Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2016; 8(6): 583-588

ISSN- 0975 1556

Research Article

Antimicrobial, Antioxidant Activity and Phytochemical Screening of *Acalypha indica* Crude Leaf Extract

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Available Online: 8th June, 2016

ABSTRACT

Medicinal plants represent a great deal of untapped reservoir of drugs. The structural diversity of their component molecules makes a valuable source of lead compounds. Plants have been the major original source of many drugs used in the treatment of diseases today. Our present study was carried out on analysis of antimicrobial, antioxidant properties and phytochemical compounds from methanolic extract of *Acalypha indica* an Indian plant known to possess different medicinal properties. The plant extracts were subjected to priliminary phytochemical screening for the presence of alkaloids, carbohydrates, glycosides, tannins, phenolic compounds, proteins, free amino acids, saponins, phytosterols, flavonoids, fixed oils and fats a total of 4 microorganisms used to assess the antimicrobial activities, it includes, two gram positive bacteria i.e. *Staphylococcus aurous* and *Bacillus subtilis*; two grams negative Bacteria: *Escherichia coli*, *Salmonella typhi*collected from department of Microbiology, Osmania University, Hyderabad. by agarwell diffusion method.Anti-oxidant activity of plant extract was performed by DPPH, FRAP, TBA and TCA method showed good results.

Keywords: Medicinal plants, phytochemicals, antibacterial, antioxidanactivity, DPPH.

INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five millennia (Thomson, W.A.R., (1978; Stockwell, C., 1988)[1]. However, a large number of useful medicinal plants have not been exploited. The plant *Acalypha indica* belongs to the family of Euphorbiaceae is a slender climbing shrub that grows to about 6 m high in marshy places (Jaures et al 2013)[2]. mostly in the backyards of houses and waste places throughout the plains of India. Extracts of *A. indica* are used as emetic, expectorant, laxative, diuretic and for the treatment of bronchitis, pneumonia, asthma and pulmonary tuberculosis. In homeopathy, the plant is used to treat severe cough associated with bleeding from lungs, haemoptysis and incipient phthisis (Zahir et al., 2013)[3]. In the continuation of the strategy of new drug discovery, we studied the photochemical composition, antimicrobial and antioxidant activities of the *A. indica* methanol leaf extracts and its derivative fractions. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids etc. that are present in the plant.

MATERIAL AND METHODS

Collection of Plant material

The fresh plant *Acalypha indica* was collected from the Yakthapur village, Mahaboob Nagar District, Andhra pradesh, India. The taxonomical identification of the plant was confirmed by Dr. Shashikanth, Department of Botany; Osmania University.

Preparation of plant extracts

Freshleaves of *Acalypha indica*, collected and washed thoroughly 2-3 times under running tap water and then with sterile water followed by shade-dried at room temperature for 1 week, grinded into uniform powder and used for extraction. 30gof the powder is mixed with 120ml of solvents in a 250ml conical flask and was kept at 25^0 c for 12h separately. The suspension was filtered through a Whatman no.4 filter paper. The organic solvents (Chloroform, ethanol, and methanol and petroleum ether) and aqueous extracts were concentrated by a rotary evaporator, while aqueous extract was dried using water bath. Finally, the extracted powder was resuspended in the respective solvents at a concentration of 1 mg/ml before it was tested for the photochemical and antibacterial activity.

Test microorganisms

The four bacterial strains used in present study were collected from microbiology department of Osmania University. The bacteria used are *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureous*.

Preparation of bacterial suspension

The screening of antimicrobial activity was carried out by Agar well diffusion method. The test organisms were sub cultured on LB broth. Take 1gram of LB broth and dissolve in 10ml of distilled water in a test tube and autoclaved. Then add 10µl of bacterial culture to the broth in laminar air flow and stored at 4°C in refrigerator to maintain the stock.

Preliminary photochemical analysis of Acalypha indica

Phytochemical screening of plant extracts was done following the standard procedure by Santarem (1983)[4],

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Table 1: Preliminary Phytochemical analysis of *Acalypha indica* Leaves.

Name of the Phytoconstituents	Hexane	Ethyl Acetate	Chloroform	Methanol
Alkaloid				
Hager's Test	-	+	-	-
Wagner's Test	+	-	-	-
Dragendorff Test	-	+	-	-
Mayer's Test	-	-	-	-
Carbohydrates				
Molish Test	+	-	+	-
Fehling Test	-	-	+	-
Benedict's Test	+	+	+	-
Proteins	-	-	-	-
Biuret Test	-	-	+	-
Xanthoprotic Test	+	-	-	-
Lead Acetate Test	-	+	-	-
Amino acids				
Ninhydrin Test	-	-	+	-
Steroids				
LibbermannBurchard Test	+	-	-	-
Salkowaski Test	-	+	-	-
Saponnins				
Foam Test	+	-	-	-
Test for Phenolic Compounds and Tannins	-	-	+	-

+ indicates presence of phytochemicals; - indicates presence of phytochemicals

Chakra et al (1984)[5] and Harbone (1998)[6]. All the prepared plant leaf extracts were subjected to preliminary phytochemical screening for the presence of alkaloids, carbohydrates, glycosides, tannins, phenolic compounds, proteins, free amino acids, saponins, phytosterols, flavonoids, terpenoids, fixed oils and fats.

Tests for Alkaloids

Dragendorff's Test

To the extract, add 1ml of Dragendorff's reagent. An orange red coloured precipitate indicates the presence of alkaloids.

Wagner's Test

To the extract, add 1ml of Wagner's reagent. Reddish brown coloured precipitate indicates the presence of alkaloids.

Mayer's Test

To the extract, add 1ml of Mayer's reagent. A dull white coloured precipitate indicates the presence of alkaloids.

Hager's Test

To the extract, add 3ml of Hager's reagent. Yellow coloured precipitate indicates the presence of alkaloids.

Tests for Carbohydrates

Molish Test

To the extract, 1ml of α-naphthol solution was added and conc. Sulphuric acid was added along the sides of the test tube. Purple or reddish violet colour at the junction between the two liquids indicates the presence of carbohydrates.

Fehling test

To the extract, equal quantities of Fehling's solution A&B were added. Upon heating gently, a brick red precipitate indicates the presence of carbohydrates.

Benedict's test

To 5ml of Benedict's reagent, 8 drops of solution under the test was added and mixed well. Then it was boiled vigorously for 2 minutes and cooled. Red precipitate indicates the presence of carbohydrates.

Tests for Proteins

Biuret test

To the extract, 1ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate solution was added. A violet color indicates the presence of proteins.

Xanthoprotic test

To the extract, 1ml of conc. nitric acid was added. When a white precipitate was formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia was added. Orange color indicates the presence of aromatic amino acids.

Lead Acetate test

To the extract, 1ml of lead acetate solution was added. A white precipitate indicates the presence of proteins.

Test for Amino acids

1. Ninhydrin Test: 2 drops of freshly prepared 0.2% ninhydrine reagent was added to the extract and heated. Development of blue color indicates the presence of proteins, peptides or amino acids.

Tests for Steroids

LibbermannBurchard Test

The extract was dissolved in 2ml chloroform in dry test tube. 10 drops of acetic anhydride and 2 drops of conc. sulphuric acid were added. The solution becomes red, and then blue and finally bluish green in color indicates the presence of steroids.

Salkowaski Test

The extract was dissolved in chloroform and equal volume of Sulphuric acid was added to it. Bluish red to cherry red color was observed in chloroform layer; whereas acid layer assumes marked green fluorescence indicates the presence of steroids.

Test for Cardiac Glycosides

Keller-killani Test

Test sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of conc. sulphuric acid. At the junction, reddish brown color was formed, which gradually becomes blue indicates the presence of cardiac glycosides.

Test for Saponnins

Foam Test

About 1ml of extract is diluted separately with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of saponnins.

Test for Phenolic Compounds and Tannins

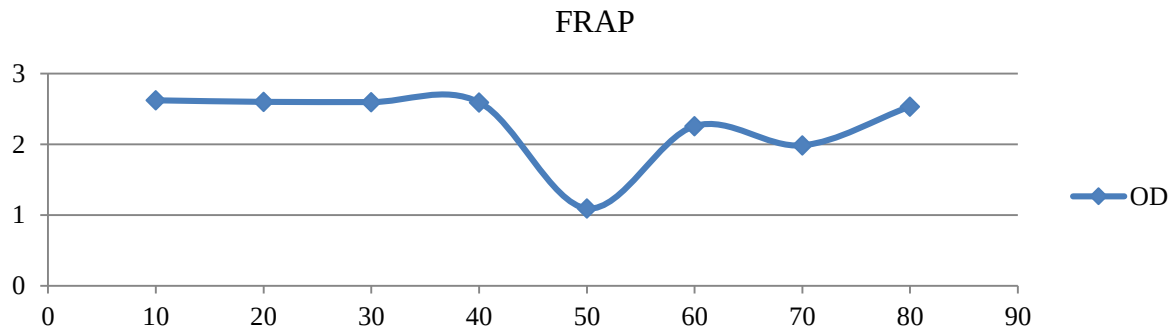

Figure 1: FRAP free radical scavenging activity of standard ascorbic acid and methanolic plant extracts.

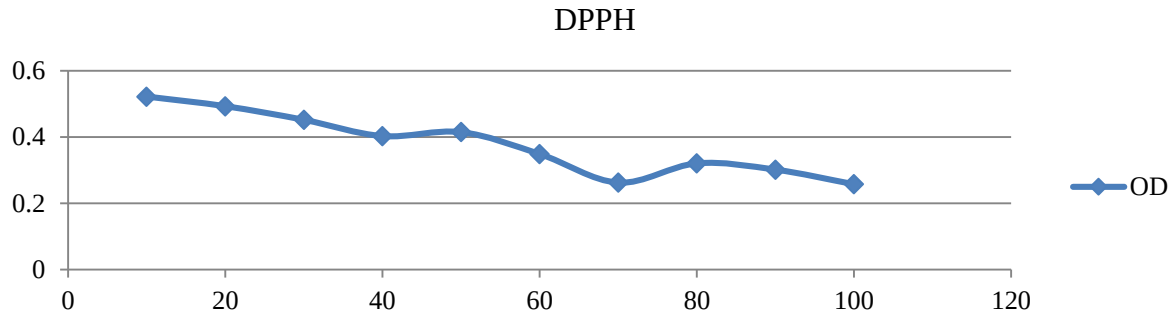

Figure 2: DPPH free radical scavenging activity of standard ascorbic acid and methanolic plant extracts.

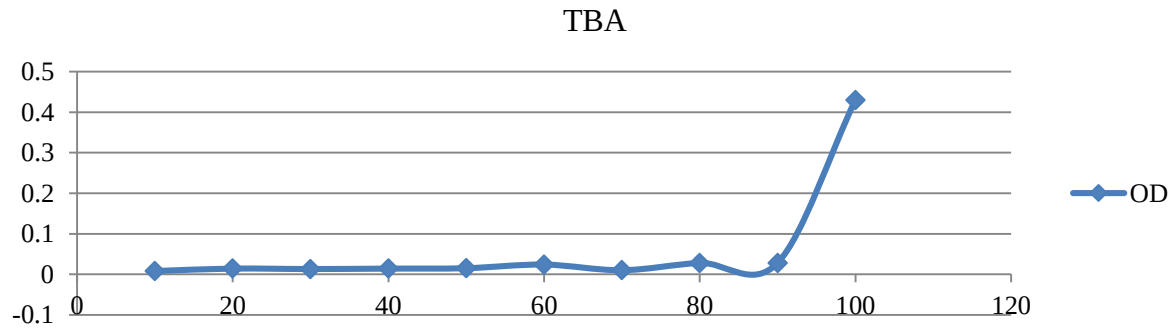

Figure 3: TBA free radical scavenging activity of standard ascorbic acid and methanolic plant extracts.

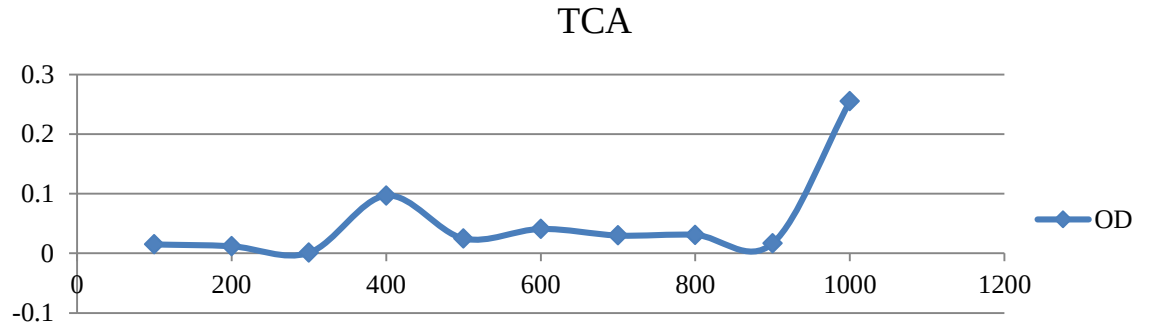

Figure 4: TCA free radical scavenging activity of standard ascorbic acid and methanolic plant extract.

Small quantities of alcoholic and aqueous extracts in water were tested for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%), 1% solution of gelatin containing 10% sodium chloride, 10% lead acetate and bromine solutions.

Anti-microbial Activity

Antimicrobial activity of A. indica extracts were evaluated by the agar well diffusion method. It is a modified method of Murray et al. Antimicrobial susceptibility was tested on solid media in petri plates. For bacterial assay nutrient agar (NA) (40 gm/L) was used for developing surface colony growth. The minimum bactericidal concentration (MBC) values were determined by serial micro dilution assay. The

Table 2: Effect of Plant extract on Bacterial Culture

Bacterial culture	Negative control (DMSO)	Positive control (Antibiotic)	Plant extract
Escherichia coli			
10µl	0	20	10
20µl	0	18	12
30µl	0	18	14
Salmonella typhi			
10µl	0	19	11
20µl	0	18	12
30µl	0	18	15
Bacillus subtilis			
10µl	0	19	10
20µl	0	18	12
30µl	0	18	15
Staphylococcus aureus			
10µl	0	19	10
20µl	0	20	12
30µl	0	20	15

Table 3: Anti-oxidant activity of methanolic extract of *Acalypha indica*.

Concentration(µl)	*O.D/(770nm)*
10µl	0.070
20µl	0.100
30µl	0.116
40µl	0.150
50µl	0.200

Table 4: Anti -oxidant activity of methanolic extract of *Acalypha indica*.

Concentration/(µl)	*O.D/(775nm)*
20µl	0.008
40µl	0.056
60µl	0.78
80µl	0.110
100µl	0.150

suspension culture, for bacterial cells growth was done by preparing 2% Luria Broth (w/v), All the media prepared was then sterilized by autoclaving the media at (121°C) for 20 min. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of each plant extract was prepared at a concentration of 1 mg/ml in different plant extracts viz. Methanol, Ethanol, Petroleum Ether, and Water. About 100 µl of different concentrations of plant solvent extracts were added by sterile pipette into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments (Positive and negative) comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded. The minimum inhibitory concentration is defined as the lowest.

In one set of petri plates, on first disc, methanol is added and it is called negative control. On second disc, *Acalypha extract* sample is added and it is called test sample. On third disc, Ampicillin antibiotic is added and it is called positive control.

Anti-oxidant Activity

DPPH method

Free radical scavenging activity of different extracts of leaves and flowers of Ageratum conyzoides Linn. Plant were measured by 1, 1- diphenyl-2-picryl hydroxyl (DPPH). In brief, 0.1 mm solution of DPPH in methanol was prepared. The plant extract at various concentrations (10-100) to each test tube with 3ml of DPPH solution was added and wrap the tubes with aluminium foil. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. Incubate 15 minutes in dark room.UV absorbance was recorded at 517nm.

TBA method

Extracts (2 ml) and standard solutions were added to 1 ml of 20% aqueous trichloroacetic acid and 2 ml of 0.67% aqueous thiobarbituric acid. After boiling for 10 min, the samples were cooled. The tubes were centrifuged at 3,000 rpm for 30 min. Absorbance of the supernatant was evaluated at 532 nm in a spectrophotometer (Saha et al., 2004)[7]. The antioxidant activity was calculated by percentage of inhibition in this method as follows: % Inhibition = 100 – [(A1 – A0) ×100] Where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts (Elmastas et al, 2007)[8]. The plant extract at various concentrations (10-100) to each test tube and add 1ml of plant extract to each test tube. To this add 2ml of TBA and 1ml of distilled water andIncubate in bath water bath for 30 minutes, Absorbance was recorded at 760nm

TCA method

The plant extract at various concentrations (10-100) to each test tube and Add plant extract methanol to each tube, Both TBA and TCA were added to each flask. Then the tubes are kept in water bath for 10 minutes at 100˚C and cool. Centrifuge at 10000 rpm for 20 minutes and supernatant in fresh tubes Absorbance was recorded at 775nm

FRAP method

This method measures the ability of antioxidants to reduce ferric iron. It is based on the reduction of the complex of ferric iron and 2, 3, 5-triphenyl-1, 3, 4-triaza-2-azoniacyclopenta-1, 4- diene chloride (TPTZ) to the ferrous form at low PH. This reduction is monitored by measuring the change in absorption at 593 nm, using a diode-array spectrophotometer. Antioxidant assay can be conducted by the method developed by Benzie and Strain (1999)[9]. Three millilitre of prepared FRAP reagent is mixed with 100 ml of diluted sample; the absorbance at 593 nm is recorded after a 30 min incubation at 37 C. FRAP values can be obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mM of Fe^{2+} equivalents per kg (solid food) or per L (beverages) of sample. The plant extract at various concentrations (10-100) to each test tube 2.5ml of 0.2M phosphate buffer was

taken in aneach test tube. To this add 2.5ml of 1%potassium Ferro cyanide. Then 1ml of plant extract was added, reaction mixture was incubated for 20 minutes at 50°C.Then 2.5ml of TCA was added and centrifuge at 3000 RPM for 10 minutes. Collect 2.5ml of supernatant, 2.5ml of distilled water and 0.5ml of Fecl3 was added. UV absorbance was recorded at 770nm

RESULTS AND DISCUSSION

Antimicrobial Activity

The petri plates were observed for zones of inhibition after incubating over night with plant compound. *Invitro* antimicrobial activity of methanol extracts of *Acalypha indica* leaves were showed significant zone of inhibition was evaluated against Gram positive (*Bacillus subtilis*) and Gram negative (*Escherichia coli*, *Salmonella typhi*) bacteria

Antioxidant Activity

FRAP Method

TBA Method

The antibacterial activities of the extracts increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the extracts for antibacterial activity the growth inhibition zone measured ranged from 11 to 20 mm for all the sensitive bacteria. Antioxidant activity of methanol extract of *Acalypha indica.*

DISCUSSION

The whole plant of *Acalypha indica* collected from the Yakthapur village, Mahaboob Nagar District, Telangana state. The taxonomical identification of the plants was done by Dr. Shashikanth, Taxonomist Department of Botany, Osmania University; Hyderabad. Review of literature explains mainly ethno botanical, ethno medicinal uses and pharmacological review. In this studies antimicrobial, antioxidant activities. The powdered whole plant of *Acalypha indica* was individually extracted with different solvents. The colours of the extracts were noted. The preliminary phytochemical screening of the extracts *Acalypha indica*, showed the presence of alkaloids, glycosides, phenols, tannins, saponins and Steroids. Preliminary organic analysis of drugs helps to undertake further studies on the isolation and identification of specific chemical constituents. Due to the presence of different phytochemical compounds of *Acalypha indica* leaf extracts has efficient anti-microbial activity against different micro-organisms (E. coli, Streptococcus, *Salmonella typhi, Bacillus subtilis)* Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. In the present work, the extracts obtained from *Acalypha indica* show strong activity against most of the tested bacterial strains. The results were compared with standard antibiotic drugs. The leaves methanolic extract of this plant showed better antioxidant potential when compare standard ascorbic acid by DPPH scavenging assay method. The absorbance at 517 nm by UV visible spectrophotometer. It means methanol extract of plant at higher concentration captured more free radicals formed by DPPH resulting into decrease in absorbance and increase in IC 50 value. The above results show that the activity of methanol extracts of *Acalypha indica* shows significant antibacterial activities. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains more or less same components like saponins, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Results show that plant rich in tannin and Phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms.

CONCLUSION

In the current investigation, the methanol extract has been selected after study of such a selected plant with water extracts and methanol extracts, extract gave higher yield of chemical constituents expected for this research work; the originality of this work is that good results have been found with methanol extract we found that most of the biologically active phytochemicals were present in the present study the plant leaf extracts of *Acalypha indica*showed an abundant production of Phytochemicals as secondary metabolites and they can be used in the pharmaceutical industries for producing a potent drug .,The methanol extracts of *Acalypha indica* were found to be active on most of the clinically isolated microorganism and bacterial, as compare with standard drugs. The present study justified the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. Methanolic extract of the antibacterial action in dose dependent on different bacterial stains however; further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.The results obtained using Four different methods to evaluate the antioxidant activity (DPPH, FRAP, TBA,TCA) *Acalypha indica* leaves extract can be considered good sources of natural compounds with significant antioxidant activity, It is very difficult to assess the antioxidant activity of a product on the basis of a single method. Antioxidant activity assessment may require a combination of different methods, and the results obtained in this study confirm the difficulty of comparing the results of the many different methods used to test antioxidant activities. The correct estimation of the antioxidant activity of a given essential oil requires the evaluation of its optimal concentration. On the other hand, the differences found in the different methodologies may, to a certain extent, be explained by the relative amounts of minor compounds in the oils, which may play a major role in the final oil antioxidant effect. In conclusion, the antioxidant power measured depends on

the chosen method, the concentration and the nature and physicochemical properties of the studied antioxidant.

ACKNOWLEDGEMENT

I have taken efforts in this Research. However, it would not have been possible without the kind support and help of some individuals and organizations. I would like to extend my sincere thanks to all of them. I am highly indebted to my professors

Prof, Rojarani for her guidance and constant supervision as well as for providing necessary information regarding the research& also for their support in completing the research. I would like to express my gratitude towards the Deportment of Genetics, Osmania University for their kind co-operation and encouragement which help me in completion of this research.

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