

Novel Anti- tuberculosis Compound

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Abstract: *Mycobacterium tuberculosis*, the causative agent of tuberculosis is quiet difficult to treat or say, recovery the patients is prolonged. Iraq has a high burden of Tuberculosis (TB) and ranks the 44th. worldwide among the countries with a high TB burden and the 5th. among the countries of the Eastern mediterranean Region. This study was designed to evaluate the Anti-tuberculosis effect of a new compound of Argan Oil 100%, which has been fetched from Morocco Kingdom combined with Sidr extract 62.5g\l against *Mycobacterium tuberculosis*.

Ten isolates *Mycobacterium tuberculosis* were isolated from patients who attended Consultants Clinic of Respiratory and Thoracic Diseases in Hilla city, Iraq. All investigated patients were preliminary diagnosed by X- ray to have TB. The effect of mixture of Argan oil 100% and sidr extract 62.5g\l was tested on *M. tuberculosis* by incorporation the mixture with Lowenstein-Jensen (L.J) medium in different ratio to get final volume mixture: L.J. medium. Accordingly ratios were; 1: 9, 0.75:9.25, 0.5: 9.5, 0.4:9.6, 0.3:9.7, 0.25:9.75, 0.2:9.8 and 0.1: 9.9ml. L.J. medium was autoclaved for sterilization and kept to cool at 45°C then the appropriate concentrations the compound were added. The medium was inoculated with the TB. The growth of TB was observed in positive control tube while no growth was seen in the tubes containing the mixture of compounds although the reading of TB. growth continued for 28 days of incubation. Similar results displayed for all other mixtures of different ratio (1: 9, 0.75:9.25, 0.5: 9.5, 0.4:9.6, 0.3:9.7, 0.25:9.75, 0.2:9.8 and 0.1: 9.9)ml. The new compound used in this study revealed valuable effect against TB. After incorporated with culture media.

Keywords: Argan oil. Sidr extract. Anti-tuberculosis.

I. Introduction

Tuberculosis (TB) is an ancient disease with a long history of co-evolution with humans and a wide geographic distribution. *Mycobacterium tuberculosis*, the primary causative agent of TB disease in humans is a member of the *M. tuberculosis* complex (MTBC), a group of pathogens with high DNA sequence similarity but different host tropism, epidemiology and pathogenicity [1,2]. TB currently infects one third of the global population, coming in second after HIV as a cause of death worldwide [3]. One of the most concerning aspects of TB epidemiology is the emergence of multi- and extensively drug resistant strains (MDR and XDR-TB), which cause of concern in many parts of the globe [4].

Iraq has a high burden of tuberculosis (TB) and ranks 44th. worldwide among countries with a high TB burden and 5th. among the countries of the Eastern Mediterranean Region. According to world health organization, there were 20 000 incident cases of TB in Iraq in 2010 [5]. The incidence rate in 2010 was 64 cases per 100 000 of population, and the prevalence rate was 117 cases per 100 000 of population, based on a total population of 32 million . In 2010, here were 3900 death cases among TB cases, this give a mortality rate of 12 death per 100 000 population. The case detection rate was 48%. The estimated multiple drug resistance-tuberculosis (MDR-TB) cases among new pulmonary drug resistance-tuberculosis (MDR-TB) notified in 2010 was 210 (3.4% of new TB cases) [6]. Emergence of MDR-TB may indicate that the traditional antibiotics already used for treatment of TB become inactive at the present time [7].

Argan oil is a plant oil produced from the kernels of the Argan tree (*Argania spinosa* L.) that is endemic to Morocco. It is used for nutritive and cosmetic purposes. The tree is extremely well adapted to drought and other environmentally harsh conditions of southwestern Morocco. The genus *Argania* is now endangered and under protection of UNESCO [8]. Argan oil remains one of the rarest oils in the world due to the small and very specific growing areas.

The fruits of the Argan tree are nut-sizes and may be round, oval or conical in shape. The fruits are covered by a thick peel which covers the fleshy pulp. The pulp surrounds a hard-shelled nut which represents approximately 25% of the weight of the fresh fruit. Argan oil is extracted from the kernels, with yields from 30% to 55% depending on the extraction method used [9]. Argan oil consist of 42.8% oleic, 36.8% linoleic, 12% palmitic, 6% stearic and <0.5% Linolenic [10]. Argan oil has a relative density at 20 °C (68 °F) ranging from 0.906 to 0.919 [11]. Argan oil contains tocopherols (vitamin E), phenols, carotenes, squalene, and fatty acids, (80% unsaturated fatty acids). The main natural phenols in Argan oil are caffeic acid, oleuropein, vanillic acid,

tyrosol, catechol, resorcinol, epicatechin and catechin[10]. Depending on the extraction method, Argan oil may be more resistant to oxidation than olive oil [12].

Ziziphusspina-christi commonly known as Christ's Thorn Jujube, is a deciduous tree and native to the warm-temperate and subtropical regions, including North Africa, South Europe, Mediterranean, Australia, tropical America, South and East of Asia and Middle East [13]. It belongs to the Rhamnaceae family in the order of Rosales that contains about 60 genera and more than 850 species. The genus Ziziphus consists of about 100 species of deciduous or evergreen trees and shrubs throughout the world [14].

The Ziziphusspina- Christi was known to be active against wide spectrum of bacteria due to presence of betulic and cyanotic acid, three cyclopeptide alkaloids as well as four saponim glycosides and several flavonoids [15].

II. Material And Methods

2.1. Preparation of the Sidrexttract: A weight of 62.5 g of fresh and clean Sider leaves was immersed in one liter of sterile distilled water and heated up to 65C° for overnight. The solution was then left in the refrigerator for 12 hours[16].The solution was filter sterilized, the solid leaves were discarded and an appropriate volume of the liquid content was mixed with concentrated Argan oil (100%) to get a combined compound of Argan oil 100%-Sider extract 62.5 g/L. to be used in necessary experiments included in this study.

2.2. Cell Toxicity of the mixture: This experiment was performed as described by [17].

2.3. Preparation of Lowenstein-Jensen (L-J) medium:L-J medium provided by HiMedia Laboratories, India was used and prepared as recommended by [18].

2.4. Bacterial isolates:Ten *Mycobacteriumtuberculosis* isolates were recovered from patients who attended the consultants clinic of respiratory and thoracic diseases in Hilla city, Iraq. all patients were preliminary diagnosed by clinicians to have TB.

2.5. Experiment:The effect of compound was tested on *M. tuberculosis* by incorporation it with L-.J medium(after autoclaving and cooling to 45C°) in universal tubes in different ratios of compound/ml. : L-J medium/ml. as; 1: 9, 0.75:9.25, 0.5: 9.5, 0.4:9.6, 0.3:9.7, 0.25:9.75, 0.2:9.8 and 0.1: 9.9.The tubes were slant inoculated and incubated for 28 days with following up the growth daily.

III. Results And Discussion

The effect of mixture (Argan oil 100% and sidr extract 62.5 g /L) on TB not studied in Iraq and most of Arabian countries yet.The growth of TB-bacilli was seen in positive control tube, while no growth was seen in tube containing the compound after an incubation of 17 days and even after 28 days. The similar result were obtained for all other mixtures of different ratio (1:9, 0.75:9.25, 0.5:9.5, 0.4:9.6, 0.3:9.7, 0.25:9.75, 0.2:9.8 and 0.1:9.9)ml. The novel compound used in this study revealed valuable inhibitory effect against TB as shown in (Table 1).

Table 1: inhibition effect of mixture in the different ratio after different times

Incubation/ Days	Ratio of(mixture : L.J. medium) ml							
	1:9	0.75:9.25	0.5:9.5	0.4:9.6	0.3:9.7	0.25:9.75	0.2:9.8	0.1:9.9
17	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-

In this study there was growth of the TB –B-bacilli in the media containing Argan oil 100% only and also growth in media containing sidr extract 62.5g/L while no growth of the TB in the mixture tube in the different ratio (1:9, 0.75:9.25, 0.5:9.5, 0.4:9.6, 0.3:9.7, 0.25:9.75, 0.2:9.8 and 0.1:9.9)ml shown in (Figure :1 – A,B,C,D,E,F,G,H).

The results Regarding the Anti-tuberculosis effect of Argan oil indicated that there was no any considerable effect for Argan oil only and Sidr extract only as well compared with the control, while when these two substances combined with each other properly, a potential compounds can be obtained.



1 2 3 4 5



1 2 3 4 5

A:B:

- 1. Positive control tube. 1. Positive control tube.
- 2. Negative control tube. 2. Negative control tube.
- 3. L-J with the compound (9:1) ml. 3. L-J with the compound (9.25:0.75) ml
- 4. L-J with Sidr extract only. 4. L-J with sidr extract only.
- 5. L-J with Argan oil only. 5. L-J with Argan oil only.



1 2 3 4 5



1 2 3 4 5

C:D:

- 1. Positive control tube. 1. Positive control tube.
- 2. Negative control tube. 2. Negative control tube.
- 3. L-J with the compound (9.5:0.5) ml. 3. L-J with compound(9.6:0.4) ml.
- 4. L-J with sidr extract only. 4. L-J with sidr extract only.
- 5. L-J with Argan oil only. 5. L-J with Argan oil only.



1 2 3 4 5

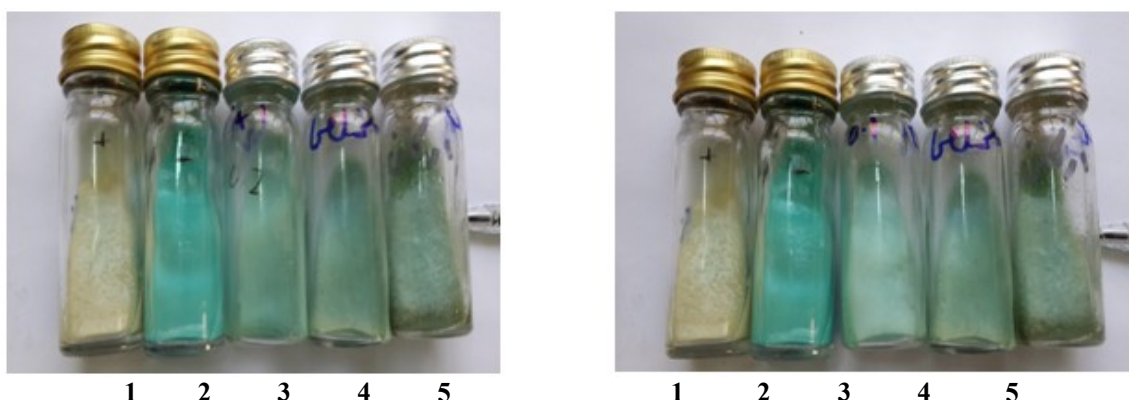


1 2 3 4 5

E:

F:

- 1. Positive control tube. 1. Positive control tube.
- 2. Negative control tube. 2. Negative control tube.
- 3. L-J with the compound (9.7:0.3) ml. 3. L.J. medium(9.75:0.25) ml
- 4. L-J with sidr extract only. 4. L-J with sidr extract only.
- 5. L-J with Argan oil only. 5. L-J with Argan oil only.



G: H:

1. Positive control tube. 1. Positive control tube.

2. Negative control tube. 2. Negative control tube.

3. L-J with the compound (9.8: 0.2) ml. 3. L.Jwith the compound (9.9: 0.1) ml.

4. L-J with sidr extract only. 4. L-J with sidr extract only.

5. L-J with Argan oil only. 5. L-J with Argan oil only.

Figure 1, (A-H) illustrate the results combined compound on TB- bacilli.

IV. Conclusion

This study settles the activity of mixture (Argan oil 100% and Sidr extract 62.5 g \ L) as anti- TB agent. The novel compound used in this study revealed valuable effect against TB. After incorporation with culture media.

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