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Antitubercular and Anthelmintic Activity of *Triumfetta rhomboidea*

E. Vadivel*, Shubham G. Arondekar
Postgraduate Chemistry Department, Dnyanprassarak Mandal`s College and Research Center, Assagao-Bardez, Goa, India
Email: velpharmamsu@gmail.com

Abstract**—***The purpose of this study was investigating experimentally the invitro Anti-tubercular and anthelmintic activity. Antitubercular activity of Triumfetta rhomboidea was investigated against Mycobacterium tuberculosis using Microplate Alamar Blue assay (MABA) and the anthelmintic activity was evaluated on adult Indian earthworms, Endrullus enguinae. Antitubercular activity was performed in eight different concentrations i.e. from 100 to 0.2 µg/ml. Anthelmintic activity was performed in three different concentrations such as 100mg/ml, 50mg/ml and 25mg/ml. Both the activities were dose-dependent. The highest concentration showed the maximum activity. Our results confirm that the methanolic and water extract of Triumfettarhomboidea exhibited significant effect against Mycobacterium tuberculosis and Endrullus enguinae respectively. Antitubercular and anthelmintic activity leads some support to the therapeutic use of Triumfetta rhomboidea for various ailments in the traditional medicine of India.*

Keywords**—***Triumfetta rhomboidea, Antitubercular activity, Mycobacterium tuberculosis, Anthelmintic activity, Endrullus enguinae.*

I. INTRODUCTION

The plant *Triumfetta rhomboidea* [1] belongs the family of *Tiliaceae* is a shrub, widely distributed in tropical and subtropical parts of India, Ceylon, Malay Peninsula, China, Africa and America. According to Ayurveda, the roots of *Triumfetta rhomboidea* is bitter, acrid, aphrodisiac, tonic and cooling, it is also useful in dysentery. The leaves and stem are used on tumors. Powdered leaf of *Triumfetta rhomboidea* isused for the treatment of anemia. The tribal in Kolli Hills, South India were using the plant for the treatment of cancer.

Tuberculosis is the main cause of morbidity in the modern era and came to existence many decades ago and has emerged as Pandemic [2] disease. The use of allopathic medicine in complex disease like tuberculosis is associated with the problem of cross-resistance and herbal drugs have proven to be most effective in this context. The exploration of therapies for the successful attenuation of the morbid condition associated with tuberculosis is the need of the day.

Tuberculosis is the most important public concern in the late 19^{th} and early 20^{th} centuries as a life-threatening disease. World Health Organisation (WHO) defines "Tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis*, which most commonly affects the lungs.It is a contagious disease. It is transmitted via droplets from the throat and lungs of people with the active respiratory disease".

The wide variety of antibiotics were used in the recent treatment of tuberculosis such as Rifampicin, Ethambutol, Isoniazid, and Pyrazinamide. Mycobacterium has a tendency to produce resistance to the drug. The emergence of the problem of multiple drug resistant (MDR) and extensively drug-resistant (XDR) strains of Mycobacterium is very common with antituberculardrugs.

The Mycobacteria have a special character that they can digest the drug by modifying their receptor structure based on the chemical structure of the drug. Thus the Mycobacteria slowly adapt and develop drug resistance. Medicinal plants whether extract or decoction used against any pathogen will not cause the problem of drug resistance because it contains many phytoconstituents. Hence an effective and suitable drug therapy from the natural source as an anti-tuberculosis drug need to be discovered which will solve the problem of cross-resistance as well as drug resistance

Helminthes infections are usually termed as helminthiasis, they are the most pervasive infection and a foremost degenerative disease affecting a large proportion of people worldwide. In developing countries, It contributes to the prevalence of malnutrition, anemia, eosinophilia, and pneumonia. It also causes a large threat to public health.

Anthelmintics are drugs that are used to treat infections caused by helminthic parasites (worm) from intestinal tract or tissue of humans and other animals. They are of very important for human tropical medicine. According to W.H.O (World Health Organization), two billion people harbour parasitic worm infections parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of significance is the infection of home pets.

Anthelmintics must be selectively toxic to the parasite [3] but not to the human cells. The toxicity may be achieved either by inhibiting the important metabolic processes of the parasite or by intrinsic pharmacokinetic properties of the compound that cause the parasite to be exposed to higher concentrations of the Anthelmintic drugs. The mode of action of many Anthelmintics is not fully understood but the sites of action and biochemical mechanisms of many of them are generally known. Parasitic helminths usually maintain an appropriate feeding site, nematodes and trematodes must actively ingest and move food through their digestive tracts to maintain an appropriate energy state. The reproductive processes require proper neuromuscular coordination.

Development of drug resistance and high cost of conventional synthetic anthelmintic drugs led to the evaluation of medicinal plants has an alternative source of for the treatment of helminthesis.

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II. MATERIALS AND METHODS

Plant collection

Triumfetta rhomboidea were collected from the North Districts of Goa in the month of October from Pernem Taluka, The plant was identified and confirmed by the botanist Dr. M. K. Janarthanam, Head, Department of Botany, Goa-University, Goa.

Extract preparation

The plants *Triumfetta rhomboidea* were shade-dried and pulverized to powder in a mechanical grinder. The powder of the plant (1 kg) was extracted by maceration with solvent methanol and water. The methanol and water extract of *Triumfetta rhomboidea* were used for the further studies.

Antitubercular activity:

The anti-tubercular activity of compounds was assessed against *Mycobacterium tuberculosis*, using Microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds was made directly on the plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink. The standards used were Pyrazinamide, Streptomycin, and Ciprofloxacin.

Anthelmintic activity:

The Anthelmintic activity [4] was performed according to the Ghosh et al method on adult Indian earthworm *Endrullus enguinae* as it has an anatomical and physiological resemblance to the intestine roundworm parasites of human beings. Endrullus enguinae was placed in Petri dish containing three different concentrations (100mg/ml, 50mg/ml, 25mg/ml) of aqueous extract of the plant *Triumfetta rhomboidea*. Each petri dish was placed with 3 worms and observed for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time taken for death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with standard compound Albendazole (20 mg/ml) treated sample.

III. RESULTS AND DISCUSSION

Nature has given the wide variety of plants for all the living creatures, which possess therapeutic important. Therefore, there is a necessity to explore their uses and to ascertain their medicinal properties.

Antitubercular activity:

The Antitubercular activity of methanolic extract of *Triumfetta rhomboidea* (METR) at eight different concentrations is presented in Table I.

TABLE I. In-vitro Anti-tubercular activity of methanolic extract of *Triumfetta rhomboidea.*

Sample (µg/ml)	100	50	25	12.5	6.25	3.12	1.6	0.8
METR	S	R	R	R	R	R	R	R
Pyrazinamide	S	S	S	S	S	S	R	R
Streptomycin	S	S	S	S	S	R	R	R
Ciprofloxacin	S	S	S	S	S	S	R	R

S=Sensitive, R=Resistant

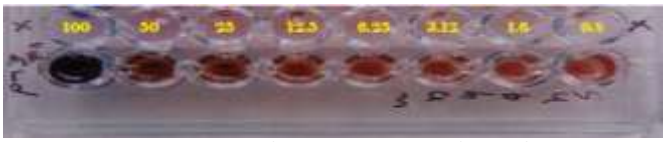

Fig. 1. Antitubercular activity of methanolic extract of *Triumfetta rhomboidea* Against *Mycobacterium tubercular.*

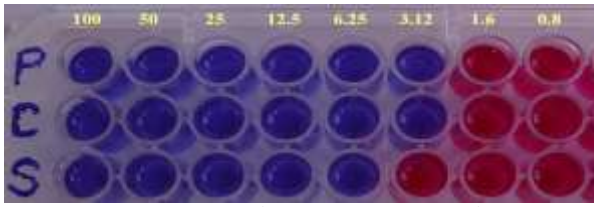

Fig. 2. Antitubercular activity of Pyrazinamide(P), Ciprofloxacin(C) and Streptomycin (S) against *Mycobacterium tubercular.*

Tuberculosis is a major health problem in many countries in the world including India. There is a need to develop newer and more potential antitubercular drug which can effective even in multi-drug resistant (MDR) and extensively drug-resistant (XDR) microbes. The Antitubercular activity of the herbal extract was estimated using Microplate Alamar Blue Assay (MABA). In this technique, The Alamar blue reagent is used to monitor the pathogenic Mycobacterial growth. If there is bacterial growth, The test solution turns pink, and if the antitubercular drug is effective enough to inhibit any bacterial growth, The test solution retains the blue colour of the reagent.

The methanolic extract of *Triumfetta rhomboidea* showed an excellent effect on the Mycobacterial growth. The extract of *Triumfetta rhomboidea* was screened from a range of 0.8 µg/ml to 100 µg/ml as presented in Table I and Figure 1. The results obtained from the preliminary phytochemical tests were very encouraging to support that the methanolic extract showed the Mycobacterial effect at a concentration of 100 µg/ml. However when compared to the standards, The plant showed activity only in the higher concentration 100 µg/ml but standard drugs such as Pyrazinamide, Ciprofloxacin, and Streptomycin showed greater activity even in less concentration. Which showed MIC at 3.12 µg/ml, 3.12 µg/ml, and 6.125 µg/ml respectively. The anti-mycobacterial activity may be due to the presence of Phenolic compounds in the methanolic extract of *Triumfetta rhomboidea*.

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Anthelmintic activity:

The Anthelmintic activity was evaluated on Indian earthworms, *Endrullus enguinae*, due to its anatomical and physiological resemblance with intestinal roundworms parasites of human beings. The progress of Anthelmintic activity of aqueous extract of *Triumfetta rhomboidea* at three different concentrations 25 mg/mL, 50 mg/mL and 100 mg/mL in earthworms are presented in Table II.

TABLE II. *In-vitro* Anthelmintic activity of the aqueous extract of *Triumfetta rhomboidea.*

Groups	Drug	Concentration (mg/mL)	Time taken for paralysis (min)	Time taken for death (mins)
Group-1	Albendazole (Standard)	20 mg/mL	6:07	7:00
Group-2	Aqueous extract	25 mg/mL	36:44	82:34
Group-3	Aqueous extract	50 mg/Ml	12:25	51:24
Group-4	Aqueous extract	100 mg/mL	5:10	7:27

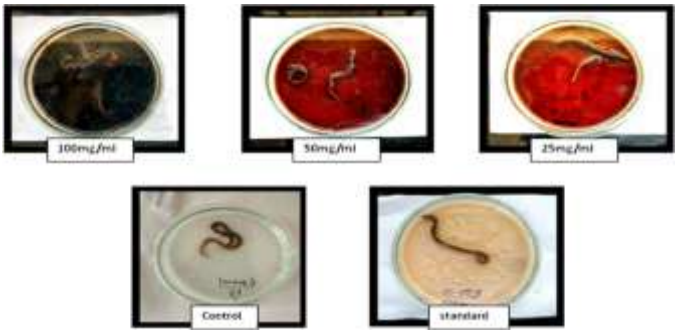

Fig. 3. Anthelmintic activity of aqueous extract of *Triumfetta rhomboidea.*

From the Table II. It is clear that the higher concentration (100 mg/ml) of extract produced a paralytic effect much earlier. The results are compared with that of the standard Albendazole. The aqueous extracts of *Triumfetta rhomboidea* possessed dose-dependent but lower concentrations such as 25mg/ml showed very less anthelmintic activity when compared to that exhibited by the standard Albendazole.

The phenolic compounds produce anthelmintic activity by uncoupling oxidative phosphorylation which hinders the energy production in the helminthic parasites and causes death. The alkaloids act on the central nervous system and are responsible for causing Paralysis of the Earthworms. The Phytochemical analysis of *Triumfetta rhomboidea* revealed the presence of phenolic compounds.

IV. CONCLUSION

This study suggests that the extract of *Triumfetta rhomboidea* posses significant *In-vitro* Antitubercular and Anthelmintic activity. It is anticipated that this plant would be a useful pharmaceutical material to treat Tuberculosis and Helminthiasis. The present investigation gives the scientific evidence that it may be a fruitful medicine of tomorrow. Future research should focus on the molecular mechanism of *Triumfetta rhomboidea*. There is a need for further investigation of this plant in order to identify and isolate its active principle(s) to treat Tuberculosis and Helminthiasis.

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