Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 4, 2011

Research Article

PHYTOCHEMICAL INVESTIGATION AND IN *VITRO* ANTI OXIDANT, ANTI MICROBIAL ACTIVITY OF DIFFERENT FRACTIONS OF *ACALYPHA INDICA LINN*

RAJESWARA RAO PRAGADA*,[1], VENKATESH VANGEPURAPU[2], SAMBASIVA RAO ETHADI[1], V.S.PRANEETH.D[1]

[1, 3, 4]A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India 530003, [2]Department of Biotechnology, Andhra University, Visakhapatnam, A.P, India. Email: profprrau@gmail.com

Received: 28 July 2011, Revised and Accepted: 6 Sep 2011

ABSTRACT

Preliminary phytochemical analysis & Quantification of Total Phenols, *In-vitro* Antioxidant and Antibacterial activities of the hydro alcoholic (70% ethanol) extract of *Acalypha indica* and it's different fractions (Hexane, Ethyl acetate, methanol) were carried out against nine selected pathogenic bacteria. The plant extract possesses steroids, Triterpinoids, saponins, flavonoides, carbohydrates, glycosides and amino acids. for total phenolic content, Gallic acid was taken as a standard and the ethyl acetate fraction contains rich phenolic content than other fractions and the Ethyl Acetate fraction showed more DPPH radical scavenging activity . In antibacterial study all extract showed good inhibition zone against only three organisms i.e. *Bacillus megaterium, Staphylococcus epidermidis, Pseudomonas aeruginosa, Priteus mirabilis, Klebsiella pneumonia, Salmonella typhimurium, Enterobacter aerogenes, Escherichia coli, Enterobacter cloacae.* The data clearly indicated that the hydro-alcoholic crude extract and their hexane, ethyl acetate and methanol fractions of *Acalypha indica* showed good antioxidant and antibacterial activity. Among the all the ethyl acetate fraction showed better activity.

Keywords: *Acalypha indica,* Antibacterial Activity, Antioxidant Activity, DPPH radical, Total Phenolic content.

INTRODUCTION

India is called Botanical Garden of the world and one of the worlds twelve leading biodiversity centers which contain over 45,000 different plant species, out of this, 15,000-20,000 species are with good medicinal properties of which only about 7,000-7,500 are being used by traditional practitioners. In India, it is estimated that there are about 25,000 licensed pharmacies of Indian system of medicine. Presently, about 1000 single drugs and about 3000 compound formulations are registered. Herbal industry in India uses about 8000 medicinal plants and the annual turnover of the Indian herbal medicinal industry is more lucrative. The Siddha system of medicine uses around 600, Ayurveda 700, Unani 700 and modern medicine about 30 plant species. After information technology, herbal technology is India's biggest revenue source[1].

Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential[2-4]. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity) [5-6]. The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs.

Though herbal medicines are effective in treatment of various ailments, very often these drugs are not scientifically exploited or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science.

A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreadful diseases.

Acalypha indica belongs to the family *Euphorbiaceae.* It occurs throughout tropical India and Sri Lanka and in South Africa, as well as in Pakistan. It has possibly been introduced elsewhere as a weed. In West and East Africa the plant is used as a medicinal plant. In West Africa the leaves are cooked and eaten as a vegetable. It is also browsed by cattle. This plant is held in high esteem in traditional Tamil Siddha medicine as it is believed to rejuvenate the body.

The purpose of the present study was to investigate the antioxidant and antimicrobial properties of *Acalypha indica*, in this study we reporting the results of such studies in order to orient future investigations towards the finding of new, potent and safe antioxidant and antimicrobial compounds**.**

MATERIALS AND METHODS

Chemicals

1,1-diphenyl-2-picrylhydrazyl and Rifampicin were purchased from Sigma Chemical Company, St. Louis, USA), Muller Hinton agar media was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. All the chemicals and reagents used were of analytical grade.

Test organisms

Nine bacterial species were used. The bacterial species were purchased from National collection of industrial micro organisms (NCIM), Pune. The Bacterial species were maintained in the nutrient broth medium on placing shaker in separate culture tubes for each species separately. Out of nine, two are Gram positive Organisms *(Bacillus megaterium, Staphylococcus epidermidis)* Gram Negative *(Pseudomonas aeruginosa, Priteus mirabilis, Klebsiella pneumonia, Salmonella typhimurium, Enterobacter aerogenes, Escherichia coli, Enterobacter cloacae)*

Culture media

For Anti bacterial activity of *Acalypha indica* Muller-Hinton Agar media (Solid and Broth) was used. For maintaining the bacterial species Nutrient both was used.

Preparation of hydro alcoholic extracts

The plant material used in present study is collected from Visakhapatnam, Andhra Pradesh and authenticated by the taxonomist Prof.M.Venkaiah, Department of Botany, Andhra University, Visakhaptnam. Freshly collected plant material was dried under shade and the dried material was milled to obtain a coarse powder. To the coarse powder (1kg) in round bottomed flask 1 litre of alcohol (70% v/v) was added and macerated for 24 hours at room temperature. The macerated powder was packed in a soxhlet apparatus and subjected to continuous extraction with 3 litre of alcohol (70% v/v).

The liquid extract was collected and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass

obtained. The mass obtained was weighed in each case. The extract was thoroughly air dried to remove all traces of the solvent. Hexane, ethyl acetate and methanolic fractions were prepared from hydro-alcoholic crude extract by successive fractionations by using hexane, ethyl acetate and methanol as solvents (analytical grade).

Phytochemical Analysis

Phytochemical studies were carried out for hydro alcoholic crude, hexane, ethyl acetate and methanol fractions of *Acalypha indica* to detect the presence of steroids, terpenoides, tannins, flavonoides, saponins, cardiac glycosides, amino acids etc following the described procedures[7-9].

Quantification of Total Phenols

Total phenolic content was determined using the Folin-Ciocalteau reagent[10]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm.The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE).

Antimicrobial Activity

The antibacterial activity of hydro alcoholic extract was determined by cup plate method[12,13].Different concentrations of the extracts were prepared by reconstituting with DMSO. The prepared Muller Hinton Agar medium was heated at 45^{0}C to get liquid state. The Muller-Hinton Agar medium was cooled at room temperature. Then, the 20 ml of Muller-Hinton Agar medium is taken in the eight test tubes, to those tubes subjected to testing bacterial inoculums (20µl). After adding the inoculums the tubes were mixed well for equal distribution of the Bacterial species in the medium wells were prepared by using metal steel borer. Different concentrations of plant extracts were placed in the wells of solidified Petri dishes.

Then the plates were incubated in incubator for 24hrs at 36^{0}C. After incubation, the zones of inhibitions were measured in mm.

DPPH Free Radical Scavenging Activity

The crude extract and different fractions (hexane, ethyl acetate and methanol) of *Acalypha indica* were screened for DPPH radical Scavenging activity. DPPH radical scavenging activity was measured according to the method of Braca *et al.*, 2003 [11]. An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed and incubated at 37°c for 30 min. and absorbance of the test mixture was read at 517nm. The All experiments were performed thrice and the results were averaged.

The percentage of inhibition of DPPH radical was calculated,

$$\text{Inhibitory ratio} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where,

A_0 is the absorbance of control;

A_1 is the absorbance with addition of plant extract/ ascorbic acid.

The optical density obtained with each concentration of test sample plotted taking concentration on X-axis and percentage inhibition on Y-axis, the graph was extrapolated to find the 50% inhibition concentration of test sample.

RESULTS

Phytochemical analysis

Qualitative chemical tests indicated that the Hydro-alcoholic crude extract *A.indica* showed positive test for Steroids, Flavonoids, Tannins, Amino acids and oils. The Methanolic fraction of *A.indica* showed positive test Saponins, Flavonoids, Amino acids and oils. The Ethyl acetate fraction of *A.indica* showed positive test Steroids, Tannins, Amino acids and oils. The Hexane fraction of *A.indica* showed positive test Steroids, oils and Amino acids.

Quantification of total phenols

The phenolic content in hydro-alcoholic crude extract, methanolic, ethyl acetate and hexane fractions of *A.indica* was found to be 1.63, 7.21 , 2.11 and 1.45 mg/g respectively. Among the selected extracts ethyl acetate fraction of *A.indica* showed high phenolic content (Table 1).

Table 1: Phenolic content present in different fractions of *Acalypha indica*

Name of the extract	GAE(Gallic acid equivalent) mg/gm
A.indica crude extract	1.63
A.indica ethyl acetate fraction	7.21
A.indica methanolic fraction	2.11
A.indica hexane fraction	1.45

DPPH Radical Scavenging Activity

The extracts hydro-alcoholic crude extract, methanolic, ethyl acetate and hexane fractions of *A.indica* showed concentration dependent percentage inhibition of DPPH radical and better percentage inhibition was produced at a concentration of 640 µg. The ethyl acetate fraction showed better activity than hydro-alcoholic crude extract, methanolic and hexane fractions of *A.indica* (Table 2 ,3 and Fig 1,2).

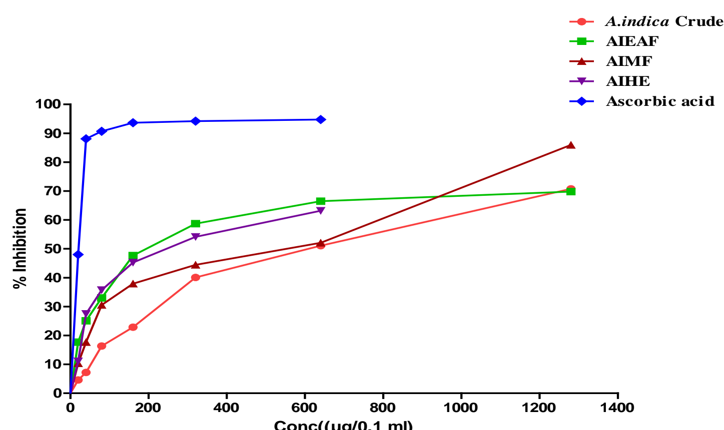

Fig. 1: Concentration dependent percent inhibition of DPPH radical by different fractions of *Acalypha indica* and Ascorbic acid in *in vitro* studies

AIEAF: *Acalypha indica* ethyl acetate fraction, AIMF: *Acalypha indica* methanol fraction, AIHF: *Acalypha indica* hexane fraction

Table 2: Concentration dependent percent inhibition of DPPH radical by different fractions of *Acalypha indica* and Ascorbic acid in *in vitro* studies

Extracts/ Fractions	Percentage inhibition of DPPH radical						
	Quantity of extracts/ ascorbic acid in micrograms						
	20(µg)	40(µg)	80(µg)	160(µg)	320(µg)	640(µg)	1280(µg)
A.indica crude extract	4.57±1.1	7.2±0.5	16.34±1.3	22.86±1.4	40.11±2.2	51.09±2.4	70.74±1.8
A.indica ethyle acetate fraction	17.71±1.3	25.06±0.7	32.99±1.7	47.69±1.3	58.71±1.5	66.5±1.3	69.8±2.2
A.indica methanolic fraction	10.36±0.6	17.71±1.1	30.57±2.2	37.91±1.4	44.45±1.3	52.09±1.3	85.97±2.2
A.indica hexane fraction	11.09±1.2	27.48±2.2	35.71±3.5	45.26±2.1	54.15±1.3	63.19±1.2	--
Ascorbic acid	48±0.5	88.08±1.0	90.68±0.3	93.63±0.5	94.21±0.3	94.74±1.1	--

Table 3: *In vitro* 50% inhibition concentration (IC_{50}) of different fractions of *Acalypha indica* on DPPH free radical scavenging activity

Name of the extract	IC_{50} value (µg)
A.indica crude extract	616.32
A.indica ethyle acetate fraction	191.25
A.indica methanolic fraction	554.2
A.indica hexane fraction	249.14
Ascorbic acid	22.0

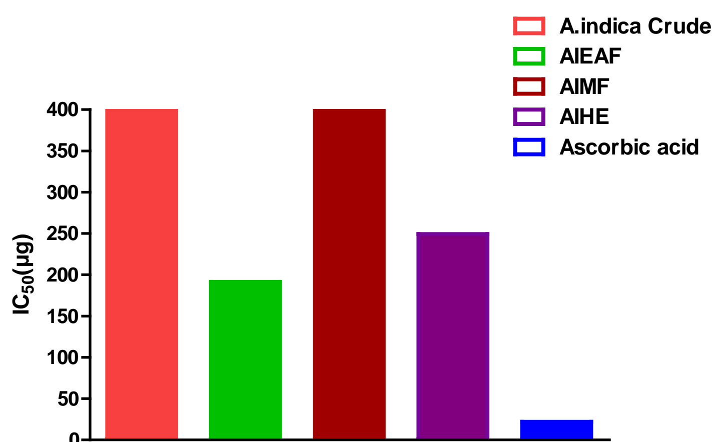

Fig. 2: *In vitro* 50% inhibition concentration (IC_{50}) of different fractions of *Acalypha indica* on DPPH radical

AIEAF: *Acalypha indica* ethyl acetate fraction, AIMF: *Acalypha indica* methanol fraction, AIHF: *Acalypha indica* hexane fraction

Based on IC_{50} values, the order of DPPH radical scavenging activity is as follows:

Ascorbic acid (22.0µg.) > ethyl acetate fraction (191.25µg) > hexane fraction (249.14µg) > methanolic fraction (554.2µg) > Hydro alcoholic crude extract of *Acalypha indica* (616.32µg).

Antimicrobial Activity

Hydro-alcoholic crude extract and their hexane, ethyl acetate and methanol fractions of *Acalypha indica* whole plant were showed significant zone of inhibition against Gram positive bacteria *Bacillus megaterium*, *Staphylococcus epidermidis* and Gram negative bacteria *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Enterobacter aerogenes*, *Escherichia coli* . Only one gram negative bacterium *Enterobacter cloacae* was slightly inhibited by all fractions (Table 4, 5, 6 &7).

Among the four (crude, hexane, ethyl acetate and methanol) fractions tested at five different doses, the ethyl acetate fraction and hydro-alcoholic crude extract 2.5 mg/50µl dose were more potent in their antibacterial activity. The order of antibacterial activity against selected Gram positive and Gram negative bacteria as follows:

Ethyl acetate fraction of *A.indica* > hydro-alcoholic crude extract of *A.indica* > methanolic fraction of *A.indica* > hexane fraction of *A.indica.*

Table 4: Anti-bacterial Activity of hydro alcoholic extract of *Acalypha indica*

Micro organisms	GRAM +Ve/-Ve	Rifampicin	Crude extract (50µl)				
			0.5mg	1.0mg	1.5mg	2.0mg	2.5mg
Bacillus megaterium	+ve	31	-	10	16	19	23
Staphylococcus epidermidis	+ve	35	-	10	14	19	25
Pseudomonas aeruginosa	-ve	41	06	11	16	20	24
Priteus mirabilis	-ve	39	-	09	13	17	23
Klebsiella pneumoniae	-ve	30	06	12	17	23	30
Salmonella typhimurium	-ve	41	06	11	16	20	24
Enterobacter aerogenes	-ve	32	06	10	16	20	23
Escherichia coli	-ve	28	-	10	14	19	23
Enterobacter cloacae	-ve	36	-	-	11	16	19

Table 5: Anti-bacterial activity of *Acalypha indica* hexane fraction

Micro organisms	GRAM +Ve/-Ve	Rifampicin	Hexane fraction (50µl)				
			0.5mg	1.0mg	1.5mg	2.0mg	2.5mg
Bacillus megaterium	+ve	32	07	09	12	15	19
Staphylococcus epidermidis	+ve	35	08	12	15	18	21
Pseudomonas aeruginosa	-ve	41	07	08	10	13	16
Priteus mirabilis	-ve	38	07	12	15	17	20
Klebsiella pneumoniae	-ve	31	08	15	18	22	26
Salmonella typhimurium	-ve	40	09	18	23	25	29
Enterobacter aerogenes	-ve	33	07	10	14	17	21
Escherichia coli	-ve	27	07	09	11	15	18
Enterobacter cloacae	-ve	35	07	11	14	16	19

Table 6: Anti-bacterial Activity of *Acalypha indica* Ethyl acetate fraction

Micro organisms	GRAM +Ve/-Ve	Rifampicin	Ethyl acetate fraction (50µl)				
			0.5mg	1.0mg	1.5mg	2.0mg	2.5mg
Bacillus megaterium	+ve	31	08	11	17	22	28
Staphylococcus epidermidis	+ve	35	10	13	19	24	29
Pseudomonas aeruginosa	-ve	41	09	13	19	23	28
Priteus mirabilis	-ve	37	07	12	18	23	29
Klebsiella pneumoniae	-ve	30	07	13	20	25	30
Salmonella typhimurium	-ve	41	10	13	19	23	30
Enterobacter aerogenes	-ve	32	08	12	18	23	28
Escherichia coli	-ve	28	10	13	19	24	28
Enterobacter cloacae	-ve	36	07	11	17	22	28

Table 7: Anti-bacterial activity of *Acalypha indica* methanolic fraction

Micro Organisms	GRAM +Ve/-Ve	Rifampicin	Methanolic fraction (50µl)				
			0.5mg	1.0mg	1.5mg	2.0mg	2.5mg
Bacillus megaterium	+ve	31	-	09	13	15	21
Staphylococcus epidermidis	+ve	34	-	08	11	15	20
Pseudomonas aeruginosa	-ve	41	-	09	13	15	21
Priteus mirabilis	-ve	37	-	07	13	17	22
Klebsiella pneumoniae	-ve	31	-	09	12	19	23
Salmonella typhimurium	-ve	41	-	08	12	15	20
Enterobacter aerogenes	-ve	32	-	09	13	16	20
Escherichia coli	-ve	30	07	11	14	19	26
Enterobacter cloacae	-ve	36	-	07	12	17	21

DISCUSSION

Qualitative investigation showed the presence of bioactive compounds like Glycosides, Saponins, carbohydrates, Alkaloids, Flavonoids, Tannins, Proteins and phenolic compounds in crude and different fractions (hexane, ethyl acetate and methanolic) of *A.indica.*

There is variability in phenolic content of crude and different fractions (hexane, ethyl acetate and methanolic) of *A.indica.*Among the hydro-alcoholic crude extract and their hexane, ethyl acetate and methanol fractions of *Acalypha indica* , the ethyl acetate fraction of *A.indica* showed better DPPH radical and the IC_{50} value was 191.25µg.Among the four (one crude, hexane, ethyl acetate and methanol) fractions tested at five different doses, the ethyl acetate fraction and hydro-alcoholic crude extract 2.5 mg/50µl dose were more potent in their antibacterial activity.

The data clearly indicated that the hydro-alcoholic crude extract and their hexane, ethyl acetate and methanol fractions of *Acalypha indica* showed good antioxidant and antibacterial activity. Among the all the ethyl acetate fraction showed better activity.

ACKNOWLEDGMENT

The authors were thankful to A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam for providing necessary facilities to carry out present research work.

REFERENCES

1. Sharma A , Shanker C, Tyagi L, Singh M, Rao CV. Herbal medicine for market potential in India: An Overview. Academic Journal of Plant Sciences 2008;1 (2): 26-36.
2. Mothana RAA , Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqotra. Journal of Ethnopharmacology 2005; 96: 177-181.
3. Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. Int J Food Sciences and Nutrition 2005; 56(4):287-291.
4. Wojdylo A, Oszmianski J , Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry 2007; 105: 940-949.
5. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants, 3rd edn. New Delhi: Council of Scientific and Industrial Research; 1992.
6. Bruneton J, Pharmacognosy, Phytochemistry, Medicinal plants. France: Lavoisiler Publishing Co;1995.
7. Faraz M, Mohammed K, Narysanna G, Hamid RV. Phytochemical screening of some species of Iranian plants. Iranian J Pharm Res 2003; 3:77-82.
8. Harborne B. Phytochemical Methods: A Guide to Modern Techniques of Plants Analysis. 3 rd ed. Chapman & Hall, London, England; 1998.
9. Edeoga HO, Okwu DE, Mbaebre BO. Phytochemical constituent of some Nigerian Medicinal Plants. Afr.J. Biotechnol 2005; 4 :685-688.
10. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic acid-phosphotungstic acid reagents. American Journal of Enology and Viticulture 1965;16:144-158.
11. Braca A, Fico G, Morelli I, De Simone F, Tome F, De Tommasi N. Antioxidant and free radical scavenging activity of flavonol glycosides from different Aconitum species. Journal of Ethnopharmacology 2003; 86: 63-67.
12. Suman acharyya, Gauri Kumar Dash, Sumanta Mondal, Santosh Kumar Dash. Antioxidative and Antimicrobial study of *Spondias mangifera* willd root. Int J Pharm Pharm Sci 2010; 2 Suppl 4:68-71.
13. Ambersingh Rajput, Suboth Chandrapal, Baghavan Patil. Phytochemical screening, Antibacterial activity and physicochemical evaluation of leaves of *Butea monosperma.* Int J Pharm Pharm Sci 2011; 3 Suppl 3:189-191.