

## PHYTOCHEMICAL INVESTIGATIONS AND ANTI-CANCER ACTIVITY OF METHANOLIC EXTRACT OF ADINA CORDIFOLIA

Jayshri H. Bairagi\*<sup>1</sup> and Dr. R. S. Ghosh<sup>2</sup>

<sup>1</sup>Research Scholar, Carrier Point University, Kota, Rajasthan.

<sup>2</sup>Dean, Dept. of Pharmacy, Carrier Point University, Kota, Rajasthan.

Received date: 12 March 2020

Revised date: 02 April 2020

Accepted date: 23 April 2020

\*Corresponding author: Jayshri H. Bairagi

Research Scholar, Carrier Point University, Kota, Rajasthan.

### ABSTRACT

*Adina cordifolia* is a deciduous tree of subfamily *Cinchonoideae*, family *Rubiaceae*, is found Southern Asia, from India and Srilanka east to southern China and Vietnam. It had been also shows anti-ulcer potential, active constituent showed interesting H<sup>+</sup>/K<sup>+</sup>ATPase inhibitory activity. The isolated stem of *Adina cordifolia* were identified as stigmasta-5,22-diene-3P-O-a-rhamnopyranosyl-(1-4)-P-Dxylopyranoside, a-amyrin, octacosanol and naringenin-7-methylether-4'-O-a-rhamnopyranoside on the basis analytical elucidation. Tannins and Phenolic compounds, Flavonoids are significantly present in 70% Hydroalcoholic and methanolic extract whereas the alkaloids, Triterpenoids, Sterols, Amino acids and Proteins presence were found negligible by the colour reactions. TLC profile of the methanolic extract for flavonoids and coumarins showed seven spots after acid spray and heated up to 110<sup>0</sup>C, whereas six spots were seen, when observed under UV light at 365 nm, before acid spray using n- Butanol: Acetic Acid: water (4:1:5) as mobile phase. Whereas TLC profile of the methanolic extract for coumarins showed three spots after acid spray and heated up to 110<sup>0</sup>C, whereas single spot was seen, when observed under UV light at 365 nm, before acid spray. using 10% Acetic Acid as mobile phase. Anti-proliferative activity of methanolic extract of *Adina cordifolia*, was determined using cell lines. Cells (5 × 10<sup>3</sup>) were used various concentrations of *Adina cordifolia*, extracts (0-100 µg/ml) were added. The IC<sub>50</sub> values were calculated by plotting the percentage survival versus the concentration of extract.

**KEYWORDS:** *Cinchonoideae*, *Rubiaceae*, *Adina cordifolia*.

### INTRODUCTION

Cancer is an immense and developing challenge, with the number of individuals around the globe who get an analysis every year expected to rise drastically, from 15 million out of 2015 to 24 million out of 2035.<sup>[1]</sup> Socio-economic factors are successively determined cancer burden of India's, as poor people are bound to pass on from melanomae earlier the time life of 70 years in comparison to prosperous people. However, estimated 600000–700000 passings in India were brought about by cancer in 2012. This partly shows late stage location and poor treatment results and other avoidable causes such as tobacco use, infections etc.<sup>[2]</sup> Ways to deal with lessen the worldwide weight of malignancy incorporate two significant systems: screening and early identification and dynamic preventive mediation. The last is the subject of this analysis and ranges a wide scope of exercises. The hereditary heterogeneity and intricacy of cutting edge malignant growths unequivocally bolster the method of reasoning for early interference of the cancer-causing

process and an improved spotlight on counteractive action as a need system to diminish the weight of malignant growth; be that as it may, the focal point of malignant growth avoidance the executives ought to be on people at high hazard and on essential limited sickness in which screening and recognition ought to likewise assume an imperative job. The planning and portion of (chemo) preventive mediation additionally influences reaction. The mediation might be inadequate if the objective populace is extremely high hazard or previously giving preneoplastic sores with cell changes that can't be switched. The field needs to move past general ideas of carcinogenesis to focused organ site aversion approaches in patients at high hazard, as is of now being accomplished for bosom and colorectal malignant growths. Setting up the advantage of new malignant growth preventive mediations will take years and potentially decades, contingent upon the result being assessed.<sup>[3]</sup> The malignant transformation is a multistep procedure related with the accumulation of numerous molecular alterations. These molecular changes impact

cellular function within the tumor and its microenvironment, and culminate in the hallmarks of cancer: sustained proliferative signaling, resistance to apoptosis, senescence, angiogenesis, invasion and metastasis, deregulating cellular energetics, avoiding immune destruction, tumor-promoting inflammation, and genome insecurity and transformation.<sup>[4]</sup> Proliferation is an important part of cancer development and progression. This is manifest by altered expression and/or activity of cell cycle related proteins. Constitutive activation of many signal transduction pathways also stimulates cell growth. Early strides in tumor improvement are related with a fibrogenic reaction and the advancement of a hypoxic situation which supports the endurance and expansion of malignancy undifferentiated cells. Some portion of the endurance procedure of malignant growth undifferentiated organisms may manifested by adjustments in cell digestion. When tumors show up, development and metastasis might be bolstered by overproduction of fitting hormones (in hormonally subordinate malignant growths), by advancing angiogenesis, by experiencing epithelial to mesenchymal change, by activating autophagy, and by submitting general direction to encompassing stromal cells genome shakiness and transformation.<sup>[5]</sup>

*Adina cordifolia* is a deciduous tree of subfamily *Cinchonoideae*, family *Rubiaceae*, is found Southern Asia, from India and Srilanka east to southern China and Vietnam. It is found scattered in deciduous forests throughout the greater part of India. It is included in threatened species and has been in use as oriental medicine since ancient times as an essential component in antiseptic and febrifuge prescriptions.<sup>[6]</sup> The bark is acrid, bitter pungent, tonic, vulnerary and aphrodisiac and is used in bilious disorders. The roots are used as an astringent in dysentery. It had been also shows anti-ulcer potential active constituent showed interesting H<sup>+</sup>/K<sup>+</sup>ATPase inhibitory activity.<sup>[7]</sup> The isolated stem of *Adina cordifolia* were identified as stigmasta-5,22-diene-3P-O-a-rhamnopyranosyl-(1-4)-P-Dxylopyranoside, a-amyrin, octacosanol and naringenin-7-methylether-4'-O-a-rhamnopyranoside on the basis analytical elucidation.<sup>[8]</sup> *Adina cordifolia* was very well established in vitro conditions in presence of MS medium supplemented 2mg/L BAP or 0.5mg/L NAA alone.<sup>[9]</sup> The major compounds identified in the extracts of *Mitragyna parvifolia* leaf (*Rubiaceae*) were butanoic acid, 2-ethylhexyl ester (19.36%), 4 methyl mannose (53.13%), mitraphylline (21.59%) and isomitraphylline (3.37%). Among these, compound mitraphylline is known for its anti-inflammatory, antiproliferative activities.<sup>[10]</sup>

## MATERIALS AND METHODS

**Table 1: Chemical and Solvents.**

Sr.No	Name	Specification	Manufacturer/Supplier
1	Petroleum ether (60-80 °C)	LR grade	Rankem, RFCL Ltd. New Delhi
2	Methanol	LR grade	Rankem, RFCL Ltd. New Delhi
3	Ethyl acetate	LR grade	Rankem, RFCL Ltd. New Delhi
4	Chloroform	LR grade	Rankem, RFCL Ltd. New Delhi
5	Acetone	LR grade	Rankem, RFCL Ltd. New Delhi
6	Acetic acid glacial	LR grade	Rankem, RFCL Ltd. New Delhi
7	Toluene	LR grade	Rankem, RFCL Ltd. New Delhi
8	Benzene	LR grade	Rankem, RFCL Ltd. New Delhi
9	n-hexane	LR grade	Rankem, RFCL Ltd. New Delhi
10	Ammonia	LR grade	Rankem, RFCL Ltd. New Delhi
11	Sulphuric acid	LR grade	Rankem, RFCL Ltd. New Delhi
12	Silica-gel (60-120) mesh size	LR grade	Rankem, RFCL Ltd. New Delhi
13	Silica gel G	LR grade	Rankem, RFCL Ltd. New Delhi
14	$\alpha$ -Naphthol	LR grade	E- merk, Mumbai, India
15	Fehling Solution A and B	LR grade	E- merk, Mumbai, India
16	Ferric chloride	LR grade	Oualigens fine chemicals, Glaxo India
17	Picric acid	LR grade	Oualigens fine chemicals, Glaxo India
18	Potassium iodide	LR grade	Oualigens fine chemicals, Glaxo India
19	Lead acetate	LR grade	Central Drug House New Delhi
20	Mercuric chloride	LR grade	Central Drug House New Delhi

**Table 2: Instruments/Apparatus.**

Sr.No	Instruments/Apparatus	Manufacturer
1	U V Cabinet	Perfit India
2	Magnetic stirrer	Remi Equipments Pvt. Ltd.
3	Water bath	Narang scientific works Pvt. Ltd.
4	Heating mantle size (1000 ml)	Perfit India
5	Oven Universal (max. temp.250 oC)	Narang scientific works Pvt. Ltd.
6	Rotary Vacuum Bath	Gupta scientific industries
7	FTIR spectrometer	Perkin Elmer
8	<sup>1</sup> H NMR spectrometer	Bruker advanced II 400 spectrometer
9	MASS spectrometer	ESI-ToF Mass spectrometer

**Procurement of Plants**

Whole Fresh plant of *Adina Cordifolia*, was collected in the month of February 2011 from the locality of Aurangabad district of Maharashtra, India.

**Selection and extraction of plant material****Cleaning, drying, powdering and extraction**

Glasswares were soaked overnight in cleaning solution and washed thoroughly with running tap water. They were then cleaned with detergent solution and rinsed several times with tap water and finally in distilled water and air dried. The glassware and media were sterilized in an autoclave at 15psi for 20 minutes, at 120°C. Plant wise Cleaning, Drying, Powdering and Extraction was carried out as below:

**Adina Cordifolia**

The collected leaves were washed with clean water and air-dried for 2 weeks. The dried leaves were powdered coarsely in a mechanical grinder and the coarsely powdered material was exhaustively macerated in a mixture of ethanol and water (50:50) for 7 days to allow for proper extraction (cold extraction). The extract was filtered with filter paper. The liquid filtrate was concentrated and evaporated to dryness in vacuo at 40 °C using a rotary evaporator to obtain good yield and hydro-alcoholic extract was kept in desiccator until further use [11].

**Qualitative Phytochemical Analysis**

Following standard protocols were used for qualitative analysis of samples to check for the presence of Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins, Terpenoids, Quinones and Proteins.

**Test for Flavonoids**

2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour was observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicated the presence of flavonoids in the sample extract.

**Test for Alkaloids**

To 1 ml of each extract, 1 ml of marquis reagent, 2ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed, appearance of dark orange or purple colour indicated the presence of alkaloids.

**Test for Saponins**

To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicated the presence of saponins.

**Test for Tannins**

To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicated the presence of tannins.

**Test for Phenols**

To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue colour indicated the presence of phenols in the sample extract.

**Test for Proteins**

To 2 ml of each extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added; formation of violet colour indicated the presence of peptide linkage molecules in the sample extract.

**Test for Cardiac Glycosides**

To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicated the presence of cardiac glycosides in the sample extract.

**Test for Terpenoids**

1 ml of extract of each solvent was taken and added 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish-brown precipitate indicated the presence of terpenoids in the extract.

**Test for Carbohydrates**

1 ml of extract was taken, added few drops of Molisch's reagent and then 1 ml of concentrated sulphuric acid was added at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or

dull violet colour indicated the presence of carbohydrates in the sample extract.

#### Quantitative Analysis

Depending on the above qualitative results the quantitative assay is carried out for Alkaloids, Tannins, Phenols, Proteins and Carbohydrates.

#### Total Tannins Content Determination

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract was added with 3.75 ml of distilled water and added 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

#### Total Phenol Content Determination

The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to the 200µl of the sample extract, 800 µl of FolinCiocalteu reagent mixture and 2 ml of 7.5% sodium carbonate added. The total content is diluted to 7 volumes with distilled water and finally kept the tubes for 2 hrs incubation in dark. The absorbance was measured at 765 nm. Gallic acid dilutions were used as standard solutions. The results of phenols are expressed in terms of Gallic acid in mg/ml of extract.

#### Total Protein Content Determination

The total proteins content was determined by using Bradford's method. Briefly, to the 100 µl of the sample extract add 3 ml of Bradford's reagent and incubate in dark for 5 minutes. The absorbance was measured at 595nm. Bovine serum albumin dilutions (0.1mg/ml to 0.5mg/ml) were used as standard solutions.

#### Total Alkaloid Content Determination

40 ml of 10% acetic acid in ethanol was added to 1g of powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

#### Total Carbohydrate determination

For estimating the polysaccharide content, 1ml of sample solution was taken and added with 1 ml of 5% phenol and then add 5 ml of concentrated sulphuric acid mixed well and left for 10 minutes. Measured the absorbance at 488 nm against blank. Then compared it with standard solution of glucose. To prepared blank, 1 ml of distilled water added to 1 ml of 5% phenol followed by 5 ml of concentrated sulphuric acid.

#### Phytochemical analysis

##### Thin layer and hptlc chromatography

Thin Layer Chromatography (TLC), were done for different concentrates to affirm the nearness of various phytoconstituents in these concentrates. TLC is a method of fluid chromatography, wherein, the concentrate is applied as a little spot or band at the starting of thin sorbent layer upheld on a glass/plastic/metal plate. The solvent relocates through the stationary phase by capillary activity. The detachment of solutes happens because of their differential adsorption/partition coefficient as for both mobile and stationary phases. Each isolated part has same travelling time yet unique travelling distance.

The mobile phase comprises of a solitary solution or a blend of solvents. In spite of the fact that, various sorbents like silica gel, cellulose, polyamide, alumina, synthetically altered silica gel and so on are utilized, Silica gel (type 60) is most ordinarily utilized sorbent. Carefully assembled plates are set up by utilizing strategies like, pouring, plunging or showering. Presently a-days, readymade precoated plates are additionally accessible. The plates should be actuated at 110°C for 1 h. This expels water/dampness approximately bound to silica gel surface<sup>[12,13]</sup>.

The Retardation Factor (Rf) can be determined as follows,

$$Rf = \frac{\text{Distance travelled by solute from } t_{\square} \text{e origin}}{\text{Distance travelled by solvent from } t_{\square} \text{e origin}}$$

- **Sorbent used:** Silica gel 60 GF<sub>254</sub> / Pre-coated TLC plates on aluminiumsheet
- **Support material:** Glass plates (for handmade TLCplates)
- **Plate size:** 10 x 10 cm / 20 x 20cm
- **Solvents:** Initially plates were developed using single solvent (100%) as per table 4.3. Based on the separation pattern, the combinations of more than two solvents were used for effective separation of phytoconstituents.

From the preliminary phytochemical investigation, the *T. indica*(seed), *C. dichotoma*(fruit) and *C. dactylon*(roots) indicated that flavonoids, saponins, triterpenoids, tannins and phenols are present as characteristic secondary plant metabolites. Furthermore, qualitative TLC/high performance TLC (HPTLC) was performed using different solvent systems and specific visualizing reagents for the separation and identification of these phytoconstituents.

##### High performance thin layer chromatography (HPTLC) fingerprint

The qualitative TLC/HPTLC analysis was performed using Linomat V sample applicator, TLC 3 densitometric scanner and WinCATs software (Camag, Switzerland; Version 1.2.3) on precoated TLC plates (Merck Ltd.; Catalogue No. 1.5554.0007).

## List of solvents based on their physico-chemical characteristics.

Solvent	Polarity index #	Dielectric constant (20 <sup>o</sup> z.25 <sup>o</sup> C)	Dipole moment	Boiling point (°C)
Cyclohexane	0.0	2.0	0	80.7
n-Hexane	0.0	2.0	0	69
Petroleum ether	0.0	-	0	-
Toluene	2.3	2.4	0	110.6
Benzene	2.7	2.28	0	80
Diethyl ether	2.8	4.34	1.15	35
Dichloromethane	3.4	9.1	1.60	40
n-Butanol	3.9	17.8	1.66	118
Ethyl acetate	4.3	6.0	1.78	78
Chloroform	4.4	4.1	-	61.7
Methanol	5.1	33	1.70	68
Ethanol	5.2	24.3	1.69	78
Acetone	5.4	20.7	2.88	56
Acetonitrile	6.2	36.6	2.91	81
Glacial acetic acid	6.2	6.15	1.75	118
Formic acid	8.6	58	1.41	100
Water	9.2	80.2	1.85	100

## Different solvents utilized for TLC study.

Solvent System No.	Solvents	Composition	Phytoconstituents to be separated
SS-1	Benzene + Ethyl acetate + CH <sub>3</sub> COOH	60 + 40 + 0.5	Terpenoids, Phenylpropanoids, Saponins, Bitter principles
SS-2	Ethyl acetate + Methanol + Water	100 + 13.5 + 10	Anthraquinones, Flavonoids, Mono- and Diterpenoids
SS-3a	Toluene + Ethyl acetate	93 + 7	Terpenoids, Phenylpropanoids, Saponins, Bitter principles
SS-3b	Toluene + Ethyl acetate	70 + 30	Terpenoids, Phenylpropanoids, Saponins, Bitter principles
SS-4	Chloroform + Methanol + Water	64 + 50 + 10	Saponins, Sapogenins
SS-5	n-Butanol + Chloroform + Ethyl acetate + Formic acid	2 + 1 + 1 + 2	Triterpenoids, Saponins, Flavonoids
SS-6	n-Butanol- Glacial acetic acid-Water	5 + 1 + 4, Upper layer	Flavonoids, Triterpenoids, Saponins, Amino acids

## Different visualizing / derivatizing reagents used for TLC study.

Reagent No.	Visualizing reagent	Detection	Phytoconstituents to be visualized
VR-1	Anisaldehyde- sulphuric acid reagent	Different visible colours	Terpenoids, Phenylpropanoids, Steroids, Saponins, Bitter principles
VR-2	Komarowski reagent	Different visible colours	Terpenoids, Sapogenins, 3-keto steroids
VR-3	Ferric (III) chloride reagent	Blue - blue green visible spot; brightfluorescence in long wave UV light (366 nm)	Tannins and Polyphenolic compounds
VR-4	Aniline phthalate reagent	Different visible colours	Sugars, Sugar derivatives, Sugar alcohols
VR-5	Potassium hydroxide reagent	Visible colours; fluorescence in long wave UV light (366 nm)	Anthraquinones, Anthrone, Coumarins
VR-6	Aluminium chloride reagent	Yellow fluorescence in long wave UV light (366 nm)	Flavonoids
VR-7	Vanillin-sulphuric acid reagent	Different visible colours	Triterpenoids, Saponins, Steroids, Phenylpropanoids

### Physicochemical Analysis & Extractive Value

The plant extracts and parts of *Adina Cordifolia*, is devoid of any visible foreign matter. The loss on drying for *Adina Cordifolia* was observed less than 10.0% w/w, it indicates that the plant parts dried properly. The acid insoluble ash values were observed to be greater than 1.0% w/w indicating that *Adina Cordifolia* contain any silicious material like sand, clay etc. The extractive values were observed to be greater than 5.0% w/w for plants with polar solvents (such as methanol and water) and below 3.0% w/w for plant parts extracted using non-polar solvents. So, it is observed that the polar phyto constituents are present in plant.

**Table 4: Quantitative pharmacognostic analysis of *Adina Cordifolia*.**

Parameter	Value (%)
Ash Value	45
Acid Insoluble Ash	13
Water Soluble Ash	25
Sulphated Ash	16
Alcohol Soluble Extractive	10
Water Soluble Extractive	48

### Phytochemical Analysis

#### Quantitative pharmacognostic analysis of *Adina Cordifolia*

It was revealed from the phytochemical studies that chemical constituents viz., Volatile oils, Glycosides and Saponins are absent in all the extracts. Tannins and

**Table 6: Qualitative TLC analysis of *Adina Cordifolia*.**

Extract	Adsorbent	Solvent system	Observation / Rf values	
			Under u v light 365 nm	After acid spray and heated at 110°C
Petroleum ether extract	Silica Gel 60GF 254 Precoated sheet	Benzene	1 spot : 0.13 (deep blue)	5spots: 0.09 (brown), 0.13 (deepBlue), 0.21, 0.27, 0.79 ( light blue)

#### TLC screening profiles of Methonolic extract for flavonoids and Coumarins

TLC profile of the methonolic extract for flavonoids and coumarins showed seven spots after acid spray and heated up to 110°C, whereas six spots were seen, when observed under UV light at 365 nm, before acid spray using n- Butanol: Acetic Acid: water (4:1:5) as mobile

**Table 7: TLC screening profiles of Methonolic extract.**

Adsorbent	Solvent system	Detecting Reagent	Observation	Inference	Rf Values	
					Under UV light 365nm	After acid spray and heated at 1100C
Silica gel 60GF 254 precoated sheet	n-Butanol: Acetic Acid:Water (4:1:5)	NP/PEG & UV	Yellow/ Orange	Flavonoids present	0.31,0.27, .041, 0.65,82, 0.92	0.27,0.31, 0.41,0.51, 0.65,0.82, 0.92

#### HPTLC Finger print analysis *Adina Cordifolia*

The characteristic HPTLC finger print profile of the chemical constituents in the ethyl acetate fraction of

Phenolic compounds, Flavonoids are significantly present in 70% Hydroalcoholic and Methanolic extract whereas the alkaloids, Triterpenoids, Sterols, Amino acids and Proteins presence was found negligible by the colour reactions. The phytochemical investigations of all the extracts is summarized in following table.

**Table 5: The phytochemical investigations of *Adina Cordifolia*.**

Sr. No	Chemical Constituents	Methonolic
1	Alkaloids	+++
2	Flavonoids	++++
3	Tannins	+
4	Terpenoids	+
5	Saponins	++
6	Cardiac glycosides	++
7	Proteins	--
8	Carbohydrates	--
9	Phenols	++

**Keywords:** ‘-‘ absent., ‘+’ presence, ‘++’ more clarity, ‘+++’ highly significant.

#### Qualitative TLC analysis of *Adina Cordifolia*

TLC profile of the petroleum ether extract showed five spots after acid spray and heated up to 1100C, whereas single spot was seen, when observed under UV light at 365 nm, before acid spray using benzene as mobile phase. The colour of the spots and Rf values are recorded in the following table.

phase. Whereas TLC profile of the methonolic extract for coumarins showed three spots after acid spray and heated up to 110°C, whereas single spot was seen, when observed under UV light at 365 nm, before acid spray. using 10% Acetic Acid as mobile phase. The colour of the spots and Rf values are recorded in the following table.

methanolic extract has been developed in solvent system Benzene: Toluene: Glacial acetic acid (3:6:1). The

developed plates were photographed under normal light, in uv chamber at 254 nm and at 366 nm (Fig.No.1).

HPTLC Finger print profile of Ethyl acetate fraction of Methanolic extract of corm in solvent system

Benzene:Toluene: Glacial Acetic Acid (3:6:1). TLC Chromatogram: a, b and c.

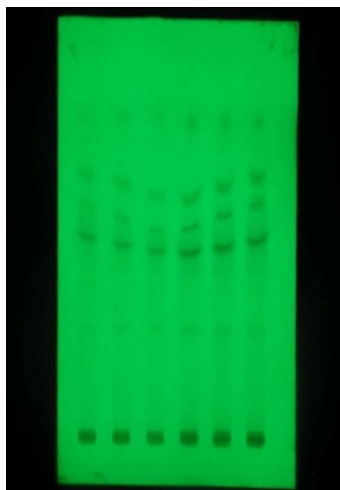


Fig. 1a: (@normal light)



Fig. 1b: (@254nm)

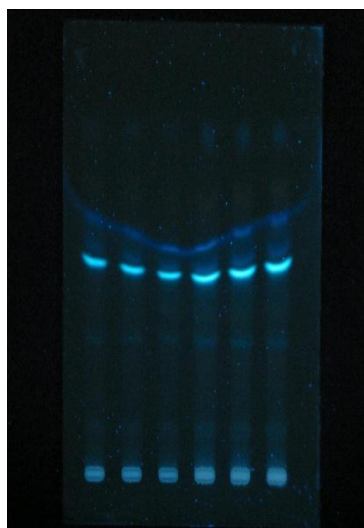


Fig. 1c: (@ 365 nm).

Figure 1: HPTLC Finger print of Extract.

### Pharmacological Evaluation

#### Anti-proliferative activity of plants

Anti-proliferative activity of methanolic extract of *Adina cordifolia*, was determined using cell lines. Cells ( $5 \times 10^3$ ) were seeded in 12-well plates containing respective medium at 37°C with 5% CO<sub>2</sub> and 95% air and in 100% relative humidity. After 24 hrs, various concentrations of *Adina cordifolia*, extracts (0-100 µg/ml) were added. At the end of 72 hrs incubation, the medium in each cell was replaced by fresh medium containing 5 mg/ml of MTT. 3 hours later, the Formosan product of MTT reduction was dissolved in DMSO, and absorbance was measured using a multi-plate reader. The IC<sub>50</sub> values were calculated by plotting the percentage survival versus the concentration of extract.

### CONCLUSION

The the presence of medicinally important constituents in the plants are studied. There are many evidences which confirms that the identified phytochemicals are bioactive. Therefore, extracts of dry leaf powder of *Adina cordifolia* plant has marked medicinal importance. This study is to investigate the phytochemical properties and their evaluation for their anti-proliferative activity. The extract of dry leaf powder of *Adina cordifolia* showed activity in the presence of Flavonoids.

### REFERENCES

1. Cancer research uk, review in, 2017/2018.
2. Mallath MK, Taylor DG, Badwe RA, Rath GK, Shanta V, Pramesh CS, Digumarti R, Sebastian P,

- Borthakur BB, Kalwar A, Kapoor S. The growing burden of cancer in India:epidemiology and social context. *The Lancet Oncology*, 2014 May 1; 15(6): e205-12.
3. Kagohara LT, Stein-O'Brien GL, Kelley D, Flam E, Wick HC, Danilova LV, Easwaran H, Favorov AV, Qian J, Gaykalova DA, Fertig EJ. Epigenetic regulation of gene expression in cancer: techniques, resources and analysis. *Briefings in functional genomics*, 2017 Aug 11; 17(1): 49-63.
  4. Kagohara LT, Stein-O'Brien GL, Kelley D, Flam E, Wick HC, Danilova LV, Easwaran H, Favorov AV, Qian J, Gaykalova DA, Fertig EJ. Epigenetic regulation of gene expression in cancer: techniques, resources and analysis. *Briefings in functional genomics*, 2017 Aug 11; 17(1): 49-63.
  5. Feitelson MA, Arzumanyan A, Kulathinal RJ, Blain SW, Holcombe RF, Mahajna J, Marino M, Martinez-Chantar ML, Nawroth R, Sanchez-Garcia I, Sharma D. Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. *In Seminars in cancer biology*, 2015 Dec 1; 35: S25-S54.
  6. Chopra, R. N., Nayar, S. L. and Chopra, I. C. Glossary of Indian medicinal plants. *Planta Medica*, 1956; 43: 59-63.
  7. Kasinadhuni, V. R. R., Rajashekhar, G., Rajagopalan, R. Sharma, V. M., Vamsi Krishna, C., Sairam, P., Sai Prasad, G., Sadhukhan, S. and Gangadhar Rao, G. Anti-ulcer potential of *Haldinia cordifolia*. *Fitoterapia*, 1999; 70: 93-95.
  8. Rokade, S. and Pawar, S. P. A Comprehensive Review on *Adina cordifolia*. *International Journal of Pharmaceutical Sciences Review and Research*, 2013; 18: 13-16.
  9. Raypa, P., Verma, A. K., Dubey, A. and Tewari, S. K. *In vitro* Establishment of a Threatened Plant Species *Adina Cordifolia*. *Octa Journal of Biosciences*, 2013; 1(2): 138-142.
  10. Vasmatkar, P., Dubey A., Tyagi, B., Baral, P., Tandon, S., Kadam, A. Antibacterial activity and GC-MS analysis of methanolic extract from stem bark and leaves of *mitragyna parvifolia* (roxb.) korth. *Indo American Journal of Pharmaceutical Research*, 2014; 4(1): 304-311.
  11. Kelly GS. Quercetin. *Alternative medicine review*, 2011 Jun 1; 16(2): 172-95.
  12. Umadevi M, Maheswari C, Jothi R, Paleti SK, Reddy YS, Narayanan RV. Hepatoprotective activity of flowers of *madhuca longifolia* (koen.) macbr. against paracetamol-induced hepatotoxicity. *Research Journal of Pharmacy and Technology*, 2011; 4(2): 259-62.
  13. Sunita M, Sarojini P. *Madhuca lonigfolia* (Sapotaceae): A review of its traditional uses and nutritional properties. *International Journal of Humanities and Social Science Invention*, 2013; 2(5): 30-6.