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Article

Compounds from *Terminalia mantaly* L. (Combretaceae) Stem Bark Exhibit Potent Inhibition against some Pathogenic Yeasts and Enzymes of Metabolic Significance

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Abstract: The chemical investigation of the anti-yeast methanol extract from the stem bark of *Terminalia mantaly* led to the isolation of seven compounds: 3-*O*-methyl-4-*O*-*α*-rhamnopyranoside ellagic acid (1), 3-*O*-mehylellagic acid (2), arjungenin or 2,3,19,23-tetrahydroxyolean-12-en-28-oïc acid (3), arjunglucoside or 2,3,19,23-tetrahydroxyolean-12-en-28-oïc acid glucopyranoside (4), 2*α*,3*α*,24-trihydroxyolean-11,13(18)-dien-28-oïc acid (5), stigmasterol (6), stigmasterol 3-*O*-*β*-D-glucopyranoside (7). Their structures were established by means of spectroscopic analysis and comparison with published data. Compounds 1-5 were

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tested *in vitro* for activity against three pathogenic yeast isolates, *Candida albicans*, *Candida parapsilosis* and *Candida krusei.* The activity of compounds 1, 2 and 4 were comparable to that of the reference compound fluconazole (MIC values below 32 µg/ml) against the three tested yeast isolates. They were also tested for inhibitory properties against four enzymes of metabolic significance: Glucose-6-Phosphate Deshydrogenase (G6PD), human erythrocyte Carbonic anhydrase I and II (hCA I and hCA II), Glutathione S-transferase (GST). Compound 4 showed highly potent inhibitory property against the four tested enzymes with overall IC_{50} values below 4 µM and inhibitory constant (Ki) <3 µM.

Keywords: Anti-yeast; Enzyme inhibitors; *Terminalia mantaly*; Combretaceae

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1. Introduction

Fungal diseases affect every year 3-4 million people worldwide. Of particular importance, the increasing resistance of pathogenic opportunistic yeasts to current drugs is a serious concern and has attracted the attention of the scientific community. New, safe, and cost-effective drugs of natural or synthetic origin are therefore actively being searched [1]. Recent epidemiological data highlight the increasing burden of pathogenic yeasts on people in poor settings [2,3,4]. *Candida* species *and Cryptococcus neoformans* are the major pathogenic yeasts and only few antifungal drugs have been developed so far to treat the invasive infections they cause [5,6]. Medicinal plants have shown credibility as sources of treatment for infectious diseases [7]. In Cameroon, extracts from medicinal plants such as *Terminalia mantaly* (Combretaceae) are widely used by traditional healers to control diverse infections or associated symptoms, including but not limited to dysentery, gastroenteritis, hypertension, diabetes, and oral, dental, cutaneous and genital affections [8]. Previous studies on the extracts of this plant have indicated antibacterial and antifungal properties, but their chemical compositions have not yet been determined. However, phytochemical studies of other species of the genus *Terminalia* have reported the presence of flavonoids, terpenoids and their glycosides derivatives, tannins, flavonones and chalcones [9-18]. Besides, in spite of the work done on *Terminalia* species, no investigation has been attempted yet on the enzyme inhibition properties of their extracts and constituents targeting Glucose-6-phosphate dehydrogenase, Carbonic anhydrase and Glutathione S-transferase.

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Glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49) enzyme catalyzes the reaction of glucose-6-phosphate into phosphogluconate, which is the first step of rate-limiting of pentose phosphate pathway. The end products of this pathway are ribose-5-phosphate and NADPH. Ribose-5-phosphate is used in DNA or RNA synthesis in cell reproduction, and NADPH is used as coenzyme for the enzymes participating in the production of reduced glutathione. Given its role in cell growth, this enzyme is of high importance to mammal cells [19,20]. However, several studies have shown that this enzyme takes an important role in the pathology of some diseases like cancer, hypertension, heart failure and type 2 diabetes. G6PD activity increases in cancer cells and its inhibition results in decrease of cell proliferation and induction of apoptosis. For example, 6-aminonicotinamide which is an inhibitor of G6PD has found use in the therapy of various tumors in the past [21].

Carbonic anhydrase (CA; carbonate hydro-lyase, EC 4.2.1.1) enzyme exists commonly in living organisms, and has various isoenzymes according to conditions and necessities of the medium. It is one of the most studied enzymes and CA-I and CA-II are the most common isoenzymes [22]. In many physiological and pathological processes, CAs catalyze the conversion of CO_2 to HCO_3^- and H^+. In addition, CA inhibitors may be used in the treatment of various diseases such as oedema, glaucoma, obesity, cancer, epilepsy and osteoporosis [23].

In living cells, the deleterious effects of free radicals and their intermediates are eliminated or minimized by various enzymatic and non-enzymatic defense systems. Enzymatic defense is provided by several enzymes such as glutathione S-transferase (GST), glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase [24]. The GSTs (EC 2.5.1.18) are a group of multifunctional enzymes that play an important role in the metabolism [25]. GSTs are important for the fight against cancer because of their interactions with carcinogens and chemotherapeutic agents. They are the target of antiasthmatic and antitumor drugs [26]. Production of excessive amounts of GST in mammalian tumor cells leads to resistance to some anticancer drugs and chemical carcinogens [27].

The reduction of drugs effects in tumor cells is an important factor limiting the therapeutic efficacy of an antineoplastic agent. The development of this resistance was associated with glutathione (GSH) and glutathione S-transferase (GST) levels in cells and changes in permeability to the drug. In this regards, G6PD, CA I, II or GST inhibitors may be useful because of their several applications, in particular for the treatment of glaucoma, epilepsy, cancer and as diuretics.

In our search for bioactive secondary metabolites from Cameroonian medicinal plants, we have investigated the MeOH extract of the stem bark of *Terminalia mantaly* L.

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(Combretaceae) that previously showed anti-yeast activity. We report in this paper the inhibitory potential of compounds isolated from this extract against some pathogenic yeasts and some enzymes of metabolic significance.

2. Materials and methods.

2.1. General Experimental Procedures

Optical rotations were measured on a JASCO digital polarimeter (model DIP-3600). UV spectra were determined on a Spectronic Unicam spectrophotometer. IR spectra were determined on a JASCO Fourier transform IR-420 spectrometer. ^{1}H and ^{13}C NMR spectra were run on a Bruker spectrometer equipped with 5 mm ^{1}H and ^{13}C probes operating at 500 and 125 MHz, respectively, with TMS as internal standard. Silica gel 230-400 mesh (Merck) and silica gel 70-230 mesh (Merck) were used for flash and column chromatography while precoated aluminum backed silica gel 60 F254 sheets were used for TLC. Spots were visualized under UV light (254 and 365 nm) or using MeOH-H_2SO_4 reagent.

2.2. Plant Material

The stem bark of *Terminalia mantaly* (Combretaceae) was collected in Yaoundé in May 2012 and identified at the Cameroon National Herbarium where a voucher specimen is deposited under the reference N° 64212/HNC (*Terminalia mantaly* H. Perrier).

2.3. Microbial isolates

Yeast isolates were generously provided by the Laboratory of Clinical Biology, Yaoundé Central Hospital and consisted of clinical isolates of *Candida albicans*, *Candida krusei* and *Candida parapsilosis.* These yeasts were maintained at room temperature and cultured at 37 °C for 24 hours on Sabouraud Dextrose Agar (Oxoid) slants prior to use.

2.4. Plant extraction and screening of anti-yeast activity

The harvested *T. mantaly* stem bark was dried at room temperature and ground using a blender. The powdered stem bark (7 kg) was extracted at r.t. with MeOH (48 h). The extract was concentrated under vacuum to afford a dark residue (250 g). Minimal Inhibitory Concentration (MIC) of the extract was determined according to the CLSI M27-A3 [6] protocol with little modifications. The RPMI 1640 supplemented with 2% glucose was used as culture medium. Briefly for the fungal susceptibility tests, 50 µL of serially 2-fold diluted concentrations of the crude extract were added in triplicate wells of a 96-wells microtiter plate.

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Fifty µL of fungal inocula standardized to a final concentration of 0.5-2.5x10^3 CFU/mL were then individually added in each well of the plate. Plant crude extract and the positive control (fluconazole) at concentrations of 0.12 to 64 µg/mL were tested in a final volume of 100 µL. So-prepared plates were incubated at 37 °C for 48 hours. MIC value was subsequently determined through macroscopic observation of plate wells, and was defined as the lowest concentration of the inhibitor that allowed no visible growth of the microorganism.

2.5. Isolation of compounds and screening for activity

A portion of 180 g of the extract was subjected to medium pressure flash chromatography over silica gel (Merck, 70 – 230 mesh) using mixtures *n*-hexane-EtOAc of increasing polarity [(70:30) – (0:100)] and EtOAc-MeOH [(95:5) – (50:50)], resulting in the collection of 75 fractions of 500 mL each, which were combined on the basis of TLC analysis to afforded 4 fractions labeled T_1-T_4. Fraction T_1 (m = 14,4 g) obtained from the mixtures of *n*-hexane-EtOAc (100:0 to 70:30) was subjected to silica gel column chromatography eluted with *n*-hexane-EtOAc and yielded oils, stigmasterol (**6**, 23 mg) and arjungenin (**3**, 7 mg). From fraction T_2 (m = 60,3 g), eluted with *n*-hexane-EtOAc [(50:50) – (25:75)], stigmasterol 3-*O*-*β*-D-glucopyranoside (**7**, 12 mg), arjungenin (**3**, 17 mg), 2*α*,3*α*,24-trihydroxyolean-11,13(18)-dien-28-oic acid (**5**, 5.0 mg) and arjunglucoside (**4**, 6 mg) were isolated. Column chromatography of fraction T_3 (m= 55.0 g) on silica gel and eluted with the mixtures of EtOAc-MeOH [(100:0) – (85:15)], yielded 3-*O*- methylellagic acid 4′-*O*-*α*-rhamnopyranoside (**1**, 32 mg), arjungenin (**3**, 12.0 mg), arjunglucoside (**4**, 3,5mg), 2*α*,3*α*,24-trihydroxyolean-11,13(18)-dien-28-oic acid (**5**, 3.5mg) and a dark mixture that was subjected to column chromatography on Sephadex LH-20 with MeOH as isocratic eluent and yielded 3-*O*-methyl ellagic acid (**2**, 12,5 mg). Fraction T_4 (m= 81.8 g) obtained with the mixtures of EtOAc-MeOH (85:15 to 65:35) was a complex mixture and thus were not studied. All the isolated compounds were screened as described above for anti-yeast activity, and as described below for enzyme inhibition activities.

2.6. Purification of Glucose 6-Phosphate Dehydrogenase and activity determination

G6PD was purified from the gill tissue of Lake Van fish according to Kuzu et al. [28], and the enzyme activity was determined spectrophotometrically using a Shimadzu spectrophotometer (UV-1800) at 25 °C, according to the method described by Beutler [29] and based on the principle of the reduction of $NADP^+$ to NADPH in the presence of glucose 6-phosphate and absorbance recorded at 340 nm.

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2.7. Purification of carbonic anhydrase isoenzymes by affinity chromatography and activity determination

The purification of hCA I and II isozymes was performed with a simple one step method by a Sepharose-4B anilinesulfanilamide affinity column chromatography as previously described [30].

The esterase activity was assessed following the change in absorbance of 4-nitrophenylacetate (NPA) to 4-nitrophenylate ion at 348 nm over a period of 3 min at 25 ^{o}C using a spectrophotometer (Beckman Coulter UV-VIS) according to the method described by Verpoorte et al. [31].

2.8. Purification of Glutathione -S Transferase enzyme and activity determination

Firstly, heamolysate from human erythrocytes was prepared according to the method of Hunaiti et.al. [32]. The prepared heamolysate was directly applied to the glutathione-agarose affinity column and washed with 10 mM KH_2PO_4 and 0.1 M KCl (pH 8.0). The washing procedure was monitored on a spectrophotometer through equal-to-blind absorbance values. After the column was stabilized, the enzyme was purified by gradient elution at +4°C [24, 33]. Elution solvent was prepared from a solvent gradient containing 50 mM Tris–HCl and (1.25–10 mM GSH, pH 9.5). Thereafter, 1-chloro-2,4-dinitrobenzene was used to determine GST enzyme activity. In fact the product obtained using this substrate, dinitrobenzene *S*-glutathione (DNB-SG) displays maximum absorbance at 340 nm. Activity measurements were thus carried out using the absorbance increment at this wavelength. [34].

2.9. *In vitro* inhibition and Kinetic studies

To determine the effects of compounds on enzymes, enzymes activities were measured with saturated substrate concentration and five different inhibitor concentrations. The 50% inhibitory concentrations (IC_{50}) were determined by plotting curves of % inhibition versus compound concentration. Results are reported as IC_{50} values. Ki constants were calculated using the Cheng-Prusoff equation [35].

3. Results and Discussion

The methanol extract of the stem bark of *Terminalia mantaly* was screened for anti-yeast activity *in vitro* against three clinical isolates consisting of *Candida albicans*, *Candida*

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krusei and *Candida parapsilosis*. The crude extract exhibited good activity with MIC values of 24 µg/mL against *C. parapsilosis* and 39 µg/mL against *C. albicans* and *C. krusei* (Table 1).

Table 1: Anti-yeast activity of *Terminalia mantaly* extract and isolates

	C. parapsilosis	*C. albicans*	*C. krusei*
	*MIC (µg/mL± SD)		
MeOH Extract	24.00± 0.21	39.00± 0.33	39.00± 0.30
Fraction T1	1250.00± 1.23	2500.00± 0.98	2500.00± 1.03
Fraction T2	39.00± 0.38	>5000	>5000
Fraction T3	0.16 ± 0.02	0.64 ± 0.12	0.02 ± 0.09
Fraction T4	>5000	>5000	>5000
1	39.00± 0.88 (80.4 µM)	9.70± 0.72 (20 µM)	>5 (10.30 µM)
2	78.00± 0.92 (247.6 µM)	156.00± 1.00 (495 µM)	19.50± 0.57 (61.9 µM)
3	>5000(9487 µM)	>5000 (9487 µM]	>5000 (9487 µM)
4	39.00± 0.13 (56.60 µM)	9.70± 0.36 (14.07 µM)	312.00± 1.04 (452 µM)
5	>5000 (9823 µM)	>5000 (9823 µM)	>5000 (9823 µM)
****Fluconazole**	2.00± 0.01 (6.53 µM)	8.00± 0.25 (26.14 µM)	32.00± 0.42 (10.45 µM)

*Plant extracts were tested using the CLSI M27-A3 [6] protocol. Activity was expressed as minimal inhibitory concentration at which there were no visible fungal growth; **Reference used as positive control.

The flash chromatography of the crude extract generated 4 fractions exhibiting varying antifungal activities. As shown in table 1, fraction T3 was the most active, with activity magnification over 100 times against *C. parapsilosis* and *C. krusei* (MIC: 0.16 µg/mL and 0.02 µg/mL respectively) and over 60 times against *C. albicans* (0.64 µg/mL). *C. krusei* was the most susceptible isolate to fraction T3. Compounds **1, 2, 3, 4,** and **5** that were isolated from fractions T1-T3 and were also tested for biological activity (Table 1; Figure 1). Compounds **6** and **7** were not tested due to reduced solubility in the culture medium. From the results achieved, compounds **1** and **4** showed the best potency against *C. albicans* with MIC of 9.7 µg/ml. They also moderately inhibited *C. parapsilosis* with an MIC of 39 µg/ml. Besides, compound **2** inhibited *C. krusei* with an MIC of 19.5 µg/ml.

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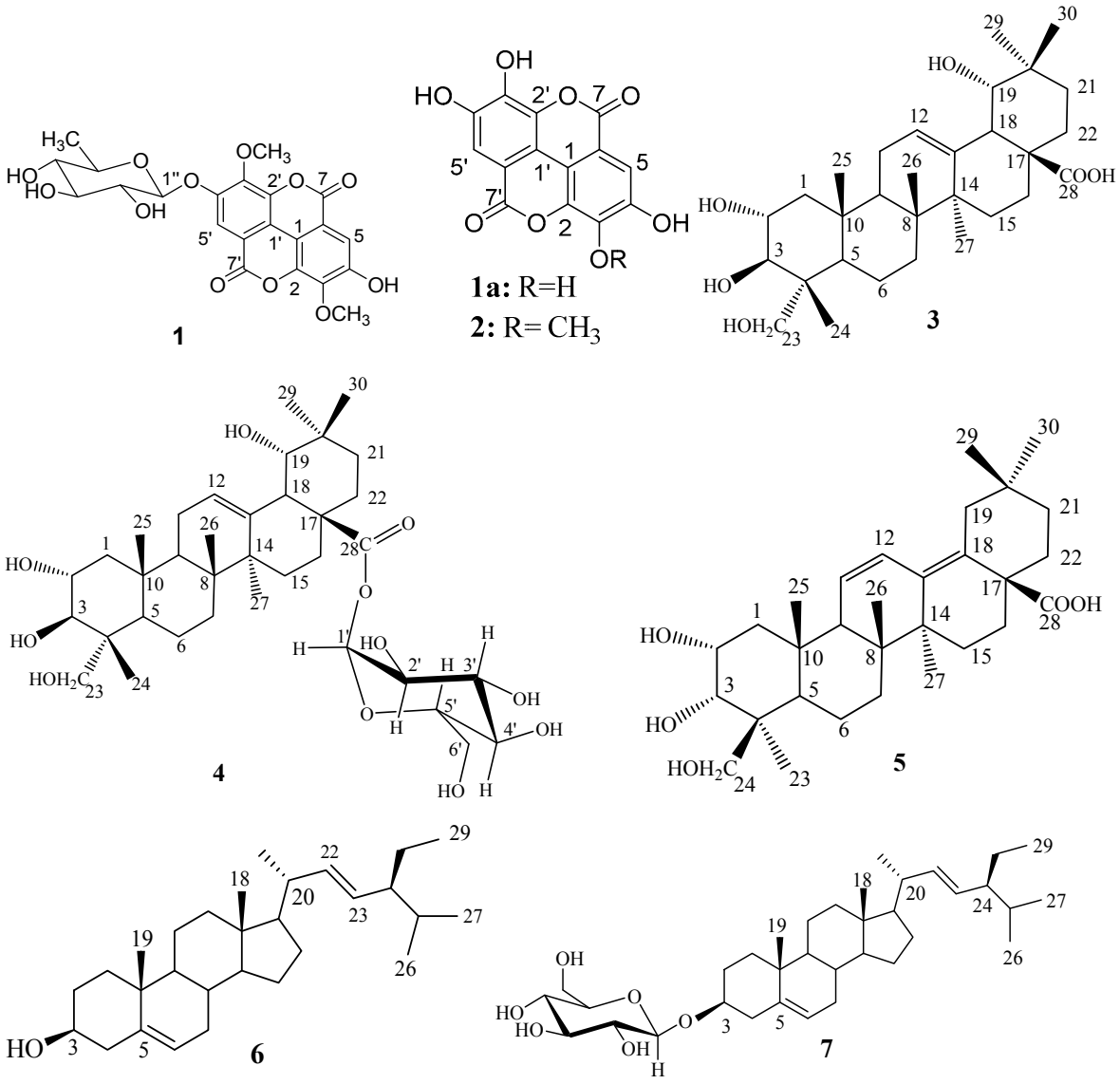

Fig.1. Structures of the isolated compounds **1-7** from *Terminalia mantaly* (Combretaceae)
The isolated compounds were tested against pathogenic yeast isolates and enzymes of metabolic significance. **1:** IC_{50}= 39 µg/mL *C. parapsilosis*; 9.7 µg/mL *C. albicans*; >5 µg/mL *C. krusei*; CAI: IC_{50}= 53.31 µM, Ki= 44.11 µM; CAII: IC_{50}= 69.11 µM, Ki= 55.78 µM; GST: IC_{50}= 63.01 µM, Ki= 42.00 µM. **2:** IC_{50}= 78 µg/mL *C. parapsilosis*; 156 µg/mL *C. albicans*; 19.5 µg/mL *C. krusei*. **3:** *C. parapsilosis*, *C. albicans*, *krusei*: IC_{50}> 5000 µg/mL; CAI: IC_{50}= 86.64 µM, Ki= 71.68 µM; GST: IC_{50}= 1.51 µM, Ki= 1.00 µM. **4:** IC_{50}= 39 µg/mL *C. parapsilosis*; 9.7 µg/mL *C. albicans*; 312 µg/mL *C. krusei*; G6PD: IC_{50}= 1.84 µM, Ki= 0.19 µM; CAI: IC_{50}= 3.28 µM, Ki= 2.72 µM; CAII: IC_{50}= 1.28 µM, Ki= 1.03 µM; GST: IC_{50}= 1.84 µM, Ki= 1.23 µM.

3. NMR spectral data of compounds 1-5

3-*O*- methylellagic acid 4′-*O*-*α*-rhamnopyranoside (1) [36]

Yellowish powder; molecular formula $C_{21}H_{18}O_{12}$; ESI-MS: $[M+Na]^+$ *m/z* 485,049 ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.13 (3H, d, CH_3, H-6″), 3.54 (1H, q, *J* = 8,0 and 12,0 Hz, H-5″), 4.01 (1H, t, H-4″), 4.04 (3H, s, OMe-3), 4.72 (1H, brd, *J* = 8,0 Hz, H-3″), 4.94 (1H, brd, *J* = 4,0 Hz, H-2″), 5.47 (1H, brs, H-1″), 7.52 (1H, s, H-5), 7.73 (1H, s, H-5′); ^{13}C NMR (125 MHz,

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DMSO-d_6), aglycone moiety: δ_C 113.4 (C-1), 140.5 (C-2), 141.8 (C-3), 153.1 (C-4), 111.9 (C-5), 113.4 (C-6), 159.1 (C-7), 114.7 (C-1′), 136.6 (C-2′), 142.2 (C-3′), 146.9 (C-4′), 112.0 (C-5′), 107.4 (C-6′), 159.1 (C-7′); rhamnose moiety: 100.5 (C-1″), 70.4 (C-2″), 70.5 (C-3″), 72.2 (C-4″), 70.3 (C-5″), 18.3 (C-6″) and 61.4 (C-3, OMe).

3-*O*-methyl ellagic acid (2) [36]

Yellowish powder; molecular formula $C_{15}H_8O_8$; ESI-MS: $[M-H]^-$ *m/z* 315,
1H NMR (DMSO-*d*6): *δ* 7.50 (1H, s,H-5), 7.44 (1H, s, H-5′), 4.02 (3H, s, 3-OMe). 13C NMR (DMSO-*d*6): *δ* 158.9 (C-7), 158.6 (C-7′), 152.2 (C-4), 148.2 (C-4′), 141.7 (C-2), 140.0 (C-3), 139.8 (C-3′), 136.1 (C-2′), 112.4(C-1′), 112.1 (C-6), 111.7 (C-1), 111.3 (C-5), 110.1 (C-5′), 107.2 (C-6′), 60.8 (3-OMe).

Arjungenin or 2,3,19,23-tetrahydroxyolean-12-en-28-oic acid (3) [37]

White powder; molecular formula $C_{30}H_{48}O_6$; ESI-MS: $[M+Na]^+$ *m/z* 527,322. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.23, 1.09, 0.90, 0.88, 0.84 and 0.65 (each 3H, s); 2.92 (1H, brs, H-18); 2.86 (1H, d, J = 8 Hz, H-3) and 3.57 (1H, m, H-2); 5.23 (1H, brs, H-12); ^{13}C NMR (125 MHz; DMSO-d_6): δ_C 16.8, 17.1, 23.9, 24.9, 28.9 and 24.5; 64.3 (C-23), 80.5 (C-3), 179.6 (C-28), 122.6 (C-12); 143.9 (C-13).

2,3,19,23-tetrahydroxyolean-12-en-28-oic acid (4) [38]

White powder; molecular formula $C_{36}H_{58}O_{11}$; ESI-MS: $[M+Na]^+$ *m/z* 689,396; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.23, 1.08, 0.89, 0.86, 0.84 and 0.63 (each 3H, s); between 2.90 and 3.80: glucose moiety with anomeric proton at 5,20 (1H, d, J = 6.9 Hz, H-1′); ^{13}C NMR (125 MHz; DMSO-d_6): *δ* 16.9, 24.5, 24.9 and 28.5; glucose moiety: 61.0, 69.9, 72.8, 77.1, 78.2, 94.5; 64.3 (C-23), 67.4 (C-2), 80.4 (C-3), 176.3 (C-28), 122.6 (C-12), 143.7 (C-13).

2*α*,3*α*,24-trihydroxyolean-11,13 (18)-dien-28-oic acid (5) [39]

Yellowish powder; molecular formula $C_{30}H_{46}O_5$; ESI-MS: $[M+Na]^+$ *m/z* 509,375 (calc. 509,324) for $C_{30}H_{46}NaO_5$); ^{1}H NMR(400 MHz; pyridin-d_5): δ 1.58; 1.06; 1.05; 1.03; 0.90 and 0.87 (each 3H, s); 6.62 (1H, d, J = 8.0 Hz, H-11) and 5.81 (1H, d, J = 8.0 Hz, H-12); 4.38 (1H, ddd, J =2.2 ;7.6 and 8.9 Hz, H-2); 3.59 (1H, d, J = 7.5 Hz, H-3); 4.43 (1H, d, J = 8.7 Hz, H-24) and 3.75 (1H, d, J = 8,76 Hz, H-24); 2.69 (1H, d, J = 12.4 Hz, H-19) and 2.15 (1H, d, J = 12.4 Hz, H-19); ^{13}C NMR (125 MHz; pyridin-d_5): δ_C 16.6, 19.5, 19.8, 23.7, 24.0 and 32.1; 65.1 (C-

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24), 68.4 (C-2), 85.5 (C-3), 178,6 (C-28) ;136,1 (C-13); 133,3 (C-18) ; 126,4 (C-12) et 125,9 (C-11).

Apart from the activity profile described above, MIC values for the other tested fractions and compounds were above 39 µg/ml. The activity of compounds **1** and **4** was comparable to that of the reference drug fuconazole against *C. albicans* and, compound **2** showed to be over 1.5 times more active than the same reference drug against *C. krusei*. Based on basic skeleton of the tested compounds, it is important to notice that one of the most active derivatives, **4** and the less active compounds, **3** and **5** are all triterpenoids. Preliminary structure-activity relationship (SAR) study clearly indicated that the glycosylation of the acidic function of compound **3** at C-28 is important for activity improvement. The other active compounds **1** and **2** are ellagic acid derivatives. Previous studies have reported the antifungal activity of ellagic acid (**1a**) against fungal strains *Trichophyton rubrum*, *T. verrucosum*, *T. mentagrophytes, T. violaceum*, *T. schoenleinii*, *Microsporum canis*, *C. glabrata*, *C. albicans* and *C. tropicalis* [40]. Also, the observed antifungal potency of compounds **1** and **2,** respectively glycosylated and methylated derivatives of **1a** highlights the potency of this class of secondary metabolites [40].

Overall, it was observed that fraction T3 exerted the more potent effect against the tested yeasts, far better than the derived compounds. This is an indication that fractionation has declined the anti-yeast activity, emphasizing the relevance of potential synergistic interactions among the components of fraction T3. Moreover, these results indicate future directions in the progression of this fraction to develop a phytodrug against yeasts infections.

Selected isolated compounds were further tested against G6PD, Carbonic anhydrase I, II and GST enzymes. The results achieved are shown in table 2.

Table 2: Inhibitory parameters of isolated compounds against G6PD, CAI, CAII, and GST

	G6PD				CAI				CAII				GST			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
[a]IC_{50}(µM)	n.a	na	na	1.84± 0.31	53.31± 1.09		86.64± 0.93	3.28± 0.13	69.31± 1.13	na	na	1.03± 0.01	63.01± 1.15	na	1.51± 0.78	1.84± 0.73
[b]Ki (µM)	n.a	na	na	0.19± 0.03	44.11± 1,12		71.68± 0.96	2.72± 0.64	55.78± 0.97	na	na	1.84± 0.11	42.00± 1.39	na	1.00± 0.03	0.19± 0.77

Enzymes were expressed and purified, and subsequently assessed for *in vitro* susceptibility to inhibitors. [a]Serially diluted triplicate concentrations were tested and activity expressed as 50% inhibitory concentration. [b]Inhibitory constant which is reflective of the binding affinity; the smaller the Ki, the greater the binding affinity and the smaller amount of medication needed in order to inhibit the activity of that enzyme.n.a= non active.

The G6PD enzyme was strongly inhibited by the triterpenoid arjunglucoside (**4**) with IC_{50} value of 1.84 µM and Ki (the inhibitor constant indicating how potent an inhibitor is; or

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the concentration required to produce half maximum inhibition) value of 0.19 µM. It has been shown that this key metabolic enzyme which catalyzes the first step of pentose phosphate pathway is expressed abundantly and very active in human tumors [21]. In contrast, G6PD-deficient tumor cell lines showed relatively slow growth and enhanced apoptosis **[41].** Previous studies also reported G6PD inhibitory properties for few compounds such as steroids and derivatives [42, 43], chalcones [28], catechin gallates [44], and some phenolic molecules [45]. In this study, the substituted ellagic acid derived compound **1** did not show any effect on the G6PD enzyme activity, although Adem et al [45] reported that ellagic acid inhibited the enzyme with an IC_{50} value of 0.072 mM. The methoxy group in compound **1** may hindrance the enzyme-inhibitor interaction. Based on the skeletal features of the tested triterpenoids **3**, **4**, **5**, the presence of hydroxyl group at C-19 and the glycosylation of C-28 carboxylic group may be both factors of activity improvement. The G6PD inhibitory potential of a terpenoid is reported here for the first time.

Compound **4** exhibited very good potency against both hCAI and hCA II enzymes with respective activity parameters of IC_{50}= 3.28 µM and Ki= 2.72 µM; and IC_{50}= 1.28 µM and Ki= 1.03 µM respectively. The other tested compounds including 3-*O*-methyl-4-*O*-*α*-rhamnopyranoside ellagic acid (**1**) and arjungenin (**3**) were found to be moderately active against hCAI and hCA II (compound **1**) with IC_{50} and Ki values globally above 44 µM. Previous studies by Sarıkaya et al [46] have indicated that ellagic acid inhibited hCA I and hCA II with K_i values of 0.207 and 0.146 mM respectively. In the present study, compound **1**, a substituted derivative of ellagic acid (**1a)** has exhibited moderate, however highly improved potency toward hCA I (Ki= 44.11 µM) and hCA II (55.78 µM) enzymes. However, this substitution has also considerably decreased the activity as observed against the G6PD enzyme. In addition to the established role of CA inhibitors (CAIs) as diuretics and antiglaucoma drugs, it has recently emerged that they could have potential as novel anti-obesity, anticancer and anti-infective drugs [47]. The high inhibitory potency of the triterpenoid **4** against CAs indicates that it is a promising compound that might be progressed for the formulation of drugs against CAIs-related diseases.

The screening of 3-*O*-methyl-4-*O*-*α*-rhamnopyranoside ellagic acid (**1**), arjungenin (**3**), and arjunglucoside (**4**) against GST enzyme showed inhibitory effects. However, the triterpenoids **3** and **4** exhibited highly potent inhibitory effects (IC_{50}s of 1.57 and 1.84 µM respectively; and Ki of 1.00 and 1.23 µM respectively). Compound **1** only exerted a moderate inhibitory effect on the enzyme (IC_{50}= 63.01 µM; Ki= 42.00 µM). These results are of higher significance as GST inhibitors are anti-cancer agents [25, 26]. Ellagic acid (**1**a) was recently

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shown to inhibit GSTs A1-1, A2-2, M1-1, M2-2 and P1-1 with IC_{50} values ranging from 0.04 to 5 µM [**48**]. Preliminary SAR studies indicate that the substitution of ellagic acid at C-3 and C-4′ gave derivative **1** which showed an IC_{50} value of 63.01 µM), thus therefore considerably decreased the activity. The inhibitory effect of this class of secondary metabolite derivatives is reported here for the first time.

Concluding remarks

The results obtained from the investigation of the methanolic extract of *Terminalia mantaly* stem bark have identified a highly potent anti-yeast fraction T3 that showed to be more promising than subsequently isolated compounds. This promising fraction deserves to be further investigated with the ultimate aim of formulating a plant-based drug against yeasts infections. Compounds **1** and **4** showed anti-yeast activity close to that of the reference drug fuconazole against *C. albicans*. Moreover, compound **2** was over 1.5 times more active than fuconazole against *C. krusei*. Besides, two of the islotated compounds, arjungenin (**3**) and arjunglucoside (**4**) were found to be very active against enzymes of metabolic significance, including G6PD (compound **4**) and GST (compounds **3** and **4**). Finally, given the anti-yeast potency of these compounds, and also the implication of the tested enzymes in some metabolic dysfunctions of public health significance (cancer, obesity, epilepsy), we envisage further SAR studies to identified potent hit derivatives that should subsequently enter drug development pipeline.

Author contributions

NS, ET, BNL, FFB, and SAN designed and supervised the study; MATT, TNK, SG, RMTK, AA, MK, VC, RD performed the chemical and biological parts of the study and drafted the manuscript; BNL and FFB critically revised the manuscript. All authors agreed on the final version of the manuscript for submission to *Medicines*.

Compliance with ethical standards

The authors declare no competing interest.

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