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RESEARCH ARTICLE

PHYTOCHEMICAL PROFILING OF *WAGATEA SPICATA* USING GC-MS TO REVEAL THE PHARMACOLOGICAL SIGNIFICANCE

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ABSTRACT

Wagatea spicata (WS), a Leguminous flowering shrub has been known for the medicinal action of its bark against skin diseases and roots against pulmonary disorders in Ethnobotanical references (Surange, 1986). 5-20 meters long plant is characterized by the presence of hard, curved, prickles on the stem (Fig 1.1), bipinnately compound leaves (Fig 1.2) and seasonal, reddish yellow, spiked inflorescence (<http://www.flowersofindia.net/catalog/slides/Candy%20Corn%20Plant.html>). In spite of its easy abundance and medicinal significance, phytochemical profile of the plant was not revealed. In the present work an effort was therefore made to elucidate the phytoconstituents of biological significance present in this plant so as to understand their role in the medicinal properties of the same. In the current study, GCMS analysis of carried out to explicate the phytochemical profile of *Wagatea spicata*. n-Hexadecanoic acid, Octadecanoic acid, τ Sitosterol, Lupeol were identified by GC-MS in the plant.

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INTRODUCTION

Plants have been used by mankind for their food, shelter, clothing and medicine since times immortal. The folklore medicine of India is a rich heritage of the country. Most of these medicines belong to the plant kingdom. However, there is no scientific characterization and documentation of many such plants of ethnobotanical origin in spite of the enormous advancements in analytical field. The present study is an effort to identify and reveal the biologically active phyto-components from one such significant plant, *Wagatea spicata* using quick and accurate modern analytical tools like GC-MS. Surange et al., (1987) reported macroscopic and microscopic studies on *W. spicata* Dalzell roots available in the drug markets of Maharashtra. During the above work, Pharmacognostic quality parameters were revealed, however the detailed phytochemical studies were needed; to establish the quality of plant, for its therapeutic use.

Although chromatographic fingerprints are the most used quality control techniques, these techniques do not reveal the phytochemical profile of the plant. Therefore, it was felt worthwhile to employ GC-MS for analysis of different extracts of *W. spicata* which will not only put emphasis on phytochemical profile but also help in identification of specific phytoconstituents. This leads to recognize the potential use of the plant under study for its pharmacological effectiveness.

MATERIALS AND METHODS

Collection of plant material

The leaves and stem of the plant were collected from Kankeshwar Hills near Alibaug. The plant materials in the form of a herbarium were identified and authenticated by Botanical Survey of India, Pune.

Drying and Pulverization

The collected plant parts were segregated as perishable aerial parts such as bark, leaves and young twigs and entire mature stem.

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Fig1. Morphology of *Wagatea spicata* Stem bark (Fig1.1), Leaves (Fig1.2) and Inflorescence (Fig1.3)

Above plant parts were shade dried for a week and after complete drying, perishable aerial parts were pulverized together using domestic mixer grinder. Considering the hardness, entire stem was broken down into small pieces using a commercial hammer mill followed by pulverization using domestic mixer grinder. The crude plant powders were sieved through 80 micron mesh and preserved in clean airtight glass containers for further analysis. Perishable aerial parts powder was labeled as whole plant powder and entire stem powder was labeled as stem powder.

Extraction

Extraction is a crucial step for assessing the phytochemical profile of the plant. To obtain maximum extraction, two different methods were employed, Maceration and Soxhlet extraction. Ethanol & water were used as solvents for maximum extraction of polar components

Soxhlet extraction

20 g of plant powder was added into 250ml of Ethanol and subjected to semi continuous Soxhlet extraction for 48 hrs at 50°C. This extract was collected and used for GC-MS analysis.

Aqueous Hot Maceration

10g of plant powder was added to 100ml of D/W and boiled for 14 hours. The concentrated extract was filtered using a Muslin cloth. The filtrate was further concentrated by evaporating to complete dryness in a water bath. 0.5g of the dried residue was reconstituted in 1ml of ethanol and subjected to GC-MS analysis.

Therefore, following four extracts were obtained after Soxhlet's extraction and hot maceration.

- Bark, Leaves, Twig (whole plant) Ethanolic Soxhlet extract,
- Bark, Leaves, Twig (whole plant) Aqueous, Hot Macerated extract,
- Stem, Ethanolic Soxhlet extract,
- Stem, Aqueous, Hot macerated Extract.

GC-MS Analysis

To obtain the Phytochemical profiling of the plant, all the four samples were individually injected on GC-MS 2010 system. The system details are provided in Table 1. The components of the samples were comprehended by Spectral comparison with the compounds enlisted in the NIST Library for GC-MS.

Table 1. Instrument Details

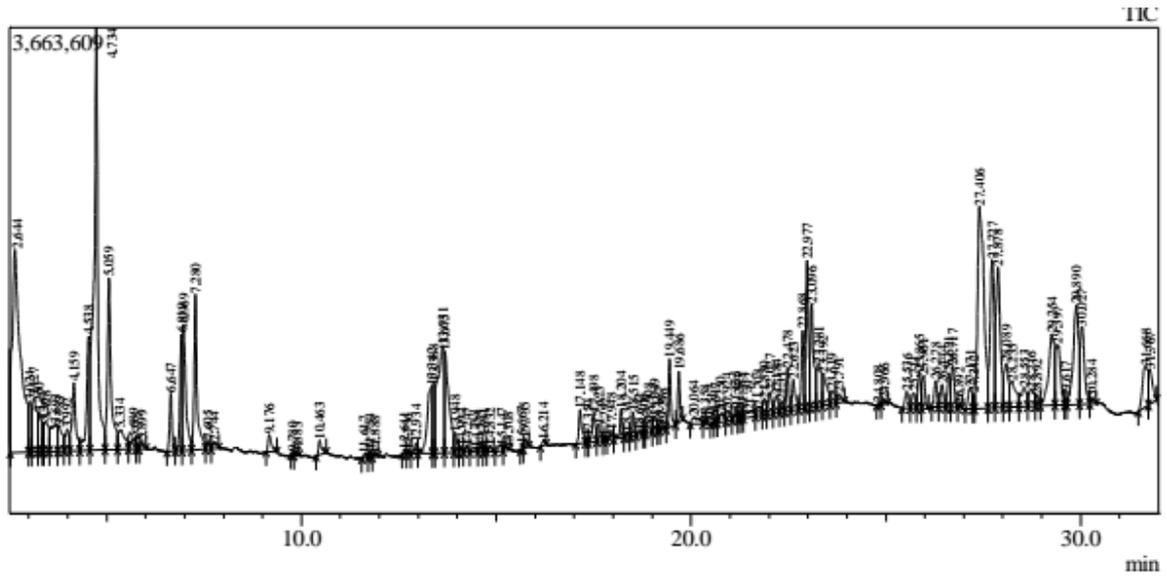
| GC-MS 2010 system | Shimadzu Analytical Pvt.Ltd. |
|-------------------------|---|
| Column | RTX-5MS |
| Carrier Gas | Helium gas |
| Mass detector | GCMS-QP2010 Ultra |
| Column oven temperature | 200°C. |
| Library Details | NIST(National Institute Standard and Technique) 8 AND NIST 8S library |

RESULTS AND DISCUSSIONS

The prime objective of the current work was to elucidate phytochemical profile of *Wagatea spicata* using GC-MS. Therefore, the Total Ion Chromatograms of all the four extracts and the detailed Peak reports containing the names of the phytochemical components identified by GC-MS along with their Retention times, Peak Areas and Peak Heights have been tabulated in figures and tables 2,3,4,5 for the extracts 1,2,3,4 respectively.

DISCUSSION

Table 2 reveal the phytochemical profile of *Wagatea spicata* thus helping in the scientific characterization of a rare plant with Ethanobotanical significance (Surange, 1986).



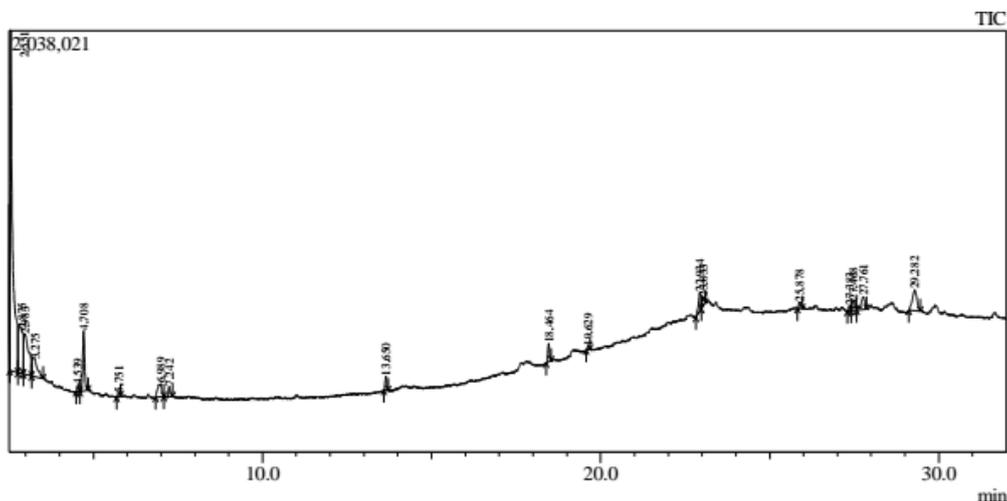


Fig. 5 Total Ion chromatogram for Extract IV

Table 2. Phytochemical components as detected by NIST Library in Extract I (Hits 1-28)

| Peak# | R.Time | Area | Area% | Height | Name |
|-------|--------|----------|-------|---------|--|
| 1 | 2.553 | 3885546 | 3.94 | 1211602 | .alpha.,.beta.-D-Glucopyranoside, 1-deoxy-1-undecyl |
| 2 | 2.623 | 19419565 | 19.68 | 1447183 | 2-O-Methyl-D-mannopyranosa |
| 3 | 3.050 | 1080039 | 1.09 | 219154 | Tetradecanoic acid |
| 4 | 3.125 | 1013632 | 1.03 | 160367 | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol |
| 5 | 3.939 | 72002 | 0.07 | 25436 | Phthalic acid, butyl tetradecyl ester |
| 6 | 3.999 | 132423 | 0.13 | 28935 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) es |
| 7 | 4.479 | 62165 | 0.06 | 19895 | Corymbolone |
| 8 | 4.592 | 60866 | 0.06 | 9103 | 1,19-Eicosadiene |
| 9 | 4.743 | 15340929 | 15.54 | 2075990 | n-Hexadecanoic acid |
| 10 | 5.048 | 476418 | 0.48 | 63556 | Hexadecanoic acid, ethyl ester |
| 11 | 5.904 | 84423 | 0.09 | 21149 | Heptadecanoic acid |
| 12 | 6.983 | 20136808 | 20.40 | 2345424 | Oleic Acid |
| 13 | 7.279 | 6885081 | 6.98 | 915147 | Octadecanoic acid |
| 14 | 10.060 | 146804 | 0.15 | 30955 | Cyclopentadecanone, 2-hydroxy- |
| 15 | 10.456 | 749659 | 0.76 | 136532 | Eicosanoic acid |
| 16 | 12.626 | 78859 | 0.08 | 22153 | Behenic alcohol |
| 17 | 12.733 | 95040 | 0.10 | 23716 | Hexadecane, 1-iodo- |
| 18 | 13.683 | 203239 | 0.21 | 55448 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) est |
| 19 | 14.475 | 68501 | 0.07 | 20720 | Tetracosane |
| 20 | 16.214 | 100310 | 0.10 | 28142 | Tetracosane |
| 21 | 17.467 | 1153 | 0.00 | 6100 | Octadecanoic acid |
| 22 | 17.658 | 436920 | 0.44 | 60815 | Sakuranin |
| 23 | 17.936 | 469438 | 0.48 | 40788 | Nonacosane |
| 24 | 18.500 | 367840 | 0.37 | 95560 | 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-I |
| 25 | 18.730 | 1843733 | 1.87 | 139264 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol |
| 26 | 19.648 | 94050 | 0.10 | 26559 | Hexatriacontane |
| 27 | 20.588 | 111683 | 0.11 | 24521 | N-Benzhydrylidene-1-(2,4,6-trimethylphenyl)ethylam |
| 28 | 21.300 | 117687 | 0.12 | 8688 | Tetracosane |

Table 3. Phytochemical components as detected by NIST Library in Extract I (Hits 29-59)

| Peak# | R.Time | Area | Area% | Height | Name |
|-------|--------|---------|-------|--------|------------------------|
| 29 | 11.754 | 230854 | 0.09 | 51312 | Behenic alcohol |
| 30 | 11.888 | 112070 | 0.05 | 26405 | Hehencosane |
| 31 | 12.641 | 123740 | 0.05 | 31564 | n-Tetracosanol-1 |
| 32 | 12.736 | 85250 | 0.03 | 20457 | Nonacosane |
| 33 | 12.934 | 320726 | 0.13 | 80605 | 1,2-Benzenedicarboxy |
| 34 | 13.342 | 4536189 | 1.85 | 502406 | Friedelan-3-one |
| 35 | 13.388 | 2089933 | 0.85 | 538966 | Friedelan-3-one |
| 36 | 13.631 | 9442216 | 3.84 | 807581 | Friedelan-3-one |
| 37 | 13.675 | 5996797 | 2.44 | 760445 | 1,2-Benzenedicarboxy |
| 38 | 13.948 | 740720 | 0.30 | 152787 | Docosanoic acid |
| 39 | 14.092 | 206089 | 0.08 | 43745 | Hexadecanoic acid, 1- |
| 40 | 14.247 | 305780 | 0.12 | 54633 | Eicosanoic acid, 2,3-b |
| 41 | 14.458 | 242781 | 0.10 | 33711 | Pentadecanoic acid, et |
| 42 | 14.575 | 267018 | 0.11 | 37769 | Oleyl Alcohol |
| 43 | 14.691 | 196406 | 0.08 | 47005 | Hexadecanoic acid, 1- |
| 44 | 14.832 | 235616 | 0.10 | 47406 | Eicosanoic acid, 2,3-b |
| 45 | 15.147 | 398158 | 0.16 | 43686 | |
| 46 | 15.308 | 178280 | 0.07 | 9365 | |
| 47 | 15.667 | 171646 | 0.07 | 48502 | 4-Methoxy-4',5'-meth |
| 48 | 15.735 | 393144 | 0.16 | 69602 | 1-Octacosanol |
| 49 | 16.214 | 171784 | 0.07 | 37834 | Octacosyl trifluoroace |
| 50 | 17.148 | 1488117 | 0.61 | 257038 | Tetracosanoic acid |
| 51 | 17.350 | 177487 | 0.07 | 40891 | |
| 52 | 17.498 | 1313799 | 0.53 | 220374 | Tetracosanoic acid |
| 53 | 17.623 | 771567 | 0.31 | 131094 | Docosanoic acid, ethy |
| 54 | 17.792 | 192693 | 0.08 | 33740 | |
| 55 | 17.948 | 388773 | 0.16 | 70523 | Docosanoic acid, ethy |
| 56 | 18.204 | 966811 | 0.39 | 223365 | Squalene |
| 57 | 18.347 | 155553 | 0.06 | 38350 | Octadecanal |
| 58 | 18.515 | 663096 | 0.27 | 184734 | Squalene |
| 59 | 18.642 | 172479 | 0.07 | 30154 | Octadecanal |

Table 4. Phytochemical components as detected by NIST Library in Extract I (Hits 60-85)

| | | | | | |
|----|--------|---------|------|---------|-------------------------|
| 60 | 18.800 | 56443 | 0.02 | 17229 | |
| 61 | 18.908 | 883235 | 0.36 | 89514 | 3,7,11,15-Tetramethyl- |
| 62 | 19.049 | 712006 | 0.29 | 114748 | Octadecanal |
| 63 | 19.167 | 137366 | 0.06 | 44973 | |
| 64 | 19.296 | 118142 | 0.05 | 26552 | Cyclohexanol, 2-(1-met |
| 65 | 19.449 | 2153645 | 0.88 | 516908 | 1-Octacosanol |
| 66 | 19.686 | 1516665 | 0.62 | 390128 | 1-Octacosanol |
| 67 | 20.064 | 594512 | 0.24 | 47441 | |
| 68 | 20.358 | 52423 | 0.02 | 13948 | |
| 69 | 20.517 | 50260 | 0.02 | 7198 | |
| 70 | 20.617 | 85310 | 0.03 | 21266 | |
| 71 | 20.750 | 621564 | 0.25 | 62622 | Hexacosanoic acid |
| 72 | 20.924 | 596373 | 0.24 | 73491 | Hexacosanoic acid |
| 73 | 21.155 | 443019 | 0.18 | 81369 | Docosanoic acid, ethyl |
| 74 | 21.233 | 188585 | 0.08 | 46391 | |
| 75 | 21.311 | 314622 | 0.13 | 70601 | Docosanoic acid, ethyl |
| 76 | 21.492 | 852942 | 0.35 | 47755 | |
| 77 | 21.708 | 694610 | 0.28 | 69205 | |
| 78 | 21.880 | 635281 | 0.26 | 124301 | Octadecanal |
| 79 | 22.027 | 1003321 | 0.41 | 165555 | Oxirane, hexadecyl- |
| 80 | 22.184 | 889167 | 0.36 | 129547 | 1-Undecene, 11,11-diet |
| 81 | 22.317 | 442788 | 0.18 | 78926 | Decane, 1,1-diethoxy- |
| 82 | 22.478 | 1501419 | 0.61 | 329179 | Cholest-22-ene-21-ol, 3 |
| 83 | 22.623 | 1339548 | 0.55 | 233216 | Tetrapentacontane, 1,54 |
| 84 | 22.868 | 2561952 | 1.04 | 592211 | Tetracosane |
| 85 | 22.977 | 5564874 | 2.27 | 1116191 | Hexacosyl heptafluorob |

Table 5. Phytochemical components as detected by NIST Library in Extract I (Hits 86-119)

| Peak# | R.Time | Area | Area% | Height | Name |
|-------|--------|-----------|--------|----------|--------------------------|
| 86 | 23.096 | 4560849 | 1.86 | 779848 | 1-Heptacosanol |
| 87 | 23.281 | 3008741 | 1.22 | 322918 | |
| 88 | 23.392 | 1762286 | 0.72 | 240307 | Octadecanal |
| 89 | 23.619 | 788605 | 0.32 | 100138 | .alpha.-Tocopherol-.bet |
| 90 | 23.791 | 449240 | 0.18 | 66458 | .alpha.-Tocopherol-.bet |
| 91 | 24.808 | 71952 | 0.03 | 19652 | Hexadecane, 1-iodo- |
| 92 | 24.966 | 134827 | 0.05 | 29054 | n-Tetracosanol-1 |
| 93 | 25.536 | 800178 | 0.33 | 126260 | Ergost-5-en-3-ol, (3.bet |
| 94 | 25.711 | 750604 | 0.31 | 119899 | Ergost-5-en-3-ol, (3.bet |
| 95 | 25.865 | 1527472 | 0.62 | 273752 | Octadecanal |
| 96 | 25.961 | 1291643 | 0.53 | 243088 | Octadecanal |
| 97 | 26.278 | 1099348 | 0.45 | 198950 | Stigmasterol |
| 98 | 26.444 | 1029352 | 0.42 | 173980 | Stigmasterol |
| 99 | 26.621 | 1376737 | 0.56 | 260233 | Z-2-Octadecen-1-ol |
| 100 | 26.717 | 1761733 | 0.72 | 307216 | Z-2-Octadecen-1-ol |
| 101 | 26.892 | 87189 | 0.04 | 20471 | |
| 102 | 27.171 | 1080034 | 0.44 | 157698 | Hexatriacontane |
| 103 | 27.242 | 325441 | 0.13 | 118428 | Tetracosane |
| 104 | 27.406 | 16977903 | 6.91 | 1523613 | 1-Triacontanol |
| 105 | 27.727 | 7834508 | 3.19 | 1115689 | .gamma.-Sitosterol |
| 106 | 27.878 | 8375082 | 3.41 | 1061400 | .gamma.-Sitosterol |
| 107 | 28.089 | 2640989 | 1.07 | 331652 | 4,4,6a,6b,8a,11,11,14b |
| 108 | 28.235 | 2001515 | 0.81 | 196192 | 1,4-Dimethyl-8-isoprop |
| 109 | 28.553 | 1518464 | 0.62 | 143648 | .alpha.-Amyrin |
| 110 | 28.716 | 1270191 | 0.52 | 131160 | .alpha.-Amyrin |
| 111 | 28.892 | 445110 | 0.18 | 74019 | |
| 112 | 29.254 | 5719878 | 2.33 | 536881 | Lup-20(29)-en-3-one |
| 113 | 29.397 | 4102998 | 1.67 | 460490 | Lup-20(29)-en-3-one |
| 114 | 29.617 | 173824 | 0.07 | 48640 | |
| 115 | 29.890 | 7007451 | 2.85 | 753615 | Lupeol |
| 116 | 30.027 | 4615518 | 1.88 | 582434 | Lupeol |
| 117 | 30.284 | 262666 | 0.11 | 44908 | 1-Naphthalenepropanol |
| 118 | 31.668 | 2875164 | 1.17 | 304642 | Stigmast-4-en-3-one |
| 119 | 31.767 | 1926941 | 0.78 | 265244 | Stigmast-4-en-3-one |
| | | 245684082 | 100.00 | 33951843 | |

119 components were detected in the Ethanolic Soxhlet extract of the whole Plant powder of which 101 phytoconstituents were identified by GCMS,NIST Library (Table 2-5).19 components were detected in the Ethanolic Soxhlet extract of the stem and all of them were identified by by GCMS(Figure 10).43 components were detected in the Ethanolic Soxhlet extract of the stem and all of them were identified by the by GCMS (Table 6).15 components were detected and identified from the aqueous, Hot macerated extract of the stem (Table 7, 8).

From Table 10 it can be elucidated that,

n-Hexadecanoic acid (Palmitic acid) is a common component in all the extracts but is observed maximum in the Ethanolic Soxhlet Extract of the Whole plant. Anti-fungal action of this component [Aparna *et al.*, 2012] explains the use of this plant in treatment of skin diseases. The anti-inflammatory activity [Aparna *et al.*, 2012] of Palmitic acid points out the potential of this plant to be used as a component of medicated oil singly or synergistically for management of Rheumatic disorders.

Table 6. Phytochemical components as detected by NIST Library in Extract II (Total Hits 1-19)

| Peak Report TIC | | | | | |
|-----------------|--------|----------|--------|---------|--|
| Peak# | R.Time | Area | Area% | Height | Name |
| 1 | 2.551 | 8680501 | 45.60 | 1644072 | 4-Trifluoromethylbenzoic acid, octadecyl ester |
| 2 | 2.875 | 2025175 | 10.64 | 211279 | Carbon dioxide |
| 3 | 2.983 | 1950907 | 10.25 | 193219 | dl-Alanyl-dl-norleucine |
| 4 | 3.275 | 969096 | 5.09 | 79298 | 2-Aminononadecane |
| 5 | 4.539 | 120981 | 0.64 | 33241 | cis-9-Hexadecenoic acid |
| 6 | 4.708 | 1187404 | 6.24 | 291216 | n-Hexadecanoic acid |
| 7 | 5.751 | 70008 | 0.37 | 19274 | cis-10-Heptadecenoic acid |
| 8 | 6.989 | 554108 | 2.91 | 61731 | Oleic Acid |
| 9 | 7.242 | 192789 | 1.01 | 50881 | Octadecanoic acid |
| 10 | 13.650 | 235636 | 1.24 | 70118 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) |
| 11 | 18.464 | 313208 | 1.65 | 89358 | 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19, |
| 12 | 19.629 | 55646 | 0.29 | 16190 | 17-Pentatriacontene |
| 13 | 22.934 | 497592 | 2.61 | 91410 | Tetracosane |
| 14 | 23.033 | 227211 | 1.19 | 42773 | 1-Pentacosanol |
| 15 | 25.878 | 130885 | 0.69 | 30119 | 14-Octadecenal |
| 16 | 27.383 | 134978 | 0.71 | 25457 | 1-Triacontanol |
| 17 | 27.468 | 318744 | 1.67 | 49290 | D-Friedoolean-14-en-3-one |
| 18 | 27.761 | 450514 | 2.37 | 60732 | .gamma.-Sitosterol |
| 19 | 29.282 | 920613 | 4.84 | 100925 | Lupeol |
| | | 19035996 | 100.00 | 3160583 | |

Table 7. Phytochemical components as detected by NIST Library in Extract III (Hits 1-28)

| Peak Report TIC | | | | | |
|-----------------|--------|----------|-------|---------|--|
| Peak# | R.Time | Area | Area% | Height | Name |
| 1 | 2.553 | 3885546 | 3.94 | 1211602 | .alpha...beta.-D-Glucopyranoside, 1-deoxy-1-undecyl |
| 2 | 2.623 | 19419565 | 19.68 | 1447183 | 2-O-Methyl-D-mannopyranosa |
| 3 | 3.050 | 1080039 | 1.09 | 219154 | Tetradecanoic acid |
| 4 | 3.125 | 1013632 | 1.03 | 160367 | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol |
| 5 | 3.939 | 72002 | 0.07 | 25436 | Phthalic acid, butyl tetradecyl ester |
| 6 | 3.999 | 132423 | 0.13 | 28935 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) es |
| 7 | 4.479 | 62165 | 0.06 | 19895 | Corymbolone |
| 8 | 4.592 | 60866 | 0.06 | 9103 | 1,19-Eicosadiene |
| 9 | 4.743 | 15340929 | 15.54 | 2075990 | n-Hexadecanoic acid |
| 10 | 5.048 | 476418 | 0.48 | 63556 | Hexadecanoic acid, ethyl ester |
| 11 | 5.904 | 84423 | 0.09 | 21149 | Heptadecanoic acid |
| 12 | 6.983 | 20136808 | 20.40 | 2345424 | Oleic Acid |
| 13 | 7.279 | 6885081 | 6.98 | 915147 | Octadecanoic acid |
| 14 | 10.060 | 146804 | 0.15 | 30955 | Cyclopentadecanone, 2-hydroxy- |
| 15 | 10.456 | 749659 | 0.76 | 136532 | Eicosanoic acid |
| 16 | 12.626 | 78859 | 0.08 | 22153 | Behenic alcohol |
| 17 | 12.733 | 95040 | 0.10 | 23716 | Hexadecane, 1-iodo- |
| 18 | 13.683 | 203239 | 0.21 | 55448 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) est |
| 19 | 14.475 | 68501 | 0.07 | 20720 | Tetracosane |
| 20 | 16.214 | 100310 | 0.10 | 28142 | Tetracosane |
| 21 | 17.467 | 1153 | 0.00 | 6100 | Octadecanoic acid |
| 22 | 17.658 | 436920 | 0.44 | 60815 | Sakuranin |
| 23 | 17.936 | 469438 | 0.48 | 40788 | Nonacosane |
| 24 | 18.500 | 367840 | 0.37 | 95560 | 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-t |
| 25 | 18.730 | 1843733 | 1.87 | 139264 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol |
| 26 | 19.648 | 94050 | 0.10 | 26559 | Hexatriacontane |
| 27 | 20.588 | 111683 | 0.11 | 24521 | N-Benzhydrylidene-1-(2,4,6-trimethylphenyl)ethylam |
| 28 | 21.300 | 117687 | 0.12 | 8688 | Tetracosane |

Table 8. Phytochemical components as detected by NIST Library in Extract III (Hits 29-43)

| Peak# | R.Time | Area | Area% | Height | Name |
|-------|--------|----------|--------|----------|--|
| 29 | 21.600 | 222878 | 0.23 | 39773 | Spiro[7H-benz[e]indene-7,1'-[2]cyclopentene]-4',9' |
| 30 | 21.749 | 1195465 | 1.21 | 131703 | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)- |
| 31 | 22.069 | 498614 | 0.51 | 38833 | Pentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)]octacos |
| 32 | 22.975 | 429490 | 0.44 | 71636 | Tetracosane |
| 33 | 23.067 | 575194 | 0.58 | 100468 | 1-Pentacosanol |
| 34 | 23.258 | 410739 | 0.42 | 37797 | Cyclodecasiloxane, eicosamethyl- |
| 35 | 23.454 | 179861 | 0.18 | 28625 | Cholesterol |
| 36 | 25.678 | 468128 | 0.47 | 72665 | Ergost-5-en-3-ol, (3.beta.)- |
| 37 | 26.402 | 424134 | 0.43 | 74246 | Stigmasterol |
| 38 | 27.828 | 1045878 | 1.06 | 163740 | .gamma.-Sitosterol |
| 39 | 28.625 | 90291 | 0.09 | 12800 | Tetracosamethyl-cyclododecasiloxane |
| 40 | 28.767 | 389878 | 0.40 | 46901 | 9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)- |
| 41 | 29.166 | 16179888 | 16.39 | 1121223 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol |
| 42 | 29.575 | 23827 | 0.02 | 7948 | 5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan- |
| 43 | 29.988 | 3023471 | 3.06 | 331975 | Lupeol |
| | | 98692549 | 100.00 | 11543235 | |

Table 9. Phytochemical components as detected by NIST Library in Extract IV (Total Hits 1-15)

| Peak Report TIC | | | | | |
|-----------------|--------|----------|--------|---------|--|
| Peak# | R.Time | Area | Area% | Height | Name |
| 1 | 2.550 | 4779724 | 10.41 | 1517818 | 1-Chloroicosane |
| 2 | 2.635 | 9559058 | 20.81 | 1158702 | N,N-Dimethylformamide trimethylene acetal |
| 3 | 4.733 | 110209 | 0.24 | 27218 | n-Hexadecanoic acid |
| 4 | 7.368 | 603133 | 1.31 | 71187 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) e |
| 5 | 8.867 | 7319879 | 15.94 | 733299 | Cyclodecasiloxane, eicosamethyl- |
| 6 | 13.675 | 7283524 | 15.86 | 831849 | Tetracosamethyl-cyclododecasiloxane |
| 7 | 15.135 | 446812 | 0.97 | 74637 | 13-Docosenamide, (Z)- |
| 8 | 16.408 | 120373 | 0.26 | 27963 | 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23 |
| 9 | 17.227 | 6132537 | 13.35 | 760039 | Tetracosamethyl-cyclododecasiloxane |
| 10 | 18.147 | 99464 | 0.22 | 23323 | Hexatriacontane |
| 11 | 20.124 | 4339984 | 9.45 | 538292 | Tetracosamethyl-cyclododecasiloxane |
| 12 | 22.658 | 2475866 | 5.39 | 346947 | Tetracosamethyl-cyclododecasiloxane |
| 13 | 25.484 | 1679209 | 3.66 | 204676 | Tetracosamethyl-cyclododecasiloxane |
| 14 | 26.895 | 61756 | 0.13 | 13284 | .gamma.-Sitosterol |
| 15 | 29.020 | 913953 | 1.99 | 112287 | Tetracosamethyl-cyclododecasiloxane |
| | | 45925481 | 100.00 | 6441521 | |

Table 10. List of biologically significant components identified from the tested extracts of *Wagatea spicata*

| S.No | Name of the component | Rt | Mol. Formula | Mol. Wt. | Area(AU) | | | | | Biological Activity |
|------|-----------------------|---------|--|----------|----------|----------|----------|----------|--|---------------------|
| | | | | | Sample 1 | Sample 2 | Sample 3 | Sample 4 | | |
| 1. | n-Hexadecanoic acid | ≈4.7min | C ₁₆ H ₃₂ O ₂ | 256 | 22276740 | 1187404 | 15340929 | 110209 | Anti-inflammatory, Antifungal, Antioxidant (Aparna, 2013) | |
| 2. | Octadecanoic acid | ≈7.2min | C ₁₈ H ₃₆ O ₂ | 284 | 6271461 | 192789 | 6885081 | -- | Antifungal, Anti bacterial, Anti tumor (Akpuaka et al., 2014) | |
| 3. | Γ Sitosterol | ≈27min | C ₂₉ H ₅₀ O | 414 | 8375082 | 450514 | 1045878 | 61756 | Antibacterial, Antifungal, Antioxidant, Anti diabetic, Anti viral (Venkata Raman et al., 2012) | |
| 4. | Lupeol | ≈29min | C ₃₀ H ₅₀ O | 426 | 4615518 | 920613 | 3023471 | -- | Anti-inflammatory, Anticancer (Mohammad Saleem, 2009) | |

Octadecanoic acid (Stearic acid): was found to be present in all extracts except the aqueous extract of stem by Hot Maceration. This could be due to the lesser content of the component in the stem and its preferable solubility in a relatively non polar solvent like ethanol [Rudi Heryanto, 2007]. Antibacterial, Antifungal [Akpuaka *et al.*, 2013] activity of this component could synergistically function with Palmitic acid in acting as a cure for skin infections.

Γ Sitosterol: is present in the whole plant extract as well the only stem extract. But the content of the component is greater in the whole plant than in the stem alone. The anti microbial, antiviral activity (Venkata Raman *et al.*, 2012) of this component thus explains the use of the bark (a component of the whole plant powder) for treating skin diseases as per the Traditional medicinal system. Ethanolic Soxhlet extraction gives maximum yield of Γ Sitosterol from this plant. This can help in using the aerial parts of the plant for therapeutic purposes without uprooting the plant.

Lupeol: was found to be present in both the whole plant extracts with a greater extraction by the Ethanolic Soxhlet Extraction.

It was also found to be present in the Ethanolic Soxhlet extract of the stem but not in the aqueous stem extract prepared by hot maceration. This could be attributed to the lesser content of Lupeol in the stem and the preferable solubility of the component in organic solvent like Ethanol (like Stearic acid)

Lupeol is well known for its anti-inflammatory activity (Mohammad Saleem, 2009) thus justifying the role of this plant in treatment of skin diseases as well as pulmonary tuberculosis which involves inflammation of the respiratory tract.

Ethanolic Soxhlet extraction can be said to be the best method of extraction of phytoconstituents from the plant parts of *Wagatea spicata*. The whole plant Ethanolic Soxhlet extract was also found to contain Squalene which is component of the skin and a common ingredient of topical formulations (Zih-Rou Huang *et al.*, 2009). The plant therefore seems to have a promising effect as a skin protecting agent when developed into a formulation. Components like α amyrene (detected in Ethanolic Soxhlet extract of the plant) and γ sitosterol (detected in all the four extracts) throw light on the anti

diabetic potential of this plant (Venkata Raman *et al.*, 2012; Singh *et al.*, 2009).

Conclusion

Thus the above study was a quick method of revealing and documenting the phytochemical profile of *Wagatea spicata*, a rare plant with a view to experimentally understand, explain and document the ethnomedical importance associated with the plant. This plant has been traditionally used in the form of its roots to cure pulmonary disorders. However in the above analysis the potential of other aerial parts of the same plant has also been brought to the fore with an environment friendly motive.

Future Prospects

The biological activities of all the components detected in the plant *Wagatea spicata* can be studied in-vivo to determine a maximum recommended starting dose for first in human clinical trials.

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