Chemical compounds from the Kenyan polypore *Trametes elegans* (Spreng:Fr.) Fr (Polyporaceae) and their antimicrobial activity

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ABSTRACT

Over the years, natural products have been used by humans in tackling infectious bacteria and fungi. Higher fungi have potential of containing natural product agents for various diseases. The aim of the study was to characterise the antimicrobial compounds from the polypore *Trametes elegans*. The dried, ground fruiting bodies of *T. elegans* were extracted with methanol and solvent removed in a rotary evaporator. The extract was suspended in distilled water, then partitioned using ethyl acetate solvent to obtain an ethyl acetate extract. The extract was fractionated and purified using column chromatographic method and further purification on sephadex LH20. The chemical structures were determined on the basis of NMR spectroscopic data from ¹H and ¹³C NMR, HSQC, HMBC, ¹H-¹H COSY, and NOESY experiments. Antimicrobial activity against clinically important bacterial and fungal strains was assessed and zones of inhibition were recorded. The polypore yielded six known compounds namely ergosta-5,7,22 trien-3-ol (1) 5α,8α–epidioxyergosta-6,9(11),22-trien-3β-ol (2), 5α,8α–epidioxyergosta-6,22-dien-3β-ol (3), ergosta-7,22-dien-3β,5α,6β-triol (4), Lupeol (5) and 9,19-cycloartane-3,30-diol (6). From this study, the isolated compounds of *T. elegans* displayed varying antimicrobial activities with zones of inhibition ranging from 8.0±0.58 to 9.7±0.33 mm at (p≤0.05). Thus, *Trametes elegans*, could be considered as a potential source of natural antimicrobials.

Keywords: Higher fungi, triterpenoids, disc diffusion assay.

INTRODUCTION

The genus *Trametes* Fr. (Polyporaceae, higher Basidiomycetes) consist of white rot polypores, that include the *Trametes versicolor* commonly known as ‘turkey tail’ fungus (Zmitrovich et al., 2012; Carlson et al., 2014). The *Trametes* polypore species play an important role in natural ecosystems as wood decomposers and have enormous potential for bioremediation and biodegradation activities, making them both ecologically and economically important. *Trametes elegans* is present in almost all forest ecosystems and are found frequently on numerous genera of deciduous hardwood forests (Carlson et al., 2014). *Trametes elegans* is well known for its...
medicinal properties, although not much research has been carried out on the medicinal properties, especially the antimicrobial activities of *T. elegans* (Awala and Oyetayo, 2015). *Trametes versicolor* is the most studied Chinese medicinal mushroom in the genus. It is known to possess a wide range of biological activities including immune-enhancing activity (Li et al., 2011), antitumor (Standish et al., 2008) and antiviral effects (Teplyakova et al., 2012). A preventive bioactive mushroom extract, known as PSK (from *T. versicolor* mycelia), demonstrated to be effective against carcinogenesis (Fisher and Yang, 2002). The extract is a protein-bound polysaccharide and was approved for use in cancer treatment by the Japanese Ministry of Health and Welfare in 1977 (Moon and Shibamoto, 2009). The need to explore natural sources for novel bioactive agents has increased in the last three decades. Fungi are among the most creative groups of eukaryotic organisms capable of producing many novel natural products that are directly used as drugs or serve as structural backbone for synthetic modifications (Stadler and Keller, 2008). The aim of the current study was to evaluate the antibacterial and antifungal activity of the compounds isolated from *Trametes elegans*.

**MATERIALS AND METHODS**

**General experimental procedures**

NMR analysis was performed on a Bruker 500 MHz NMR spectrophotometer and spectra were recorded in CDCl₃ at the University of Surrey, United Kingdom. Structures of compounds were elucidated and they were confirmed by comparison of their NMR data against literature values.

**Collection of fruiting body**

The sample fruiting body of *Trametes elegans* was collected from rotten wood logs and stumps along from Kerio Valley, Elgeyo Marakwet County and Kabarnet forest, Baringo County in Kenya. The sample material was collected in July 2013, when the rains had ended though with high humidity and the temperatures were between 18-26 °C. The identification of the polypore mushroom was done through the examination of morphological features and further molecular identification by Dr. Leung Siu Han from Mushroom Initiative, Hong Kong. The voucher specimen number JO 13020/59 of *Trametes elegans* species was kept as herbarium in Integrated Biotechnology Research Laboratory at Egerton University.

**Extraction and isolation**

The *Trametes elegans* macro fungi samples were brush-cleaned to remove any attached soil and humus, chopped into small pieces and then air-dried under shade to constant weight. The cleaned, dried fruiting bodies were ground using a mechanical blender and stored in an air-tight container for further use. The sample material (400 gm) was extracted with methanol for 72 hours in dark with occasional stirring. The extract was filtered through Whatman No. 1 filter paper and the filtrate evaporated by rotary evaporator under reduced pressure and then partitioned with ethyl acetate solvent. The extract was fractionated (Figure 1) and purified using column chromatography with silica gel (0.063–0.200 mm, Merck 9385). The purity was checked using TLC plates (Merck Art 554, 20 cm x 20 cm, silica gel 60 F254 coated). The Ethyl acetate extract (4 gm) was eluted with hexane–diethyl ether gradient elution to obtain fractions, Fr 1 to Fr 6 (Figure 1). The fractions were further subjected to repeated silica gel column chromatography eluted with dichloromethane, ethyl acetate gradients to afford six sub-fractions that were also repeatedly chromatographed to yield the first four pure fractions. The last two were obtained on further purification on Sephadex LH20.

**Antimicrobial activity**

The antibacterial assay was based on the disc diffusion assay according to (CLSI, 2012) The pathogens included gram negative strains; *Salmonella typhi*, *Shigella*, *Escherichia coli* (E. coli), *Citobacter enterocolitica* and *Klebsiella pneumonia*. Gram positive bacteria; *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Staphylococcus aureus* and *Entero feacalis*, Fungi; *Candida albicans*, and *Cryptococcus neoformans*. Antimicrobial activity against clinically important strains was evaluated and zones of inhibition were reported as mean±SEM.
Ethyl acetate extract 4 gm

Hex:Et<sub>2</sub>O gradient elution

Fr 1 Fr 2 Fr 3 Fr 4 Fr 5 Fr 6
90/10 DCM:EtOAc 85/15 DCM:EtOAc 80/20 DCM:EtOAc 70/30 DCM:EtOAc 50/50 DCM:MeOH
(1) 6.2 mg (2) 6.3 mg (3) 5.0 mg (4) 4.9 mg (5) 3.2 mg (6) 2.9 mg

Figure 1: Flow chart showing the isolation of compounds from *T. elegans*.

**RESULTS**

Compound 1 (Figure 2) was obtained as a white amorphous powder. Its <sup>13</sup>C-NMR (Table 1) spectrum together with DEPT-135 revealed 28 carbon signals that included one oxygenated methine carbon at δ<sub>c</sub> 70.7 and six olefinic carbon signals at δ<sub>c</sub> 116.6, 119.8, 132.2, 135.8, 140.3 and 141.8. The <sup>1</sup>H NMR spectrum of compound was indicative of two tertiary methyls (δ<sub>H</sub> 0.94, 0.63) and four secondary methyl signals δ<sub>H</sub> 0.83, 0.84, 0.91 and 1.03. The other key proton resonances are olefin protons at δ<sub>H</sub> 5.57, 5.39 and a multiplet signal between 5.19-5.21. A broad deshielded proton signal at δ<sub>H</sub> 5.17 and 5.23. Two oxygenated tertiary carbons were observed at δ<sub>c</sub> 78.6 (C-8), 82.9 (C-5) and one secondary at δ<sub>c</sub> 66.6 (C-3) ppm. The oxygenated methine carbon was assigned to C-3 due to the HMBC cross signal between a proton at δ<sub>H</sub> 4.02 (1H-3) with the C-5 resonance (δ<sub>c</sub> 82.9). The <sup>1</sup>H NMR of compound 2 also exhibited signals deshielded coupled H6–H7 pair at δ<sub>H</sub> 6.23 and 6.50 and H22–H23 pair at δ<sub>H</sub> 5.17 and 5.23. Two oxygenated tertiary carbons were observed at δ<sub>c</sub> 79.6 (C-8), 82.4 (C-5) and one secondary at δ<sub>c</sub> 66.7 (C-3) ppm. The carbon at δ<sub>c</sub> 82.4 (C-5) had cross correlations with δ<sub>H</sub> 6.50 (H-6), 6.23 (H-7), 1.91(H-4), 1.68(H-1) and 0.88(3H-19). In addition, the δ<sub>c</sub> 66.7 had HMBC correlations with δ<sub>H</sub> 2.11/1.91 (2H-4), 6.50 (1H-6), 1.69(H-1) and 1.53(H-2). The carbon at δ<sub>c</sub> 79.6 (C-8) had HMBC correlations with δ<sub>H</sub> 6.50 (H-6). The vinyl proton signal of compound 2 at δ<sub>H</sub> 5.40 was lacking in the proton spectrum of compound 3. The <sup>13</sup>C spectrum of the two compounds 2 and 3 was almost similar except for the presence of a double bond signals at δ<sub>c</sub> 142.8 and 119.7 (C-9-C-11) for compound 2. The structures were concluded as sterols and identified as 5α,6α-epidioxyergosta-6,9(11),22-dien-3β-ol (2) and as 5α,6α-epidioxyergosta-6,22-dien-3β-ol (3).
Compound ergosta-7,22-dien-3β,5α,6β-triol 4 was obtained as an amorphous powder. The 13C NMR (Table 1) demonstrated 28 carbon signals. The 1H NMR exhibited resonance of six methyl groups including two tertiary methyl groups at δH 0.92 (3H, s, H-18) and 1.08 (3H, s, H-19). The existence of two double bonds were indicated by the proton signals between 5.30 and 5.16 ppm in the 1H NMR spectrum for three vinyl protons (including one trans double bond at δH 5.16 1H, dd, J= 8.4, 15.3 Hz, H-22). The 1H NMR spectrum contained broad-proton signals at δH 4.08 and 3.63 ppm, consistent with the presence of two oxygenated methines. The observed unusual downfield signal of δH 4.08 (1H-3α) was due to through space interaction with the hydroxyl group at C-5 typical of 3β-hydroxysterols bearing a 5α-hydroxyl group (Ahmed et al., 2006). By comparison of its spectroscopic data with those reported in the literature the compound was identified as ergosta-7,22-dien-3β,5α,6β-triol.

Compound 5 was obtained as a white solid. The 13C NMR spectra of the displayed thirty carbon resonances including two oxygenated carbons. The 1H and 13C NMR, DEPT, and HSQC data supported the presence of eight methine carbons including an oxymethine (δC 76.8/δH 3.22), twelve methylenes including one oxygenated (δC 63.4/δH 3.63) and four sp3 quaternary carbons. 1H-NMR spectrum showed signals due to three secondary methyl groups at 0.86, 0.88 and 0.87, three tertiary methyl singlet signals at 0.98, 0.96 and 0.89 and characteristic doublets at δH 0.38 (J=3.98 Hz) and at δH 0.14 (J=4.16 Hz) for non-equivalent protons of a cyclopropyl methylene. The confirmation of the structure of 9, 19-cycloartane-3, 30-diol was accomplished through 2D NMR experiments (COSY and HMBC).

![Structures of compounds 1-6.](image-url)
Table 1: $^{13}$C-NMR chemical shifts of the triterpenoids from *Trametes elegans*.

| C | 1   | 2   | 3   | 4   | 5   | 6   | C | 1   | 2   | 3   | 4   | 5   | 6   |
|---|-----|-----|-----|-----|-----|-----|---|-----|-----|-----|-----|-----|-----|
| 1 | 38.6| 32.8| 34.9| 33.2| 38.9| 30  | 16| 29.9| 28.8| 28.8| 28.1| 35.8| 27.2|
| 2 | 32.2| 30.8| 30.2| 31  | 27.4| 35  | 17| 56  | 56.1| 56.4| 56.2| 43  | 52.1|
| 3 | 70.7| 66.6| 66.7| 68  | 79.2| 76.8| 18| 12.3| 13.2| 13.1| 12.5| 48.3| 18  |
| 4 | 41  | 36.3| 37.2| 39.6| 38.9| 40.4| 19| 16.5| 25.7| 19.8| 19.9| 48.2| 27.5|
| 5 | 141.6| 82.9| 82.4| 76.2| 55.5| 43.5| 20| 40.6| 40.1| 40.1| 40.7| 151.2| 36.7|
| 6 | 119.8| 135.7| 135.6| 73.8| 18.5| 24.9| 21| 21.3| 20.9| 21.2| 21.3| 30  | 18.6|
| 7 | 116.6| 130.9| 130.9| 117.7| 34.5| 28.3| 22| 135.8| 135.3| 135.2| 136.9| 40.2| 35.6|
| 8 | 140.3| 78.5| 79.6| 144.2| 41  | 47.1| 23| 132.2| 132.7| 134.3| 132.2| 28.2| 24.3|
| 9 | 46.6| 142.8| 51.3| 43.6| 50.6| 23.1| 24| 43.1| 43.8| 43  | 43  | 15.6| 39.8|
| 10| 37.2| 38.2| 37.1| 37.2| 37.4| 25.9| 25| 33.4| 33.3| 33.3| 33.3| 16.1| 28.2|
| 11| 21.3| 119.9| 23.6| 22.3| 21.1| 25.4| 26| 20.1| 20.1| 20.1| 20.2| 16  | 22.8|
| 12| 39.3| 41.1| 39.5| 39.4| 25.3| 35.6| 27| 19.9| 19.8| 19.8| 19.9| 14.8| 23.1|
| 13| 43.1| 43.8| 44.8| 43.4| 38.3| 45.6| 28| 17.8| 17.7| 17.7| 17.7| 18  | 17.9|
| 14| 54.8| 48.3| 51.9| 55  | 43  | 49.1| 29| 109 | 14.6 |       |       |       |       |
| 15| 23.2| 21.1| 20.8| 23.2| 27.7| 33.1| 30| 19.5| 63.5 |       |       |       |       |

Solvent: CD$_3$Cl, δ in ppm, 125 MHz.

**DISCUSSION**

Compound 1 had earlier been isolated from *Lentinula edodes*, *Tricholoma matsutake*, *Paecilomyces sp. J300* (Ohnuma et al., 2000; Kwon et al., 2002) and from the Mangrove fungus *Aspergillus awamori* (Gao et al., 2007). The compound is one of the important pharmaceutically relevant compounds, a vitamin D precursor (Hu et al., 2017). The compound was previously found to be effective against *Staphylococcus aureus* and *Bacillus subtilis* with MIC value of 2.5–5 mg/ml (Vazirian et al., 2014). The compounds 5α,6α-epidioxyergosta-6,9(11),22-dien-3β-ol (2) had been reported by (Kobori et al., 2007; Fangkrathok et al., 2013) and 5α,6α-epidioxyergosta-6,22-dien-3β-ol (3) by (Lee et al., 2006). Compound 3 was earlier isolated from *Ganoderma applanatum* and proved to be weakly active against many gram-positive and gram negative microorganisms (Lindequeist et al., 2005).

Compound 4 was previously reported in the literature by (Li et al., 2005; Lee et al., 2006; Gao et al., 2007). Compounds 5 and 6 have been reported to be present in diverse species of the plant kingdom (Khan et al.,
1994; Inada et al., 1995; Burns et al., 2000; Jamal et al., 2008; Jain and Bari, 2010) but rare reports in the fungi and animal kingdoms. Nevertheless the antimicrobial activity of compound 5 is well documented (Gallo and Sarachine, 2009; Siddique and Saleem, 2011).

Compound 1 had the most notable inhibition against Streptococcus pyogenes by (9.7±0.58 mm), and a mixture of 2 and 3 by (9.0±0.58) at (p≤0.05). Compounds 2 and 3 did not inhibit individually any of the tested strains but a mixture of the two compounds inhibited Streptococcus pyogenes, probably due to synergism. Compound 4 also inhibited the growth of Staphylococcus aureus by 8.0±0.58 mm. All the Gram-negative bacteria and fungal pathogens were generally found to be more resistant to test compounds. Compounds 5 and 6 were not tested due to low yields. By and large, the tested compounds indicated moderate growth inhibition of a number of strains, particularly against gram-positive bacteria with the zones of inhibitions ranging from 8.0–9.7 mm.

Conclusion

The aim of the study was to isolate antimicrobial compounds from T. elegans. This has been evidenced by the moderate activity observed for the isolated and tested triterpenoid compounds. It is worthy to note that very few studies had been carried out on isolation and antimicrobial properties of the compounds, particularly from the species Trametes elegans. Therefore there was need for proper investigation, documentation of ethno-mycological importance and scientific validation of the medicinal properties of this natural resource.

COMPETING INTERESTS

The authors declare that there is no competing interests.

AUTHORS CONTRIBUTIONS

RKM was the principal investigator, MKL, AWN, JOO, PKC contributed fully to the work. All authors read and approved the manuscript.

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