Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

ISOLATION AND CHARACTERIZATION OF PHYTOCONSTITUENT FROM *LIPPIA NODIFLORA* LINN

T. Regupathi[*1] and K. Chitra[2]

[1*]Department of Pharmaceutics, Ultra College of Pharmacy, Madurai, Tamil Nadu, India.

[2]Department of Pharmaceutical Chemistry, Sri Ramachandra University, Chennai, Tamil Nadu, India.

ABSTRACT

In view of the conventional and medicinal uses of the plant, studies have been conducted on its chemical constituents by various workers. Uptill now, several flavones glycosides including lippiflorin A and B, nodiflorin A and B, nodifloritin A and B, alkaloids, essential oil, resin, sugars and stigmasterol have been reported. In view of the reported biological and medicinal significance, present studies were undertaken on the Ethyl acetate soluble fraction of ethanolic extract of *Lippia nodiflora* was isolated by column chromatography followed by characterization of isolated compounds by UV, IR, NMR and Mass spectroscopy. The repeated fractionation of active ethyl acetate fraction of ethanolic extract of *Lippia nodiflora* by silica gel column chromatography yielded, yellowish red amorphous powder obtained by concentrating the eluent fractions (20-28 fractions) and this compound designated as LN - 1. The compound LN - 1 which isolated from this column chromatography was subjected into spectral studies for the determination of the structure. Characterization of isolated compounds by UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT 90, DEPT 135, HMBC, HSQC, ^{1}H - ^{1}H COSY and Mass spectroscopy. Isolated compound from *Lippia nodiflora* was determined as 3´´´ acetyl verbascoside, chemically β (3, 4 - dihydroxy phenyl) ethyl) 3 - O α - 3´´´ acetyl rhamno pyranosyl) 4’ - O caffeoyl β - D glucopyranoside.

KEYWORDS

Lippia nodiflora, Column chromatography, TLC, IR, NMR, UV and Mass spectroscopy.

Author for Correspondence:
Regupathi T,
Department of Pharmaceutics,
Ultra College of Pharmacy, Thasildhar Nagar,
Madurai, Tamil Nadu, India.

Email: poornareg@yahoo.co.in

INTRODUCTION

Lippia nodiflora is the main member of the family verbenaceae showing a variety of medicinal uses. It can be the source of the home - grown medicine. In India, it is found in the warmer parts including Andhra Pradesh, Karnataka, Kerala, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal. It is common in wet places along bunds or irrigation channel boundaries and sliver banks. The

plant is rich in many important medicinal useful compounds. The plant has a variety of components such as triterpenoids, flavonoids, phenols, steroids, and many others. Among these flavonoids were the most frequently found constituent. Nodifloretin (3), *β*-sitosterol glycoside and stigmasterol glycoside from the leaves of *L. nodiflora*)[1]. Nodifloridin A (1) and Nodifloridin B (2) along with lactose, glucose, fructose and maltose were isolated from the plant[2]. The plant is used as gastro protective effect[3], anti-inflammatory, antineoplastic[4], antioxidant[5] and diuretic[6]. The plant is used for the treatment of diuretic, aphrodisiac, diseases of heart, ulcers, bronchitis, fever and colds[7].

The chemicals which are responsible for colours and smell into the plant are known as secondary metabolites or pytochemicals. The term, phytochemicals, is generally used to refer to chemicals that may have life significance but have not been established as essential nutrients. Phytochemicals have been used as drugs since long in the earlier period. Numerous of the secondary metabolites have been screened from medicinal plants and have been used in herbal therapy. In view of the traditional and medicinal uses of the plant, studies have been carried out on its chemical constituents by different workers. Recent studies are involved in the identification and isolation of novel healing compounds of medical importance from the plants for specific diseases[8-10]. The plants possesses secondary metabolites such as alkaloids, tannins, flavonoids, sterola, triterpenes, etc., noted to have the most important role in nourishment, physiology and control of diseases[11,12]. The first record of the chemical works on *Lippia* goes back to 1927 when C. Rimington and J. I. Quin isolated icterogenin, a triterpenoid, from the aerial parts of *Lippia.* In view of the reported biological and medicinal significance, present studies were undertaken on the Ethyl acetate soluble fraction of ethanolic extract of *Lippia nodiflora* was isolated by column chromatography followed by characterization of isolated compounds by UV, IR, NMR and Mass spectroscopy.

MATERIAL AND METHODS

Collection and confirmation of plant materials

The plant specimens for the proposed study *Lippia nodiflora* Linn. was collected from the rice fields and other nearby irrigated fields in and around Madurai District, Tamil Nadu, India during the month of October 2009. The herbarium of these plants was identified and authenticated by Dr. D. Stephen, American college of Arts and Science, Madurai.

Preparation of plant extracts

The fresh whole plant of *L. nodiflora* was cleaned with distilled water to separated unnecessary foreign materials like soil and dusts. After, washed plant material was dried in shadow at room heat without direct exposure of sunrays. It was then coarsely grounded by using automatic device. The pulverized plant substance was passed through sieve no 40 and stored in an airtight container for future use.

The roughly crushed plant materials of *L. nodiflora* (2000 g) were extracted separately to exhaustion in a soxhlet equipment for 72 hours by using Petroleum ether (60-80˚C) and Methanol (95 %) solvent (Merk and Spectrum Chemicals, India) systems. All the extracts were filtered through a cotton plug followed by what mann filter paper (No.1) and concentrated by using a rotating evaporator at low down heat (40-50˚C) and reduced pressure to get 20.6 g and 100.2 g respectively. The extracts were conserved in sealed containers and kept at 4˚C until further use.

Column chromatography

The ethyl acetate fraction of ethanolic extract of *Lippia nodiflora* (EELN) was subjected to column chromatography based on phytochemical screening. The fractions collected were further evaluated by Thin Layer Chromatography (TLC) to know the number of constituents present. Silica gel G was used as stationary phase.

Column chromatography was done by using a glass column. The dimensions of the column were 100 cm in length and 4 mm in diameter. The column was packed with silica gel by wet packing method where in the padding of cotton was placed at the

bottom of the column and then it is filled with the eluting solvent of lowest polarity (Pet. ether). Then the required amount of stationary point (silica gel) was transferred into the column to form a bed of silica, a thin pad of cotton was placed over it. The rest of the column was filled with the solvent of the lowest polarity (Pet. ether) and eluted gradually.

THIN LAYER CHROMATOGRAPHY (TLC)

Among the various methods of separation of the plant constituents the chromatographic procedure is one of the most commonly used techniques. A chromatography is essentially a technique for the separation of the components of the mixture by a continuous distribution of the components between the two phases, one of which is moving faster than other. TLC has become widely adopted technique for the standardization of the herbal products. TLC provides drug fingerprint and it is a valuable technique for the rapid and reliable monitoring of the identity and purity of drugs. It is also used for the detection of adulteration and substitution. Furthermore TLC offers a wide choice of mobile phases. TLC has certain advantages over paper chromatography. Fractionations can be effected more rapidly with smaller quantities of the mixture; the separated spots are usually more compact and more clearly demarcated from one another and the nature of the film is often such that drastic reagents such as concentrated sulphuric acid, which would destroy a paper chromatogram, can be used for the location of separated substances.

The ethyl acetate extract concentrate (15 g) of *Lippia nodiflora* was chromate graphed in silica gel (60 - 120 mesh, 300 gm, 100 x 5 cm) column. Elution of the column was carried out with different ratios of toluene and ethyl acetate mixture. The fractions of 100 ml were collected each time. The fractions were monitored by using TLC (pre coated TLC plate, silica gel G_{60} F_{254} with fluorescent indicator, Merck, India). The spots on TLC were observed using UV chamber.

Fractions from first column of toluene and ethyl acetate (70: 30) solvent system were pooled and a re - column was built (3 g mixture) with silica gel G (200 - 400 mesh, 0.5 x 100 cm Merck India). This column was eluted with toluene, chloroform and ethyl acetate (6: 2: 2 v/v/v). The 20 ml fractions were collected and monitored by TLC.

RESULTS AND DISCUSSION

Structure elucidation of isolated compound from ethyl acetate extract of *L. nodiflora*

Isolation of phytoconstituents from *L. nodiflora*

On column chromatography of ethyl acetate extract of *Lippia nodiflora*, the reddish residue was obtained by concentrating the fractions (81 -120) of toluene and ethyl acetate (70: 30) solvent system which was found to be a mixture (on testing by TLC).

On re - column chromatographic separation of *Lippia nodiflora* the reddish amorphous solid obtained by concentrating the eluent fractions (20 - 28 fractions) of toluene, chloroform and ethyl acetate (6: 2: 2) was found to be homogenous (on testing by TLC) and gave positive result characteristic of polyphenolic and glycoside. This compound was designated as isolated compound LN - 1 and it responded positively for phenol test ic substance ($FeCl_3$ test) and glycoside (Molisch's test).

The compound LN - 1 was reddish amorphous powder and gave positive result for characteristic test for phenol and glycoside. Phenylerthanoid glycoside was isolated from the aerial parts of *Lippia nodiflora* by fractionation of the ethyl acetate extract through a silica gel column.

Phenylerthanoid glycosides are characteristic compounds of the order of Lamiales to which the Lamiaceae and Verbanaceae families belong. In the genus *Lippia*, this verbascoside has previously been isolated from *L. multiflora*, *L. citriodora* and *L. dulcis.* This is the first report on isolation of LN - 1 compound from *Lippia nodiflora.*

LC - MS CHROMATOGRAM OF LN -1

The eluted fractions 20 - 28 were evaluated by LC-MS analysis. The chromatogram revealed that eluted fraction contains a single compound. It

showed the retention time of 2.685. The chromatogram is given in Figure No.1.

The UV, ^{1}H-NMR, ^{13}C-NMR, DEPT 135, ^{1}H-^{1}H COSY, HSQC, HMBC and ESI-MS were recorded for LN - 1.

UV Spectrum of IN - 1

The LN-1 showed an intense λ_{max} at 236, 262 and 268 nm. The UV spectrum is shown in Figure No.2.

IR Spectrum of LN - 1

The IR spectrum is shown in Figure No.3. The spectral data of compound LN-1 and their group assignments are tabulated in Table No.3.

The IR (KBr) showed shallow absorption bands at 3380 cm^{-1} to 3000 cm^{-1} indicated the presence of OH stretching and the aromatic stretching. The peak at 2925.48 cm^{-1} was due to the aliphatic CH stretching. The peaks at 1612.2, 1511.92 and 1450. 21 cm^{-1} indicated the presence of C = C stretching. Peaks at 1159.01 and 1112.73 indicated the C - O stretching and C - O - C stretching of ester and ether respectively. The signal at 970.0 and 833 indicated the bending vibrations of C - H. The peak at 732.817 cm^{-1} was due to the deformation of - OH group.

^{1}H - NMR Spectrum of LN-1

The ^{1}H - NMR spectral data of LN-1 and corresponding signal assignments are given in Table No.4.

^{13}C - NMR Spectrum of LN - 1

The ^{13}C - NMR spectral data of LN-1 and corresponding signal assignments are given in Table No.5.

The ^{13}C NMR spectrum showed 31 carbon resonances suggesting this compound appeared to contain two aromatic rings. These deductions were confirmed by the presence of some aromatic peaks in the region of the ^{13}C spectrum. This was further confirmed by the peaks at δ 154.19, 153.8, 145.6 and 145.3 which were more deshielded and showed no correlation with proton in the HSQC experiment to be the hydroxyl carbon of aromatic ring. The presence of carbonyl carbon was indicated by the signal of δ 168.3 and 178.1 ppm which is characteristic of ester carbonyls.

Two anomeric protons signals were observed at δ_H 4.39 (d J = 7.9 Hz), 5.20 (d J = 1.1 Hz) indicating the presence of sugar moieties. This was confirmed by their chemical shifts which were consistent with the β - glucopyranose and α - rhamnopyranose data respectively. The presence of methyl doublet at δ_H 1.3 ppm confirmed that one of the sugar residues was rhamnose. This was further confirmed by the corresponding anomeric carbon resonance at δ 104.2 and 94.0. From the 31 carbon resonance, 12 were assigned to these sugar moieties. An acyl group determined to be a caffieoyl moiety was assumed to be positioned at C - 4′ of glucose unit due to the strong deshielding of the ^{1}H – ^{1}H signal (δ_H 4.63 d, J = 9.5 Hz) caused by the carbonyl carbon due to acylation and also the HMBC correlation at 6.98 (H - β′) and the carbon signal at 168.3 confirmed the acylation of caffeoyl moiety. The downfield shift on H - 1′′ (δ_H 5.80) of rhamnose and C – 3′ (δ_c 81.3) of glucose was also observed, indicating an inter glycosidic linkage of C - 1′′ (rham) → C - 3′(glu).

Mass Spectrum of isolated compound lN -1

The ESI - MS mass spectral data are given in Table No.6.

This compound was isolated as a reddish amorphous solid with a molecular formula (calculated) $C_{31}H_{37}O_{16}$ with a mass of *m/z* 665.21 $(M - 1)^{-}$, 624.60 $(M - 1)^{-}$ - $COCH_3$ and 461 (M - 1) - $COCH_3$ - Glu. UV (MeOH) spectra gave λ_{max} at 232 nm, 262 nm and 268 nm.

The complete assignment of all proton and carbon resonance was based on COSY, HSQC and HMBC experiment on the basis of NMR data and literature survey. Compound I was found to be β (3, 4 - dihydroxy phenyl) ethyl) 3 - O α - 3′′′ acetyl rhamno pyranosyl) 4’ - O caffeoyl β - D glucopyranoside known as 3′′′ acetyl verbascoside.

Table No.1: Chromatographic fraction of *l. Nodiflora*

S.No	Fractions Collected	Eluent Composition	Remarks
1	1-20	100% Toluene	Double compound mixture
2	21-40	90 : 10 Toluene: Ethyl acetate	Double compound mixture
3	41-60	80 : 20 Toluene: Ethyl acetate	Mixture of four compounds
4	61-80	80 : 20 Toluene: Ethyl acetate	Mixture of three compounds
5	81-100	70 : 30 Toluene: Ethyl acetate	Mixture of three compounds
6	101-120	70 : 30 Toluene: Ethyl acetate	Mixture of two compounds

Table No.2: Re - chromatographic fractionation of *l. Nodiflora*

S.No	Fractions Collected	Eluent Composition	Remarks
1	1 - 4	6 : 2 : 2	Mixture of two compounds
2	5	6 : 2 : 2	Single compound low yield
3	6 – 12	6 : 2 : 2	Mixture of three compounds
4	13 – 19	6 : 2 : 2	Single compound low yield
5	20 - 28	6 : 2 : 2	Single compound; compound - I

Table No.3: IR spectral data of isolated compound lN - 1

S.No	Peak - Wave Number (cm^{-1})	Group assignment
1	3380.6	OH - strectching
2	2925.48	CH - stretching - alkane
3	1612.2 1511.0 1450.0	C = C stretching
4	1222 1195 1112	Ester - C - O stretching Ether - C - O - C stretching
5	1033	C - O stretching
6	970, 833	C - H bending deformation
7	732.8	OH deformation

Table No.4: ^{1}H – NMR spectral data of isolated compound lN - 1

S.No	Chemical shift value (δ_H ppm)	Signal assignment
Aglycone		
1	6.25	1H (d, J = 2.0 Hz) H - 2
2	7.83	1H (d, J = 8.1 Hz) H - 5
3	8.83	1H (d, J = 8.1 Hz) H - 6
4	3.92	2H (m) H- α
5	2.5	2H (t, J = 7.3 Hz) H- β
Caffeoyl		
6	7.08	1 H, (d, J = 2.0 Hz) H –2´
7	6.85	1 H, (d, J = 8.1 Hz) H –5´
8	7.25	1 H, (d, J = 8.1 Hz) H –6´
9	7.04	1 H (d, J = 15.9 Hz)H –α´
10	6.98	1 H, (d, J = 15.9 Hz) H -β´
Glucose		
11	6.15	1H, (J = 7.8 Hz) H – 1´´
12	4.74	1H, H – 2´´
13	4.45	1H, H – 3´´
14	4.63	1H, (d, J = 9.5 Hz) H – 4´´
15	5.0	1H, H – 5´´
16	3.82	2H, H – 6´´
Rhamnose		
17	5.8	1H, (d, J = 1.3 Hz) H – 1´´´
18	3.94	1H, H – 2´´´
19	4.38	1H, H – 3´´´
20	4.68	1H H – 4´´´
21	4.65	1H H – 5´´´
22	1.31	3H (d, J = 6.1 Hz) H – 6´´´
23	1.20	3H – acetyl CH_3

Table No.5: ^{13}C - NMR spectral data of isolated compound lN - 1

S.No	Chemical shift value (^{13}C δ ppm)	Signal assignment
Aglycone		
1	132.4	C - 1
2	108.6	C - 2
3	145.59	C - 3
4	145.3	C - 4
5	129.9	C - 5
6	125.3	C - 6
7	72.5	α
8	39.3	β
Caffeoyl		
9	128.1	C – 1´
10	127.4	C – 2´
11	154.19	C – 3´
12	153.8	C – 4´
13	115.0	C – 5´
14	127.0	C – 6´
15	145.7	C - α´
16	114.5	C - β´
17	168.3	C = O
Glucose		
18	105.9	C – 1´´
19	77.0	C – 2´´
20	81.4	C – 3´´
21	73.5	C – 4´´
22	78.2	C – 5´´
23	66.3	C – 6´´
Rhamnose		
24	94.0	C – 1´´´
25	71.8	C – 2´´´
26	72.6	C – 3´´´
27	78.0	C – 4´´´
28	72.4	C – 5´´´
29	22.0	C – 6´´´
Acetyl		
30	29.5	CH_3
31	178.1	C = O

Table No.6: Mass spectrum of isolated compound lN -1

S.No	Mass Peak *m/z*		Assignment
1	*m/z*	665.2	$(M - H)^-$
2	*m/z*	623.6	$(M - H) - COCH_3$
3	*m/z*	461.2	$(M - H) - COCH_3$ - Glu

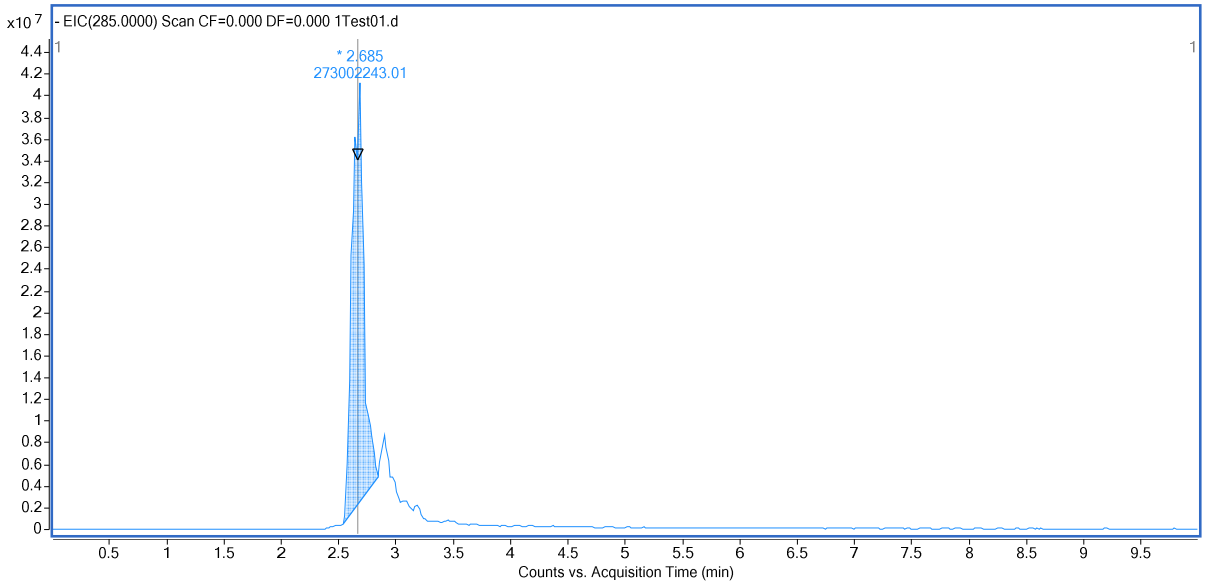

Figure No.1: LC - MS chromatogram of isolated compound from *L. nodiflora*

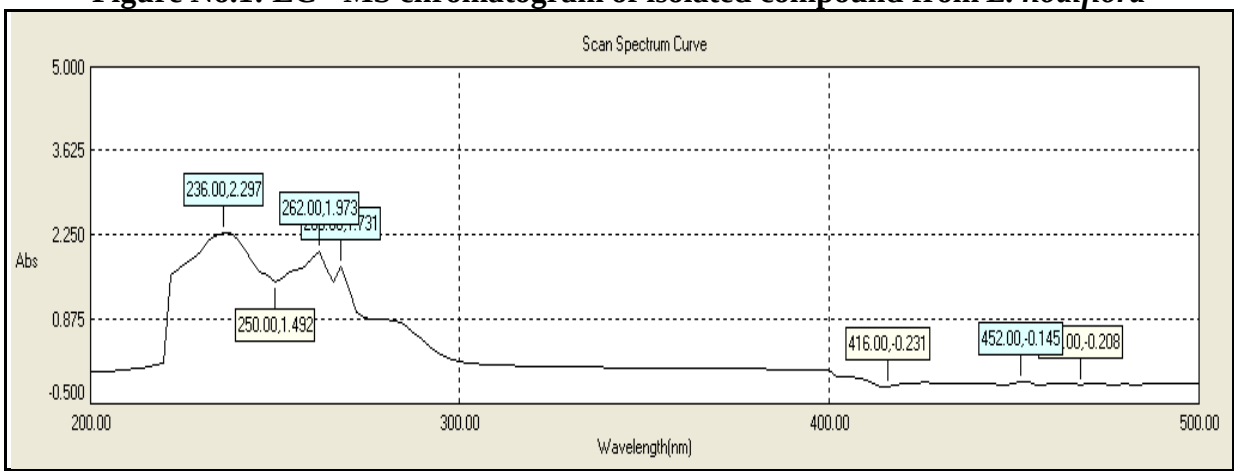

Figure No.2: UV spectrum of isolated compound from *L. nodiflora*

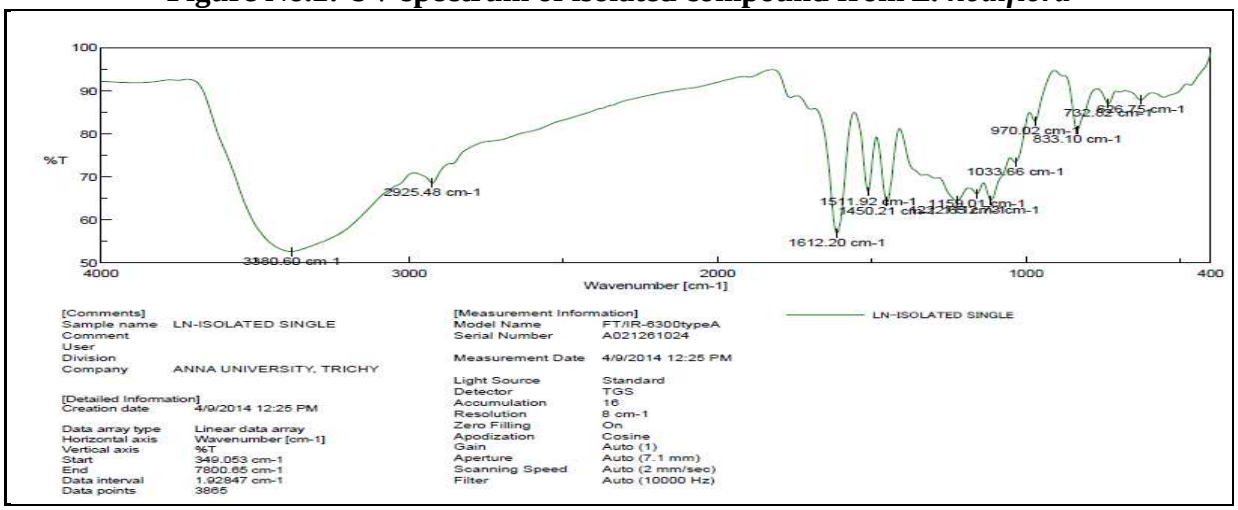

Figure No.3: IR spectrum of isolated compound from *L. nodiflora*

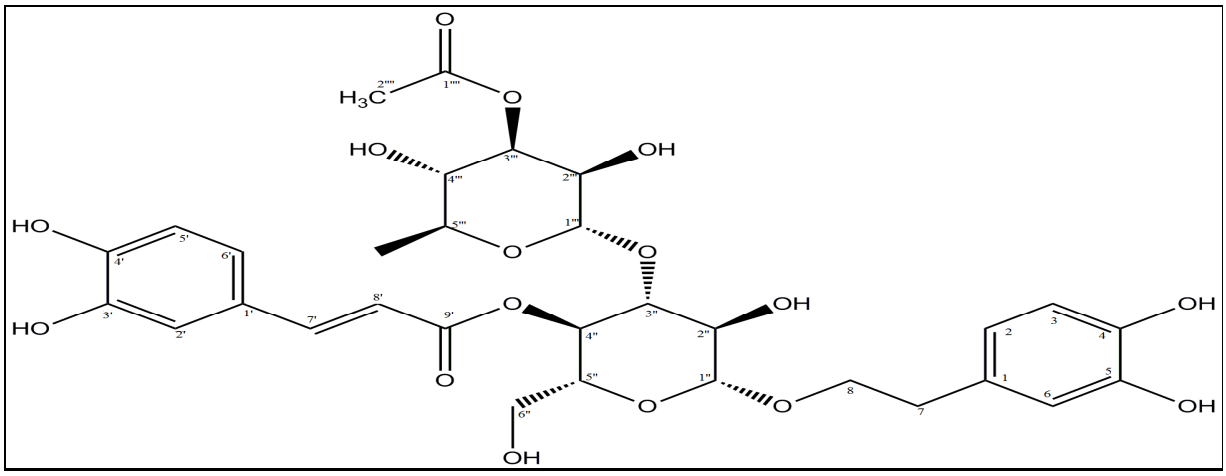

Figure No.4: Structure of the isolated compound 3´´´ acetyl verbascoside

CONCLUSION

The present study was projected to isolate and characterize the phytoconstituent from the medicinal plant *Lippia nodiflora.* From the above procedural workout, it can be clearly concluded that the plant *L. nodiflora* does contain the compound LN - 1. Characterization of isolated compound LN - 1 by UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT 90, DEPT 135, HMBC, HSQC, ^{1}H - ^{1}H COSY and Mass spectroscopy. Based on the spectral studies, isolated compound from *Lippia nodiflora* was determined as 3´´´ acetyl verbascoside, chemically β (3, 4 - dihydroxy phenyl) ethyl) 3 - O α - 3´´´ acetyl rhamno pyranosyl) 4' - O caffeoyl β - D glucopyranoside.

ACKNOWLEDGEMENT

Authors are thankful to Department of Pharmaceutics, Ultra College of Pharmacy, Madurai, Tamil Nadu, India for providing necessary facilities to execute this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Barua A K, Chakrabarti P. Structure of nodifloretin, new flavone from *Lippia nodiflora, Transactions of the Bose Research Institute (Calcutta),* 34(3), 1971, 5-8.
2. Joshi B C. Chemical examination of *Lippia nodiflora Vijnana Parishad Anusandhan Patrika*, 11(4), 1970, 214-219.
3. Khalil H, Ismail H. Gastroprotective effect of *Lippia nodiflora* L, extracts in ethanol induced gastric lesions, *Phcog. Mag*, 3(12), 2007, 259-262.
4. Ahmed F, Selim M S T. Anti-inflammatory and antineoceptive activities methanolic extract of *Lippia nodiflora* Linn, *Die pharmazie*, 59(4), 2004, 329-30.
5. Durairaj A, Tamilselvan V. Antioxidant and free Radical Scavenging Effects of *Lippia nodiflora*, *Pharmaceutical Biology*, 46(10-11), 2008, 762-771.
6. Sangita Shukala, Rashmika patel. Study of Phytochemical and diuretic potential of methanol and aqueous extracts of aerial parts of *Phyla nodiflora* linn, *Internat. J. Pharm. and Pharmaceut. Sci*, 1(1), 2009, 85-91.
7. Gamble J S. Flora of the Presidency of Madras, *Calcutta, India: Botanical survey of India*, 7(2), 1957, 87-88.
8. Khan R A, Khan M R, Sahreen S. Evaluation of *Launaea procumbens* use in renal disorders: A rat model, *J. Ethnopharmaco*, 128(2), 2010, 452-461.
9. Khan R A, Khan M R, Sahreen S, Bukhari J. Prevention of CCl_4 induced nephrotoxicity with *Sonchus asper* in rat,

Food Chem. Toxico, 33(7), 2010, 1304-1321.

10. Sahreen S, Khan M R, Khan R A. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits, *Food Che*, 122(4), 2010, 1205-1211.
11. Sahreen S, Khan M R, Khan R A. Hepatoprotective effects of methanol extracts of *Carissa opaca* leaves on CCl4 induced damage in rat, *BMC Compl. Altr. Med*, 2011, 11-48.
12. Khan R A, Khan M R, Sahreen S. Protective effect of *Sonchus asper* extracts against experimentally induced lung injuries in rats: A novel study, *Exp. Toxicol. Pathol*, 68(10), 2011, 1-7.

Please cite this article in press as: Regupathi T and Chitra K. Isolation and characterization of phytoconstituent from *lippia nodiflora* Linn, *Asian Journal of Phytomedicine and Clinical Research*, 4(4), 2016, 149 - 158.