an inhibition at the lowest corresponding concentrations (130.42 ± 2.48, 120.25 ± 1.17 and 125.00 ± 1.75) µg/mL (see Table 3).

Group B, comprised of compounds (3) and (4), which showed inhibition at moderately higher concentrations (400.64 ± 2.75, 325.90 ± 2.75) µg/mL. Group C consisted of compounds (5), (7), and (9) at corresponded to the highest concentrations (552.80 ± 3.15, 736.00 ± 5.66, 920.56 ± 8.15) µg/mL. Among the synthetic compounds (4), (5), (7), (9), (10) and (11), no significant inhibitory effect was detected for the heterocycles (10) and (11). Oxadiazole 4 showed the best α-amylase inhibition.

4. CONCLUSIONS

The oil extract (1) (ricinoleate triglyceride) reacted over 40% from original castor oil and was closest to highest percentage reported in the literature (45%).
The synthetic compounds showed better biological effects on Gram-negative bacteria. The presence of amine functions (NH₂) of the tested molecules provided all synthesized compounds with higher activity on mushrooms. All synthesized compounds showed high antioxidant activity. The compounds with the greatest anti-radical activity were noted as: Castor oil (1) > Ester (2) > Amino-oxadiazole (10) > Amino-triazole (7) > Amino-thiadiazole (11) > oxadiazole (4) > triazole (9) > thiadiazole (11). The intermediate and diazole derivatives 1-11 were evaluated for in vitro α-amylase inhibitory activity and showed good to moderate inhibitory activity compared to standard acarbose. The synthesized compounds can be ranked in descending order of their anti-diabetic activity as follows: Acarbose > Ester (2) > Castor oil (1) > thiosemicarbazide (8) > hydrazide (3) > oxadiazole (4) > thiadiazole (5) > Amino-triazole (7) > triazole (9) > Amino-oxadiazole (10) > Amino-thiadiazole (11).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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