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SHORT COMMUNICATION

Antimycobacterial activity of ethanolic extract of *Artemisia absinthium* L.

T. Hojageldiyev[1], Y. Bolmammedov[1,*], S. Gurbanaliyev[2]

[1]State Medical University of Turkmenistan, Ashgabat, Turkmenistan

[2]Velayat Tuberculosis Hospital of Balkan velayat, Balkan, Turkmenistan

*E-mail address: yklym95@gmail.com

ABSTRACT

It is known that, drugs used in the treatment of tuberculosis show toxic effect to organism especially to liver beside its therapeutic effect. Because of ineffectiveness of drugs used in the treatment regimen of tuberculosis against multidrug resistance (MDR) and extensively drug-resistance (XDR) tuberculosis requires the development of new treatment methods and new novel drugs. Considering the usage of *Artemisia absinthium* in traditional medicine in treatment of wounds which suggests its antibacterial activity it seems that, also it may have significant antimycobacterial activity. The objective of present study was to evaluate antibacterial activity of ethanolic extract of *A. absinthium* against M. tuberculosis. In this study, the effect of ethanolic extract of *A. absinthium* was tested against tuberculosis and pharmaco-toxicological properties evaluated on laboratory animals. The 20%, 40%, 70% and 96% ethanolic extracts of *A. absinthium* prepared then its bacteriostatic and bactericidal activities were evaluated by validated methods. Data were analyzed by GraphPad Prism 7.0 at the level $P<0.05$. Results showed that ethanolic extracts of *A. absinthium* show no toxicological properties with having high LD50. All concentrations of extract show high bacteriostatic activity on M. tuberculosis. 96% ethanolic extract has highest bactericidal effect among other concentrations. By conducting further studies, as a results of our study, antimycobacterial drug can be prepared from *A. absinthium.*

***Keywords*:** *Artemisia absinthium*, antimycobacterial, ethanolic extract, Mycobacteria tuberculosis, *Mycobacterium tuberculosis*

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1. INTRODUCTION

Tuberculosis (TB) is an ongoing public health challenge with an estimated 10.0 million new TB cases worldwide in 2017 [1]. Despite progress in prevention and treatment of tuberculosis, multidrug-resistant (MDR) tuberculosis has become increasingly prevalent, and the extensively drug-resistant (XDR) form is emerging [2]. The most distinguishing characteristic of all Mycobacterium species is that the cell wall is thicker than in many other bacteria, which is hydrophobic, waxy, and rich in mycolic acids/mycolates. The cell wall consists of the hydrophobic mycolate layer and a peptidoglycan layer held together by a polysaccharide, arabinogalactan [3].

Mycolic acids are major and specific lipid components of the mycobacterial cell envelope and are essential for the survival of agents of genus [4].

Drugs used in the treatment of tuberculosis is cumbersome, due to its long duration and having hepatotoxicity leads to poor patient compliance [5, 6]. The current first-line four-drug regimen for drug-susceptible TB takes six to nine months to complete, and has significant side effects. Treatment for drug-resistant TB may take up to 30 months [7].

Other serious adverse effects after hepatotoxicity include dermatological, gastrointestinal, hypersensitivity, neurological, ototoxicity, neuropsychiatric manifestations, haematological and renal reactions. They can lead to drug discontinuation (in up to 10% of patients) or even more serious morbidity or mortality [8, 9].

This needs for more effective and tolerable treatment of TB infection. New drugs are required to shorten and simplify treatment, to improve the efficacy and tolerability of treatment for resistant strains of Mycobacterium tuberculosis.

Drugs made from medicinal plants affect to organism like endobiotic which reduces adverse effects to body. They are not only indispensable in health care, but form the best hope of source for safe future medicines [10, 11].

Historically, natural products have proved to be the most prolific and diverse source of antibiotics including some of those used for the treatment of TB. Current studies have indicated the urgent need for the development of new, safe and efficacious drugs to help reduce the global burden of tuberculosis. Novel antimycobacterial scaffolds from natural products have recently been reported. Natural products of plant biodiversity have received considerable attention as potential anti-TB agents since they are a proven template for the development of new molecules against tuberculosis. Many antitubercular compounds that may prove to be useful leads for TB drug discovery have been derived from medicinal plants [12].

Artemisia is a fairly large genus within the family of the Asteraceae (Compositae), with more than 200 individual species [13]. The well-known compound, artemisinin is also comes from this genus known as Artemisia annua, and its identification as an effective malaria treatment led to a Nobel prize for Chinese pharmaceutical chemist Tu Youyou in 2015. *Artemisia absinthium* or wormwood best known as the principal ingredient in the infamous Absinthe drink, has been used medicinally since the times of ancient Greece, and also in western European systems of traditional medicine. It is considered to produce most medicinally important secondary metabolites [14].

It has always been of great botanical and pharmaceutical interest and is useful in traditional medicines for the treatment of a variety of diseases and complaints [15, 16]. Extracts of the plant have shown to exhibit strong antimicrobial activity, especially against Gram-positive pathogenic bacteria [17].

Moreover aqueous extracts of *A. absinthium* are rich in caffeoyl and dicaffeoylquinic acids, which are known to inhibit HIV-1 integrase from integrating the reversibly transcribed viral DNA into host cell DNA [18]. This study was conducted to investigate the antimycobacterial activity of the ethanol extract of root of the *A. absinthium.*

2. MATERIALS AND METHODS

2. 1. Plant Material

The root of the *A. absinthium* were collected from different areas of hills near to Ashgabat in September 2017. Voucher specimens were deposited in the herbarium of State Medical University of Turkmenistan, Ashgabat, Turkmenistan.

2. 2. Preparation of the Extracts

The root of the plant material was made to become dry in an open air and protected from direct exposure to sunlight. The dried plant material was pulverized to particles of about 200 μm by using grinding machine. The air dried and powdered root of plant extracted with 96%, 70%, 40% and 20% ethanol by cold maceration at room temperature for 5 days to yield a powder after filtration and evaporation under vacuum. The dry powder was made up to required concentration in distilled water, filter sterilized and stored at 20 °C until use.

2. 3. Evaluation of Toxicity

Toxicological properties of prepared extracts studied on laboratory animals. Mice with weight 20-25 gr, rats with weight 180-200 gr, rabbits with weight 2 kg used in the study. Acute and chronic toxicity evaluated by administration of extracts orally to the laboratory animals. Cage side observations, daily food and water intake, body weight and mortality of the animals were recorded.

2. 4. Microbial Strain and Growth Media

Mycobacterium tuberculosis is employed in the study. For this, sputum specimens were collected from patients of Velayat tuberculosis hospital of Balkan velayat. Specimens tested for drug susceptibility and only drug susceptible strain *Mycobacterium tuberculosis* (H37Rv as control) and MDR isolates used in the study. Sodium hydroxide (Modified Petroff) method was used for the digestion and decontamination of the specimens [19].

Löwenstein-Jensen (L-J) medium is used for tuberculosis culture. The 96%, 70%, 40% and 20% ethanolic plant extracts were incorporated in the medium prior to inspissation. The medium set inoculated with the standard bacterial suspension and incubated at 37 °C for 6 weeks. Reading was taken weekly.

2. 5. Determination of Colony Forming Units

Determination of Colony forming units (cfu) on L-J - The ten-fold dilution of standard 1 mg/ml *M. tuberculosis* suspension were streaked on L-J medium for determining cfu in the presence and absence of plant extracts [20]. Percentage inhibition was calculated by mean reduction in number of colonies on extract containing as compared to extract free controls.

2. 5. Statistical Analysis

Statistical analysis was performed using independent t test and analysis of variance by GraphPad Prism 7.0. Data considered significant when $p<0.05$.

3. RESULTS

According to results of the study conducted on laboratory animals, the 20%, 40%, 70% and 96% ethanolic extract of root of A. absinthium showed a LD50 was very high, to be greater than 1 g/ kg. No toxic effects were reported other than lethal dose value.

Table 1. Results of anti-tuberculosis assay using plant extracts in Lowenstein Jensen (L-J) medium.

Medium	Mean cfu		% Inhibition	
	Drug-susceptible TB	MDR-TB	Drug-susceptible TB	MDR-TB
1. L-J (control)	54	32	0	0
2.L-J + 20% ethanolic extract	33	22	39	32
3. L-J + 40% ethanolic extract	26	17	52	47
4. L-J + 70% ethanolic extract	10	7	82	78
5. L-J + 96% ethanolic extract	0	0	100	100

Average growth and percentage inhibition of drug-susceptible TB and MDR isolates by L-J proportion method on extract containing and extract free control L-J slants after 6 weeks of incubation at 37 °C were recorded (Table 1). Inhibition of M. tuberculosis isolates was observed for all concentrations of extract. In the present study, ethanolic extracts of selected medicinal plant was observed to have anti-tuberculosis activity against MDR M. tuberculosis and drug-susceptible reference strain M. tuberculosis H37Rv. These MDR isolates were earlier found to be resistant against at least rifampicin and isoniazid, in addition to some other first line and second line drugs. As inhibition of growth by these extracts was observed in both the systems, inference about their anti-tuberculosis activity appears to be meaningful.

4. DISCUSSION

Plant derived products have been at the origin of many pharmaceutical drugs still in use. Their potential to offer new molecules and drugs is fully recognized today. The importance of

phytotherapy in the pharmaceutical field is steadily increasing. It is related to the belief of many people that natural products are safer.

A. absinthium is regarded as a broad remedy for all diseases owing to its curative medical powers. It contains absinthin, artabsin, essential oil, anabsinthin, anabsin, matricin, organic acids, lactones and resins [21]. Wormwood also possesses flavonoids such as rutin, quercetin and other flavonoid glycosides including quercitin-3- O-d-glucoside, isoquercitrin, quercitin-3-O-rhamnoglucoside, isorhamnetin-3-glucoside, isorhamnetin-3-O-rhamnoglucoside and phenolic acids (syringic, chlorogenic, coumaric, vanillic acids and salicylic acid) which are possibly involved in the mechanism of free radical scavenging assay [22]. Thujone is considered as most important component. Thujone is less soluble in water as compared to ethanol, only 8% of thujone is recovered in water as compared to extraction in 90% ethanol [23]. It was an important chemical, which is responsible for the hallucinogenic action of banned alcoholic drinks which is made from *A. absinthium* at the beginning of 20th century. Because of its medicinal potential, the medical use of *A. absinthium* is also proved by the monograph of the European Medicines Agency [24].

It is believed that the antimicrobial activity of hydroalcoholic extract of aerial parts of A. absinthium can be attributed to the presence of the major (camphor, p-cymene, caryophyllene) or minor (a-pinene, b-pinene) components of the plant or synergy between these compounds [25].

Phytochemical analysis of root of *A. absinthium* shows that polyacetylenes and sesamines are bioactive compounds which could play a significant role in its different kinds of action [26]. These natural polyacetylenes are compounds whose structures contain two or more triple bonds [27]. More than 2000 different acetylenes and biologically related substances have been extracted and identified in higher plants, of which more than 1100 have been found in species of the Asteraceae family. Polyacetylene compounds of the Asteraceae family have cytotoxic, antimicrobial, anti-inflammatory, neurotoxic, phototoxic, and several other types of activity [28].

Currently there are many opinions on combating against M. tuberculosis and its drug-resistance strains. A study suggests that, when tuberculosis bacteria covered by granuloma as result of our immune system, it shifts to survival mode and become dormant which is highly tolerate form of bacteria to antibiotics, and it is believed that this dormancy can be overcome by disrupting oxygen sensor of *M. tuberculosis* [29]. In this study it is hypothesized that root of wormwood contains chemical substance, might be polyacetylenes, which alters the mycolate layer of *M. tuberculosis*. For this reason, for confirming exact mechanism of inhibition of growth of *M. tuberculosis* strains by ethanolic extracts of root of *A. absinthium* further studies should be conducted including phytochemical analysis.

5. CONCLUSION

In conclusion, 20%, 40%, 70% and 96% ethanolic extracts of root of *A. absinthium* shows high antimycobacterial activity, that is the growth inhibition percent increases relatively to solvent concentration with having 100% growth inhibition of *M. tuberculosis* strains on 96% ethanolic extract. Therefore, by conducting further studies, it can be used in the management of tuberculosis.

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