In vitro study for antifungal compounds from \textit{Parinari curatellifolia} (Chrysobalanaceae) and \textit{Terminalia sericea} (Combretaceae)

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ABSTRACT

\textit{Parinari curatellifolia} (Chrysobalanaceae) and \textit{Terminalia sericea} (Combretaceae) have been traditionally used in Southern Highlands of Tanzania for treatment of various infectious disorders. The present study aimed to evaluate antifungal activity of the isolated compounds from \textit{Parinari curatellifolia} and \textit{Terminalia sericea} plant species. The ethyl acetate extract of the root barks from \textit{Parinari curatellifolia} and \textit{Terminalia sericea} were fractionated using column chromatography. The structures of compounds were established using both 1D and 2D-NMR spectroscopic techniques while antifungal activities of the fractions and isolated compounds were evaluated using broth microdilution assay against \textit{Candida albicans}, \textit{Cryptococcus neoformans} and \textit{Aspergillus niger} species. Two known compounds toddalolactone (1) and 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-ol acid 1-lactone (2) from \textit{P. curatellifolia} and two compounds Sericic acid (3) and sericoside (4) from \textit{T. sericea} were isolated and their structures identified and confirmed by spectral data obtained and from the literatures. Strong antifungal activity was shown by Sericic acid (3) with MIC value of 0.07 mg/ml against \textit{C. albicans} and \textit{C. neoformans}. Isolation of toddalolactone (1) from \textit{Parinari curatellifolia} as well as the antifungal activity of Sericic acid (3) from \textit{Terminalia sericea} is being reported for the first time. Bioactivity of these compounds support traditional use of the studied plants.

Keywords: Sericic acid, toddalolactone, fungi, antifungal, \textit{Parinari curatellifolia}, \textit{Terminalia sericea}. 

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INTRODUCTION

Plants have been used traditionally over decades in the treatment of fungal infections or related ailments in most parts of the world including Tanzania. Medicinal herbs constitute an indispensable component of traditional medicine practiced worldwide due to the low cost, perceived safety, bio-degradability, easy access and ancestral experiences (Ogunnusi et al., 2010). A number of plants used in traditional medicines are currently being investigated for treatment of fungal infections which are common opportunistic infections among people with immunocompromised conditions like HIV/AIDS and cancer patients (Disse et al., 2017; Fokou et al., 2017). Some of the already identified plants are used as mouth preparations (Fyhrquist et al., 2002) and others are made in the form of ointments or creams for topical use on the skin (Chhetri et al., 2010). Ethnobotanical study undertaken in Iringa and Njombe regions has revealed that, the plant species namely Terminalia sericea Burch. Ex. DC (Combretaceae) locally known as “Mpululu” and Parinari curatellifolia (Chrysobalanaceae) locally called “Msaula” among the Hehe tribe of Iringa, Tanzania, are used for the treatment of anaemia, diabetes, diarrheaa, gonorrhrea and vaginal douches (Mbunde et al., 2016). Previously, T. sericea aqueous and methanol extracts of T. sericea was reported to have no antibacterial activity against Bacillus subtilis, Staphylococcus aureus and Staphylococcus epidermidis, but more recent studies have reported the presence of antibacterial activity in the leaves, roots and stem barks (Fyhrquist et al., 2002). Also, antifungal activity against Candida albicans was reported in the methanolic extract of T. sericea (Masoko et al., 2005). Reports on biological assays against various pathogenic organisms revealed that, Parinari capensis contains antimalarial (Uys et al., 2002), antifungal, antibacterial (Chukwudi et al., 2014; Angaman et al., 2018), and antioxidant (Nukarume et al., 2009) while ethyl acetate root extract of Parinari curatellifolia exhibited anticancer activity (Lee et al., 1996).

Based on the wide use of these plants in Southern Tanzania, the present study was carried out to isolate pure compounds from Parinari curatellifolia and Terminalia sericea plants and evaluate the antifungal effect against selected pathogenic fungi like moulds and yeast.

MATERIALS AND METHODS

Reagents

Methanol, Ethanol (absolute), Dichloromethane, Ethyl acetate, Petroleum ether were purchased from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands), Dimethyl sulfoxide (DMSO) was from Sigma® (Poole, Dorset, UK), TLC silica gel pre-coated plates (Merck-Germany), Silica gel-60 for column (0.063-0.200 mm; 70-230 mesh) (Merck-Germany), Sabour dextrose agar and broth from HIMEDIA® (Himedia Laboratories Pvt Ltd, Mumbai, INDIA), p-Iodonitrotetrazolium chloride was bought from SIGMA® (Sigma- Aldrich®, St Louis, USA), Microtitre plates (Clear Flat Bottom Polystyrene, TC-Treated, Individually Wrapped, Sterile, 1 x 8 Stripwell™) was from Sigma® were supplied by TechnoNet solutions, Tanzania.

Collection of plant materials

The roots of Terminalia sericea and Parinari curatellifolia were collected from Mafinga, Iringa in Southern Tanzania in June 2015. Identification and preparation of the voucher specimens was done with the aid of a Botanist from University of Dar es Salaam and voucher specimens (number MNM15 for T. sericea and voucher specimen number MNM10 for P. curatellifolia) kept at the Herbarium of the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences.

Extraction and isolation of pure compounds

The root barks of Parinari curatellifolia and Terminalia sericea were air-dried, cut into small pieces, ground using a machine grinder and subsequently soaked at room temperature.
for 48 hours using ethyl acetate then followed by ethanol respectively. The crude extracts were filtered and concentrated in vacuo using a rotary evaporator with the bath temperature maintained at 40 °C.

Ethyl acetate extract of *P. curatellifolia* root bark (30 g) was packed to column chromatography on silica gel (100 g) initially eluting with 100% petroleum ether adding ethyl acetate to get several fractions which were then combined based on similarity in the TLC to give 16 fractions. Repeated column chromatography of fraction 3 on silica gel eluted with 1:1% v/v Ethyl acetate and Dichloromethane obtained three sub-fractions which were 3A, 3B and 3C. Sub fraction 3B was then purified by preparative TLC to yield Toddalolactone (1) (14 mg). Fraction 4 was purified by crystallization in methanol to yield 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid t-lactone (2) (20 mg).

The ethanol extract of root bark of *Terminalia sericea* was sequentially fractionated by vacuum liquid chromatography (VLC) using petroleum ether, dichloromethane, ethyl acetate and methanol. The ethyl acetate fraction (20 g) was packed in a column in silica gel eluting in gradient with solvents of different polarities such as petroleum ether, dichloromethane and ethyl acetate. Several fractions were collected and combined to get 9th fractions. The 8th fraction was dissolved in ethyl acetate and formed two layers thus decanted to separate soluble and insoluble fraction. Insoluble fraction was crystallized with methanol to yield the compound named Sericic acid (3). Sericoside (4) (90 mg) was obtained as pure compounds after eluting methanol fraction with ethyl acetate then chromatographed in sephadex LH-20 to get fractions in which three fractions were subjected to crystallization using methanol to yield the compound.

**Spectroscopic analysis**

Spectroscopic analysis of the isolated compounds from *P. curatellifolia* was done at Stockholm University, Sweden while compounds from *T. sericea* were analysed at Dortmund University, Germany. Both 1D’s and 2D’s NMR analyses (1H, 13C-NMR, COSY, HSQC, HCCOSW, DEPT and HMBC spectra) were recorded on a Bruker Avance DRX-600 (600 MHz) NMR spectrometer. NMR data were processed using Mestre Nova Software (Mestre lab Research S.L.) and Topspin software (Bruker BioSpin Corporation).

**Test for antifungal activity**

**Preparation of microorganisms**

Pure cultures of human pathogenic fungi: *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 90112), *Aspergillus niger* (AZN 8240) and *Candida albicans* clinical isolates, were obtained from the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS). The fungal species were maintained on sabouraud dextrose agar at 4 °C. The fungal species were inoculated in sabouraud dextrose broth (SDB) and Muller Hinton broth and incubated at 35 °C prior to screening tests. Clotrimazole was prepared from solution of distilled water and used as positive controls.

**Determination of Minimum Inhibitory Concentration (MIC) by broth micro dilution method**

The MIC for the crude extracts and isolated compounds was determined by broth microdilution tests performed in a sterile flat bottom 96-well polystyrene microtiter plates. The extracts and isolated compounds were tested against standard and clinical isolates of fungi like *Candida albicans, Cryptococcus neoformans* and *Aspergillus niger*. Microorganism’s inocula were prepared from 24 hours grown cultures. Serial two-fold dilution method was performed as follows; 100 µl of broth (Sabouraud’s dextrose broth) were pipetted into each well. To each well of the first row 100 µl of the compound solution or fluconazole or solvent was added. Each of the test materials were tested in duplicate. After thorough mixing, 100 µl of the mixture was drawn and transferred to the second well in the
row and after mixing well again 100 µl of the mixture was drawn and transferred to the third well in the row. This procedure was repeated until the last well in the row. Then 100 µl of the mixture was discarded from each last well of the row. One additional row was used as growth control, in which no drug was added; instead a blank culture medium was added. The inoculated microtiter plates were incubated at 37 °C for 24 hours for moulds and 48 hours for yeast like fungus. MIC values were detected using tetrazonium salt indicator, (Iodonitrotetrazolium chloride) which changes colour from pink (for presence of growth) to colourless (for no growth). Before recording of experimental results in the MIC assay plate, a volume of 40 µl of a 0.2% INT was pipetted into each well and incubated at 37 °C for 2 hours. The lowest concentration at which no growth observed indicated by colourless colour and this was taken as MIC value.

RESULTS
Isolation and characterization of compounds
Toddalolactone (1) was observed to be white crystals, UV/VIS on positive TLC and purple in colour when the TLC is sprayed with vanillin Sulphuric acid mixture. It was soluble in Dichloromethane and Ethyl acetate, insoluble in Methanol. Rf value 0.57 (100%DCM). Reported Molecular Weight 308.33 g/mol, C₁₆H₂₃O₆ (Yu et al., 2017). ¹H and ¹³C NMR data are presented in Table 1.

10-hydroxy-13-methoxy-9- methyl-15-oxo-20-norkaur-16-en-18-oic acid t-lactone (2) was observed to be white with needle-like crystals structures, UV positive in TLC and purple in colour when the TLC is prayed with vanillin Sulphuric acid mixture. It was soluble in Dichloromethane and Ethyl acetate, insoluble in Methanol. They were white needle crystals with Rf value 0.45 (100%DCM). Reported Molecular Weight 344.0 g/mol, C₂₁H₂₃O₄ (Garo et al., 1997). ¹H and ¹³C NMR data are presented in Table 2.

Sericic acid, (2α,3β,19α,24 – tetrahydroxy-olean-12-en-28-oic acid) (3) white amorphous, soluble in Dichloromethane and Ethyl acetate. Reported Molecular Weight 504.345 g/mol, C₃₀H₄₆O₆ (Hess and Monache, 1999). ¹H and ¹³C NMR data are presented in Table 3.

Glucosyl - 2α,3β,19α,24 – tetrahydroxy-olean - 12 - en - 28-oate (Sericoside) (4) was white in colour, soluble in Ethyl acetate. Molecular Weight 666.8 g/mol, C₃₀H₃₆O₁₁ (Terreaux et al., 1996). ¹H and ¹³C NMR data are shown in Table 4.

Antifungal activity of isolated compounds
Minimal Inhibitory Concentration values of the different crude extracts and isolated compounds against the pathogenic yeasts and moulds were determined and reported in Table 5. According to Gibbons (2004), antimicrobial activity is classified into the following categories: MIC <1 mg/ml: Good antimicrobial activity, MIC >1 mg/ml or <4 mg/ml: Moderate good antimicrobial activity, MIC = 4 mg/ml or < 6 mg/ml: Moderate antimicrobial activity, MIC > 6 mg/ml: Poor antimicrobial activity.

Table 1: A table showing NMR (CDCl₃, 600 MHz) data for Toddalolactone (1) from Parinari curatellifolia

| Position | ¹³C NMR CDCl₃ | ¹H NMR CDCl₃ | Literature CDCl₃ (Yu et al., 2017) |
|----------|---------------|---------------|----------------------------------|
| 2        | 161.6         | 6.26 (1H, d, J = 12.0 Hz) | 160.9                            |
| 3        | 112.9         | 7.85 (1H, d, J = 12.0 Hz) | 112.8                            |
| 4        | 138.9         | 3.88 (6H, d, J=6.0 Hz) | 138.7                            |
| 4a       | 107.4         |                | 107.3                            |
| 5        | 156.1         | 3.88 (6H, d, J=6.0 Hz) | 156.0                            |
| 6        | 118.0         |                | 117.9                            |
|    | 7   | 161.1 | 161.5 |
|----|-----|-------|-------|
| 8  | 95.9| 6.65  | 95.8  |
| 8a | 155.2|      | 155.1 |
| 1" | 26.3| 2.95  | 26.1  |
| 2" | 78.2| 3.59  | 78.1  |
| 3" | 73.0|       | 72.9  |
| 4" | 23.8| 1.30  | 23.7  |
| 5" | 26.25| 2.75  | 26.2  |

OMe-C 63.4
OMe-C 56.4

Figure 1: Structures of the isolated compounds toddalolactone (1) and 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid i-lactone (2), Sericic acid (3) and sericoside (4).
Table 2: A table showing NMR (600 MHz) data for 10-hydroxy-13-methoxy-9- methyl-15-oxo-20- norkaur-16-en-18-oic acid 1-lactone (2) from *Parinari curatellifolia*.

| Position | $^{13}$C NMR CDCl$_3$ | $^1$H NMR CDCl$_3$ | Literature CDCl$_3$, (Garo et al., 1997) |
|----------|----------------------|-------------------|---------------------------------|
| 1        | 31.3                 | 1.16 (2H, m)      | 31.0                            |
| 2        | 20.2                 | 2H (1.60, m)      | 19.9                            |
| 3        | 35.4                 | 1.72 (2H, m)      | 35.1                            |
| 4        | 47.6                 |                   | 47.3                            |
| 5        | 52.0                 | 2.52 (1H, dd, $J=6.0, 12.0$ Hz) | 51.7 |
| 6        | 18.3                 | 1.27 (3H, s)      | 18.0                            |
| 7        | 25.6                 | 1.50 (2H, m)      | 25.3                            |
| 8        | 54.5                 |                   | 54.2                            |
| 9        | 43.2                 |                   | 42.9                            |
| 10       | 87.5                 |                   | 87.2                            |
| 11       | 31.8                 | 1.87 (2H, m)      | 31.5                            |
| 12       | 34.9                 | 2.17 (2H, m)      | 34.5                            |
| 13       | 79.9                 |                   | 79.6                            |
| 14       | 40.7                 | 2.17 (2H, m)      | 40.4                            |
| 15       | 208.3                |                   | 208.0                           |
| 16       | 147.6                |                   | 147.3                           |
| 17       | 116.7                | 5.36 (1H, s)      | 116.4                           |
| 18       | 180.6                |                   | 180.3                           |
| 19       | 17.2                 | 1.10 (3H, s)      | 16.9                            |
| 20       | 18.7                 | 1.72 (2H, m)      | 18.4                            |
| 21       | 50.3                 | 3.25 (3H, s)      | 50.0                            |

Table 3: A table showing NMR (CD$_3$OD, 600 MHz) data for Sericic acid (3) from *Terminalia sericea*.

| Position | $^{13}$C NMR CD$_3$OD | $^1$H NMR CD$_3$OD | Literature Pyridine-d$_5$ (Hess & Monache, 1999) |
|----------|----------------------|-------------------|---------------------------------|
| 1        | 44.8                 |                   | 47.4                            |
| 2        | 67.7                 | 3.70 (1H, m)      | 68.6                            |
| 3        | 84.1                 | 2.96 (2H, m)      | 85.7                            |
| 4        | 43.2                 |                   | 43.9                            |
| 5        | 55.4                 | 0.90 (1H, s)      | 56.5                            |
| 6        | 18.1                 | 1.36 (2H, m)      | 19.3                            |
| 7        | 32.3                 | 1.22 (1H, m)      | 33.6                            |
| 8        | 40.7                 |                   | 40.0                            |
| 9        | 47.6                 | 1.70 (3H, m)      | 48.4                            |
| 10       | 38.9                 |                   | 38.4                            |
| 11       | 26.7                 | 0.88 (2H, s)      | 28.8                            |
| Position | $^{13}$C NMR CD$_3$OD | $^{1}$H NMR CD$_3$OD | Literature CDCl$_3$ (Terreau et al., 1996) |
|----------|----------------------|----------------------|-----------------------------------------|
| 1        | 47.9                 | 0.80 (2H, t, $J=11.9$ Hz) | 47.5                                    |
| 2        | 69.7                 | 3.69 (2H, m)          | 68.6                                    |
| 3        | 86.1                 | 2.95 (1H, d, $J=9.544$ Hz) | 85.7                                    |
| 4        | 44.5                 |                      | 43.9                                    |
| 5        | 57.4                 | 0.88 (3H, m)          | 56.6                                    |
| 6        | 20.1                 | 1.34 (2H, m)          | 19.5                                    |
| 7        | 34.2                 | 1.23 (2H, m)          | 33.5                                    |
| 8        | 41.0                 |                      | 40.2                                    |
| 9        | 49.5                 | 1.68 (IH, m)          | 48.5                                    |
| 10       | 39.4                 |                      | 38.4                                    |
| 11       | 25.2                 | 1.88 (2H, dt, $J=4.9$, $J=8.7$) | 24.5                                    |
| 12       | 124.8                | 5.23 (1H, t, $J=6.96$) | 123.8                                   |
| 13       | 144.6                |                      | 144.3                                   |
| 14       | 42.7                 |                      | 42.0                                    |
| 15       | 29.6                 | 1.69 (2H, m)          | 28.9                                    |
| 16       | 28.5                 | 2.23 (IH, sept)       | 27.9                                    |
| 17       | 47.2                 |                      | 46.4                                    |
18  45.2  2.95 (2H, d, $J=9.54$ Hz)  44.6
19  82.5  3.17 (1H, d, $J=3.78$ Hz)  80.9
20  36.1  35.5
21  29.5  0.91 (2H, m)  29.0
22  33.4  1.56 (2H, m)  33.0
23  25.1  1.19 (3H, s)  24.6
24  66.3  3.93 (1H, d, $J=11.2$ Hz)  65.6
25  17.6  0.85 (3H, d, $J=6.36$ Hz)  17.2
26  17.8  0.63 (3H, s)  17.5
27  23.9  24.1
28  178.7  177.2
29  28.8  0.85 (3H, d, $J=6.36$ Hz)  28.7
30  25.3  0.85 (3H, t, $J=9.2$)  24.8

Glucose
1'  95.9  5.27 (1H, d, $J=8.16$ Hz)  95.8
2'  74.1  3.22 (2H, m)  74.1
3'  78.9  3.29 (2H, m)  79.3
4'  71.2  3.26 (2H, d, $J=7.08$ Hz)  71.0
5'  78.5  3.29 (2H, m)  78.9
6'  62.5  3.72 (2H, m)  62.1

Table 5: MIC results (mg/ml) for crude extract and compounds isolated from *Parinari curatellifolia* and *Terminalia sericea*

| Fraction/compound (mg/ml) | C. albicans ATCC 90028 | C. albicans clinical | C. neoformans ATCC 90112 | A. niger AZN 8240 |
|--------------------------|------------------------|----------------------|--------------------------|------------------|
| Compound (1)              | Nt                     | Nt                   | Nt                       | Nt               |
| Compound (2)              | 3                      | 3                    | 1.5                      | 0.75             |
| Compound (3)              | 0.07                   | 0.07                 | 0.07                     | -                |
| Compound (4)              | 1.6                    | 3.1                  | 6.2                      | 1.2              |
| Crude extract TSR         | 0.16                   | 2.5                  | 0.63                     | -                |
| Crude extract PCR         | 1.25                   | 0.31                 | 0.04                     | 0.04             |
| Clotrimazole              | 0.09                   | 0.19                 | 0.19                     | 0.05             |

TSR = *Terminalia sericea* root crude extract, PCR = *Parinari curatellifolia* root crude extract, Nt = not tested
DISCUSSION

Isolated compounds

The $^1$H and $^{13}$C NMR data in Table 1 and information from the literature revealed that compound (1), was a known compound 6-[(2R)-2,3-dihydroxy-3-methyl-butyl]-5,7-dimethoxy-coumarin a toddlerlac-tone (Yu et al., 2017). The presence of this diterpene lactone (1), in Parinari species which belongs to the family Chrysobalanaceae is noteworthy, since this type of compounds were mostly reported in the Rutaceae family (Hisashi et al., 1991)

The $^{13}$C-NMR spectrum of compound (2) indicated the presence of carbonyl characteristic for a ketone at $\delta$ 208.3 ppm, and a carboxyl functional group at $\delta$ 180.6 ppm, as well as an exocyclic methyldiene group at $\delta$ 147.6 (quaternary C-atom) and at $\delta$ 116.7 (=CH,) ppm. In the $^1$H-NMR, two olefinic protons were clearly visible at $\delta$ 5.36 and $\delta$ 6.13 ppm. From these data, compound (2) was assigned as a kaurenoid diterpene. Spectral data of this compound was confirmed to be similar with already reported data by Garo et al. (1997).

The structures of Sericic acid (3) and Sericoside (4), indicated the characteristic feature of oleanene moiety based on extensive spectroscopic analysis in the $^1$H and $^{13}$C NMR including two- and three-dimensional NMR techniques. The structures for compounds were deduced from the comparison of the observed NMR spectral features (Table 2) with those reported in the literature for these compounds (Terreaux et al., 1996; Hess and Monache, 1999). Also, confirmation of chemical shift of Sericic acid was done by comparing $^{13}$C NMR chemical of related compounds arjungenin (Aimée et al., 2017) and tomentosic acid (Mahato et al., 1990) both found in Terminalia species. Comparing carbon and proton spectral data for Compound (3) and (4) indicates that, the only difference between the two was the presence of glucoside moiety. Therefore, Compound (4) is a glycoside of compound (3) been referred to as sericoside due to observed $J$ value on the attachment of the hexose moiety of the $\beta$-glucose at C-l axial proton.

Antifungal activity of fractions and compounds

The results in Table 5 showed that, crude extracts and isolated compounds from Parinari curatellifolia and Terminalia sericea were active against the tested pathogenic fungi demonstrating varying antifungal activity. Crude extract from Parinari curatellifolia revealed strong antifungal activity (MIC = 0.75 mg/ml) against C. albicans comparatively higher compared to the isolated compound 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid $\tau$-lactone (2) (MIC = 3 mg/ml) from the same plant extract. The observed lower activity for the 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid $\tau$-lactone, Sericic acid and Sericoside compounds compared to crude extracts from the respective plants (Mbunde et al., 2019) can be explained by mechanism of interaction induced by several compounds in fraction or crude form that my enhance activity positively synergism) and negatively (antagonism) against the fungal pathogens.

Interestingly, to the best of our knowledge bioactivity of these compounds against human pathogenic microbes Candida albicans, Cryptococcus neoformans and Aspergillus niger is being reported for the first time. Antifungal activity has only been reported for 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid $\tau$-lactone in phytopathogenic fungus Cladosporium cucumerin (Garo et al., 1997). Other biological activities for 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid $\tau$-lactone reported in malaria against Plasmodium falciparum (Uys et al., 2002). Toddalolactone (1) on the other hand was not subjected to assay because the amount isolated was very small. However, it has been reported in the literature to have anti-hypertension, anti-inflammatory, antifungal activities (Upadhyay, 2016), larvicidal, anti-fibrotic and anti-thrombotic activity (Yu et al., 2017).

Findings from our study revealed that, Sericic acid (3) from T. sericea had stronger antifungal activity (MIC = 0.068 mg/ml) than...
the tested known standard drug clotrimazole (MIC = 0.09 mg/ml). This may be attributed by the presence of conjugated carbons, number of phenolic, hydroxyl and carboxyl groups and the number of acceptor atoms of hydrogen bonds in the compound which are known as important structural descriptors in the antimicrobial activity of terpenes (Sieniawska and Rafał, 2017). However, weak activity of Sericoside (4) contradicts this fact hence; further studies are needed to elucidate the mechanism of actions. The two compounds from Terminalia sericea has been previously reported to contain antibacterial activity against Bacillus subtilis and Pseudomonas fluorescens with equal MIC values of 5 µg/ml and >40 µg/ml respectively (Conrad et al., 1998) while Hess et al. (1995) reported antibacterial activity of Sericic acid against Staphylococcus aureus (MIC = 1.0 mg/ml).

Conclusion
This study reports the antifungal activity of Sericic acid for the first time while toddalolactone (1) is being isolated for the first time in P. curatellifolia and the family Chrysobalanaceae. Sericic acid (3) had comparable activity with the standard antifungal drug clotrimazole against Candida albicans and Cryptococcus neoformans. The observed biological activities in the crude extracts and isolated compounds justify, its popular use in Southern Tanzania for the treatment of infectious diseases.

COMPETING INTERESTS
The authors declare that they have no competing interests in the publication of this work.

AUTHORS’ CONTRIBUTIONS
All authors contributed to the realization of this work. They also read and approved this manuscript.

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