



PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF EUPHORBIACEAE FAMILY PLANT LEAVES- *ACALYPHA INDICA* L., *CROTON BONPLANDIANUM* BAILL

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ABSTRACT

Objective: Plants are the most important sources of medicines. Now a day the large number of drugs in use is derived from plants. The important advantages for therapeutic uses of medicinal plants in various ailments are their safety besides being effective, economical and easy availability. The present study investigates the phytochemical, and pharmacological activity of two medicinal plants *Acalypha indica* L., *Croton bonplandianum* BAILL.

Methods: Leaves of two plants from the family Euphorbiaceae - were collected, dried and powdered. The powdered material is subjected to soxhlet extraction using various solvents and allowed to evaporation. The crude extracts thus obtained were used for further investigation of phytochemicals, in vitro antioxidant and antimicrobial activity of aqueous, methanol and chloroform extracts from euphorbiaceae plants- *Acalypha indica* L., *Croton bonplandianum* BAILL. leaves.

Results: The phytochemical analysis revealed the presence of carbohydrates, amino acids, proteins, tannins, flavanoids, anthocyanins and β -cyanins, quinones, glycosides and phenols in aqueous, methanol and chloroform extracts. Terpenoids and coumarins are present in aqueous and methanol extracts and absent in chloroform extracts. Alkaloids present only in the chloroform extract. Quantitative analysis demonstrated that presence of high concentration of proteins, sugars, phenol and tannins in methanol extracts of screened plants. The antioxidant activity of the extracts was evaluated by DPPH Radical Scavenging Assay. The antioxidant activity was compared with standard antioxidant such as ascorbic acid. All the extracts showed significant antioxidant activity. Methanol extract of *Acalypha indica* L. has high antioxidant activity and low IC₅₀ value. The plant preparations were also screened individually for antimicrobial activity against three gram negative (*E.coli*, *S.typhimurium*, *S.paratyphi*) and three gram positive (*S.epidermis*, *B.subtilis*, *S.aureus*) organisms by disc diffusion method.

Conclusion: Results showed that all the extracts were effective against both gram positive and gram negative organisms. The methanol extracts of *Acalypha indica* L. shows more effectiveness than the other extracts against bacterial species tested.

Key words: Euphorbiaceae, phytochemicals, antioxidant, IC₅₀, antimicrobial.

INTRODUCTION

According to World Health Organization 75 - 95% of the world populations of developing countries were chiefly rely on traditional medicines and major part of traditional therapies involves the use of plant extract products or their active constituents [1]. Traditional medicine usage is a common practice in developed and developing countries at the primary healthcare level [2]. Due to increased and indiscriminate use of antibiotics for treatment of humans and animals there develops the antibiotic resistance and multidrug resistance microorganisms which has increased a great deal in developing countries [3].

The Euphorbiaceae is a very large, widely distributed family including around 300 genera and 7700 species found in temperate, sub-tropical and tropical. The euphorbiaceae plants are shrubs, trees, herbs or rarely lianas[4]. The Euphorbiaceae family provides food [4, 5] and varied medicinal properties used in ethno botany[6-10]. They are useful in the treatment of human diseases such as respiratory infections, rheumatism, venereal diseases, toothache, cough, ulcer and wounds [11]. The Euphorbiaceae have numerous economic values many species have physiological actions because of their toxic chemical components [12] like diterpenoids, triterpenoids, various alkaloids and flavanoids. For a long time this family has been recognized and reported for antimicrobial, anti oxidant, anticancer components the medicinal value of many the genera of Euphorbiaceae plants has been known and utilized by ancient people[13]. The present study investigates the phytochemical, and pharmacological activity of two medicinal plants - *Acalypha indica* L., *Croton bonplandianum* BAILL. of euphorbiaceae family.

MATERIALS AND METHODS

Plant materials

Leaves of two plants from the family Euphorbiaceae - *Acalypha indica* L., *Croton bonplandianum* BAILL were collected from out fields of Guntur district, Andhrapradesh, India. The plants were identified and authenticated by a taxonomist.

These leaves were washed with distilled water, and shade dried till it is crisp (approximately 15 days). These dried samples were powdered and stored at 4°C until further use.

Extraction procedure

The powdered material is weighed in a selected quantity and is subjected to soxhlet extraction using, Chloroform, Methanol and Water in successive mode respectively for 48 hrs. The solvent was recovered using Rotary Vacuum Evaporator and the concentrated extract was further evaporated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of phytochemical screening, and Pharmacological evaluation.

PHYTOCHEMICAL ANALYSIS

I. Qualitative Analysis

Chemical tests were carried out on the Chloroform, Methanol, and aqueous, extracts using procedures to identify the phytochemicals as described by Sofowara [14], Trease and Evans [15] and Harborne [16].

Test for Carbohydrates

To 2ml of extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Formation of Purple colour at the inter phase of the two layers indicated the presence of carbohydrates.

Test for Amino acids and Proteins

2ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Test for Tannins

To 1ml of extract, 2ml of 5% ferric chloride was added. Formation of greenish black color indicated the presence of tannins.

Test for Saponins

To 2ml of extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.

Test for Flavonoids

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of extract followed by addition of concentrated sulphuric acid. Appearance of yellow colouration indicated the presence of flavonoids.

Test for Alkaloids

To 2ml of extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color indicated the presence of alkaloids.

Test for Anthocyanin and Betacyanin

To 2ml of extract, 1ml of 2N sodium hydroxide was added and heated for 5 minutes at 100°C. Formation of yellow color indicated the presence of betacyanin.

Test for Quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicated the presence of quinones.

Test for Glycosides

To 1 ml of the extract add few drops of HCl, allowed for 5 minutes for hydrolysis and neutralized with NaOH solution. A few drops of Fehling's solution A and B for few minutes. An orange red precipitate indicates the presence of glycosides.

Test for Terpenoids

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Red brown color formation at the interface indicated the presence of terpenoids.

Test for Phenols

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of green color indicated the presence of phenols.

Test for Coumarins

To 1 ml of extract, 1ml of 10% Sodium hydroxide was added. Formation of yellow color indicated the presence of coumarins.

II. Quantitative analysis

Estimation of Total Proteins

Total protein in the plant extracts was determined using colorimetric method described by [17]. Plant extract (0.4 ml) was mixed with 4 ml of copper sulphate solution and incubated at room temperature for 10 minutes. Then, 4 ml of phenol reagent was allowed to react for 30 minutes. The absorbance was measured at 600 nm against reagent blank. Bovine serum albumin (1mg/ ml) was used as standard and then 15, 30, 60, 90, 120 and 150 µg were taken from the standard solution and these readings were used to calculate the total amount of proteins.

Estimation of Total Sugars

Total sugar in the plant extract was determined by [18]. Plant extract (1 ml) was mixed with 1 ml of 2% phenol and 5 ml of concentrated sulphuric acid, allowed to react for 30 minutes and absorbance was measured at 430 nm against reagent blank. For total sugar estimation glucose (1mg/ml) was used as a standard and then 20, 40, 60, 80, 100 µg were taken from the standard solution and readings were used to know the total sugars present in extraction samples.

Determination of total phenol content

The amount of total phenol content, in various solvent extracts of *Acalypha indica* L., *Croton bonplandianum* BAILL., leaves was determined by Folin-Ciocalteu's reagent method [19]. 0.5 ml of

extract and 0.1 ml (0.5 N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg of extracted compounds). The assay was carried out in triplicate and presented as the mean ± SD.

Determination of tannins

The tannins were determined by Folin and Ciocalteu method. 0.1 ml of the sample extract was added with 7.5 ml of distilled water and adds 0.5 ml of Folin Phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solutions of gallic acid is treated in the same manner as described earlier and read against a blank. The results of tannins are expressed in terms of gallic acid mg/g of extract [20]

PHARMACOLOGICAL EVALUATION

I Anti oxidant activity

DPPH(2,2-diphenyl-1-picryl hydrazyl) Radical Scavenging Assay

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging protocol. DPPH solution (0.004% w/v) was prepared in 95% ethanol. A stock solution of aqueous extract, methanol extract, chloroform extract, and standard ascorbic acid were prepared in the concentration of 10mg/100ml (100µg/ml). From stock solution 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes respectively. With same solvent made the final volume of each test tube up to 10 ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 15 min and thereafter the optical density was recorded at 523 nm against the blank. For the control, 2 ml of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution was recorded after 30 min. The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation [11-13]

$$\text{DPPH Scavenged (\%)} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

Where "A control" is the absorbance of the control reaction and "A test" is the absorbance of the sample of the extracts. IC 50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Antimicrobial activity by disc plate method

Authentic pure cultures of gram negative bacteria - *E.coli*, *S.typhimurium*, *S.paratyphi* and gram positive bacteria - *S.epidermidis*, *B.subtilis*, *S.aureus* were collected from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh.

Preparation of Discs

From the plant extracts, 100 mg of crude extracts were dissolved in 1 ml of 4 % dimethyl sulphoxide (DMSO) and 0.2 ml of the prepared extracts were loaded on to the filter paper discs (Sterilized Whatmann No. 1 filter paper discs of 6 mm diameter) to get 20 µg / disc concentration and allowed to dry at room temperature in laminar air flow chamber [21-24].

Preparation of media

Muller Hinton Agar (MH, Hi media) was used. The formula (gm/litre) Beef extract 2g, casein acid hydrolysate 17.5g, starch

1.5 g and agar 17g; pH 7.4 ± 0.2. About 38g of MH agar was weighed and dissolved in 1000 ml of distilled water and adjusted to pH 7.4 ± 0.2, sterilized by autoclaving at 121 °C for 15 minutes at 15 psi pressure and was used for sensitivity tests [21-24].

II Antimicrobial activity

The antimicrobial activity of the extracts was evaluated by disc diffusion method [25]. Previously prepared paper discs containing different extracts were placed individually on the surface of the petriplates, containing 20 ml of respective media seeded with 0.1 ml of previously prepared microbial suspensions individually (10 CFU/ml). Standard antibiotic Streptomycin (20 µg/disc) obtained from Hi-media, Mumbai, was used as positive controls. The discs containing water, Methanol, and chloroform served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 h at 37°C and the diameter of the inhibition zones was recorded [34].

Table 1: Phytochemical screening.

Compound	<i>Acalypha indica</i> L.			<i>Croton bonplandianum</i> BAILL.		
	Water extract	Methanol extract	Chloroform extract	Water extract	Methanol extract	Chloroform extract
Carbohydrates	+	+	+	+	+	+
Amino acids and proteins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Saponins	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Alkaloids	-	-	+	-	-	+
Anthrocyanins & β-Cyanins	+	+	+	+	+	+
Quinones	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	-
Phenols	+	+	+	+	+	+
Coumarins	+	+	-	+	+	-

II. Quantitative analysis

Based upon the preliminary phytochemical analysis qualitative determination of phyto constituents were carried out for various

extracts of *Acalypha indica* L. and *Croton bonplandianum* BAILL. by various standard methods and found that high amount of total proteins, total sugars, total phenols and total tannins were present in the methanol extracts of those plants (table-2)

Table 2: Quantitative analysis of Proteins, Sugars, Phenols and tannins.

	<i>Acalypha indica</i> L.			<i>Croton bonplandianum</i> BAILL.		
	Aqueous extract	Methanol extract	Chloroform extract	Aqueous extract	Methanol extract	Chloroform extract
Total protein (µg/mg)	91.45±0.95	162.08±1.91	142.29±1.57	78.96±0.95	142.92±1.91	129.37±1.08
Total sugars (µg/mg)	111.67±2.89	281.11±1.92	239.44±2.54	108.33±3.33	271.11±1.92	223.88±3.47
Total Phenols (µg/mg)	14.11±0.07	19.78±0.04	16.29±0.03	9.82±0.02	15.19±0.10	12.54±0.09
Total tannins (µg/mg)	9.04±0.07	23.5±0.43	21.92±0.14	9.75±0.25	15.67±0.07	10.42±0.14

*Each value is presented as mean ± S.D. (n=3)

PHARMACOLOGICAL EVALUATION

I Anti oxidant activity

On the basis of phytochemical investigation all extracts were

chosen for the antioxidant studies. DPPH Radial Scavenging Activity of *Acalypha indica* L. and *Croton bonplandianum* BAILL. were represented in table-3 and table-4 respectively. The graphical representations were given in figure 1 and 2

Table 3: DPPH Radial Scavenging Activity of *Acalypha indica* L.: Table -3

Concentration (µg/ml)	Water extract		Methanol extract		Chloroform extract		Standard (Ascorbic acid)	
	% inhibition	IC ₅₀ (µg/ml)	% inhibition	IC ₅₀ (µg/ml)	% inhibition	IC ₅₀ (µg/ml)	% inhibition	IC ₅₀ (µg/ml)
20	10.46±0.12		14.36±0.12		12.33±0.31		25.8±0.6	
40	17.33±0.06		20.06±0.23		19.23±0.21		42.86±0.23	
60	23.13±0.35		26.66±0.06		25.46±0.51		56.66±.58	
80	29.43±0.06		37.23±0.12		33.63±0.06		72.36±0.32	
100	34.56±0.06	149.9	44.16±0.06	116.3	42.16±0.49	123.4	85.46±0.42	51.34

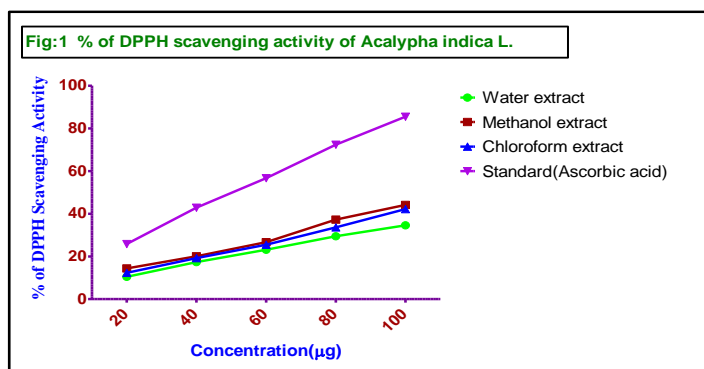


Figure 1: % of DPPH scavenging activity of *Acalypha indica* L.

Table 4: DPPH Radial Scavenging Activity of *Croton bonplandianum* BAILL.

Concentration (µg/ml)	Water extract		Methanol extract		Chloroform extract		Standard (Ascorbic acid)	
	% inhibition	IC ₅₀ (µg/ml)	% inhibition	IC ₅₀ (µg/ml)	% inhibition	IC ₅₀ (µg/ml)	% inhibition	IC ₅₀ (µg/ml)
20	7.86±0.06		10.86±0.12		9.23±0.15		25.8±0.6	
40	11.86±0.12	239.45	14.73±0.12	191.08	13.63±0.06	200.58	42.86±0.23	51.34
60	14.46±0.06		19.06±0.12		17.23±0.21		56.66±.58	
80	19.4±0.2		23.83±0.06		21.93±0.23		72.36±0.32	
100	23.46±0.06		29.53±0.06		27.96±0.21		85.46±0.42	

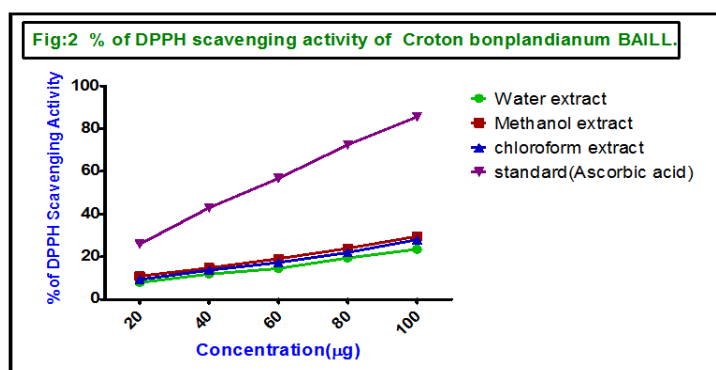


Figure 2: % of DPPH scavenging activity of *Croton bonplandianum* BAILL.

II Antimicrobial activity

Antimicrobial activity of various extracts of screened plant species leaves were assayed in vitro by disc diffusion method [25] against three gram negative (*E.coli*, *S.typhimurium*, *S.paratyphi*) and three gram positive (*S.epidermis*, *B.subtilis*, *S.aureus*) bacterial species. The antimicrobial activity of aqueous, methanol, and chloroform extracts of *Acalypha indica* L. and *Croton bonplandianum* BAILL.

were shown in Fig:3 and 4 respectively. All the extracts exhibited the antimicrobial activity towards gram positive and gram negative organisms. The methanol extracts of the investigated plants showed maximum antimicrobial activity than the others. The inhibitory zones of different extracts varied with the type of microorganism involved in the work. These inhibitory zones were compared with the standard antibiotic Streptomycin (20µg).

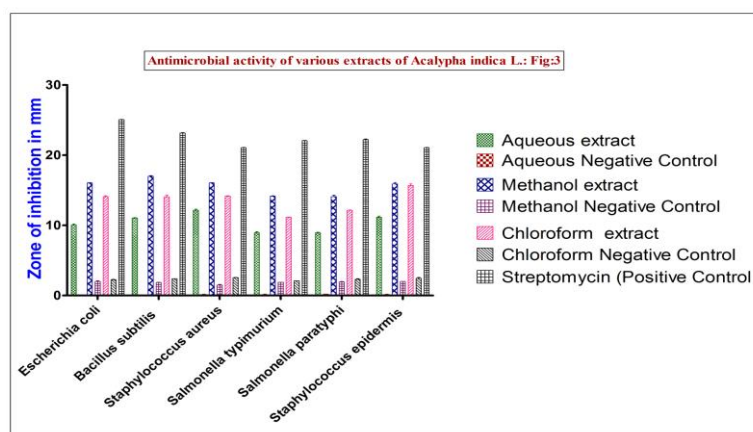


Figure 3: Antimicrobial activity of various extracts of *Acalypha indica* L.

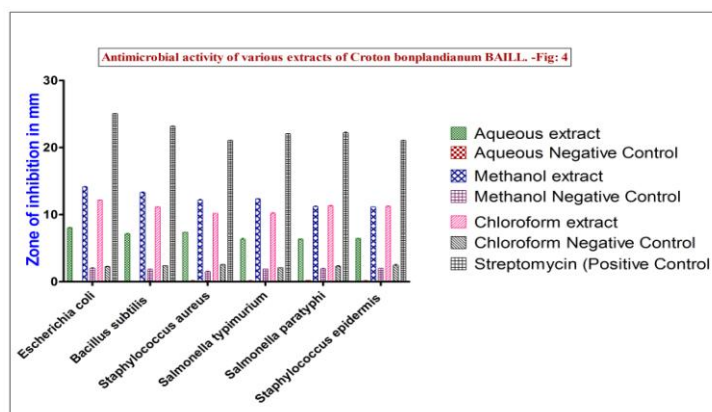


Figure 4: Antimicrobial activity of various extracts of *Croton bonplandianum* BAILL.

DISCUSSION AND CONCLUSION

Secondary metabolites of the medicinal plants are known to be bioactive compounds and they responsible for different activities like antimicrobial, antioxidant and anti cancer [26-27]. Most of the secondary metabolites of the plants displayed the antimicrobial and antioxidant activities through different biological mechanisms. The phytochemical screening of of aqueous, methanol, and chloroform extracts of *Acalypha indica* L., *Croton bonplandianum* BAILL. revealed that the crude extracts contains carbohydrates, amino acids, proteins, tannins, flavanoids, anthocyanins and β -cyanins, quinones, glycosides and phenols. Terpenoids and coumarins are present in aqueous and methanol extracts and absent in chloroform extracts. Alkaloids present only in the chloroform extract. Saponins are completely absent in all the extracts. The detected different bioactive compounds in different extracts of leaves of *Acalypha indica* L., *Croton bonplandianum* BAILL. are responsible for the antioxidant and antimicrobial activities. In the quantitative studies of various extracts of *Acalypha indica* L., *Croton bonplandianum* BAILL. showed the presence high amount of total proteins (162.08 ± 1.91 and 142.92 ± 1.91), total sugars (281.11 ± 1.92 and 271.11 ± 1.92), phenols (19.78 ± 0.04 and 15.19 ± 0.10) and tannins (23.5 ± 0.43 and 15.67 ± 0.07) in the methanol extracts.

Antioxidant activity

The antioxidant activity through free radical scavenging activity (DPPH) method of aqueous, methanol and chloroform extracts from *Acalypha indica* L., *Croton bonplandianum* BAILL. leaves 20 to 100 $\mu\text{g/ml}$ concentrations was determined and compared with ascorbic acid (standard 20 to 100 $\mu\text{g/ml}$). The principle of antioxidant activity is their interaction to produce oxidative free radicals. The role of DPPH method is that the antioxidants react with the stable free radical. During the free radical reaction, DPPH (α, α -diphenyl- β -picrylhydrazyl) is converted into α, α -diphenyl- β -picrylhydrazine with colour change. The rate of colour change gradually decreases to indicate the scavenging potentials of the sample antioxidant. The crude extracts of the screened plants contain flavonoid, tannins, phenolics and aromatic compounds. All these bioactive compounds were able to discolour DPPH solution by their hydrogen donating ability [28-33]. From the results it appears that the extracts of *Acalypha indica* L., *Croton bonplandianum* BAILL. possess hydrogen donating capabilities and it will act as an antioxidant. The results from this experiment reveals that the methanol extracts of screened plants showed high antioxidant capacity. These results indicated that the phenolic compounds (phenols and tannins) had a major contribution to the antioxidant activity of the plants. The parameter IC_{50} (—efficient concentration value), is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color). The methanol extracts of these plants showed low IC_{50} concentration (table 3 and 4).

Antimicrobial activity

Generally, the antimicrobial activity of plant crude extracts depends on the dose and the type of bacterial strains employed.

Also this antibacterial actions could be related to their chemical components in the crude extracts [28- 33]. The antimicrobial activity of various extracts from *Acalypha indica* L., *Croton bonplandianum* BAILL. leaves against three gram negative (*E.coli*, *S.typhimurium*, *S.paratyphi*) and three gram positive (*S.epidermidis*, *B.subtilis*, *S.aureus*) organisms examined was assessed by the size of inhibition zone. The results obtained from this experiment revealed that all the extracts of the screened plants showed broad antibacterial activity against tested organisms. Out of which methanol extract of *Acalypha indica* L. shows highest antimicrobial activity against both gram positive and gram negative organisms. According to the results of antibacterial activity of *Acalypha indica* L., *Croton bonplandianum* BAILL. leaves may be attributed to the presence of phenolic components and their concentration.

When we compare the results obtained during the course of the present study, demonstrated that *Acalypha indica* L. shows presence of high amount of phytochemicals like phenols, tannins, total proteins and total sugars. It also proved that *Acalypha indica* L has high potential antioxidant and antimicrobial activity than *Croton bonplandianum* BAILL.

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