Pleione 8(2): 283 - 292. 2014. **ISSN: 0973-9467**

Bioactivity Assay of 1,3-Dimethyl-5-(2,5,13,17-tetramethyl octadeca-6,16-dien-8-yl) benzene isolated from *Clerodendrum infortunatum* Linnaeus (Lamiaceae) an ethnomedicinal plant of NE-India

Pinak Pani Nath Choudhury[1,5], Biswajit Nath[2], Sudip Choudhury[3], S.B.Paul[3] and **M. Dutta Choudhury[4]**

[1]Department of Zoology, S.S. College, Hailakandi, 788151, Assam. India
[2]Department of Chemistry, R.K.Vidyapith, R.K.Nagar, Karimganj, 788166, Assam, India
[3]Department of Chemistry, & [4]Department of Life Science and Bioinformatics, Assam University, Silchar, 788011, Assam, India
[5]*Corresponding author; E-mail*: pinaknath@yahoo.com

[Received 12.09.2014; Revised 08.12.2014; Accepted 09.12.2014; Published 31.12.2014]

Abstract

Clerodendrum infortunatum Linnaeus (Lamiaceae), a perennial under-shrub is being traditionally used by the people of Bangladesh and North-East India for the treatment of various life threatening diseases. Crude methanolic extract of the leaves of the plant has been reported by several scientists to possess Antimicrobial, Antioxidant, Analgesic activity etc. The present study documented isolation of a yellow oily substance from the methanolic leaf extract of the plant by column chromatography which is having profound antioxidant activity. The isolated substance was tested for antimicrobial activity but no significant antimicrobial activity was observed at least against the microbes under investigation but shows moderate insecticidal activity against *Periplaneta americana* Linnaeus. The isolated yellow substance was subjected to HPLC, which resolves that the substance to be a pure compound. To explore the structural elucidation, Electron Impact Mass Spectral data, Fourier Transform Infrared data and ^{1}H NMR data were recorded. The structure of the substance was elucidated with the help of the spectral data. The IUPAC name of the isolated compound is *1,3-Dimethyl-5-(2,5,13,17-tetramethyl octadeca-6,16-dien-8-yl) benzene*

Key words**:** *Clerodendrum infortunatum*, Antioxidant, Insecticidal, Chromatography, EIMS, FTIR, NMR,

INTRODUCTION

Animals, including humans, and most microorganisms depend directly or indirectly on plants as a source of food. Plants produce numerous chemicals for defence and communication. These chemicals may have general or specific activity against key target sites in bacteria, fungi, viruses, or neoplastic diseases. Discovery of vincristine and vinblastine (Cutler*et al*. 2000) in 1963 by R. L. Noble and his Canadian co-workers (Neuss *et al*. 1990) and its successful use patent by Eli Lilly launched the pharmaceutical industry in quest of natural product leads for the treatment of various cancers. Products of natural origins can be called ‘‘natural products.’’

Despite the rise of combinatorial chemistry as an integral part of lead discovery process, the natural products still play a major role as starting material for drug discovery (Lahlou 2013). Natural products have been especially successful as lead structures for antibacterial

therapies (Lahlou 2007). In a study of the pharmaceuticals on the market from 1981-2002, only 43% of the drugs were purely synthetic, while the remaining 57 % were obtained from a natural source (Newman *et al*. 2003).

Plants under the genus, *Clerodendrum* has been reported to exhibit considerable ethnomedicinal properties (Sajem & Gosai 2006). A number of compounds of diverse classes have been isolated and their medicinal properties have been investigated by different groups of researchers. In particular, the plant *Clerodendrum infortunatum* (Synonym: *C. viscosum* Ventenat), widely available in North-East India, is of special interest (Shrivastava & Patel 2007). *Clerodendrum infortunatum* is a perennial under-shrub with bluntly 4-angled stem. Leaves opposite, long petioled, rounded ovate, serrate, hairy, chartaceous**.** Flowers with white corolla in pyramidal terminal panicles (Ghani 2003, Das & Choudhury 2009). The plant being traditionally used by the people of Bangladesh and North-East India for the treatment of various life threatening diseases (Anisuzzaman 2007; Pavel & Hossain 2007). Repellent response of *Clerodendrum infortunatum* to adult and larvae of *Tribolium castaneum* was studied by Husain & Rahman, (2006). Methanolic extract of the leaves of the plant has been reported by several scientists to possess Antimicrobial (lobo *et al*. 2010), Antioxidant, (Dey *et al*. 2012) and Analgesic activity (Rao & Chandrashekar, 2012). In spite of such important bio-activity, to our knowledge, no systematic investigation has been done to isolate the active principle(s) from the plant. A few secondary metabolites has been isolated, but the medicinally responsible compounds of this plant still remained to be unveiled.

MATERIALS AND METHODS

Based on ethno-botanical information, the plant *Clerodendrum infortunatum* Linnaeus (Lamiaceae) was collected and was identified consulting Assam University Herbarium.

Green leaves of the plants were collected from several places in Barak Valley. The leaves were air dried at ambient temperature and then powdered. Grinded aerial parts of the plant was macerated consecutively with petroleum ether, ethyl acetate and methanol at room temperature and by Soxhlet apparatus. The uniformity was tested by TLC and thereby the extraction at large scale was performed exhaustively by Soxhlet apparatus.

In each batch of exhaustive extraction 35g material was taken in soxhlet apparatus. The material was first defatted by extraction with petroleum ether (300 ml). The defatted plant material was then extracted with ethyl acetate (300 ml) and then with methanol. In the present study only the methanol extract were dried *in vacuo* using rotary evaporator to get the crude extract. Standard disc diffusion method was used to determine the zone of inhibition for antimicrobial study. The sterile molten nutrient agar cooled to 45^0C was inoculated with different organism. The inoculums used were the young cultures. Under aseptic technique the inoculums was uniformly inoculated over the molten agar by using sterile cotton swab. Whattmann No. 2 filter paper disc of 6 mm diameter containing 200 µl/disc of sample was placed over the inoculated medium. The plates were allowed to remain in the room temperature for 2 hours. After 2 hours the plates were incubated at 37^0C for 24 hours. The zone of inhibition was measured using zone reader. The standard antibiotic used were Ciprofloxasin. Pathogenic strains of microbes were collected from Silchar Medical College Hospital, Silchar, Assam.

From the crude extract pure fractions were obtained through column chromatography and bioactivity assay as mentioned above was again performed with purified fractions. Product purity was monitored on Parkin Elmer CLARUS 500 GC-MS system. IR spectra were recorded on a Schimadzu Prestige 21 FTIR spectrophotometer using KBr pellets. ^{1}H NMR (400 MHz) spectra were recorded on a BRUCKER-ACF 300 spectrometer with

tetramethylsilane (TMS) (ä 0.00) as an internal standard in $CDCl_3$ (as solvent) at room temperature. All Chemical shift are quoted in ppm. GC-MS were recorded with Parkin Elmer CLARUS 500 GC-MS instrument and peaks were recorded in mass unit (m/z).

Direct toxicity analysis with *Periplaneta americana* was performed following the method described by Talukdar and Howse& Rahman *et al*. (Talukdar & Howse 1993; Rahman *et al*. 2007)

Free radical scavenging ability of the isolated compound, M1 was tested qualitatively by DPPH radical scavenging method as described by Choi *et al.* (2000) with necessary modifications.

RESULT

Characterisation of crude extract and isolated compound

The methanol extract was fractionized by column chromatography using petroleum ether: ethyl acetate in the ratio 9.5:0.5 as eluent. One yellow oily fraction was obtained. This fraction will hereafter be noted as M1 was collected on the basis of TLC analysis. The isolated fraction was subjected to HPLC which resolved the material to be a single and pure compound at least the solvent system used and the $ë_{max}$ set for the compound (Figure 1).

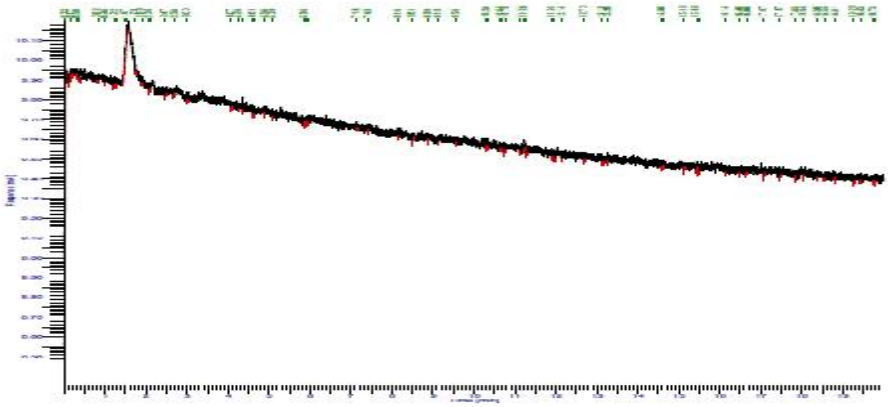


Figure 1: HPLC plate of M1 showing single peak

Study of Antimicrobial Activity

The activity of the crude and isolated compound was monitored against different microorganisms such as *Bacillus subtilis. Klebsilla pneunomae*, *Escherichia coli*, and *Staphylococcus aureus*. The material does not exhibited any antimicrobial activity against the microbes under investigation. The photographs of the culture plates were taken and are presented below (Figure 2) and the zone of inhibition recorded are tabulated in the Table 1.

Insecticidal effect of Crude Methanolic Extract and the isolated compound (M1) against Periplanata americana

As the plant possesses repellent activity against floor beetle so the insecticidal effect of the crude methanolic extract and the isolated compound was carried out on *Periplanata americana* – a common insect of this locality.

Insects were chilled at 4^0 C for a period of 10 minutes. The immobilized insects were individually picked up and 1 ml each of crude Methanolic extract, isolated compound from

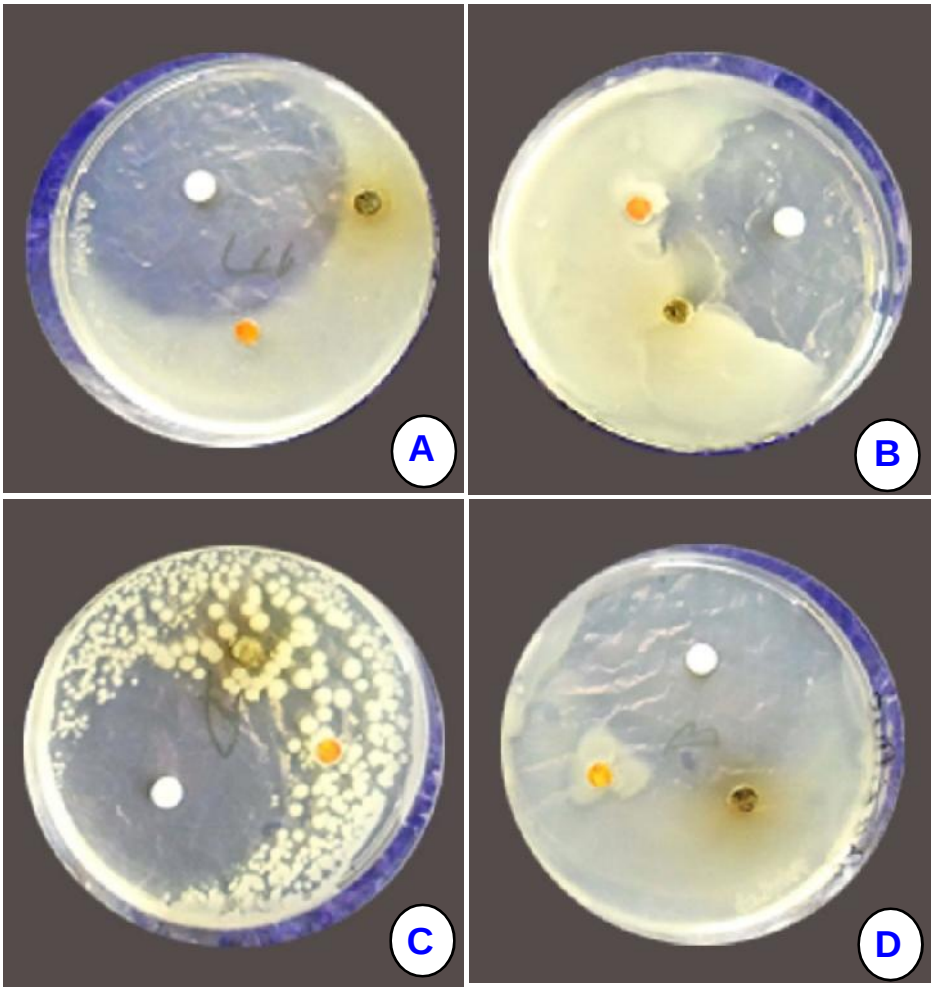


Figure: 2: Photographs of culture plates showing antimicrobial activity of crude and fractions obtained from methanol extract of *C. infortunatum* leaves [A. *Escherichia coli* (Migula) Castellani & Chalmers; B. *Klebsiella pneumoniae* (Schroeter) Trevisan; C. *Staphylococcus aureus* Rosenbach; D. *Bacillus subtilis* (Ehrenberg) Cohn]

Table 1: The results of the antimicrobial experiments of methanolic extract

Organisms	**Crude Extract**	**Isolated Compound**	**Standard (Ciprofloxacin)**
Bacillus subtilis	0	0	6 cm
Klebsilla pneumoniae	0	0	7 cm
Staphylococcus aureus	0	0	5 cm
Escherichia coli	0	0	8 cm

methanolic extract (M1) and Methanol as standard were applied to the dorsal surface of the thorax of each insect using a micro-capillary tube. 12 insects were taken for this experiment.

Four insects were kept in each petridis.

- In Petridish 1, the insects were treated with Methanol as standard,
- In Petridish 2, the insects were treated with crude methanolic extract and
- In the 3rd Petridish, the insects were treated with isolated compound.

The experiment was carried out in qualitative mode and the concentration of the sample was not studied. The result obtained are presented in Table 2.

Table 2. The results of the insecticidal effect of methanolic extract

Substance Used	**Time**	**No of *P. americana* taken**	**Mortality**
Methanol	After 30 minutes	4	0
	After 60 minutes	4	0
	After 90 minutes	4	0
Crude Extract	After 30 minutes	4	0
	After 60 minutes	4	1
	After 90 minutes	4	1
Isolated Compound (M1) using methanol	After 30 minutes	4	0
	After 60 minutes	4	2
	After 90 minutes	4	2

The result shown above indicates the Crude extract possess some insecticidal effect and the isolated compound, M1 is probably one of the chief constituents responsible for this activity of the extract in particular and the plant as a whole.

Anti-oxidant activity of M1

A solution of 0.1 mM DPPH in methanol was prepared. From this stock, 2.5 mL solution was taken and to it 2.5 mL methanol was added. UV-visible spectrum of the solution was takenusing Shimadzu UV 1601PC Spectrophotometer, where the characteristic absorption of DPPH at 515 nm was observed. To analyse the effect of the isolated compound, to 2.5 mL of the DPPH stock solution, 2.5 mL of methanolic solution of the Compound M1 (100µg/mL) was added. The reaction mixture was vortexed thoroughly and allowed to stand in the dark for 30 minutes at room temperature. The UV-visible spectrum of the solution was again taken.

Distinct decrease in the DPPH absorption indicated considerable Antioxidant property of the compound (Figure 3). Reduction in DPPH absorbance (keeping the concentration of DPPH same) was observed, which gave the indication that the compound M1 possess free radical scavenging property and therefore, is capable to exhibit antioxidant activity.

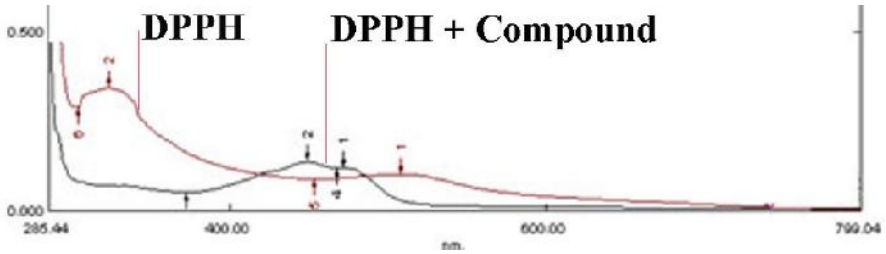


Figure 3: UV spectra visualizing exhibit antioxidant activity of M1

As the compound itself absorbs in a region very near to that of DPPH, quantitative assay by this method may not give proper result and therefore was not performed.

As the isolated compound has shown free radical scavenging properties, the structure of the compounds needs to be known, to explore the potential bio-activities and to design the subsequent level of experimental setup.

Elucidation of structure of compound M1

M1 exhibited clean molecular ion peak at m/z 410 in the mass spectra obtained from GC-MS (Table 3). The peak was associated with peaks at m/z 411 (33 % of the molecular ion peak) and m/z 412 (5 % of molecular ion peak) indicating that they were the peaks corresponding to [M+1] AND [M+2] respectively. The compound exhibited peak at m/z 395 corresponding to [M-15] peak due to dissociation of CH_3 moiety from the compound. As the compound does not seem to contain nitrogen as even number of nitrogen atom (according to nitrogen rule) could not be justified in MS fragmentation or from the ^{1}H NMR spectrum of the compound. Thus the molecular formula of the compound seems to be $C_{30}H_{50}$ and hence the compound is having 5 (five) double bond equivalents (DBE) (Fig. 4). The FT-IR spectrum (Table 4) of the compound exhibited band at about 2950 cm^{-1} due to í(C-H) asymmetric stretching of methyl group. The peak at 2926 cm^{-1} is due to í(C-H) asymmetric stretching of methylene moiety (-CH_2-). The peak at 2850 cm^{-1} is characteristic to í(C-H) symmetric stretching of methylene moiety (-CH_2-). The band at 1630 cm^{-1} is due to í(C=C). The band with moderate intensity cantered at about 759 cm^{-1} corresponds to C-H bending in 1,3,5-trisubstituted benzene ring. The ^{1}H NMR spectrum of the compound (Table 5) exhibited peaks at ä 7.0 to ä 7-5 indicative of the presence of 1,3,5-trisubstituted benzene ring. The peak at ä 4.7 is due to vinylic proton. The peak at ä 3.4 seems to be due to the benzal proton. The peak centred at ä 0.9 correspond to terminal methyl proton. The ^{1}H NMR spectrum assignment of the compound is presented in figure 4. The ^{13}C NMR spectrum of the compound (Figure 5) exhibited a number of peaks at from 127 to 138 indicative of the presence of the benzene ring carbons and the peaks at 131 and 126 and that of 133 and 137 is due to olefinic carbon. A number of peaks between 19.6 to 38.0 are due to methyl and methylene carbons. The ^{13}C NMR spectrum assignment of the compound is presented in figure 6. The EIMS fragmentation of the compound are presented in figure 7. The high percentage abundance of the peak at m/z 243 indicates the high stability of the corresponding fragment. This cationic fragment seems to get its high stability due to resonance. The structure of M1 tentatively assigned from chemical and spectroscopic studies is presented in figure 8 with the IUPAC name 1,3-Dimethyl-5-(2,5,13,17-tetramethyl octadeca-6,16-dien-8-yl) benzene.

(A) Electron Impact Mass Spectral Data

Table 3: EI MS Data of Compound M1

EI MS Peaks (m/z)	
410 (Molecular ion peak)	*167*
367	*83*
339	*71*
327	*69*
325	*43*
243 (Base Peak)	*32*

(B) Fourier Transform Infrared Data

Table 4: FTIR Data of Compound M1

IR Bands (cm^{-1}); KBr pellet
2950
2926
2850
1630
759

(C) ^{1}H NMR (Nuclear Magnetic Resonance) Data

Table 5: ^{1}H NMR Data of Compound M1

Chemical Shift (Internal reference TMS); in δ ppm; $CDCl_3$ solvent
7.0
7.5
4.7
3.4

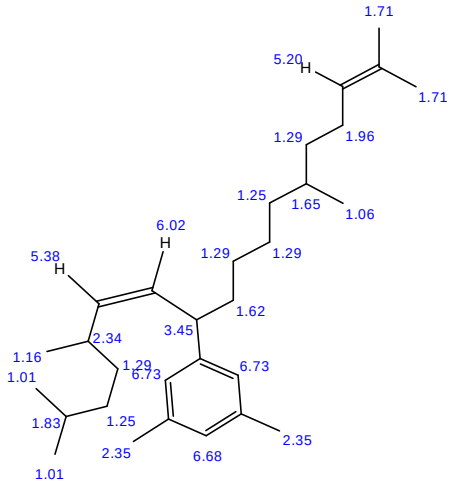


Figure 4: ^{1}H NMR spectrum assignment of M1

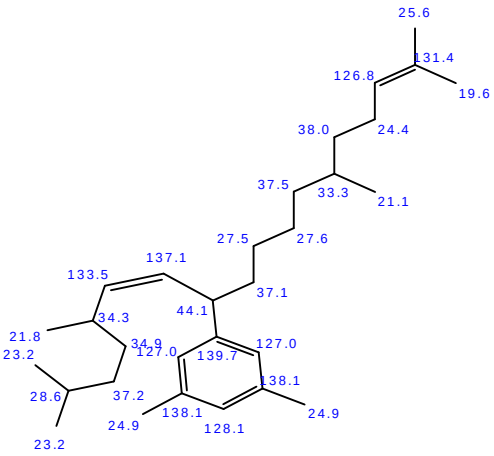


Figure 6: **^{13}C NMR spectrum assignment of compound IC**

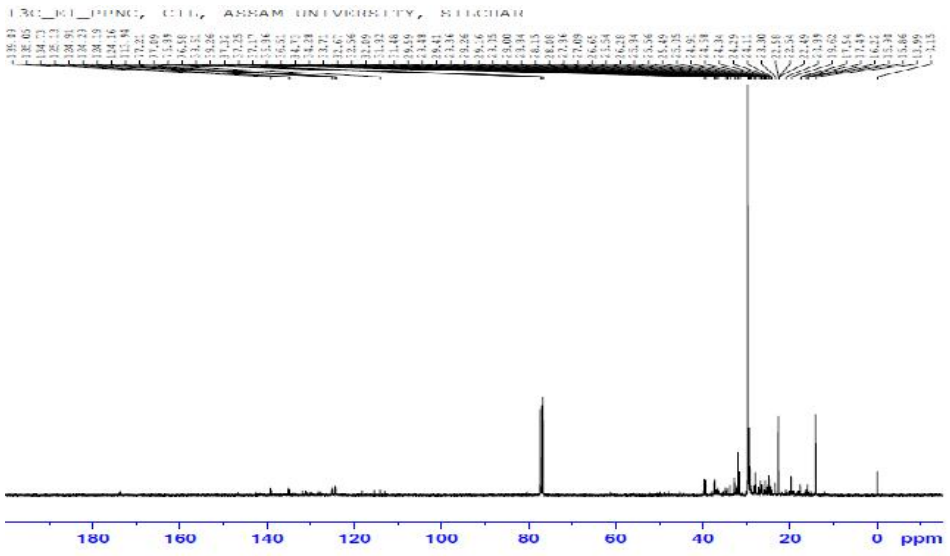
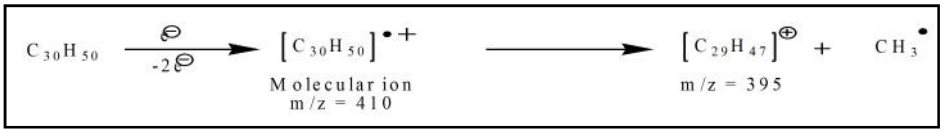


Figure 5: ^{13}C NMR spectrum of compound IC

The EIMS fragmentations of the compound are presented below:



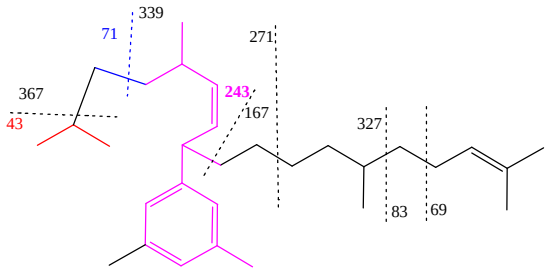
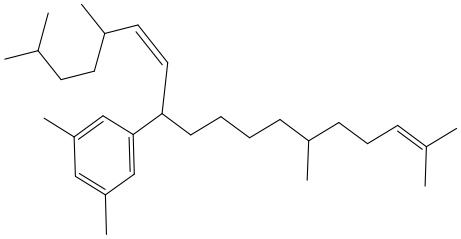


Figure 7: Mass Spectral fragmentation of compound M1



1,3-Dimethyl-5-(2,5,13,17-tetramethyl octadeca-6,16-dien-8-yl) benzene

Figure 8: The tentative structure of the isolated compound

DISCUSSION AND CONCLUSION

Methanolic extract of the leaves of the plant has been reported by several scientists to possess Antimicrobial (Lobo *et al*., 2010), Antioxidant, (Dey *et al*. 2012) Analgesic (Rao & Chandrashekar 2012). Though the present study showed that the methanolic leaf extract of the plant and the isolated pure compound M1 does not show any significant antimicrobial activity but the isolated pure compound M1 shows profound free radical scavenging activity as it was reported by Dey *et al*. (2012) that the crude methanolic leaf extract possesses antioxidant property. Abbaszadeh *et al*. (2014) reported three pure compounds from the plant vizclerodin, 15-methoxy-14, 15-dihydroclerodin, and 15-hydroxy-14, 15-dihyroclerodin. All these compound possesses very low insecticidal effect (Abbaszadeh *et al*. 2014). The present study documented one new compound viz. 1,3-Dimethyl-5-(2,5,13,17-tetramethyl octadeca-6,16-dien-8-yl) benzene from the methanolic leaf extract of *Clerodendrum infortunatum,* which have moderate insecticidal effect.

Acknowledgements

The study is supported by Department of Life Science & Bioinformatics and Department of Chemistry, Assam University, Silchar and Institutional Biotech Hub of S.S. College Hailakandi.

The financial support from UGC NERO under minor research project is also gratefully acknowledged.

LITERATURE CITED

Abbaszadeh, G.; Srivastava, C.; & Walia, S. 2014. Insecticidal and Antifeedant Activities of ClerodaneDiterpenoids Isolated from the Indian Bhant Tree, *Clerodendroninfortunatum* , Against the Cotton Bollworm, *Helicoverpaarmigera*. *J. Insect Sci.* 14(29): 1 – 13.

Anisuzzaman, M. Rahman; A.H.M.M. Harun-Or-Rashid; M. Naderuzzaman, A.T.M. Islam, A. K. M. R. 2007. An Ethnobotanical study of Madhupur, Tangail. *J. Appl. Sci. Res.* *3*(7): 519 – 530.

Choi, H.Y.; Jhun, E.J.; Lim, B.O.; Chung, I.M.; Kyung, S.H. & Park, D. K. 2000. Application of flow injection—chemiluminescence to the study of radical scavenging activity in plants. *Phytoth. Res. / : PTR* 14(4): 250 – 253.

Cutler, S.J. & Cutler, H.G. 2000. *Biologically active Natural Product*. CRC Press, Boca Raton, London. Pp. 14 – 15

Das, S. & Choudhury, M.D. 2009. Ethno-medicobotanical observations on some tribal communities of Tripura , India. *Pleione 3*(1), 89 – 95.

Dey, P.; Choudhury, D.; Tamang, S.; Choudhury, T.K. & Mandal, N. 2012. *In Vitro* Antioxidant And Free Radical Scavenging Potential of *Clerodendrumviscosum. Int. J. Pharm. Bio. Sci*. *3*(3): 454 – 471.

Ghani, A. 2003. *Medicinal Plants of Bangladesh* (2nd ed., pp. 7, 176–177). The Asiatic Society of Bangladesh, Dhaka.

Husain, M.M. & Rahman, M.M. 2006. Repellent Effect of Indigenous Plant Bhat (*ClerodendronnViscosum* Vent.) Leaf on *Triboliumcastaneum* Herbst. *Bangladesh J .Sci. Ind. Res*. 41(1-2): 67.

Lahlou, M. 2007. Screening of natural products for drug discovery. *Expert Opin. Drug Disc.* 2(5): 697 – 705.

Lahlou, M. 2013. The Success of Natural Products in Drug Discovery. *Pharmaco. Pharmacy*, 04(03): 17 – 31.

Lobo, R.; Chandrshakar, K.S.; Jaykumar, B. & Ballal, M. 2010. In vitro antimicrobial activity of *Clerodendrum viscosum* (Vent.). *Der Pharmacia Lettre, 2*(6): 257 – 260.

Neuss, N.; Neuss, M.N.; Brossi, A. & Suffness, M. 1990. Therapeutitic use of bisindole alkaloids from Catharanthus. In. *The Alkaloids*, 37: 229 – 239.

Newman, D.J.; Cragg, G.M. & Snader, K.M. 2003. Natural Products as Sources of New Drugs over the Period 1981-2002. *J. Nat. Prod. 66*: 1022.

Pavel, P. & Hossain, A.B.M.E. 2007. Ethnobotanical Investigation into the Mandi Ethnic Community in Bangladesh. *Bangladesh J. Pl. Taxon*. 14(2): 129 – 145.

Rahman, S.S.; Rahman, M.M.; Khan, M.M.R.; Begum, S.A.; Roy, B. & Fakruddin Shahed, S.M. 2007. Ethanolic extract of melgota (*Macaranga postulata*) for repellency, insecticidal activity against rice weevil (Sitophilusoryzae). *Afr. J. Biotech.* 6(4): 379 – 383.

Rao, S. N. Chandrashekar, R. (2012). Acute Central and Peripheral Analgesic Activity of Ethanolic Extract of The Leaves of *Clerodendrum viscosum* (EECV) in Rodent Models. *J. Drug Deliv. Therap.* 2(5): 105 – 108.

Sajem, A.L. & Gosai, K. 2006. Traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India, *J. Ethnobio. Ethnomed.J. Ethnobio. Ethnomed.* 2: 33.

Shrivastava, N. & Patel, T. 2007. *Clerodendrum* and Heathcare: An Overview. *Med. Arom. Plant Sci. Biotech.* 1(1): 142.

Talukdar, F.A. & Howse, P.E. 1993. Deterrent and insecticidal effect of extract of pithraj, Aphanamixispolystacha against *Tribolium castaneum*. *J. Chem. Ecel*. 19: 2463 – 2471.