Anti-Tuberculosis Activity and GC-MS Analysis of Leaf Extract of *Plantago lanceolata L.*

Shuma Fayera*

Department of Chemistry, Dambi Dollo University, Oromia, Ethiopia

Corresponding Author*

Shuma Fayera
Department of Chemistry,
Dambi Dollo University
Oromia, Ethiopia.
E-mail: shumafayera2018@gmail.com

Received: 24-Feb-2022, Manuscript No. NPCR-22-16009; **Editor assigned:** 05-Mar-2022, **PreQC No.** NPCR-22-16009 (PQ); **Reviewed:** 18-Mar-2022, QC No. NPCR-22-16009; **Revised:** 21-Mar-2022 (R), Manuscript No. NPCR-22-16009; **Published:** 10-Apr-2022, DOI: 10.37532/npcr.22.10.3.01-04

Abstract

Leaves of *Plantago lanceolata* were traditionally used to treat wounds, burns, inflammations, fevers, diabetes, and cancer. The present study was carried out on the Gas Chromatography-Mass Spectrometry (GC-MS) Analysis and Antitubercular Activities of Leaf Extract of *Plantago lanceolata* since the plant was used for wound healing in Ethiopia. The n-hexane extracted powdered leaves of Plantago lanceolata herb oil were investigated by GC-MS and showed the presence of 26 compounds. Among these, the major constituents of leaf oil were 9-Octadecenoic acid (24.20%) and hexadecanoic acid (23.65%). hence the present work may support the medicinal use of *Plantago lanceolata*. The Methyl Isocyanate (MIC) of the crude extracts showed an inhibitory effect on *M. tuberculosis* H37Rv was determined at different concentrations of 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.12 µg/ml, 1.56 µg/ml, and 0.78 µg/ml. Accordingly, the Methyl Isocyanate (MIC) of the pet, the ether extract was 12.5 µg/ml; while that of $CHCl_3$: MeOH (1:1) and MeOH extract was 6.25 µg/ml and 1.56 µg/ml respectively. It can be concluded that the present study provided scientific support for the traditional use of *Plantago lanceolata* leaf extract for the treatment of tuberculosis.

Keywords: Analysis • Leaf oil • Antitubercular • *Plantago lanceolata*

Introduction

It has been discovered that higher plant extracts are a promising source of anti-TB compounds. Herbal medicine is based on the fact that a single plant may contain thousands of constituents with the possibility of discovering a new drug [1]. Tuberculosis is a highly infectious disease in about one-third of the world's population. However, this problem has become serious as *Mycobacterium tuberculosis* developed resistance against both the first line and also the second-line drugs. Due to this, there is the emergence of Multi-Drug Resistant (MDR) and Extensively Drug-Resistant (XDR) strains of M. tuberculosis all over the world including in Ethiopia [2].

Tuberculosis (TB) is one of the oldest diseases known to humanity. It remains one of the major deadliest infectious diseases for humans. The global Tuberculosis (TB) epidemic situation has been further aggravated by the emergence of Human Immunodeficiency Virus (HIV) infection and strains of drug-resistant Tuberculosis (TB). Multidrug-Resistant TB (MDR-TB) has been reported in almost all parts of the world, primarily as a consequence of poor treatment services, which have not only increased the costs of treatment but also increased the risk of transmission of these resistant strains of the bacilli as it stated in [3]. Among the medicinal species of therapeutic interest is included *Plantago lanceolata* L. which is a species of the genus Plantago in the Plantaginaceae botanical family that originated from Europe, widely spread throughout the world. *Plantago* lanceolata L. is a common perennial weed of arable fields and grassland abundant throughout Europe, North and Central Asia (Figure 1).

Figure 1. Image of Plantago lanceolata herb taken by Shuma.

Recently, in many countries including Mexico and Argentina, a lot of reports on the use of Plantago species in traditional medicine against cancer have been presented [4]. In Ethiopia, common plantain is a plant known as a weed, but it also has an old traditional medicinal value of wound healing and has got an application for many skin disorders [5-6].

Materials and Methods

Collection and Identification of the Plant Material

The leaves of *Plantago lanceolata* L., the herb were collected from Haramaya University's main campus, Haramaya in December 2016. The botanical specimen of the plant was identified and the voucher specimen was deposited at the Herbarium, Haramaya University as stated in [7].

Extraction of the Plant Material

The collected leaf was washed repeatedly and dried in an open-air protected from direct exposure to sunlight. Air-dried leaves of *Plantago lanceolata* L., were grinded by the analytical mill and packed in polyethylene bags. A 210 g of the powdered *P. lanceolata* L., leaves were then extracted with petroleum ether for 8 h in soxhlet apparatus (60 g of each) and soaked with 300 mL of petroleum ether and heated with heating mantle at 45°C for 8 h, and filtered with filter paper. The filtrate was collected and concentrated at 40°C under reduced pressure using a Rotary evaporator. After air-drying at room temperature, the defatted marc was subjected to a soxhlet extractor with $CHCl_3$: MeOH (1:1) for 8 hours. Then filtered using Whatman No.1 filter paper and concentrated using a Rotary evaporator at 40°C under reduced pressure. The marc collected after $CHCl_3$/MeOH extraction was dried at room temperature for further extraction with Methanol (MeOH) by the same procedure. This procedure was repeated until sufficient crude extract was collected and the crude extract was kept at 4°C until analysis as stated in [7].

Extraction of n-hexane Crude Oil

The dried and powdered leaf of *Plantago lanceolata* was extracted with n-hexane using a soxhlet apparatus at 60°C for 3 h to obtain the low polar components. During the extraction procedure, 98% n-hexane ACS grade was used as an extracting solvent. The obtained extracts were concentrated by a rotary evaporator under reduced pressure at 40°C.

Methylation of n-hexane Crude Oil

After removing hexane using a rotary evaporator the oily mixtures were derived from their methyl esters according to the general guidelines given by the International Olive Oil Council (IOOC) and the International Union of

Pure and Applied Chemistry (IUPAC) described by the transesterification process as reported in [8]. In this process dried n-hexane extracts (1 gm)were dissolved in hexane and then allowed to react with a methanolic solution (5 mL) of KOH (2M) at room temperature for 30 min. The upper phase was subsequently analyzed by means of Gas Chromatography-Mass Spectrometry (GC-MS) systems.

GC-MS Analyses of Plantago lanceolata n-hexane Leaf Extract Crude Oil

The crude oils from the leaf of *Plantago lanceolata* were analyzed with a Gas Chromatography-Mass Spectrometry (GC-MS) system consisting of an Agilent Technology model 7820A GC using HP-5 Column (30 m length, 0.25 mm internal diameter × 0.25 µm film thickness) which was coated with 5% phenyl 95% methyl polysiloxane as the stationary phase. The syringe was washed with 5 µL of dichloromethane and 1 µL of the esterified sample in dichloromethane was injected through an autosampler and analyzed with the HP-5 Column. The column temperature was programmed as follows: 50% to 120°C at 20°C/min (with 10 min hold time) and 4 min solvent delay. The inlet temperature was fixed to 275°C. The carrier gas was helium with a constant flow of (1.0 mL/min). The interface temperature was 2800°C. The sample was injected in splitless mode. The mass spectra were recorded in electron ionization mode at 70 eV with scanning from 40 m/z to 600 m/z. The ion source temperature was set at 150°C and the quadruple temperature was 230°C. The composition of each constituent in the oil was determined based on GC peak areas. The constituents of the essential oil were identified by their retention time MH\ Mass Hunter\Library search\NIST 14.

Testing for Antitubercular Activity

Drug susceptibility and determination of MIC of the test *Plantago lanceolata* crude extracts against M. tuberculosis H37Rv was performed by agar microdilution method where twofold dilutions of each test compound were added into 7H10 agar supplemented with Oleic Acid-Albumin-Dextrose-Catalase (OADC) and organism. A culture of *M. tuberculosis* H37Rv growing on L-J medium (Lowenstein-Jensen medium) was harvested in 0.85% saline with 0.05% Tween 80. A suspension of 1 µg/mL concentration of extracts/compounds was prepared in Dimethyl Sulfoxide (DMSO). This suspension was added to (in tubes) 7H10 Middle brook's medium (containing 1.7 mL medium and 0.2 mL OADC supplement) at different concentrations of extract keeping the volume constant, that is, 0.1 mL. The medium was allowed to cool keeping the tubes in a slanting position. These tubes were then incubated at 37°C for 24 h followed by streaking of M. tuberculosis H37Rv (5×10^4 bacilli per tube). These tubes were then incubated at 37°C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where the medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies occurred was taken as Minimum Inhibitory Concentration (MIC) of test extract [9]. The MIC of the crude extracts showed an inhibitory effect on M. tuberculosis H37Rv was determined at different concentrations of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.12 µg/mL, 1.56 µg/mL, and 0.78 µg/mL. Rifampin, Ethambutol, and Isoniazid were used as standard drug against M. tuberculosis.

Results and Discussion

GC-MS Characterization of Plantago lanceolata n-hexane Extracted Oil

The oil composition was assessed through analysis by combined gas GC-MS. Identification of chemical constituents of the oil was determined by their GC retention index and interpretation of their mass spectra and confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) as reported by [8]. The gas chromatogram of n-hexane extracted oil of the leaf of the plant showed the presence of 26 compounds and 100.00 % of components of n-hexane extracted oil has been identified in table 1. It was found that the major constituents of n-hexane extracted oil were 9-octadecenoic acid(24.20%), hexadecanoic acid (23.65%), methyl tetradecanoate (6.69%), 2,5-dimethoxy-4-(methylsulfonyl) amphetamine (6.15%), N-(1-cyclohexyl ethyl) benzene ethanamine (5.60%), N-(aminocarbonyl)-2-acetamide (4.73%) and 2-cyano-1-methyl-2-phenoxy ethylamine (4.53%).

Structures of Major Compounds of Plantago lanceolata n-hexane Extracted Oil

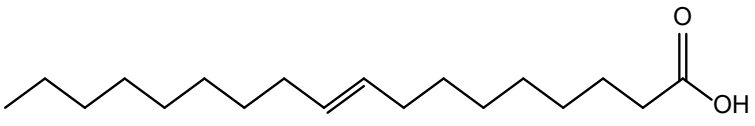
Plantago lanceolata leaf showed the presence of twenty-six compounds using GC-MS (Table 1 and Figure 2). 9-Octadecenoic acid (24.20%) and hexadecanoic acid (23.65%) were the most abundant compounds of the n-hexane extracted oil of the plant leaves.

Antitubercular Activity of Plantago lanceolata crude extracts

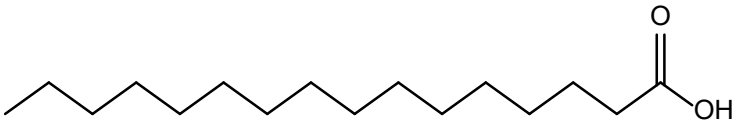
The MIC of the crude extracts showed an inhibitory effect on M. tuberculosis H37Rv was determined at different concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg/ml. Accordingly, the MIC of pet. The ether extract was 12.5 µg/ml; while that of CHCl3: MeOH (1:1) and MeOH extract was 6.25 and 1.56 µg/ml respectively (table 2). One can observe that the antitubercular effects increased as the polarity of extracted

Table 1. GC-MS analysis result of Plantago lanceolata n-hexane extracted Oil.

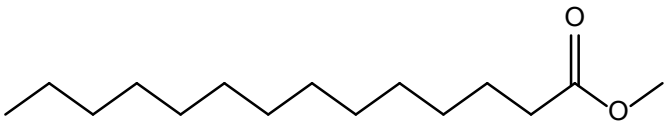
Compound Identified	Rt	M.F	Area (%)
α-Pinene	5.37	$C_{10}H_{16}$	2.69
Bicyclo[3.1.0] hexane	6.06	C_6H_{10}	1.72
β-Pinene	6.04	$C_{10}H_{16}$	2.83
2-cyano-1-methyl-2phenoxyethylamine	6.20	$C_{10}H_{12}NO$	4.53
N-methyl-1-(3,5-dimethyl-1-adamantanoyl)semicarbazide	6.81	$C_{15}H_{25}NO_2$	0.64
3-methoxyamphetamine	6.95	$C_{10}H_{15}NO$	2.05
N-(aminocarbonyl)-2-chloro-Acetamide	7.00	$C_3H_5ClN_2$	4.73
2-chloro-Benzenemethanol	7.40	C_7H_7ClO	0.53
dl-Alanylglycylglycine	12.56	$C_7H_{13}N_3O_4$	0.64
2-Aminononadecane	13.636	$C_{19}H_{41}N$	2.04
N-methyl-1-octanamine	13.953	$C_9H_{21}N$	0.32
1-Octanamine	14.849	$C_8H_{19}N$	0.61
5-(2-Aminopropyl)-2-methylphenol	14.909	$C_{10}H_{15}NO$	0.55
dl-Alanyl-dl-valine	15.627	$C_8H_{16}N_2O_3$	0.37
Amphetamine	15.938	$C_9H_{13}N$	1.94
1-Propanamine	15.995	C_3H_9N	0.90
Methyl tetradecanoate	16.299	$C_{15}H_{30}O_2$	6.69
R-(-)-Cyclohexylethylamine	17.163	$C_8H_{17}N$	0.51
(2-Aziridinylethyl) amine	17.524	$C_4H_{10}N_2$	0.85
Acetic acid	17.707	CH_2CHOH	0.49
N-methyl-2-Formylhistamine	18.623	$C_7H_{11}N_3O$	1.55
Hexadecanoic acid	18.909	$C_{16}H_{32}O_2$	23.65
2-Ethoxyamphetamine	21.690	$C_{11}H_{17}NO$	3.24
9-octadecenoic acid	21.798	$C_{18}H_{34}O2$	24.20
2,5-dimethoxy-4-(methylsulfonyl) amphetamine	22.018	$C_{12}H_{19}NO_4S$	6.15
N-(1-cyclohexylethyl) benzene ethanamine	22.241	$C_{16}H_{25}N$	5.60



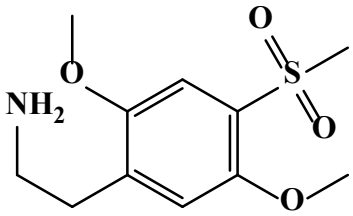
9-Octadecenoic acid



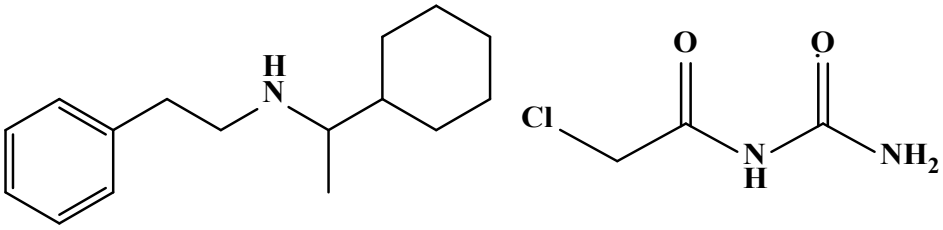
Hexadecanoic acid



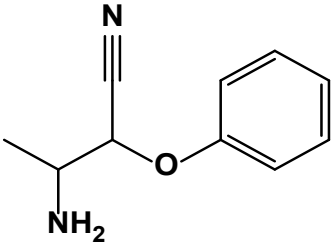
Methyl tetradecanoate



2, 5-Dimethoxy-4-(methylsulfonyl) amphetamine



N-(1-cyclohexylethyl) benzeneethanamine N-(aminocarbonyl)-2-chloro-Acetamide



2-cyano-1-methyl-2-phenoxyethyl amine

Figure 2. Structures of the Major Constituents of Plantago lanceolata n-hexane extracted Oil.

Table 2. Antitubercular activity of Plantago lanceolata crude extracts.

Sample	Pet. Ether extracts	CHCl3: MeOH (1:1) extracts	MeOH extracts	Rifampicin	Ethambutol	Isoniazid
MIC (µg/ml)	12.5	6.25	1.56	0.75	3.25	0.65

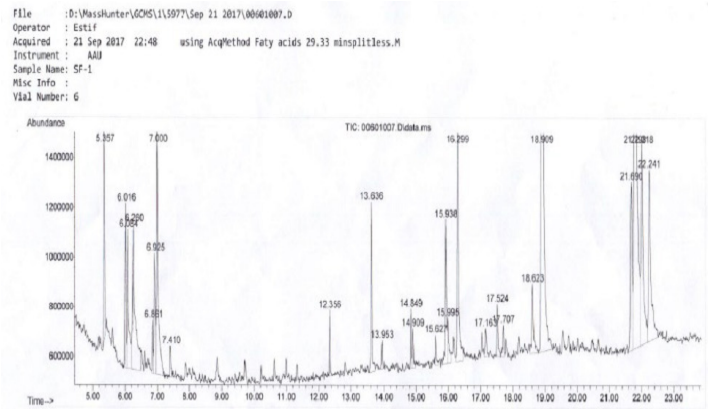


Figure 3. GC-MS of Plantago lanceolata n-hexane extracted Oil.

solvents increased. From the previous study high presence of tannins and saponins observed in MeOH extracts may enhance the Antituber activity of MeOH extracts [7]. Hence, the findings indicate that *Plantago lanceolata* extracts could be used as adjuvant therapy for TB (Figure 3).

Conclusion

It can be concluded that the present study provided scientific support for the traditional use of *Plantago lanceolata* leaf extract for the treatment of tuberculosis.

References

1. Hostettmann, K., et al. Phytochemistry of Plants used in Traditional Medicine. In: Hostettmann K., A. Marton, M. Mailard, M. Hamburger, (ed). Clarendon Press, Oxford, 17 (1995): 17.
2. Gupta R., et al, Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant Mycobacterium tuberculosis isolates., *Indian J Med Res* 131 (2008): 809-813.
3. Balcha, E., et al. Evaluation of *in-vitro* antimycobacterial activity of selected medicinal plants in Mekelle, Ethiopia. *World Appl Sci J*, 31.6 (2014): 1217-20.
4. Haddadian K., et al. A review of Plantago plant. *Indian J Tradit Knowl*, 13.4 (2014): 681-685.
5. Fekadu Fullas, Interactions of Ethiopian Herbal Medicines and Spices with Conventional Drugs. A Practical Guide. Sioux City, USA. (2006): 109.
6. Kesatebrahan H. Antimicrobial activity and phytochemical screening of crude extract of medicinal plants grown in Eastern Ethiopia. *Int J Pharma Bio Sci*, 4.4 (2013): 326-333.
7. Fayera S, et al. Phytochemical Investigation and Antimicrobial Study of Leaf Extract of *Plantago lanceolata*. *Nat Prod Chem Res* 6 (2018): 311.
8. Shafaghat A., et al. (2017) Synthesis of biodiesel from Reseda luteolin L., seeds and its chemical characterization using GC and GC-MS instrumentation. *Trends Phytochem Res*, 1(2): 77-82.
9. Collins L. & Franzblau S.G. Microplate alamar blue assay versus BACTECB 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. *Antimicrob Agents Chemother*, 41.5 (1997): 1004-1009.

Cite this article: Fayera S. Anti-Tuberculosis Activity and GC-MS Analysis of Leaf Extract of *Plantago lanceolata L*. Nat Prod Chem Res. 2022, 10 (2), 1-4.