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Chemical Investigation on Berries of Embelia Schimperi from Oromia Region, Ethiopia

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Abstract
The aims of this study were to investigate the chemical constituents of *Embelia schimperi. Embelia* is a genus of climbing shrubs in the family Myrsinaceae. The plant has been much known as local medicine for the treatment of tape worms. In this project extraction, isolation and characterization of *Embelia schimperi* has been under taken and chemical investigation on the solvent extract of the plant has been conducted. The methanol extracts of the berries lead to isolation of one new compound. The compounds were identified to be alkaloid benzoate coded as F_4. Structural elucidation of isolated compounds was accomplished by means of spectroscopic methods (NMR, UV, IR and LC-MS) technique.
Keywords: *Embelia schimperi*, chemical constituents, LC-MS; alkaloid

Introduction

Chromatographic separation of an ethyl acetate extract of dried ground stem bark of *Embelia schimperi* led to the isolation of a new compound identified as 2,5-dihydroxy-3-methyl-1,4-benzoquinone (3) on the basis of spectroscopic and physical data. The plant's crude extract and pure compound 3 were assayed for *in vitro* antimicrobial activity against clinical strains of *Salmonella* spp., Proteusspp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Cryptococcus neoformans*, *Shigella dysentriae* and *Staphylococcus aureus*. Disc diffusion method was used and zones of inhibition, after respective incubation periods, were used to quantify antimicrobial activity. Standard antibiotics namely: augmentin, cotrimoxazole, gentamycin, tetracycline and lyncomycin were used as controls. Compound (4) as exhibited by the zones of inhibition which ranged from 10-20 mm, the extracts were most active against *P. aeruginosa* as shown by the largest zone of inhibition of 20 mm, which was comparable to that of the control antibiotic gentamycin, with an inhibition diameter of 21 mm on the same microorganism [1]. Chromatographic analysis of air-dried berries of the plant from cold ethyl acetate extract led to the isolation of methyl vilangin (5) and Biembelin (6). Methyl vilangin was found to be lethal against 2nd instar larvae of *Aedes aegypti* (yellow fever vector) by first stopping the process of metamorphosis from the 2nd instar stage to the other stages and finally causing mortality to the larvae [2]. An investigation of MeOH extract of stem part of the plant led to the isolation of a new resorcinol derivative, 5-(7'Z-pentadecenyl) resorcinol (7), along with the known compounds lupeol (8) and β-sitosterol glucoside (9). Compound 5 exhibited moderate in vitro cytotoxic activity against human Hela cell line [3]. Fractionation of the methanolic extract of leaves of the plant has led to the isolation of novel flavonol glycosides (10). The compounds were characterized as isorhamnetin 3-O-β-galactoysyl (1→ 4)-β-galactoside and quercetin 3-O-[α-rhamnosyl (1→2)] [α-rhamnosyl (1→ 4)]-α-rhamnoside. Also reported from the same extracts were known compounds quercetin, myricetin, quercetin 3-O-α-rhamnoside, quercetin 3-O-β-glucoside, quercetin 3-O-rutinoside, myricetin 3-O-β-xyloside, isorhamentin 3-O-β-glucoside and myricetin 3-O-β-glucoside. Their structural elucidation was accomplished using spectral measurements and chemical methods [4].

Materials and Methods

Plant Collection and Identification

The berries of *Embelia schimperi* were collected from Oromia region, Horo Guduru Wellaga Zone in Horo woreda, Loti-Ano kebele, which is 334 km west of Addis Ababa on December 13, 2015. The plant was identified by botanists in the Department of Biology, Addis Ababa University, as shown below figure 1.

Fig.1 Embelia schimperi locally called Enkoko in Amharic and Hanku in Afan Oromo plant Photo taken by researcher on November 2014

Extraction and Isolation of the Compound

The berries of *Embelia schimperi* was dried with air and ground using mortar and pestle. 100 g of powdered berries of the plant were soaked with 100 mL of *n*-hexane, 100 mL of ethyl acetate and 100 mL of methanol for 72 hr separately; the filtrate was concentrated with Rota vapor to yield 3.6 g, 4.2 g and 8.5 g solid, respectively. By TLC monitoring 7.5 g of the crude extract of methanol was applied on to a column packed with n-hexane silica gel 210 g. The column was eluted using the following solvent system :- fractions 1-5 (100 mL x 5) by 100 % pure *n*-hexane, *n*-hexane/EtOAc (9:1) fractions 6-10 (100 mL x 5), *n*-hexane/EtOAc (8:2) fractions 11-16 (100 mL x 6), *n*-hexane/EtOAc (7:3) fractions 17-20 (100 mL x 4), *n*-hexane/EtOAc (6:4) fractions 21-23 (100 mL x 3), *n*-hexane/EtOAc (1:1) fractions 24-26 (100 mL x 3), pure chloroform fractions 27-28 (100 mL x 2), chloroform/methanol (9:1) fractions 29-30 (100 mL x 2), chloroform/methanol (8:2) fractions 31-32 (100 mL x 2), chloroform/methanol (7:3) fractions 33-34 (100 mL x 2), chloroform/methanol (6:4) fractions 35-36 (100 mL x 2), chloroform/methanol (1:1) fractions 37-38 (100 mL x 2), pure methanol fractions 40-41 (100 mL x 2), 41 fractions were collected.

Analysis of the Isolated Compound

NMR spectra were recorded on a Bruker Advance instrument (400 MHz and 100 MHz) and with TMS as an internal standard (chemical shifts in δ, ppm). The isolated compound was dissolved in DMSO-d_6 and analyzed with one-dimensional NMR (proton ^{1}H, carbon ^{13}C) and LC-MS.

Results and Discussion

Ground berries part of *Embelia schimperi* (100 g) were subjected to exhaustive extraction with n-hexane, ethyl acetate and methanol separately and concentrated under reduced pressure using Rota vapor and yielded n-hexane extract (3.6 g), ethyl acetate extract (4.2 g) and a methanol extract (8.5 g). Based on TLC analysis and yield the methanol extract was selected for further isolation. Chromatographic purification of the methanol extract gave compounds coded F_4 and the phytochemical tests were conducted from the methanol extract of berries of the plant using standard procedures. (Table 1)

Table 1. Phytochemical screening test results

Extract	Class of secondary metabolites	Present(+) and Absent (-)
Methanolic extract of *Embelia schimperi* berries	Saponins	-
	Terpenoids	+
	Anthraquinones	+
	Flavonoides	-
	Alkaloides	+
	Steroids	-
	Glycosides	-
	Tannins	+

Characterization of Compound F_4

Compound F_4 was obtained as an amorphous powder from MeOH and its molecular formula, $C_{21}H_{33}O_2N$ was determined by negative LC-MS and NMR spectra. In the negative LC-MS spectrum, the quasi-molecular ion peak was at m/z 331.25 [M-H].

Compound F_4 was obtained as an amorphous brown substance isolated from MeOH extract and the

compound was characterized as follows.

In the UV spectrum at λ_{max} (in MeOH) absorption maximum at 224nm revealed the molecule has unsaturated carbonyl chromophores ($\pi \rightarrow \pi^*$) conjugation. In the IR (KBr disk) spectrum showed absorption band at 3436 cm^{-1} due to the presence of secondary amine (R_2NH). Strong absorption band at 2925 cm^{-1} and medium absorption band at 1464 cm^{-1} due to saturated C-H stretching, strong absorption band at 1744 cm^{-1} due to ester group and absorption band at 1255 cm^{-1} and 1112 cm^{-1} due to C-N stretching. ^{1}H-NMR δ_H (400 MHz, DMSO-d_6): spectrum (appendix 9 and table 5) revealed the presence of proton signals at δ_H 7.75 (2H, dd J=6.4 and 13.6 Hz), δ_H 7.575 (2H, dd J=4 and J=6 Hz) and δ_H 7.705 (1H, dd J=3.2 and 12.8 Hz) attributed to aromatic protons with a mono substituted phenyl ring, signals at δ_H 4.12 (2H, dd, J=4 Hz) due to oxygenated methylene group and signals at δ_H 1.28-1.62 (m, 8H) due to methylene protons. The signals at δ_H 2.75 (1H, dd J=4.2 Hz) showed proton of methine attached to carbon bearing of amine part. δ_H 2.25, 1.98, 1.68 and 1.5 with multiplet and integrated for four proton indicate methine. The presence of sharp singlet peak at δ_H 3.15 (3H) suggest N- methyl proton, two methyl group proton signal at δ_H 1.22 (6H, d) and one methyl group proton signal at δ_H 0.88 (3H, d, J=4 Hz).^{13}C-NMR spectrum and revealed a total of twenty-one carbon signals. Signals at δ_C 132.17, 130.05 and 129.05 attributed to mono substituted benzene ring, signals at δ_C 167.45 due to ester carbonyl group in the structure, and signal at δ_C 67.88 due to the presence of oxymethylene carbon. The signals at δ_C 30.26, 28.82, 23.71, and 22.85 were assigned to aliphatic carbons. The signal at δ_C 49.05 was assigned to the carbons that bearing amine part and signals at δ_C 38.55, 31.74, 29.44, and 27.06 were assigned for tertiary carbons of the compound. Signal δ_C 40.21 was assigned to the methyl carbon attached to nitrogen. Signals at δ_C 14.33 and δ_C 11.22 were assigned for the methyl carbons. The multiplicity of each carbon atom was determined using DEPT-135 experiment, which revealed the presence of four methyl groups (one is attached to nitrogen and three of them is attached to carbons), five methylene, five aromatic methane, five aliphatic methane, and two quaternary carbons. (Table 2)

Table 2. ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectral data of F_4 in DMSO-d_6

Position	^{1}H-NMR (ppm)	^{13}C-NMR(ppm)	DEPT-135
1	2.75(1H, dd)	49.05	CH
2	1.68(1H, dt)	29.44	CH
3	1.35(2H, dd)	28.82	CH_2
4	1.98(1H, m)	31.74	CH
5	1.62(2H, dd)	30.26	CH_2
6	2.25(1H, m)	38.55	CH
7	0.88 (3H, d, J=4 Hz)	14.33	CH_3
1``	1.28(2H, dt)	23.71	CH_2
2``	1.28(2H, dt)	22.85	CH_2
3``	1.5(1H, m)	27.06	CH
4``,5``	1.22(6H, d)	11.25	CH_3
N-CH_3	3.15(3H)	40.21	CH_3
1`	4.12 (2H, dd, J=4 Hz)	67.88	CH_2
2`	-	-	-
3`	-	167.45	Quaternary
4`	-	132.17	Quaternary
5`, 9`	7.75(2H,dd J=6.4 and 13.6 Hz)	130.05	CH
6`, 8`	7.575(2H,dd J=4 and J=6 Hz)	129.05	CH
7`	7.705(1H,dd J=3.2 and 12.8 Hz)	132.05	CH

Based on the NMR (1D), UV, IR and LC-MS spectra and table 2 the structure of compound F_4 was 2-methyl-4-(3-methyl butane)-6-(methyl benzoate)-N-methyl cyclohexylamine. (Fig 2)

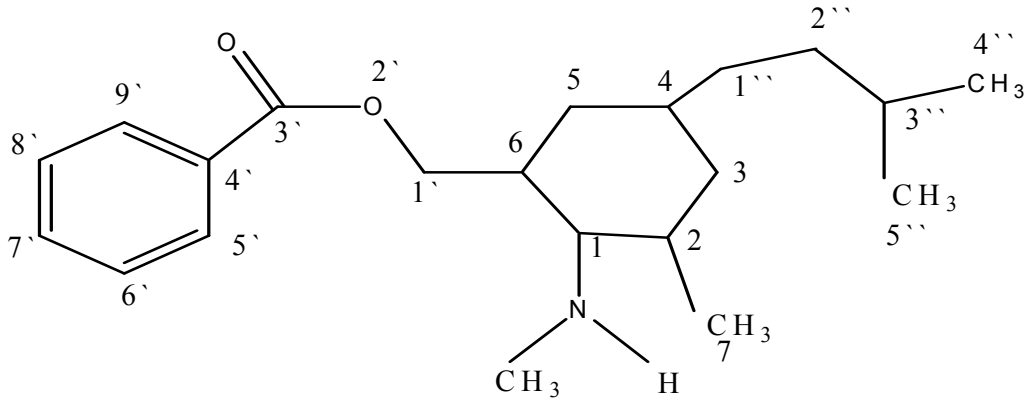

Fig. 2 The structure of compound (F_4)

Conclusion and Recommendation

This work resulted in the isolation of one new alkaloid compound [2-methyl-4-(3-methyl butane)-6-(methyl benzoate)-N-methyl cyclohexylamine (F_4)] isolated for first time, from the berries of *Embelia schimperi*. The structures of the compound were characterized on the basis of spectral data (UV, ^{1}H-NMR, ^{13}C-NMR, DEPT-135, IR and LC-MS) as well as comparison with the literature data. Based on TLC analysis the plant contains several polar chemical constituents which were not isolated in this study because of financial and time constraints. It is possible to isolate more polar compounds using advanced chromatographic techniques such as MPLC and HPLC techniques with the help of reverse phase column. It is also important to evaluate the crude extracts, fractions and isolated pure compounds for antimicrobial activities to check their potential as lead compounds in the development of antimicrobial agents. Therefore, much more Phytochemical and biological study should be carried out on the plant in future.

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