

Chemical Constituents of *Dissotis perkinsiae* (Melastomaceae) and Their Antimicrobial Activity

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ABSTRACT

The phytochemical study of the Methanol/dichloromethane extract of the leaves of *Dissotis perkinsiae* led to the isolation and identification seven compounds: Ursolic acid (1); Sitosterol- β -D-glucoside (2); Isoquercitrin (3); Quercetin-3-O- β -galactoside (4); Kaempferol-3-O- β -D-glucoside (5); Kaempferol-7-O- β -D-glucoside (6); Trans-Tiliroside (7). Their structures were elucidated on the basis of spectroscopic analysis and by comparison of their spectral data with those reported in the literature. The results of antimicrobial activity indicated that the MIC vary from >0.5 to 0.00078 mg/mL on yeasts and from >0.5 to 0.25 mg/mL on bacteria. Sitosterol- β -D-glucoside was the most active with the broad spectrum (MIC=0.125mg/mL on *C. albicans*, MIC=0.0625 mg/mL on *C. krusei* and MIC=0.0078 mg/mL on *C. parapsilosis*). Kaempferol-7-O- β -D-glucoside was the most active on *C. krusei* (MIC=0.0039 mg/mL). The anti yeast activity of Sitosterol- β -D-glucoside and Kaempferol-7-O- β -D-glucoside were better than fluconazole (0.032mg/mL) on *C. parapsilosis* (MIC 0.0078 mg/mL) and *C. krusei* (0.0039 mg/mL) respectively. Sitosterol- β -D-glucoside and Trans-Tiliroside showed weak inhibitory activity against *S. enteric* and *S. aureus* with MIC value of 0.5mg/mL. Respectively this inhibitory effect was lower activities observed with Chloramphenicol and Ampicillin (0.000488 mg/mL). The results of this study suggest that *Dissotis perkinsiae* represent an untapped source of compounds with potential antimicrobial activity that could be explored in the development of new therapeutic natural products.

INTRODUCTION

Application of natural products with therapeutic properties is as old as human civilization. These products have been exploited for human use from thousands of years, and plants have been the chief source of compounds used for medicine. In fact, plants represent the largest sources of active substances that can be used in medical therapy due to the large structural diversity that these metabolites exhibit, being perhaps the oldest

source of medicines for man (Brandão *et al.*, 2010). Drugs derived from natural products with antibacterial, antifungal, anticoagulant, antiparasitic, immune suppressive and anticancer activity are capable of treating 87 % of categorized human diseases (Newman *et al.*, 2003). Of the 520 new drugs approved between 1983 and 1994, 39 % were natural products or derived from natural products and 60–80 % of antimicrobial were derived from natural products (Cragg *et al.*, 1997). Such data justifies work in the area of natural products, particularly in view of its importance in the search for new drugs against bacterial and fungal infections. The genus *Dissotis*, member of the Melastomaceae family is one of the most important genus in tropical forests which represent about 140 species in Africa (Loigier, 1994).

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They are climbing shrubs, shrubs or small trees of up to 2 m, and are found in countries as Democratic Republic of Congo, Benin, Nigeria, Cote d'Ivoire, Togo and Cameroon (Maluma, 2005). Several species are used in folk medicine, mainly as anti-diarrheic, antimicrobial, skin diseases, antioxidant, fever, malaria, cleansing, antitumoral, anti-rheumatic, and anti-inflammatory agents, as well as to lower blood cholesterol. *Dissotis perkinsiae*, found in tropical areas of Nigeria, Togo and Cameroon is a shrub with the height of 90-150 cm. The leaves are purple or pink with a diameter of about 10.5 cm (Hutchinson and Dalziel, 1954). It is used in traditional medicine for the treatment of the skin diseases and malaria (Haxaire, 1979). In previous studies, some triterpenoids, flavonoids and tannins were identified (Ndjateu *et al.*, 2014). Such data have encouraged the investigation of *Dissotis perkinsiae* with the aim to search for compounds with antibacterial and antifungal activities.

MATERIAL AND METHODS

General experimental procedures

Mass spectral data [Electrospray ionization mass spectrometry (ESI-MS)] were measured on a Waters Synapt HDMS spectrometer. NMR spectra were recorded with a Varian spectrometer at 400 MHz. Chemical shifts (δ) were quoted in parts per million (ppm) from the internal standard tetramethylsilane (TMS). Deuterated solvents dimethyl sulfoxide (DMSO-*d*₆), and chloroform (CDCl₃) were used as solvents for the NMR experiments. Column chromatography was performed on silica gel 60 [(0.2-0.5 mm) and (0.2-0.063 mm)] mesh (Sigma-Aldrich, Germany). Pre-coated silica gel 60 F254 thin layer chromatography (TLC) plates (Merck, Germany) were used for monitoring fractions and spots were detected with UV light (254 and 365 nm) and then sprayed with 30% sulphuric acid (H₂SO₄) followed by heating to 110 °C.

Sample collection

Dissotis perkinsiae leaves were harvested in Bangoua-Cameroon, (May, 2012) and identified by Mr. Victor Nana (Plant taxonomist) of the Cameroon National Herbarium (HNC), where a voucher specimens are deposited (24719/SRF/Cam). Then, leaves were dried at room temperature and powdered.

Extraction and isolation

1700 g of powdered leaves were extracted by maceration in seven liters (7L) of dichloromethane/methanol (DCM/MeOH, 1:1, v/v) mixture at room temperature for 72 h. The filtrate was concentrated in rota vapor (Büchi R-200) under reduce pressure at 65 °C to yield 240g of extract. Dried extract was dissolved in water and successively extracted with hexane, dichloromethane, ethyl acetate and n-butanol. All these extracts collected were preserved for chemical analysis.

Hexane fraction (18.0 g) was subjected to vacuum liquid chromatography (VLC) over TLC grade silica gel (GF254) and eluted with *n*-hexane/AcOEt mixture with increasing polarity from

n-hexane to AcOEt. Ten fractions (F1-F10) were obtained after combining subfractions according to their TLC profiles. Fraction F2 obtained at a polarity of *n*-hexane/AcOEt (85:15), yield Ursolic acid (2,15mg). Fraction F8 obtained with *n*-hexane/AcOEt (20:80) was subjected to other column chromatography with *n*-hexane/AcOEt increasing polarity to yield Sitosterol-3-*O*- β -D-glucoside (3.26 mg).

The ethyl acetate soluble fraction was subjected to column chromatography (CC) over silica gel (4x150 cm, 250 g, 70 - 230 mesh) and eluted with *n*-hexane/AcOEt mixture with increasing polarity to afford 66 subfractions combined in 6 fractions (F1-F6) according to their TLC profiles. Fraction F5 was subjected to other column chromatography to yield Isoquercitrin (4.17 mg), Quercetin-3-*O*- β -galactoside (5.9 mg), Kaempferol-3-*O*- β -D-glucoside (6,14 mg) and Kaempferol-7-*O*- β -D-glucoside (7.12mg). Fraction F6 was subjected to column chromatography (CC) and eluted with *n*-hexane/AcOEt mixture with increasing polarity to yield Trans-Tiliroside (8.18 mg).

Antimicrobial activity of Extracts/Fractions and Compounds Tested microorganisms

The micro organisms include bacteria (*Staphylococcus aureus* NR46374, *Klebsiella pneumonia* NR41916, *Salmonella enterica* NR13555, *Shigella flexneri* NR518) and fungal strains (*Candida albicans* ATCC P37037, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019) originally obtained from BEI resources and American Type Culture Collection respectively. The organisms were maintained on agar slope at 4°C and sub-cultured for 24h and 48 h respectively for bacteria and yeasts before use.

Preparation of stock solution of plants crude extracts, fractions and compounds

The different stock solution of crude extracts, fractions and compounds were prepared by dissolving 2 mg in 1 mL of DMSO 10 % for a final concentration of 2 mg/mL. Reference antibiotics were prepared in the same condition by dissolving 512 μ g of Fluconazole (Sigma Aldrich) and 2mg of Chloramphenicol and Ampicillin (Sigma Aldrich) in 1 mL of DMSO 10 % to yield 512 μ g/mL and 2 mg/mL respectively. After preparation, the different stock solutions were sterilized with 0.20 μ M Syringe Filter and store at -20°C before use.

Antifungal activity

Determination of minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

The minimum inhibitory concentration (MIC) was determined according to Clinical Laboratory Standards Institute M27-A3 microdilution method (CLSI, 2008) using (12 x 8 wells) microtitre plates. In the well of the first line (1-12), 100 μ L of RPMI 1640 (Sigma Aldrich) medium were introduced and 100 μ L in the other well of the plates. Later on, 100 μ L of stock solution of crude extracts, fractions and compounds were added to the first well. The medium and extract/compound in the first well were mixed thoroughly before transferring 100 μ L of the resultant

mixture to the well of the second line. Serial two-fold dilutions of the test samples were made and 100 μL of inoculum standardized at 2.5×10^4 cells/mL were introduced in the entire well containing the test substances except the column of blank which constitute the sterility control. The concentrations ranged from 0.000488 to 0.5 mg/mL and from 1.25 $\mu\text{g/mL}$ to 128 $\mu\text{g/mL}$ for crude extracts/fractions/compounds and fluconazole respectively. After 48 hours of incubation at 37°C, the turbidity was observed as indication of growth. MIC was defined as the lowest concentration inhibiting the growth of yeasts. The MFC was determined by transferring 50 μL aliquots of the clear wells into 150 μL of freshly prepared broth medium and incubating at 37°C for 48 hours. The MFC was regarded as the lowest concentration of test sample which did not produce turbidity as above, indicating no microbial growth. All tests were performed in triplicates.

Antibacterial activity

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

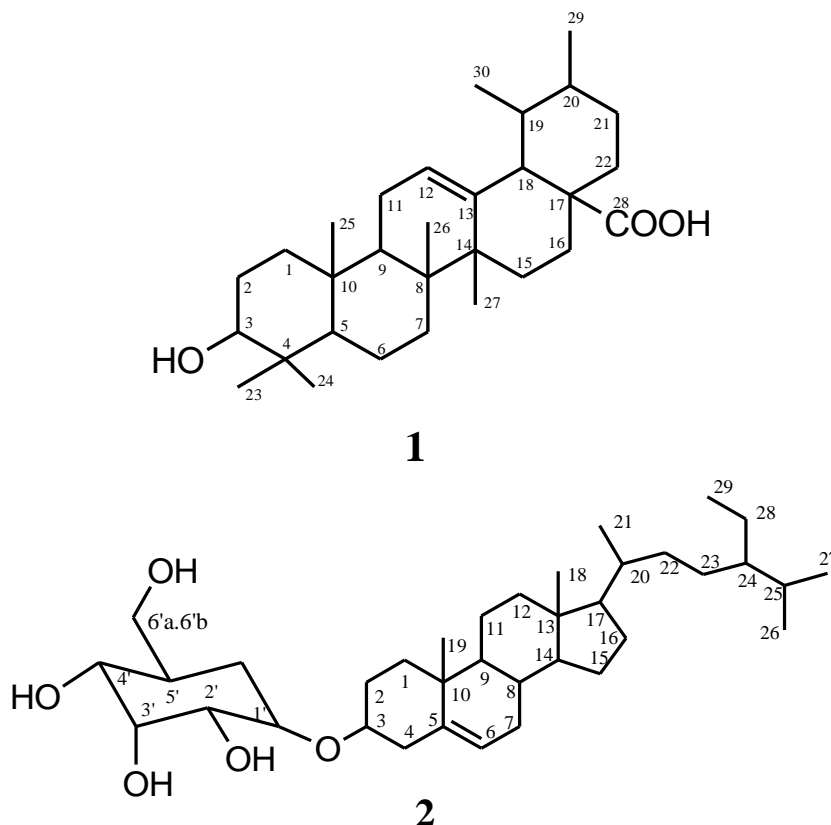
The MIC was determined according to Clinical laboratory Standards Institute (CLSI) M38-A microdilution method (CLSI, 2008) using 96 wells microtitre plates. 100 μL of Muller Hinton Broth (Lab M Limited Topley House) was introduced in the wells of column 1 (1A-1H), and 100 μL in the remaining wells. Later on, 100 μL of stock solutions of plants extracts/compounds at 2 mg/mL were added to the first well. The medium and sample in the first well were mixed thoroughly and serially diluted by transferring 100 μL from wells of column 1 to wells of column 2, and so on till column 11.

Thereafter, 100 μL of the inoculums were introduced in each well containing the test substances. The wells of column 12 are used as blank. The concentration of substances range from 0.5 mg/mL to 0.000488 mg/mL. After an incubation period at 37°C for 24 hour, turbidity was observed as indication of growth. Thus, the lowest concentration inhibiting the growth of bacteria was recorded as the MIC. The MBC was determined by transferring 50 μL aliquots of the clear wells into 150 μL of freshly prepared broth medium and incubating at 37°C for 24 hours. The MBC was regarded as the lowest concentration of test sample which did not produce turbidity as above, indicating no microbial growth. All tests were performed in triplicates.

RESULTS

Fractionation and isolation of compounds

The extracts from *Dissotis perkinsiae*, were fractionated by silica gel column chromatography to afford eight compounds (1-7) identified as Ursolic acid (1) (Seebacher *et al.*, 2003); Sitosterol- β -D-glucoside (2) (Mizanur *et al.*, 2009; Nono *et al.*, 2014; Mai *et al.*, 2012); Isoquercitrin (3) (Mortada *et al.*, 2010; Danielly *et al.*, 2007; Agus *et al.*, 2014); Quercetin-3-O- β -galactoside (4) (Mortada *et al.*, 2010; (Wang Z *et al.*, 1998; Chen L *et al.*, 2005); Kaempferol-3-O- β -D-glucoside (5) (Ferreira *et al.*, 2010; Abdelaaty *et al.*, 2005); Kaempferol-7-O- β -D-glucoside (6) (Xiaoshu Zhang *et al.*, 2013); Trans-Tiliroside (7) (Mekhelfi *et al.*, 2014). The structures of the compounds were determined by analysis of their NMR data and comparison with those reported in the literature (Figure 1).



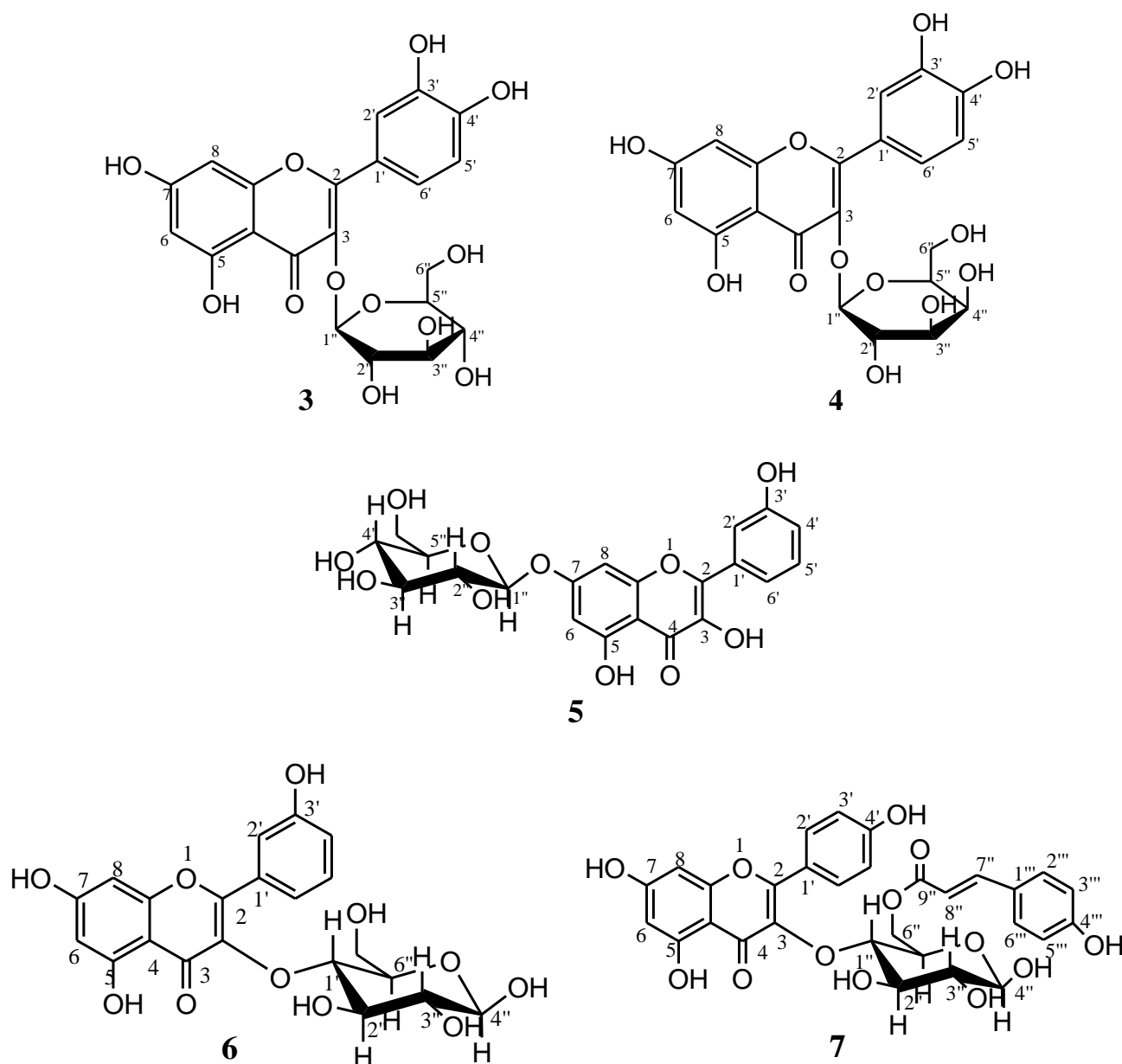


Fig. 1: Compounds isolated from *Dissotis perkinsiae*.

Antimicrobial results

The results of antibacterial and antifungal activities of extracts, fractions and compounds from *Dissotis perkinsiae* are presented in tables 1, 2, 3 and 4. The results indicate that the MIC vary from >0.5 to 0.00078 mg/mL on yeasts and from >0.5 to 0.25 mg/mL on bacteria. This inhibition was found to be extracts, compounds and microorganism dependent. The activity of ethyl acetate and hexane fractions were better than crude extract on the three yeast species. Compounds 2 (Sitosterol- β -D-glucoside) was the most active with the broad spectrum. It is the most active on *C. albicans* and *C. parapsilosis* while, compounds 6 (Kaempferol-7-O- β -D-glucoside) was the most active on *C. krusei*. The antiyeasts

activity of Sitosterol- β -D-glucoside and Kaempferol-7-O- β -D-glucoside were better than fluconazole (0.032 mg/mL) on *C. parapsilosis* (MIC 0.0078 mg/mL) and *C. krusei* (0.0039 mg/mL) respectively. Sitosterol- β -D-glucoside and Kaempferol-7-O- β -D-glucoside exhibited fungicidal activity on the tested microorganisms, high light the ability of these compounds to kill *Candida* species. On bacteria, the activity of crude extract was better than fractions. Compounds 2 and compounds 7 showed weak inhibitory activity respectively against *S. enterica* and *S. aureus* with MIC value of 0.5 mg/mL. This inhibitory effect was less than activity of Chloramphenicol and Ampicillin (0.000488 mg/mL).

Table 1: MIC of extract, fractions and compounds on yeasts strains (mg/mL).

Microorganisms	Minimal Inhibitory Concentration										
	Extracts			Compounds							Reference
	DP	DPAE	DPH	1	2	3	4	6	7	Fluconazole	
<i>C. albicans</i> P37037	>0.5	0.25	0.5	0.5	0.125	>0.5	0.5	0.5	>0.5	0.032	
<i>C. krusei</i> 6258	>0.5	0.5	0.5	>0.5	0.0625	0.25	0.125	0.0039	0.25	0.032	
<i>C. parapsilosis</i> 22019	>0.5	0.5	0.5	>0.5	0.0078	>0.5	>0.5	0.5	0.25	0.032	

DP: Methanol/dichloromethane extract of leaves of *Dissotis perkinsiae*; DPAE: Ethylacetate fraction from DP extract; DPH: Hexane fraction from DP extract; Ursolic acid (1); Sitosterol- β -D-glucoside (2); Isoquercitrin (3); Quercetin-3-O- β -galactoside (4); Kaempferol-7-O- β -D-glucoside (6); Trans-Tiliroside (7).

Table 2: MFC of extract, fractions and compounds yeasts strains (mg/mL).

Microorganisms	Minimal Fongicidal Concentration										
	Extracts			Compounds							Reference
	DP	DPAE	DPH	1	2	3	4	6	7	Fluconazole	
<i>C. albicans</i> P37037	>0.5	>0.5	>0.5	>0.5	0.5	>0.5	>0.5	0.5	>0.5	0.032	
<i>C. krusei</i> 6258	>0.5	>0.5	>0.5	>0.5	0.25	>0.5	>0.5	0.0312	>0.5	0.032	
<i>C. parapsilosis</i> 22019	>0.5	>0.5	>0.5	>0.5	0.0312	>0.5	>0.5	0.5	>0.5	0.032	

DP: Methanol/dichloromethane extract of leaves of *Dissotis perkinsiae*; DPAE: Ethylacetate fraction from DP extract; DPH: Hexane fraction from DP extract; Ursolic acid (1); Sitosterol- β -D-glucoside (2); Isoquercitrin (3); Quercetin-3-O- β -galactoside (4); Kaempferol-7-O- β -D-glucoside (6); Trans-Tiliroside (7).

Table 3: MIC of extract, fractions and compounds on bacteria strains (mg/mL).

Microorganisms	Minimal Inhibitory Concentration											
	Extracts			Compounds							Reference	
	DP	DPAE	DPH	1	2	3	4	6	7	Choremphenicol	Ampicillin	
<i>S. aureus</i> NR46374	0.25	0.25	0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.5	0.000488	0.000488	
<i>K. pneumonia</i> NR41916	0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.0039	0.000488	
<i>S. enterica</i> NR13555	>0.5	>0.5	>0.5	>0.5	0.5	>0.5	>0.5	>0.5	>0.5	0.000488	0.000488	

DP: Methanol/dichloromethane extract of leaves of *Dissotis perkinsiae*; DPAE: Ethylacetate fraction from DP extract; DPH: Hexane fraction from DP extract; Ursolic acid (1); Sitosterol- β -D-glucoside (2); Isoquercitrin (3); Quercetin-3-O- β -galactoside (4); Kaempferol-7-O- β -D-glucoside (6); Trans-Tiliroside (7).

Table 4: MBF of extract, fractions and compounds on bacteria strains (mg/mL).

Microorganisms	Minimal Bactericidal Concentration											
	Extracts			Compounds							References	
	DP	DPAE	DPH	1	2	3	4	6	7	Choremphenicol	Ampicillin	
<i>S. aureus</i> NR46374	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.000488	0.000488	
<i>K. pneumonia</i> NR41916	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.0039	0.000488	
<i>S. enteric</i> NR13555	>0.5	>0.5	>0.5	>0.5	0.5	>0.5	>0.5	>0.5	>0.5	0.000488	0.000488	
<i>S. flexineri</i> NR518	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.000488	0.000488	

DP: Methanol/dichloromethane extract of leaves of *Dissotis perkinsiae*; DPAE: Ethylacetate fraction from DP extract; DPH: Hexane fraction from DP extract; Ursolic acid (1); Sitosterol- β -D-glucoside (2); Isoquercitrin (3); Quercetin-3-O- β -galactoside (4); Kaempferol-7-O- β -D-glucoside (6); Trans-Tiliroside (7).

DISCUSSION

Ursolic acid, quercetin, β -sitosterol glucoside, were previously reported from the same plant by (Ndjateu *et al.*, 2014).

The antimicrobial activity of Sitosterol- β -D-glucoside and Kaempferol-7-O- β -D-glucoside can be explained by the substitution of hydroxyl group in the β -Sitosterol and Kaempferol skeletons a glucoside group which enhance their activity. In fact, the glucoside group is known to be responsible for antimicrobial activity due to their ability to form a complex with cell membrane of microorganisms, and thus, inhibiting microbial growth (Cowan, 1999). The difference in activity of the tested compounds on yeasts and bacteria can be explained by genetic distance or specific mechanisms of action on the cell membrane of the microorganisms. In the same way as many antifungal drugs, these compounds could act on ergosterol biosynthesis. Absent in bacteria, ergosterol is one of the key components of the fungal cell membrane and the main sterol of yeasts and other fungi. It is necessary for the growth of cells and normal membrane function. It serves as a bioregulator of membrane fluidity, asymmetry and

membrane integrity and contributes to the proper function of membrane-bound enzymes. Thus, the inhibition of ergosterol biosynthesis lead to the death of fungi cell (Lupetti *et al.*, 2002).

CONCLUSION

The results provide justification for the use of *Dissotis perkinsiae* in folk medicine to treat various diseases. This study might be considered as a prelude to discover new antimicrobial agents to the problematic pathogenic bacteria and fungi. Moreover, the broad spectrum activity of isolated compounds as Sitosterol- β -D-glucoside gives the opportunity for possible discovery of new, effective components for downstream clinical development. Whereas, many studies have to be carry out to obtain insight into its mode of action and antifungal activity.

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