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# Terpenoids from *Taraxacum officinale*

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## ABSTRACT

*Taraxacum officinale*, commonly known as dandelion is reported to exhibit antimicrobial properties. The study was conducted to isolate the dichloromethane soluble constituents of the plant which may contribute to this property. The dichloromethane extract of the leaves of *Taraxacum officinale* afforded taraxasteryl acetate (**1a**), lupeol acetate (**1b**), taraxinic acid (**2a**), 11,13-dihydrotaraxinic acid (**2b**), phytyl fatty acid ester (**3**), and squalene (**4**). The structures of **2a** and **2b** were elucidated by extensive 1D and 2D NMR spectroscopy, while **1a**, **1b**, **3**, and **4** were identified by comparison of their $^{1}H$ and $^{13}C$ NMR data with those found in the literature. The mixture of **1a** and **1b** indicated low activities against the bacteria: *E. coli, S. aureus, P. aeruginosa* and fungi: *C. albicans* and *T. mentagrophytes.* It was inactive against *B. subtilis* and *A. Niger.*

Keywords: *Taraxacum officinale,* Asteraceae, taraxasteryl acetate, lupeol acetate, taraxinic acid, 11,13-dihydrotaraxinic acid, squalene, phytyl fatty acid ester

## INTRODUCTION

*Taraxacum officinale*, commonly known as dandelion is found only in Benguet. The leaves are used for fomentations and as bitter depurant. The pounded leaves are used for poulticing and applied to remove warts (Quisumbing, 1978). A current study reported that the methanol extract of *T. officinale* exhibited antimicrobial activity against eleven out of the thirty-two tested microorganisms (Sengul *et al.*, 2009). A previous study on the roots of *T. officinale* reported the isolation of 14-O-β-D glucopyranosyl-11,13-dihydro-taraxinic acid and 14-O-β-D glucopyranosyl-taraxinic acid which were obtained from the extract that exhibited inhibitory activity on the formation of leukotriene B4 from activated human neutrophils (Yoshiki, *et al.,* 2001). Other studies on the roots reported the isolation of a new triterpene, 3β-hydroxylup-18(19)-ene-21-one (Kisiel, *et al.,* 2000) and a new γ-butyrolactone glycoside, taraxacoside Rauwald and Huang, 1985). The leaves afforded a sesquiterpene, lettucenin A which was found to be a fungitoxin (Tahara, *et al.,* 1988). The aerial part of the plant afforded scopoletin and esculetin (Komissarenko and Derkach, 1981). *T. officinale* also afforded flavoxanthin and chrysanthemaxanthin (Cadosh, *et al.,* 1978).

We now report the isolation of taraxasteryl acetate **1a**, lupeol acetate **1b**, taraxinic acid **2a**, 11,13-dihydrotaraxinic acid **2b**, squalene **3**, and phytyl fatty acid ester **4** from the leaves of *T. officinale* (Figure 1). The structures of **2a** and **2b** were elucidated by extensive 1D and 2D NMR analyses. The antimicrobial test results of a mixture of **1a** and **1b** are also reported.

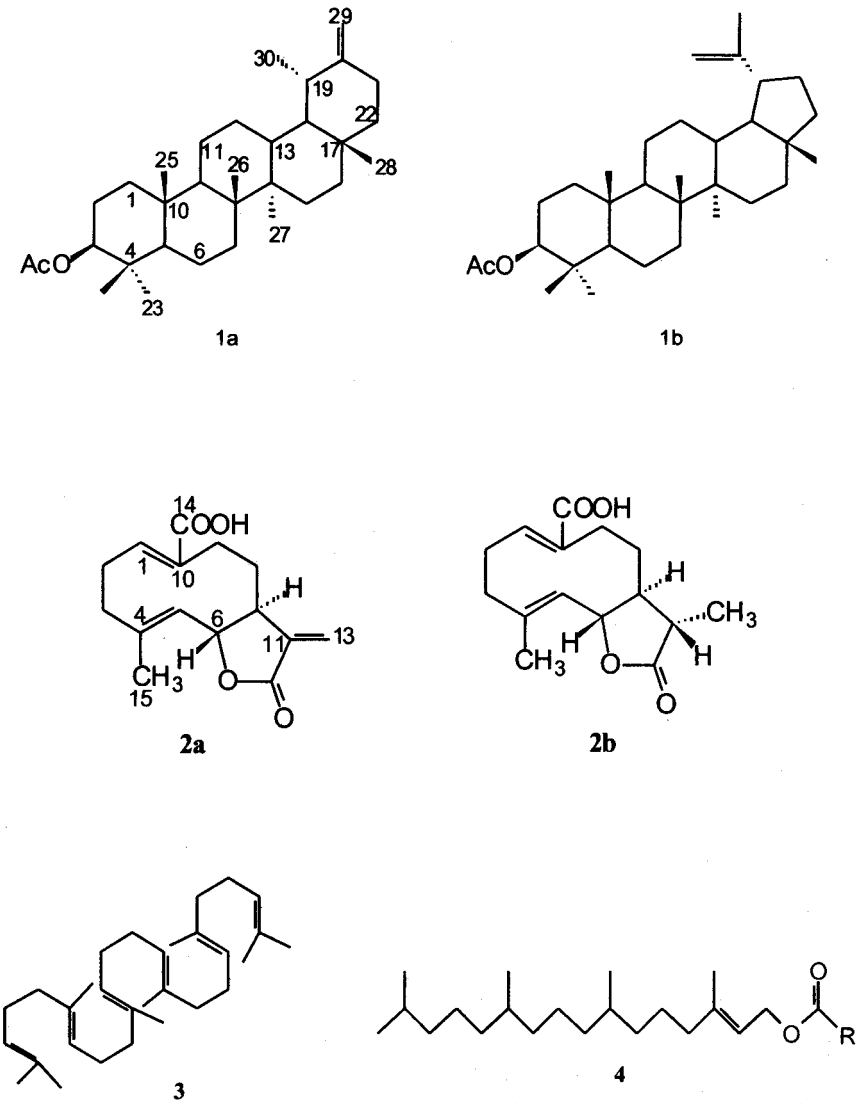



Figure 1. The six terpenoids isolated from *Taraxacum officinale*: taraxasteryl acetate **1a**, lupeol acetate **1b**, taraxinic acid **2a**, 11,13-dihydrotaraxinic acid **2b**, squalene **3**, and phytyl fatty acid ester **4**.

## MATERIALS AND METHODS

### General Experimental Procedures

NMR spectra were recorded on a Bruker Avance 400 in $CDCl_3$ at 400 MHz for $^1H$ NMR and 100 MHz for $^{13}C$ NMR Spectra. Column chromatography was performed with silica gel 60 (70-230 mesh); TLC was performed with plastic backed plates coated with silica gel $F_{254}$ ; plates were visualized by spraying with vanillin sulfuric acid and warming.

### Sample collection

The plant material was collected from Baguio City in September 2006. It was identified as *Taraxacum officinale* at the Philippine National Museum. A voucher specimen no. 109 is deposited at the Chemistry Department, De La Salle Univesity-Manila.

### Isolation

The air-dried leaves of *Taraxacum officinale* (476 g) *were groun*d in an osterizer, soaked in dichloromethane for three days, then filtered. The filtrate was concentrated under vacuum to afford a crude extract (77 g) which was chromatographed in increasing proportions of acetone in dichloromethane at 10 % increment. The dichloromethane and 10% acetone in dichloromethane fractions were rechromatographed by gradient elution from petroleum ether to 1% ethyl acetate in petroleum ether, then finally 2.5% ethyl acetate in petroleum ether. The petroleum ether fractions were rechromatographed (5×) in 2.5% ethyl acetate in petroleum ether to afford squalene (4, 17 mg). The 2.5% ethyl acetate in petroleum ether fractions were rechromatographed (7×) in 2.5 % ethyl acetate in petroleum ether to afford 1a and 1b (10 mg, colorless solid) and phytyl fatty acid ester (3, 12 mg). The 70% acetone in dichloromethane fraction was rechromatographed (8×) in dichloromethane:diethyl ether: acetonitrile (1:1:8) to afford 2a and 2b (7 mg, colorless oil).

### Antimicrobial Tests

The microorganisms used were obtained from the University of the Philippines Culture Collection (UPCC). These are *Pseudomonas aeruginosa* (UPCC 1244), *Bacillus subtilis* (UPCC 1149), *Escherichia coli* (UPCC 1195), *Staphylococcus aureus* (UPCC 1143), *Candida albicans* (UPCC 2168), *Trichophyton mentagrophytes* (UPCC 4193) and *Aspergillus niger* (UPCC 3701). The test compound was dissolved in 95% ethanol. The antimicrobial assay reported in the literature was employed (Guevara and Recio, 1985). The activity index was computed by subtracting the diameter of the well from the diameter of the clearing zone divided by the diameter of the well.

## RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried leaves of *Taraxacum officinale* afforded a mixture of triterpenes (**1a** and **1b**), a mixture of germacranolides (**2a** and **2b**), phytyl fatty acid ester (**3**) and squalene (**4**). Their structures were elucidated as follows.

A 2:1 ratio of a mixture of **1a** and **1b** was deduced from the integrals and disparity in single hydrogen peaks for the vinylic protons in the $^{1}H$ NMR spectrum. The spectrum indicated the following resonances for the major compound **1a**: vinylic protons at $\delta$ 4.61 (2H), an oxymethine proton at $\delta$ 4.50, a methyl which appeared as a doublet at $\delta$ 1.03, six methyl singlets, and an acetoxy methyl at $\delta$ 2.05. The minor compound **1b** gave resonances for vinylic protons at $\delta$ 4.57 and 4.68, an allylic methyl at $\delta$ 1.68, six methyl singlets, and an acetoxy methyl at $\delta$ 2.04. The rest of the resonances were attributed to methylene and methine protons which were mostly overlapping in the shielded region of the spectrum. These were characteristic resonances for triterpenes with vinylic methylene and acetoxy substituents.

The $^{13}C$ NMR data of **1a** and **1b** (Table 1) gave some resonances which were overlapping or closely spaced doublets with the following functionalities: a carbinyl carbon at $\delta$ 81.0; vinylic carbon (doublets) at $\delta$ 154.6 and 151.0 and 109.3 and 107.1; closely spaced acetoxy methyls $\delta$ 21.5 and 21.3; and an acetoxy carbonyl at $\delta$ 171.0. The rest of the resonances were due to other methyl, methylene, and methine carbons in the compounds.

The COSY spectrum of **1a** gave four isolated spin systems as follows: $H_2$-1/$H_2$-2/H-3; H-5/$H_2$-6/$H_2$-7; H-9/$H_2$-11/$H_2$-12/H-13/H-18/H-19/$H_3$-30, $H_2$-29/$H_2$-21/$H_2$-22; H-5/$H_2$-6/$H_2$-7; $H_2$-15/$H_2$-16 (Figure 2). The COSY of **1b** also gave four isolated spin systems as follows: $H_2$-1/$H_2$-2/H-3; H-5/$H_2$-6/$H_2$-7; H-9/$H_2$-11/$H_2$-12/H-13/H-18/H-19/$H_2$-29($H_3$-30)/$H_2$-21/$H_2$-22; H-5/$H_2$-6/$H_2$-7; $H_2$-15/$H_2$-16 (Figure 2).

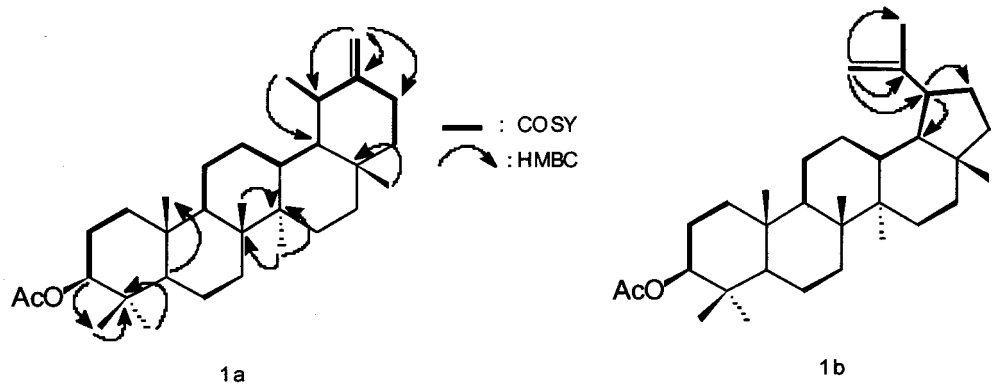



Figure 2. $^{1}H$-$^{1}H$ COSY and Key $^{1}H$-$^{13}C$ long-range correlations of **1a** and **1b**.

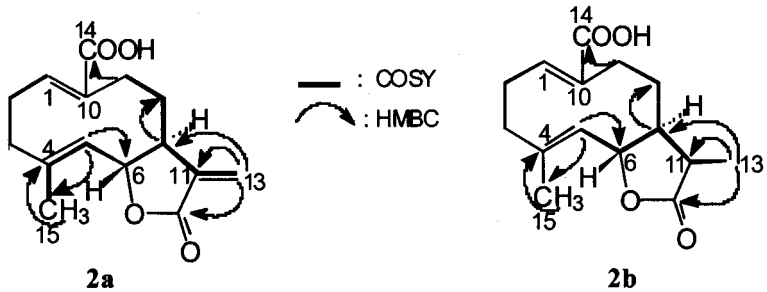
The $^{1}H$ and $^{13}C$ connectivities in **1a** and **1b** were verified by HMQC. The structures of **1a** and **1b** were elucidated by analysis of the HMBC 2D NMR data with key HMBC correlations shown in Figure 2. Thus, for **1a** and **1b**, the vinylic protons $H_2$-29 were long-range correlated to C-19, C-20 and C-21. The acetate was attached to C-3 since long-range correlations were observed between H-3 and the carbonyl carbon of the acetate. Correlations were also observed between H-3 and C-4, C-23 and C-24. All long-range correlations were consistent with the structures of **1a** and **1b**.

Literature search revealed that **1a** is taraxasteryl acetate (Reynold and Enriquez, 1996), while **1b** is lupeol acetate (Perfecto, *et al.*, 2005) as evidenced by similar $^{13}C$ NMR spectral data.

The $^{1}H$ NMR data of a mixture of **2a** and **2b** in a 3:2 ratio is presented in Table 2. The major compound **2a** indicated resonances for exocyclic methylene protons at $\delta$ 5.53 and 6.28, a vinylic proton at $\delta$ 4.93, an oxymethine proton at $\delta$ 4.60, and an allylic methyl at $\delta$ 1.64. The minor compound **2b** gave similar resonances to **2a** except in the regions where their structures differ. Thus, instead of the exocyclic methylene protons at $\delta$ 5.53 and 6.28 in **2a**, a methyl doublet at $\delta$ 1.25 (J = 7.2 Hz) and a methine multiplet at $\delta$ 2.25 for the proton to the carbonyl appeared in **2b**.

The $^{13}C$ NMR spectrum of **2a** (Table 2) gave resonances for fifteen carbons with the following functionalities: a carbonyl carbon of a lactone at $\delta$ 170.3 and a carboxylic acid at $\delta$ 178.4; olefinic carbons at $\delta$ 143.03, 126.05, 139.78, 119.60, 150.0, 130.0; an oxymethine carbon at $\delta$ 81.84; a methine carbon at $\delta$ 50.36; methylene carbons at $\delta$ 26.67, 39.3, 29.68, 30.03; and methyl carbons at $\delta$ 16.90. These resonances indicated a sesquiterpene lactone with three olefins and a carboxylic acid. The minor compound **2b** gave similar resonances to **2a** except in the region where their structures differ. Thus, the exocyclic methylene carbons at $\delta$ 139.8 and 120.0 were replaced by the methyl carbon at $\delta$ 13.2 and the methine carbon at $\delta$ 42.4. The change in structure also caused the carbon resonance at C-7 to be deshielded from $\delta$ 50.36 to 54.8.

The COSY spectrum of **2a** and **2b** showed two isolated spin systems as follows: H-1/$H_2$-2/$H_2$-3; H-5/H-6, H-15/H-7/$H_2$-8/$H_2$-9 (Figure 3).




**2a** **2b**

Figure 3. $^{1}H$-$^{1}H$ COSY and Key $^{1}H$-$^{13}C$ long-range correlations of **2a** and **2b.**

The $^{1}H$ and $^{13}C$ connectivities in **2a** and **2b** were verified by HMQC. The structure of **2a** was elucidated by analysis of the HMBC 2D NMR data with key HMBC correlations shown in Figure 3. Thus, the carboxylic acid was assigned to C-14 due to long-range correlations between the vinylic proton H-1 and $H_2$-9 and this carbon. The carbonyl of the lactone was attributed to C-12 since long-range correlations were observed between H-6, H-7, $H_2$-13 and this carbon. The exocyclic methylene was attributed to C-13 on the basis of long-range correlations between $H_2$-13 and H-7, H-11, H-12 and this carbon. The allylic methyl was assigned to C-15 since long-range correlations were observed between H-5, $H_2$-3 and this carbon. All long-range correlations were consistent with the structure of **2a**. Compound **2b** has similar long-range correlations to **2a**, except in the region where their structures differ. Thus, $H_3$-13 is long-range correlated to C-7, C-11, and C-12.

The relative stereochemistry of **2a** was deduced by NOESY (Figure 4). The allylic methyl ($H_3$-15) was close in space to the lactonic proton (H-6). This suggests that they are on the same face of the molecule. On the opposite face of the molecule, the methine proton (H-7) was close to the vinylic proton proton (H-5), which was in turn close to the vinylic proton (H-1).

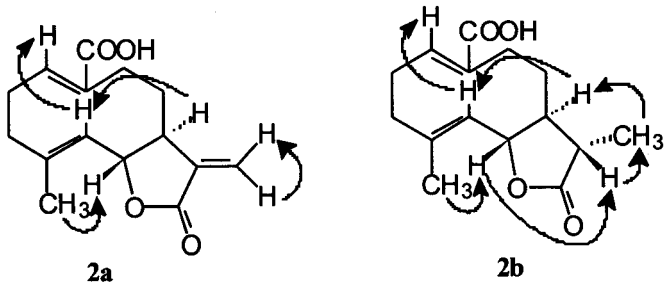



Figure 4. NOESY Correlations of **2a** and **2b**

Literature search revealed that **2a** is taraxinic acid as evidenced by similar $^{1}H$ NMR data (Hansel, *et al.*, 1980). This compound exhibited potent antiproliferative activity against human leukemia-derived HL-60. This suggests that taraxinic acid may have potential as a therapeutic agent in human leukemia (Choi, *et al.*, 2002).

The relative stereochemistry of **2b** was deduced by NOESY (Figure 4). The allylic methyl ($H_3$-15) was close in space to the lactonic proton (H-6), which was in turn close to the methine proton (H-11). This suggests that they are on the same face of the molecule. On the opposite face of the molecule, the methyl (H-13) was close to the methine proton (H-7), which was in turn close to the vinylic proton (H-5).

Based on literature, **2b** was identified as 11,13-dihydrotaraxinic acid (Tanaka, *et al.*, 2001). The 14-O-$\beta$-D-glucoside of **2b** significantly down regulated the cell surface expression of either intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion -1 (VCAM-1), or both, suggesting that it may be effective in inhibiting allergic inflammation or rejective reactions by transplants (Tanaka, *et al.*, 2001).

Compounds **3** and **4** were identified by comparison of their $^1$H NMR spectral data with those reported in the literature for squalene (Inte, *et al.,* 1998) and phytyl fatty acid ester (Ragasa, *et al.,* 2004), respectively. Squalene significantly suppresses colonic ACF formation and crypt multiplicity. This strengthens the hypothesis that squalene possesses chemopreventive activity against colon carcinogenesis (Rao *et al.* 1998). Squalene has cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties (Farvin *et al.* 2006). Phytyl fatty acid ester exhibited low antifungal activity against *A. niger* and *C. albicans* (Ragasa, *et al.,* 2004).

As part of our continuing search for antimicrobial compounds from Philippine medicinal plants, the mixture of **1a** and **1b** were tested for possible antimicrobial activities by the agar well method. Results of the study (Table 3) indicated low activities against the bacteria: *E. coli, S. aureus, P. aeruginosa* and fungi: *C. albicans* and *T. mentagrophytes.* It was inactive against *B. subtilis* and *A. Niger.*

## Acknowledgement

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Table 1. 100 MHz $^{13}C$ NMR Spectral Data of **1a** and **1b** in $CDCl_3$.

| Position | δc, 1a | δc, 1b |
|---|---|---|
| 1 | 38.5 | 38.4 |
| 2 | 23.7 | 23.7 |
| 3 | 81.0 | 81.0 |
| 4 | 38.1 | 37.1 |
| 5 | 55.5 | 55.5 |
| 6 | 18.2 | 18.2 |
| 7 | 34.0 | 34.2 |
| 8 | 40.93 | 40.9 |
| 9 | 50.35 | 50.4 |
| 10 | 37.0 | 37.1 |
| 11 | 21.3 | 21.0 |
| 12 | 25.5 | 25.1 |
| 13 | 37.1 | 38.1 |
| 14 | 42.2 | 42.8 |
| 15 | 27.9 | 27.4 |
| 16 | 36.3 | 35.6 |
| 17 | 43.0 | 43.0 |
| 18 | 48.7 | 48.3 |
| 19 | 39.0 | 48.0 |
| 20 | 154.6 | 151.0 |
| 21 | 25.6 | 29.8 |
| 22 | 38.9 | 40.0 |
| 23 | 28.0 | 28.0 |
| 24 | 16.5 | 16.5 |
| 25 | 16.3 | 16.2 |
| 26 | 15.9 | 16.9 |
| 27 | 14.7 | 14.7 |
| 28 | 18.0 | 18.0 |
| 29 | 107.1 | 109.3 |
| 30 | 25.5 | 19.3 |
| Oac | 21.5 | 21.3 |
| | 171.0 | 171.0 |

Table 2. 400 MHz $^{1}H$ NMR and 100 MHz $^{13}C$ NMR Spectral Data of **2a** and **2b** in $CDCl_3$.

| Position | $\delta_C$, **2a** | $\delta_H$ *mult.*[a] (*J* Hz) (**2a**) | $\delta_C$, **2b** | $\delta_H$ *mult.* (*J* Hz) (**2b**) |
|---|---|---|---|---|
| 1 | 149.7 | 5.79 dd (4.4, 12.8) | 149.7 | 5.78 (4.4, 12.8) |
| 2 | 26.7 | 2.35, 3.40 | 26.6 | 2.35, 2.40 |
| 3 | 39.3 | 2.28, 2.38 | 39.3 | 2.28, 2.38 |
| 4 | 143.0 | - | 143.0 | - |
| 5 | 126.0 | 4.93 d (10) | 126.1 | 4.83 d (10) |
| 6 | 81.8 | 4.60 dd (8.8, 10) | 81.4 | 4.58 dd (8.8, 10) |
| 7 | 50.4 | 2.58 | 54.8 | 1.65 |
| 8 | 30.0 | 1.95 (2H) | 30.0 | 1.95 (2H) |
| 9 | 29.7 | 2.20 (2H) | 29.7 | 2.20 (2H) |
| 10 | 130.0 | - | 130.0 | - |
| 11 | 139.8 | - | 42.4 | 2.25 |
| 12 | 171.9 | - | 170.3 | - |
| 13 | 120.0 | 5.53 d (3.2), 6.28 d (3.2) | 13.2 | 1.25 d (7.2, Me) |
| 14 | 178.4 | - | 178.4 | - |
| 15 | 16.9 | 1.64 d (1.2, Me) | 16.8 | 1.64 d (1.2, Me) |

[a]multiplet unless otherwise indicated

Table 3. Antimicrobial Test Results on 1a and 1b

| Organism | Sample (30 µg) | Clearing Zone (mm)[a] | Antimicrobial Index (AI) |
|---|---|---|---|
| *E. coli* | **1a and 1b** | 11 | 0.1 |
| | Chloramphenicol[a] | 23 | 2.8 |
| *P. aeruginosa* | **1a and 1b** | 11 | 0.1 |
| | Chloramphenicol[a] | 8 | 0.3 |
| *S. aureus* | **1a and 1b** | 11 | 0.1 |
| | Chloramphenicol[a] | 25 | 3.2 |
| *B. subtilis* | **1a and 1b** | - | 0 |
| | Chloramphenicol[a] | 20 | 2.3 |
| *C. albicans* | **1a and 1b** | 12 | 0.2 |
| | Canesten, 0.2g[b] | 18 | 0.8 |
| *T. mentagrophytes* | **1a and 1b** | 12 | 0.2 |
| | Canesten, 0.2g[b] | 55 | 4.5 |
| *A. niger* | **1a and 1b** | - | 0 |
| | Canesten, 0.2g[b] | 23 | 1.3 |

[a]Average of three trials [a]chloramphenicol disc - 6 mm diameter, [b]contains 1% chlotrimazile