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Extraction and Isolation of active constituents from *Ixora chinensis* Lam leaves

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ABSTRACT

The plants belonging to Rubiaceae family are generally a rich source of substances of phytochemical interest, used in traditional system of medicine. Ixora species are generally used as the ornamental plants in gardens and parks. The literature review showed the presence of many important phytoconstituents present in the various parts of the other species. Hence an effort was made to investigate the chemical constituents of the different extracts of the leaves of Ixora chinensis Lam. Freshly collected leaves were shade dried and subjected to successive Soxhlet extraction with different solvents of increasing polarity (petroleum ether (60-80 ^{0}), ethyl acetate and n-butanol), then the extracts were concentrated by evaporating to dryness. Preliminary phytochemical investigation indicated the presence of glycosides, steroids, flavonoids, triterpenoids and alkaloids. By performing column chromatography, pure compounds were isolated from different extracts. The chemical investigation indicated the presence of ***Ixorene (1)*** *and* ***Oleanolic acid (2)*** *from the petroleum ether extract,* ***Catechin (3), Quercetin 3-O-Rhamnoside (4)*** *and* ***Kaempferitrin (5)*** *from the ethyl acetate extract and* ***Rubiothiagepine (6)*** *from n-butnol extract, for the first time. The structures of the isolated compounds (1- 6) was realized on the basis of the spectral data (IR, ^{1}H & ^{13}C NMR and Mass).*

Key words: *Ixora chinensis* Lam, Ixorene, Oleanolic acid, Catechin, Kaempferitrin.

INTRODUCTION

In the global context, herbal medicines flourish as the method of therapy of choice in many parts of the world. In recent years, the increasing demand for herbal medicines is being fueled by a growing consumer interest in natural products. Now it is finding new popularity as an alternative conventional medicine even in the industrialized countries and the adoption of crude extracts of plants for self-medication by the general public is in the increase. Thus, phytotherapy acts as a bridge between traditional medicine and modern medicine.

Plants contain active constituents that are used in the modern medicine for the treatment of many human diseases. Ixora is a genus of flowering plants belonging to the family, Rubiaceae with around 500 species worldwide [1].

Most of the species are grown as ornamental plants. Phytochemical studies of other species like *I. coccinea* indicates the presence of important phytochemicals [2-5] such as lupeol, ursolic acid, oleanolic acid, sitosterol, rutin, lecocyanadin, anthocyanins, proanthocyanidins, glycosides of kaempferol and quercetin. Hence another plant of the same genus were selected for the phytochemical studies.

Ixora chinensis Lam [6], commonly known as Chinese Ixora [7] (santan-tsina) is a shrub or a small tree native to China and Thailand. Leaves are short stalked, obviate -oblong, waxy, 6-10 cm long pointed at both ends and borne on short petioles. Flowers are densely arranged, with 4 petals in pink (Fig. No.1). Traditionally, the plant is used to treat hypertension, rheumatism, abscesses, bruises and wounds. It is also beneficial to bone marrow and pregnant uterus. Fatty acids found in the seed oil of *Ixora chinensis* are ixoric acid and crepenynic acid [8].

Fig No.1 Ixora chinensis Lam leaves and flowers

From the literature survey, it was revealed that no substantial work was carried out on the leaves of *Ixora chinensis* Lam both in the chemical investigation and pharmacological activities. Hence an effort was made to investigate the chemical constituents of the different extracts of the leaves of *I. chinensis* Lam.

MATERIALS AND METHODS

2. Experimental procedure

2.1. Materials and Methods:

Solvents: Petroleum ether (60-80 ^{o}), ethyl acetate, n-butanol were used for soxhilation process.

Chemical and Reagents: Laboratory grade (L.R) chemicals were used for isolation. Analytical grade (A. R) reagents were used for analytical work.

Absorbents: 1. Silica Gel (ACME Chemical works, Mumbai) was used for TLC.
2. Silica Gel of mesh size 60-120 (Merck, Bombay) was used for column chromatography.

Equipments: All the Melting points were recorded in a Toshiwal electrically heated melting point apparatus and were uncorrected. I.R spectra of the compounds were recorded using Thermo Nicolet Nexus 670 I.R spectrophotometer. ^{1}H NMR spectra were taken on varian EM-360 (300 MHZ) NMR spectrometer using $CDCl_3$ as solvent. ^{13}C NMR was recorded on Bruker instrument with $CDCl_3$ as solvent at 300 MHz. Mass spectra were recorded on a GC-MS, data on E:/ISO/21184-1.QGD.

2.2. General Experimental Procedures:

2.2.1. Collection and Authentication *Ixora chinensis* Lam.

The leaves of ***Ixora chinensis* Lam** was collected from local areas of Hyderabad and authenticated by P.Venu, Additional Director, Office-in-charge, Botanical Survey India, Deccan Regional Center, Hyderabad-500048 and Voucher specimen (MRCP/25/2014) was kept at Malla Reddy College of Pharmacy, Dhullapally, Hyderabad, Telangana.

2.2.2. Extraction and Fractionation of *Ixora chinensis* Lam. leaves

The shade dried leaves of ***I. chinensis* Lam** (5 Kg) were grinded to fine powder and were subjected to continuous successive extraction with different solvents like petroleum ether (60-80º), ethyl acetate and n-butanol into 15 batches of each 250 g to 280 g in soxhlet extractor. After complete extraction, the different (Petroleum ether, chloroform, ethyl acetate and ethanol) solvents were concentrated and finally dried under reduced pressure to the dryness in flash evaporator. After drying the respective extracts were weighed and percentage yield was calculated. The yield was found to be 44 g, 39 g and 50 g respectively.

2.2.3. Preliminary qualitative chemical investigation:

Preliminary qualitative chemical investigation, indicated the presence of glycosides, steroids flavonoids, carbohydrates, triterpenoids and alkaloids.

2.2.4. Isolation of compounds:

From Petroleum ether extract:

The concentrated petroleum ether extract (30 g) of *I. chinensis* Lam was dissolved in a small quantity of the same solvent and adsorbed on silica gel and then charged into column. The chromatogram was allowed to develop overnight, taking care to prevent the drying of the column by plugging the open end with adsorbent cotton. The elution of solvent system was started after complete saturation of the column. The column being successively eluted with Petroleum ether: Acetone in graded mixture i.e., 90:10, 85:15. From above elution's, two different fractions were collected (i.e. fraction A, B).

Fraction A was eluted from petroleum ether: acetone (90:10), resulted in a single compound which was confirmed by TLC (CH_3OH: $CHCl_3$, 7:3). The product was designated as **compound ICL-1.**

Fraction B was eluted from petroleum ether: acetone (85:15), resulted in a single compound which was confirmed by TLC (CH_3OH: $CHCl_3$, 7: 3). On evaporation gave a crystalline powder. This elute was collected and concentrated, about 10 ml of crude residue was obtained. It was recrystallized with acetone. The product was designated as c**ompound ICL-2.**

From Ethyl acetate extract

The concentrated ethyl acetate extract (35 g) was dissolved in chloroform (15 ml) and chromatographed through a column of silica gel 60-120 mesh LR (diam. 4 cm X length 45 cm). The column being successively eluted with Ethyl acetate: chloroform in graded mixture i.e. 85:15, 80:20, 70:30. From above elution's, three different fractions were collected (i.e. fractions **A, B & C**).

Fraction A was eluted from Ethyl acetate: chloroform (85:15), resulted a single compound which was confirmed by TLC (MeOH: $CHCl_3$, 8:2). The product was designated as **compound ICL-3.**

Fraction B was eluted from Ethyl acetate: chloroform (80:20), resulted a single compound which was confirmed by TLC which by evaporation, gave a solid compound. This elute was collected and concentrated, about 10 ml crude residue substance is obtained. The product was designated as **compound ICL-4.**

Fraction c was eluted from Ethyl acetate: chloroform (60:40), resulted another single compound which was confirmed by TLC. On evaporation, gave a solid compound. This elute was collected and concentrated to about 10 ml crude residue substance. The product was designated as **compound ICL-5**.

From n-butanol extract

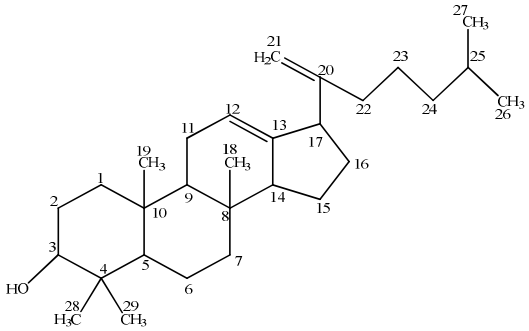
Column being successively eluted with increasing polarities, n-butanol: methanol. The elution was carried out with n - buanol: methanol in graded mixture i.e. 80:20 and finally carried out with 100% methanol. From above elution's, one fraction was collected (i.e. fraction **A**).

Fraction A was eluted from n-butanol: methanol (80:20) resulted finally a single compound which was confirmed by TLC (chloroform: methanol 8.5:1.5). This elute was collected and concentrated to about 10 ml crude residue substance. It was recrystallised with acetone. The product was designated as compound **ICL-6**.

RESULTS AND DISCUSSION

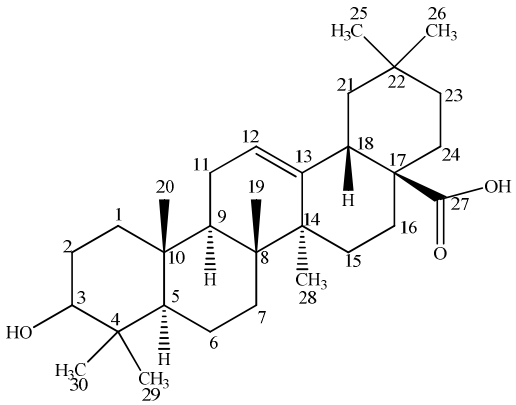
Ixorene (1): Amorphous powder; IR (KBr, cm^{-1}): 3358.12 (O-H stretching), 2916.10 (C-H stretching), 1735.9 (CH_2 stretching), 1375.80 (O-H deformation) 1166.82 – 1093.09 (C-O stretching), 835.14 to 566.31 (C=C deformation); ^1H NMR ($CDCl_3$, 500 MHz, δ ppm): 5.35 (m, 1H vinylic proton, H-12), 5.12 (d , 1H, CH_2 proton , H-21) , 4.95 (d ,1H , CH_2 , proton , H-21), 3.46 (s , 1H , OH group , H-3), 3.37 (m , 1H , H-3), 2.42 to 1.92 (m , CH & CH_2 protons),1.14 (s , 6H, 2(CH_3) group, H-18 & 19), 1.11 (s , 12 H , 4 (CH_3) group , H-26 , 27, 28, 29); ^{13}C NMR ($CDCl_3$, 125 MHz, δ ppm): Table No.1; $C_{29}H_{48}O$; EIMS (m/z)(%): 412 [M^+].

Fig No.2 Ixorene



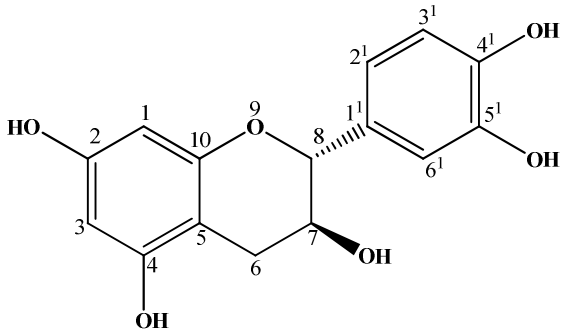
Oleanolic acid (2): Crystalline powder; IR (KBr, cm^{-1}): 3410.37 (O-H stretching), 1736.86 (CH_2 stretching), 1376.60 (O-H deformation), 1032.39 (C-O stretching), 899.91 to 556.65 (C=C deformation); ^1H NMR ($CDCl_3$, 500 MHz, δ ppm): 10.5 (s, 1H, COOH H-28), 5.2 (d, 1H, vinyl proton H-12), 3.6 (s, 1H, OH group), 3.21 (m, 1H ,CH proton, H-3), 2.37 to 1.25 (m, 25H, CH & CH_2 protons) ,1.00 (m, 9H, 3 X CH_3 group , H- 25, 26, 27) ,0.98 (m, 12H, 4 X CH_3 group H-23, 24, 29 & 30); ^{13}C NMR ($CDCl_3$, 125 MHz, δ ppm): Table No.1; $C_{30}H_{48}O_3$; EIMS (m/z)(%): 456[M^+].

Fig No.3 Oleanolic acid



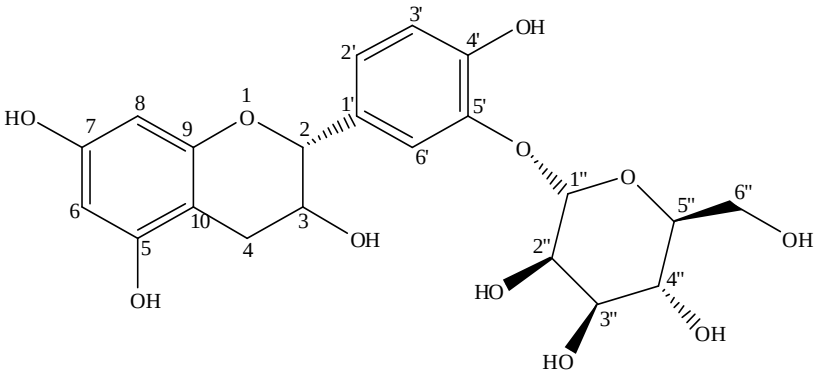
Catechin (3): light green colour crystals; IR (KBr, cm^{-1}): 3362.15 (OH stretching), 2916.10 (C-H stretching) 1160.97 (phenolic OH stretching) and 777.49 to 575.39 (C=C deformation). ^1H NMR ($CDCl_3$, 500 MHz, δ ppm): 11.92 (s,1H, OH, H-7), 8.15(d, 1H, H-2), 6.53 (d, 2H, H-3, H-6), 5.92 (d, 1H , H-1), 5.73 (s, 4H,4 x OH, H-1.4, 4 & 5), 5.71 (d, 1H, H-3), 3.21 (s, 2H, H-6); ^{13}C NMR ($CDCl_3$, 125 MHz, δ ppm): Table No.2; $C_{15}H_{12}O_6$; EIMS (m/z) (%): 288 [M^{-1}].

Fig No. 4 Catechin



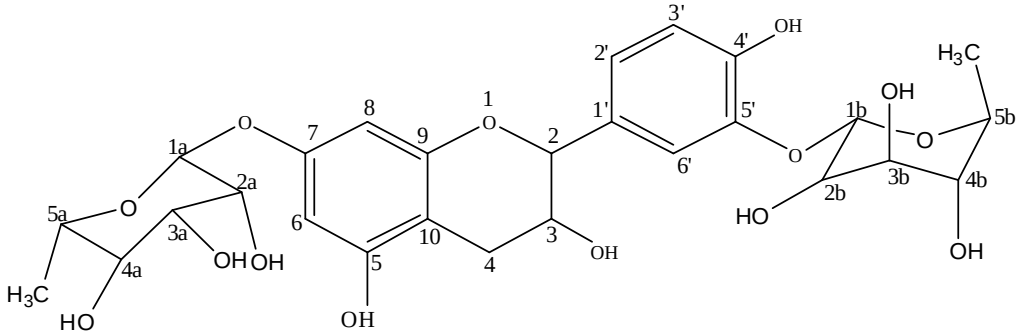
Quercetin 3-O-alpha-Rhamnoside (4): light green crystals; IR (KBr, cm^{-1}): 3452.62, 2916.57, 2848.74, 1709.03, 1376.04, 1164.90, 719.20-569.21; ^{1}H NMR ($CDCl_3$, 500 MHz, δ ppm): 7.41 to 6.05 (d, 5H, aromatic protons, H C-8), 5.42 (s, 4H, OH group H-5, 3, 3' & 4'), 5.71 (d, 1H, H-1"), 4.05 to 3.37 (m, 5H, CH group of pyranose ring), 3.49 (s, 4H, OH group of pyranose ring); ^{13}C NMR ($CDCl_3$, 125 MHz, δ ppm): Table No.2; $C_{21}H_{15}O_{11}$; EIMS(m/z)(%): 465[M^{+1}].

***Fig No. 5* Quercetin 3-O-alpha-Rhamnoside**



Kaemferitrin (5): green crystals; IR (KBr, cm^{-1}): 3326.52, 2916.90, 2848.98, 1734.46, 1376.67 and 728.53 – 561.62; ^{1}H NMR ($CDCl_3$, 500 MHz, δ ppm): 7.15 (d, 2H, H -2'&6'), 6.71(d, 1H, H-2) , 6.65 (d, 1H, H-3' & 5'), 6.21 (d, 1H, H-1) , 5.78 (m, 1H, H -1a), 5.63 (m, 1H, H-1b), 5.33 (s, 2-OH group, H-5 & 4'), 4.56 to 3.75 (m, 6H, CH group of 2 pyranose ring), 3.65(s, 6H, 6 x OH group of 2 pyranose ring), 1.93 (m, 6H, 2 X CH_3 group of pyranose ring); ^{13}C NMR ($CDCl_3$, 125 MHz, δ ppm): Table No.2; $C_{27}H_{30}O_{14}$; EIMS(m/z)(%): 578[M^{+1}].

Fig No. 6 Kaemferitrin



Rubiothiagepine (6): green crystals; IR (KBr, cm^{-1}): 3320.51 (O-H stretching), 2972.89 & 2877.57 (CH stretching), 1649.75 (C=N), 1044.82 (C-O ether stretching); ^{1}H NMR ($CDCl_3$, 500 MHz, δ ppm): 6.05(d, vinylic proton, H-5)

,5.89 (d, 1H vinylic proton, H-5), 5.35 (d, 1H ,H-9) , 4.46 (m,1H , H-3), 3.82(m, 2H,CH_2 protons, H-14), 3.75 to 3.45 (m, 2H, CH proton H-10, 13), 3.32 & 3.21 (m, 2H,CH proton, H-11,12), 3.68 (s, 4H,OH group), 3.35(s, 3H,CH_3 group, 4-8), 2.82 (s,1H,OH group); ^{13}C NMR ($CDCl_3$, 125 MHz, δ ppm): Table No.1; $C_{12}H_{19}NO_8S$; EIMS(m/z)(%): 359 [M^{-1}].

Fig No. 7 Rubiothiagepine

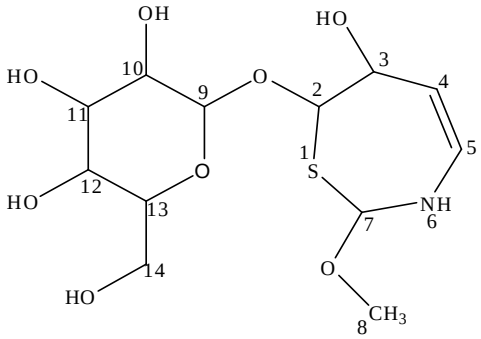


Table No. 1: ^{13}C-NMR Spectral data of isolated compounds

Carbon No.	Compound No.1	Compound No.2	Compound No.6
1	38.1	38.5	C-S
2	27.5	27.5	71.1
3	80.1	79	79.2
4	38.8	38.8	121.7
5	55.1	55.1	134.5
	18.2	18.3	C-N
6	33.5	33.3	162.3
7	34.4	39.7	62.9
8	47.7	47.7	112.2
9	36.8	37.9	70.5
10	23.1	23.6	74.3
11	122.5	124.4	73.5
12	135.1	145.1	85.5
13	50.9	42	68.5
14	31.3	29.2	
15	20.7	23.5	
16	64.1	46.8	
17	14.5	41.7	
18	19.3	47.2	
19	150.5	31.1	
20	109.1	34.7	
21	37.3	32.4	
22	27.7	23.3	
23	42.1	23.2	
24	29.5	16.8	
25	22.2	17.4	
26	22.3	26.1	
27	24.1	180.3	
28	24.2	26.9	
29	-	26.6	
30			

Table No.2 ^{13}C-NMR Spectral data of isolated compounds

Carbon No.	Compound No.3	Compound No.4	Compound No.5
1	109.1	C-O	C-O
2	152.1	155.6	91.3
3	95.7	135.1 179.1	165.9
4	159.9	160.7 100.1 160.5	98.5
5	111.5	95.2	162.5
6	23.5	155.4 104.9	173.6
7	140.5	124.8	134.2
8	122.2	123.1	156.9
9	C-O	117.2	158.9
10	174.3	141.9	104.1
1'	130.1	140.2	122.3
2'	121.2	114.5	126.7
3'	118.9	110.1	117.3
4'	147.2	73.5	159.9
5'	145.8	72.9	117.5
6'	115.7	68.9	127.3
1''	-	81.6	-
2''	-	62.6	-
3''	-	-	-
4''	-	-	-
5''	-	-	-
6''		-	-
1a		-	106.9
2a		-	72.9
3a		-	70.9
4a		-	73.1
5a		-	73.7
CH_3		-	18.9
1b		-	112.7
2b		-	72.2
3b			73.9
4b			73.6
5b			73.5
CH_3			19.1

DISCUSSION

The above obtained isolated compounds (1-6) were realized on the basis of spectral evidence (IR, ^{1}H NMR, ^{13}C NMR and Mass).

Compound (1) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{29}H_{48}O$ on the basis of positive-ion peak EIMS (m/z) 412 $[M]^+$. IR spectral data showed the absorption bands for hydroxyl groups (3358.12 cm^{-1}), C-H stretching of methyl groups (2916.10 cm^{-1}), CH_2 stretching (1735.9 cm^{-1}) and C=C deformation (835.14 to 566.31 cm^{-1}); ^{1}H NMR data of **1** indicated the presence of vinylic proton at δ 5.35 as multiplet. A peak at δ 3.46 indicated the presence of hydroxyl proton as singlet and a peak at δ 5.12 of CH_2 protons as doublet. ^{13}C NMR spectrum of **1** exhibited the presence of 29 carbon signals at their respective δ ppm (table no.1).

Compound (2) was obtained as yellow colour crystals. Its molecular formula was found to be C_{30} $H_{48}O_3$ on the basis of positive-ion peak EIMS (m/z) 456 $[M]^+$. IR spectral data showed absorption bands for hydroxyl groups (3410.37 cm^{-1}), carboxylic group C-O stretching (1084.41 cm^{-1}) and ethylenic deformation (899.91 to 556.65 cm^{-1}). ^{1}H NMR data of **2** reveals, the presence of vinyl proton at δ 5.2 as multiplet. A peak at δ 10.5 indicated the presence of carboxyl proton as singlet and a multiplet at δ 0.98 indicated the presence of four methyl group protons at positions 23, 24, 29 and 30. ^{13}C NMR spectrum of **2** exhibited the presence of 30 carbon signals at their respective δ ppm (table no.1).

Compound (3) was obtained as light green color crystals. Its molecular formula was determined to be $C_{15}H_{12}O_6$ as M^{-1} peak by EIMS (m/z) 288 $[M^{-1}]$. IR spectral data showed absorption bands for hydroxyl groups (3362.15 cm^{-1}), C-H stretching (2916.10 cm^{-1}) phenolic hydroxyl group (1160.97 cm^{-1}) and aromatic group (777.49 to 575.39 cm^{-1}). ^{1}H NMR data of **3** reveals, the presence of a peak at δ 11.92 as singlet indicated the presence of hydroxyl group

proton, a doublet at δ 5.92 due to H-1 proton and a singlet at δ 5.73 indicates the presence of four hydroxyl group protons. ^{13}C NMR spectrum of **3** exhibited the presence of 15 carbon signals at their respective δ ppm (table no.2).

Compound (4) was obtained as light green sticky mass. Its molecular formula was found to be $C_{21}H_{15}O_{11}$ on the basis of positive-ion peak EIMS (m/z) 465 $[M]^{+1}$. IR spectral data showed absorption bands for hydroxyl groups (3452.62 cm^{-1}), ethylene group (2916.90 cm^{-1}), C-H stretching of methyl group (2848.98 cm^{-1}), carbonyl group (1709.09 cm^{-1}) and aromatic group (719.20 to 569.21 cm^{-1}) functionalities. ^{1}H NMR data of **4** reveals, the presence of a doublet at δ 7.41 to 6.05 due to aromatic protons, a singlet at δ 5.42 indicates the presence of hydroxyl group protons, a multiplet at δ 4.05 to 3.37 indicates the presence of CH group of pyranose ring and a singlet at δ 3.49 is due to hydroxyl group of pyranose ring. ^{13}C NMR spectrum of **4** exhibited the presence of 21 carbon signals at their respective δ ppm (table no.2).

Compound (5) was obtained as green crystals. Its molecular formula was found to be $C_{27}H_{30}O_{14}$ on the basis of positive-ion peak EIMS (m/z) 578 $[M]^{+1}$. IR spectral data showed absorption bands for hydroxyl group (3326.52 cm^{-1}), C-H stretching (2916.90 cm^{-1}), carbonyl group (1734.46 cm^{-1}) and aromatic functionalities (728.67 to 561.62 cm^{-1}). ^{1}H NMR data of **5** reveals, the presence of a doublet at δ 7.15 due to two protons at 2nd and 6th positions, the presence of a multiplet at δ 5.78 indicates the presence of 1a proton, a singlet at δ 3.65 indicates the presence of two pyranose rings hydroxyl group protons and a multiplet at δ 1.93 is of 6 protons of two methyl groups. ^{13}C NMR spectrum of **5** exhibited the presence of 27 carbon signals at their respective δ ppm (table no.2).

Compound (6) was obtained as green colored crystals. Its molecular formula was found to be $C_{12}H_{19}NO_8S$ on the basis of M^{-1} peak at EIMS (m/z) 359 $[M]^{-1}$. IR spectral data showed absorption bands for hydroxyl group (3320.51 cm^{-1}), C=N group (1649.75 cm^{-1}) and C-O ether stretching groups (1044.82 cm^{-1}). ^{1}H NMR data of **6** reveals, the presence of a doublet at δ 6.05 due to vinylic protons, the presence of another doublet at δ 5.35 indicates the presence of a proton at 9th position , a singlet at δ 3.68 indicates the presence of four hydroxyl group protons and a singlet at δ 2.82 is of hydroxyl group proton. ^{13}C NMR spectrum of **6** exhibited the presence of 12 carbon signals at their respective δ ppm (table no.1).

CONCLUSION

The investigation of chemical compounds from Natural products is fundamentally important for the development of new drugs, especially in view of the vast worldwide flora. Based on the results, the obtained compounds of *I. chinensis* Lam leaves are effective pharmaceutical compounds which will serve as a better alternative to chemical based pharmaceuticals. Further these compounds have to be screened for different activities based on literature available..

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REFERENCES

[1]. A. R. Kharat, V.V. Nambiar, Y. S. Tarkasband, R. R. Pujari, *Inter. J. Res. Pharm. Chem*., **2013**, 3, 628-635.
[2]. M. S. Baliga, P. J. Kurian, *Chin. J. Integr. Med*., **2012**, 18 (1), 72-79.
[3]. U. O. Gloria, U. N. Gibson, *Int. J. Acad. Res*. **2011**, 3 (2), 381.
[4]. I. Ambreen, M. A. Versiani, S. Shumaila, S. A. Khalid, T. A. Syed, F. Shaheen, *Rec. Nat. Prod.*, **2013**, 7 (4), 302-306.
[5]. C. Y. Ragasa, F. Tiu, J. A. Rideout, *Nat. Prod. Res.*, **2004**, 18 (4), 319-23.
[6]. C. P. Khare, Indian Medicinal Plants. Springer private limited, **2007**. p.338-339.
[7]. L.Y. Chen, Y. C. Chien, M. C. Huang, *Journal of the American Society for Horticultural Science*, **2003,** 128, (1), 23-28.
[8]. H. Minquan, *Phytochemistry*, **1990**, 29 (4), 1317-19.