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Antibacterial Stilbene Derivative from the Leaves of Combretum paniculatum

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Abstract
Combretaceae family comprises 20 genera with 600 species of which the genus *Combretum* and *Terminalia* contain the largest number of species, 370 and 200 species, respectively. The genus *Combretum* is used in folk medicine for the treatment of various diseases such as stomach pain and diarrhea. Silica gel column fractionation of the leaf extract of the leaves of *C. paniculatum* led to the isolation of two compounds; myristic acid and a new stilbene derivative (1-(2',6'-dihyroxphenyl-2-(4"-hdroxyphenyl))-1,2-ethane-diol). The structures of these compounds were determined with the help of spectroscopic methods (UV-Vis, IR, and NMR). Phytochemical screening tests revealed the presence of terpenoids, tannins, saponins, alkaloids and phenols. Antibacterial analysis of the hexane, EtOAc and methanol extracts using paper disc diffusion method against *Staphylococcus aureus, Escherichia coli, Klebsella preumanin* and *Proteus mirabilis* revealed the *n*-hexane and ethyl acetate extracts were active against *S. aureus*, *E. coli* and *P. mirabilis* at concentration of 1.5mg/mL each. On the other hand, the methanol extract is not active against *E. coli* but showed activity against *K. preumanin.* The stilbene derivative (**2**) was found to be active against all bacterial strains tested.
Keywords: Phytochemical, antibacterial, *C. paniculatum*, Myristic acid, stilbene derivative

INTRODUCTION

Medicinal plants have been used since ancient times in virtually all cultures as a source of medicines [Richardson, 2001; Farnsworth and Soejarto, 1991; Verpoorte, 2000]. The use of these medicinal plants is increasing and this is evident by the fact that 75 % of new anticancer drugs marketed between 1981 and 2006 have been derived from plant sources [Cock, 2011]. The Combretaceae family is distributed in approximately 20 genera with 600 species. The largest genera are *Combretum* and *Terminalia,* with about 370 and 200 species, respectively [Martini et al., 2004; Sowemimo et al., 2009]. *Combretum paniculatum* belongs to the Combretacea family. It is ascadent shrub with tailing branches. The leaves of the plants are used in folk medicine for the treatment of various diseases such as stomach pain and diarrhea [Banskota et al., 2000; Abera, 2014; Asres et al., 2001].

The ethanol extract of the leaves of *C. paniculatum* showed significant cytotoxic activity against breast cancer cells [Pettit et al., 1988]. Combretastin A-4 compound is an anticancer molecule, which have a potent target in cancer chemotherapy in cell division [Pettit et al., 1988]. In Ethiopia, *C. paniculatum* (fig 1) is used for treatment of ringworm and wounds [Abera, 2014]. Despite the traditional use of this plant against wide range of diseases, there is limited scientific report on the chemical constituents and antibacterial activities of the leaves of *C. paniculatum.*

Fig 1: Photo taken in July 2016 and February, 2017 by Fekadu Asefa from Debeso, Sayo Nole, West Wollega, Oromia, Ethiopia

We hereby report the isolation and characterization of two compounds from the leaves of *Combretum paniculatum*; myristic acid and stilbene derivative (1-(2',6'-dihyroxphenyl-2-(4"-hdroxyphenyl))-1,2-ethane-diol). The antibacterial activities of the extracts and stilbene derivative isolated from this species for the first time is also incorporated herein.

MATERIALS AND METHODS

Chemicals, instruments and apparatus

Melting points were recorded using digital melting point apparatus. Analytical thin layer chromatograms were run

on a readymade 0.2mm thick layer of silica gel GF254 (Merck) coated on aluminium plate. Column chromatography was performed using silica gel (230-400 mesh) Merck. ^{1}H, ^{13}C and DEPT-NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz in $CDCl_3$ and DMSO-d_6. The ultraviolet visible (UV/Vis) spectra were taken on T60 UV-Visible spectrophotometer (200-600 nm). Infrared (IR) spectra were obtained on Perkin-Elmer 65FT ((IR ν_{max} KBr (4000-400) cm^{-1}) infrared spectrometer using KBr pellets. Solvents used including n-hexane, EtOAc, chloroform and methanol were analytical grade and all of them were purchased from Fine Chemical General Trading PLC, Addis Ababa, Ethiopia

Plant material ccollection

The leaves of *C. paniculatum* were collected in February, 2017 from Debeso, Sayo Nole Woreda, Western Wollega zone, Oromia region, Ethiopia, Which is 569 km from Addis Ababa found to Western part of the country. The plant material was authenticated by Mr Shambel Alemu and voucher specimen (Voucher No-CP 001) was deposited in the National Herbarium of Ethiopia, Addis Ababa University. The freshly collected plant leaves were washed with tap water, taken and then dried in air under shade. The dried leaves of the plant were grinded using mortar and pestle.

Extraction and isolation of ccompounds

The powdered leaves of *C. paniculatum* (300 g) were successively extracted with each 1.5 mL of *n*-hexane, EtOAc and MeOH for 72 hours at room temperature, filtered and concentrated to give 4.51 g (1.5%), 8.86 g (2.9%) and 21.5 g (7.2%), respectively. Ethyl acetate extract (8 g) was adsorbed and subjected to silica gel (150 g) column chromatographic using ethyl acetate in n-hexane. A total of 41 fractions each 50 mL were collected. Fraction 4, eluted with *n*-hexane:EtOAc (7:3) as eluent, which showed 4 spots on TLC was rechromatographed over silica gel column chromatography using n-hexane:EtOAc:MeOH as an eluent to furnish 31 fractions each 10 mL. Fraction 26 (8 mg) showed a single spot and found to be compound **1**. Fraction 15-19 (360 mg) of the ethyl acetate extract, eluted with *n*-hexane:EtOAc (1:4), after silica gel column chromatography furnished compound 2 (36 mg).

Phytochemical screening of crude extracts

The preliminary phytochemical screening tests for the presence of tannins, saponins, alkaloids, terpenoids, flavonoids, anthraquinones and phenols were qualitatively determined using literature protocols [Mamta and Jyoti, 2012].

Evaluating antibacterial activities

The *in vitro* anti-bacterial activity of the extracts of the leaves of *C. paniculatum* was evaluated using the American Test Culture Collection (ATCC) bacterial strains. Four bacterial strains were obtained from Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory Center, Adama, Ethiopia. Three Gram-negative bacterial strains (*Escherichia coli* ATCC 25922, *Klebsella preumanin* ATCC 35032, *Proteus mirabilis* ATCC 25923) and one Gram–positive (*Staphylococcus aureus* ATCC 25923) human bacterial pathogens were selected for the study. The antibacterial activities were determined using well diffusion method against different strains of bacteria [Zwadyk, 1992]. Ciprofloxacin and chloroform were used as a positive and negative control, respectively.

Media preparation

Mueller Hinton agar was prepared by dissolving the solid media in distilled water. The solution was then sterilized in autoclave at 121^{o}C for 15 minutes, cooled then poured in Petri dishes. The solution was then left to solidify.

Inoculation and incubation

Antibacterial activity was done on Mueller Hinton agar. 1 mL of bacteria suspension was uniformly spread on the sterile Mueller Hinton Agar Petri dish. Standard solutions of 1.5 mg/mL concentration of the extracts and isolated compounds were prepared and 10 µL solutions from the concentration were loaded to the discs in different replications. 6 mm-diameter wells were cut from the agar using a sterile cork-borer and the sample were placed in the wells. The Petri dish was then placed in an incubator for 24 hours at 37^{o}C. At the end of incubation period, the inhibition diameter was measured and expressed in millimeters. 3 drops of each bacterial suspension were applied on the Petri dish to compare with 1 drop of chloroform applied on it. Ciprofloxacin were used as a positive control while chloroform was used as a negative control group. Antibacterial activity was determined by measuring the inhibition zone diameter (mm) against each test organism.

RESULTS AND DISCUSSION

Extraction yield

The leaves of *C. paniculatum* were successively extracted with n-hexane, EtOAc, and MeOH to give 4.51 g (1.5%), 8.86 g (2.9%) and 21.5 g (7.2%), respectively. This indicates that the secondary metabolites present in the leaves are mainly polar compounds.

Phytochemical screening of crude extracts

Phytochemical screening of *n*-hexane, ethyl acetate and methanol extracts of the leaves of *C. paniculatum* revealed the presence of secondary metabolites (table 1). The present study revealed that n-hexane leaves extract of *C. paniculatum* contains terpenoids, whereas alkaloids, tannins, saponins, anthraquinones and phenols were not detected. Ethyl acetate extract contains alkaloids, tannins, phenols, flavonoids, terpenoids and anthraquinones,

however, saponins were not detected. The methanol leaves extract of *C. paniculatum* contains alkaloids, terpenoids, tannins, anthraquinones, saponins and phenols (table 1). The presence of flavonoids, phenolics, alkaloids, anthraquinones and terpenoids responsible for the treatment of various diseases is one positive attributes of this plant.

Characterization of isolated compounds

Compound **1** showed absorption band at 3430 cm^{-1} attributed to O-H stretching of carboxyl group, strong absorption band at 2915 cm^{-1} showed the presence of the C-H stretching of alkyl groups. The absorption band at 1730 cm^{-1} due to C=O stretching of carboxyl, and a strong absorption band at 1381 cm^{-1} attributed to C-O stretching carboxylic acids. The ^{1}H-NMR spectrum (table 2) showed a signal at δ_H 2.36 integrating for two protons is accounted to methylene protons adjacent to a carboxyl. The multiplet signal observed at δ_H 1.69 is ascribed to methylene protons on carbon flanked between two methylene carbons. The intense broad singlet signal at δ_H 1.27 integrated for eighteen protons is attributed to protons on overlapping methylene carbons. The presence of terminal methyl group is evident at δ_H 0.9 (3H). The proton decoupled ^{13}C-NMR spectrum with the aid of DEPT-135 revealed the presence of eleven well resolved carbon resonances. The signal at δ_C 177.9 (C-1) is accounted to a carbonyl group of carboxyl moiety. The terminal methyl group is evident at δ_C 14.1 (C-14). An intense signal at δ_C 29.6 is characteristics of the presence of many overlapping methylene groups. The remaining carbon signals were observed at δ_C 33.6, 31.9, 24.7 and 22.6. Thus, the spectral data generated is in agreement with literature reported for myristic acid [Huang and Yang, 2004].

Compound 2 was obtained as obtained as solid crystal (36 mg) with golden color melting at 183-184^{o}C. Its TLC showed one spot at R_f 0.54 with ethyl acetate:methanol (9:1) as a mobile phase. The IR (KBr) spectrum of showed broad absorption band due the O-H stretching at 3430 cm^{-1}, strong absorption band at 2925 cm^{-1} due to C-H stretching, sharp absorption band at 1633 cm^{-1} attributed to C=C stretching and absorption band at 1381 cm^{-1} due to C-C stretching. The ^{1}H-NMR spectrum (table 2) showed the presence of nine proton signals of which seven are on the aromatic ring and the remaining two are on the oxygenated aliphatic carbons. The peak at δ_H 7.32 (2H, H-2'', *d*, *J* = 8.8 Hz) and δ_H 6.82 (2H, H-3'', *d, J* = 8.8 Hz) are due to symmetrically placed protons on unsymmetrically *p*-substituted aromatic ring. The other signal observed in the aromatic region at δ_H 6.12 (2H, *d, J* = 2) is accounted to the proton on C-3',5'. The triplet signal at δ_H 6.08 (1H, *t*,) is due to aromatic proton at C-4' position. The peaks at δ_H 5.37 and 3.61 with *J* = 1.6 Hz each are attributed to protons on oxygenated sp^3 methine carbons.

The proton decoupled ^{13}C-NMR spectrum with the aid of DEPT-135 of compound **2** showed the presence of ten well resolved carbon resonances of which four are due to quaternary carbons. The high intense peaks at δc 107.7 (C-3',5'), 114.9 (C-3'',5''), 127.7 (C-2''6'') and 157.7 (C-2',6') suggest the presence of two phenyl groups with AA'XX' spin system and hence only eight aromatic signals are observed of which three are oxygenated sp^2 quaternary carbons (C-2',6' and C-4'') and two are nonoxygenated sp^2 quaternary carbons (C-1' and C-1''). On the other hand the signals characteristics of oxygenated sp^3 methine were observed at δ 58.0 and 84.5. The remaining peaks were observed signals are at δ 100.7 (C-4'), 133.2 (C-1''), 141.2 (C-1'), 84.5 (C-2) and 58.0 (C-1). The whole NMR spectral data is shown in Table 2. Thus, based on the above spectral data the compound fits with the stilbene derivative (**2**) whose structure is shown in Figure 2. To the best of our knowledge, compound **2** (Fig 2) is reported here for the first time.

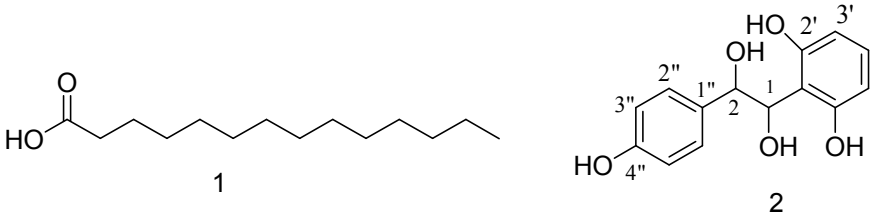

Fig 2: Structures of isolated compounds from *C. paniculatum*

Physical property and spectral data of compounds

Compound 1: Amorphous (8 mg); 53-54^{0}C M. p; IR ν_{max} KBr (4000-400) cm^{-1}, 3430(OH), 2915(C-H), 1730(-COOH). ^{1}H-NMR (400MHz, $CDCl_3$): δ_C(ppm) 2.36(2H, *t*, C-2), 1.69(2H, m, C-3), 1.27(18H, m, C-4 -C11), 1.65(2H, m, C-13) –all CH_2, 0.9(3H, t,C-14) -CH_3. ^{13}C- NMR (100MHz, $CDCl_3$): δ_C(ppm) 179.2 (C-1), 34 (C-2), 22.7 (C-3), 29.0-29.7 (C4-C11), 31.9(C-12), 22.7(C-13), 14.1(C-14).

Compound 2: Golden solid crystal (35.5 mg); 183^{0}C-184^{0}C M. p; IR ν_{max} KBr (4000-400) cm^{-1}, 3430(OH), 2925(C-H), 1633(C=C), 1481(C-C); ^{1}H-NMR (400MHz, Acetone-d6): δ_C(ppm) 7.32(2H, C-2'', C-6''), 6.82(2H, C3'',C5''), 3.61(1H, C-1), 5.37(1H, C-2), 6.12(2H, C-3', C-5'), 6.08(1H,*t*, C-4'), ^{13}C- NMR (100MHz, Acetone-d6): δ_C(ppm) 133.2(C-1''), 127.7(C-2'', C-6''), 114.93 (C-3'', C-5''), 156.7 (C-4''), 58.0 (C-1), 84.5 (C-2), 141.2 (C-1'), 157.8 (C-2',C-6'), 107.9 (C-3', C-5'), 100.7 (C-4').

Antibacterial activity

The inhibitory effects of *C. paniculatum* extracts were examined against *S. aureus*, *E. coli*, *K. pneumonin* and *P. mirabilis* strains (Table 3). The *n*-hexane and EtOAc extracts were inactive against *K. preumanin* while the methanol extract was inactive against *E.coli.* The extracts demonstrated significant difference in antibacterial activity against *S. aureus* and other bacterial strains with zone of inhibition ranging from 6-12 mm. Stilbene derivative (2) demonstrated zone of inhibition of 19 mm against *S. aureus* which was turned out to be comparable with ciprofloxacin (23 mm zone of inhibition). The results obtained from the antibacterial screening of the leaves in a good agreement with the ethnobotanical survey of the plant in the literature [Abera et al., 2014]. Based on our findings the traditional use of the plant *C. paniculatum* to treat wound caused by *S. aureus* is validated suggesting the plant can be used as a remedy for its wound healing activity.

CONCLUSION

In conclusion, the phytochemical study on the chemical constituents of the leaves of *C. spinarum* led to the isolation of two compounds; myristic acid and a stilbene derivative (1-(2',6'-dihyroxphenyl-2-(4"-hdroxyphenyl))-1,2-ethane-diol). To the best of our knowledge the stilbene derivative (**2**) was isolated for the first time and its antibacterial study also exhibited pronounceable activity against *S. aureus*. The antibacterial activity displayed the leaves extracts and compound 2 substantiate the traditional use of this plant against bacteria.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

Abera B, (2014). *Abera B*. Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest Ethiopia. *J. Ethn bio*, 1: 10-40.

Asres K, Bucar F, Kartnig T, Witvrouw M, Pannecouque C, De Clercq E, (2001). Antiviral activity against HIV-1 and HIV-2 of ethnobotanically selected Ethiopian medicinal plants. *J. Phyt Res*, 15:62-69.

Banskota A.H, Tezuka Y, Kim Q.T, Tanaka K, Saiki L, Kadota S, (2000). Thirteen novel cycloartane-type triterpenes from *Combretum quadrangulare*. *J. Nat. Prod*. 63:57-64.

Cock I, (2011). "The Scope of Pharmacognosy, *Pharm Commun.*, 1(1):156-171.

Farnsworth N.R. Soejarto D.D, (1991). "Global importance of medicinal plants", In: Akerele, O; Heywood, V. and Synge, H (Eds.), Conservation of Medicinal Plants, Cambridge University Press, Cambridge.

Huang X.A,Yang R.Z, (2004). A new hydroquinone diglucoside from *Lysimachia fordiana*. *Chemistry of Natural Compounds*, 4(5): 457-459.

Martini N.D, Katerere D.R.P, Eloff J.N, (2004). Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae*). J. Ethn. pharm.*, 93: 207-212.

Mamta S, Jyoti S, (2012). Phytochemical screening of *Acoruscalamus* and *Lantana camara*. *J. Int Res Pharm*. 3(5): 119-123.

Martini N, Katerere D.R.P, Eloff J.N, (2004). "Biological activity of five antibacterial flavonoids isolated from *Combretum erythrophyllum* (Combretaceae)", *J Ethn. pharm*., 93:207-212.

Pettit G.R., Singh S.B., Schmidt J.M., Niven M.L., Hamel E., Lin C.M, (1988). Isolation, structure, synthesis, and antimitotic properties of combretastatins B-3 and B-4 from *Combretum caffrum*. *J. Nat. Prod*., 51(3): 517-527.

Richardson M.A, (2001). Biopharmacologic and herbal therapies for cancer: research update from NCCAM, *J. Nutr,* 131: 3037-3040.

Schwikkard S, Bing-Nan Z, Glass T.E, Sharp J.L, Mattern M.R, Randall K.J, Kingston D.G., (2000). Bioactive compounds from *Combretum erythrophyllum*. *J. Nat. Prod.,* 63: 457-560.

Sowemimo A.A, Van de Venter M, Baatjies L, Koekemoer T, (2009). Cytotoxic activity of selected Nigerian plants. Afr. *J. Trad. Comp. Alt. Med.* 6(4), 526-528.

Verpoorte R, (2000). Pharmacognosy in the New Millenium: Lead finding and Biotechnology*, J. Pharm.*, 52, 253-262.

Zwadyk P, (1992). Enteriobactericeae in Zinsser Microbiology, 20th ed. Gerongthieme Verlag, Stuggart. 87

Table 1: Phytochemical analysis of n-hexane, ethyl acetate and methanol extracts of *C. paniculatum*

Phytochemical	Tests/Reagents	n-hexane Extract	EtOAc Extract	MeOH extract
Saponins	Froth test	-	-	-
Flavonoids	NaOH Test	-	+	+
Phenols	10% $FeCl_3$	-	+	+
Tannins	Ferric chloride test	-	-	+
Alkaloid	Wagner's test	-	+	+
Anthraquinones	Bontrager's test	-	+	+
Terpenoids	Salkowski Test	+	+	+

+ means presence; - means absence

Table 2: ^{1}H-NMR (400MHz, acetone-d_6) and ^{13}C-NMR (100 MHz, acetone-d_6) spectral data of stilbene derivative (**2**)

Carbon position	^{1}H-NMR δ *in* ppm and *J* in Hz	^{13}C-NMR data δ *in* ppm	Multiplicity
1''	-	133.2	Q
2",6"	7.32 (2H, *d*, *J* = 8.8)	127.7	CH
3'',5"	6.82 (2H, *d*, *J* = 8.8)	114.93	CH
4"	-	156.7	Q
1	3.61 (1H, *d*, *J* = 1.6)	58.0	CH
2	5.37 (1H, *d*, *J* = 1.6)	84.5	CH
1'	-	141.2	Q
2',6'	-	157.8	Q
3',5'	6.12 (2H, *d*, *J* = 2)	107.9	CH
4'	6.08 (1H, *t*)	100.7	CH

Table 3: Inhibition zone diameter of the three successive plant extract, compound 2, antibiotics and chloroform

Extract/control	Zone of inhibition (mm)			
	S. aureus	*E. coli*	*K. preumanin*	*P. mirabilis*
n-hexane extract	8	10	-	9
Ethyl acetate extract	15	12	-	14
Methanol extract	7	-	10	8
Compound 2	19	12	11	10
Ciprofloxacin (positive control)	23	21	19	24
Chloroform (negative control)	0	0	0	0

(-) no zone of inhibition