Nat. Volatiles & Essent. Oils, 2021; 8(6): 5572-5580

NVEO
Natural Volatiles &
Essential Oils

Investigation Of Antitubercular Activity Using Alamar Blue Dye, In Vitro Antioxidant Activity Of Different Extracts Of Grewia Tiliaefolia Vahl Leaf

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ABSTRACT

In the current study, the antioxidant activity and Anti-TB activity of medically important plant Grewia tiliaefolia Vahl leaf different extracts were evaluated. Antioxidants present in the plant was a determination by 2, 2 diphenyl- 1-picrylhydrazyl (DPPH), Hydrogen peroxide assay, Reducing power assays, Anti-TB activity using Alamar Blue Dye. The selected plant extracts showed the concentration-dependent percentage inhibition on the free radicals. The extracts hexane, ethyl acetate, and methanol showed the concentration-dependent percentage of inhibition compared with ascorbic, and the 50% Inhibition Conc. (IC_{50}) for DPPH radical 343, 260.79,204.8, 41.89 µg/ml hydroxyl radical 367.8, 324.95, 292.81, 78.47 µg/ml and reducing power380.39, 282.01, 233.91, 44.79 µg/ml respectively. In the Alamar Blue Dye for anti-TB activity, the MIC for standard drugs was found as Pyrazinamide-3.125µg/ml, Ciprofloxacin3.125g/ml, Streptomycin 6.25µg/ml and 6.25 µg/ml for both methanol and ethyl acetate extract activity similar to that of standard Streptomycin, 12.5 for hexane extract.

Keywords: Grewia tiliaefolia, Quantification, Antioxidant activity, Anti-TB activity using Alamar Blue Dye.

Introduction

Free radicals and receptive oxygen species (ROS) in science deliver a medicinal insurgency that guarantees another period of wellbeing and malady on the board [1]. Ironically oxygen, a component imperative forever under specific circumstances effectively affects the human body [2]. Most of the conceivably destructive impacts of oxygen are because of the arrangement and movement of various synthetic mixes, known as ROS, which tend to give oxygen to different substances. Free radicals and cell reinforcements have turned out to be generally utilized terms in current exchanges of infection components [3].

The secondary metabolite obtained from plants is phenolics one of the most significant jobs is their cancer prevention agent movement. Cell reinforcements have won approval for their touted medical advantages as cardiovascular protectants, hostile to maturing impacts and conceivable enemy of malignant growth movement and so on [4] one such therapeutic plant is Grewia tiliaefolia Vahl has a place with the family Tiliaceae ordinarily found in the sub-Himalayan area from Jammu to Nepal and found in the focal locale of Chennai, Burma, Bihar, Orissa. It is usually known as Dhamani, Dhaman. It is a notable herb in Ayurvedic arrangement of medication and has been utilized in vitiated states of

pitta and Kapha, consuming sensation, hyperdipsia, rhinopathy, pharyngoplasty, hack, skin illnesses, pruritus, wounds, ulcers, looseness of the bowels, haematemesis, and general debility [5]. The watery concentrate of Grewia tiliaefolia Vahl leaves demonstrated the Analgesic and antipyretic action [6]. The bark of the Grewia tiliaefolia demonstrated the nearness of three tri-terpenoids, viz. Betulin, Friedelin, and Lupeol. Roots demonstrated the nearness of Friedelin and Lupeol. Tri-terpenoids separated Grewia tiliaefolia bark at higher focuses showed cytotoxic movement against LEUK-L1210 cells [7]. The stem bark of Grewia tiliaefolia demonstrated semen coagulant and cardiovascular impacts [8]. Even though Grewia tiliaefolia is broadly utilized in ethnomedicine, its pain-relieving and antipyretic properties have not yet been pharmacologically assessed. Henceforth the present examination was attempted to the cancer prevention agent action of Grewia tiliaefolia Vahl leaves.

Materials and Methods

Chemicals and Drugs

2, 2-diphenyl-1-picrylhydrazyl, Hydrogen peroxide, All other chemicals and reagents utilized were of analytical grade obtained from Sigma Chemical Company, St.Louis, USA, and Fine Chemicals Ltd., Mumbai, India. Mycobacterium tuberculosis H37Rv ATCC 25618 obtained from Microbiology Department of Maratha Mandal's N. G. H. Institute of Dental Sciences and Research, Belgaum and EAC cell lines maintained at H. S. K. College of Pharmacy, Bagalkote were used in this study.

Instruments

Rotavap Buchi (Buchi Labortechnik AG, CH-929 Flawil 1, Switzerland), Vaccum Pump (Millipore) Vaccum PR, Pump 4 BAR Uv-Visible Spectrophotometer (Thermo Scientific UV-10)

Collection, authentication, and Processing of Plant Material

The plant material was gathered in 2016 from the Kambala Konda backwoods region, Andhra Pradesh, India, and verified by Dr. B. S. Padal, taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. The Voucher examples A.U. (B.D.H), N0.22231 were saved in the herbarium, A.U. School of Pharmaceutical Sciences, Andhra University.

Preparation of the extracts

The leaves of Grewia tiliaefolia were shade dried and ground into coarse powder and were extracted using the extractor Soxhlet mechanical assembly. The extraction was done by utilizing solvents of expanding extremity beginning from low polar hexane, ethyl acetate, and methanol. After extraction, the separated dissolvable was vacuum sifted utilizing Whatman no: 1 channel paper and the filtrate was concentrated by a rotary evaporator. The acquired concentrates were put away in a desiccator until use.

2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by [9]. An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.1 ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of the respective vehicle in place of plant

extract/ascorbic acid. The percentage inhibition was calculated as [(A0-A1)/A0] ×100. where A0 was the absorbance of the control, and A1 was the absorbance of the plant sample (Test drug) / ascorbic acid.

Hydrogen Peroxide assay

The hydrogen peroxide scavenging assay was carried out following the procedure of [10]. A solution of hydrogen peroxide (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The extract at different concentrations in 3.4 ml phosphate buffer was added to 0.6 ml of hydrogen peroxide solution (0.6 ml, 43 mM). The absorbance estimation of the reaction mixture was recorded at 230 nm was determined after 10 mins against a blank solution in phosphate buffer without sample. The percentage of scavenging of hydrogen peroxide of plant extract and standard compounds were calculated.

Reducing power assay method

Various samples in methanol at various concentrations each 2.5 was mixed with a phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5 ml, 1%), and the mixture was incubated at 50°C for 20 min. Next, 2.5ml of trichloroacetic acid (10%) was added to the reaction mixture, which was then centrifuged at 650 RPM for 10 min [10]. The upper layer of the solution (5 ml) was mixed with distilled water (5ml) and ferric chloride (1 ml, 0.1%), and the absorbance was measured at 700 nm. A stronger absorbance will indicate higher reducing power.

Anti-TB activity using Alamar Blue Dye

The counter mycobacterial action of extracts was evaluated against M. tuberculosis utilizing the microplate Alamar Blue test (MABA). This approach is non-dangerous, utilizes a thermally steady reagent, and shows a great connection with the proportional and BACTEC radiometric techniques. Sterile deionized water of 200µl was added to all external border wells of sterile 96 wells plate to limit dissipation of medium in the test wells during hatching. The 96 wells plate got 100 µl of the Middlebrook 7H9 broth and sequential weakening of mixes were made straightforwardly on a plate. The last medication focuses tried were 100 to 0.2 µg/ml. Plates were secured and fixed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly arranged 1:1 blend of Almar Blue reagent and 10% tween 80 was added to the plate and hatched for 24 hrs. A blue shading in the all-around was deciphered as no bacterial development, and pink shading was scored as growth. The MIC was characterized as the least

MIC was defined as the lowest drug concentration which prevented the color change from blue to pink [11]. Standard Strain utilized: Mycobacteria tuberculosis (Vaccine strain, H37 RV strain) ATCC No-27294. Standard used for the Anti-Tb test which was performed.Pyrazinamide-3.125µg/ml, Ciprofloxacin3.125g/ml, Streptomycin6.25µg/ml

Statistical analysis

Statistical analysis and graphs were plotted using ms excel and GraphPad Prism 5 software (GraphPad Software, inc., USA), and the results are expressed as mean ±Standard error of the mean.

Results and Discussion

The percentage inhibition of successively extracted hexane, ethyl acetate, and methanolic leaf

extracts of Grewia tiliaefolia Vahl, and ascorbic acid on DPPH free radicals at 400 µg were 57.27±0.32%, 66.30±0.16 %, 73.23±0.4%, and 90.61% for hydrogen peroxide assay 51.16±1.36 %, 55.04±0.47 %, 58±0.38 %, and 90.61±0.30% respectively and for reducing power assay 50.25±0.2% for hexane extract 60.89±0.24%, 66.28±0.29% for ethyl acetate extract and 97.19±0.24 % for ascorbic acid and the results were given Table 5, 6, 7 and fig.5, 6, 7 respectively. The mean IC_{50} values on DPPH radical of Grewia tiliaefolia were found to be 211µg, 267 µg, 367.7 µg, 326.18µg, and 295.5µg respectively. The mean IC_{50} values of hexane, ethyl acetate, methanolic extract of Grewia tiliaefolia were found to be 343 µg/ml, 260.79 µg/ml, and 204.8 µg/ml respectively for DPPH radical, 367.8 µg/ml, 324.95 µg/ml, 292.81 µg/ml respectively for hydroxyl radical and 380.39 µg/ml, 282.01 µg/ml, 233.91 µg/ml respectively for Reducing power. The mean IC50 value of ascorbic acid was found to be 41.89 µg/ml, 78.47 µg/ml for hydroxyl radical, and 44.79 µg/ml for reducing power assay. The results were given in Table 8 and fig. 8. The predominance of ''all-natural antioxidants has intensified the search for novel antioxidants of natural origin. A lot of natural substances and mixtures have been investigated and recognized as antioxidants. Some natural products have been exploited commercially. Reduction of the radicals by antioxidant molecules is crucial to the protection of cells against various disorders [12]. Nature has given a fantastic storage house of solutions to cure all the ailments of mankind. Plants are rich sources of natural antioxidants, the best known are tocopherols, carotenoids, vitamin C, flavonoids, and diverse other phenolic mixes [13]. Recently, among natural antioxidants, flavonoids have received increasing attention. As compared with vitamin C and E, dietary flavonoids are considered to be more powerful antioxidants [14]. The successively extracted hexane, ethyl acetate, and methanolic leaf extracts of Grewia tiliaefolia Vahl showed a dose-dependent free radical scavenging activity on Dpph radical, hydroxyl radical, and reducing power assay. The standard drug ascorbic acid also showed similar dose-dependent activity and produced maximum scavenging activity. The selected plant extracts produced concentration-dependent percentage inhibition of free radicals and produced maximum activity. Among the extracts of Grewia tiliaefolia Vahl methanolic extract showed more percentage inhibition on oxidants[15] (Free radicals /ROS). The result showed that the methanolic extract contains some compounds which are responsible for antioxidant activity as compared to other extracts.

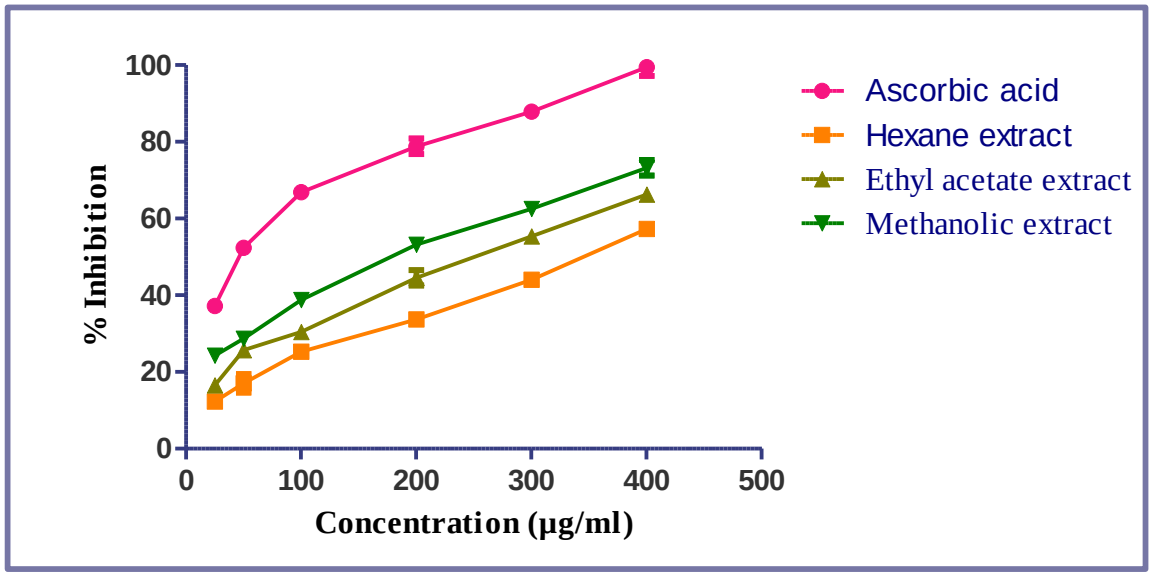


Fig.1. Concentration-dependent percentage inhibition of different extracts of Grewia tiliaefolia on DPPH radical

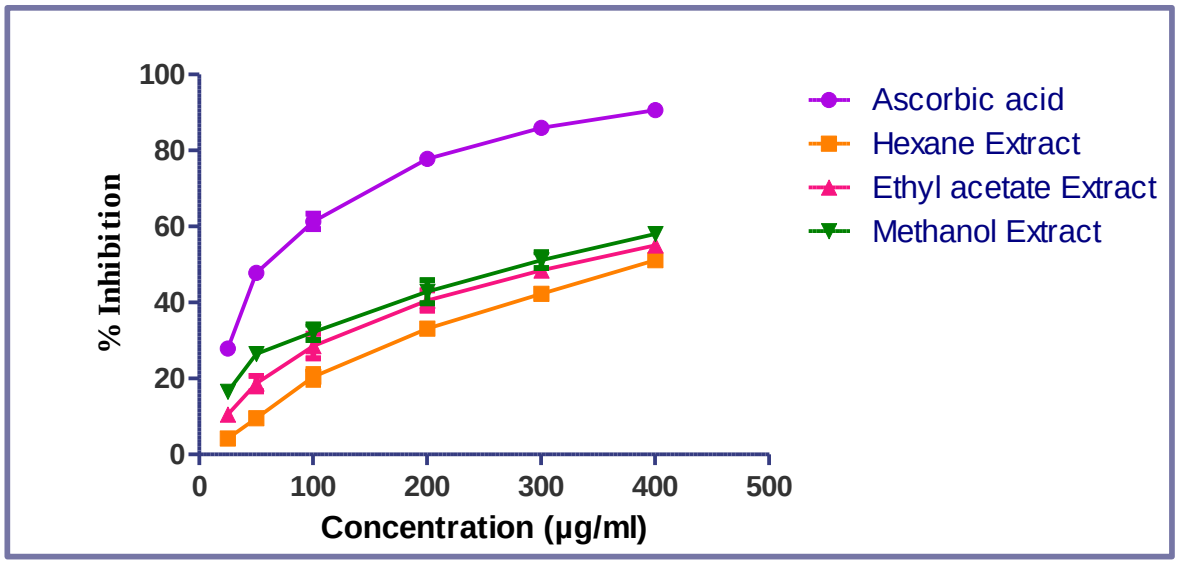


Fig. 2. Concentration-dependent percentage inhibition of different extracts of Grewia tiliaefolia on Hydrogen peroxide radical

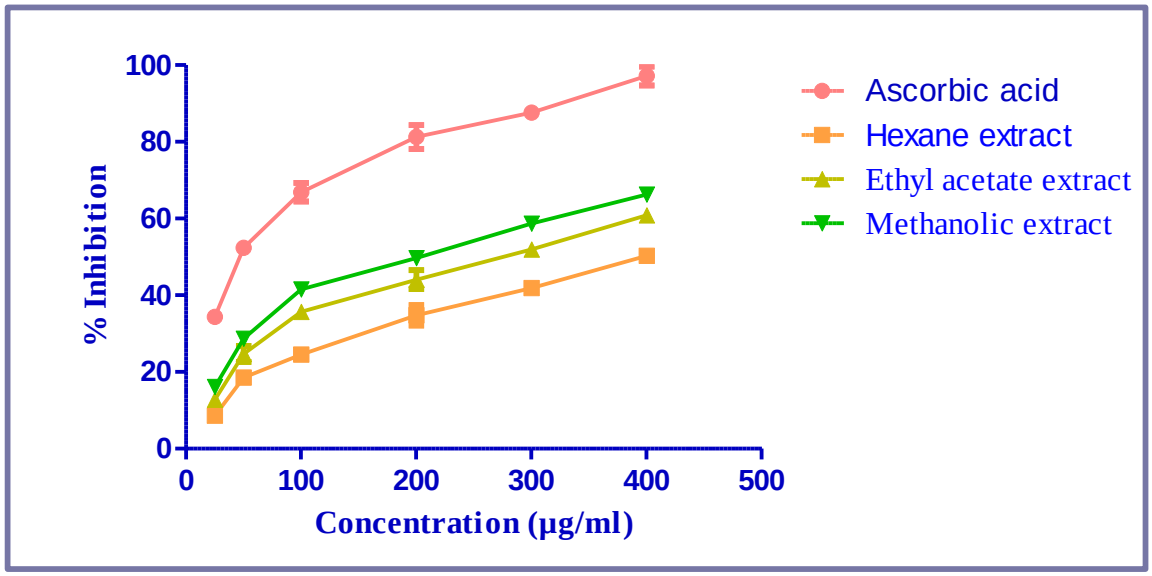
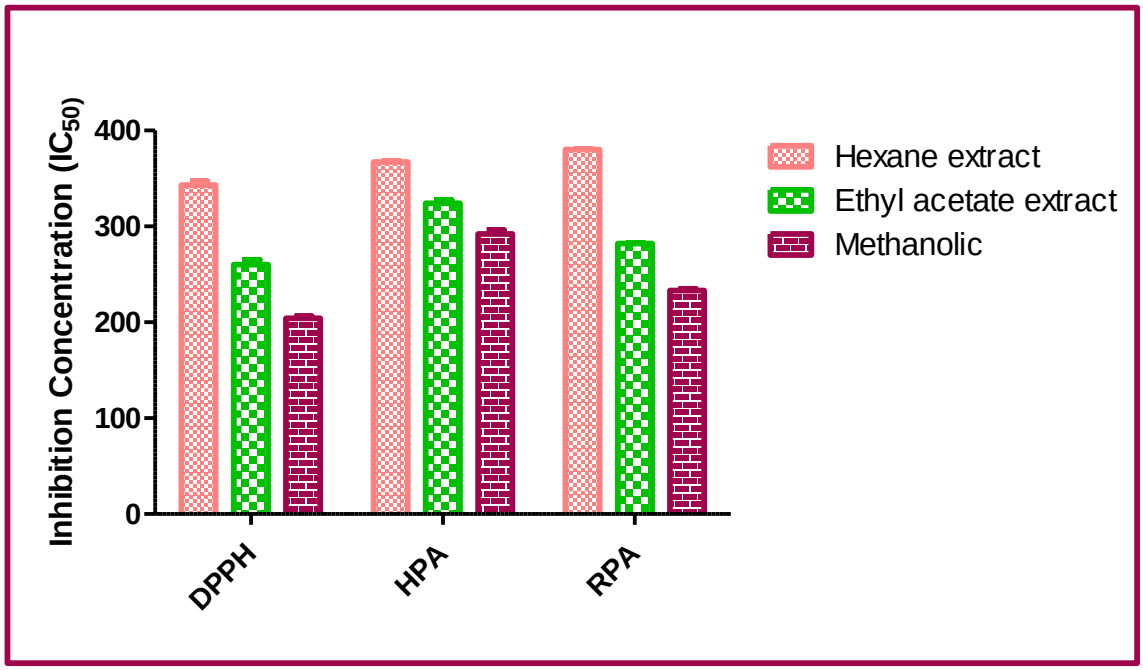


Fig.3. Concentration-dependent percentage inhibition of different extracts of Grewia tiliaefolia by Reducing power assay



DPPH Assay; RPA-Reducing Power Assay; HPA-Hydrogen Peroxide Assay

Fig.4. 50% Inhibition concentration (IC_{50}) of different extracts of Grewia tiliaefolia against DPPH, Hydrogen peroxide radicals and Reducing Power Assay

Table 1. Anti-TB activity on standard drugs using Alamar Blue Dye

Figure No	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4	0.2
01	Pyrazinamide-	S	S	S	S	S	S	R	R	R	R
02	Ciprofloxacin	S	S	S	S	S	S	R	R	R	R

03	Streptomycin	S	S	S	S	S	R	R	R	R	R

NOTE: S - Sensitive R- Resistant

Table 2. Anti-TB activity on different extracts of Grewia tiliaefolia using Alamar Blue Dye

Figure No	Sample/Extract	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
08	Hexane	S	S	S	S	R	R	R	R
09	Ethyl acetate	S	S	S	S	S	R	R	R
10	Methanolic	S	S	S	S	S	R	R	R

NOTE: S - Sensitive R- Resistant

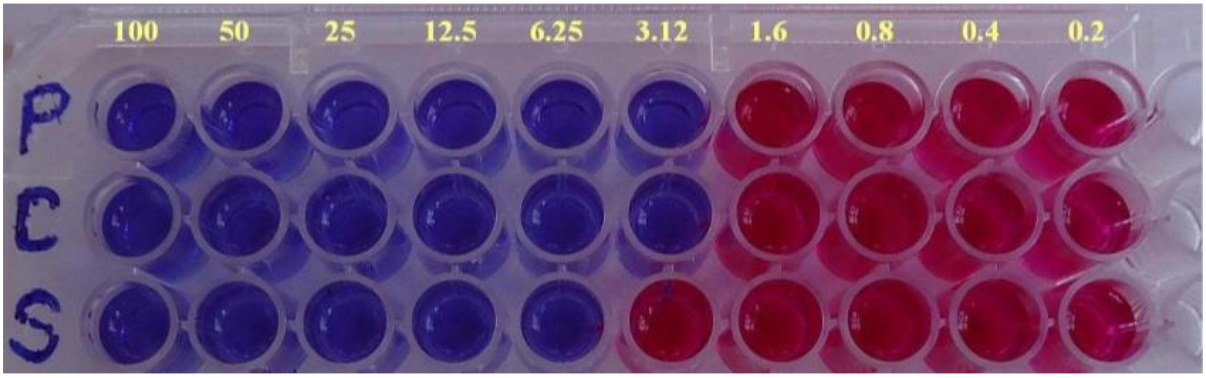


Fig.5. Standard Drug

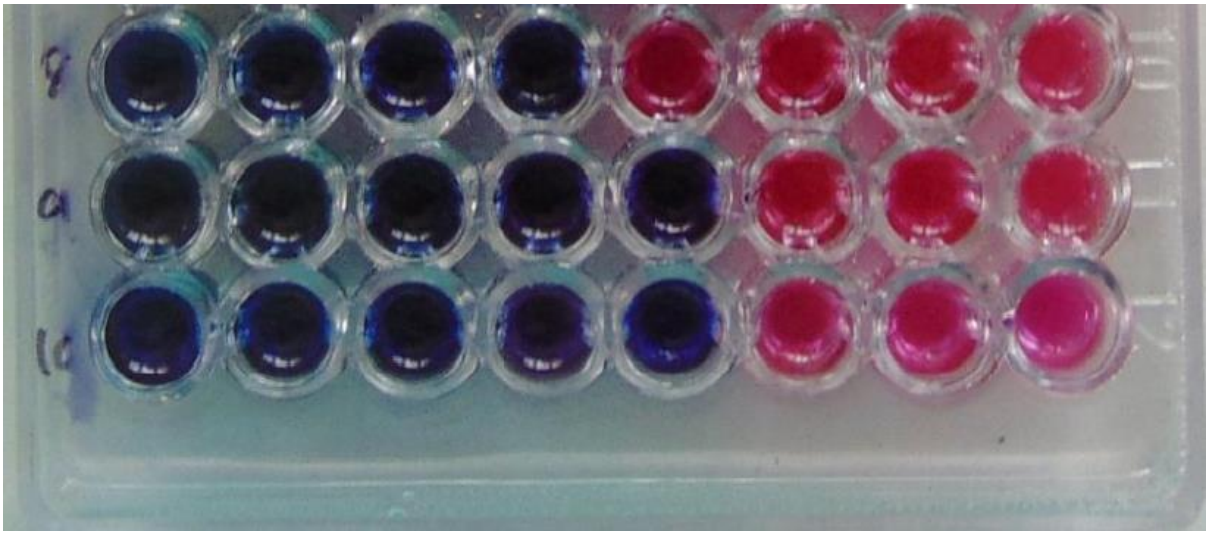


Fig.6. Anti-TB activity of hexane, ethyl acetate, methanol extracts

In antitubercular activity, the MIC was defined as the lowest drug concentration which prevented the color change from blue to pink. The MIC for standard drugs was found as Pyrazinamide-3.125µg/ml, Ciprofloxacin3.125g/ml, Streptomycin 6.25µg/ml, and 6.25 µg/ml for both methanol and ethyl acetate extract activity similar to that of standard Streptomycin, 12.5 for hexane extract. The blue color in the well was interpreted as no bacterial growth, and the pink color was scored as growth. The MIC was defined as the lowest drug concentration which prevented the color change from blue to pink. Herbs contain free radical scavengers like polyphenols, flavonoids, and phenolic compounds that act as natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing

the deleterious consequences of oxidative stress.

Conclusion

The results of the present study suggested that the tested extracts have potent antioxidant activity and/or free radical scavenging activity and also scientifically proved that they can be used as anti-tubercular drugs. The present study provides scientific evidence of the plant about antioxidant and anti-tubercular activity which can be used for ayurvedic formulations.

Acknowledgments

The authors are thankful to AU College of Pharmaceutical Sciences and School of Chemistry, Andhra University for providing the necessary laboratory facilities and UGC for their financial support through the Rajiv Gandhi National Fellowship (RGNF).

Conflict of interest

The authors have no conflict of interest.

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