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Antibacterial, Antioxidant, Anthelmintic, Antioxidant activity of *Abrus precatorious* Linn.

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ABSTRACT:

Tuberculosis is an infection caused by bacilli mycobacterium tuberculosis, a gram positive, non-motile, catalyse positive, rod like microorganism of family *mycobacteraceae*. Present study was undertaken to explore antimycobacterial and antioxidant potential of ethanolic seed extract of Abrus precatorius. Previously ethanolic seed extract have been studied for its phytochemical constituents and antimicrobial activity, seed extract demonstrated to present alkaloids, glycosides, flavonoids and triterpines in ample amount, researcher have also reported family fabaceae to have antimycobacterial activity. However their antimycobacterial potential against mycobacterium species have not investigated scientifically.

Our study analysed antimycobacterial and antioxidant potential of ethanolic seed extract of Abrus precatorius against three different strains of mycobacterium by cup diffusion method and antioxidant potential by DPPH and H2O2 assay method. ethanolic seed extract have significant potential to inhibit mycobacterium culture growth. The zone of inhibition produced by 50 mg/ml concentration of seed extract was closely correlating to that of standard rifampicin. An sample view on the outcomes of this study gives insight in to strategy of chemical constituents of ethanolic seed extract for treatment of mycobacterium infection.

KEYWORDS: (tuberculosis, antioxidant, cup diffusion method, antioxidant, mycobacterium.)

I. INTRODUCTION

Tuberculosis, one of the biggest killers among the infectious diseases and one of the oldest recorded human afflictions, today infection with Myco bacterium tuberculosis accounts for up to two million deaths annually and ranks as the second leading cause of death from an infectious disease worldwide, after the human immune deficiency virus HIV [1]. It has evaluated for phytochemical constituents and this study have demonstrated that it contains glycosides, flavonoids and triterpenes in ample amounts [2]. Some of these chemicals are responsible for antimicrobial activity [3]. So, we hypothesize that phytochemicals present in ethanolic seed extract may inhibit the growth of mycobacterium species. This will be useful to overcome resistance developed by mycobacterium species to existing drug and will help to avoid side effects associated with high doses of drugs and long term therapy of multiple drug regimens.

Introduction to Tuberculosis

Tuberculosis, one of the oldest recorded human afflictions, is still one of the biggest killers among the infectious diseases, today infection with *Mycobacterium tuberculosis* accounts for up to two million deaths annually and ranks as the second leading cause of death from an infectious disease worldwide, after the human immune deficiency virus HIV[1]. TB is caused both by the tissue-damaging actions of the invading pathogen and by the alterations effected by the host's immune response in an attempt to cope withthe invasive pathogen [4].

Anti-tubercular activity-

Anti-mycobacterial activity of plant extracts is usually done by culturing mycobacteria in ranging types of Agar and broth based media[5]. These methods are: Agar well/disc diffusion method, Macro and micro dilution method and Micro plate alamar blue assay. The Agar well/disc diffusion method is one of the most commonly used methods [5].

MATERIAL AND METHODS

Collection and Authentication of plant material-
Seeds were collected from the village Makhani (dist- Parbhani), identified and Authenticated by Dr. B. D. Gacchande, Dept. of Botany science college, Nanded.

Preparation of extract-
Ethanolic seed extract of *Abrus precatorius* was prepared by process of cold extraction. Seeds were

Seeds were coarsely powdered with the help of grinder and 300 gm of powder was soaked in 750 ml of ethanol in conical flask for 1 week and content were filtered through muslin cloth and then with filter paper in order to obtain clear filtrate. The filtrate was then evaporated and dried at room temperature. The thick mass gathered at bottom of flask was then scraped and stored at cool condition. From this extract tests sample of different concentrations were prepared [6]

Fig. no.1 *Abrus precatorius*

Sr.No	Phytoconstituents	Aq.Ext.	Methanolic Ext.	Ethanolic extract
1	Alkaloids	-	+	+
2	Glycosides	+++	+++	+++
3	Flavonoids	+	-	+++
4	Triterpenes	-	-	+++
5	Tannins	++	+	++
6	Saponins	+++	-	+

(Table No.1): phytoconstituents

Chemicals for Antimycobacterial Test-

L-Asparagine: High media
Monopotassium Phosphate: Rankem Ltd
Magnesium Sulphate: Rankem Ltd
Magnesium Citrate: Rankem Ltd
Potato Flour: High media
Malachite Green: High media
Standard: Rifampicin: Lupine Laboratories.
Instrument- Autoclave (Mic), Incubator (Remy), Antibiotic zone scale: High media, Micropipette.

Chemicals for Antioxidant Activity-
Methanol (100ml): Rankem Ltd.
DPPH: High media
Potassium dihydrogen phosphate (0.2M): Rankem Ltd .Hydrogen peroxide: Rankem Ltd.

Sodium hydroxide (0.2M): Rankem Ltd.
Ascorbic Acid (AA): OXFORD Lab.
Instrument*:* UV-Spectroscopy (Shimadzu)

EXPERIMENT METHODS :-

Antimycobacteral Activity

Microbial culture- Standard cultures of different species of gram positive mycobacterium such as *Mycobacterium tuberculosis H37Ra ATCC No. 25177, Mycobacterium Smegmatis (ATCC No.14468) and Mycobacterium Phlei (ATCC No. 17420)* were procured from School of Life Science, Swami Ramanand Teerth Marathwada University, Vishnupuri, Nanded (MH).

Procedure-
Preparation of medium-
All components of medium weighing 37.3 g were dissolved in 600 ml of purified water containing 12 ml of glycerol. It was then heated with frequent agitation to completely dissolve the medium. Lastly medium was sterilized by autoclave at temperature 121°C and pressure 15 psi, for 15 minutes [7].

Preparation of plates-
Nutrient medium was prepared and sterilized at 15 psi for 15 minute in the autoclave, it was allowed to cool bellow 450C and seeded with turbid suspension of 24 hours old bacterial slant culture. This seeded preparation was then poured in sterile Petri plates under aseptic condition and allowed it to solidify. Cups of 7 mm diameter were bored in the medium plates with sterile cork borer. 100 micro litre of extract solution of 25 and 50 µg/ml concentration prepared in dimethyl sulphoxide was added in the cup under aseptic condition with the help of micropipette. 100 micro litre of DMSO was placed in one cup of the blank (negative control) a standard antibiotic (25µg/ml) was placed in the seeded nutrient medium as reference antibiotic (positive control). The plates were kept in the refrigerator for 15 min to allowed diffusion of the compound from the cup in to the medium. Then the plates were shifted to incubator at 370C and incubated for 24 hours. After incubation, plates were observed for the zone of inhibition of the bacterial growth around the cup.
Results were recorded by measuring the zone of inhibition in millimetre (mm) using zone recorder [8].

Antioxidant Activity
Evaluation of Antimycobacterial and Antioxidant activity of *Abrus Precatorius. Linn.*

Procedure-DPPH assay
The free radical scavenging activity of methanolic extract dissolved in DMSO was measured by DPPH. The 0.2 mmol /L solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of DMSO solutions with plant extract at concentrations of 0.2, 0.4, 0.8, and 1 mg/ml. After 30 min absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as inhibition percentage and was calculated using the formula. Inhibition of free radical DPPH in percent was calculated as follows [9]

% Radical Scavenging Activity = A –B / A x 100
Where A is the absorbance of DPPH solution and B is the absorbance DPPH solution with extract.
H2O2 radical scavenging assay-
A solution of hydrogen peroxide (2 mm) was prepared in phosphate buffer (pH 7.4) and concentration was determined UV-Vis. Methanol extract having different concentration (0.2, 0.4, 0.8, and 1 mg/ml) in distilled water was added to a hydrogen peroxide solution (0.6 ml, 2 mM) respectively. The absorbance of hydrogen peroxide at 230 nm was determined after 20 min against a blank solution in phosphate buffer without hydrogen peroxide (Bansiwal et al. 2009).
% Radical Scavenging Activity = A –B / A x 100

OBSERVATIONS AND RESULTS

Percentage yield of extract
Plant Name- *Abrus precatorius Linn*
Part used- Seeds
Solvent for extraction- Ethanol
Wt. of powder used for extraction- 300 gm
Yield of extract- 12.57 gm
Percentage yield of extract- 4.19%

Antimycobacterial activity

The antimycobacterial activity of ethanolic extract was determined by screening them against *Mycobacterium tuberculosis (ATCC No.27294), Mycobacterium smegmatis (ATCC No. 14467), and Mycobacterium phlei (ATCC No. 17420)* using cup diffusion method. The basic principle of antimycobacterial assay lies in the comparison of inhibition of growth of microorganism produced by the known concentration of extract to be tested with that produced by known concentration of standard antitubercular agent having known activity.The antimycobacterial activity of extract at two different concentrations was carried out by using cup diffusion method against Rifampicin as a standard drug. The results obtained are presented in table no. 2.

Sr.No	Concentration	Zone of Inhibition in (mm)		
		M. tuberculosis	***M.smegmatis***	***M.phlei***
Test 1	25 mg/ml	11 ±1.8	09.5±1.5	10±1.3
Test 2	50 mg/ml	14±1.3	12.5±1.2	12.2±1.7
Rifampicin	10 µg/ml	16±1.1	15±1.4	15±1.5

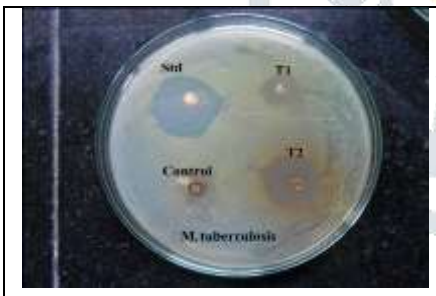
(Table No. 2) : Antitubercular activity)

Activity was performed in triplicate and standard deviation was calculated for both extract and standard concentration. The extract of concentration 25 mg/ml showed 11±1.8 mm, 09.5±1.5 mm, 10±1.3mm zone of inhibition against *M. tuberculosis, M. smegmatis and M. Phlei*respectively. While50 mg/ml concentration of seed extractshowed significant activity against *M. tuberculosis,* which is nearer to the zone of inhibition produced by 10 µg/ml of rifampicin

Photographs of plates

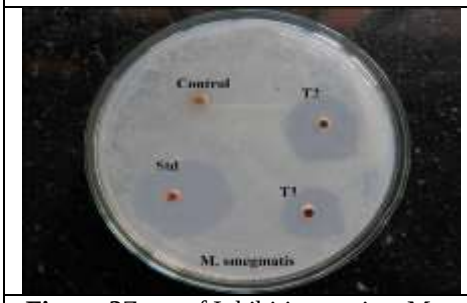
1] M. tuberculosis-

(Table no.3)



T1= Ethanolic seed extract at concentration 25 mg/ml.
T2= Ethanolic seed extract at concentration 50 mg/ml.
Std=Standard at concentration 10 µg/ml.

Figure-2 Zone of Inhibition against M. tuberculosis.



T1=Ethanolic seed extract at concentration 25 mg/ml.
T2=Ethanolic seed extract at concentration 50 mg/ml.
Std=Standard at concentration 10 µg/ml.

Figure-3 Zone of Inhibition against M. smegmatis.

	**T1=** Ethanolic seed extract at concentration 25 mg/ml. **T2=**Ethanolic seed extract at concentration 50 mg/ml. **Std=**Standard at concentration 10 µg/ml.
Figure-4 Zone of Inhibition against M. phlei.	

Antioxidant Activity

Antioxidant activity of ethanolic seed extract was carried out by most commonly used methods such as DPPH assay and H_2O_2 radical scavenging method. Results for individual method are summarised as follows.

DPPH Radical scavenging assay [10]

In-vitro free radical scavenging activity by DPPH assay was based on the reduction of methanolic solution of coloured free radical DPPH by antioxidant. Percentage radical scavenging activity was found to increase with increase in concentration of extract. Highest % of radical scavenging activity by extract was noted to be 74.46±0.55 at concentration 1mg/ml and lowest value (40.42±2.07) was obtained at concentration 0.2 mg/ml. It clearly indicates that there exists dose response relationship between concentration of extract and free radical scavenging activity. Activity of extract was closely corresponds to the activity of standard antioxidant Ascorbic acid at dose 1 mg/ml. IC_{50} (50 % inhibition) of extract was found to be 0.381± 1.8mg/ml. which is shown in Table 5.

Concentration (mg/ml)	**Radical scavenging activity (in %)**	
	Ethanol extract	**Ascorbic acid**
0.2	40.42±2.07	48.93±0.86
0.4	55.31±0.66	59.57±0.88
0.8	63.82±2.17	68.08±0.13
1	74.46±0.55	76.59±1.15
IC_{50}	0.381±1.8(mg/ml)	

Table no. 3 : radical scavenging activity

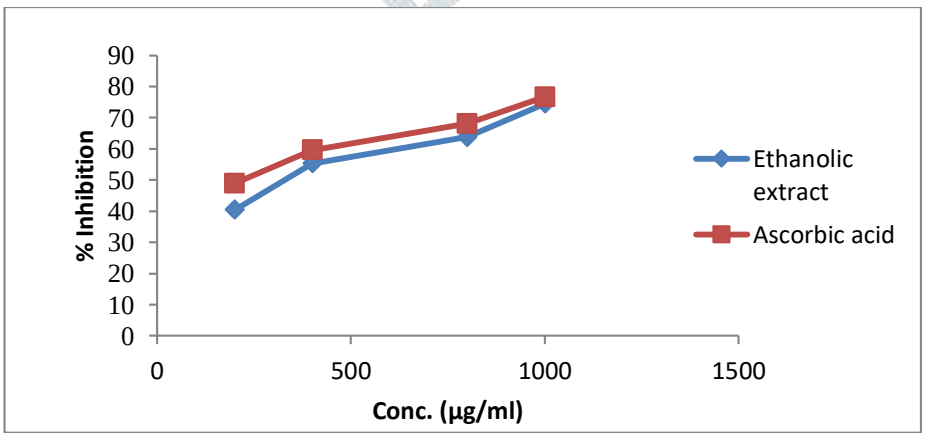


Figure 2- %Inhibition due toethanolic seed extract by DPPH assay method.

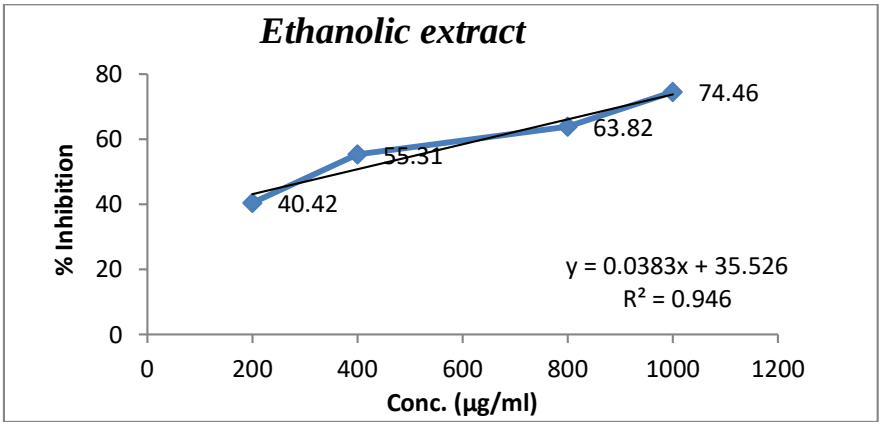


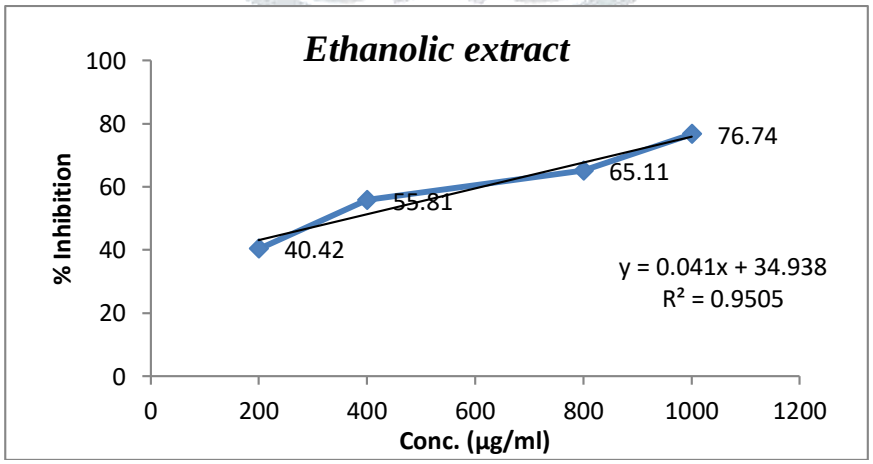
Fig. No.3 : Ethanolic extract DPPH Assay

H_2O_2 radical scavenging assay-

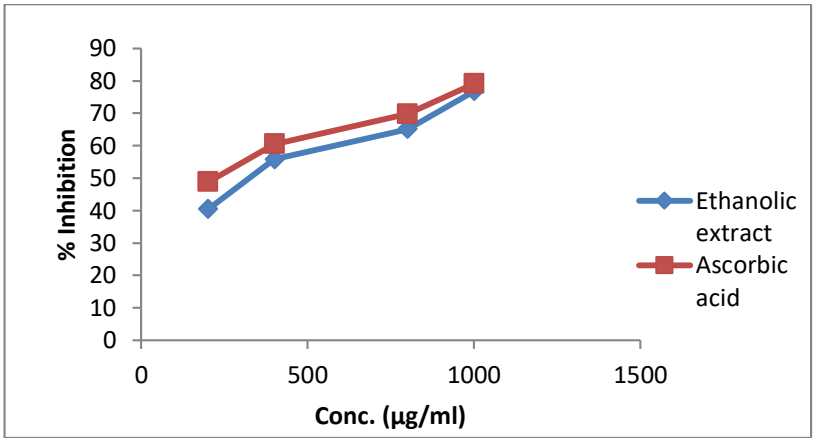
The H_2O_2 radical scavenging activity was based on inhibition of hydroxyl radical generated due to the reaction of H_2O_2 with phosphate buffer by free radical scavenger. Extract showed dose dependant radical scavenging activity and % inhibition values of extract at concentration 0.2, 0.4, 0.8 and 1 mg/ml were 40.42±1.12, 55.81±0.64, 65.11±1.19, 76.74±1.36 and for standard values were 48.83±1.57, 60.46±1.06, 69.76±1.32, 79.06±1.41 respectively. IC_{50} value of test compound comes across 0.367± 1.27 (mg/ml). The detailed result of test has shown in Table 6.

Concentration (mg/ml)	**Radical scavenging activity (in %)**	
	Ethanol extract	**Ascorbic acid**
0.2	40.42±1.12	48.83±1.57
0.4	55.81±0.64	60.46±1.06
0.8	65.11±1.19	69.76±1.32
1	76.74±1.36	79.06±1.41
IC_{50}	0.367± 1.27 (mg/ml)	

Table no.6) ethanolic seed extract of *Abrusprecatorius.*



(Fig No. 4 : IC_{50} of ethanolic seed extract by H_2O_2 assay method)



(**Fig.No. 5 :** -% Inhibition due to ethanolic seed extract by H_2O_2 assaymethod

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