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Anti-tubercular Evaluation of *Acalypha indica* Linn. Fractions AgainstH37Rv Strain

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Abstract : Tuberculosis (TB) is a major global health problem caused by *Mycobacterium tuberculosis (M.tb)*. The present investigation deals with the anti-tubercular activity of different fractions of methanol extract of *Acalypha indica* against *Mycobacterium tuberculosis*H37Rv strain at different concentrations (0.8µg/ml to 100µg/ml) by Microplate Alamar Blue assay (MABA) method.The results revealed that ethyl acetate fraction (F1) and aqueous fraction (F3)of *A. indica* methanol extract have exhibited sensitivity at 100 µg/ml concentration when compared with the standard pyrazinamide. However, n-butanol fraction (F2)has shown resistivity even at 100 µg/ml concentration. The test results indicate the presence of active ingredients in ethyl acetate and aqueous fractions but not in n-butanol fraction of *A. indica.*

Keywords : Pyrazinamide,in vitro, MABA, Micobacterium tuberculosis, Euphorbiaceae.

Introduction

Tuberculosis is amajor public health problem caused by *Mycobacterium tuberculosis*, a leading cause of death worldwide and produces serious health complications[1,2]. In 2015 WHO estimated that 10.4 million new TB cases global reported, of which 5.9 million aremen, 3.5 million are women and 1.0 million are children[3]. New cases around 60% accounted from six countries; India, Indonesia, China, Nigeria, Pakistan, and South Africa[3]. The quantity of TB deaths can be reduced with a timely diagnosis and correct treatment. Moreover, delay in the treatment giverise to multidrug resistant tuberculosis (MDR-TB), which does not react to first-line drugs. MDR-TB strains are resistant to the regularly usedanti-tubercular drugs like isoniazid and rifampicin[4]. Recently about 5 % of new cases of tuberculosis are due to MDR strains, with more than half from China and India. It was also estimated that there are 50,000 cases of extensively drug-resistant tuberculosis(XDR-TB), which does not reactto second-line drugs[5].Thus, there is an urgent need to search for newer anti-tuberculosis agents, which are safe, effective and affordable. Medicinal plants provide a valuable source of compounds for identifying and optimizing new drug leads[6,7]. India has a unique wealth of medicinal plants with huge traditional knowledge of use of herbal medicine for curing several diseases[8,9]. Earlier studies revealed that only few plants have shown anti-TB activity such as *Salvia hypargeia, Euclea natalensis*[10-14].

Acalypha indica Linn.(Family Euphorbiaceae) is a common annual shrub grown throughout India as a weed. It iserect,herbaceous plant and can grow up to 100 cm in height. It has been traditionally used for diuretic, anthelmintic, respiratory problems, rheumatoid arthritis, to cure scabies and other skin infections[15].The previous studies reported that *A. indica* has investigated for different pharmacological activities such as anti-inflammatory[16,17],anti-bacterial[18,19], anti-fungal[20],hepatoprotective[21],anthelmintic[22],anti-fertility[23],antiulcer activity[24],antioxidant and anticancer activity[25].

The chemical investigation of *A.indica* revealed the presence of β-sitosterol and its β-D-glucoside were isolated from the leaves and twigs of *A. indica*[26,27].Potassium brevifolincarboxylate, 1-O-galloyl-β-D-glucose, 1,2,3,6-tetra-D-galloyl-β-D-glucose, corilagin, geraniin, acaindinin, acetonylgeraniin A, euphormisin M2, repandusinic acid A, and chebulagic acid, as well as two flavonoid glycosides quercetin 3-O-β-D-glucoside and rutin[28].Chrysin, and galangin were isolated from the whole plant extract[29].Acalphin,acyanogenic glycosidewas also isolated from the same plant[30].Kaempferol glycosides, mauritianin, clitorin, nicotiflorin and biorobin isolated from the flowers and leaves of *A. indica*[31].Based on the literature survey of *A. indica*, the phytochemicals such as phenolics and flavonoids are important components of the plant and some of their biological activities could be imputed to the presence of these constituents. In viewof the above biological activities of *A. indica*, no systematic anti-tubercular study has been carried outeven though earlier report indicated the anti TB potential of the aqueous extract[32].In our previous study, the crude extracts of *A. indica* have shown activity against *M. tuberculosis* H37Rv strain[33]. Hence, in the present study we made further work to systematically predict the anti-tubercular potential of various fractions of methanol extract of *A. indica* on drug resistant strain and further take up the study to isolate the active ingredient responsible for the activity.

Materials and Methods

Chemicals

The chemicals and reagents used for investigation were of analytical grade. Diastase, Almar blue reagent, and chemicals used were from Hi Media, Mumbai, India.

Plant material

Whole plant of *A. indica* was collected in the month of Jun 2015around Kadur town of Chikmagalur Dist., Karnataka state, India. The collected plant was authenticated with voucher specimen no.KUYLK4411at the Herbarium, Department of Botany, Kuvempu University, Shankaraghatta, Shimoga Dist. Karnataka State, India.

Preparation of extracts

The collected plant materials was cleaned to remove dirt from the roots and washed thoroughly with water to get rid of dust and soil particles and immediately sprayed with ethanol to cease enzymatic degradation of secondary metabolites. The shade dried plant material (100 gm) was chopped into smaller fragments of 1-2 inches and subjected for successive extraction with n-hexane, dichloromethane and methanol in soxhlet extractor for 72 h. The solvents were removed under reduced pressure and controlled temperature of 40-50 °C using a rotary evaporator. The yield of the extracts was found to be n-hexane (3.5 gm), dichloromethane (2.8 gm), and methanol (12 gm), respectively.

Fractionation using various solvents

5gm of methanolic extract was taken in a 100ml separating funnel with 50ml of ethyl acetate and the contents were shaken vigorously foraround5minutes time interval and kept for half an hour for separation. Ethylacetate soluble fraction F1 was collected separately and the solvent was evaporated using rotary evaporator under reduced pressure and controlled temperature of 45$\pm$ 5 ^{o}C. Insoluble fraction was retained in the separating funnel and to this,50ml of n-butanol was addedand similar procedure was repeated as ethyl acetate fraction. Butanol soluble fraction F2 was collected separately and the solvent was evaporated. The insoluble fraction of methanol extract was finally dissolved in water and designated as F3. All the fractions were stored at -20^{o}C until further use[34].

Anti-tubercular activity by MABA method

The anti-tubercular activity of the fractions (F1, F2 and F3) is carried out on *M. Tuberculosis* H37Rv strain, by microplate alamar blue assay (MABA) method. In comparison with the BACTEC and fluorometric MABA methods, visual MABA is an inexpensive alternative, providing identical and rapid results without the use of specialized equipment. In addition to the above mentioned merits, visual MABA method was adopted for the screening of test extracts. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration that prevented a colour change from blue (no growth) to pink (growth) and the drug pyrazinamide

drug was used as positive standard for comparison. The procedure involves by taking 200µl of sterile deionized water and was introduced into all outer perimeter wells of sterile 96 well plates to avoid evaporation of medium in the test wells during incubation. The 96 well plate received 100 µl of the 7H9Middle brook broth and serial dilution of compounds were made directly on plate. The final concentrations of the fractions tested were of 0.8 to100 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for 5 days.After incubation, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10 % Tween-80 was added to the plate and incubated for 24 h. After incubation, the change in colour was observed[35].

Results

Anti-tubercular activity of fractions

The result for anti-tubercular activity by different fractions of methanol extract of *A. indica* on *M. tuberculosis* revealed that ethyl acetate and aqueous fractions exhibited sensitivity at100 µg/ml whereas n-butanol fraction was resistant. The analysis was carried out by MABA method as shown in Table l.

Table l. Anti-TB activity of various fractions of *A. indica.*

Sl. No.	Samples	Concentration in µg/ml							
		100	**50**	**25**	**12.5**	**6.25**	**3.12**	**1.6**	**0.8**
1	F1	S	R	R	R	R	R	R	R
2	F2	R	R	R	R	R	R	R	R
3	F3	S	R	R	R	R	R	R	R

F1: ethyl acetate fraction of methanol extract of *A. indica*, F2:n-butanol fraction of methanol extract of *A. indica*, F3:aqueous fraction of methanol extract of *A. indica*.
Resistivity (R). Sensitivity (S).

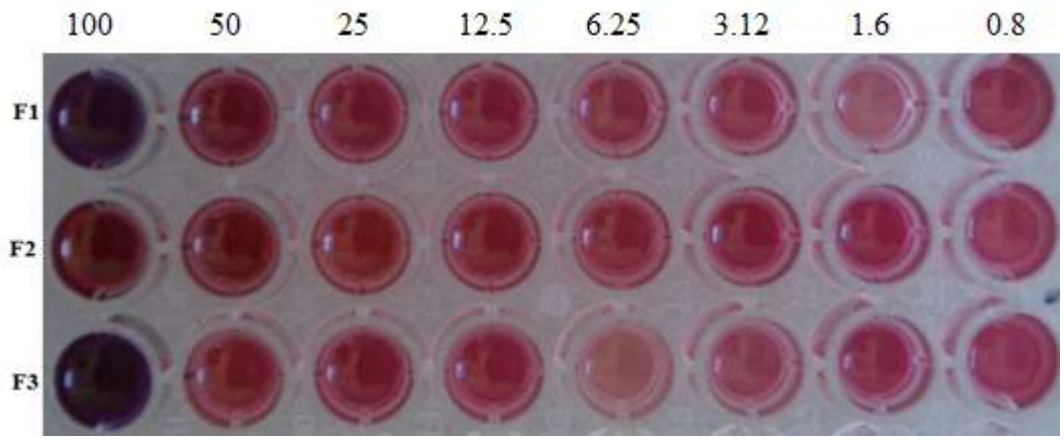

Fig. 1. Anti-TB result of F1: *A. indica* ethyl acetate fraction, F2: *A. indica* n-butanol fraction and F3 :*A. indica* aqueous fraction with concentration in µg/ml.

Discussion

The current study is to assess the anti-tubercular potential of different solvent fractions of methanol extract of *A. indica.* The ethyl acetate and aqueous fraction (F1 and F3) has shown sensitivity at 100 µg/ml concentration when compared with standard pyrazinamide at 3.125 µg/ml while n-butanol fraction (F3) has notshown any sensitivity even at100µg/ml concentration as shown in the Table l and Fig 1. The above activity generated by F1 and F3 may be due to the presence of phytochemicals in the fractions viz flavonoids, alkaloids, saponins and steroids. Fractions F1 and F3have shown moderate activity may be due to flavonoids and saponins because polyphenols are known to effect on microbial metabolism and growth, based on concentration of active compounds[36].Flavonoids show activity by damaging cytoplasmic membrane with the generation of hydrogen peroxide, inhibition of nucleic acid synthesis and inhibition of ATP synthase[37]. This might be the reason for

mechanism of action of saponins and flavonoids present which may inhibit *M. tuberculosis*. Hence, the further study of ethyl acetate and aqueous fractions will be carried out to separate the phytochemical responsible for the anti-TB activity. Our earlier report on anti-TB activity by *A. indica* indicated that the methanol extract was found to be active at lower concentration, whereas the fractions generated have not found to be as active as the crude extract. This might be because of synergistic effect due to the presence of many phytochemicals in the crude methanol extract compared with the fraction. Further separation of individual compounds from the fractions of F1 and F3 will through some light in dictating the compound which actually has considerable degree of anti-TB activity against H37Rv strain.

Conclusion

The present investigation conclude that the ethyl acetate and aqueous fractions of methanol extract of *A. indica* have shown anti-tubercular activity when compared with n-butanol fraction at different concentrations, which may be due to the presence of phytochemicals in the fractions. Hence, the inhibition might have performed by one or combination of phytochemicals which can be considered for further fractionation and isolation of the active ingredients responsible for anti-tubercular activity.

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Conflict Of Interest

No conflict of interest

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