Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 805-810
Received: 28-05-2019
Accepted: 30-06-2019

Nandini G
Department of Bioanalytical Sciences, Ramnarain Ruia Autonomous College, Mumbai, Maharashtra, India

Vikas Vaidya
Department of Chemistry, Ramnarain Ruia Autonomous College, Mumbai, Maharashtra, India

Correspondence
Nandini G
Department of Bioanalytical Sciences, Ramnarain Ruia Autonomous College, Mumbai, Maharashtra, India

Isolation of active constituents from *Wagatea spicata* using preparative HPTLC and structural elucidation using FTIR and NMR and GCMS techniques

Nandini G and Vikas Vaidya

Abstract
Wagatea spicata (synonymously known as *Moullava spicata*) is a perennial plant of Leguminosae family having ethnomedical significance as a skin disease healer [1]. It exhibits a diverse concentration of biologically active constituents such as Lupeol, Bergenin, Stigmasterol, Friedelin, n-Hexadecanoic acid, Palmitic acid, Gamma sitosterol [2]. However the systematic study based on qualitative and quantitative detection of several marker compounds from *Wagatea spicata* is essential to reveal the entire phytochemical profile of the plant. Therefore, the primary objective of this work was to isolate and characterize the phytoconstituents from *Wagatea spicata* which may possibly be contributing to its biological activity. Additionally this will contribute towards establishment of quality of the aerial plant material.
Preparative HPTLC method was developed for the rapid and less laborious, greener isolation of components from the methanolic leaf extracts of *Wagatea spicata*. Techniques such as Fourier Transform Infra Red Spectroscopy, Nuclear Magnetic Resonance Spectroscopy & Gas Chromatography Mass Spectrometry (GCMS) were employed for structural elucidation & identification of isolated components.

Keywords: Isolation, GCMS, preparative HPTLC, structural elucidation, phytoconstituents

Introduction
Indians have a rich heritage of natural medicines of plant, animal and mineral origin. Generations after generations have benefitted by using these formulations from the tribal sources. In order to unfold these natural medicines to more people for greater good, it is necessary to standardize these formulations and document their uses with scientific evidence. As a fundamental step towards this goal it is essential to define and document the plants and other raw materials that are required make these formulations. Isolation and identification of phytoconstituents is a quintessential thread that can help weave the quality of the end product.
HPTLC is the most commonly chosen method for analysis of phytoconstituents due its simplicity, quickness, affordability, discontinuous (offline) and open nature. In our prior work, the HPTLC fingerprint of *Wagatea spicata* leaves has already been reported [3]. An effort towards the identification of components was also made. However, the current work was performed to practically isolate components from leaf extracts of *Wagatea spicata*, that reportedly possess antimicrobial or antioxidant activities, using Preparative HPTLC and elucidating their structure using FTIR, GCMS and NMR.

Materials and methods
Plant processing: The plant was collected from Kankeshwar Hills near Alibaug, Maharashtra and was identified and authenticated by the Botanical Survey of India, Pune. Leaves being site of synthesis of metabolites were selected and shade dried for one week followed by pulverization using domestic mixer grinder. The coarse leaf powder was sieved through 80 micron mesh and preserved in clean airtight glass container, in a clean dry place, away from light.

Extraction of phytoconstituents from leaf: 1g of fine leaf powder in 25ml of methanol was subjected to accelerated maceration by ultrasonication for 30 minutes, followed by overnight steady state extraction. After filtration using Whatman filter paper number 41, the clear extract was used for isolation by preparative HPTLC.

Isolation of components using preparative HPTLC
To separate the leaf components with an aim of isolating them, following steps were performed:

a. Separation using HPTLC
b. Collection and extraction of isolates
A detailed description of each of the above steps is as under:

a. Separation using HPTLC: The leaf extract was run separately on ten commercially coated TLC plates (Merck Ltd). The mobile phase used was Toluene: Ethyl acetate: Formic acid in the ratio 2:7:1(v/v/v) [3]

Table 1: Experimental Details

HPTLC system used	CAMAG HPTLC with WinCATS software
Stationary Phase	TLC Silica Gel 60 F 254 plates
Detector	CAMAG TLC Scanner 4
Mobile Phase	Toluene: Ethyl acetate: Formic acid 2:7:1 v/v/v
Volume of application	180ul
Solvent front	70 mm
Rate of application	5 sec/µl
Band length	180mm

b. Collection of isolates: Three well separated bands at R_f 0.30, R_f 0.63 and R_f 0.70 showing UV absorbance were selected for isolation. These bands were marked, excised and extracted in methanol. Bands of similar R_f from different plates were pooled together in three separately labelled, clean conical flasks. The extract was filtered and concentrated and the isolates so obtained were used for further analysis using FTIR, NMR and GCMS techniques.

FTIR Analysis of isolates
FTIR fingerprints of all the three isolates were recorded at room temperature on IRAffinity-1 with DRS-8000 accessory at Shimadzu India Pvt. Ltd.

Table 2: Experimental Details

FTIR system used	IRAffinity-1
Scan range	400-4000cm-1
Resolution	4cm-1
Apodization	Happ-Ganzel
No. of scans	45

NMR analysis of isolates
Proton NMR analysis followed by 13 C HSQC was carried out for all the three liquid state samples at National NMR Facility, TIFR.

Table 3: Experimental Details

NMR system used	Bruker 800MHz spectrometer (Bruker Ltd. Switzerland).
Processional frequency	800MHz
Solvent used	$CDCl_3$

GCMS Analysis of isolates
The three isolates were individually injected on GC-MS 2010 system. The identity of the samples was deduced by Spectral comparison with the compounds enlisted in the NIST 11 Library for GC-MS.

Table 4: Experimental Details

GCMS system used	GC-MS 2010 Shimadzu Analytical Pvt.Ltd.
Column	RTX-5MS
Column oven temperature	200 °C
Carrier Gas	Helium
Mass detector	GCMS-QP2010 Ultra
Library Details	NIST (National Institute Standard and Technique) 11

Results and Discussion
Images of plate of Preparative HPTLC run have been documented below. Also, the GCMS chromatograms and spectra with the detailed peak reports containing the R_f values, peak areas and peak heights of the individual components with component identity, IR and NMR spectra obtained during the analysis, have been tabulated in figures below.

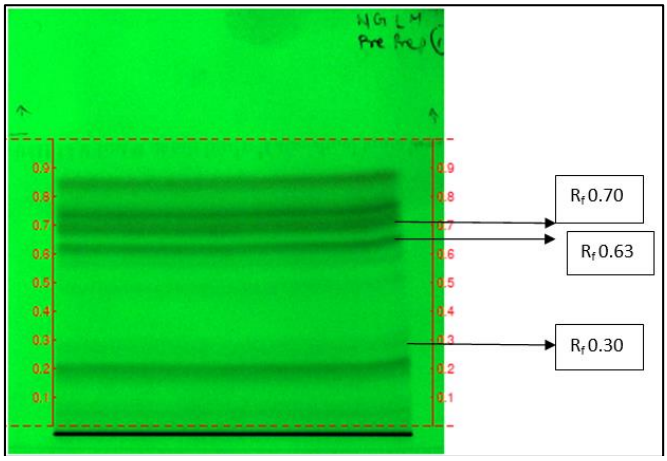


Fig 1: Pre-preparative separation (Pilot run) of leaf extract of *Wagatea spicata* in the mobile phase Toluene: Ethyl Acetate: Formic Acid 2:7:1 v/v/v observed at 254 nm

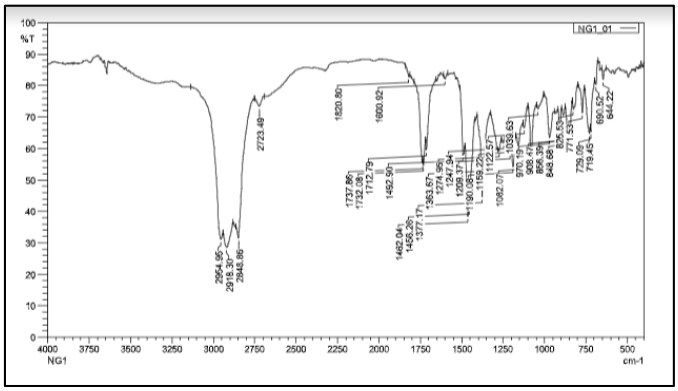
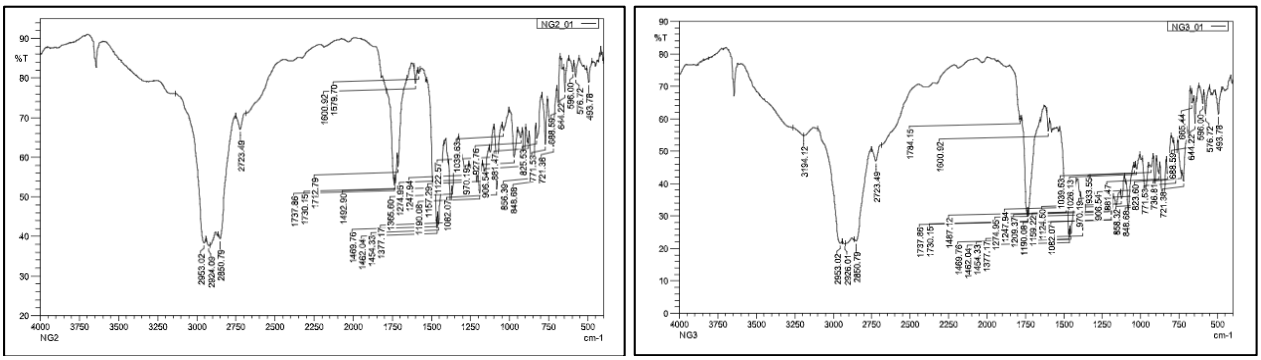


Fig 2: FTIR spectrum of component isolated at R_f 0.30



R_f 0.63 R_f 0.70

Fig 3: FTIR spectrum of component isolated at R_f 0.63 and 0.70

Table 5: List of peaks exclusively detected in each sample

NG1(R_f 0.3)	NG2(R_f 0.63)	NG3(R_f 0.70)
690.52	825.53	665.44
719.45	856.39	736.81
729.09	927.76	823.6
825.53	1122.57	858.32
856.39	1157.29	933.55
908.47	1365.6	1026.13
1122.57	1492.9	1124.5
1363.67	1579.7	1487.12
1456.26	1712.79	1784.15
1492.9	2924.09	2926.01
1712.79		3194.12
1732.08		
1820.8		
2848.86		
2918.3		
2954.95		

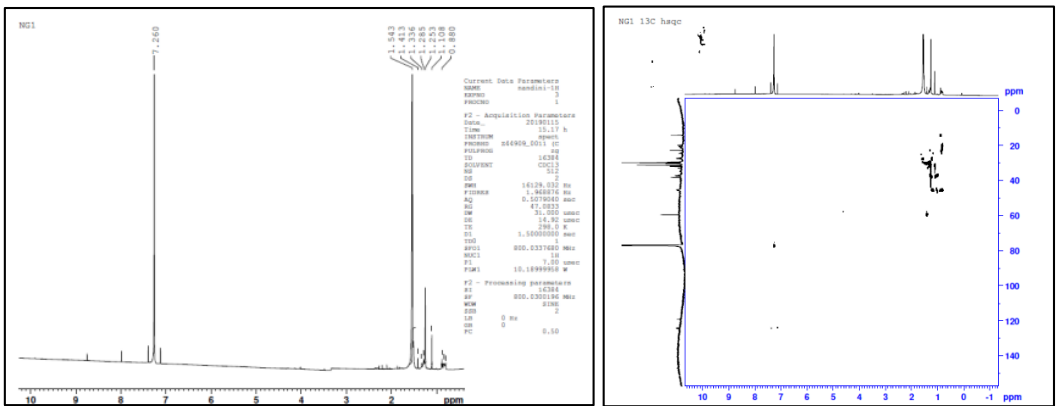


Fig 4: H1NMR spectrum of component isolated at R_f 0.30

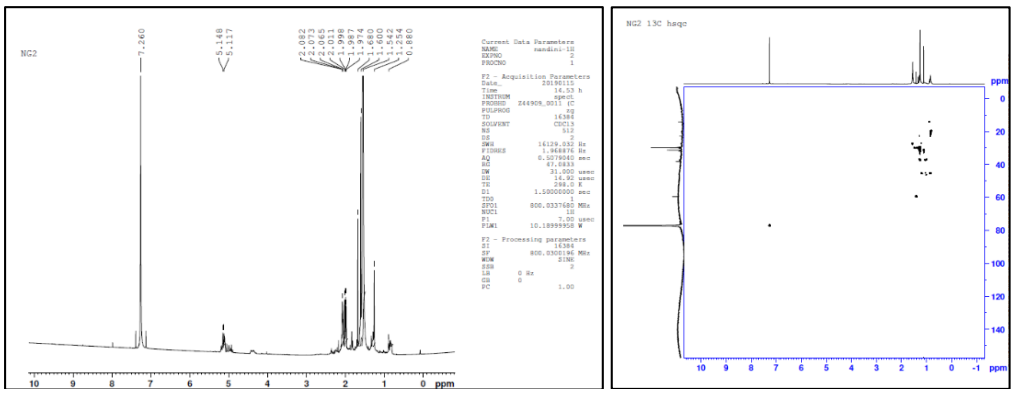


Fig 5: NMR spectrum of component isolated at R_f 0.63

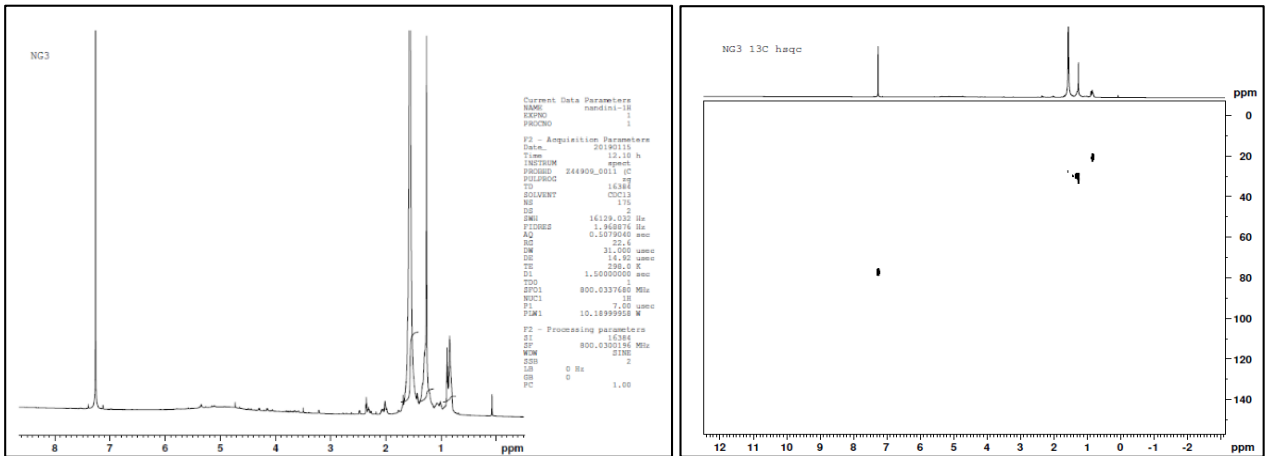


Fig 6: NMR spectrum of component isolated at R_f 0.70

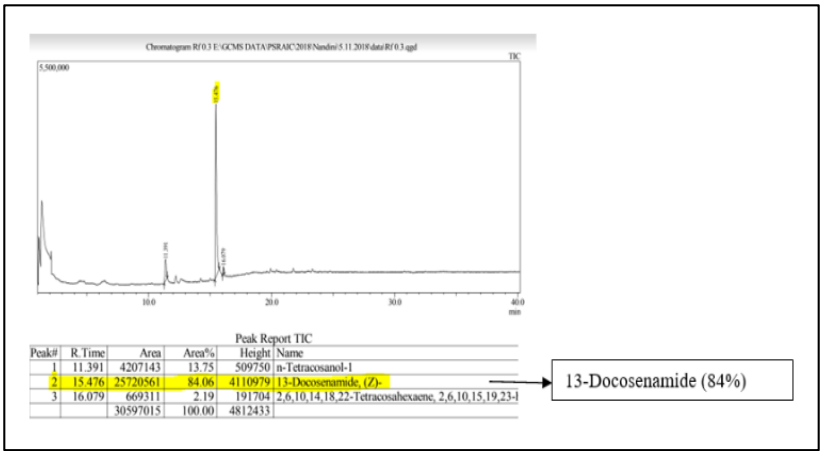


Fig 7: TIC and peak table of component isolated at R_f 0.30

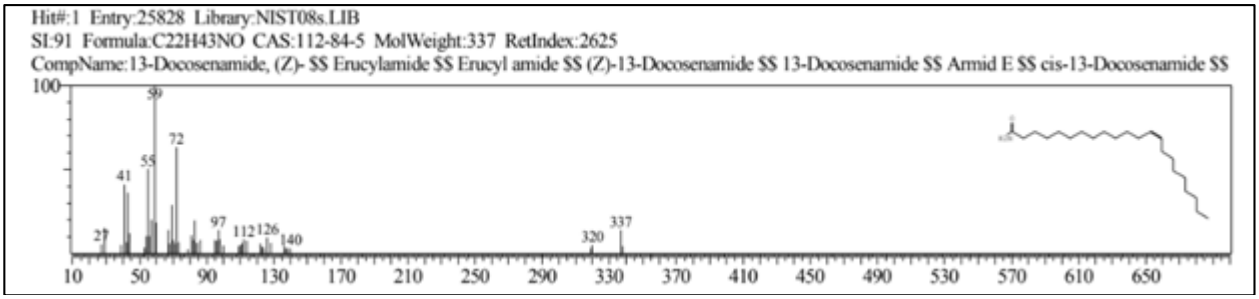


Fig 8: GCMS library report of component isolated at R_f 0.30

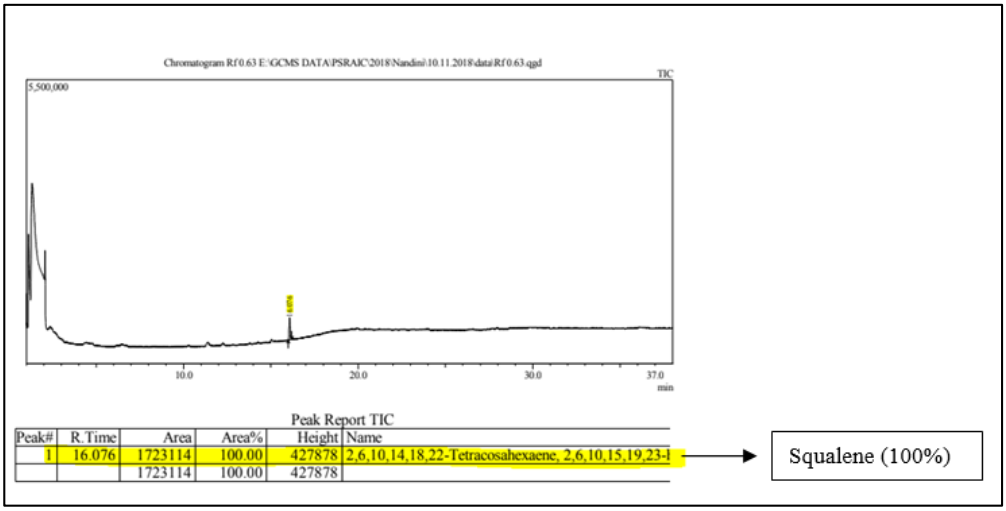


Fig 9: TIC and peak table of component isolated at R_f 0.63

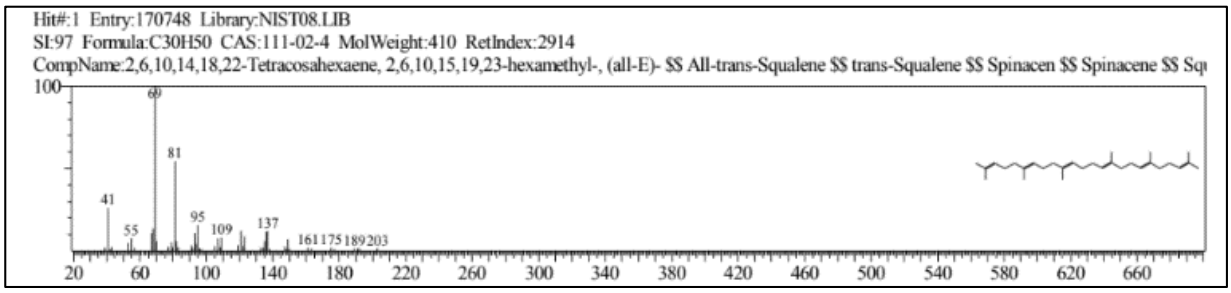


Fig 10: GCMS library report of component isolated at R_f 0.63

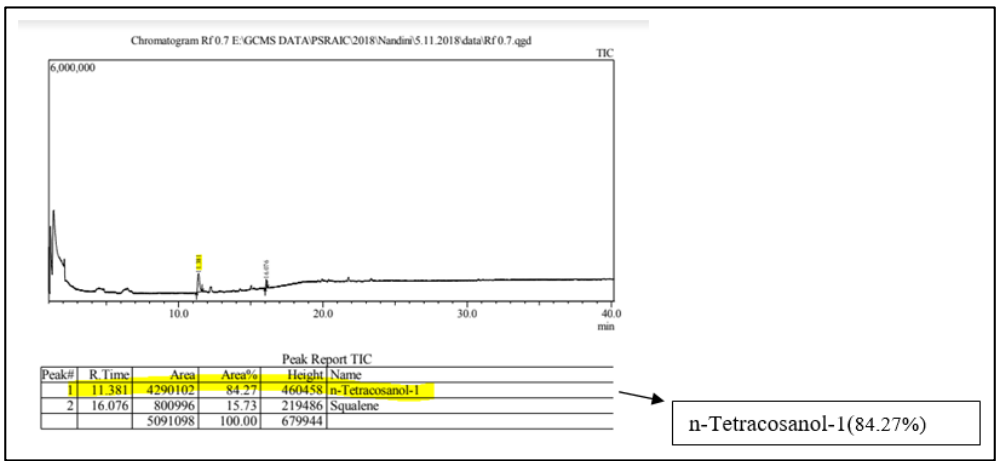


Fig 11: TIC and peak table of component isolated at R_f 0.70

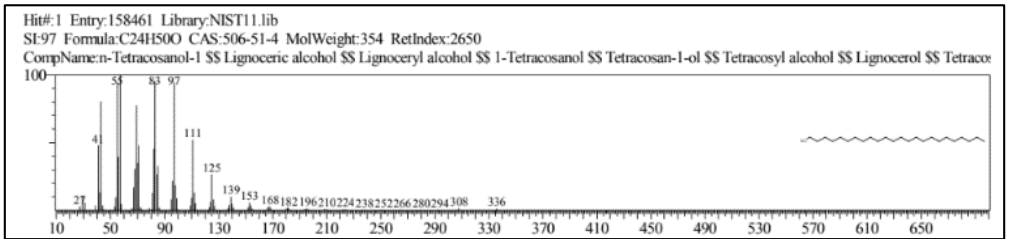


Fig 12: GCMS library report of component isolated at R_f 0.70

Discussion

1. Preparative HPTLC analysis: HPTLC fingerprint of the leaves of *Wagatea spicata* has already been reported[3].The present work focuses on isolation of the components with a view of their structural elucidation. From the above data, it is evident that preparative HPTLC served as not only an effective tool but also greener, less laborious and sophisticated tool for separation and isolation of components from *Moullava spicata.*

2. FTIR analysis: As deduced from the FTIR fingerprints (1300-cm^{-1}-900 cm^{-1}) of all the three isolates, table 5 states exclusive peaks detected in each sample. The isolate R_f 0.3 shows the presence of the frequencies at 690.52, 719.45, 729.09 $cm^{-1,}$ which are characteristic of OCN deformation when amide is present as stated in the standard reference chart (770-620 cm^{-1})[4]. This finding confirms the presence of amide group. The isolate also shows the presence of the frequency 825.53 that falls in the range 840-790 cm^{-1} which is a result of C-H out of plane bending when R1R2C=CHR3 present.

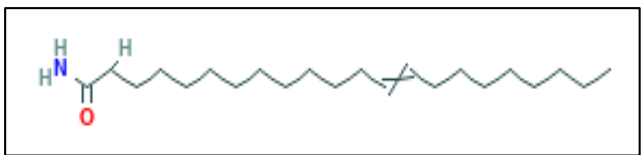
In the FTIR spectrum of the isolate at R_f 0.63, the presence of the frequency 825.53 that falls in the range 840-790 cm^{-1} which is a result of C-H out of plane bending when R1R2C=CHR3 present. The isolate shows a signal at 1579.7 cm^{-1} also which falls in the range 1580-1650 due to C=C stretching in polyenes.

The isolate R_f 0.70 shows a response at 3194 cm^{-1} which is very closely in the range 3400-3200cm-1 for polymeric hydroxy compounds. This substantiates the presence of a hydroxyl group [4]

3. NMR analysis: NG1 shows the following signal as observed in the PMR and HSQC COSY spectrum.

0.88(t,3H, CH_3), 1.108-1.413(m,38H,-CH_2,- CH=CH), 8.8(s,2H,-NH_2)

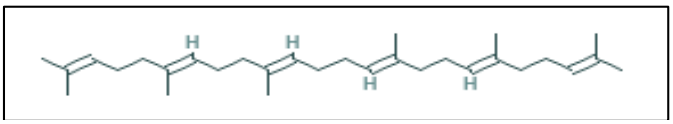
The above results indicate that the molecule isolated at R_f 0.30 is 13-Docosenamide and the structure is:



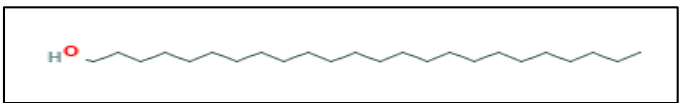
NG2 shows the following signal as observed in the PMR and HSQC COSY spectrum.

0.85-0.88(4s, 24H, 8CH3), 1.25-1.4 (10t,20H,10 CH2), 1.1(3t,6H, 6CH=)

The above results indicate that the molecule isolated at R_f 0.63 is Squalene. The results obtained are in accordance with bis molecule of squalene.



NG3 shows the following signal as observed in the PMR and HSQC COSY spectrum.
0.88 (t, 3H, CH3), 1.26-1.4 (m,46H,21CH2, CH_2-OH), 1.68(s,1H, -OH) The above results indicate that the molecule isolated at R_f 0.70 is n-Tetracosanol



4. Identification using GCMS
From the figures 7 and 8, it can be observed that the component at R_f 0.3 has a molecular weight 337 g/mol which is the molecular weight of 13-docosenamide and shows 91% similarity index with the same. In the fragmentation pattern, detection of fragment at m/z 320 suggests amide group loss from the structure.
From the figures 9 and 10, it can be seen that the component R_f 0.63 has a molecular weight 410 g/mol which is the molecular weight of 2, 6, 10, 14, 18, 22-Tetracosahexaene (IUPAC name for Squalene) and shows more than 95% similarity index with same. In the fragmentation pattern, detection of fragment at m/z 69 can be mainly due to the loss of multiple CH_2 groups.
From the figures 11 and 12 we understand that the component at R_f 0.70 has a molecular weight 354 g/mol which is the molecular weight of n- Tetracosanol-1 and shows 97% similarity with the same. In the fragmentation pattern, detection of fragment at m/z 336 can be due to probable loss of H_2O.
From the above interpretation, it can be manifested that the isolates collected at R_f 0.30, R_f 0.63 and R_f 0.70 are 13-docosenamide, squalene and n-Tetracosanol-1 respectively.
Three Phytoconstituents, namely, 13 -Docosenamide, Squalene and n-Tetracosanol-1 from the leaf extract of *Moullava spicata* (*Wagatea spicata*), were isolated using Preparative HPTLC and identified using contemporary spectroscopic as well as hyphenated techniques. Further quantification of each component is essential for the ultimate quality establishment of the plant under study.

Acknowledgements
The authors hereby convey profound gratefulness towards Anchrom Enterprises, Shimadzu India Pvt. Ltd. National NMR Facility, TIFR, Mumbai for their kind cooperation and constant guidance during present work.

References
1. Surange SR, Deokule SS. Pharmacognistic studies on *Wagatea spicata* Dalzell, Ancient Science of Life, 1986; VI(4):238-243
2. Nandini G, Palekar S, Vaidya V, Shinde M. Phytochemical profiling of *Wagatea spicata* using GC-MS to reveal the pharmacological significance, International Journal of Current Research. 9(12):62197-62204
3. Nandini Girish, Akshay Charegaonkar, Vikas Vaidya. Phytochemical Marker Based Quality Evaluation of *Wagatea spicata* using HPTLC and LCMS, International Journal of Research and Analytical Reviews, 2018; 5(3) (E-ISSN 2348-1269, P- ISSN 2349-5138)
4. John coates, Interpretation of Infrared spectra, a practical approach, Encyclopedia of analytical chemistry, R.A. Meyers (Ed.) John Wiley & Sons ltd, Chichester. 2000, 10815-10837
5. Hasanain Khaleel Shareef, Haidar J, Muhammed, Haider Mashkoor Hussein, Imad Hadi Hameed. Antibacterial Effect of Ginger (*Zingiber officinale*) Roscoe and Bioactive Chemical Analysis using Gas Chromatography Mass Spectrum, Oriental Journal of Chemistry. 32(2):817-837
6. e reference- http://shodhganga.inflibnet.ac.in/jspui/bitstream/10603/133706/13/13_chapter%206.pdf
7. Zih-Rou Huang, Yin-Ku Lin, Jia-You Fang, OrcID. Biological and Pharmacological Activities of Squalene and Related Compounds: Potential Uses in Cosmetic Dermatology, Molecules 2009; 14(1):540-554
8. Cynthia Ekwy Ogukwe, Chris O Akalezi, Brendan Okechukwu Ezugwu, Henry Ebuka Ojukwu Evaluation of the anti-tumour agents in *Annona muricata* (Linn.) Leaves using column chromatography and gas chromatography - mass spectrometry, World Journal of Pharmaceutical Research. 5(2):5-17.
9. Vashisht Sushma, Singh Manish Pal, Chawla Viney. GC-MS Analysis of Phytocomponents in the Various Extracts of *Shorea robusta* Gaertn F., International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(6): 783-788
10. Sheik Uduman MST, Rathinam P, Karuru Y, Obili G, Chakka G, Janakiraman AK. GC-MS Analysis of Ethyl Acetate Extract of Whole Plant of *Rostellularia diffusa*. Pharmacog J. 2017; 9(1):70-2.
11. Deore SL, Khadabadi SS. Isolation and characterization of phytoconstituents from *Chlorophytum borivilianum*. Pharmacognosy research. 2010; 2(6):343-349. doi:10.4103/0974-8490.75452
12. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary, and alternative medicines*:* AJTCAM. 2010; 8(1):1-10.
13. Nagappan R. Evaluation of aqueous and ethanol extract of bioactive medicinal plant, *Cassia didymobotrya* (Fresenius) Irwin & Barneby against immature stages of filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific journal of tropical biomedicine. 2012; 2(9):707-711. doi:10.1016/S2221-1691(12)60214-7
14. Sytar O, Hemmerich I, Zivcak M, Rauh C, Brestic M. Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. Saudi journal of biological sciences. 2016; 25(4):631-641. doi:10.1016/j.sjbs.2016.01.036
15. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese medicine. 2018; 13:20. doi:10.1186/s13020-018-0177-x
16. Kagan IA, Flythe MD. Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. Journal of visualized experiments: JoVE, 2014; (85):51411. doi:10.3791/51411
17. Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants. 2015; 4:196. doi:10.4172/2167-0412.1000196