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Phytochemical Study of Methanolic Extract of Indian Bhant Tree, *Clerodendrum infortunatum*

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ABSTRACT

Plants are the reservoirs of many secondary metabolites and other phytochemical constituents. Many of these compound have their own particular properties. Bioefficacy of these compound can be used to develop suitable drugs, fungicide, insecticide, vermicide etc. *Clerodendrum infortunatum* is traditional medicinal plant used for the treatment of various diseases. The present study focussed on the phytochemical characterization of leaves the plant. The air dried leaves of *C. infortunatum* was used to prepare fine powders. From this, the extract was collected using methanol as solvent. After this, preliminary phytochemical tests were conducted and found that the presence some active compounds such as terpenoids, flavonoids, alkaloids, sterols etc..So further studies were carried out through GC-MS chromatography. It showed the presence of several compounds having different activity. Identification of these compounds should provide new possibilities in pharmaceutical and pest management systems.

KEYWORDS; Clerodendrum infortunatum, GC-MS, terpenes, secondary metabolites, flavonoids

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INTRODUCTION

The ethno-botanical importance of *Clerodendrum infortunatum*(Indian Bhant tree) is due to its usage as traditional medicine to cure common illness such as bronchitis, asthma, fever, diseases of blood, inflammation, burning sensation, tuberculosis, hepatic diseases etc. The people of North-East India especially Kuki and Rongmai Naga tribes use orally plant leaves extract of this for fever and bowel troubles. For removing Ascarides, fresh leaf juice is used. Like that, leaves and flowers are used to cure scorpion sting (Das *etal*., 2014). Ramba, Rajbanshi, Polia and Lepcha tribes of North Bengal use fresh root bark of *C. infortunatum* to cure diarrahoea. Kachari, Hmar and Riang tribes of Baragk Valley and North- Cachar Hills use leaf extract in stomach pain and in diabetes (Baid, 2013). The root paste is also used as a bandage in swelling (Barbhiliya and Sharma, 2009). Fresh juice of the leaves has been used as vermifuge and in the treatment of malaria (Chopra *etal.*, 1992).

Phytochemical aspects including, leaves of the plant were reported to contain saponin, alkyl sterols, some enzymes (Khatri, 2005), and 2,-(3, 4-dehydroxyphenyl) ethanol- 1-O-α-2 rhamnopyranosyl (1→3)-β-D-(4-O-caffeoyl) glycolpyranoside (acetoside) (Prajapati*etal*., 2000; Kapoor, 2001). It was also found that the leaves contain a fixed oil which consists of glycerides of lenoleic, oleic, stearic and lignoceric acid (Kapoor, 2001). The chemical compound isolated from the roots are Luperol, β- sitosterol, the sterol known as Clerosterol identified as 5, 25-sigmastadien_3β-ol, Clerodolone as lup_20 (30)-en- 3β-diol-12-one and clerodone as 3β-hydroxylupan- 12-one and a steroidal glycoside. It is also reported the isolation of three compounds identified as clerodin, 15-methoxy-14, 15-dihydroclerodin and 15-hydroxy-14, 15-dihydroclerodin from this plant (Abbaszadeh *etal*., 2014). Besides the above major chemicals found in the *Clerodendrum* genus the other constituents are carbohydrates, phenolic, flavonoids, terpenoids, sugars and steroids (Sharma *etal*., 2008).

Methanolic extract of the dried powdered leaves of *C. infortunatum* Linn. (MECI) showed DPPHradical scavenging activity, nitric oxide scavenging activity,superoxide anion scavenging assay, hydroxy radical scavenging activity. Oleanolic acid and clerodinin A found after HPLC analysis ofthe Methanolic extract of leaves of *C. infortunatum* Linn. shows anticancer activity against Ehrlich's ascitescarcinoma (EAC) bearing Swiss albino mice. The anticancer effects of the plant extract were thought to bedue to the suppression of lipid peroxidation and increase in thecontent of the enzymatic defence system (Sannigrahi *etal*., 2012). Itisreported that isolation of three compounds from *C. infortunatum* Linn. extracts which are identified as clerodin (CD), 15-methoxy-14, 15-dihydroclerodin (MDHC) and 15-hydroxy-14, 15-dihydroclerodin (HDHC). Compounds CD and MDHC showssignificantly higher antifeedant activity compared to the keyingredient in many commercial pesticides, azadirachtin, at itshighest concentration. The test was

performed on a highlypolyphagous pest, the cotton bollworm, *Helicoverpa armigera* (Abbaszadeh *etal*., 2014). Also, methanolic extracts of *C. infortunatum* Linn. Showsnoo tropic potential (memory enhancing effects) on adult Swiss albino wistar mice at higher dose (200 mg/kg) of the plant extract.

In this review, different activities of *C. infortunatum* Linn. Wasree valuated for their respective pharmacological activities with current research chapter. Apart from this most of the studies on *C. infortunatum* was conducted in North East part of India and least in South especially the plants collected from South Kerala. Because it is traditionally used from past decades by different tribes of North-East India, North Bengal etc. to treat various common disorders and current traumas like scorpion sting, snake bite etc. Such activities can be established by experimental procedures. Human clinical trials may be performed on the existing pharmacological activities of this plant to establish this plant as medicinal drug. Chemical constituents obtained from different parts and their medicinal uses have been established, but many bioactive constituents and pure compounds have so far been neglected by phytochemists and pharmacologists and a large amount of work has been done only on extracts and not on the isolated fractions. Also, identification and isolation of new compound should be helpful in agricultural sector for introducing new strategies of pest control which promote yield and environmental protection. With this point of view the present chapter aims at focusing on the unexplored and untouched areas related with *Clerodendrum infortunatum* Linn.

MATERIALS AND METHODS

Plant collection and preparation of extract

The fresh leaves of C. Infortunatum was collected loacally. This can allowed for air drying on 7 days and made as fine powder using electric motor. The fine powder is used for extract preparation using methanol as solvent in soxhlet extraction apparatus. Then solid extract is prepared by evaporating the solvent and kept for future use.

Pliliminary qualitative assay of methanol leaf extract of C. infortunatum

1. *Test for Sterols:*

Salkowski test: 2 ml of extract was mixed with 2 ml of chloroform and 2 ml of concentrated H2SO4 was added carefully and shaken well. The chloroform layer appeared red and acid layer fluorescent greenish yellow. This strongly supports the presence of sterols from the extract.

2. *Test for Terpinoids:*

Salkowski test: 2 ml of extract was mixed with 2ml chloroform and 3 ml concentrated H2SO4 was added carefully and shaken well. Positive result for the presence of terpinoides was noted by the appearance of reddish brown colour at interphase.

3. *Test for Alkaloids Mayer's test:*

Extract was warmed with 2% H2SO4 for two minutes, filtered and few drops of Mayer's reagents were added. White coloured precipitation appeared giving a positive result.

4. *Test for flavonoides*:

Ammonium Test: A small quantity of the extract heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture filtered and the filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. Waited for colouration in Ammonia layer. Yellow colouration at ammonia layer was observed which indicates the presence of flavonoid from the extract.

5. *Test for Carbohydrate*:

Fehling Test: Equal quantity of Fehling solution A and Fehling solution B are mixed and few drops of extract was added and boiled. Brick red precipitate of cuprous oxide confirms the presence of carbohydrate.

6. *Test for Phenols:*

Ellagic Acid Test: The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) NaNO2 solution. Niger brown precipitate occur and the solution turned muddy. Hence the presence of Phenols were observed from the extract.

7. *Test for Glycosides:*

Concentrate H2SO4 Test: In 5ml extract, 2ml glacial acetic acid, one drop of 5% FeCl3 and conc. H2SO4 was added. The brown ring colouration in interphase marks the presence of Glycosides.

8. *Test for Protein:*

Biuret Test: 2ml of Biuret reagent was added to 2ml of extract. The mixture was shaken well and warmed on water bath. Presence of protein was noted as violet colouration was observed.

9. Test for Saponin:

Foam Test: The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A thin foam layer was observed. This indicates that Saponin is weakly present in the extract.

GC- MS Analysis

Methanolic extract of *C. infortunatum* leaf was passed through anhydrous Na2SO4 and activated charcoal (2:1; w/w) to remove any trace of moisture and colour. The samples were analysed using Thermo Scientific Trace 1300 gas chromatography instrument attached with Thermo Scientific ISQ QD single quadrupole mass spectrophotometer. The GC was equipped with TG-5MS column (30 m × 0.25 mm × 0.25 μm). The inlet temperature was maintained at 250°C. The initial temperature was set at 60°C (solvent delay 5 min) with a hold of 2 min, followed by a ramp of 5°C to 290°C with a hold of 6 min (54 min programme). Samples were (1 μl) injected in a splitless mode (split flow 50 ml/min) with split less time of 0.80 min, using a Thermo Scientific AI-1310 auto-sampler. The carrier gas was helium, with a constant flow of 1 ml/min. MS transfer line temperature was set at 290°C with an Ion source temperature of 230°C (electron ionization). The individual samples were analysed at electron energy 70 eV (vacuum pressure- 2.21e-0.5 Torr). The mass analyser range was set to 50-650 amu.

GC-MS data analysis

All samples were analysed thrice for confirmation. MS data analysis was performed by Automated Mass Spectral Deconvolution and Identification System (AMDIS) version 2.70. The major and essential compounds were identified by mass fragmentation patterns using the database of National Institute Standard and Technology (NIST) with a MS library version 2011.

RESULTS

Plliminary qualitative assay of methanol leaf extract of *C. infortunatum*

1. *Test for Sterols:*

Salkowski test: The chloroform layer appeared red and acid layer fluorescent greenish yellow. This strongly supports the presence of sterols from the extract.

2. *Test for Terpinoids:*

Salkowski test: Appearance of reddish brown colour at interphase supports the presence of terpenes.

3. *Test for Alkaloids Mayer's test:*

In the presence of Mayer's reagents, white coloured precipitation appeared means that alkalids are present in the extract.

4. *Test for flavonoides:*

Ammonium Test: Yellow colouration at ammonia layer was observed which indicates the presence of flavonoid from the extract.

5. *Test for Carbohydrate:*

Fehling Test: Brick red precipitation indicated the presence of carbohydtrates.

6. *Test for Phenols:*

Ellagic Acid Test: Niger brown precipitate occur and the solution turned muddy. Hence the presence of Phenols were observed from the extract.

7. *Test for Glycosides:*

The brown ring colouration in interphase marks the presence of Glycosides.

8. *Test for Protein:*

Presence of protein was noted as violet colouration was observed.

9. ***Test for Saponin:***

A thin foam layer was observed. This indicates that Saponin is weakly present in the extract.

GC- MS Analysis

Gas chromatography-Mass spectroscopy showed 46 major and minor compounds present in methanolic leaf extract of C. infortunatum. In that Caryophyllene (Area 772872 and retension time, 16.624), 4,7,10,13,16.19-Docosache (Area 452471 and RT 16.624) etc. possess majority and followed by some recognised compounds such as Seychellene, cyclopentane, fluoranthene, benezene compound, copaene, dodecane, benzoic acids etc.

Biocompounds	Present/absent
Sterols	Present
Terpenoids	Present
Alkaloids	Present
Flavonoids	Present
Carbohydrate	Present
Phenol	Present
Glycosides	Present
Protein	Present
Saponins	Present

Table 1:Preliminary qualitative assay of methanol leaf extract of *C. infortunatum*

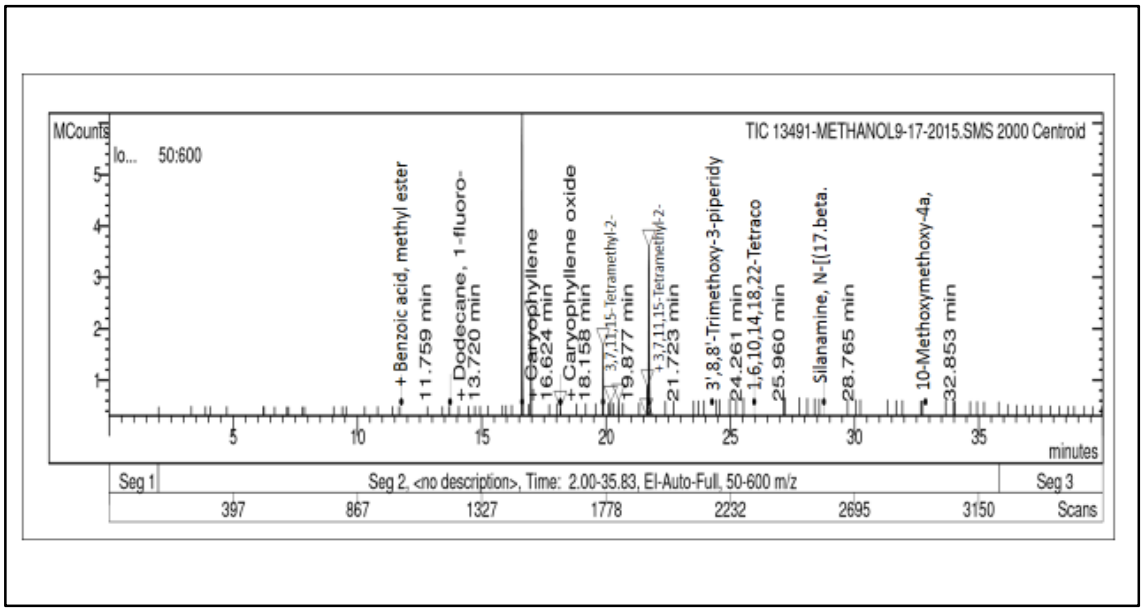

Figure1: Chromatogram shows the compounds present in methanol leaf extract of *C. infortunatum*

No.	Compound	RT	Area
1	Benzoic acid, methyl est	11.759	81316
2	Cyclopentasiloxane, deca	12.416	19969
3	Dodecane, 1-fluoro-	13.720	15005
4	2-(4'-Trimethylsilyloxyp	13.993	3504
5	2H-1,4-Benzodiazepin-2-o	14.122	1879
6	3-Chloro-N,N-dimethyl-6-	14.158	1879
7	Naphthalene, 1,2,3,4-tet	14.637	1561
8	2,5-Diethylphenol	15.554	3517
9	3,5-Diisopropoxy-1,1,1,7	15.919	24834
10	Copaene	16.122	10319
11	Nonadecane, 1-chloro-	16.253	15719
12	4,7,10,13,16,19-Docosahe	16.624	452471
13	Caryophyllene	16.624	772872
14	1,4,7,-Cycloundecatriene	16.978	442767
15	Seychellene	16.986	3755
16	2,6-Difluorobenzoic acid	17.214	28762
17	Phenol, 2,4-bis(1,1-dime	17.351	2607
18	Naphthalene, 1,2,3,5,6,8	17.508	6597
19	Methyl eicosa-5,8,11,14,	17.879	9080
20	Tetracontane, 3,5,24-tri	18.075	11529
21	Caryophyllene oxide	18.158	44131

22	3-Buten-2-ol, 4-(2,6,6-t	18.385	14943
23	Benzene, (1-propyloctyl)	18.475	17642
24	9-Desoxo-9-x-acetoxy-3-d	18.605	3608
25	Methyl eicosa-5,8,11,14,	18.857	14211
26	Benzene, (1-methyldecyl)	18.967	33986
27	Benzene, (1-propylnonyl	19.257	13303
28	Cyclopenta[1,3]cycloprop	19.386	5741
29	Tetracontane, 3,5,24-tri	19.610	7193
30	Ethane, 1-(9-borabicyclo	19.734	28165
31	3,7,11,15-Tetramethyl-2-	19.877	213811
32	3,7,11,15-Tetramethyl-2-	20.049	22159
33	3,7,11,15-Tetramethyl-2-	20.181	85539
34	Pentadecanoic acid, 14-m	20.500	101725
35	Estra-1,3,5(10)-trien-17	20.784	6133
38	9,12,15-Octadecatrienoic	21.662	146034
39	3,7,11,15-Tetramethyl-2	21.723	634249
40	Fluoranthene	22.867	16245
41	3',8,8'-Trimethoxy-3-pip	24.261	11769
42	1,6,10,14,18,22-Tetracos	25.960	44288
44	Silanamine, N-[(17.beta	28.765	28626
46	Methoxymethoxy-4a,6a,	32.853	14345

Table 2: GC-MS analysis shows the compounds present in methanol leaf extract of *C. infortunatum*

DISCUSSION

Phytochemical profiles of plants vary heavily depending on variation in season, soil constituents as well as cultivar. Complexity and variation in metabolites regarding polarity, molecular weight, abundance and different physiochemical properties makes it impossible to extract the whole metabolome using a single solvent extraction method.*C. infortunatum* is an ethno pharmacological plant which is extensively utilized in traditional medicinal system to ameliorate a wide range of diseases.

Moreover, several evidence based reports are available which have already demonstrated the therapeutic potentialities of *C. infortunatum* (Das *etal*., 2014). Previously, in a preliminary phytochemical analysis, Dey*etal*. (2014) had quantified some major chemical species such as tannin, phenol, ascorbic acid, riboflavin, thiamine, alkaloid, flavonoid, sugar, lipid, protein etcthat have a wide range of anti-insect properties, including insecticidal, repellent, antifeedant, and insect growth inhibitory activities (Ahmad 2007; Dhaliwal and Koul 2011).

Terpenoids are a large class of natural products that includes various types, such as monoterpenes, sesquiterpenes, triterpenoids, and diterpenes. Considerable attention has been paid to the insect antifeedant activity of some natural clerodanediterpenoids isolated from several plant families (Wagner *etal*. 1983). Moreover, Ghosh and his group (2015) studied the phytochemical profile of the methanolic extract of leaves using chromatographic approaches and identified few compounds. Limonene, catechol, p-vinylguaiacol (2-methoxy-4-vinylphenol), 5,8,11-eicosatriynoic acid, stigmasterol, desulphosinigrin, guaiacol (2-methoxyphenol), tyrosol (4-hydroxy-benzeneethanol), vaccenic acid, hexadecanoic acid, phytol, betulin, hydroxymethyl furfural were the major bioactive constituents recognized in *C. infortunatum.*

Metabolic intermediates and derivatives of several compounds such as vitamin D (9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-); eugenol (phenol, 2,6-dimethoxy-4-(2-propenyl)-); cinnamic acid (hydrocinnamic acid, o-[(1,2,3,4-tetrahydro-2-naphthyl)methyl]-); and vanillic acid (benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester) were identified as well. Previously, Dey*etal*. (2014) also reported the presence of high amount of thiamine, riboflavin and ascorbic acid in the leaves of *C. infortunatum*. Moreover, Erukainure (2011) also reported the presence of different vitamins including vitamin D in *Clerodendrum* species.

Identification of hydrocinnamic acid has profound importance because cinnamic acid derivatives are integral part of our diet and has been attributed to the prevention of different diseases related to oxidative stress like atherosclerosis, inflammatory injury, cancer, and cardiovascular diseases (Teixeira*etal*., 2013). The presence of 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- and n-hexadecanoic acid was supported by a previous study which reported both the compounds in *C. infortunatum* leaves (Ghosh *etal*., 2015).

Previous phytochemical investigation of the plant revealed the presence of alkyl sterols and 2,-(3, 4-dehydroxyphenyl) ethanol 1-O-α-2 rhamnopyranosyl-(1→3)-β-D- (4-O-caffeoyl) glycopyranoside or acteoside (Akihisa *etal*. 1989; Sinha *etal*., 1981). Some reports indicated hepatoprotective, anti-inflammatory, antinociceptive, and neuropharmaco logical activities of *C. Infortunatum* (Ahmad *etal*., 2007; Das *etal*., 2010).

A diterpene (clerodin), ethylcholestatrieneol, scutellarin-7-O-glucuronide, hispidulin-7-O-glucuronide have been reported from the leaves. Lupeol, p-sitosterol, clerosterol, clerodolone, clerodone and a steroidal glycoside have been reported to be isolated from die roots of C. infortunatum (Raslogi and Mehrotra, 1990). Acacetin, apigenin, methyl ester of acacetin-7-Oglucuronide, clerodin, hentriacontane, (24S)-ethyl-cholesta-5, 22, 25-triene- 3f3-ol, fumaric acid and esters of caffeic acid, p-sitosterol and p-sitosterolgluco side have been isolated from the flowers of *C. infortunatum.* Flavonoids isolated from the roots of *C. infortunatm* have been reported to

exhibit anti-fungal activity (Roy *etal*., 1996). Medicarpin and d Jindkylmcdicmpin have been isolated from the leaves and cabruvin and quercetnn, from the seeds of *C. infortunatum*.

All these components act together (synergistic) resulting in promising toxic effects against the tested insect. From this study, it is also revealed that plant secondary metabolites act together and result in better toxic effect than the individual components. That explains why, crude extract exhibited much higher level of insecticidal and antifeedant effect than isolated fractions.

CONCLUSIONS

The present study shows the presence of more than 40 active compounds in methanol extract of C. *infortunatum*. This confirms the application of *C. infortunatum* in medical and pest management fields. Further plan of stufies include application of the plant in control of agricultural pests

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