

Antimycobacterial Activity and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Henna (*Lawsonia Inermis*) Leaves extract.

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Abstract: *Lawsonia inermis* or (henna) is used as a cosmetic dye and medicinal plant for its strong antimycobacterial and other therapeutic effects. In this study, we investigated the phytochemical and antimycobacterial activity of *L. inermis* leaves methanol extract against Rifampicin sensitive and resistant *mycobacterium tuberculosis (M.tb)* strains. Fresh Sudanese henna leaves were collected from the University of Gezira campus garden, shade dried, ground and was extracted by methanol. Crude henna methanolic extract was then tested for antimycobacterial activity against eight *M.tb* strains sensitive and resistant to rifampicin, using drug susceptibility test. The phytoconstituents were identified by using gas chromatograph interfaced to a mass spectrometer (GC-MS). GC-MS analysis revealed the presence of seven phytochemical components, with prevailing of squalene (34.433%), Lawsone (20.185%) and Coumarin-4-carboxylic acid (23.328%). The crude methanolic extract of henna leaves demonstrated an inhibitory effect on all tested *M.tb* strains, with antimycobacterial activity being ≤ 50 mg/ml. Henna leaves crude methanolic extract is rich in phytoconstituents that may involve in its remarkable antimycobacterial activity against rifampicin sensitive and resistant *M.tb* strains.

Key words: Antimycobacterial, Extract, Henna, *Lawsonia inermis*, Sudan

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Introduction

Tuberculosis (TB) is a major threat to the health of millions of populations in the developing and developed countries (Drobniewski et al. 2007). Despite the introduction of the inexpensive and effective four-drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) treatment regimen, TB continues to cause considerable morbidity and mortality worldwide. The second line drugs such as capreomycin, kanamycin, and amikacin were found to have a lower efficacy, expensive, and toxic (Ibekwe and Ameh 2014) There is no doubt that natural products, having specific chemical structures and powerful antimicrobial effects remain important participants in the development of new generations of antimycobacterial drugs. New safe herbal drugs are urgently required to counteract the growing resistance towards currently available drugs (Kumar et al. 2010).

Henna or hina (*Lawsonia inermis*, family Lythraceae) is a flowering plant or shrub native to tropical and subtropical regions of Africa, Southern Asia, and the Middle East (Emin and Mehmet 2012). Henna has been used for more than 4000 years as a cosmetic by African, Mediterranean, Middle Eastern, and Asian cultures. In many circumstances, the dye is applied over extensive areas of the body to create a variety of designs (Kandil et al. 1996). The widespread use of henna clearly shows that it is considered to be safe for external application. The reported tuberculostatic and antimicrobial activity of *Lawsonia inermis* Linn is probable due to lawsone, the major bioactive constituent of this plant (Rahmoun et al. 2012).

In Sudan, few studies have been undertaken on TB, although the prevalence of drug resistance is not known (Sharaf Eldin et al. 2011). Furthermore, the emergence of drug resistant tuberculosis has the potential to be a serious public health problem, hence strengthened tuberculosis control and improved monitoring of therapy is needed. In the present study, we report on *Lawsonia inermis* leaf methanolic extract, phytoconstituents therein, and their inhibitory effect against *mycobacterium tuberculosis* (*M.tb*) strains resistant and sensitive to rifampicin.

Experimental

Plant material:

Fresh Henna or "*lawsonia inermis*" leaves were collected from the garden of the University of Gezira (Elrazy Campus), Wad Medani City, Gezira State, Central Sudan. The plant material was botanically identified based on ethnomedical data and interviewed by the Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Sudan. Leaves washed thoroughly with tap water then rinsed three times with distilled water. After leaves were, shade dried, coarsely powered and packed in airtight bottle for the preparation of extract.

Preparation of crude methanol extract

The method as described by Gull et al. 2013. In brief, a coarsely shade dried powder (750 g) of crude henna leaves was extracted by maceration using 2500 mL methanol (LOBA chemie-India) for 72 hours in dark at room temperature with occasional shaking, filtered using a Whatmans No. 1 filter paper, and evaporated to dryness at 60°C by a rotary vacuum evaporator (Heidolph, UK). The produced green-gummy extract 170 g (23%) was placed into airtight amber glass container and kept refrigerated until use.

Phytochemical investigation by GC-MS analysis

The crude methanolic extract was phytochemically investigated on Agilent 7890A GC system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument (Agilent Technologies, USA) employing the following conditions: the fused silica capillary column was packed with HP-5MS (5% Phenylmethylsulphoxane) of 30 m x 250 µm x 0.25 µm dimensions, operating in electron impact mode at 70 eV. Helium gas was used as carrier gas at a constant flow of 1.2 ml/min and injection volume of 2 µl was employed (split less). An aliquot of 1 µl of sample was injected into the column at injector temperature 280°C with split ratio of 20:80. The oven temperature was programmed from 100°C (Isothermal for 5 min), with an increase of 10°C/min up to 200 °C, then 5 °C/min to 300°C, ending with 10 min, isothermal at 300°C. The mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 45-450 Da. Total GC-running time was 36 min. Compounds were identified by comparison of their mass spectra with those from the National Institute of Standards and Technology (NIST'98) mass spectral database.

Preparation of mycobacterium tuberculosis suspension for biological tests

Antimycobacterial activity against 8 M.tb strains; four rifampicin sensitive (H37, 150, 1303, and 150904) and four rifampicin resistant (153, 1214, 1240, and 1175) were tested, and were kindly obtained from the National Reference Laboratory for Tuberculosis (NRL-TB), Sudan. Where in vitro bioassay has been conducted. The tested strains were all maintained on Löwenstein-Jensen medium placed into a sterile McCartney bottle (a 15 mL screw-capped bottle) containing 1.5-2 mL distilled water and ten glass beads (3.0 mm diameter). The mixture was homogenized on a vortex mixer for approximately 5 minutes with brief interruptions and then the opacity of the suspension was adjusted by the addition of distilled water to that of a standard suspension of McFarland standard No. 1.

Drug susceptibility test by proportion method

The antimycobacterial activity of henna extracts was determined by employing Löwenstein-Jensen medium (LJ media) prepared using the method adopted by Hans et al. 2007. In brief; the ingredients of 600 mL mineral salt solution, 20 mL malachite green solution, and 1 L homogenized eggs were aseptically pooled in a large, sterile flask and mixed well. Fifteen milliliter (15 mL) of the henna extracts (50 mg/ml) containing-medium was dispensed into screw-capped glass bijou bottle, and then inspissated once at 85°C for 45 min. The strains suspensions were scraped by a standard wire loop (3 mm external diameter) of which 2 µl was used to inoculate both the henna free and henna-containing media. All tubes were then incubated at 35-37°C for 4-6 weeks. Susceptibility or resistance was recorded at day 28 and day 42 when the proportion of bacteria in drug-containing medium to that of drug free medium is < 1 or ≥ 1 respectively (Ani et al. 2009). Reference strain H37Rv was used as internal control for each batch of medium produced.

Ethical clearance:

The ethical approval of this study was obtained from The Ethical Committee of the University of Gezira (Wad-Medani, Sudan) and from Gezira Ministry of Health.

Results

GC-MS analysis and phytoconstituents identified in henna leaf methanolic extract:

GC-MS analysis showed the presence of seven components extracted from Sudanese henna leaves. Their names, retention time, molecular weight, molecular formula, and the area (%) are presented in **Table I**, together with their chemical structure (**Figure I**).

Antimycobacterial activity of henna leaf methanolic extract

The obtained crude methanolic extract was green in color, gummy in nature, oily in texture, with percentage yield of 23%. In the view of the well-defined antimicrobial activity of henna, the methanolic henna leaf extract demonstrated an inhibitory effect on all tested *M.tb* strains (**Table II**) at a concentration ≤ 50 mg/ml. The major secondary metabolites namely, squalene, lawsone and coumarin carboxylic acids, and/or the sum of phytoconstituents are likely responsible for the observed remarkable antimycobacterial activity being ≤ 50 mg/ml of the crude extract.

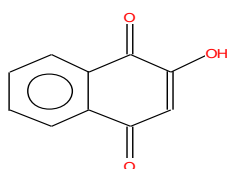
Table I Phytoconstituents identified in *Lawsonia inermis* methanolic extract of the leaf by GC-MS analysis.

No	Compound name / Synonyms	Retention Time	Molecular Weight	Molecular Formula	Area %
1	1,4-Naphthalenedione, 2-hydroxy (lawsone)	12.707	174	C ₁₀ H ₆ O ₃	20.185
2	2H-1-Benzopyran-3-carboxylic acid, 2-oxo-, methyl ester (Coumarin-3-carboxylic acid, methyl ester)	14.647	204	C ₁₁ H ₈ O ₄	5.131
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	16.049	296	C ₂₀ H ₄₀ O	4.991
4	Coumarin-4-carboxylic acid, methyl ester	16.606	204	C ₁₁ H ₈ O ₄	23.328
5	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- (Squalene)	29.611	410	C ₃₀ H ₅₀	34.433
6	dl- α -Tocopherol	33.592	430	C ₂₉ H ₅₀ O ₂	5.985
7	γ -Sitosterol	35.922	414	C ₂₉ H ₅₀ O	5.947

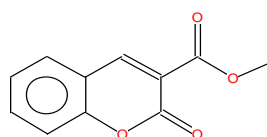
Table II Antimycobacterial activity of crude henna leaf methanolic extract against rifampicin sensitive(S) and resistant(R) strains.

TB Strain	RIF S/R	Extract concentration (50 mg/ml)
H37	S	S
150	S	S
1303	S	S
150904	S	S
153	R	S
1214	R	S
1240	R	S
1175	R	S

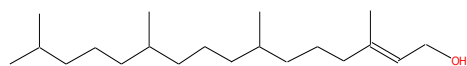
RIF, rifampicin; S, sensitive; R, Resistance.



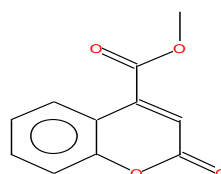
(a) 1,4-Naphthalenedione, 2-hydroxyl (lawsone).



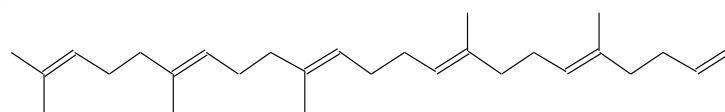
(b) Coumarin-3-carboxylic acid, methyl ester.



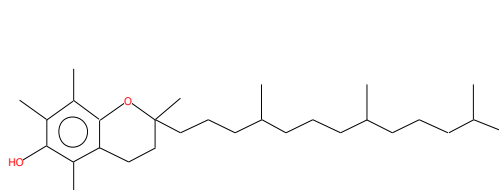
(c) 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (Phytol).



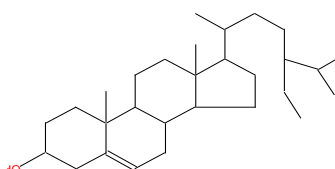
(d) Coumarin-4-carboxylic acid, methyl ester.



(e) 2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-, (all-E)-(Squalene).



(f) dl-α-Tocopherol



(g) γ-Sitosterol

Figure I Chemical structure of phytoconstituents identified by GC/MS in *Lawsonia inermis* methanolic extract of the leaf.

Discussion:

In this study, the methanol extraction method was used according to the notion that henna active components identified for their antimicrobial activity were aromatic compounds or organic compounds and were better obtained with methanol or ethanol extraction (Zhang and Lewis 1997). Our results showed that, the crude methanolic extract of henna leaves, demonstrated an inhibitory effect against all tested *M.tb* strains. This growth inhibitory activity towards rifampicin sensitive and resistant *M.tb* of henna extract or chemical derivatives therein may play crucial roles in the modern day chemotherapy of tuberculosis, especially as drug-resistant tuberculosis have become a great threat to global health. The tuberculostatic activity of the herb henna was previously documented *in vitro* and *in vivo* on guinea pigs and mice following infection with *M.tb* H37Rv, where the herb at a dose of 5 mg/kg led to significant resolution of experimental tuberculosis (Sharma 1990).

Natural products derived from medicinal plants have been an important source of *M.tb* therapeutics (Gupta et al. 2010). Our data identified seven components in Sudanese henna leaves extract, where squalene, Lawsone, and Coumarin carboxylic acid esters are the major chemical components with (34.433%, 20.185% and 23.328% occurrence respectively). In parallel to our result, Nasir et al. 2014 reported that, lawsone (2-hydroxy-1,4-naphthoquinone) was the major chemical component identified in both Nigerian and Egyptian henna.

Hema et al. 2015 identified six compounds in henna, and the prevailing compounds were α -D-glucopyranoside, methyl (51.73%) and 1, 4-naphthoquinone, 2-hydroxy lawsone (19.19%). Moreover, 7 different compounds were identified using GC-MS analysis of henna methanol fraction, and the prevailing compounds were squalene (71.49%) and vitamin E (76.85%) as reported Sharma and Goel 2018. Based on the study of the bioactive compounds found in *Lawsonia inermis*, it was reported that the plant contains mainly lawsone, flavonoids, tannins, alkaloids, terpenoids, quinines, coumarins, xanthenes and carbohydrates as active components (Arpita et al. 2014). The discrepancy in the type and amount of henna extract chemical components is most likely due to variation in geographical region, the age of the plant, local climate; seasonal variations, experimental conditions and genetic differences (Nasir et al. 2014).

Our data concluded the phytochemical components and the antimycobacterial activity of crude methanolic extract of henna leaves against *M.tb* strains tested (50 mg/mL). Further studies are needed to investigate the antimycobacterial activity of the extract chemical constituents and their *in vitro* and *in vivo* bio tests for realistic prospects in the future clinical use.

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