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TLC BIOAUTOGRAPHY AND LCMS-MS ANALYSIS FOR IDENTIFICATION OF COMPOUNDS HAVING INHIBITORY ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS* IN *ABIES WEBBIANA* LEAVES EXTRACT

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Keywords:

Abies webbiana, HPTLC, TLC bioautography, LCMS-MS, Antimicrobial activity

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ABSTRACT: This study was performed to identify the antimicrobial compounds in *Abies webbiana* leaves. The antimicrobial compounds were identified by LCMS-MS. The microbial inhibitory activity was evaluated on *Staphylococcus aureus ATCC 6538* using REMA technique and Agar-overlay TLC bioautography. The chloroform extract of *Abies webbiana* leaves was used for the study. The MIC against *Staphylococcus aureus* was found to be 195.3 µg/ml- 390.6 µg/ml. A mobile phase comprising of toluene: ethyl acetate: glacial acetic acid (9:1:0.3) was used for identification of antimicrobial compounds present in the extract by TLC bioautography. Totally 16 bands were observed in the HPTLC chromatogram when the plate was observed at 254 nm. The band observed at R_f = 0.58 showed the antimicrobial activity. Preparative TLC technique was used to isolate this active band. The compounds present in the isolated band were then identified by LC-QTRAP. Totally 7 compounds were identified to be present in the isolated bands of chloroform extract. The compounds identified include Betuloside, 2,7-Dihydroxy-4'-methoxyisoflavone, Geinstein 7-O-beta-D-glucoside, β-Sitosterol, Abietane, Coniferol, and 1-(3,4-Dihydroxyphenyl)-1-decene-3,5-dione-Pos. This study was useful in identifying the compounds that show synergistic antimicrobial activity. Structural characterization of plant metabolites in *Abies webbiana* leaves exhibiting synergistic growth inhibition of *S. aureus* might serve as 'lead' molecule for developing antimicrobial agents.

INTRODUCTION: Traditional medicinal plants play a significant role in dealing with all the health-related problems in our day to day life.

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Fighting against microorganisms and developing alternate medicines for microbes that are resistant to antibiotics have become one of the major areas of research. Herbal medicines are advantageous due to a reduced risk of side effects, low cost, and effective in chronic conditions.

The WHO has stated that about 70-80% of the people in developing countries have resorted to the use of complementary or alternative medicines at one point in time or another [1].

Abies webbiana is a plant belonging to family Pinaceae and commonly known as Talispatra in Bengali and Hindi, Talispatrum in Sanskrit & Indian fir in English. Talispatra leaves have been reported to act as a febrifuge, central nervous system depressant, antibacterial, antifungal, antitumour, antispasmodic, anti-tussive, female anti-fertility, mast cell stabilizing, anxiolytic, anti-rheumatic and is useful in treating dental problems, The major chemical constituents reported in the leaves extract were 1-(4'-methoxyphenyl)-aziridine, Betuloside, β-Sitosterol, N- triacontanol, Abietane, Abeisin [2-7].

Certain chemical constituents, mainly monoterpenes (from essential oil), flavonoids, biflavonoid glycosides, phytosterols and diterpene glycosides (taxol-like compounds) were isolated from *A. webbiana* leaf. The anti-inflammatory effect was exhibited by (+)-pinitol which was isolated from the leaf [4, 7-12]. A new aziridine alkaloid (1-(4'-methoxyphenyl)-aziridine) was isolated from the leaves of *A. webbiana* [3]. The predominant components reported in the methanolic extract of *Abies webbiana* were benzenepropanol, 4-hydroxy-à-methyl, 2-furancarboxaldehyde, and 5-(hydroxymethyl) [13]. Isolation of 4'-hydroxy quercetin was done from ethyl acetate extract of *Abies webbiana* leaves [14]. Chemical fingerprinting of *Abies webbiana* was performed using different analytical techniques to differentiate this plant from others [15].

Although, there are several studies focusing on identification of various phytochemical constituents present in the *Abies webbiana*, the main objective of this study was to identify the active compounds in the *Abies webbiana* leaves extract that were responsible to demonstrate significant activity against Gram-positive *S. aureus*, using Agar overlay TLC bioautography and further structural identification by LCMS-MS.

MATERIALS AND METHODS:

Collection of Plant Material: *Abies webbiana* as a sample of whole leaves & powdered leaves was procured from Yucca Enterprises and was authenticated from Dr. Harshad M. Pandit, formerly Head and Associate Professor of Botany, G. N. Khalsa College, Mumbai (Authentication number: ada p 1050418)

Preparation of Chloroform Leaves extract: *Abies webbiana* leaves powder was extracted using Successive cold maceration technique. The successive extraction was done using various solvents in the following sequence- Hexane, Toluene, Chloroform, Acetone, Methanol and Distilled water. About 25 grams of crude powder was weighed and transferred in a 500 ml beaker, and 250 ml of each solvent was added for successive extraction. Maceration was carried out for 24 h, and at the end of each extraction cycle, the marc was separated out by vacuum filtration and was taken ahead for further extraction with the next solvent. The filtrate of each solvent was reduced under pressure using rotary evaporator, and the dry powder obtained was stored in the refrigerator at 4 ºC. The stock solution was prepared by dissolving the dried extract in DMSO of 1000 µg/ml concentration and was further analyzed for its antimicrobial activity using the REMA technique.

Phytochemical Tests: [16] The phytochemical tests for the presence of secondary metabolites were performed on each solvent extract as per the standard procedures.

Bacterial Strains: The bacteria used was the Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538. The bacterial culture was maintained on Mueller Hinton (MH) agar (Oxoid, Basingstoke, UK) at 4 °C and cultured in MH broth at 37 °C.

Anti-Microbial Activity testing by Resazurin Microtitre Assay (REMA) Method: Resazurin dye (0.02% w/v) was prepared by dissolving 10 mg of resazurin sodium in 50 mL sterile water. The solution was homogenized using cyclomixer. Resazurin is an oxidation-reduction indicator which shows the growth of microorganisms in cytotoxicity assays. It is purple, non-fluorescent and non-toxic dye. It is reduced to Resorufin by the viable cells and turns pink and fluorescent. Resazurin Microtiter assay method (REMA) was performed under aseptic conditions using 96 well microtitre plates (Tarson). Minimum inhibitory concentration (MIC) of the extract was also determined by REMA [17].

The first column wells were filled with 200 µL of sterile water. All other wells of the microtiter plate were filled with 100 µL of MH broth.

Ciprofloxacin was taken as the standard antibacterial agent, and a solution of 1000 ppm was prepared for the assay. Ciprofloxacin stock solution (100 µL) was added in well A2 and serial dilution was achieved by transferring 100 µL of Ciprofloxacin solution from row A2 to the subsequent wells and till well B12. The solution volume of 100 µL was discarded from the last well B12. Thus, the total volume maintained in each well was 100 µL. *Abies webbiana* leaves chloroform extract (50 mg/ml) was added (100 µL) in wells C2, D2 and E2 and serial dilution were achieved by transferring 100 µL from C2 to C12, D2 to D12 and E2 to E12 respectively. From the last well, 100 µL solution was discarded and the total volume maintained in the wells was 100 µL. DMSO (100 µL) was added in well F2 and serially diluted till well F12. The solution volume of 100 µL was discarded from well F12, thus maintaining the total volume of 100 µL in these wells. The broth volume of 200 µL was added in wells H2-H12.

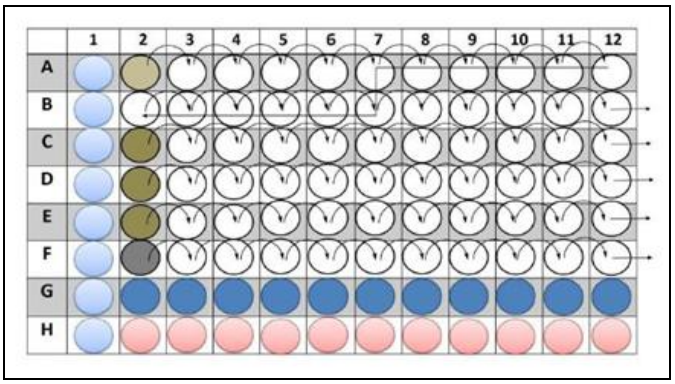


FIG. 1: MICROTITER PLATE FOR STUDYING ANTIMICROBIAL ACTIVITY BY REMA

Each microtiter plate had the following set of controls:

Column A1-H1: Sterile water (200 µL).

Row A2-A12 & Row B2-B12: Standard antibacterial agent (Ciprofloxacin).

Row C2-C12 & D2-D12 & E2-E12: Test extract (*Abies webbiana* leaves chloroform extract).

Row F2-F12: Vehicle control (DMSO).

Row G2-G12: Negative control (200 µL of broth).

Row H2-H12: Growth control (100 µL broth + 100 µL microbial suspension).

Stabilizing microbial count is an essential step to get accurate results as color development depends on cell density. After the attainment of some visible turbidity, stabilization of microbial count was done by taking OD at 550 nm and was adjusted to 0.1. The diluted microbial suspension (100 µL) was added to all the wells except negative control (well G2-G12) and Positive control (well H2-H12). The broth solution (100 µL) was added to negative control wells. Sterile Resazurin solution (30 µL) was added to each well. The microplates were covered with sterile aluminum foil to prevent evaporation of solvents and cross contamination. The microtiter plates were incubated for 24 h at 37 °C in a temperature-controlled incubator. The microtiter plates were observed for the color change visually under laminar air flow unit. The color change from purple to pink indicated the growth of micro-organisms. The microtiter plate depicting serial dilutions is shown in **Fig. 1**. All the experiments were performed in triplicates.

Identification of Antibacterial Compound using HPTLC: HPTLC analysis for *Abies webbiana* leaves chloroform extract was carried out using a CAMAG HPTLC system. Sample solutions were applied onto the plates with semi-automatic TLC sampler Linomat V (Camag, Muttenz, Switzerland) and were controlled by Win CATS software 1.4.7. Plates were developed in 10 cm × 10 cm twin trough chamber (Camag, Muttenz, Switzerland). A TLC scanner 4 was used for scanning the TLC plates and CAMAG Reporter 3 system for photo documentation of TLC plates. Chromatographic separation of the phytochemical constituents was achieved on pre-coated silica gel aluminum plates 60F254 (E.Merck, Darmstadt, Germany) having 0.2 mm thickness. The plates were prewashed with methanol and activated at 60 ºC for five minutes before chromatography. The plates were air dried after development and were scanned under UV 254 nm and 366 nm to observe the separation obtained. The optimized mobile phase comprised of toluene: ethyl acetate: glacial acetic acid (9:1:0.3 v/v) and the concentration of extract taken was 5 mg/ml.

TLC Bioautography: The localization of antibacterial compounds present in *Abies webbiana* leaves chloroform extract were identified by Agar-overlay TLC bioautography technique. The developed TLC plates were placed in a sterile Petri

plate (150 mm). The inoculum of *S. aureus* containing 10^8CFU/ml in 10 ml of molten Mueller-Hinton agar was poured over the TLC plate. After solidification, the TLC bioautographic plate was incubated for 24 h at 37 ºC. The solution of Iodonitrotoluene (20% w/v) was prepared as an indicator, and 5 mL was poured onto the above TLC bioautographic Petri plate. No incubation was done for these as the color change was instantaneous. The region of the white patch against the pink background on the TLC bioautographic plate indicated the bacterial growth inhibition and thereby the presence of active compounds. The R_f of this inhibitory band was recorded, and further isolation was done by Preparative TLC method. Ciprofloxacin was taken as the standard anti-bacterial agent. Preparative TLC plates of 1.0 mm thickness were made using the same stationary phase, and the plate was developed using the optimized mobile phase. The band of interest was isolated and reconstituted in 10 ml of chloroform. This solution was centrifuged using ultra-centrifuge for 20 min at the speed of 4000 rpm. The supernatant was filtered using Whatman filter paper. The excess solvent in the filtrate was kept for evaporation that resulted in the dried isolated band containing active compounds of the extract.

Identification of Unknown Compounds using LCMS-MS: The isolated band was then further analyzed using LCMS-MS technique. The SCIEX QTRAP® 4500 system coupled to the SHIMADZU NEXARA HPLC System was used for the analysis.

HPLC Conditions: The isolated band was injected through the SHIMADZU NEXARA LC System at a flow rate of 0.5ml/min. The mobile phase A consisted of 5 mM Ammonium formate with 0.1% Formic acid in Water and mobile phase B was 5 mM Ammonium formate with 0.1% Formic acid in Methanol. The column used for the analysis was Phenomenex Kinetex C18, 100 × 3 mm, 2.8 µ column. The column oven temperature was maintained at 40 ºC and the autosampler temperature was maintained at 10 ºC. The samples were run for 30 min at a flow rate of 500 µL/min with the gradient program mentioned below. The injection volume was 10 µL.

Mass Spectrometric Conditions: The SCIEX QTRAP® 4500 system was used with the IonDrive™ Turbo V ion source and the electrospray ionization probe for the analysis. The QTRAP® 4500 system is a Triple Quadrupole Linear Ion Trap system with improved data acquisition by Information Dependent Acquisition (IDA) workflow. A survey Enhances MS (EMS) scan was performed to obtain the precursor masses present in the extract. This was followed by Enhanced Product Ion (EPI) scan. EPI scans are called 'enhanced' because fragments are accumulated in Q3 of the mass spectrometer, giving better signal-to-noise for the detected MS/MS spectra. The details of the MS parameters used are mentioned in **Table 2**.

The extract was injected in both positive and negative polarity. Data were acquired over a mass range of 100 - 1000 Da m/z with IDA MS/MS performed over the mass range of 50-1000 Da m/z with a collision energy of 35 eV and a spread of ±15 eV. The ionspray capillary voltage was kept at 5500V, GS1 and GS2 was 50 psi and 50 psi respectively. Curtain gas was maintained at 25 psi. The source temperature was maintained at 550 °C. The LC gradient program and MS/MS parameters used for the studies are given in **Table 1** and **Table 2,** respectively.

TABLE 1: GRADIENT PROGRAM

Time (min)	Flow (mL/min)	%B
0.01	0.5	10
3	0.5	15
10	0.5	40
15	0.5	60
22	0.5	80
26	0.5	80
27	0.5	10
30	0.5	stop

TABLE 2: MS PARAMETER DETAILS

Parameters	Period-1-Experiment-1	Period-1-Experiment-2	Period-1-Experiment-3
Scan Type	Enhanced MS (EMS)	Enhanced Resolution (ER)	Enhanced Product Ion (EPI)
Polarity	Positive	Positive	Positive
Scan mode	Profile	Profile	Profile
Ion source	Turbo Spray	Turbo Spray	Turbo Spray
#Scans to sum	1	1	1

		Resolution Q1 Open	30.00 Da Resolution Q1Unit
Scan rate	10000 Da/s	250 Da/s	10000 Da/s
Intensity Thres	0.00 cps	0.00 cps	0.00 cps
Settling Time	0.0000 msec	0.0000 msec	0.0000 msec
MR Pause	1.5000 msec	15.0000 msec	1.5000 msec
Q0 trapping	No	No	No
MCA	No	No	No
Center / Width	No	Yes	No
LIT fill time	5.00 msec	5.00 msec	20.00 msec
Dynamic fill time	On	-	On
Mass range	100 to 1000 Da	-	50-1000 Da
CUR	25	25	25
CAD	-2	-2	-2
IS	5500	5500	5500
TEM	550	550	550
GS1	50	50	50
GS2	50	50	50
DP	80	80	80
EP	10	10	10
CE	10	10	35
CES	0	0	15

The targeted analysis was done for identification and confirmation of unknown compounds by structural elucidation using PeakView® software and MasterView™ Software.

RESULTS AND DISCUSSION:

Phytochemical Tests: Initially, phytochemical testing of *Abies webbiana* leaves chloroform extract was performed to identify the types of secondary metabolites present in the extract and is shown in **Table 3**. Secondary metabolites such as alkaloids, flavonoids, carbohydrates, and tannins were found to be present in the extract.

TABLE 3: PHYTOCHEMICAL TESTING FOR THE CHLOROFORM EXTRACT OF *ABIES WEBBIANA* LINN.

Test		Result + (Present) / - (Absent)
Test for Alkaloids	Dragendroff's test	+
	Meyer's test	+
	Hager's test	+
Test for Flavanoids	Shinoda test	+
Test for Saponin	Foam test	-
Test for Steroids	Salkowski reaction	-
	Lieberman Burchard reaction	-
Test for Carbohydrates	Molisch's test	+
Test for Tannins	Lead acetate test	+
	Dilute HNO_3 test	-

Anti-Microbial Activity Testing of *Abies webbiana* leaves Chloroform extract by REMA Method: Out of all the extracts, chloroform extract showed comparatively better antibacterial activity against *Staphylococcus aureus* ATCC 6538. The MIC values obtained for the extract is shown in **Fig. 2** and was found to be in the range from 195.3125 µg/ml - 390.625 µg/ml. Ciprofloxacin was taken as the standard antibacterial agent. After the overall observation, it can be concluded that the chloroform extract of *Abies webbiana* leaves have the potential to inhibit *Staphylococcus aureus ATCC 6538* and can be used as a potential source of antimicrobial compounds.

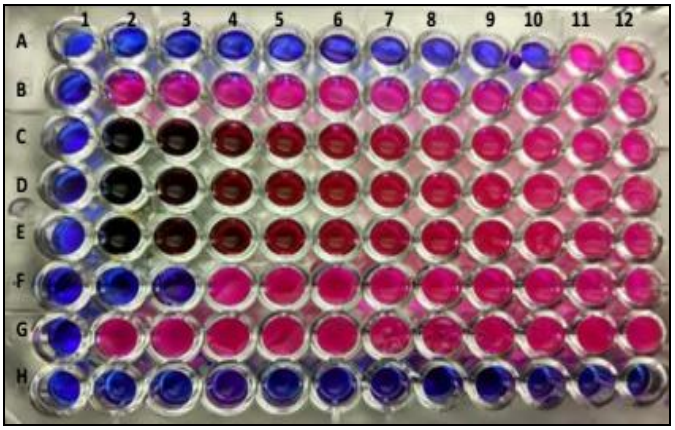


FIG. 2: ASSESSMENT OF REMA RESULTS FOR *ABBIES WEBBIANA* CHLOROFORM EXTRACT (50mg/mL) AGAINST *STAPHYLOCOCCUS AUREUS* ATCC 6538

*pink color well- indicates the growth of micro-organisms

*blue color well- indicates inhibition of micro-organisms

Observation: MIC of Chloroform extract was found to be 195.3125 µg/ml - 390.625 µg/ml against *Staphylococcus aureus* ATCC 6538.

Thin Layer Chromatography: HPTLC was performed initially to separate the various components present in the chloroform extract of *Abies webbiana* leaves. The total number of bands observed in the extract was found to be 16 when viewed under short UV radiation (254 nm), and 7 bands when viewed under long UV radiation (366 nm). The TLC plate observation is shown in **Fig. 3**, and the HPTLC chromatograms obtained are shown in **Fig. 4** and **Fig. 5**.

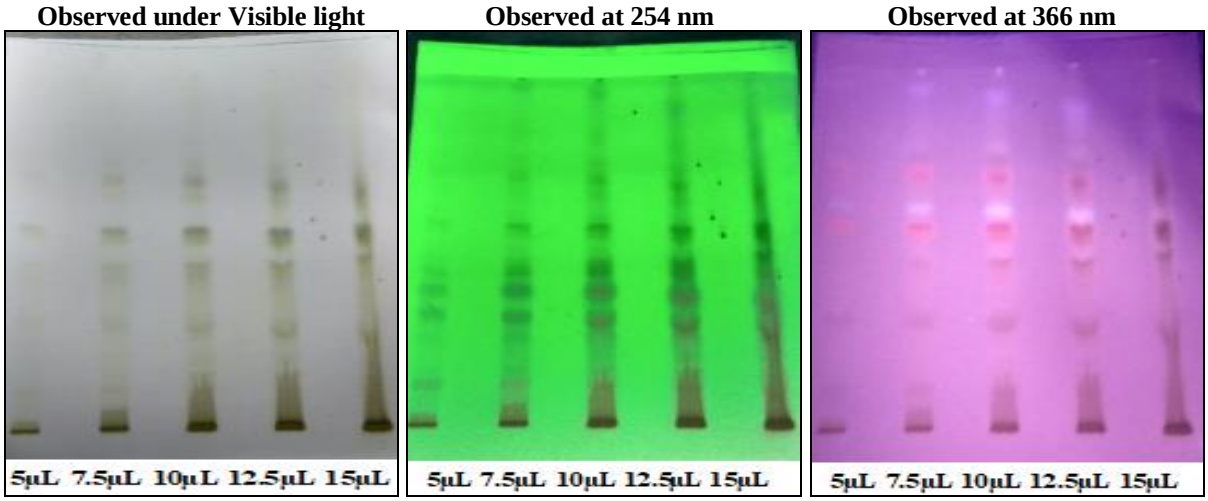


FIG. 3: TLC PLATE OBSERVATION OF *ABIES WEBBIANA* LEAVES CHLOROFORM EXTRACT (50 mg/mL) AT DIFFERENT WAVELENGTHS

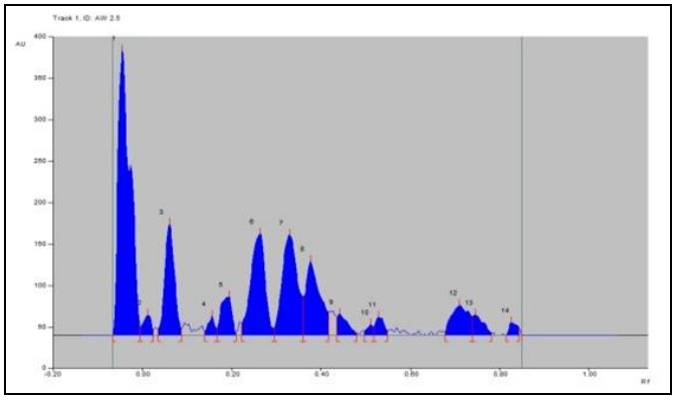


FIG. 4: HPTLC CHROMATOGRAM OF *A. WEBBIANA* LINN. CHLOROFORM EXTRACT (AT 254 nm)

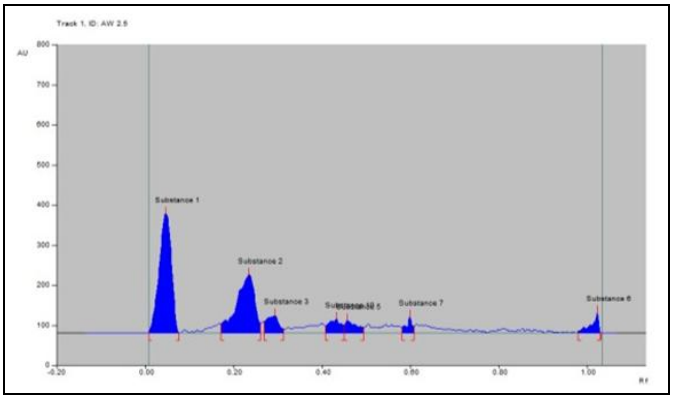


FIG. 5: HPTLC CHROMATOGRAM OF *A. WEBBIANA* LINN.CHLOROFORM EXTRACT (AT 366 nm)

TLC-Bioautography: The bands responsible for showing antibacterial activity against *S. aureus* ATCC 6538 were observed by using Agar-overlay TLC Bioautography technique. The band observed at R_f = 0.58 showing white color patch was found to have inhibitory activity against *Staphylococcus aureus* ATCC 6538 and is shown in **Fig. 6**. This band was further isolated by using preparative TLC and LCMS-MS analysis was performed for structural identification of the antimicrobial compounds in chloroform extract of *Abies webbiana* leaves. The microtiter plate depicting a set of controls for studying antimicrobial activity against *Staphylococcus aureus* is shown in **Fig. 1.**

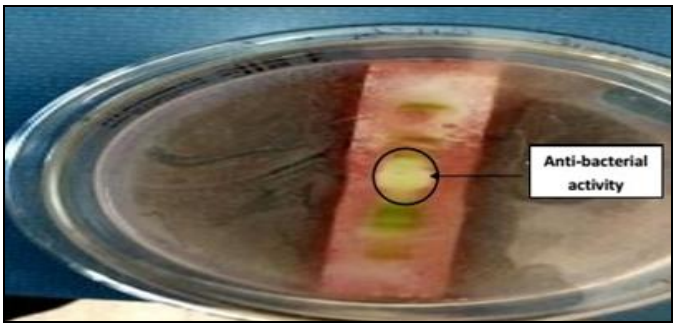


FIG. 6: TLC BIOAUTOGRAPHY DEPICTING THE BAND SHOWING ANTIBACTERIAL ACTIVITY

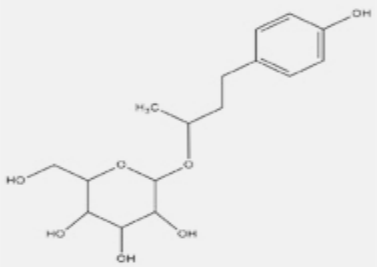
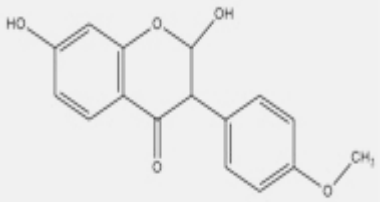
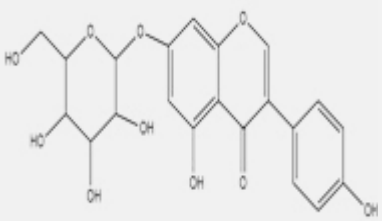
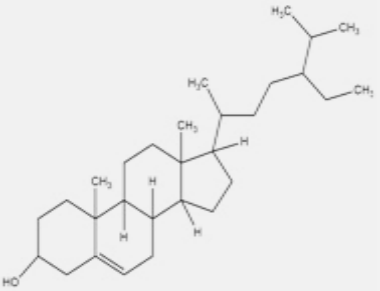
Identification of Unknown Compounds using LC-MS-MS: The IDA MS and MS/MS data was used for simultaneous identification of metabolite

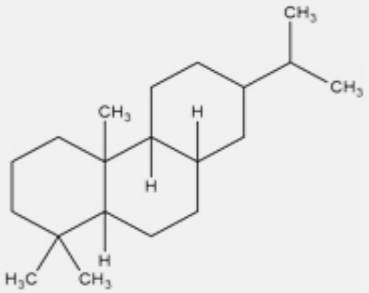
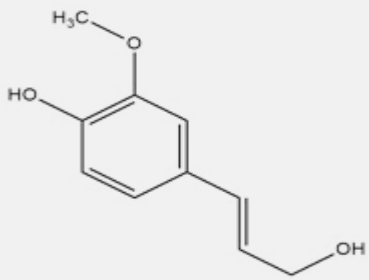
precursor masses followed by confirmation of metabolites and MS/MS fragmentation pattern matching. Using the accurate mass elemental composition analysis by FormulaFinder in the PeakView® and MasterView™ Software, the suggested formulas were linked to ChemSpider to automatically generate the list of possible compounds with the predicted formula. The metabolite structure was imported by ChemSpider for identification and confirmation. This was done for structural elucidation by theoretical and experimental fragmentation pattern matching. This workflow helped to identify and confirm the presence of the metabolites mentioned in **Table 4** in both the positive and negative ionization mode. Thus, the 7 metabolites identified were: Betuloside, 2,7-Dihydroxy-4'-methoxyisoflavanone, Genstein 7-O-beta-D-glucoside, β-Sitosterol, Abietane, Coniferol, and 1-(3,4-Dihydroxyphenyl)-1-decene-3,5-dione-Pos, and their reported activities is listed in **Table 5.**

TABLE 4: LIST OF PLANT SECONDARY METABOLITES IDENTIFIED

S. no.	Compound Name	m/z	RT (min)	Ionization Mode
1	Betuloside	327.1	21.16	Negative
2	2,7-Dihydroxy-4'-methoxyisoflavanone	285.0	18.68	Negative
3	Genistein7-O-beta-D-glucoside	431.2	19.69	Negative
4	β-Sitosterol	415.1	18.98	Positive
5	Abietane	277.1	20.2	Positive
6	Coniferol	181.1	13.64	Positive
7	1-(3,4-Dihydroxyphenyl)-1-decene-3,5-dione-Pos	277.1	20.13	Positive

TABLE 5: REPORTED ACTIVITIES OF THE IDENTIFIED SECONDARY METABOLITES IN *ABIES WEBBIANA* LEAVES CHLOROFORM EXTRACT

S. no.	Compound- phytochemical class	Structure	Reported Activity
1	Betuloside- arylbutanoid glycoside)[18, 19]		Anti-inflammatory, Analgesic, Diuretic
2	2,7-Dihydroxy-4'-methoxyisoflavanone- isoflavone[20]		Selective Growth Inhibitory Effect of Biochanin A (5,7-Dihydroxy-4'-methoxyisoflavanone) Against Intestinal Tract Colonizing Bacteria
3	Genistein7-O-beta-D-glucoside-isoflavone[21]		Antiviral activity
4	β-Sitosterol- phytosterols[22]		*Staphylococcus aureus, Escherichia coli*

5	Abietane- diterpene[23]		Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria
6	Coniferol- monolignols[24]		*E. coli*
7	1-(3,4-Dihydroxyphenyl)-1-decene-3,5-dione-Pos		No information

CONCLUSION: The study confirms the efficacy of chloroform extract of *Abies webbiana* leaves as natural antimicrobial effective against *S. aureus*. The results obtained in our study proves that TLC-bioautography was effective in the identification of active bands exhibiting growth inhibitory activity against *S. aureus*.

The active bands subjected to LC-MS/MS analysis helped in the identification of metabolites as bioactive compounds. These metabolites can be concluded as 'lead' molecules exhibiting synergistic inhibitory activity against *S. aureus* and may lead to the development of plant-derived antimicrobial agents.

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CONFLICT OF INTEREST: The authors confirm that this article content has no conflicts of interest.

REFERENCES:

1. http://www.searo.who.int/entity/health_situation_trends/who_trm_strategy_2014-2023.pdf?ua=1(accessed 26 October 2018)
2. Gautam S and Sachan N: Evaluation of the antidepressant activity of ethanolic extract of *Abies webbiana* and *Berberis aristata* in laboratory animals. Journal of Drug Delivery and Therapeutics 2019; 9(1): 244-47.
3. Ghosh A, Sen and Bhattacharya S: A new alkaloid isolated from *Abies webbiana* leaf. Pharmacognosy Res 2010; 2(3): 186-89.
4. The wealth of India, raw materials, New Delhi Publication and Information Directorate, CSIR 1A, Anonymous, 2010.
5. Kirtikar KR and Basu BD: Indian medicinal plants. Second edition, Vol. 3 1993.
6. Nadkarni K, Nadkarni A and Chopra R: Indian Materia Medica. Popular Prakashan, Vol. 1: 2007.
7. Khare CP: Indian medicinal plants- An illustrated dictionary. Springer publisher 2007.
8. Ghosh A and Bhattacharya S: A Nitrogenous Compound Isolated from Abieswebbiana Leaf. Der Pharma Chemica 2010; 2(3): 205-08.
9. Chatterjee A, Kotoky J, Das K, Banerjee J and Chakraborty T: Abiesin A, bioflavonoid of *Abies webbiana*. Phytochemistry 1984; 23(2): 704-05.
10. Singh R, Pandey B, Tripathi M and Pandey V: Antiinflammatory effect of (+)-pinitol isolated from *Abies pindrow* leaves. Fitoterapia 2001; 72: 168-70.
11. Chatterjee A and Pakrashi SC: The Treatise on Indian Medicinal Plants. Council of Scientific & Industrial Research. Publications & Informative Directorate, India: CSIR 13, 1991.
12. Tiwari K and Minocha P: A chalcone glycoside from *Abies pindrow*. Phytochemistry 1980; 19: 2501-03.
13. Rajalakshmi P, Vadivel V, Ravichandran N, Sudha V and Brindha P: Pharmacognostic evaluation of *Abies webbiana* leaf: a Siddha herbal ingredient. Asian Journal of Pharmaceutical and Clinical Research 2016; 9(4): 213- 19.
14. Yadav D and Ali M: Isolation of flavonoid from *Abies webbiana* leaves and its activity. Pharmacognosy Journal 2016; 8(4): 341-45.

15. Vadivel V and Anand P: Chemical Fingerprints of an Indian Traditional Herbal Drug Talisapatra (*Abies webbiana*) and Comparison with English yew (*Taxus baccata*). International Journal of Pharmacognosy and Phytochemical Research 2018; 10(2): 84-91.
16. Khandelwal K: Practical Pharmacognosy. Nirali Prakashan, 19th edition 2008.
17. Khalifa R, Nasser M, Gomaa A, Osman N and Salem H: Resazurin microtiter assay plate method for detection of the susceptibility of multidrug-resistant *Mycobacterium tuberculosis* to second-line anti-tuberculous drugs. Egyptian Journal of Chest Diseases and Tuberculosis 2013; 62(2): 241-47.
18. Kim M, Nugroho A, Choi J, Park J and Park H: Rhododendrin, an analgesic / anti-inflammatory arylbutanoid glycoside, from the leaves of *Rhododendron aureum*. Archives of Pharmacal Research 2011; 34(6): 971-78.
19. Zhang B, Li J, Zhang D, Ding Y and Du G: Analgesic and anti-inflammatory activities of a fraction rich in gaultherin isolated from *Gaultheria yunnanensis* (Franch.), Rehder Biol Pharm Bull 2007; 30(10): 465-69.
20. Sklenickova O and Flesar J: Selective Growth Inhibitory Effect of Biochanin A (5,7-Dihydroxy-4'-methoxy-isoflavanone)Against Intestinal Tract Colonizing Bacteria. Molecules 2010; 15: 1270-79.
21. Donovan S, Andres A, Mathai R, Kuhlenschmidt T and Kuhlenschmidt M: Soy formula and isoflavones and the developing intestine. Nutr Rev 2009; 67: S192-S200.
22. Ododo M, Choudhury M and Dekebo A: Structure elucidation of β-sitosterol with antibacterial activity from the root bark of *Malva parviflora*. Springer Plus 2016; 5(1): 1210.
23. González M: Aromatic abietane diterpenoids: their biological activity and synthesis. Natural Product Reports 2015; 32(5): 684-04.
24. Makwana S, Choudhary R, Haddock J and Kohli P: *In-vitro* antibacterial activity of plant-based phenolic compounds for food safety and preservation. LWT - Food Science and Technology 2015; 62(2): 935-39.

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