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Antibacterial and Antioxidant Phenylpropanoid Derivative from the Leaves of *Plantago lanceolate*

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Abstract

Plantago lanceolata (Plantaginaceae) is a perennial cosmopolitan species traditionally used for blood clotting and healing of wound. The powdered leaves were successively extracted with n-hexane, ethyl acetate and methanol to give 1.55, 2.16, and 8.2%, yield, respectively. Silica gel column chromatography afforded one phenylpropanoid derivative named verbascoside. The structure of the compound was determined using spectroscopic methods (UV-Vis, IR, NMR). The extracts, and verbascoside were evaluated *invitro* for antibacterial activities by using the disc diffusion method against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumania* and *Proteus miabilis*. The promising inhibition zone (20 mm) was observed by verbascoside against *S. aureus* compared to standard Ciprofloxacin (23 mm). The radical scavenging activity of the methanol and ethyl acetate extract, and verbascoside compounds were 64.2%, 79.2 and 83.9, respectively suggesting that verbascoside displayed powerful radical scavenging activity indicating the potential of the plant as herbal remedies.

Keywords: Plantago; *P. lanceolate;* Verbascoside; Antioxidant; Antibacterial

Introduction

Natural products and their related moieties have historically been incredible as a source of therapeutic agents. The past century, however, has seen an increasing role played by microorganisms and marine organisms in the production of drugs for the treatment of serious diseases. Natural products will continue to play a crucial role in meeting this demand through the expanded investigation of the world's biodiversity, much of which remains unexplored [1]. Traditional remedies for wound healing also have a wide usage among the people living in the rural parts of Ethiopia. Several medicinal plants have been reported to be used for the treatment of wounds and ulcers [2]. Among these plants, Plantago species were reported to be used as wound healing agent with its severe, haemostatic and antimicrobial properties.

Plantago lanceolata (ribwort plantain) belonging to the genus Plantago and family Plantaginaceae is a perennial cosmopolitan species which shows high ecological plasticity, being found naturally in grassy areas on roadsides, in pastures and in crops as weeds. *P. lanceolata* is locally termed as "gurteb" in Amharic and "kortobe" in Afan Oromo (Ethiopia). Those Plantago is a genus of about 265 species of small, inconspicuous plants commonly called plantains. Previous studies have shown that Plantago species have analgesic, anti-inflammatory, antimicrobial, antioxidant, hepatoprtective activities, and cytotoxic effect on the cancer cells [3]. Despite the traditional use of this plant against various diseases, to the best of our knowledge there is limited report on the chemical constituents, antibacterial and antioxidant studies of the leaves extract of this plant. Hence, the current study was undertaken primarily to isolates, characterizes compounds from the leaves of *P. lanceolata* plant and examine for antibacterial and antioxidant studies of the leaves extract and compounds.

Materials and Methods

Plant material

Fresh leaves of *P. lanceolata* were collected in November 2016 from Mugher town, Adea Berga Woreda, west shoa zone, Oromia, Ethiopia. The plant was authenticated by a botanist Shambel Alemu at the Biology Department of Addis Ababa University and specimen stored (Voucher no: F001/2016) in the National Herbarium of Ethiopia, Addis Ababa University, Addis Ababa, Ethiopia. The leaves were washed with water without squeezing to remove trash and dust particles and then air-dried at room temperature (26°C) for one week. The air-dried leaves were chopped into small pieces and finally grounded using a mortar and weighed.

Extraction and isolation

Air dried leaves powder (300 g) of *P. lanceolata* was first soaked in n-hexane (1.5 L) for 72 h at room temperature. The mixtures were filtered and concentrated under reduced pressure at a temperature of 40°C using rotary evaporator to afford 4.65 g bright green crude extract. The marc was then extracted with ethyl acetate (1.5 L), after soaking for 72 h at room temperature. Then filtered and concentrated in rotary evaporator to furnish 6.5 g deep green crude extract. Finally the marc remaining was extracted using methanol following similar procedure and afforded 24.6 g of dark green crude extract. The methanol extract (13.6 g) was subjected to silica gel column chromatography (silica gel 150 g) and eluted with increasing gradient of ethyl acetate in n-hexane. A total of 27 fractions were collected. Fractions 22-23 (2.35 g) were combined and further purified by column chromatography (Silica gel 40 g) using methanol/Ethyl acetate

(5:95, isocratic mode) as eluent to give five fractions. Fraction 3 (66 mg, compound 1) afforded compound 1.

Antibacterial activity

The EtOAc and methanol extracts of the leaves and verbascoside (1) were evaluated *invitro* for antibacterial activity by using the disc diffusion method against one gram positive bacterium *Staphylococcus aureus* (S. aureus) and three gram negative bacterium *Escherichia coli*, *Klebsiella pneumania* and *Proteus miabilis*. The bacterial cultures were inoculated into the Muller Hinton Agar (MHA). Ciprofloxacin was used as positive control. Approximately, 20 mL of sterile MHA were poured into sterile culture plates and allowed to set wells of about 6 mm in diameter which were punched on the plates. 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. The plates were incubated at 37°C. The antibacterial activity of the plant extracts were evaluated by measuring the zone of inhibition against the test organism after 24 hrs [4].

Antioxidant activity

DPPH assay: The free radical scavenging activity of the EtOAc, MeOH extract and isolated compounds were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method [5]. With this method it is possible to determine the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. As a result of the color changing from purple to yellow the absorbance is decreased when the DPPH radical is scavenged by an antioxidant through donation of hydrogen to form a stable DPPH-H molecule [6]. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity [7].

The EtOAc extract was dissolved in four vials containing methanol to give 500, 250, 125 and 62 µg/mL. To each 1 mL of the above EtOAc extracts was added each 4 mL of 0.04% DPPH which gave 100, 50, 25 and 12 µg/mL. The resulting solution was placed in an oven at 37°C for 30 minutes and subjected to UV-Vis spectrophotometer to record absorbance at 517 nm. This was repeated for the methanol extracts and isolated compounds. The absorbance of 0.04% DPPH in MeOH solution was found to be 1.06. The percentage DPPH inhibition was calculated according to the following formula [8].

$$\% \text{ of radical scavenging activity} = Ab_{standard} - Ab_{analyte} / Ab_{standard} \times 100$$

Results and Discussion

Silica gel column chromatography of the leaf extract of *Plantago lanceolata* afforded one compound. The FT-IR spectrum displayed absorption bands at 3429 cm^{-1}, 1692 cm^{-1}, 1604 cm^{-1} and 2923 cm^{-1} attributed to hydroxyl moiety, α, β-unsaturated carbonyl carbon, carbon-carbon double bond and methyl C-H stretching vibrations.

The ^1H NMR spectrum of compound 1 (Table 1) exhibited six aromatic protons (δ_H 7.20-6.58 region) with two sets of ABX multiplicity pattern, two trans-olefinic protons (J=15.99 Hz), and a benzylic methylene at δ_H 2.77 (2H, t, J=7.39 Hz) suggesting the presence of a caffeic acid unit and 3', 4'- dihydroxyphenethyl alcohol moiety. In addition, two anomeric proton signals at δ_H 4.46 (d, J=7.9 Hz) and 5.31 (d, J=1.6 Hz) were attributed to β-glucose and α-rhamnose units, respectively, suggesting the presence of disaccharide moiety. The acyl group was positioned at the C-4" position of the glucose unit, on the basis of the deshielding of the H-4" signal (δ_H 4.95 t, J=9.4 Hz) of the glucose unit. The methyl group of acetyl attached to the rhamnose was observed at δ_H 1.98 whereas methyl of the ethyl moiety attached to the glucose were observed at δ_H 1.20.

Position	δ_H	δ_c	Position	δ_H	δ_c
1		130.3	1"	4.46 (1H, d, J=8.00)	102.8
2	6.78 (1H, d, J=1.99)	116	2"		75.2
3		143.4	3"		79.4
4		144.8	4"	4.94 (1H, t, J=9.6,9.1)	70.7
5	6.74 (1H, d, J=7.99)	115.1	5"		72.7
6	6.57 (1H, dd, J=10.00)	120.2	6"	3.62(2H, m)	61.4
7	2.76 (2H, m)	35.3	CH_2CH_3	3.64(2H, m)	59.76
8		71.1	CH_2CH_3	1.202(3H, t)	13.6
1'		126.6	1"'	5.31(1H, d, J=1.6)	100.9
2'	7.21 (1H, d, J=1.99)	114.4	2"'		71.3
3'		148.3	3"'		69.4
4'		145.5	4"'	4.82 (1H, br)	74.7
5'	6.89 (1H, d, J=7.99)	115.6	5"'		68.6
6'	7.05 (1H, dd, J=9.99)	122.1	6"'	1.11(3H, d, J=5.99)	17.6

7'	7.62 (1H, d, J=16)	146.3	CH_3CO	1.97(3H, s)	20
8'	6.33 (1H, dd, J=15.99)	113.9	CH_3CO		170.3

Table 1: Spectral data of compound 1.

The proton decoupled ^{13}C-NMR spectrum (Table 1) with the aid of DEPT-135 (Appendix 4) revealed the presence of thirtythree carbon resonances of which eight are quaternary, eighteen methines, four methylenes and three methyl groups. The spectrum showed signal at δ_C 170.3 due to acetyl carbonyl carbon and δ_C 166.4 due to α, β-conjugated carbonyl carbon. The presences of four oxygenated aromatic carbons were evident at δ_C 143.4, 144.8, 145.5, 148.3. The signal observed at δ_C 146.3 is ascribed to the β-carbon of the α, β-unsaturated carbonyl carbon. The spectrum also showed the presence of two sugar moieties at δ_C 61.4, 68.6, 69.4, 70.7, 71.1, 72.6, 74.8, 75.2, 79.4, 100.9 and 102.8. Among these, the signal characteristics of anomeric carbons were appeared at δ_C 100.9 (C-1''') and 102.8 (C-1''). The methyl group of acetyl attached to the rhamnose was observed at δ_C 20.0, methylene and methyl of the ethyl moiety attached to the glucose were observed at δ_C 59.8 and δ_C 13.6, respectively. Another diagnostic signal due to methyl group was observed at δ_C 17.6 suggesting one of the sugar moieties as rhamnose. Signals corresponding to two monosaccharides were found bearing 4'''-acetyl and 6''-O- ethyl group. Based on the above spectral evidence, compound 1 was found to be 6''-O-ethyl-4'''-acetyl verbascoside (1, Figure 1).

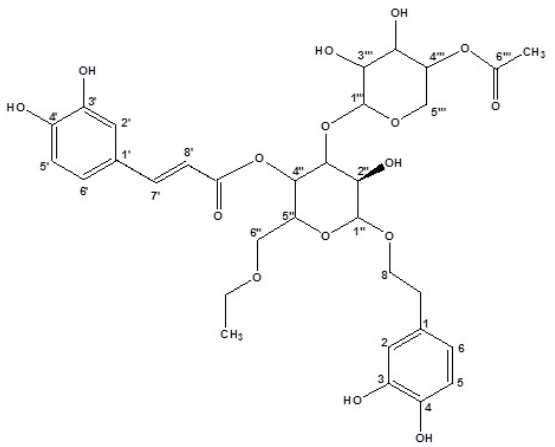

Figure 1: Structure of compound 1.

The antibacterial tests showed considerable antibacterial activity against the bacterial species used in the study. verbascoside (1) showed promising activity against the tested strains except for *K. pneumoia*. The methanol extract was found to inhibit *S. aureus* and *P. miabilis* compared with EtOAc extract. On the other hand, the EtOAc extract displayed better activity than the MeOH extract against *E. coli* and *K. pneumonia*. verbascoside (1) showed promising inhibition diameter (20 mm) against *S. aureus* as compared to standard drug (23 mm) (Table 2).

Sample	Types of bacteria with mean inhibition diameter (mm)			
	S. aureus	***E. coli***	***K. pneumonia***	***Proteus miabilis***
Ethyl acetate extract	-	8	8	-
Methanol extract	7	-	-	8
Verbascoside	20	13	7	9
Chloroform	-	-	-	-
Ciprofloxacin	23	21	19	24

Table 2: Zone of bacterial growth inhibition diameter (mm).

The ethyl acetate, methanol extracts and verbascoside (1) were examined for its radical scavenging activities. The DPPH radical scavenging activity was found to be 64.2%, 79.2, 87.7 and 83.9, respectively, at 100 µg/mL (Table 3). The IC_{50} values of the EtOAc, MeOH, and verbascoside (1) were 36.6, -59.1 and 76.1, respectively. The result obtained was found to be promising as compared to ascorbic acid which is used as positive control with percent inhibition of radical by 97% at 100 µg/mL. The promising activity of verbascoside (1) may be attributed to the presence of phenolic hydroxyl groups. The result suggests that the leaves of *P. lanceolata* can be used as a natural antioxidant.

Concentration	samples					
	EtOAc extract		**MeOH extract**		**Verbascoside**	
	Absorbance	**% Scavenging activity**	**Absorbance**	**% scavenging activity**	**Absorbance**	**% Scavenging activity**
100	0.38	64.2	0.2	79.2	0.2	83.9
50	0.45	57.5	0.3	76.4	0.3	76.4

25	O.56	47.2	0.3	71.7	0.3	67.9
12	0.62	41.5	0.5	57.5	0.4	60.4

Table 3: % scavenging activity of the extracts and isolated compounds of *P. lanceolate.*

Conclusion

For decades traditional medicines have been used and continue to be an alternative approach on treatment for various diseases. Currently, the growing interest of consumers in substances of natural origin in association with the increasing concern surrounding potentially harmful infections disease has directed to a rising interest in the use of plant extracts as functional ingredients in many pharmaceutical products. Silica gel column chromatography of the methanol extract furnished a phenylpropanoid derivative, verbascoside (1) which showed promising antibacterial activity against *S. aureus* and antioxidant activity. The findings support the traditional use of the plant to treat various infectious diseases.

Conflict of Interest

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

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Supporting Information

UV-Vis, IR, ^{1}H NMR, ^{13}C NMR, and DEPT-135 spectral data of compound 1 are all included in supporting information for further reference.

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