The Effect of Some Sudanese Medicinal Plant Extracts on Some Clinically Isolated Pulmonary Tuberculosis Bacteria

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ABSTRACT

Plants are an abundant source of biologically active compounds that have been shown to be effective antimicrobial agents. Many plants have traditionally been used to treat Mycobacterium infections. The goal of this research was to evaluate the effect of local medicinal plants on *Mycobacterium tuberculosis* isolates. The investigation was carried out on 130 pulmonary tuberculosis specimens from humans, obtained from Abo Anga and Al-Shaab hospitals. There were 103 infected males and 27 infected females, all of whom were between the ages of 20 and 30. The specimens were smeared, fixed, and stained directly with Ziehl-Neelsen. The acid-fast bacilli (AFB) were visible as red, straight, or slightly curved rods, singly or in small groups against a blue background.

The seven isolates were tested against extracts of Sudanese medicinal plants that had previously been shown to inhibit the growth of other microorganisms. The most active extracts were those of six plants particularly four extracts, belonging to two families that demonstrated activity against clinical isolates, and the minimum inhibitory concentrations were determined. Phytochemical screening was performed on the plants that demonstrated high anti-TB activity. It was concluded that specific tannins, saponins, and flavonoids play a significant role in anti-TB activity.

Key Words: *Sudanese Medicinal plants, Tuberculosis, Isolates, Phytochemical screening*.

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INTRODUCTION

Tuberculosis (TB) is still a major public health concern in various parts of the world, particularly in developing countries [1]. It is a contagious disease that is spreading in some parts of the world. It is estimated that in developing countries, 30-60% of adults have *Mycobacterium tuberculosis* [2]. Each year, about 8-10 million people develop clinical TB, and 3 million people die as a result of the disease [3].

More than half of patients do not recover, the organisms become resistant to the medicines used, and since patients typically live in close quarters, drug-resistant organisms may be transmitted to others. The primary aim of anti-TB treatments are to cure each patient and thus reduce disease mortality and morbidity. The burden of TB is almost entirely carried by those living in low-income countries, with the disease accounting for a quarter of all avoidable deaths in these countries [4]. TB primarily affects young adults who are the economically productive segment of society [5]. The worldwide emergence of MDRTB is a major threat to TB control that is defined as TB caused by bacteria resistant to at least the 2 most important first-line medicines, isoniazid and rifampicin [6]. It is well understood that the emergence of drug resistance is linked to poor treatment practices, particularly erratic drug intake or monotherapy in the early months of treatment. Patients' noncompliance with treatment, especially failure to adhere to multi-drug regimens and repeated interruptions can

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result in the selection of drug-resistant mutants of tuberculosis bacteria in such cases [7].

The Combretaceae family contains about 20 genera and 600 species. This plant is found primarily in tropical and subtropical regions such as Brazil and Africa [8]. This family is extensively used as traditional medicine [9]. Most African communities use these plants to treat snake and scorpion bites, as well as pneumonia, chest coughs associated with tuberculosis, fever, colds and sore throats, mental problems, and venereal diseases such as syphilis [10]. Phytochemical constituents found in the genus include alkaloids, saponins, tannins, and cardiac glycosides [11, 12]. To date, over 27000 alkaloid-based compounds have been recorded in the Dictionary of Natural Products [13].

This study aimed at evaluating the effect of local medicinal plants on *Mycobacterium tuberculosis* isolates.

MATERIALS AND METHODS

Study area and sample collection

One hundred and thirty sputum specimens were collected from patients of various ages and genders at Khartoum State's Abo-Anga and Al-Shaab hospitals who were clinically suspected of being infected with *M. tuberculosis*. The specimens were transferred to the microbiology lab for staining by Ziehl-Neelsen technique and culturing in Lowenstein-Jensen (L.J) medium [14, 15].

Chloroform and methanol extraction

Before testing for anti-TB activity, 50 grams of each plant sample were powdered and extracted. To extract substances, chloroform was added and heated at 35°C for 24 hours. The dry samples were placed in a soxhlet containing methanol (96%). To extract substances, samples were packed into soxhlet with methanol (96%) and heated at 40°C for 24 hours.

Testing of extracts for anti-TB activity

Using serial dilution, six different concentrations were created. Before solidification, 3 ml of each concentration were added to seven bottles of L.J. medium. Finally, six different concentrations of the seven extracts were tested on each isolate.

Preliminary phytochemical screening of samples having antibacterial activity

Phytochemical analysis of the active morphological sample is extremely informative in terms of the nature of the constituents of each plant sample. It was thought essential to correlate the nature of chemical constituents and antibacterial activity tests for the detection of the various chemicals.

Preparation of the extracts

For 4 hours, 10 grams of powdered plant material were refluxed with 100ml of 80% ethanol. The cool solution was filtered, and 100 ml of 80% ethanol was passed through the filtrate. The extract was used in a variety of tests, including Unsaturated Sterols, Triterpenes, Alkaloids, Flavonoids, Saponins, Cyanogenic glycoside, Anthraquinone glycoside, Coumarins, and Anthraquinone glycoside.

RESULTS AND DISCUSSION

Clinical isolates

One hundred thirty sputum isolates were obtained from patients suspected of having *Mycobacterium tuberculosis* based on their symptoms.

Microscopical examination

Microscopically, 90 (69.23%) specimens showed positive Acid-Fast Bacilli (AFB) by Ziehl Neelsen stain, that appeared as red, straight or slightly curved, and 40 (30.77%) showed negative result.

Age and gender

The age group ranged between 10-60 years. Most of them were males, 62 (68.89%) males and 28 (31.11%) females **(Table 1)**. Some of them were treated for more than two months.

Cultural examination

The cultivation of the obtained specimens on slopes of L.J. medium resulted in pure positive growth for *M. tuberculosis* in 15 (16.67%) and contamination in 32 (35.56%), and 43 (47.78%) were considered negative after the optimal period of incubation.

Thermal, photo, and biochemical activities of the isolates

According to the tests, seven isolates (46.67%) were identified as *M. tuberculosis* and tested against antibacterial agents (antibiotics and medicinal plant extracts).

Susceptibility testing to commonly used antibiotics

The in vitro studies of the susceptibility to anti-tuberculosis drugs were performed on L.J. medium and the inoculums of the isolated species were prepared. The resistance of the isolates to Streptomycin (SM), Rifampicin (RMP), Ethambutol (EMP), and Isoniazid (INH) was 3 (42.86%), 3 (42.86%), 4 (57.14%), and 2 (28.57%), respectively **(Table 2)**. Multi-drug resistant (MDR) strains were 5 (71.43%), and those resistant to two or three drugs were 4 (57.14%) and 1 (14.29%), respectively. None of them showed resistance to all drugs **(Table 3)**.

Screening for the anti-TB activity of some Sudanese plants

In the preliminary screening for the anti-TB activity of six Sudanese medicinal plants belonging to two families, the total number of extracts examined against the tested organisms was seven. All of them were methanol extracts and exhibited inhibitory effects against one or more of the isolates **(Table 4)**.

Determination of minimum inhibitory concentration (MIC)
The MIC of seven extracts belonging to six medicinal plants, which showed antibacterial activity, was determined against the clinical isolates of *M. tuberculosis*. The results were summarized in **Table (5)** as mg/ml of crude extract.

Preliminary phytochemical screening of selected Sudanese medicinal plants with a relatively high anti-TB activity
Seven samples of different parts of six plants belonging to two families that proved to have a high anti-TB activity were examined phytochemically. The presence or absence of various types of secondary metabolites (alkaloids, flavonoids, terpenoids, saponins, anthraquinones, and cyanogenic glycosides) was tested. The results were summarized in **Table 6**. The susceptibility of the isolates to the extracts of the medicinal plants is shown in **Table 7**.

Table 1. Gender and age rates of TB Patients

Less than 10 Years	11-20	21-30	31-40	41-50	51-60	Over 60 Years	Total
(n)	3	50	40	17	12	8	130
(%)	2.31%	38.46%	30.77%	13.08%	9.23%	6.15%	100%
Gender	Male/Female 3 0	Male/Female 38 12	Male/Female 32 8	Male/Female 13 4	Male/Female 10 2	Male/Female 7 1	Male/Female 103 27

Table 2. Drug resistance Patterns in TB patients

Drug	Streptomycin	Rifampicin	Ethambutol	Isoniazid
Seven Isolates	42.86 %	42.86 %	57.14 %	28.57 %

Table 3. Drug resistance rate in TB patients

R- rate	R_1	R_2	R_3	R_4	S
Strains	1	4	1	0	1
	14.29 %	57.14 %	14.29 %	0	14.29 %

Key: R_1: Resistant to one drug, R_2: Resistant to two drugs, R_3: Resistant to three Drug, R_4: Resistant to four Drug, S: Sensitive

Table 4. Preliminary screening for the anti-TB activity of some Sudanese plants

Family/botanical name/vernacular name	Folkloric uses (Local uses)	Area of collection	Part used	Solvent used	Yield %
Combretaceae *C.aculeatum* Ver. Shiheit	For wounds healing	W.S	R	$CHCL_3$	1.82
				MeOH	10.68
Combretaceae *C.adenogonium*		W.S	L	$CHCL_3$	4.6
				MeOH	16.12
Combretaceae *C.glutinosum*	For hepatic disease	W.S	L	$CHCL_3$	2.94
				MeOH	22.52
Combretaceae *C.hartmannianum* Ver. Habiel	For jaundice	W.S	L	$CHCL_3$	9.8
				MeOH	10.6
			S	$CHCL_3$	6.6
				MeOH	8.6
Combretaceae *T.laxiflora* Ver. Daroot	For eye-wash	W.S	L	$CHCL_3$	3.24
				MeOH	18.6
Loranthaceae	For wound healing	C.S	L	$CHCL_3$	7.8

P.acaciae			MeOH	9.0
Ver.Inab elnabag				

Key: R: Root, L: Leaf, S: Seed, Ver.: Vernacular name, W.S: Western Sudan, C.S: Central Sudan

Table 5. Minimum inhibitory concentration (mg/ml) of the crude extracts against the clinical isolates

Plant	Part used	Solvent used	Clinical isolates						
C.aculeatum	R	MeOH	75.0	37.5	37.5	75.0	75.0	75.0	75.0
C.adenogonium	L	MeOH	18.75	37.5	18.75	37.5	18.75	9.38	18.75
C.glutinosum	L	MeOH	150.0	150.0	150.0	75.0	150.0	150.0	75.0
C.hartmannianum	L	MeOH	18.75	18.75	37.5	37.5	18.75	18.75	9.38
	S	MeOH	75.0	75.0	37.5	75.0	75.0	37.5	75.0
T.laxiflora	L	MeOH	37.5	37.5	37.5	37.5	75.0	37.5	9.38
P.acaciae	L	MeOH	9.38	37.5	9.38	9.38	9.38	37.5	9.38

Key: R: Root, L: Leaf , S: Seed

Table 6. Preliminary Phytochemical Screening of the most active plants sample

Family	Botanical name	Part used	Alkaloids	Flavonoids	Coumarins	Saponins	Tannins	Anthra-quinones	Cyanogenic-glycosides	Sterols and/or triterpenes
Combretaceae	C. aculeatum	R	_	+	+	_	+	+	+	+
	C. adenogon-ium	L	_	+	+	+	+	_	+	+
	C. glutinosum	L	_	+	+	+	+	+	_	+
	C. hartmann-ianum	L	+	+	+	+	+	+	+	+
		S	_	+	+	+	+	+	_	+
	T. laxiflora	L	_	±	+	+	++	+	+	++
Loranthaceae	P. acaciae	L	_	+	+	+	+++	+	_	±

Key: L: Leaf (-): Not detectable, R: Root (±): Traces, S: Seed (+): Low concentration, (++): Medium concentration (+++): High concentration

Table 7. Susceptibility of the isolates to extracts of the medicinal plants

Medicinal – Plants / Isolates	MIC of Medicinal Plant Extracts (mg/ml)						
	C. ac (R)	C. ad (L)	C. gl (L)	C. h (L)	C. h (S)	T. lax (L)	P. ac (L)
TB 1	75.0	18.75	150.0	18.75	75.0	37.5	09.38
TB 2	37.5	37.5	150.0	18.75	75.0	37.5	37.5
TB 3	37.5	18.75	150.0	37.5	37.5	37.5	09.38
TB 4	75.0	37.5	75.0	37.5	75.0	37.5	09.38
TB 5	75.0	18.75	150.0	18.75	75.0	75.5	09.38
TB 6	75.0	09.83	150.0	18.75	37.5	37.5	37.5
TB 7	75.0	18.75	75.0	09.38	75.0	09.38	09.38

Key: MIC: Minimum Inhibitory Concentration, C. ac: Combretum aculeatum, C. ad: Combretum adenogonium, C. gl: Combretum glutinosum, C. h: Combretum hartmannianum, T. lax: Terminalia laxiflora, P. ac: Plicosepalus acaciae, R: Root, L: Leaf, S: Seed

Given that *Mycobacterium tuberculosis* is the cause of tuberculosis for more than 100 years and that efficient chemotherapy against the disease has been available for more than 50 years, it is a threat against humanity that this disease is still one of the world's most serious public health issues today [16]. TB treatment has been reshaped, and current therapy regimens are based on multidrug therapy, with 3-4 anti-tuberculosis drugs typically used. Even so, multidrug-resistant tubercle bacilli are becoming a problem for a variety of drugs, including isoniazid, ethambutol, rifampin, and streptomycin [17].

The drug resistance patterns of SM, RMP, EMP and INH were: (42.86%), (42.86%), (57.14%), (28.57%), respectively.

In the present study, the in vitro drug susceptibility testing showed that out of seven isolates, 1(14.29%) was fully sensitive to all the four drugs under investigation. However, 6 (85.71%) were resistant to one or more drugs. One (14.29%) isolate was resistant to a single drug, while 5 (71.43%) were multi-drug resistant (MDR), 4 (57.14%) were resistant to two drugs, and 1(14.29%) was resistant to three drugs. In another study, it was reported that primary and secondary drug resistance strains were 2.4% and 14.3%, respectively [18].

Drug-resistant tuberculosis is extremely difficult to treat, which necessitates the use of more and different medications over a longer period of time. Surgery is often required to remove areas of demolished lungs that are strongly infected with mycobacteria and are inaccessible to drugs [19]. Because mycobacteria are becoming increasingly resistant to traditional anti-tuberculosis drugs, there is an opportunity for new anti-tuberculosis agents. According to a recent WHO report, 2% of all tuberculosis cases worldwide are multi-drug resistant by description, meaning they are resistant to isoniazid + rifampicin (plus/minus other resistances). These cases can be cured in the United States and other high-resource countries, but at a high cost and with long courses of rather toxic medicines, posing a serious compliance dilemma [20]. It is critical to developing new anti-tuberculosis agents, preferably those that can be easily and cheaply manufactured from local sources. The use of antimicrobials derived from natural plants has a significant effect on human health care in developing countries.

Local healers have used herbal remedies in rural areas for centuries, and it has been developed in industrialized countries. Medicinal plant research has yielded a number of substances used in advanced medication to cure serious diseases [21]. In the situation of *C. aculeatum*, the root extract had the same effectivity as the seeds extract of *C. hartmannianum*, and both *C. hartmannianum and C. aculeatum* rank fifth in effectivity against the seven clinical isolates when compared to other plant extracts. In the case of *C. glutinosum*, the leaves extract had the lowest efficacy against clinical isolates when compared to all other extracts. C. aculeatum aerial part water decoction has anti-mycobacterial activity [22]. Martini et al. 2004 investigated the antibacterial activity of *C. erythrophyllum* and discovered that seven antibacterial flavonoids had antimicrobial activity against Gram-negative and -positive strains in another study [23].

In the case of *C. hartmannianum*, the leaves extract was more effective than the *Plicosepalus acaciae* extract against the seven clinical isolates, with a lower MIC comparable to *C. hartmannianum* seeds extract. *C. hartmannianum* leaves extract exhibited lower MIC in five clinical isolates out of seven samples, while *C. hartmannianum* seeds extract showed lower MIC in two clinical isolates. In the case of *C. adenogonium*, the leaves extract demonstrated lower MIC in one clinical isolate and moderate affectivity in the remaining six isolates. *T. laxiflora* leaves extracts exhibited moderate effectivity in five clinical isolates, higher effectivity in one, and lower effectivity in the last. The leaves extract of *P.acaciae* demonstrated high activity against six isolates in the current study. El- Shafeia et al. (2017) analyzed *P.acaciae* for antibacterial activity and discovered that the plant contains a high concentration of flavonoids, tannins, and alkaloids [24].

El- Shafeia et al. (2017) evaluated the antimicrobial activity of chloroform, methanol, and aqueous extracts of *P. acaciae* leaves and stem, and discovered that the leaf methanol extract exhibited the highest level of activity against a variety of Gram-positive and Gram-negative clinical isolates from Sudanese patients [24].

Except for *C. adenogonium*, all of these plant extracts contained anthraquinones. Except for the leaves of *C. hartmannianum*, none of them were found to contain alkaloids. Saponins, which have been linked to antibacterial activity in some plants, were found in all plant extracts except *C. aculeatum*. Tannins were abundant in *T. laxiflora* and *P. acaciae. P. acaciae* leaves, on the other hand, were high in sterols and triterpenes. All plant extracts contained flavonoids and coumarins, and four plant samples contained cyanogenic glycosides [25].

These chemical groups may vary even within the same plant species' morphological parts. At this point, it is impossible to say whether the activity of these samples was caused by one or more of these chemical groups, or by other groups of compounds that were not tested. Further research is required to identify the compounds responsible for the activity in each sample.

CONCLUSION

The most effective extracts (7) from six plants (7 parts) belonging to two families demonstrated activity against clinical isolates, particularly four extracts, and the minimum inhibitory concentrations were determined. Phytochemical screening was performed on the plants that demonstrated high anti-TB activity. It can be stated that specific tannins, saponins, and flavonoids play a significant role in the anti-TB activity.

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