

# Evaluation of Anti-tuberculosis activity of *Cassia occidentalis* seeds Extracts in Kassala state- Sudan

Dr. Mohamed Elamin Almahy

Dr. Abdelrhman Mohamed Sidahmed

## Abstract:

This study aimed to evaluate the *anti-tuberculosis* activates of extracts *Cassia occidentalis* seeds. The investigation was carried out on 202 pulmonary tuberculosis specimens from human, obtained from Kassala Hospital from January 2014- up to January 2016. The prevalence of tuberculosis is higher in male (70 %) than females (30%) and most patients aged 20 – 30 years old. The positive specimens were cultured on Löwenstein-Jensen 'L.J.' medium media. Eighteen (13%) pure positive mycobacterial Seven (39%) specimens were identified. The seven isolates were treated with Chloroformic and aqueous extract of *Cassia occidentalis* seeds (Soreib), The Minimum Inhibitory Concentration (MIC) results revealed the Chloroformic extracts of *C. occidentalis* seeds had high activity compared with Rifampicin. The aqueous extracts of *Cassia occidentalis* had low MICs less activity compared with Rifampicin.

**Keywords:** Anti-tuberculosis, *Cassia occidentalis*, extracted Aqueous, Chloroform.

تقييم فعالية مستخلصات بذور نبات السوريب ضد الميكروب المسبب للدرن

بولاية كسلا - السودان

د. محمد الأمين الماحي مصطفى اسحاق-- قسم الأحياء - كلية التربية - جامعة كسلا

د. عبد الرحمن محمد سيد أحمد -قسم الكيمياء السريرية - كلية الطب -جامعة كسلا

المستخلص:

هدفت هذه الدراسة إلى تقييم فعالية مستخلصات نبات السوريب ضد الميكروب المسبب للدرن. أجريت هذه الدراسة علي ( 202 ) عينة مأخوذة من مرضي يشته في إصابتهم بميكروب داء السل الرئوي وجمعت العينات من مستشفى كسلا في الفترة من يناير 2014م حتى يناير 2016م . أوضحت الدراسة عدد الذكور المصابين (70%) ذكر بينما عدد الإناث المصابات (30%) أنثى. لوحظ أن معظم الإصابات تكثر وسط

المرضى الذين تتراوح أعمارهم بين العشرين والثلاثين سنة. زرعت العينات الموجبة للصبغة علي وسط (Löwenstein-Jensen media) ، كان عدد العينات النامية ثمانية عشر عينة ( 13% ). تم التعرف علي سبع عينات (39%) من التي تسبب داء السل الرئوي. عوملت العزلات السبعة ببذور السوريب المستخلصة بالكلورفورم والمستخلص المائي. حدد أقل تركيز مثبط لنمو البكتيريا ,وجد أن أكثر مستخلص نباتي فعال وله أعلى تركيز مثبط لنمو البكتيريا هو مستخلص بذور السوريب بالكلورفورم مقارنة بالمضاد الحيوي ريفامبين. أما المستخلص المائي لبذور السوريب فكان اقل تركيز مثبط لنمو البكتيريا مقارنة بالريفامبين 1.

### Introduction:

Tuberculosis (TB) is an infectious disease caused by TB (*Mycobacterium tuberculosis*), (Putra *et al.*,2021 ).Most of the TBC germs attack the lungs, but can also attack other organs of the body (Rosandali ,2016).It continues to be one of the greatest challenges to global health. In 2015, 10.4 million people were estimated to have developed TB and 1.4 million died of the disease (Yeon *et al.*,2017). Of these 10.4 million new cases, 56% were men, % 34were women, and 10% were children. People with human immunodeficiency virus (HIV) accounted for 1.2 million (%11) of all new TB cases, and although the number of TB associated deaths fell by %22 between 2000 and 2015, TB remained one of the top 10 causes of death worldwide in 2015(Yeon *et al.*,2017).

The estimated adult HIV prevalence of 1.5% remains lower than that of its African neighbors to the south and a report from 2002 suggested %4 of tuberculosis patients were co-infected with HIV ( Ghada et al., 2016).

The Republic of Sudan is the largest country in the African is divided between five geographic regions Eastern, Western, Southern, Northern and Central Sudan which includes the Capital Khartoum. It has high burden of tuberculosis (TB) with a prevalence of 209 cases per 100,000 of the population and 50,000 incident cases during 2009(Almahy,2018).

M. tuberculosis of infection is transmitted from person to person by droplet nuclei containing the organism and spread mainly by coughing. In most cases, the initial infection is clinically silent because host immunity adequately limits further multiplication of bacilli (Yeon *et al.*,2017).

The Sudan National TB Program treatment policy was for an intensive phase of Rifampicin, Isoniazid, Pyrazinamide and Streptomycin daily under direct supervision for two to three months until the patient became smear negative followed by eight months of Isoniazid and Ethambutol (Almahy,2018). The objective of this study is to verify the activity of the active extracts against *Mycobacterium tuberculosis* clinical isolates to determine the minimum inhibitory concentrations ( MICs ) of active extracts.

### **Materials and Methods:**

#### **Plant material:**

The plants Seeds of *Cassia occidentals* were used in this study were collected between July and October 2015 from Gash Delta in Kassala state They were authenticated by Medicinal and Aromatic.

Plants and Traditional Medicine Research Institute (MAPT-MRI). The voucher specimens were deposited at the herbarium of that institute.

#### **Preparation of the extracts:**

Extraction was carried out according to method described by Sukhdev *et al.* (2008):

Specific weight of each plant sample was coarsely powdered using mortar and pestle. Coarsed sample were successively extracted with Chloroform and methanol using soxhelt extractor apparatus. Extraction carried out for about six hours for chloroform and eight hours for methanol till the colour of the solvents at the last siphoning time became colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts exposed to air in Petri dishes till completely dried and the yield percentages were calculated as follows:

Weight of extract obtained / weight of plant sample X100.

#### **Antimycobacterial testing**

Evaluation of antimycobacterial activity was carried out as a susceptibility test of *M.tuberculosis* on LJM following the propor-

tional method as described in Din-Norm 58, 943, part 8(1996).In short, extracts, were dissolved in Dimethyl sulfoxide(DMSO) and added to the medium before heating at 85°C for 35 min. Test tubes containing the medium were inoculated with a strain of *M. tuberculosis* with the following characteristics: *in vivo* sensitivity against Streptomycin, Rifampicin, Ethambutol and Isoniazid; and with minimum inhibitory concentrations of 2, 4, 0.5 and 0.06  $\mu$ g/ml LJM, respectively. In the case of punicalagin a patient strain has also been used. Asres *et al.*(2001) .Cultures were incubated for 4 weeks in a vertical position at 36°C. Cultured tubes were examined visually and sample tubes showing less growth than control tubes were considered to be inhibited.

### Incubation and Reading

Snedecor and Cochran. (1989).The slopes were incubated at 37°C.Read the proportion tests were read at 28 days and again at 42 days. Growth was recorded as:3+ confluent growth ,2+ more than 100 colonies, 01-99 cols. The actual number of colonies. When the number of colonies in a given dilution is less than 15, the number of colonies was counted with the next larger inoculum, or estimate if more than 100. (Make no attempt to estimate the number of colonies if the growth is 3+).

### Statistical analysis

Analysis of variance (ANOVA) was conducted to test the effect of the different concentrations of the plant extracts.

### Results and Discussion

*C. occidentalis* of family caesalpiniaceae. All parts of the plant were used medicinally (Ali,2015). Leaf extracts of this plant were found to be active against different types of microorganisms .The seeds of this plant possess a strong antibacterial activity against *S. aureus*, *B. subtilis*, *B. proteus* and *Vibrio cholerae* and against fungi *A. flavus*, *A. niger* and *Trichophyton mentagrophytes*(Singh *et al.*,2016).

The Anti- *Mycobacterium* activity of extracts of Seeds *Cassia occidentalis* were assessed against seven clinical isolates of

*Mycobacterium tuberculosis*, four different concentrations (100, 50, 25 and 12.5mg/ml) were tested, Rifampicin concentrations (40µg/ml). The results obtained by *Cassia occidentalis* Chloroformic extract of the seeds showed high activity while aqueous extract showed moderate activity at concentrations 100 and 50mg/ml. Table(1) & figure(1).

**Table ( 1 ).The activity percent of *Cassia occidentalis* extracts against isolate**

Plant	Part Used	Solvent	Concentrations (mg/ml)				Rifampicin
			100	50	25	12.5	
<i>Cassia occidentalis</i>	Seeds	Chloroform	100	100	100	100	100
		Aqueous	86	71	71	71	

**Figure( 1 ):Inhibition percent of *Cassia occidentalis* and Rifampicin against *M.t* isolates**

**Key:**

Ch : Chloroform , Aq : Aqueous

RIF : Rifampicin , *M.t* : *Mycobacterium tuberculosis*

The minimum inhibitory concentration (MIC) results revealed the Chloroformic extracts of *C. occidentalis* seeds had high activity compared with Rifampicin. The aqueous extracts of *Cassia occidentalis* had low MICs less activity compared with Rifampicin.

The plant contained crude protein %2.3, crude fiber %20.8 , lipid %14.9 and carbohydrates 48.1% (Al-Snafi,2015). The phytochemical analysis showed that the different parts of the plant contained different chemical groups including alkaloids, anthocyanosides, phenolics, proteins, phlobatannins, steroids, tannins, flavonoids, anthroquinone, saponins, terpenes, resins, balsams, amino acids, carbohydrates, sugars and cardiac glycosides (Ali,2015).

The(MIC) of extracts of Seeds *Cassia occidentalis* belonging to both extracts which showed anti-mycobacterial activity were determined against the clinical isolates of *M.tuberculosis*. The results were summarized in table (2) as mg/ml of extracts.



**Table (2).Minimum Inhibitory Concentrations (MICs )( mg/ml) of *Cassia occidentalis* for Seeds.**

Isolates	MIC (mg/ml)	
	Aqueous	Chloroform
M.t <sub>1</sub>	<12.5	<12.5
M.t <sub>2</sub>	12.5	<12.5
M.t <sub>3</sub>	100	<12.5
M.t <sub>4</sub>	>100	<12.5
M.t <sub>5</sub>	>100	25
M.t <sub>6</sub>	25	50
M.t <sub>7</sub>	25	25

M.t<sub>1-7</sub> =*Mycobacterium tuberculosis* isolates

The seeds and oil of *C. occidentalis* was evaluated against *C. maculatus*. At the rate of 10 % (w/w), both fresh and dry leaves as well as whole and ground seeds had no contact toxicity on the cow-pea beetle. In contrast (Ali,2015).This plant is also used in another poly herbal formulation Liv.52 tablet and syrup used extensively in the management of Hepatitis A (HA)( Vijay *et al* ., 2016).

**Conclusions**

Isolation identification of active substances from the extracts which exhibited promising activities need to be carried out. The results of this study have further shown that there is potential to develop new compounds against multi drug resistant TB from Plants.

**Recommendations**

- The active constituents of the plant should be isolated, identified and characterized. These should then be tested on the resistant strains of *Mycobacterium tuberculosis*.
- Toxicity studies should be carried out on the active plant to determine safety of the extracts.

## References:

- (1) **ALmahy, M.E. (2018).** Evaluation of Anti-tuberculosis activity and phytochemical screening of some Sudanese Medicinal Plants, Ph.D. thesis, Sudan Academy of Sciences.
- (2) **Ali, E. A. (2015).** The Therapeutic importance of *Cassia occidentalis* –An overview
- (3) Indian Journal of Pharmaceutical Science & Research, p159.
- (4) **Al-Snafi, A.E. (2015).** The chemical contents and pharmacological effects of *Anagallis arvensis* - A review. International Journal of Pharmacy, 5(1), 37-41.
- (5) **Asres, F. K., Bucar, S., Edelsbrunner, T., Kartnig, G., Hoöger, and W. Thiel. (2001).** Investigations on Antimycobacterial Activity of Some Ethiopian Medicinal Plants. *Phytother. Res.*, 15: 323–326.
- (6) **Ghada, S. Sharaf, Eldin, ImadFadl- Elmula, Mohammed, S., Ali, Ahmed, B. Ali, Abdel Latif, G.A Salih, Kim, Mallard, Christian, Bottomley and Ruth McNerney .(2016).** Tuberculosis in Sudan: a study of *Mycobacterium tuberculosis* strain genotype and susceptibility to anti-tuberculosis drugs. *BMC Infectious Diseases*, 11:219.
- (7) **Putra, A. S., Yulia, K. A., Reni, R. A. H., Fauziah, N., Fitri, H and Nofi, S. (2021).** Improvement of Knowledge and Attitudes on Tuberculosis Patients with Poster Calendar and Leaflet. Universitas Islam Negeri Sumatera Utara, Indonesia .Journal of Health Education, 6 (1), p39.
- (8) **Rosandali, A. (2016).** Hubungan Antara Pembentukan Scar Vaksin Bcg Dan Kejadian Infeksi Tuberkulosis. Jurnal Kesehatan Andalas, 5(1), 1–5.
- (9) **Singh, V.V., Jain, J., Mishra, A.K. (2016).** Pharmacological and Phytochemical profile of *Cassia occidentalis* L: A review. Journal of Drug Delivery and Therapeutics, p 95.
- (10) **Snedecor , G.W. and Cochran, W.C.(1989).** Statistical Methods, 8<sup>th</sup>edn.Iowa State University Press, Ames, Iowa.

- (11) **Sukhdev. S. H., Suman. P. S. K., Gennaro. L and Dev. D. R. (2008).** Extraction technologies for medicinal and aromatic plants. United Nation Industrial Development Organization and the International Center for Science and High Technology. pp 116.
- (12) **ijay, V. S., Jainendra, J and Arun , K. M.(2016).**pharmacological and phytochemical profile of *Cassia occidentals* L : A Review. Journal of Drug Delivery and The rapeutics , 6(5), p 94.
- (13) **Yeon, J. J., Kyung, S. L., Joon. J.Y.(2017).** The diagnosis of pulmonary tuberculosis: a Korean perspective. Precision and Future Medicine, 1(2): 77-87.