

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.711.345>

Analysis of Medicinally Important Phytochemicals from *Adina cordifolia* Leaves

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ABSTRACT

The present study was aimed to evaluate the bioactive phytochemical constituents of *Adina cordifolia* leaves, with this broad objectives the total phenolic contents (TPC), total flavonoid contents (TFC), total antioxidant potential and GC-MS analysis of leaf extracts were carried out. For the identification of medicinally important compounds in *Adina cordifolia* leaf, extracts were prepared using cold extraction method in different solvents of varying polarities viz., chloroform, ethyl acetate, acetone and methanol. Total phenolic content of plant extracts was analysed using Folin-ciocalteu reagent, total flavonoid content was estimated by using aluminium chloride and total antioxidant activity was estimated by formation of a green phosphate Mo(V) complex at acid pH. Gas Chromatography Mass Spectroscopy was performed to identify phytochemicals present in plant extracts using National Institute of Standards and Technology (NIST) library. The highest total phenolic content and flavonoid were found in acetone extract of *Adina cordifolia* leaf. Total antioxidant activity was highest in methanol extract. A wide range of fatty acids and phytochemicals were also identified having antibacterial, antifungal and anti-inflammatory activities. The study concludes that *Adina cordifolia* have many biologically important compounds, so it can be recommended as a plant of pharmaceutical importance.

Keywords

Adina cordifolia, Phenol,
Flavonoid, trans-
squalene, GC-MS

Article Info

Accepted:

22 October 2018

Available Online:

10 November 2018

Introduction

Several plants contain a variety of phyto-pharmaceuticals with vital applications among the fields of agriculture and medical speciality. Plants have great potential uses as drugs and pharmacopoeil medication as a large proportion of the world population depends on traditional medicines of plant origin due to the inadequate supply and high

prices of conventional modern medicine. These medicinal plants have provided the numerous plants derived therapeutic agents which play a very important role for the development of novel drug leads for the treatment and hindrance of diseases.

Haldu (*Adina cordifolia*), deciduous tree of subfamily *Cinchonoideae*, family *Rubiaceae*, is native of which 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, ascending to an altitude of 900 m in sub-Himalayan tract. *A. cordifolia* is included in threatened species (www.fes.org.in).

A. cordifolia has been used in oriental medicine since ancient times as an essential component of various antiseptic and febrifuge prescriptions (Chopra *et al.*, 1956). The bark is acrid, bitter pungent, tonic, vulnerary and aphrodisiac and is used in biliousness. The roots are used as an astringent in dysentery (Chadha, 1985).

A. cordifolia had been also evaluated for its anti-ulcer potential active constituent showed interesting H⁺/K⁺ ATPase inhibitory activity (Kasinadhuni *et al.*, 1999). Four compounds isolated from the 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-methyl ether-4'-O-a-rhamnopyranoside on the basis of spectral and chemical evidence (Rokade and Pawar 2013).

In vitro propagation through apical buds is the best possible means for *in situ* conservation of *Adina cordifolia*, a threatened species, to produce a large numbers of plants in a short span of time. *Adina cordifolia* was very well established *in vitro* conditions in presence of MS medium supplemented 2mg/L BAP or 0.5mg/L NAA alone (Raypa *et al.*, 2013).

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 (Vasmatkar *et al.*, 2014).

Materials and Methods

Materials

The leaves of *Adina cordifolia* were collected from Agro-forestry Research Centre (AFRC) Haldi (29. 06441° N, 79.82281° E) G. B. Pant University of Agriculture and Technology, Pantnagar in the month of March.

Experimental work

Preparation of plant material

Leaves were washed thoroughly and shade dried for one week.

Then dried leaves were grounded to make powder and stored in air tight container.

Preparation of samples

Plant extracts were prepared in four different solvents viz., chloroform, ethyl acetate, acetone and methanol. Powdered plant material was used for respective solvent (1:4 w:v) extraction. The extracts were filtered, evaporated and dried then respective extracts were used for further experimentation.

Analysis of total phenol content

The Folin-Ciocalteu Method (McDonald *et al.*, 2001) with minor modifications was used to determine the total phenolic contents of extracts using catechol as standard. The results were then expressed as g of catechol per 100 g of sample in dried weight (DW).

Analysis of total flavonoid content

The total flavanoid content was determined by the method of Mandal *et al.*, (2009) using catechin as a standard. The total flavonoid contents were expressed as catechin equivalents in gram per 100g dried sample.

Determination of total antioxidant activity in *A. cordifolia* leaf extracts

The assay was performed according method described by Oueslati *et al.*, 2012. The assay was based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate Mo(V) complex at acid pH. The total antioxidant activity was expressed as the number of equivalence of ascorbic acid.

Statistical analysis

All the analyses were performed in triplicates and results were reported as means \pm standard deviation (SD). The significance of differences among treatment means were determined by one way analysis of variance (ANOVA) with a significant level of ($p < 0.05$). One-way analysis of variance (ANOVA) was carried out using SPSS 16.

Gas Chromatography based mass spectroscopic analysis of *A. cordifolia* extracts

Interpretation of mass spectrum

The GC-MS analysis was carried out by using GCMS-QP2010 Plus with following experimental conditions: Initial temperature 60 °C with RAMP of 3 °C/min, final temperature 210 °C, final hold time 10 min, carrier gas He, flow rate 1 mL/min. Column, silica DB-5, capillary (30nm X 0.25mm ID X 0.25 μ mdf). MS were recorded under electron ionization (EI) condition (70eV) with split mode of 40:1. The compounds were identified by matching their mass spectra with those recorded in NISTMS Wiley Library.

Results and Discussion

The Phenolic compounds contribute to the antioxidant potential of plants by neutralizing

free radicals and preventing decomposition of hydroperoxides into free radicals. Hence, it is important to quantify phenolic derivatives and to assess its contribution to antioxidant activity. Plant extracts were prepared in four different solvents viz., chloroform, ethyl acetate, acetone and methanol having polarity index 2.7, 4.4, 5.1 and 5.1 respectively. Total Phenolic content was expressed as g catechol equivalents/100g dry weight. The total phenolic contents of *Adina cordifolia* extracts ranged from 1.86 to 8.47g/100g in chloroform and acetone extracts respectively (Table 1). Highest total phenolic content was found in acetonic extract of leaf. The content is significantly higher than the phenolic contents of chloroform and ethyl acetate solvents used. Significant differences were recorded in the total phenolic contents of chloroform and ethyl acetate except methanol. Phenol was highest in acetone and lowest in chloroform due to polarity index of solvents. Kumari *et al.*, (2017) reported the total phenolic content in *Adina cordifolia* leaves (determined as gallic acid equivalents or GAE) in ethyl acetate fraction 29.82 ± 2.51 mg GAE/g and in methanolic extract 45.32 ± 2.67 mg GAE/g.

Total flavonoid content was expressed as g catechin equivalents/100g dry weight. The total flavonoid content of *Adina cordifolia* was recorded ranging from 2.21g/100g to 9.23g/100g in chloroform and acetone extracts respectively. The acetone extract exhibited a total flavonoid content that is significantly higher than chloroform and methanol extracts. The flavonoid contents of the rest of the solvents are also significantly different from each other except for ethyl acetate extract whose flavonoid contents are not significantly different. Flavonoid was highest in methanol and lowest in chloroform due to polarity index of solvents. Kumari *et al.*, (2017) reported total flavonoids content in methanolic extract (39.94 ± 3.02 mg Rutin/g). The difference in amounts of phenols and flavonoids are

probably related to geographical and environmental factors, processing methods which may play role in such a large variation.

Total antioxidant potential expressed as ascorbic acid equivalents (AAE) g/100g and in methanol extract of *Adina cordifolia* leaf had highest total anti-oxidant activity i.e. 36.08 ± 0.33 g/100g DW (Table 1). The total antioxidant potential of *Adina cordifolia* were recorded ranging from 23.28 g/100g to 36.08 g/100gm. Significant differences were recorded in all the extracts.

The dependency of antioxidant activity obtained through assay, in relationship to the Total Phenolic Content, was also evaluated. Although, there is a positive linear correlation ($r=0.9221$) among the total antioxidant activity and TPC assay for $R^2 = 0.8504$. The results indicated that the phenolic compounds in the different extracts of leaf could be the main contributor to the antioxidant activities.

Identification of phytochemicals by GC-MS

Identification of phytochemicals was based on the principles of molecular weight (MW), retention time (RT), molecular formula (MF) and concentration (peak area%).

It was done in order to determine some compounds present in plants having any medicinal value. The Gas chromatography mass spectrum of the sample were interpreted using the database of National Institute Standard and Technology (NIST) having more than 2,00,000 patterns. For identification of any unknown compound, its spectrum is compared to spectrums database stored in NIST-11 library for similarity.

A total of 66 constituents, contributing 61.74% of the chloroform extract, 80.42% of the ethyl acetate extract, 60.88% of the

acetonic extract and 45.59% of the methanolic extract were identified. Pursual of Table 2 indicates the names and respective percentage of identified constituents while figure 1 represents the major compounds.

All the extracts were different in their qualitative and quantitative make-up of major and minor constituents.

In present communication the dominating constituents in the leaves extracts of Haldu were trans squalene (15.4-42.1%), vitamin E (2.9-5.8%), phytol (1.1-9.42%), and neophytadiene (2.0-2.4%) in respective extracts.

Trans squalene and vitamin E possess antioxidant power.

The compounds identified in leaf *A. cordifolia* chloroform extracts are tetradecanal (0.93%), Neophytadiene (2.46%), Trans-squalene (42.13%), Phytol isomer (2%), Vitamin E (4.22%), Ergost-5-en-3-ol (3.38%), Campesterol (1.71%), Naphthalene (3.48 %). Trans-squalene (15.42 %), Neophytadiene (2.05 %), Hexadecanoic acid methyl ester (1.10%), Phytol isomer (9.42%), Tetradecanal (1.09%), Vitamin E (5.84%), Gamma-sitosterol (4.11%) were reported in *A. cordifolia*'s ethyl acetate extract.

Trans-squalene (27.44%), Tetradecanal (0.68%), Neophytadiene (2.09%), Trimethylsilyl palmitate (3.36%), Phytol isomer (1.18%), Vitamin E (2.99%), Campesterol (1.05%), Phenol (7.33%), Naphthalene (3.77%) were reported in *A. cordifolia*'s acetonic extract whereas phenol (1.14%), naphthalene (1.16%), Epiglobulol (3.23%), caryophyllenoxide (4.14%), loliolide (1.32%), pentyl octanoate (3.44%), behenyl behenate (6.53%) were identified in leaf *A. cordifolia* methanolic extract.

Table.1 Total phenolic content, total flavonoid content and total antioxidant activity in leaves of *Adina cordifolia* in different solvent extracts

Leaf Extracts	Total Phenolic content (g CalE/ 100gdw)	Total flavonoid content (g CE/ 100gdw)	Total antioxidant Potential (g/100gm)
Chloroform	1.86 ± 0.43 ^a	2.21 ± 0.46 ^a	23.28 ± 0.63 ^a
Ethyl acetate	3.51 ± 0.33 ^b	8.38 ± 0.89 ^c	28.92 ± 0.74 ^b
Acetone	8.47 ± 0.61 ^c	9.23 ± 0.91 ^c	33.22 ± 0.32 ^c
Methanol	7.54 ± 0.39 ^c	4.96 ± 0.27 ^b	36.08 ± 0.33 ^d

CalE= Catechol equivalent CE = Catechin equivalent Each value is expressed as mean ± S.D (Standard Deviation) (n = 3).

Table.2 Some phyto compound and their important uses

S. N.	Name of compound	Activity	References
1.	3,5-di-tert-butylphenol	Antifungal activity	Rathna, <i>et al.</i> , 2016
2.	Trans-chrysanthemic acid	Important component of pyrethrins (Natural pesticide)	Xu H. <i>et al.</i> , 2018
3.	Loliolide	Astringent, antipyretic, anti-inflammatory and vasodilatory effects	Fujita <i>et al.</i> , 1972
4.	Neophytadiene	Antipyretic, Analgesic, And Anti-Inflammatory, Antimicrobial	Duke and Beckstrom-Sternberg (1994)
5.	Linolenic acid	Important in prevention of coronary heart disease	Lorgeril <i>et al.</i> , 2001
6.	N-hexadecanoic acid	Anti-inflammatory property	Aparna <i>et al.</i> , 2012
7.	Vitamin E	Antiageing, Analgesic, Antidiabetic, Antiinflammatory, Antioxidant	Duke and Beckstrom-Sternberg (1994)
8.	Trans-squalene	Neutralize xenobiotics, anti-inflammatory, anti-atherosclerotic and anti-neoplastic, role in skin aging and Adjuvant activities	Duke and Beckstrom-Sternberg (1994)
9.	Ergost-5-en-3-ol	Anti-inflammatory, Analgesic and Antipyretic property	Kantheil <i>et al.</i> , 2014
10.	Campesterol tms	Antimicrobial Anti-inflammatory Anticancer Antiarthritic Antiasthma Diuretic	Duke and Beckstrom-Sternberg (1994)
11.	Gamma-sitosterol	Anti-diabetic, Anti-angeogenic, anticancer, antimicrobial, anti-inflammatory, antidiarrhoeal and antiviral	Duke and Beckstrom-Sternberg (1994))
12.	9-octadecenoic acid, methyl ester	Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge	Duke and Beckstrom-Sternberg (1994)
13.	Beta-sitosterol trimethyl silyl ether	Antimicrobial Anti-inflammatory Anticancer Antiarthritic Antiasthma Diuretic	Duke and Beckstrom-Sternberg (1994)

Fig.1 *Adina cordifolia*'s leaf total phenolic and flavonoid content in different solvent extracts

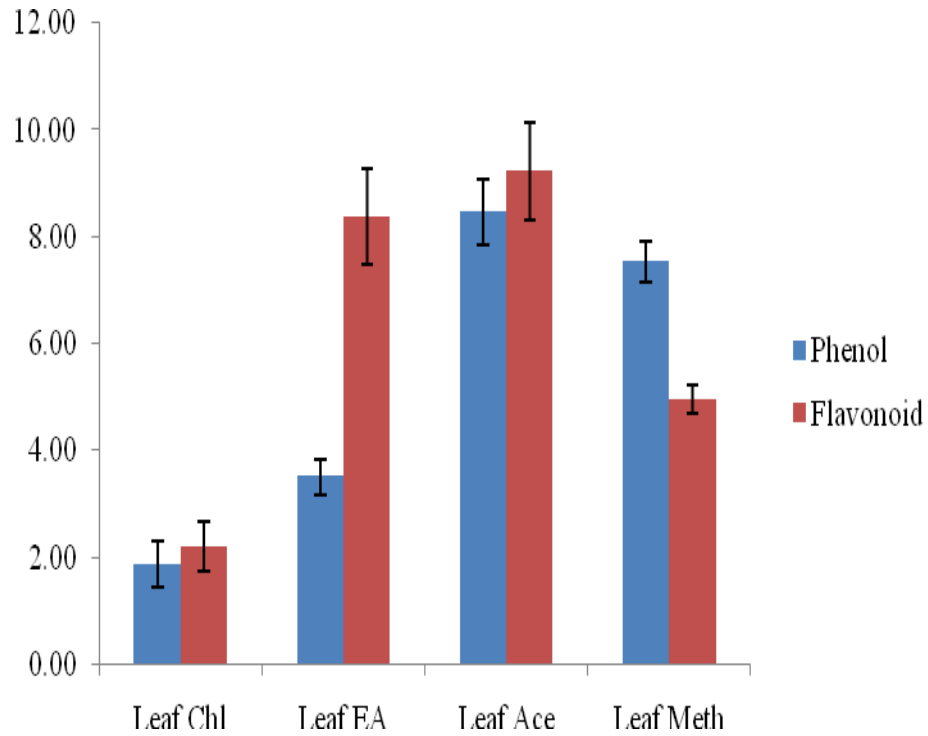


Fig.2 *Adina cordifolia*'s leaf total total antioxidant activity in different solvent extracts

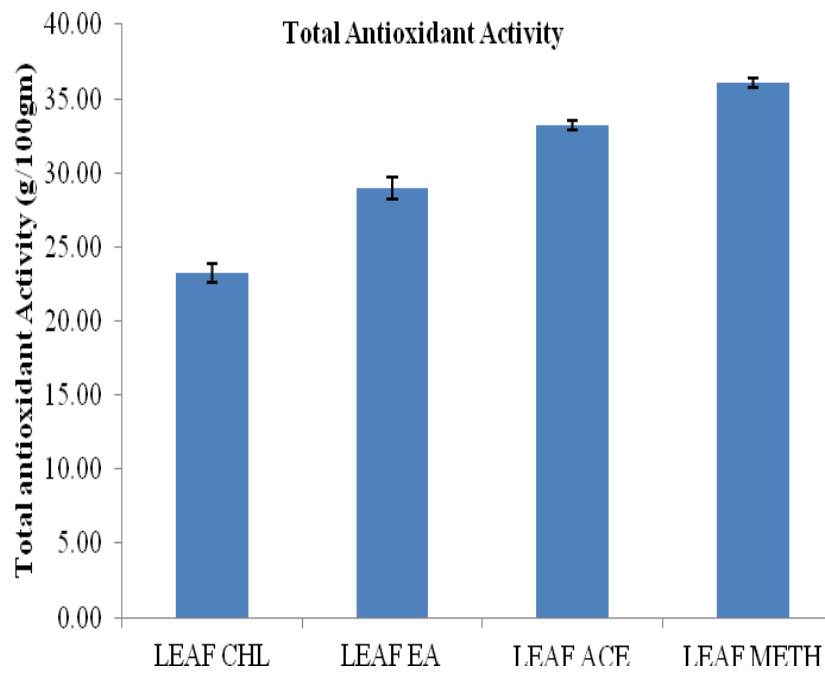


Fig.3 GC MS analysis chromatogram for *Adina cordifolia*'s leaf Chloroform extract

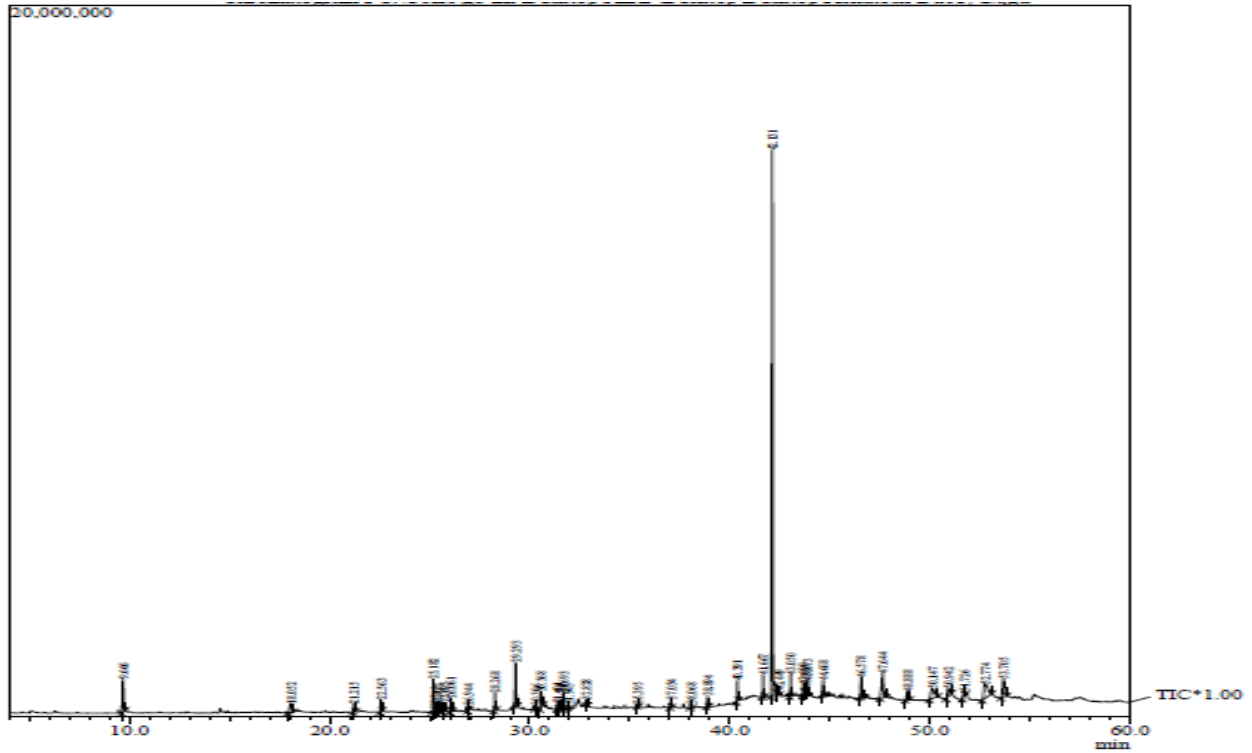


Fig.4 GC MS analysis chromatogram for *Adina cordifolia*'s leaf Ethyl acetate extract

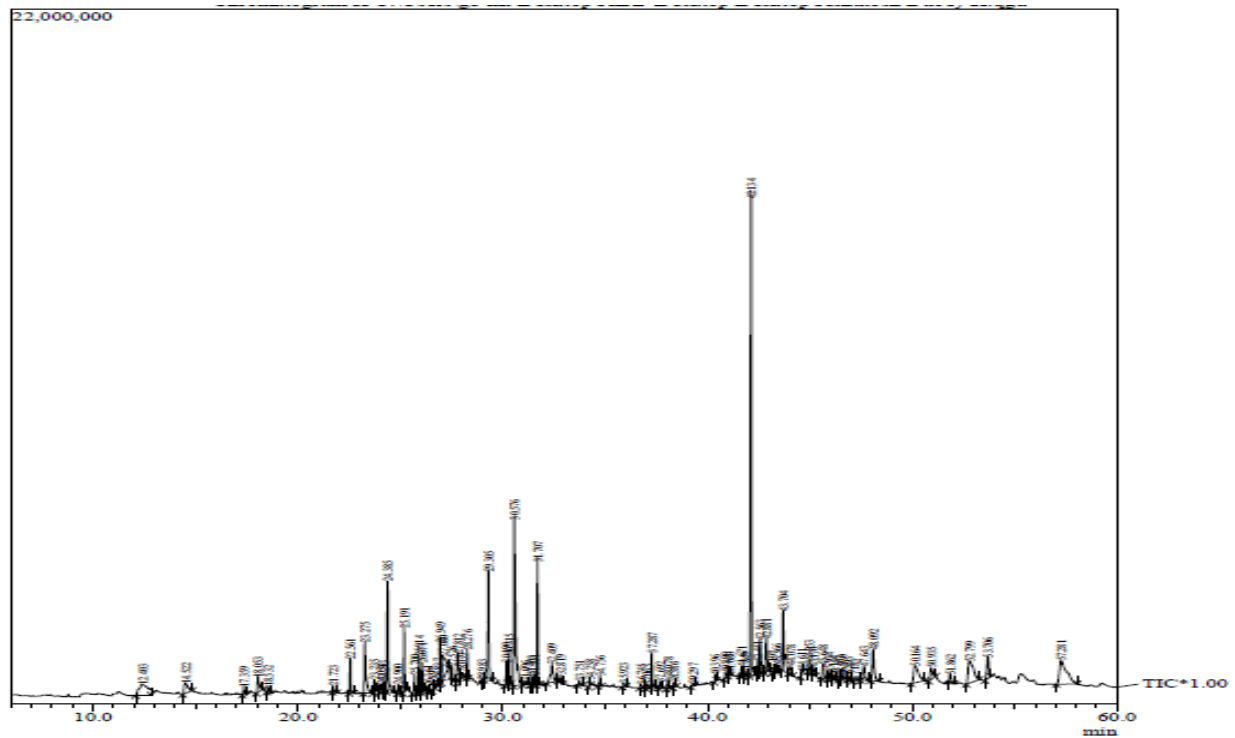


Fig.5 GC-MS analysis chromatogram for *Adina cordifolia*'s leaf Acetonic extract

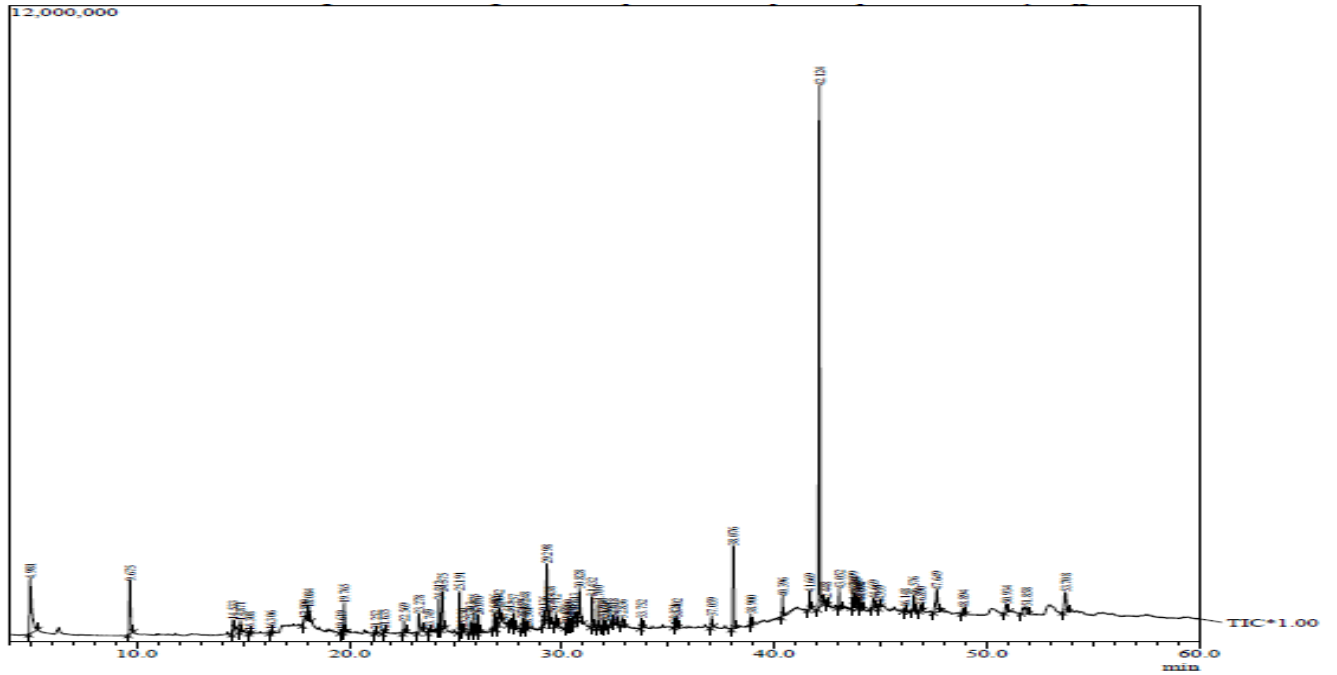
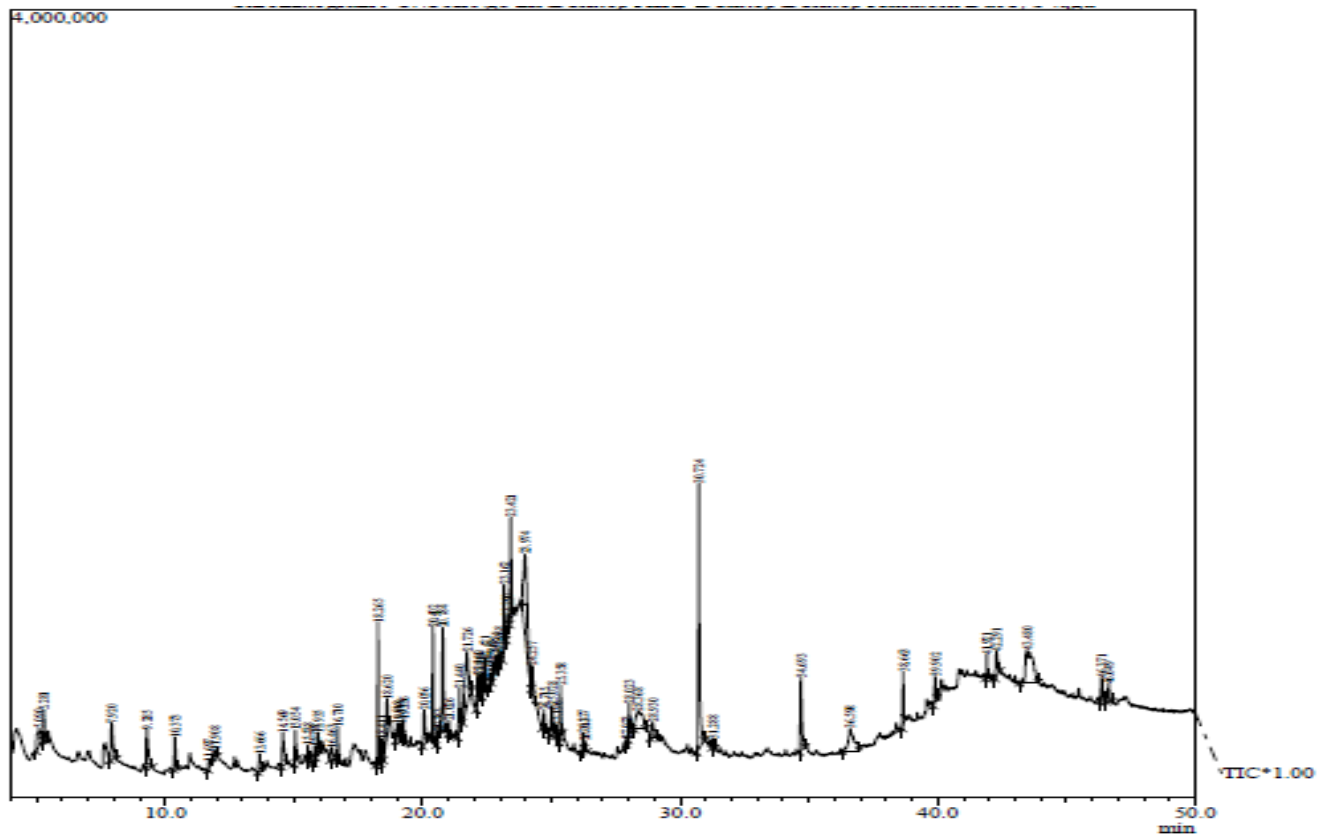


Fig.6 GC-MS analysis chromatogram for *Adina cordifolia*'s leaf methanolic extract



Comparative analysis of *Adina cordifolia*'s Leaves Metabolites

Compound	Molecular Formula	Nature of Compound	% Contribution in Chloroform Extract	% Contribution in Ethyl acetate extract	% Contribution in Acetonic extract	% Contribution in Methanol extract
Methyl 4-ethylbenzoate	C ₁₀ H ₁₂ O ₂	Monoterpenoid		1.33		
3,5-di-tert-butylphenol	C ₁₄ H ₂₂ O	Phenol		1.09	0.82	
Trans-chrysanthemic acid	C ₁₀ H ₁₆ O ₂	Monoterpenoid		0.33		
Tetradecanal	C ₁₄ H ₂₈ O	Aldehyde	0.93	1.09	0.68	
P-menth-3-en-9-ol	C ₁₀ H ₁₈ O	Monoterpenoid		0.49		
Loliolide	C ₁₁ H ₁₆ O ₃	Carotenoid		0.34		1.32
2,5,5,8a-tetramethyl-6,7,8,8a-tetrahydro-5h-chromen-3-one	C ₁₃ H ₂₀ O ₂	Ketone		4.51		
Neophytadiene	C ₂₀ H ₃₈	Diterpenoid	2.46	2.05	2.09	
2-methyl-octadecyne	C ₁₉ H ₃₆	Hydrocarbon		0.66		
Dodecanal dimethylacetal	C ₁₄ H ₃₀ O ₂	Aldehyde		1.10		
3,7,11,15-tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	Diterpenoid		1.66		
Linolenic acid	C ₁₈ H ₃₀ O ₂	Fatty acid		0.18		
Citronellol acetate	C ₁₂ H ₂₂ O ₂	Hydrocarbon		0.39		
Hexadecanoic acid methyl ester	C ₁₇ H ₃₄ O ₂	Ester	0.32	1.10		
Dimethyl{bis[(2z)-pent-2-en-1-yloxy]}silane	C ₁₂ H ₂₄ O ₂ Si	Hydrocarbon		2.96		
N-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	Fatty acid		1.38		
2-hydroxyisocaproic acid, trimethylsilyl ester	C ₉ H ₂₀ O ₃ Si	Ester		0.37		
Ethyl pentadecanoate	C ₁₇ H ₃₄ O ₂	Hydrocarbon		0.74		
Trimethylsilyl palmitate	C ₁₉ H ₄₀ O ₂ Si	Hydrocarbon		4.60	3.36	
Linoleic acid, methyl ester	C ₁₉ H ₃₄ O ₂	Ester		0.65		
Phytol isomer	C ₂₀ H ₄₀ O	Diterpenoid	2.00	9.42	1.18	
N-propyl 9,12-octadecadienoate	C ₂₁ H ₃₈ O ₂	Hydrocarbon		0.28		
Phytol, trimethylsilyl ether	C ₂₃ H ₄₈ OSi	Hydrocarbon	1.11	3.35		
N-octadecyl isocyanate	C ₁₉ H ₃₇ NO	Hydrocarbon		1.35		
Hexadecanal	C ₁₆ H ₃₂ O	Aldehyde		0.27		
Isooctyl phthalate	C ₂₄ H ₃₈ O ₄	Hydrocarbon		0.40		
Tetradecanal	C ₁₄ H ₂₈ O	Aldehyde		0.25		
Nonadecane	C ₁₉ H ₄₀	Hydrocarbon		0.29		
Trans-squalene	C ₃₀ H ₅₀	Triterpene	42.13	15.42	27.44	
Solanesol	C ₄₅ H ₇₄ O	Alcohols		1.75		
Geranyl linalool isomer	C ₂₀ H ₃₄ O	Diterpenoid		0.56		

Neryl linalool isomer	C ₂₀ H ₃₄ O	Diterpenoid		0.38		
Beta.-tocopherol	C ₂₈ H ₄₈ O ₂	Phenols		0.80		
Gamma.-tocopherol	C ₂₈ H ₄₈ O ₂	Phenols		0.36		
1-bromotetracosane	C ₂₄ H ₄₉ Br	Hydrocarbon		0.23		
Vitamin E	C ₂₉ H ₅₀ O ₂	Phenols	4.22	5.84	2.99	
Methoprene	C ₁₉ H ₃₄ O ₃	Hydrocarbon		2.25		
Ergost-5-en-3-ol	C ₂₈ H ₄₈ O	Sterols	3.38	2.79		
Campesterol tms	C ₃₁ H ₅₆ OSi	Sterols	1.71	0.99	1.05	
Gamma.-sitosterol	C ₂₉ H ₅₀ O	Sterols		4.11		
Beta.-sitosterol trimethylsilyl ether	C ₃₂ H ₅₈ OSi	Sterols		1.58		
Phenol	C ₆ H ₆ O	Phenol			7.33	1.14
Naphthalene	C ₁₀ H ₈	Monoterpenoid	3.48		3.77	1.16
4-trimethylsiloxybenzaldehyde	C ₁₀ H ₁₄ O ₂ Si	Monoterpenoid			1.02	
9-octadecene	C ₁₈ H ₃₆	Hydrocarbon			0.57	
9-eicosene	C ₂₀ H ₄₀	Diterpenoid			1.44	
Dodecylcyclohexane	C ₁₈ H ₃₆	Hydrocarbon			0.20	
Alpha-octadecene	C ₁₈ H ₃₆	Hydrocarbon			1.47	
Phthalic acid, butyl octyl ester	C ₂₀ H ₃₀ O ₄	Diterpenoid			1.01	
Palmitic acid, methyl ester	C ₁₇ H ₃₄ O ₂	Fatty acid			0.39	
Alpha.-d-xylofuranose, 1,2-o-isopropylidene-5-(t-butyl)dimethylsilyl)-	C ₁₄ H ₂₈ O ₅ Si	Carbohydrate			1.53	
1-heneicosanol	C ₂₁ H ₄₄ O	Alcohols			0.74	
Tetratriacontane	C ₃₄ H ₇₀	Hydrocarbon			0.13	
Trimethylsilyl 3-phenoxypropanoate	C ₁₂ H ₁₈ O ₃ Si	Ester			1.01	
2,4'-bis(trimethylsilyloxy)diphenylmethane	C ₁₉ H ₂₈ O ₂ Si ₂	Hydrocarbon			0.66	
Ethyl 9,12-hexadecadienoate	C ₁₈ H ₃₂ O ₂	Ester		0.23		
9-octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	Ester		0.33		
Methyl ricinoleate	C ₁₉ H ₃₆ O ₃	Fatty acid		0.17		3.12
Behenyl behenate	C ₄₄ H ₈₈ O ₂	Ester				6.53
mome inositol	C ₇ H ₁₄ O ₆	Alcohol				8.18
Pentyl octanoate	C ₁₃ H ₂₆ O ₂	Ester				3.44
13-Hexyloxacyclotridec-10-en-2-one	C ₁₈ H ₃₂ O ₂	Ketone				9.81
Epiglobulol	C ₁₅ H ₂₆ O	Sesquiterpene				3.23
Caryophyllenoxid	C ₁₅ H ₂₄ O	Sesquiterpene				4.14
Pentyl octanoate	C ₁₃ H ₂₆ O ₂	Ester				3.44

The compounds 3,5 ditert-butylphenol, trans-chrysanthemic acid, loliolide, neophytadine, Hexadecanoic acid, Vitamin E, Trans-squalene, Ergost-5-en-3-ol, Campesterol, Gamma.-sitosterol, 9- octadecanoic acid methyl ester, beta sitosterol trimethyl silyl ether etc. identified in present study have been reported to used as starting material for the synthesis of industrially important component of natural pesticide (Xu *et al.*, 2018), possess antifungal activity, astringent power, antipyretic property, inflammatory effect, analgesic effect, vasodilatory effect, antimicrobial property, antiageing and antidiabetic property, antioxidant activity, adjuvant activities, antiarthritic, antidiarrhoeal and antiviral effect (Rathna *et al.*, 2016; Fujita *et al.*, 1972; Lorgeril *et al.*, 2001; Aparna *et al.*, 2012; Kanthal *et al.*, 2014; Duke and Beckstrom-Sternberg, 1994).

Based on these findings it can be concluded that the plant may be good natural source for many industrially important phytoconstituents. The plant can also be a source of herbal medicine and natural antioxidant.

The results of present study indicated considerable amount of total phenolic content and total flavonoid content. The highest total phenolic and flavonoid content were recorded in leaf acetone extract. According to Verma *et al.*, (2010) flavonoids and alkaloids seem to be most likely compounds eliciting *in vitro* cytotoxicity effect. The phenolic compounds are reported to show as scavengers of Reactive Oxygen Species (ROS), antioxidant and anti-inflammatory activities (Sivanandham, 2011). The flavonoids are also medicinally important and exhibit analgesic, anti-inflammatory, antioxidant, anti-arthritis and immunomodulatory properties (Gill *et al.*, 2011). Total antioxidant potential was found highest in methanol extract of leaf *A. cordifolia* due to some compounds having

antioxidant property such as loliolide, methyl ricinoleate.

In this study different extracts of *Adina cordifolia* leaves were analyzed for the presence of active bioactive compounds by GC-MS analysis with their spectrum, retention time, molecular weight and similarity index. The mass spectrum of each compound was compared with NIST-11 database and gave more than 90% match resulting in confirmatory compound match. Major component found in the extracts is squalene, isoprenoid compound that possesses antioxidant activities and widely produced in plants. Squalene protects cells against radicals, strengthens the immune system and decreases the risk of various cancers (Kalinova *et al.*, 2006). Squalene is not very susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen, protecting human skin surface from lipid peroxidation due to exposure to UV and other sources of ionizing radiation.

Chloroform is best solvent for extraction of squalene followed by acetone and ethyl acetate while methanol is not capable of extracting squalene. Table 2 contains important phytochemicals found in *Adina cordifolia* leaf and their biological activity. The activity of compound was identified from Dr. Duke's Phytochemical and Ethnobotanical database (Duke and Beckstrom-Sternberg, 1994).

Leaf juice of *Adina cordifolia* is used to treat boils and eye disorders like conjunctivitis (Hossan 2009). There are no reports available on the identification of biologically important compounds from *Adina cordifolia*. In this study, chloroform, ethyl acetate, acetonetic and methanolic extract of *Adina cordifolia* leaf were quantitatively analyzed for total phenolic and flavonoid content and total antioxidant activity and then active bioactive

compounds of plants were evaluated by GC-MS analysis. The important compounds identified by GC-MS belong to diterpene, triterpene and fatty acids. These identified phytochemicals are assumed to be responsible for eliciting the traditional medicinal activity of *A. cordifolia*. The present study is significant because there is less literature available on *Adina cordifolia* phytochemical analysis.

Acknowledgement

Authors are grateful to Dept. of Biochemistry, GBPUA&T, Pantnagar to carry out this research work. Our sincere thanks to DST-FIST for providing equipment facility and Directorate of Experiment Station (DES) Pantnagar for utilization of infrastructure. Pratima acknowledge UGC for fellowship.

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How to cite this article:

Pratima Raypa, A.K. Verma, Salil Tewari and Ashutosh Dubey. 2018. Analysis of Medicinally Important Phytocompounds from *Adina cordifolia* Leaves. *Int.J.Curr.Microbiol.App.Sci.* 7(11): 3007-3019. doi: <https://doi.org/10.20546/ijcmas.2018.711.345>