International Journal of Phytomedicine 8 (2016) 444-452
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Phytochemical analysis, anti-oxidant and anti-microbial activity of *"Acalypha indica"* leaf extracts in different organic solvents

Sudhakar Chekuri[1], Narendar Vankudothu[1], Shivaprasad Panjala[1], Nirmala Babu Rao[1], Roja Rani Anupalli[1*]

***Corresponding author:**

Roja Rani Anupalli

[1]Department of Genetics, University College of science, Osmania University, Hyderabad Telangana, India-500007.

Abstract

The herb *Acalypha indica* which belongs to Euphorbiaceae family has multiple medicinal properties which include anti-oxidant, anti-bacterial, anti-fungal, anti-inflammatory, antiulcer, anti-helmintic, anti-cancerous, anti-venom, and neuro-protective activity. The present study was designed to evaluate the phytochemical, antimicrobial and anti-oxidant activity of *Acalypha indica* leaves extracts in different solvent extractions like methanol, hexane, ethyl acetate and petroleum ether. Fresh leaves of the plant were collected and shade dried. Dried leaves were milled to obtain powder. Powder was subjected to soxhlet extraction using solvents and extracts were successively obtained. Phytochemical analysis was conducted following standard methods. Phytochemical analysis showed the presence of Alkaloids, Phenols, Saponins, Flavanoids and Amino acids. Leaf extract of methonal have shown the highest anti-oxidation capacity than hexane, ethyl acetate and petroleum ether. Anti-microbial activity has been performed on microbes like *Bacillus sps*, *E.coli, Psuedomonas sps* and *Streptococcus sps.* A highest value of zone of inhibition was found in methanol extract against *E.coli.* These results provide evidence that *Acalypha indica* leaf extract possesses vital phytochemicals, antimicrobial and antioxidant properties. Hence this plant can be studied further for drug analysis for finding potent medicines for diseases.

Keywords: *Acalypha indica,* Euphorbiaceae, Phytochemical analysis, Antimicrobial activity, Antioxidant activity, FRAP.

Introduction

Plant derived drugs have been a part of the evolution of human healthcare for thousands of years .Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced in India such as Ayurveda, Unani and Siddha. Medicinal components from plants play an important role in conventional as well as western medicine. Plant based drugs are commonly used in India and China. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. These substances are usually found in several parts of plants like root, leaf, shoot and bark.

The effects of plants extracts on microbes have been studied by a very large number of researchers in different parts of the world. In recent years, multiple drug resistance has developed in many microbes,(*Bacillus sps E.coli Psuedomonas sps Streptococcus sps*) which has resulted in search for new antibiotic sources [1] *Acalypha indica* (English: Indian acalypha, Indian nettle, three-seeded mercury) is an erect, simple or branched, slightly hairy annual herb. Acalypha indica flowers are green, unisexual found in catkin inflorescence. It occurs throughout tropical Africa and South Africa, in India and Sri Lanka, as well as in Yemen and Pakistan. It has possibly been introduced elsewhere as a weed. 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 Siddha medicine as it is believed to rejuvenate the body.

Taxonomic Classification of plant is
Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Euphorbiales
Family: Euphorbiaceae
Subfamily: Acalyphoideae
Genus: *Acalypha*
Species: *Acalypha indica*

DOI:10.5138/09750185.1882

Vernacular names

Sanskrit: Haritha manjari, Muktavarchas, Rudra, Aritamanjari
Telugu: Moorkonda, ,Kuppi chettu, Pappantichettu, Mulakan-dachettu
Kannada: Kuppigidda, Chalmari, Tuppakire
Hindi: Khokli or Kuppi
Malayalam and Tamil: Kuppameni

Medicinal value of *Acalypha indica*

Several chemical and biological investigations have been carried out on this plant. The juice of *Acalypha indica* is added to oil or lime and used to treat a variety of skin disorders. The dried leaves of *Acalypha indica* are made into a poultice to treat bedsores and wounds. The leaves of Acalypha grandis have also been reported to possess contraceptive activity. The leaf extracts reduce mutagenicity in E. coli. and posses anti periodic and laxative properties .Leaf is also used for curing jaundice, piles, rheumatism, ulcers, externally skin eruptions, ring worms, eczema. The leaf extract is applied to pustules, insect bites. A decoction of the leaves gives relief in earache.

The *Acalypha indica* root is prescribed as a tonic, astringent, febrifuge and strong purgative. Alcohol Extract of the root bark can be used externally as emollient; a poultice (a soft, moist mass of material, typically consisting of bran, flour, herbs, etc., applied to the body to relieve soreness and inflammation and kept in place sore with a cloth) is used for chilblains (a painful, itching swelling on a hand or foot, caused by poor circulation in the skin when exposed to cold), in insect bites, swelling rheumatism and facial paralysis. The roots are used in chest pain, joint pain, migraine, blood dysentery and the root extract lowers the blood sugar level up to 30%. Methanol extract of the whole plant has potential analgesic and anti-inflammatory actions in rats and mice [2-4]. The Root extracts of Acalypha Indica Linn have potential Nitric Oxide Scavenging Activity [5]. The ethanol, chloroform and hexane extracts of Acalypha indica were known for inhibition of the -amylase activity [6].

Acalypha indica is a natural diuretic agent [7]. In medicine, diuretics are used to treat heart failure, liver cirrhosis, hypertension, water poisoning, and certain kidney diseases. Some diuretics, such as acetazolamide, help to make the urine more alkaline and are helpful in increasing excretion of substances such as aspirin in cases of overdose or poisoning. Whole plant extract of Acalypha indica has capacity of Post-coital antifertility activity (can be used as a contraceptive). The petroleum ether and ethanol extracts were found to be most effective in causing significant anti-implantation activity [8]. Vasoconstriction is the narrowing of the blood vessels resulting from contraction of the muscular wall of the vessels, in particular the large arteries and small arterioles, which is particularly important in staunching hemorrhage and acute blood loss. When blood vessels constrict, the flow of blood is restricted or decreased, thus retaining body heat or increasing vascular resistance. Leaf extracts of Acalypha indica has natural vasoconstrictor activity [9]. A nosocomial
infection is an infection whose development is favored by a hospital environment, such as one acquired by a patient during a hospital visit or one developing among hospital staff. Acalypha indica has Antibacterial Activity against Human Pathogens Causing Nosocomial Infection [10] and several other microbes [11,12].

Acalypha indica has acaricidal activity [13]. Acaricides are pesticides that kill members of the arachnid subclass Acari, which includes ticks and mites. Acaricides are used both in medicine and agriculture, although the desired selective toxicity differs between the two fields. This herb has anti-cancerous activity against various types of cancers [14] [3]. Other properties of the herb include anti-inflammatory, anti-helmintic [15], anti-bacterial [16], anti-fungal [17], anti-oxidant [18], neuro-protective, anti-venom and antiulcer activity.

Materials and Methods

Materials required for phytochemical screening

Chemicals and Reagents required: Dilute Hydrochloric Acid, Mayer's Reagent (Potassium Mercuric Iodide), Wagner's Reagent (Iodine in Potassium Iodide), Distilled Water, Benedict's reagent, Alkali, Fehling's A & B solutions, Chloroform, Conc. Sulphuric acid, Ferric Chloride Solution, Sodium Hydroxide Solution, Dilute Acid, Lead Acetate Solution and 25% W/V Ninhydrin Reagent. Equipment Required: Water Heater. (All the chemicals were procured from Hi media)

Materials required for Anti-Oxidant Activity

Test Tubes, Test Tube Stand, Plant Extract, Micropipette, Micro Tips, 0.2M Phosphate Buffer (p^H 6.6), 1% Potassium Ferricynide, Eppendorf Tubes, Solvents (Methanol, Ethyl Acetate, Petroleum Ether and Hexane), TCA (Trichloroaceticacid), Distilled Water, Fecl3. Equipment Required: Water Heater, Centrifuge and Spectrophotometer. (All the chemicals were procured from Hi media)

Materials required for Anti-Microbial Activity

LB Media Mix, Agar, Distilled Water, Conical Flask, Cotton Plug, Newspaper, Rubber Bands, Petri plates, Spirit, Cotton, Flame, Paraffin Marker, Scale, Tissue Paper, Micropipette, Micro Tips, Crude Compound, Microbial Cultures, Gel Puncher and Spreader. Equipment Required: Weighing Machine, Autoclave, Laminar Airflow Chamber and Biological incubator.

Methods

Collection of Plant Sample

Different plant materials of *Acalypha indica* were collected from CIMAP (Central Institute of Aromatic and Medicinal Plants) and authenticated by department of Botany, Osmania University, Hyderabad, Telangana. The leaves were separated and were washed in a tray and shade dried for 3-5 days. After three days this shade dried leaves were milled to obtain a fine powder. Always shade drying is preferred as it prevents denaturation of important phytochemicals when compared to sun drying.

Extraction from the plant powder using soxhlet apparatus

This fine powder was subjected to extraction using soxhlet apparatus. About 250ml of solvent and 10 grams of dried fine plant powder was taken for extraction. The whole setup of soxhlet extraction unit was subjected to continuous extraction for 48 hours. Four different kinds of solvents were used individually for extraction namely methanol, ethyl acetate, petroleum ether and hexane. Liquid plant extract was obtained in the round bottom flask. This plant extract obtained from four different solvents were collected in four different glass bottles. These solvent extracts were used for Phytochemical Analysis, Anti-Oxidant Activity and Antimicrobial Activity.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods [19].

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered [19]. The following tests were carried out.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids [19].

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids [19].

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates [19].

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars [19].

Fehling's Test: Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars [19].

Detection of phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes [19].

Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols [19].

Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids [19].

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids [19].

Detection of proteins and amino acids

Ninhydrin Test: To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid [19].

Anti-Oxidant Activity

Anti-oxidant activity was done by following the FRAP (Ferric Reducing Antioxidant Power) method. 10 test tubes were taken and to each 2.5ml of 0.2M phosphate buffer (pH 6.6) was added. 2.5ml of 1% potassium ferricynide was added to all test tubes. 10 eppendorf tubes were taken and volumes of 10ul, 20ul to 100ul of plant extract was added to tubes individually and volume was made upto 1ml by adding respective solvent. This makes a total 1ml of plant extract. Then this each 1ml plant extract in 10 different eppendorf tubes were added to respective test tubes and reaction mixture was incubated for 20mins at 50 C. Then 2.5ml of TCA was added to all tubes and centrifuged at 3000rpm for 10 minutes. After centrifuging, 2.5ml of supernatant liquid was collected and 2.5ml of distilled water and 0.5 ml of FeCl3 was added to all test tubes. UV absorbance was recorded at 770nm.

Collection of plant compound

Solvents with leaf extracts were subjected to rotary evaporator to obtain the plant compound. This plant compound is used for studying anti-microbial activity.

Anti-Microbial Activity

LB Agar medium was prepared by taking a conical flask and dissolving 4 grams of agar and 4 grams of luria-beurtni medium mix in 250ml of distilled water and was subjected to autoclaving at 121 C temperature and 15lb pressure for 15 minutes. After autoclaving, in each Petri plate around 10ml of LB Agar media was poured the Petri plates were closed and paraffin was wrapped to each Petri plate and media in Petri plates were subjected to solidification for about 24 hours.

In the present work four microbial cultures were used for study namely *Bacillus sps E.coli Psuedomonas sps* and *Streptococcus sps.* After solidification of media in Petri plates, 100ul of each culture was taken and spread plate technique was performed. Using gel puncher, in each Petri plate 4 wells were punched and into each well respective labeled amount of plant crude (like 10ul, 25ul and 50ul) extract was added. Then the Petri plates were closed and sealed using parafilm. These Petri plates were subjected to incubation in a biological incubator at 25 degrees centigrade for 48 hours after which results were examined.

Results

Phytochemical Analysis

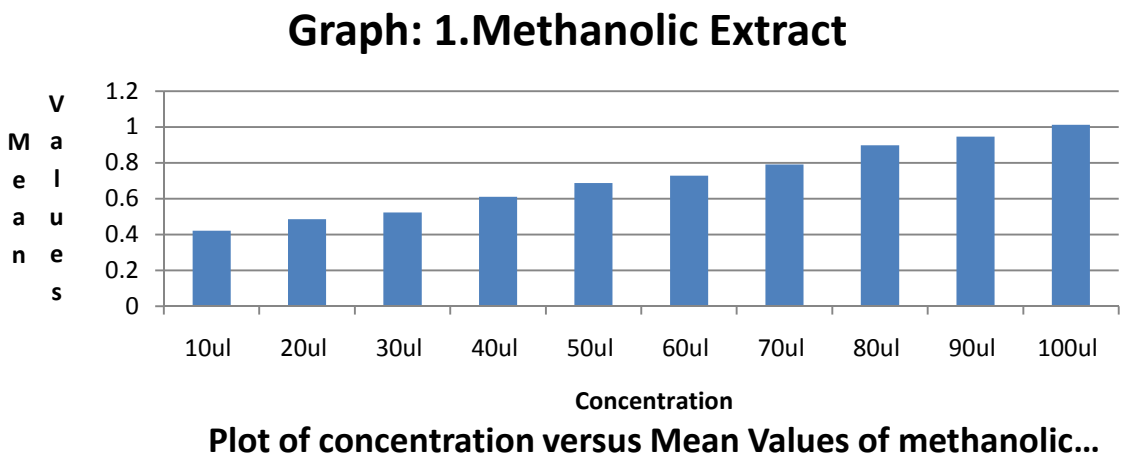
Table: 1 Phytochemical analysis performed using standard protocols.

Test	Phytochemicals	Methanolic extract	Ethyl acetate extract	Petroleum ether extract	Hexane extract
Mayer's test	Alkaloids	++	++	+	++
Wager's test	Alkaloids	++	++	++	++
Benedict's test	Carbohydrates	-	-	-	-
Fehling's test	Carbohydrates	-	-	-	-
Salkowski's test	Phytosterols	-	-	-	-
Ferric chloride test	Phenols	-	++	++	+++
Foam test	Saponins	+	++	-	-
Alkaline Reagent Test	Flavanoids	++	-	-	+++
Lead acetate test	Flavanoids	-	+	+	-
Ninhydrin test	Amino acids	+	+++	-	+

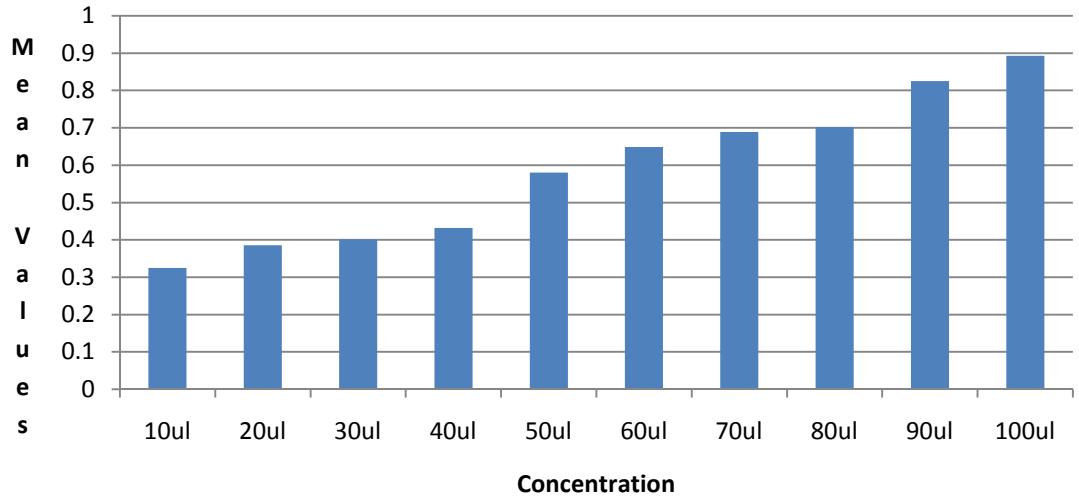
Table 1: - = Absence + = Presence ++ = Moderate Presence +++ = More Presence

Anti-Oxidant Activity

Graphs showing the plots of concentration versus Mean Values of particular extract

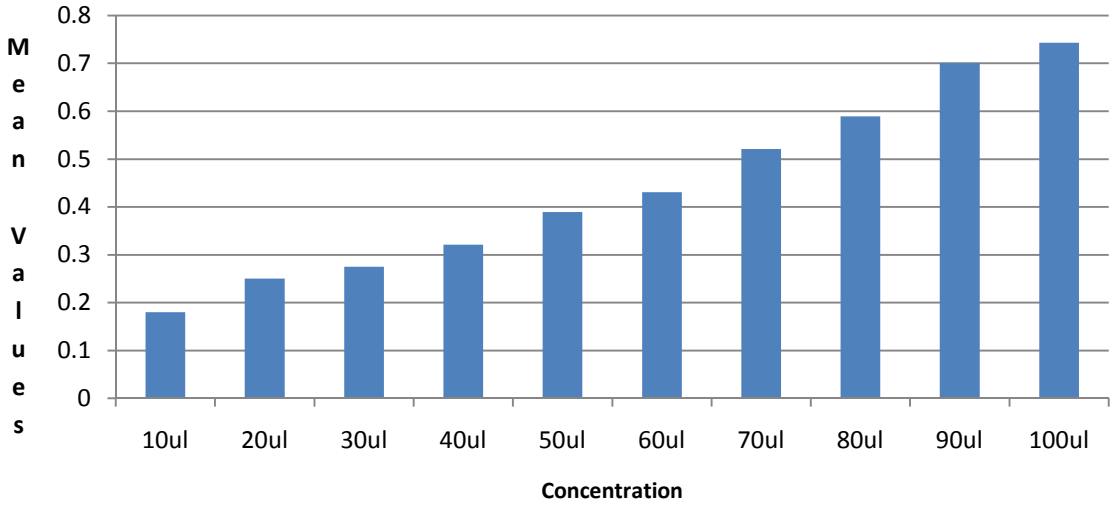


Graph:2. Ethyl Acetate



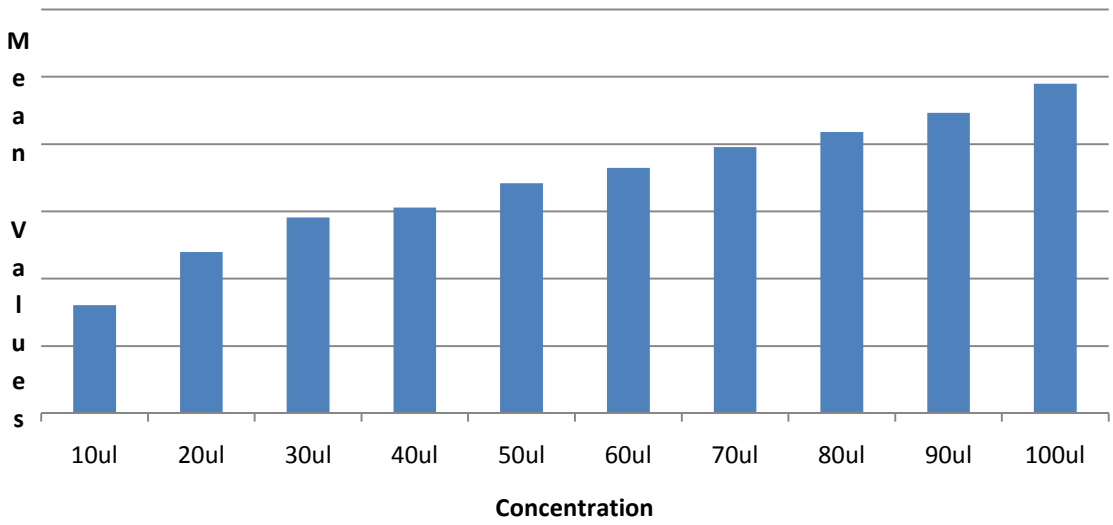
Plot of concentration versus Mean Values of Ethyl Acetate extract

Graph: 3.Petroluem Ether



Plot of concentration versus Mean Values of Petroleum Ether extract

Graph: 4.Hexane



Plot of concentration versus Mean Values of Petroleum Ether extract

Anti-Oxidant Activity

Graphs showing the plots of concentration versus Mean Values of particular extract

Table: 2 Methanolic extract

MICROBIAL CULTURE	Negative control (DMSO)	Positive control (Ampicillin)	CONCENTRATION OF PLANT EXTRACT	ZONE OF INHIBITION (in mm)
Bacillus sps	0	11	50ul	6±1
	0	10	25ul	4±1
	0	12	10ul	2±1
E.coli	0	08	50ul	7±1
	0	10	25ul	4±1
	0	11	10ul	3±1
Psuedomonas sps	0	12	50ul	4±1
	0	10	25ul	2±1
	0	11	10ul	1±1
Streptococcus sps	0	11	50ul	4±1
	0	12	25ul	4±1
	0	08	10ul	2±1

Table: 3 Hexane extract

MICROBIAL CULTURE	Negative control (DMSO)	Positive control (ampicillin)	CONCENTRATION OF PLANT EXTRACT	ZONE OF INHIBITION (in mm)
Bacillus sps	0	12	50ul	5±1
	0	13	25ul	5±1
	0	11	10ul	2±1
E.coli	0	09	50ul	5±1
	0	11	25ul	4±1
	0	10	10ul	1±1
Psuedomonas sps	0	12	50ul	4±1
	0	11	25ul	3±1
	0	10	10ul	1±1
Streptococcus sps	0	12	50ul	5±1
	0	11	25ul	3±1
	0	11	10ul	1±1

Table: 4 Petroleum Ether extract

MICROBIAL CULTURE	Negative control (DMSO)	Positive control (Ampicillin)	CONCENTRATION OF METHANOLIC PLANT EXTRACT	ZONE OF INHIBITION (in mm)
Bacillus sps	0	12	50ul	2±1
	0	13	25ul	1±1
	0	10	10ul	1±1
E.coli	0	11	50ul	2±1
	0	11	25ul	2±1
	0	12	10ul	1±1
Psuedomonas sps	0	13	50ul	3±1
	0	09	25ul	1±1
	0	10	10ul	1±1
Streptococcus sps	0	13	50ul	2±1
	0	11	25ul	1±1
	0	10	10ul	1±1

Table: 5 Ethyl Acetate extract

MICROBIAL CULTURE	Negative control (DMSO)	Positive control (ampicillin)	CONCENTRATION OF METHANOLIC PLANT EXTRACT	ZONE OF INHIBITION (in mm)
Bacillus sps	0	11	50ul	5±1
	0	09	25ul	3±1
	0	10	10ul	1±1
E.coli	0	08	50ul	4±1
	0	10	25ul	3±1
	0	11	10ul	1±1
Psuedomonas sps	0	12	50ul	4±1
	0	11	25ul	2±1
	0	10	10ul	1±1
Streptococcus sps	0	11	50ul	4±1
	0	12	25ul	1±1
	0	12	10ul	1±1

Antimicrobial Activity

Antimicrobial activity was performed and tables 2, 3, 4 and 5 shows the results of methanol, hexane, petroleum ether and ethyl acetate extracts respectively.

Results

In phytochemical analysis alkaloids and phenols are more in methanol,ethyl acetate,petrolium ether and hexane crude extracts. Carbohydrates and phytosterlos are absent in four organic crude extracts. Saponins are present in only methanol and ethyl acetate crude extracts, absent in petroleum ether and hexane crude extracts. Flavanoids are present in only ethyl acetate and petroleum ether crude extracts, absent in methanol and hexane crude extracts. Amino acids are highly present in only ethyl acetate crude extract absent in methanol, petroleum ether and hexane crude extracts(table :1). Antioxidant activity is done by four different organic crude extracts (methanol, ethyl acetate, petroleum ether and hexane extracts) shows that methanol extract has high free radical scavenging activity and hexane extract and ethyl acetate extracts, performing medium level of free radical scavenging activity and petroleum ether extract shows the low free radical scavenging activity (Graph 1, 2, 3, and 4).

Anti microbial activity done by four organic crude compounds (methanol, ethyl acetate, petroleum ether and hexane crude extracts.) with different microbes like *Bacillus sps, E.coli, Psuedomonas sps, Streptococcus sps* with different concentrations like 10ul,25ul,and 50ul.In methanolic crude compound (50 ul of concentration) shows highest level of zone inhibition in *E.coli* medium level of zone inhibition in *Bacillus sps, and Streptococcus sps* lowest level of zone inhibition (10 ul of concentration) in *Psuedomonas sps*. In hexane crude compound (25 ul and 50 ul of concentration) shows highest level of zone inhibition in *E.coli ,Bacillus sps* and *Streptococcus sps* and lowest level of zone inhibition (10 ul of concentration) in *Psuedomonas sps*. In petroleum ether crude compound (50 ul of concentration) shows highest level of zone inhibition in *Psuedomonas sps*. And lowest level of zone inhibition (10 ul of concentration) *E.coli ,Bacillus sps and Streptococcus sps* .In ethyl acetate crude compound (50 ul of concentration) shows highest level of zone inhibition in *,Bacillus sps* and medium level of zone inhibition in *E.coli* and *Psuedomonas sps*. And lowest level of zone inhibition in *Streptococcus sps*.All the four crude compounds treated with different microbial cultures as a negative control DMSO while as a positive control ampicillin (antibiotic) (Table 2, 3, 4 and 5)

Discussion

Phytochemical analysis shows that most of the phytochemicals got dissolved in methanol solvent followed by hexane, ethyl acetate and petroleum ether. A particular phytochemical has its own affinity to a particular solvent. In the above result hexane has high affinity towards alkaloids and flavanoids and low affinity towards amino acids. Ethyl acetate has high affinity towards amino acids and good affinity towards alkaloids and saponins. Methanol has a good affinity towards alkaloids, phenols and flavanoids and low affinity towards saponins and amino acids. Petroleum ether has a good affinity towards alkaloids phenols and low affinity towards flavonoids. The phytochemical constituent which is common in all the 4 solvents are phenols and alkaloids. Antioxidant activity was performed for the extracts from 4 solvents. Absorbance value is highest for methanol extract followed by hexane, ethyl acetate and petroleum ether. This shows high antioxidation capacity for methanol extract. Higher the absorbance values, higher the antioxidation capacity. Anti-microbial activity for the extracts of methanol and hexane showed better results than to that of ethyl acetate and petroleum ether. A highest value of zone of inhibition was found in methanol extract against *E.coli.*

Phytochemical analysis was performed in 4 solvents. The results are in table 1. Methanol extract has the more active compounds. Plotted graphs (graphs 1, 2, 3 and 4) show the results in different solvents. Antioxidant activity of methanol extract has high free radical scavenging activity which can be noticed in graph 1. Antimicrobial activity (tables 2, 3, 4 and 5) results in various solvents. Table 3 shows that methanol extract has highest zone of inhibitions against various type of microbes and table 5 shows that petroleum ether extract has least zone of inhibitions.

Conclusion

Phytochemical analysis shows that methanol extract has the more active compounds, ethyl acetate extract and hexane extracts having moderate active compounds and petroleum ether extract has least active compounds. Antioxidant activity shows that methanol extract has high free radical scavenging activity and hexane extract and ethyl acetate extracts performing moderate free radical scavenging activity and petroleum ether extract shows the low free radical scavenging activity. Antimicrobial activity shows that methanol extract has high zone of inhibitions against various type of microbes like *Bacillus sps, E.coli, Psuedomonas sps, Streptococcus sps*, ethyl acetate extract and hexane extracts shows medium level of zone of inhibitions and petroleum ether extract has least zone of inhibitions. Hence for further analysis, like drug designing, methanol extracts must be examined.

Aknowledgement

My heartiest thanks to DST-SERB, Non UGC and UGC, New Delhi, for providing fellowship and giving financial support my research work.

References

[1]. Tamil R, Selvan, Sultan Mohideen AK, Asrar Sheriff M. and Azmathullah NMD.: Phytochemical Screening of Acalypha indica L. Leaf Extracts: International journal of applied biology and pharmaceutical technology [IJABPT] Volume 3 Issue 2 April/June 2012 Pg 158- 161.

[2]. Rahman MA, Bachar SC, Rahmatullah M (2010). Analgesic and antiinflammatory activity of methanolic extract of Acalypha indica Linn. Pak. J. Pharm. Sci., 23(3): 256-258.

[3]. [14]. T. Reddy Prasad Reddy, R. Srinu Venkat Rao, A.V.N.Swamy, P.Reddanna, G.Pulla Reddy, D.V.Rami Reddy: Exploring the Anti-inflammatory and Anti-cancer compounds from the leaves of Acalypha indica : IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) ISSN: 2278-3008. Volume 4, Issue 2 (Nov. – Dec. 2012), PP 01-07

[4]. Mohana Vamsi N, Venkata Sunil Kumar M,Kodandaram N, Padmanbha Reddy Y. Evalution of Anti-inflamatory activity of Acalypha indica. Ind pharm.2008; 7:89-91.

[5]. Balakrishnan N, Panda A B, Raj N R, Shrivastava A and Prathani R : The Evaluation of Nitric Oxide Scavenging Activity of *Acalypha indica* Linn : Root Asian J. Research Chem. 2(2): April.-June, 2009 ISSN 0974-4169 Pg.148-150.

[6]. [5]. Nandhakumar M, Tamil Iniyan G, Senthilkumar M, Dinesh Kumar B, Mitra A.In vitro Assay of alpha amylase inhibitory activity of indian medicinal herb acalypha Indica.J ournal of Clinical and Diagnostic Research 2009 April; 3:1475-1478.

[7]. Das AK, Ahmed F, Biswas NN, Dev S, Masud MM. Diuretic Activity of Acalypha indica. Dhaka Univ J Pharm Sci. 2005; 4:1-2.

[8]. Shivayogi PH, Rudresh K, Shrishailppa B, saraswati BP,Somanth RP. Post-coital antifertility activity of Acalypha indica L.J ethno pharmacol. 1999; 67:253-58

[9]. Krishna Madhuri. Marri. Prasad Rao. Machineni, Vineela.sathuluri, V.Narasimha Rao and Bathula Praveen Kumar. Bhogavalli : Studies on Phytochemical screening and vasoconstrictor activity of leaf extracts of Acalypha indica on frog blood vessels: Scholars Research Library Annals of Biological Research, 2011, 2 (2) : 337-340

[10]. T.Murugan and P.Saranraj: Antibacterial Activity of Various Solvent Extracts of the Indian Herbal Plant Acalypha indica against Human Pathogens Causing Nosocomial Infection : International Journal of Pharmaceutical & Biological Archives 2011; 2(5):1473-1478.

[11]. Farah Dayana Ishak, Siti Zaiton Mat So'ad, Anis Hazirah Asmali Jauhari, Nini Nadira Mashud and Norazian Mohd Hassan In Vitro Study of Antimicrobial Activity of Acalypha Indica Linn. Extract The Open Conference Proceedings Journal, 2013, 4, (Suppl-2, M14) 57-60 57 2210-2892/13 2013

[12]. Rajaselvam J, Benila smily J.M and Meena R: A Study of Antimicrobial Activity of Acalypha Indica against Selected Microbial Species: International Journal of Pharma Sciences and Research: Vol 3 No 9 Sep 2012 473-476.

[13]. Singh DAP,Raman M, SaradhaV, Jayabharathi P,Kumar VRS, Acarcidal Property of kuppaimemeni (Acalypha indica) against natural Psoroptes cuniculi infestion in broiler Rabbits. Indian J Anim Sci. 2004; 74(10):1003-6.

[14]. Sanseera D, Niwatananun W, Liawruangrath B, Liawruangrath S, Baramee A, Trisuwan K. & Pyne S.G. (2012). Antioxidant and anticancer activities from aerial parts of Acalypha indica Linn: Chiang Mai University Journal of Natural Sciences, 11 (2), 157-168.

[15]. Shivakar YM and kumar VL . Anthelmintic activity of latex of calotopis procera.Pharm.Biol.41 (4) .2003:.263-265.

[16]. Govindarajan M, Jebanesan A, Reetha D, Anisath R, Pushpanathan T, Samidurai K. Antibacterial activity of Acalypha indica L. Eur Rev Med Pharmacol Sci. 2008;12(5):299-2.

[17]. Alade PI, Irobi ON (1993). Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. J. Ethnopharmacol., 39(3): 171-174.

[18]. Prasad Paindla, Estari Mamidala: Phytochemical and Chromatographic Studies in the Leaves Extract of Acalypha Indica: Online International Interdisciplinary Research Journal, {Bi Monthly}, ISSN2249-9598, Volume-IV, Issue-I, Jan-Feb 2014 Pg 175-182.

[19]. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur : Phytochemical screening and Extraction: A Review Internationale Pharmaceutica Sciencia Vol 1 Issue 1 Jan-Mar 2011 Pg 98-10.

[20]. Sonwane Pradeep, Navghare Vijay, Ingole Parag, Pawale Sachin, Khadbadi Somshekhar, Gond Namdev. Antidiabetic activity of acalypha indica linn in alloxan induced diabetic rats IAJPR. 2013; 3(9): 7081-7086