

Phytochemical and Chromatographic Studies in the Leaves Extract of *Acalypha Indica*

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Abstract

Acalypha indica is herb found in tropical countries. This plant used traditionally for treats various diseases. In the present study we carried out phytochemical analysis and TLC profiling were performed on different solvent extractions like n-hexane, chloroform, ethyl acetate, acetone and methanol of *Acalypha indica* leaves extracts. Fresh matured leaves were collected and shade dried. The leaves powder was successively extracted with different solvents. This study involves the preliminary screening of phytochemical and the qualitative thin layer chromatographic separation of secondary metabolites from the leaves of *Acalypha indica*. TLC profiling of the *Acalypha indica* was carried out using sequential extracts of solvents with varying polarity; n-hexane, ethyl acetate and acetic acid respectively. Qualitative phytochemical analysis of this plant confirm the presence of various phytochemical like alkaloids, carbohydrates, glycosides, saponins, proteins, tannins, phenols, amino acids, and starch in their leaf extracts. The TLC chromatograms constituted different coloured phytochemical compounds with different R_f values. It can be conveniently used to evaluate the quality of different area samples. For TLC, new solvent system developed for the best separation of the phytoconstituents present in the extracts.

KEYWORDS: *Acalypha indica*, Phytochemicals, TLC, Plant extracts, Retention factor (R_f)

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Prashant Tiwari and Bimlesh kumar, 2011). Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. Many of the indigenous medicinal plants are used as spices (Amin Mir and Sawhney, 2013).

The plant *Acalypha indica* linn. is commonly known as *Indian acalypha* and it belongs to the family Euphorbiaceae, is a weed widely distributed throughout the plains of India. It has been reported to be useful in treating pneumonia, asthma,

rheumatism and several other ailments. (Chopra, 1956). The dried leaves of *Acalypha indica* was made into a poultice to treat bedsores and wounds and the juice of *Acalypha indica* is added to oil or lime and used to treat a variety of skin disorders (Donw et al 1938). The leaves of *Acalypha grandis* have also been reported to possess contraceptive activity (Bourdy et al 1992; Rajendra Prasad et al, 2013). Several chemical and biological investigations have been carried out on this plant (Bauer et al 1923). The *Acalypha indica* root is prescribed as a tonic, astringent, febrifuge and strong purgative (Khare, 2007). The leaves extract reduced mutagenicity in *E. coli* (Gupta et al 2008). Extract of the root bark with alcohol can be used externally as emollient; a poultice is used for chilblains, in insect bites, swelling rheumatism and facial paralysis (Kirtikar et al 2006). Leaves possess anti periodic and laxative properties, the leaves are used in jaundice, piles, rheumatism ulcers and also externally skin eruptions, ring worms, eczema (Prasad Paindla and Estari Mamidala, 2013). The leaves extract are applied to pustules, insect bites (Nadkarni, 2009). The roots are used in chest pain, joint pain, and migraine and blood dysentery and the extract of the root lowered the blood sugar level up to 30% (Chopra et al 2006; Rajendra Chary, 2013). In the present study, we have concentrated on the preliminary screening and qualitative separation of secondary metabolites from leaves of *Acalypha indica* through TLC.

MATERIALS AND METHODS

Plant Collection

Based on our previous research work (Prasad Paindla and Estari Mamidala, 2013) and its traditional medicinal use the *Acalypha indica* plant was selected for this study. Plant leaves were collected from the rural areas of Jannaram forest, Adilabad district, Andhra Pradesh, India in the month of October 2012. The plant voucher specimens identification was done with the help of Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal and the same was deposited at Infectious Diseases & Metabolic Disorders Research Lab, Department of Zoology, Kakatiya University, Warangal.

Preparation of Plant Extracts

The collected plant leaves were left at room temperature for two weeks to dry. Samples chopped into smaller pieces and then ground into powder. The powdered drug (approx. 600 gm) were then packed in the Soxhlet apparatus and was extracted successively with hexane (H) (Merck, India), chloroform (C) (Merck, India), ethyl acetate (EA), acetone (A) and methanol (M) (Merck, India). After completion of total, the extracted powder was discarded and the different extracts so obtained were further processed. The excess solvent in the extracts were removed by distillation and the concentrated extracts so obtained were further dried at a temperature not exceeding 40° C in rotary evaporator. The yield values and other physical properties were observed (De, Dey & Ghosh 2010).

Preliminary Phytochemical analysis

Preliminary phytochemical screening of the extracts for alkaloids, carbohydrates, glycosides, saponins, proteins, phenolic compounds, tannins using standard phytochemical screening methods (Pongtip Sithisarn, 2006, Yan-Ling Wang, 2010, Wagner 2004, Britto and Sebastian, 2011).

1. Test for the Alkaloid:

Mayer's test:

To detect the presence of alkaloids, a few drops of Mayer's reagent is added in solvent free extracts. Alkaloids solution produces cream colored precipitate in presence of Mayer's reagent.

Hager's test:

Solvent free extract 50mg is stirred with few ml of dilute HCl and filtered take few ml of filtrate and add 1 or 2 ml of Hager's reagent. A prominent yellow precipitate indicates the presence of Alkaloids.

2. Test for the carbohydrates:

Fehling's (A and B) test:

Reducing sugars two methods were used to test for reducing sugars. First, the ethanol extract (1 ml) was added to 1ml of water and 20 drops of boiling Fehling's solution (A and B) in a test tube was added too. The formation of a precipitate red-brick in the bottom of the tube indicates the presence of reducing sugars. Second, added to 2 ml of aqueous solution, 5-8 drops of boiling Fehling's solution. A red-brick precipitate showed the presence of reducing sugars.

Benedict's test:

The extract (100mg) is dissolved in 5ml of water and filtered. To 0.5ml of filtrate, 0.5ml of Benedict's reagent is added. The mixture is heated on boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugars.

Iodine test:

The aqueous extract 5ml was treated with the reagent of the starch (iodine). Any shift to blue violet indicates the presence of starch.

3. Test for the Glycosides:

Borntrager's test:

50mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered, to 2ml of filtered 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia is added to it. Pink colour indicates the presence of glycosides.

Brown ring test:

To test the cardiac glycoside phytochemicals presence, in a test tube 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride (FeCl_3) solution. Afterwards it was underplayed with 1 ml concentrated sulphuric acid (H_2SO_4). A brown ring of the interface indicates a de-oxy sugar characteristic of cardenolites.

4. Test for the Saponins:

To test the saponin phytochemicals presence in various extract, the extract was diluted with 20 ml distilled water and was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicates the presence of saponin.

5. Test for proteins:

Million's test:

Crude extract when mixed with 2ml of million's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Biuret test:

An aqueous sample is treated with an equal volume of 1% strong base like sodiumhydroxide (or) potassium hydroxide followed by drops of aqueous copper(II) sulfate. If the solution turns purple, protein is present.

Ninhydrin test:

Crude extract when boiled with 2 ml of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

6. Test for the Phenolic compounds:

Lead acetate test:

The extract (50mg) is dissolved in distilled water and to this 3ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

7. Test for the Tannins:

To test the tannin phytochemical presence, in a test tube 1 ml of 5% ferric chloride added to solvent free extract. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate.

TLC PROFILE:

The TLC was performed on precoated 20×20 cm and 0.25 mm thick plates. The plates were prepared by using silica gel for TLC, were left overnight for air drying. The reference TLC plates were then developed in five different solvent systems like hexane (H): ethyl acetate (EA): acetic acid (AC) (90:10, 60:30:10, 40:40:20, 30:60:10 and 10:80:10) and visualized under Iodine chamber. Different bands were observed and corresponding R_f values are determined.

R_f value of each spot was calculated as:

Distance Travelled by the Solute / Distance Travelled by the Solvent.

RESULTS & DISCUSSION:

Percentage yield of extracts:

The yield of sequential extracts shown in table 1.

Table 1: Percentage yield of different extracts of *Acalypha indica* leaves

S.No	Solvent	Initial Weight (gm)	Yield of the extract (in gm)	Percentage yield (%w/w)
1	Hexane (H)	600	1.910	0.31
2	Chloroform (C)	600	4.570	0.76
3	Ethyl Acetate (EA)	600	1.900	0.31
4	Acetone (A)	600	3.800	0.63
5	Methanol (M)	600	0.880	0.14

The amount obtained from hexane, chloroform, ethyl acetate, acetone and methanol extracts are 0.910 gm (0.31%), 4.570 gm (0.76%), 1.900 gm (0.31%), 3.800 gm (0.63%), and 0.880 gm (0.14%) respectively. More yield obtained from chloroform crude extracts.

Phytochemical analysis:

Phytochemical screening of the present study revealed the presence of alkaloids, carbohydrates, starch, glycosides and proteins. Slightly present of phenols and tannin, while it give the negative results to saponins. Among these phytochemicals, alkaloids, carbohydrates, starch, glycosides present in all extracts. Phenols present in hexane, chloroform and acetone extracts. Tannin present in hexane, chloroform and acetone extracts. Saponins are absent in all solvent extracts.

Table-2: Phytochemical studies of *Acalypha indica* leaves

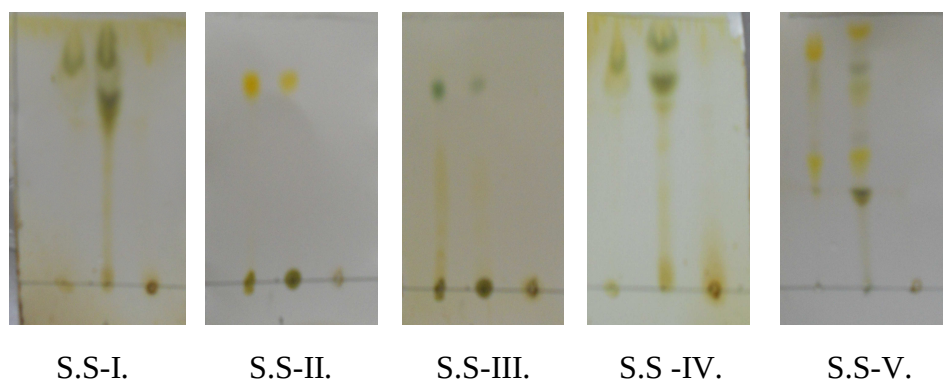
Phytochemicals	Test conducted	Extracts				
		(H)	(C)	(EA)	(A)	(M)
Alkaloids	Mayer's test	++	+	-	-	-
	Hager's test	+++	+++	+++	+++	+++
Carbohydrates	Fehling's test	+++	+++	+++	+++	+++
	Benedict's test	-	+	++	+++	+++
Starch	Iodine test	+++	+++	+++	+++	+++
Glycosides	Borntrager's test	+++	+++	+++	+++	+++
	Brown ring test	+++	+++	+++	+++	+++
Saponins	Saponification test	-	-	-	-	-
Proteins	Millon's test	++	+	-	-	-
	Biuret test	+++	++	+	-	-
	Nin hydrin test	+++	+++	+++	+++	+++
Phenols	Lead acetate test	+++	++	-	+	-

+ = Presence, ++= Moderate presence, +++=More presence, - = Absence,

TLC profile

Thin layer chromatographic technique is a useful analytical tool for the isolation and identification of organic compounds. The chromatogram of *Acalypha indica* leaves extracts shows in Figure-1.

Figure-1. Chromatogram of *Acalypha indica* leaves extracts in different solvent systems (S.S). [S.S-I=H: AA (90:10), S.S-II=H: EA: AA (60:30:10), S.S-III= H: EA: AA (40:40:20), S.S-IV= H: EA: AA (30:60:10), S.S-V= H: EA: AA (10:80:10)].



S.S-I.

S.S-II.

S.S-III.

S.S-IV.

S.S-V.

Table-3: TLC solvent systems for different extract of *Acalypha indica* leaves

S.No	Extracts Name	Solvent system I		Solvent system II		Solvent system III		Solvent system IV		Solvent system V	
		No. of spots	R _f Value	No. of spots	R _f Value	No. of spots	R _f Value	No. of spots	R _f Value	No. of spots	R _f Value
1	Hexane extract	1	0.75	4	0.35 0.47 0.79 0.87	1	0.92	1	0.8	2	0.71 0.81
2	Chloroform extract	2	0.28 0.48	7	0.16 0.34 0.44 0.5 0.56 0.66 0.76	5	0.16 0.35 0.5 0.58 0.7	4	0.2 0.31 0.54 0.68	5	0.22 0.5 0.56 0.72 0.86
3	Ethyl Acetone extract	1	0.77	6	0.37 0.52 0.56 0.64 0.81 0.95	2	0.84 0.94	7	0.3 0.44 0.6 0.66 0.7 0.84 0.92	4	0.51 0.65 0.73 0.89
4	Acetone extract	2	0.31 0.55	4	0.32 0.44 0.56 0.8	6	0.18 0.35 0.62 0.68 0.72 0.83	3	0.31 0.41 0.83	4	0.25 0.5 0.63 0.84
5	Methanol extract	1	0.38	2	0.22 0.46	1	0.35	3	0.52 0.64 0.93	2	0.51 0.65

The data of quantitative separation of secondary metabolites from leaves of *Acalypha indica* by thin layer chromatography is tabulated (Table-3). R_f values obtained by thin layer chromatography patterns are useful to establish their identity and purity of the herbs. The plates were kept in iodine chamber to observe the variously colored bands. Out of five extracts the highest spots (7) obtained in chloroform and ethyl acetate extracts. TLC of chloroform extracts of *Acalypha indica* revealed the presence of 7 spots having R_f values of 0.16, 0.34, 0.44, 0.5, 0.56, 0.66 and 0.76 respectively in solvent system II H: EA:AA (60: 30: 10). TLC of ethyl acetate extracts of *Acalypha indica* revealed the presence of 7 spots having R_f values of 0.3, 0.44, 0.6, 0.66, 0.7, 0.84 and 0.92 respectively in solvent system IV, H: EA:AA (30: 60: 10).

DISCUSSION

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, carbohydrates, starch, glycosides, proteins, phenols and tannin etc. This study results indicate that, the leaves of the *Acalypha*

indica are rich in carbohydrates, starch, glycosides, alkaloids, proteins. They are known to show medicinal potential and physiological activities (Sofawara, 1993). Chandra Mohan and Dinakar, 2010 did the preliminary phytochemical analysis in *Acalypha indica*. Qualitative phytochemical analysis of these plant confirm the presence of various phytochemical like as alkaloids, carbohydrates, starch, glycosides, proteins, phenols and tannin in their chloroform extracts. The phytochemical found in *Acalypha indica* are known to have antibacterial activities (Govindarajan and Samidurai 2008), anti-inflammatory effects (Mohana Vamsi and Sunil kumar 2008; Lingaiah, 2013), acaricidal effects (Singh and Raman,2004), diuretic effects(Das et al,2005), anti-diabetic activities (Manisha masinet al 2011). Antioxidant activities (Ruchi et al, 2007), wound healing effects (Suresh Reddy et al, 2002).

CONCLUSION

The phytochemical analysis of the leaves of *Acalypha indica* showed the presence of secondary metabolites including Alkaloids, carbohydrates, glycosides, starch, proteins, slightly present of phenols, tanins, which has great medicinal properties. The results of the chloroform and ethyl acetone extracts show that at least four different phytoconstituents were present in each extracts of *Acalypha indica* leaves. Hence the presently studied *Acalypha indica* leaves extract could be of considerable interest to the development of new life saving drugs. However, further research is required to isolate the bioactive principle of this plant as well as further studies on its bioefficacy against human pathogens.

ACKNOWLEDGEMENT

The authors acknowledgement the kindness and cooperation of the informants and local administrators in the study area, and the support of Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal for identification of the plant species. Our thanks also to tribal people in study area.

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