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Available online at www.ijit.net**PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *BUDDLEJA POLYSTACHYA* (ETHANOL EXTRACT)****BERHANEMESKEL ATSBEHA¹, FIKRE MAMMO², BELAYHUN KIBRET³**¹*Department of Chemistry, College of Natural and Computational Sciences, Aksum University, Axum, P.O.Box: 1010, Ethiopia, North East Africa.*²*Department of Chemistry, College of Natural and Computational Sciences, Hawassa University, Hawassa, Ethiopia.*³*Department of Biochemistry, College of Health Sciences, Hawassa University, Hawassa, Ethiopia.*Corresponding author mail id: bma174@yahoo.com**ABSTRACT**

The ethanol extract of *Buddleja polystachya* leaves was extracted using ethanol as a solvent and its yield was found to be about 22.16% dark green residue. Fractionation of crude ethanol extract afford a new 5-ethyloctyl octyl phthalate compound and its structure has been elucidated by UV-visible, FTIR, ¹H and ¹³C NMR, DEPT spectroscopic data. Phytochemical screening was performed on the ethanol crude extracts using standard procedures and the plant was rich in Flavonoides, Alkaloids, Terpenoides, Cardiac glycosides, oils and Saponin compounds.

KEYWORDS: *Buddleja polystachya*, solvent, Fractionation, Structure elucidation and phytochemical.**INTRODUCTION**

Buddleja polystachya is belong to the family Buddlejaceae, is commonly called Butterfly Bush.^{1, 4, 5} It is a multi-branched shrub or occasionally small tree endemic to the semi-arid highlands flanking the Red Sea in Eretria, Ethiopia, Saudi Arabia, and Yemen, where it grows in secondary scrub or around forest at elevations of between 2,200 and 3,600 m. it usually grows to less than 5 m, but can occasionally reach 12 m in favorable conditions. The bark can be either red-brown or grey in color. The bright orange flowers form dense panicles less than 20 cm long; the scent is acrid and generally considered unpleasant. The leaves are about 15 cm long and narrow, with a pointed tip, the upper surface a pale grey-green. The fruit is a small dry orange capsule.^{2, 3} In Ethiopia, traditionally the dried or fresh leaves of the plant was used for the treatment of malaria, the leaves powder with Butter smeared on the skin topically for treatment of wounds/diseases of the skin. Besides, the juice obtained by soaking with water used for eye infection applied by dropping at night times, for Dingetegna applied in Nasal. The genus *Buddleja* used for wound healing and the infusion of the leaves is used topically for the treatment of burns, external and internal ulcers. It also used traditionally in the form of a poultice or lotion to promote wound healing, was able to stimulate the growth of dermal fibroblasts and reduce inflammation.^{6, 7, 8, 9}

MATERIALS AND METHODS**Plant Material**

The plant was selected in the Botanical garden of Addis Ababa University and collected in March 10, 2011 from Dawuro (SNNPRS, Ethiopia). The voucher specimen was deposited at the National Herbarium (Ethiopia), Department of Biology, Addis Ababa University, Faculty of Natural Sciences.

Extraction of Plant Material

Air-dried and powdered leaves of *Buddleja polystachya* (250g) were soaked with n-hexane (900ml) for 48 h. After filtration, the extract was evaporated under reduced pressure and temperature of 40⁰c using rotary evaporator. The air dried marc was extracted twice with ethanol (2x1.3 litre) at room temperature for 96 h. The combined extracts were evaporated under reduced pressure afforded 55.4 g (22.16%) dark green residue.

Fractionation of Crude Ethanol Extract

About 90.00g of silica gel was measured and mixed with 250ml of petroleum ether (100%), packed into a column chromatography. 20.0g of the crude ethanol extract was subjected to column chromatography on silica gel using petroleum ether, with a gradient of petroleum ether up to 100% followed by mixture of

chloroform, ethyl acetate and ethanol in increasing order of polarity.

RESULTS AND DISCUSSIONS

Phytochemical investigation of *Buddleja polystachya* leaves yielded to the isolation of BPE-9 from the ethanol extract. Structure elucidation of the compound was done based on the spectroscopic analysis (UV-Vis, ^1H , ^{13}C -NMR, DEPT and IR spectrum) data obtained for the compound and in relationship with data's of comparable literature for equivalent compound.

Characterization of Compound BPE-9

Compound BPE-9 was isolated as oil. The UV-Vis spectrum at λ max using CHCl_3 as solvent shown absorption bands at 223 and 274nm indicating ester substituted aromatic ring.

IR analysis indicated that the presence of ester carbonyl band (1732 cm^{-1}), aromatic -C=C-H stretching (2952 and 2922 cm^{-1}), methoxy (2853 cm^{-1}), benzene derivative (1652 cm^{-1}), C-H bending vibration of methyl (1461 cm^{-1}), symmetrical C-H bending vibration of the methyl (1377 and 1366 cm^{-1}), C-O stretching bands of the ester group (1271 , 1071 , 1040 cm^{-1}) and weaker bands (721 cm^{-1}) represented the methylene rocking vibration in which all methylene groups rock in phase. Besides, this band was generally appeared for straight-chain alkanes containing at least four adjacent methylene groups.

The ^1H - NMR shown three characteristics signals namely aromatic region at δ 6.5- 8.4, oxygenated region at δ 2.8-4.5 and aliphatic region at δ (1.0- 2.3). Four protons in the aromatic region were observed. Doublet of doublets (dd) at δ 7.73 integrated for two protons and another doublet of doublets at δ 7.53 which were integrated for two protons showed in the aromatic region. Multiplet peaks at δ 4.26 integrating for four protons assigned to the two methylene groups which were geminal to the ester alcohol group. Multiplet peaks at δ 2.3 integrating for four protons each corresponding to two methylene group's β - position to oxygen attached methylene of the ester group. Multiplet small peaks at δ 1.46 displayed for the methine proton. Broad singlet peaks between δ 1.25-1.37 integrating for twenty protons indicative of long chain hydrocarbons. Triplet peaks at δ 0.9 integrating for nine protons corresponding to the terminal methyl groups.

The ^{13}C NMR spectrum showed at four different chemical shift regions. The carbonyl ester region at δ 160-180, the aromatic carbon at δ 140-120, the carbon - oxygen at δ 100-50 and the aliphatic region from δ 40-10.

The ^{13}C - NMR spectra showed signal at δ 167.77 overlapped of two carbons assigned to the carbonyl saturated ester. The signal at δ 68.17 overlapped of two carbons, was assigned to the oxygenated methylene group. The region from δ 140-120 was corresponding to the aromatic carbons showed at δ 132.47, 130.88 and 128.81, each of the signals was overlapped of two carbon atoms. The aliphatic regions run from δ 40-10 have 16 carbons. The longest peak at δ 29.73 was for the overlapping of five carbon atoms. Therefore, from the ^{13}C - NMR spectrum showed 26 carbons out of which 3 CH_3 , 5 CH , 16 CH_2 and 4 quaternary carbon signal with molecular formula of $\text{C}_{26}\text{O}_4\text{H}_{42}$ were identified (Table 1).

The DEPT spectrum showed 15 peaks corresponding to 22 carbons. Compound BPE-9 has three methyl groups at δ 10.99, 14.08, and 14.15. Fourteen methylene groups were showed at δ 68.18, 31.95, 30.39, 29.73, 29.39, 28.95, 23.77, 23.016, and 22.72. Peaks at 68.18 showed that overlapping of two oxymethylene groups. In addition, the longest peak at δ 29.73 notified that overlapping of five methylene carbons which were at different chemical shifts confirmed by the presence of IR spectra at 721 cm^{-1} which was generally appeared for straight-chain alkanes containing at least four adjacent methylene groups. Five methine groups were observed out of which one was in the aliphatic region at δ 38.76 and four in the aromatic regions at δ 130.89 and 128.81. The peaks at δ 130.89 and 128.81 were overlapping of two methine groups.

The difference in carbon numbers of the ^{13}C -NMR and DEPT spectra was four. As a result, compound BPE-9 has four quaternary carbon atoms at δ 167.77 and 132.47 out of which, peaks at δ 167.77 would be for overlapping of two carbonyl groups and peaks at δ 132.47 indicated for the overlapping of two quaternary carbon atoms of the aromatic ring.

By Comparison of the ^{13}C -NMR and ^1H -NMR data to those published in literature^{10, 11}, compound of **BPE-9** was identified to have an ester of phthalate moiety. In addition, the comparative study of its NMR data with reported data revealed its identity as Octoil reported in the literature¹² showed a good agreement with compound **BPE-9**.

Based on the spectroscopic data of the compound and literature data, the proposed structure of compound BPE-9 is:

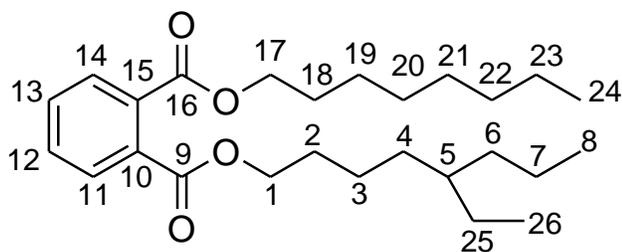


Figure 1: 5-ethyloctyl octyl phthalate

CONCLUSION

Thin layer chromatography analysis of the ethanol extract showed more compounds. The presence of oily components made separation difficult. However, through repeated use of solvent-solvent extraction and column chromatography, compound BPE-9 was isolated and characterized using spectroscopic data and confirmed as 5-ethyloctyl octyl phthalate. To the best of our knowledge the compound isolated is newly reported for the first time from the leaves of the plant.

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Table 1: ^1H (400 MHz, CDCl_3) and ^{13}C (400 MHz, CDCl_3) NMR data of compound BPE-9

Position	Carbon type	^{13}C NMR (δ_c)	^1H NMR		
			(δ_H , ppm)	No. Hydrogen	multiplicity
1	CH_2	68.17	4.26	2	m
2	CH_2	30.37	2.30	2	m
3	CH_2	23.78	1.25-1.37	2	m
4	CH_2	29.72	1.25-1.37	2	m
5	CH	38.74	1.46	1	m
6	CH_2	29.72	1.25-1.37	2	m
7	CH_2	22.71	1.25-1.37	2	m
8	CH_3	14.12	0.9	2	t
9	Quaternary carbon	167.77	-	-	-
10	Quaternary carbon	132.47	-	-	-
11	CH	128.81	7.73	1	dd
12	CH	130.89	7.53	1	dd
13	CH	130.89	7.53	1	dd
14	CH	128.81	7.73	1	dd
15	Quaternary carbon	132.47	-	-	-
16	Quaternary carbon	167.77	-	-	-
17	CH_2	68.17	4.26	2	m
18	CH_2	29.38	2.30	2	m
19	CH_2	29.72	1.25-1.37	2	m
20	CH_2	29.72	1.25-1.37	2	m
21	CH_2	29.72	1.25-1.37	2	m
22	CH_2	31.95	1.25-1.37	2	m
23	CH_2	23.01	1.25-1.37	3	t
24	CH_3	14.06	0.9	2	m
25	CH_2	28.95	1.25-1.37	2	m
26	CH_3	10.98	0.9	3	t