Polyphenolic profile and evaluation of the antimicrobial, antidiabetic, anti-Alzheimer and larviscidal activities of Linum trigynum L.

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Abstract

In this study, we investigated the polyphenolic composition of the *n*-butanol fraction of *Linum trigynum* L. (BELTr), a medicinal plant from the Linaceae family that grows in Algeria, using RP–UHPLC–ESI–QTOF–MS technique and evaluated its antimicrobial, larviscidal and inhibition of α-amylase and butyrylcholinestrase (BChE) potentials. Fifty six polyphenols were identified in the BELTr with isomers of vicenin-2 and orientin, and isovitexin as the major compounds. The extract showed a significant inhibition of BChE (IC_{50}: 112.45 ± 3.93 µg/mL) and a good inhibition of α-amylase (IC_{50}: 2.25 ± 4.05 mg/mL). In addition, the BFLTr exhibited antimicrobial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538P, *Salmonella enterica* ATCC 13076, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC 13883, *Enterococcus faecalis* ATCC 19433, and *Pseudomonas aeruginosa* ATCC 27853, as well as a yeast strain, *Candida albicans* ATCC 1031, with MICs values ranging between 250 and 500 µg/mL and a weak larviscidal effect. Hence, the extract could be used as alternative treatment for the management of oxidative stress and for prevention from diabetes and Alzheimer's diseases.

Introduction

Linum genus, belonging to the Linaceae family, comprises about 230 species, distributed worldwide (Kartal et al. 2004). In Algeria, there are 7 species of this genus, *L. tenue* Desf., *L. strictum* L., *L. corymbiferum* Desf., *L. suffruticosum* L., *L. usitatissimum* L., *L. tryginum* and *L. numidica* (Quèzel and Santa 1962). In traditional medicine, *L. grandiflorum* seed oil is used to improve fertility and has laxative, emollient, analgesic, resolutive, and expectorant properties (Bown 1995). Additionally, *L. usitatissimum* L. has been utilized in folk medicine to promote abscess maturation and other ailments (Fujita et al. 1995). Previous studies have revealed that *Linum usitatissimum* possess significant pharmacological effects, including antiulcer, antioxidant, antibacterial, antifungal, hepatoprotective, antiurolithiatic, antidiabetic, immunoregulatory, and anticancer activities (Dugani et al. 2008; Herchi et al. 2016; Mahmood et al. 2019; Xu et al. 2008; Sun et al. 2009; Prathyusha et al. 2019; Kapuriya et al. 2018; Ge et al. 2009; Keykhasalar et al. 2021). Many other *Linum* species have been studied for their anticancer activity (Mouna et al. 2022; Mouna et al. 2023a; Esfandiari et al. 2016). However, few phytochemical and biological studies have been conducted on other species. A study carried out by Asad et al. 2021 demonstrated that *Linum grandiflorum* L has antioxidant and anti-inflammatory activities. *L. Arboreum* L fractions have shown dose-dependent cytotoxic activity against HCT-116 also with antioxidant and antimicrobil proprieties (Sultan et al. 2021). *Linum lewisi* has also an antioxyidant activity due to the high content from aryltetralin lignan such as s justicidin B (Dougué Kentsop et al. 2022). *Linum* species are rich in secondary metabolites, particularly arylnaphthalene and aryldihydroarylnaphthalene lignans (Konuklugil et al. 2007; Schmidt et al. 2012). and flavonoids *C*-glycosides, which possess diverse biological properties such as antioxidant potential (Dar et al. 2017), prevention of ovariectomy-induced osteoporosis (Zhang et al. 2020) and anticancer activities (Czemplik et al. 2016; Vasilev et al. 2006). The characterization of the phytochemical content confirmed the presence of isoflavones, sterols and

antioxidant agents such as α-tocotrienol, γ-tocotrienol, α-tocopherol, γ(β)-tocopherol, δ-tocopherol, retinol, zeaxanthin, lutein and β-carotene also with fatty acids (Mattioli et al. 2019). Our works concern *Linum trigynum* L. which has seven synonyms (*Cathartolinum gallicum* Rchb., *Chrysolinum gallicum* Fourr., *Linum aureum* Waldsr & Kit., *Linum gallicum* L., *Linum procumbens* Gaterau., *Linum strictissimum* Pall., *Numisaureum petiolatum* Raf.). Previously, we have reported the phytochemical profile, the anticancer and antioxidant activities of two Algerian *Linum* spesies, L. *trigynum* L and *L. numidicum* (Mouna et al. 2022; Mouna et al. 2023a, Mouna et al. 2023b). In this work, we focused on the polyphenolic composition of the *n*-butanol extract of *Linum trigynum* and its antimicrobial, andiabtetic and larviscdal activities which are reported here for the first time.

Experimental

Plant Material

Linum trigynum L. was collected from Djebel El-Ouahch-Constantine (North-Eastern Algerian), in May 2022. The plant material was authenticated by Pr. Gérard de Bélair at the National GdB Herbarium, and a voucher code of 011_42 was assigned to the sample.

Extaction

The aerial parts and roots of *L. trigynum* L. (100 g), previously dried and pulverized, were subjected to hydroalcoholic extraction with a mixture of methanol/water (70/30: v/v) three times over 24 hours. The macerate was filtered and concentrated under vacuum at 40°C. The resulting extract was diluted with 300 mL of distilled water before being subjected to liquid-liquid extractions using solvents of increasing polarity (petroleum ether, chloroform, ethyl acetate, and *n*-butanol, successively). After concentration to drynes, 0.2 g of petroleum ether extract (PELTr), 0.8 g of chloroform extract (CELTr), 0.4 g of ethyl acetate extract (EAELTr), and 2 g of *n*-butanol extract (BELTr) were obtained.

RP–UHPLC–ESI–QTOF–MS analyses

LC analyses were performed using an Agilent HPLC model 1200 coupled a 6540 Agilent Ultra-High-Definition (UHD) quadrupole-time-of-flight (QTOF) mass analyzer (Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer was equipped with in Agilent Dual Jet Stream electrospray ionization (EIS) source. A Zorbax Eclipse plus C18 column (1.8µm, 150mm×4.6 mm) (Agilent Technologies, Palo Alto, CA, USA) was used and using the following solvents: Solvent A 0.1% formic acid H_2O, Solvent B 0.1% formic acid acetonitrile. The gradient used was 5% B for 5 min, 5-100% for 20 min, and 100% for 7 min. The flow rate used was 0.150 mL/min and the column temperature at 45°C. Internal mass correction of each sample was performed with a continuous infusion of Agilent TOF mixture containing trifluroacetic acid ammonium salt and hexakis (1H,1H,3H-tetrafluoropropoxy) phosphazine. The mass scan between m/z 70 and 1100, the acquisition mode was using Auto MS2 and in the profile mode *via* the Agilent Mass Hunter Workstation B.05.01. Mass Hunter Qualitative Analysis B.06.00 software (Agilent Technologies) was employed for data analysis.

Butyrylcholinesterase inhibition

The inhibition of butyrylcholinesterase (BChE) was evaluated using Ellman et al. 1961 method. A mixture of 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of extract solution at different concentrations, and 20 µL of BChE solution (6.85_10 − 3 U) was incubated at 25 ^{0}C for 15 minutes. Then, 10 µL of DTNB (0.5 mM) and 10 µL of butyrylthiocholine chloride (0.2 mM) solution were added to the incubated mixture. The absorbance was measured at 412 nm at 0 and 15 minutes using a PerkinElmer Multimode Plate Reader EnSpire in a 96-well microplate format. Galantamine was used as positive control. The percentage of BChE enzyme inhibition was determined in relation to the blank by the formula (E - S)/E 100 (E: The activity of the enzyme without extract/ S: The activity of the enzyme with extract).

α-Amylase inhibition

The inhibitory activity of α-amylase was evaluated using Zengin et al 2014 method. In a 96-well microplate, 25 µL of extract solution was added to 50 µL of α-amylase solution (1U prepared in phosphate buffer with 6 mM NaCl) and incubated at 37°C. After 10 minutes of preincubation, 50 µL of a 0.1% starch solution was added. The reaction mixture was incubated at 37°C for 10 minutes and then stopped by adding 25 µL of HCl (1 M), followed by 100 µL of iodine-potassium iodide solution. The absorbance was measured at 630 nm. Acarbose was used as a standard α-amylase enzyme inhibitor. Inhibition activity was determined against a blank containing a sample without the enzyme.

Antimicrobial activity

The antimicrobial activity was evaluated using standard bacterial strains, including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538P, *Salmonella enterica* ATCC 13076, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC 13883, *Enterococcus faecalis* ATCC 19433, and *Pseudomonas aeruginosa* ATCC 27853, as well as a yeast strain, *Candida albicans* ATCC 1031. Materials and reagents used in the determination of antimicrobial activity included Ciprofloxacin (HiMEDIA), Muller Hinton Agar Medium, Dimethyl sulfoxide (DMSO) (Sigma-Aldrich D8418), a 96-well microplate (Greiner, VWR), an incubator (Memmert BE500), and a spectrophotometer (JENWAY 6300, UK).

Well diffusion method

Microorganism inoculums were standardizer to a turbidity equivalent to that of 0.5 McFarland standard (10^{8} UFC/mL for bacterial cells or 10^{7} yeasts). A volume of microbial inoculum was spread over the surface of the nutritive medium (HM), and then a well with a diameter of 6 nm was made aseptically using a sterile rod. Finally, 60 µL of the antimicrobial agent (*n*-butanol extract 100 mg/mL) to be tested was added to the well. The inhibition diameter was measured after 24 h for bacteria and 48 h for yeast at 37°C (Hsouna et al. 2011).

Determination of the MICs

The Minimum Inhibitory Concentrations (MICs) of the extract and reference (Ciprofloxacin) were determined using the Broth Microdilution Techniques (Gulluce et al. 2007). Extract concentration ranging from 1000 µg to 7.81 µg/mL were prepared with 90 µL of the nutritive medium (HM) in each well, and then 20 µL of bacterial suspension was added. The mixture was incubated for 24 h at 37°C for bacteria and 48 h at 37°C for yeast, with each test carried out in triplicate. The positive control was a mixture of Mueller–Hinton Broth, Ciprofloxacin, and 20 µL of bacterial suspension. Two negative controls were prepared: the first was a mixture of Mueller–Hinton Broth and 20 µL of bacterial suspension, and the second was the Mueller–Hinton Broth only. After incubation, 20 µL of 0.01% resazurin was added to each well and the microtiter plate was left to incubate for additional 4 hours. The indicator solution changes from blue to pink in the presence of bacterial activity; the MIC was defined as the lowest concentration of extract that remained blue.

Larviscidal effect

The larviscidal effect was tested on mealworms by caudally direct injection of 3 µL of extract solution at different concentration (16, 8, 4 and 2 mg/ mL) in the ventral side of the larvae (*Tenebrio molitor)*, lateral to the midline. Batches of larvae were incubated at room temperature for 7 days (each batch contains fifteen larvae that have the same weight 150 mg) for each concentration). Mortality was evaluated by the discoloration of the larvae (Van der Valk and Van der Meijden 2014).

Statistical analysis

Results were expressed as mean ± SD. All experiments were performed in triplicate.

Results and Discussion

Polyphenolic profile

Thirty-one flavonoids, including flavones, flavanols, flavanone, anthocyanidin and isoflavone, were identified in the *n*-butanol extract of *Linum tryginum* (BELTr), by the use of RP–UHPLC–ESI–QTOF–MS technique (Fig. 1, Table 1) with orientin isomer1 (**22**), isovitexin (**30**), orientin isomer 2 (**25**) and vicenin 2-isomer 4 (**19**), as the major compounds, accounting for 19.22%, 17.58%, 11.28% and 9.017%, respectively. Additionally, malvidin 3-*O*-β-D-glucoside (**41**) and 8-*C*-disaccharide genistein (**27**) were found to be important constituents, representing 4.09% and 4.29% respectively.. Furthermore, the extract contained twelve quinic acids and phenylethanoid derivatives, along with two lignans (**12** and **13**) and a coumarin derivative (**43**). Feruloyltyramine, 8-*C*-Disaccharide genistein, malvidin 3-*O*-glucoside and 5-*O*-β-D-glucoside-7-methoxy-3',4'-dihydroxy-4-phenylcoumarin were detected as important constituents for the first time in *Linum* genus. In our first previous report, we showed that the ethyl acetate extracts of *Linum trigynum* L. and *Linum numidicum* Murb. were rich in flavones *C*-glycosides (mainly homoorientin and cirsiliol for *L. trigynum* extract and vicenin-2 isomer 1 and vitexin for *L. numidicum*) and aglycons (tetrahydroxyflavone in the two extracts). Chicoric acid was the major phenolic acid in ethyl acetate extract of *L. numidicum* while 4-caffeoylquinic acid and chlorogenic acid were more abundant in *L.*

trigynum extract. A few number of lignans was also detected in the two extracts (Mouna et al. 2022). In the second report, which was conducted on the phytochemical composition of VLC fractions of *L. numidicum n*-butanol extract, the results revealed that flavone *C*-glycosides (vicenin-2 isomers, isovitexin, orientin isomer 1, homoorientin, vitexin, malvidin 3-*O*-galactoside, genkwanin and luteolin 7-*O*-β-D-glucoside) were the major class of compounds in addition of feruloyltyramine, which was the major compound in the two VLC fractions. Only three lignans were detected (lanicepside B, olivil 4'-*O*-glucoside and podophyllotoxin-β-D-glucoside) (Mouna et al. 2023a). Morover, LC-MS/MS analysis of aqueous and ethanolic extracts of flaxseed shell showed the main presence of phenolic acids (*p*-hydroxybenzoic acid, vanillin, *p*-coumaric acid, ascorbic acid, ferulic acid and ellagic acid) (Han et al. 2018). The richness in *C*-glycosides flavones was confirmed by Tchoumtchoua et al. who separed vitexin, isovitexin, orientin, isoorientin, vicenin-1, vicenin-2 (Tchoumtchoua et al. 2019).

Table 1

Polyphenols characterized by RP-HPLC–ESI-QTOF-MS analyses of BELTr[a]

Peak	Compounds	Rt[b]	*m/z*	Intensity peak area (%)	Major fragments m/z (intensity %)
1	Chlorogenic acid	18.02	355.1008	123 507 597 (2.36)	163.0 (100) 162.0 (1.98)
2	Caffeoylquinic acid	18.40	353.0862	94 520 491 (1.81)	193.100 160.01
3	Vicenin 2-isomer1	18.94	595.1646	1 072 864 (0.02)	383.5 (100), 503.2 (100) 473.3 (100), 353.7 (100)
4	Feruloyltyramine	19.07	265.1540	186925 826 (3.57)	314.1364 (99.9), 315.1383 (21.9), 336.1194 (18.7), 337.1166 (29), 316.1437 (23)
5	3-*O*-coumaroylquinic acid isomer 1	19.17	339.1057	1 764 380 (0.034)	163.039246 (110), 119.050407 (37), 191.057541 (8), 221.221786 (4)
6	Methyl chlorogenate isomer1	20.30	369.1171	44 160 745 (0.84)	177.0536 (100), 117.0327 (94.36), 145.0277 (70.13), 149.0594 (54.84), 134.0351 (21.59)
7	4-caffeoylquinic acid	20.40	353.0863	52 248 621 (0.99)	163.038132 (100), 164.044006 (11.58), 355.09799 (1.08)
8	7,2'-Dihydroxyflavone	20.50	253.0708	106 129 (1.85)	117.0350 (100), 135.0083 (7.72), 129.0299 (5.56), 242.0195 (5.30), 133.0310 (5.22)
9	Vicenin 2-isomer2	20.70	593.1488	2 135 910 (0.04)	383.5 (100), 503.2 (100) 473.3 (100), 353.7 (100)
10	3-Feruloylquinic acid	21.00	367.1017	118751307 (2.27)	163.0389 (100), 324.3259 (95.07), 145.0284 (79.45), 368.4248 (63.35), 177.0546 (51.73)

[a]BELTr : *n*-Butanol Extract of *Linum trigynum;* [b]Rt: Retention Time

Peak	Compounds	Rt[b]	*m/z*	Intensity peak area (%)	Major fragments m/z (intensity %)
11	6,8-di-*C*-glucosyl naringenin	21.20	595.1656	21 990 168 (0.42)	385.0889 (69.08), 355.0808(67.50) 475.1242 (38.18) 313.0695(16.66)
12	Lanicepside B	21.36	561.1933	3 576 895 (0.067)	358 (57), 328 (8), 297 (6), 206 (98), 188 (55), 175 (100), 151 (54), 137 (58), 131 (19), 124 (18), 115(14), 91 (10), 73 (11), 60 (20).
13	Olivil-4'-*O*-β-D-glucoside	21.50	583.2010	977 240 (0.01)	375.1(78), 360.1(36), 345.1, 327.1(44), 309.1 (8)
14	Vicenin-2 isomer 3	21.60	593.1488	25 498 132 (0.48)	383.5 (100), 503.2 (100) 473.3 (100), 353.7 (100)
15	5,3'-dihydroxyflavone	21.70	253.0708	535 809 (0.01)	255.0639 (99.9), 176.9873 (18.4), 137.0237 (23), 177.0158 (8), 177.0318 (7)
16	Coumaroyl-fructofuranosyl-glucoside	21.80	487.1436	4 871 436 (0.09)	144.8436 (52), 307.1420(48.8)
17	Luteolin-7,3'-di-*O*-β-D-glucoside	22.10	609.1438	6 033 541 (0.12)	609.1514 (99.9), 447.0971 695, 610.1549 (27.8), 448.1015 (13.6), 285.0424 (51)
18	3-*O*-Coumaroylquinic acid isomer 2	22.10	337.0916	1 257 786 (0.024)	163.039246 (110), 119.050407 (37), 191.057541 (8), 221.221786 (4)
19	Vicenin-2 isomer 4	22.40	593.1488	471675740 (9.017)	383.5 (100), 503.2 (100) 473.3 (100), 353.7 (100)

[a]BELTr : *n*-Butanol Extract of *Linum trigynum;* [b]Rt: Retention Time

Peak	Compounds	Rt[b]	*m/z*	Intensity peak area (%)	Major fragments m/z (intensity %)
20	Methyl chlorogenate isomer 2	22.44	369.1171	43 758 833 (0.84)	177.0536 (100), 117.0327 (94.36), 145.0277 (70.13), 149.0594 (54.84), 134.0351 (21.59)
21	3-O-Feruloylquinic acid isomer 2	22.50	367.1020	1 468 310 (0.028)	163.0389 (100), 324.3259 (95.07), 145.0284 (79.45), 368.4248 (63.35), 177.0546 (51.73)
22	Orientin isomer 1	22.85	449.1066	1005174319 (19.22)	447.0931 (99.9), 445.0777 (84.8), 895.1959 (40.8), 893.181 (23.0), 448.0962 (18.6)
23	Homoorientin	23.00	447.0912	99 946 322 (1.91)	329.0663 (100), 299.0555 (85.52), 339.087 (20.53), 300.0601 (20.38), 353.0663 (20.37)
24	Quercetin-3-methoxy-3`-*O*-glucoside isomer 1	23.05	479.1173	981352 (0.019)	332.03 (100) 366.8458(7.92)
25	Orientin isomer 2	23.20	447.0914	589852465 (11.28)	447.0931 (99.9), 445.0777 (84.8), 895.1959 (40.8), 893.181 (23.0), 448.0962 (18.6)
26	Tricin-5- *O*-*β*-D - Glucoside	23.45	493.1326	2 817 350 (0.05)	491.1205 (99.9), 492.1233 (28.7), 983.2467 (26.7), 984.2501 (14.4), 490.1116 (10.3)
27	8-C-Disaccharide genistein	23.53	565.1543	224 195 406 (4.29)	432.34 270.241
28	4,5-Dicaffeoylquinic acid isomer 1	23.70	515.1211	1 157 371 (0.022)	163.0418 (99.66), 499.1293 (5.66), 145.0305 (2.66), 337.0962 (2.33), 319.0909 (6)
29	Methyl chlorogenate isomer 3	23.80	367.1020	136 168 592 (2.60)	177.0536 (100), 117.0327 (94.36), 145.0277 (70.13), 149.0594 (54.84), 134.0351 (21.59)

[a]BELTr : *n*-Butanol Extract of *Linum trigynum;* [b]Rt: Retention Time

Peak	Compounds	Rt[b]	*m/z*	Intensity peak area (%)	Major fragments m/z (intensity %)
30	Isovitexin	24.10	433.1117	919460511 (17.58)	433.1133 (99.9), 434.1178 (31.7), 415.1024 (98), 435.12 (65), 313.0705 (31)
31	Isovitexin 2"-*O*-Arabinoside	24.20	563.1388	57 995 431 (1.11)	283.0586 (100), 313.0683 (82.44), 337.0703 (34.72), 309.0693 (20.01), 349.0706 (17.69)
32	Vitexin	24.20	431.0966	1 725 028 (0.03)	433.1134 (19.32), 313.0744 (16.75), 283.0638)(13.39), 284.0718 (8.20), 415.1061 (6.56)
33	kaempferol-7-O-hexoside	24.40	447.0915	8 561 729 (0.16)	447.0934 (100), 285.0398 (44.46), 448.0973 (21.20), 284.0323 (8.25), 286.0429 (7.24)
34	Vitexin-2"-rhamnoside	24.50	577.1544	10 746 057 (0.21)	577.1559 (100), 298.0451 (17.17), 327.0591 (9.29), 576.1379 (7.88), 473.1141(6.26)
35	Hyperoside	24.50	463.0864	175 164 (0.003)	399.0713 25.68, 447.0928 24.94, 369.0605 19.74, 345.0604 12.41, 429.0826 6.45
36	Rutin	25.00	609.1437	80 675 226 (1.54)	611.0 (100), 610.0 (23.75), 303.0 (17.17), 302.0 (12.51), 612.0 (9.16)
37	Violanthin	25.00	577.1542	6 196 120 (0.12)	353.0665 (100), 383.0763 (72.63), 413.0853 (21.47), 457.1133 (13.83), 325.0714 (12.45)
38	Spiraeoside	25.20	463.0858	84 440 584 (1.61)	303.0534 (100), 153.0207 (53), 257.0449 (50.60), 229.0497 (48.50), 201.0535 (24.80)
39	Luteolin	25.37	287.0544	2 648 849 (0.05)	153.0182 (65.44), 241.0497 (17.70), 245.0497 (16.86)

[a]BELTr : *n*-Butanol Extract of *Linum trigynum;* [b]Rt: Retention Time

Peak	Compounds	Rt[b]	*m/z*	Intensity peak area (%)	Major fragments m/z (intensity %)
40	Rosmarinic acid	25.40	359.0753	74 379 (0.0014)	161.0257 (100), 133.0295 (93.60), 135.0453 (88.60), 72.9923 (69.40), 123.0455 (37.50)
41	Malvidin 3-*O*-β-D-galactoside	25.44	493.1326	214198979 (4.095)	331.0812(100) 333.0969(37.80) 475.1236(69.41)
42	Quercetin-3-methoxy-3'-*O*-β-D-glucoside isomer 2	25.50	477.1020	24 717 242 (0.47)	332.03 (100) 366.8458(7.92)
43	5-*O*-β-D-glucopyranosyl-7-methoxy-3',4'-dihydroxy-4-phenylcoumarin	25.51	463.1225	225569645 (4.31)	222.24(100), 432.0283(56), 429.1134(17), 191.1217(8.1), 188.1021(5)
44	Afzelin	25.52	433.1119	30 742 554 (0.59)	287.055 (100), 71.0499 (36.94), 85.0291 (25.73), 57.0344 (8.34), 153.0174 (5.49)
45	Luteolin-7-*O*- β -D-glucoside	25.74	449.1066	119703204 (2.29)	287.0556(100) 431.0978(47.53), 269.0450(40.45), 163.0606(8.89)
46	Quercitrin	25.90	447.0913	26984954 (0.515)	413.0872 (38.40), 303.0498 (22.53), 431.0976 (20.21), 345.0604 (14.33), 369.0601 (4.53)
47	Isorhamnetin 3-*O*-β-D-glucoside	26.00	477.1019	6 039 717 (0.11)	314.0432(100), 315.0510(49.00), 255.0299(17.00) ,285.0405(14.00)
48	8,3'4'-trihydroxyflavone-7-*O*-6(6"-*O*-*p*-coumaroyl-β-D-gluoside	26.30	593.1487	14 980 326 (2.86)	270.246(100), 448.8187(100)
49	3-*p*-Coumaroylquinic acid isomer3	26.30	337.0917	18 536 319 (0.35)	163.039246 (110), 119.050407 (37),

[a]BELTr : *n*-Butanol Extract of *Linum trigynum;* [b]Rt: Retention Time

Peak	Compounds	Rt[b]	*m/z*	Intensity peak area (%)	Major fragments m/z (intensity %)
					191.057541 (8), 221.221786 (4)
50	Genkwanin	26.59	285.0752	427 746 (0.008)	283.0592 (100), 284.0623 (12.71), 268.0361(2.30), 285.0626 (1.80), 269.0339 (1.30)
51	Cirsiliol	26.60	331.0804	16 997 315 (0.32)	331.087 (99.9), 332.0844 (26.4), 353.0698 (9.8), 333.0834 (4.9), 683.1542 (3.5)
52	Tricin 5-glucoside isomer 2	26.90	491.1174	5 133 191 (0.098)	491.1205 (99.9), 492.1233 (28.7), 983.2467 (26.7), 984.2501 (14.4), 490.1116 (10.3)
53	Quercetin-3-methoxy-3'-*O*-β-D glucoside isomer 3	27.40	477.1016	3 021 362 (0.058)	332.03 (100) 366.8458(7.92)
54	Pectolinarin	28.21	623.1957	150947696 (2.88)	298.0527 (100), 313.0549 (45.89), 314.0704 (32.28), 148.9620 (32.04), 497.1583 (30.48)
55	Tetrahydroxy-Flavone	28.40	285.0394	81 881 (0.0015)	255.0371(100), 285.0482(23.92), 256.0398(13.51), 133.0331(8.01)
56	6,4'-dimethoxy scutellarein-7-neohesperidiside	29.20	667.1756	7 977 542 (0.15)	340.8786(80), 285.0414(67.47) 117.0352(10), 326,297(8.04)

[a]BELTr : *n*-Butanol Extract of *Linum trigynum;* [b]Rt: Retention Time

Butyrylcholinesterase Inhibition

Existing treatments for Alzheimer's disease (AD) target both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) to enhance choline transmission and improve patient's cognitive abilities. The anti-Alzheimer activity of the BELTr was tested against BChE. A significant activity was observed (IC_{50}:112.45 ± 3.93 µg/mL) (Table 2), compared to the standard Galantamine (IC_{50}:35.04 ± 1.28 µg/mL). The inhibitory activity of the BELTr against BChE is reported here for the first time for the species.This activity can be attributed to the polyphenols. In establishing a structure-activity relationship for flavonoids, previous research by Conforti et al. 2009 demonstrated that flavones *C*-glycosides exhibit

good inhibitory potential against AChE and BchE, compared to other types of flavonoids. This type of structure represents more than 50% of the polyphenolic composition of the BELtr, particularly isovitexin (17.58%), which has shown a higher activity than the standard the standard quercetin (Choi et al. 2014). Molecular docking studies have also shown that orientin exhibited a lower binding energy with AChE compared to the standard drug Donepezil, suggesting its potential as a favored compound for AChE inhibition (Qader et al. 2020).

Table 2
αAmylase and butyrylcholinesterase (BChE) inhibitory activities of BELTr[a]

Extract/standards	Inhibition of BChE	Inhibition of α-amylase
	IC_{50} (µg/mL)	
BELTr	112.45 ± 3.93	2255.84 ± 4.05
Galantamine[b]	35.04 ± 1.28	-
Acarbose[b]	-	3650 ± 10.70
[a]BELTr : *n*-Butanol Extract of *Linum trigynum*,[b]: Standards		

α-Amylase Inhibition

α-Amylase is an enzyme of critical importance due to its starch hydrolysis activity. It was suggested that inhibition of this enzyme can efficiently control the postprandial hyperglycemia. In this study, the BELTr was assessed for its in vitro α-amylase inhibitory effect, for the first time. It exhibited a good inhibitory activity (IC_{50}:2.25 ± 4.05 mg/mL) which was higher than that of the standard Acarbose (IC_{50}:3.65 ± 10.70 mg/mL) (Table 2). This may be due to the presence of the polyphenols, particularly the major compounds, orientin isomer 1 and 2, isovitexin and vicenin 2, according to Proença et al. who demonstrated that the flavones possessing a C2 = C3 double bond of C-ring and OH group at the 3'- and 4'-positions of B-ring and at 5- and 7- positions of the A-ring, exhibit the highest α-amylase inhibitory activity through competitive inhibition (Proença et al. 2019). Flavone *C*-glycosides have been found to possess better therapeutic properties than *O*-glycosylated flavones due to their increased stability and reactivity. Moreover, Yang et al. reported that flavone *C*6-glycosides (such as isovitexin) have more α-amylase inhibitory activity than flavone *C*8-glycosides (such as orientin) (Yang et al. 2014). These results are confirmed for isovitexin by Abu Bakar et al. 2018 who found that flavone *C*-glycosides are among the most active flavones againt α-amylase by forming a stable isovitexin-α-amylase complex. Thus, the α-amylase inhibitory capacity of the BELTr can be attributed to its flavone *C*-glycosides through direct and synergistic effects.

Antimicrobial Activity

The antimicrobial activity of the BELTr was evaluated against gram (+) and gram (-) bacteria namely, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538P, *Salmonella enterica* ATCC 13076, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC 13883, *Enterococcus faecalis* ATCC 19433, and *Pseudomonas aeruginosa* ATCC 27853 and a fungi strain, *Candida albicans*. The potency was determined based on the MIC values (in µg/mL) by the use of Well diffusion method and the Broth Microdilution Techniques. The BELTr exhibited a good activity against *Candida albicans* and bacteria strains namely, *S. aureus* ATCC 25923, *K. pneumoniae*, *P. aeruginosa* and *E. faecalis* with an MIC value of 250 µg/mL (Table 3). However, the inhibition of the growth of *S. aureus* ATCC 6538P, *B. subtilis E. Coli* and *S. enterica* was lower (MIC:500 µg/mL). This antimicrobial activity could be attributed to the presence of polyphenols, particularly flavonoids such as orientin, which has previously demonstrated varying levels of antibacterial activity against tested bacteria (Lin et al. 2004). The combination of orientin with vicenin 2 resulted in a synergistic effect that increased their efficiency (100%) compared to when they are used individually (Ali and Dixit 2012). Additionally, isovitexin and 4-phenylcoumarin derivative have shown good antimicrobial activity (Verotta et al. 2004).

Table 3
Antimicrobial activity of BELTr

Microorganisms	MIC (µg/mL)
Escherichia coli ATCC 25922	500
Staphylococcus aureus ATCC 25923	250
Staphylococcus aureus ATCC 6538P	500
Klebsiella pneumoniae ATCC 13883	250
Salmonella enterica ATCC 13076	500
Bacillus subtilis ATCC 6633	500
Pseudomonas aeruginosa ATCC 27853	250
Enterococcus faecalis ATCC 19433	250
Candida albicans ATCC 10231	250

Larviscidal activity

The toxicity assay revealed that the BELTr has a low larviscidal activity with a Median lethal dose LD_{50} value of 8 mg/mL (Table 4). Previous study revealed that ether extract of *Linum usitatissimum* showed moderate larviscidal activity against larvae of *Aedes albopictus* (Bilal et al. 2012). Additionaly, Ferreira et al. 2019 showed that flavonoid extract possessed moderate larviscidal activity. We can deduce from these results that larviscidal effect must be tested using different types of extracts and larvea.

Table 4
Larviscidal effect of BELTr

BELTr	% Mortality rate					
	2 mg	4 mg	8 mg	16 mg	LD_{50}[a] mg/mL	Toxicity
Tenebrio molitor larvae	0.00 ± 0.00	0.00 ± 0.00	8.00 ± 0.00	10.±1.77	8	-

[a]LD_{50}: Median Lethal Dose

Conclusion

This study aimed to to evaluate the anti-Alzheimer, anti-diabetic, antimicrobial and larviscidal activities of the *n*-butanol extract obtained from aerial parts of *L. trigynum* L. and to identify the polyphenols which may be responsible of these activities, by LC-HRMS/MS. The major chemical class of identified polyphenols was favone *C*-glycosides, which account for more than 50% of the extract including, orientin isomer 1, isovitexin, orientin isomer 2 and vicenin 2-isomer 4. The extract exhibited an excellent α-amylase inhibitory activity which was higher than the used standard and significant anti-Alzheimer, antimicrobial and larviscidal activities.

Declarations

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Author contributions

SM conducted the experiments and manuscript writing. AK and ZK supervised the project, conceived the study and participated in the manuscript writing. DG performed the antimicrobial activity. All authors read and approved the final manuscript

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Availability of data and materials All data generated or analyzed during this study are included in this published article.

Conflict of interest The authors declare that there is no confict of interest

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

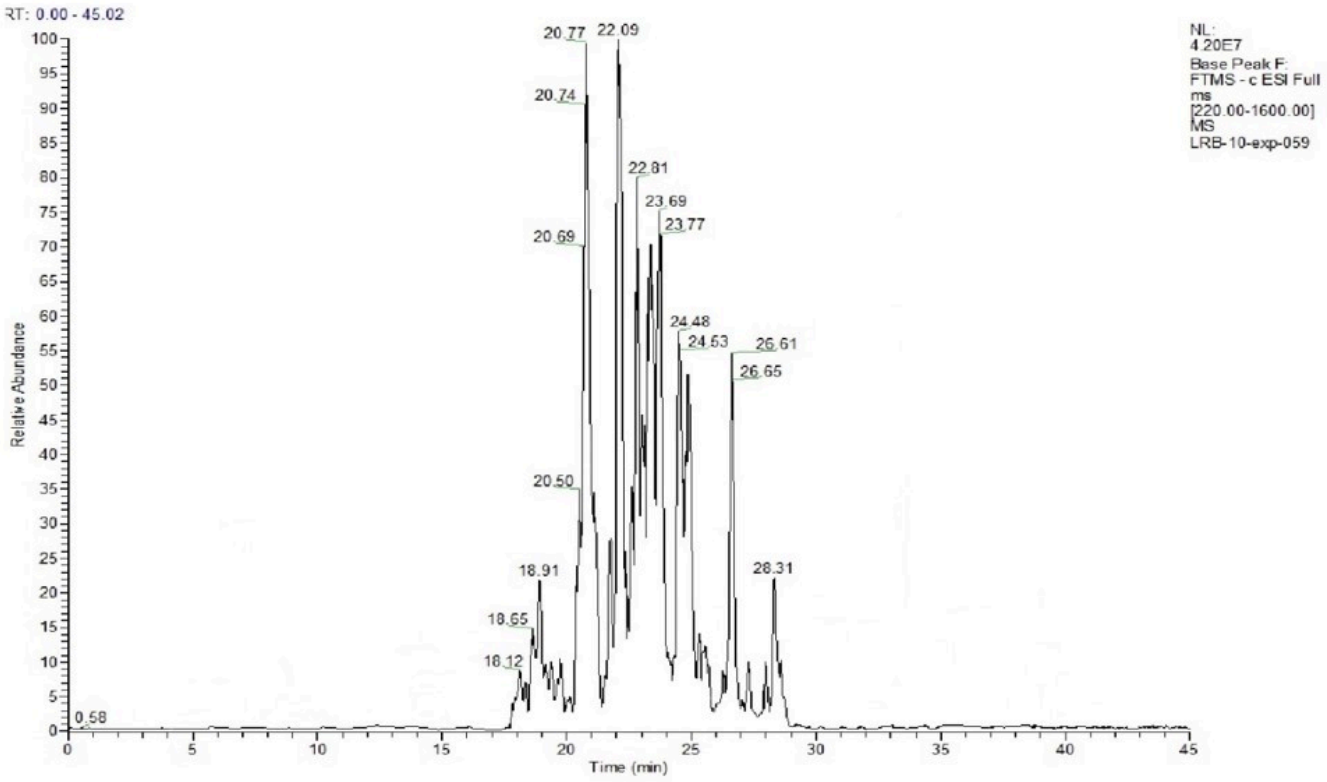

Figure 1

Base peak chromatogram of BELTr by RP-HPLC–ESI-QTOF-MS