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Antimicrobial Activity and Isolation, Identification, Characterization of Phytochemicals from *Seetzenia lanata* (Willd.) Bullock

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Abstract

Multiple drug resistance in microorganisms is increasing day by day. It is imperative to search for some novel, effective, safer chemical compound that is capable of inhibiting the growth of pathogenic microorganisms. Plants are one of the major sources of biologically active chemical compounds. Phytochemicals and their biological activities in many plants are not fully known. Likewise nonpolar phytochemicals present in *Seetzenia lanata* (Willd.) Bullock has not yet been reported. The objective of the present study is to report the presence of primary metabolites, ash content, moisture content, and antimicrobial activity in *Seetzenia lanata*. In conjunction with that GC-MS analysis was also conducted to identify and characterize nonpolar phytochemicals in the plant. Resulting in the study reports a total of 28 phytochemicals in *Seetzenia lanata* for the first time. Acetone crude extract is capable to inhibit the growth of *T. rubrum*, *M. canis*, *E. floccosum*, *P. chrysogeum*, *C. albicans* and *B. subtilis*.

***Key words*:** Antimicrobial, Medicinal plant, Primary metabolites, GC-MS, *Seetzenia lanata* (Willd.) Bullock

Multiple drug resistance in pathogenic microorganisms has been a dire issue for many years. It is one of the major causes of premature death in many impoverished and developing countries. Nowadays even developed countries are also reporting this type of premature death. A new study on death related to pathogenic microorganisms estimated approximately 4.95 million deaths by multiple drug-resistant microorganisms in 2019 [1]. Term multiple drug resistance Is used for those pathogenic microorganisms that had developed themselves to survive in presence of antimicrobial compounds. In many populations, herbal remedies are one of the major alternative pathways to treat pathogenic microorganisms and many medical conditions [2]. Antibiotic resistance is now becoming a global problem and it is affecting global health, food security and economy. Population from many poor and developing countries used herbal remedies as a primary treatment to treat medical conditions [3-5]. Plants are a tremendous source of biologically active phytochemicals [6-10]. These phytochemicals are reported to treat many medical conditions. However, the Identification and characterization of phytochemicals and antimicrobial activity present in many plants like *S. lanata* are not yet reported. *S. lanata* belongs to the family of Zygophyllaceae reported as a rare plant species in many literatures. It is commonly known by the name 'dhakri' in Rajasthan, India [11-13]. Objective of the present study is to identify the presence of water content, non-burnable ash content, and primary metabolites in *S. lanata*. Simultaneously antimicrobial activity with less toxic acetone crude extract was also calculated with eight microorganisms (*Trichophyton rubrum*, *Microsporum canis*, *Epidermophyton floccosum*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Penicillium chrysogenum*, *Aspergillus niger* and *Candida albicans*). Apart from that GC-MS analysis was also conducted to identify and characterize the phytochemical present in *S. lanata*.

MATERIALS AND METHODS

Collection of plant material: *S. lanata* was harvested from Thar desert, Latitude: 27.143876 and Longitude: 72.328292 (Bagga, Phalodi, Rajasthan, India). After harvesting the plants were washed thoroughly under tape water followed by distilled water. Cleansed plant samples were shade dried (Non-flowering and fruiting, complete plant), followed by the dried plant material being powdered in pulverized machine. Identification of plant was done by the Botanical Survey of India (BSI), Jodhpur, Rajasthan, India.

Estimation of moisture content percentage

Moisture content is used to determine the amount of water in the plant sample. It was calculated by taking five grams of freshly collected plant (triplicate) followed by drying it at

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room temperature (10 days). After drying, the dried plant sample was weighed. Moisture content percentage was calculated by formula [14].

$$\text{Percentage moisture content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Estimation of Ash content percentage: The measurement of non-burnable ions and material can be calculated by this method. It was calculated by taking five grams of powdered dried plant (triplicate) samples in crucibles. Followed by that the crucibles were kept in muffle furnace. After that muffle furnace was heated for 7 hrs at 650°C (1202°F). After heating, the blazed plant material was weighed. The percentage ash content was calculated by the following formula [15-16].

$$\text{Percentage ash content} = \frac{\text{Pre weight} - \text{Ash weight}}{\text{Pre weight}} \times 100$$

Estimation of carbohydrates: Free sugar was estimated by crushing 0.5-gram powder plant material in 5 ml 80% ethanol. Followed by that the crushed mixture was Centrifuged for 15 minutes at 12000 RPM. After that, the supernatant was collected and assorted with 5 ml sulphuric acid. After that 1 ml 5% phenol was mixed and the mixture was incubated for 20 minutes at room temperature. Finally, the reading was taken with a spectrophotometer. 490 NM wavelength was used. 80% ethanol was used as a blank. The standard was prepared by glucose [17].

Estimation of protein: Total protein was estimated by Folin- Colcalteu reagent-based Lowry method. 10 ml of 0.2 molar phosphate buffer (28% of 0.2 M, diabetes sodium phosphate and 72% of 0.2 M, monosodium phosphate) was used to crush 100 mg of plant sample. The standard was prepared by bovine serum albumin (BSA). 660 NM Wavelength was used to estimate protein content in the plant sample [17-18].

Estimation of lipid: Total lipid was estimated by crushing 1 gram powder plant sample in 10ml distilled water. After crushing 20 ml Chloroform was added and crushed. Now 10 ml methanol was added, mixed and incubated for 20 minutes in room climate. Followed by that 10 ml distilled water was added and mixed. Finally, the mixture was filtered with filter paper and collected in Petri plate and dried. The dried filtrate was measured by weighing balance [19].

Antimicrobial activity: Agar well diffusion method was adopted to test antimicrobial activity in the extract of *S. lanata*. The crude extract was prepared by maceration method. In the ratio of 1:10, 10 grams of the powdered plant was assorted in 100 ml acetone. Followed by that the mixture was incubated for 24 hours at 25 °C and subsequently, filtrate was collected. Now the solid Residue was again mixed with acetone (1:10) and incubated for 24 hours at 50°C. After incubation, the filtrate was again collected and mixed with previously extracted filtrate. Finally, the mixture of the filtrate was dried in a petri plate. Nutrient agar was used to grow bacteria and fungus was grown on potato dextrose agar. To study the antimicrobial activity three different concentrations of crude extract was loaded in well (5 mg/ml to 1 mg/ml). Microorganisms selected for antimicrobial activity are collected from Sawai Man Singh Medical College (SMS), Jaipur, Rajasthan, India and Institute of Microbial Technology, Chandigarh, India [2], [20-21].

Gas chromatography mass spectrometry analysis: For isolation of phytochemicals, 10 grams of powdered plant sample was added in a conical flask. After that 100 ml of acidic solution (30 ml HCL and 70 ml distilled water) was added and mixed thoroughly. After mixing the mixture was incubated for 4 hours at 37°C (98.6 °F). After incubation, the filtrate was discarded and the solid residue was again collected in a new erlenmeyer flask. To neutralize the pH of the solid residue from acidic to 7, the solid residue was washed with distilled water. Followed by that solid residue was again collected and dried at room temperature. After drying the solid Residue was collected in a flat bottom flask and 100 ml benzene was added. The mixture was incubated for 24 hours at 37° Celsius. Finally, after incubation filtrate was collected and dried in a Petri plate. Dried plant extract was mixed with HPLC grade chloroform and GC MS analysis was conducted. 2 µl of the sample was injected in GC-MS. The total time of GC-MS was 30 minutes. "Rxi5 Si MS" GC column was used with a thickness of 0.25 um, diameter of 0.25 mm, and length of 30.0 m. Due to its low boiling point, density, and solubility helium was used as a carrier gas. Pressure was maintained at 66.8 kPa, total flow was kept at 23.9 mL/min. flow in column was at 1.18 mL/min, the linear velocity was at 39.4 cm/sec, and purge flow was at 5.0 ml/min. Ion source temperature of mass spectrometry was 250 °C.

RESULTS AND DISCUSSION

Water plays an important role in every biochemical process of living organisms. The majority of the weight of any living organism is due to the presence of water. Commercially, the weight of the plant also impacts storage and transportation. After drying the freshly harvested 1 gm plant sample weighed 0.270± 0.015 gm (average). Resulting in 27% of dry weight and water weighing approximately 73%.

The amount of non-blazable compounds can easily be identified by ash content. It is helpful in the identification of inorganic compounds. Many traditional medicines use ash as an herbal preparation. 1 gram of dried plant sample remains 0.121 ± 0.018 gm after blazing, resulting in 12.2% ash and 87.9% blazable compounds.

Macromolecules are one of the major currency of biological activities. It helps in storage and metabolism. Carbohydrates, lipids, and proteins are some of the major macromolecules that play an important role in nutrition and diet. One gram of powder plant sample contains approximately 0.022 ± 0.011 gm of free sugar which is less than 10%, fat weighs approximately 0.113 ± 0.016 gm or 11.3%, and Protein content in 1 gram powder plant sample is 0.284 ± 0.040 gm.

Table 1 Antifungal activity with acetone crude extract

	T. rubrum			*M. canis*			*E. floccosum*			*P. chrysogeum*			*C. albicans*			*A. niger*		
Concentrations (mg/ml)	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1
Zone of inhibition size is in millimetre	12	8	2	4	8	10	4	6	8	8	14	16	6	8	10	00	00	00

00- No zone of inhibition

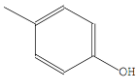
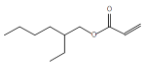
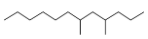
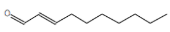
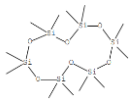
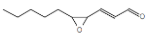
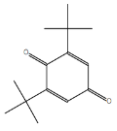
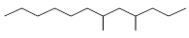
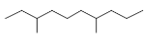
Antimicrobial activity: The antimicrobial activity of acetone crude extract was tested with 8 microorganisms (6 fungus and 2 bacteria). Zone of inhibition was observed with *T. rubrum, M. canis, Epidermophyton floccosum, P. chrysogeum, Candida albicans,* and *Bacillus subtilis*. The highest zone of inhibition was reported with *P. chrysogeum* (1 mg/ml Concentration) and the lowest with T. rubrum (1 mg/ml Concentration). No zone of inhibition was recorded with *A. niger* and *P. aeruginosa* (Table 1-2). Zone of inhibition with *T. rubrum* was observed in all concentrations of acetone crude extract (12 mm with 5 mg/ml, 8 mm with 2.5 mg/ml, and 1mg/ml showing 2 mm zone). *T. rubrum* is a fungus of Ascomycota phylum [22] known for infections like ringworm, nail infection, athlete's foot, jock itch, etc. [23]. Acetone crude extract with *M. canis* is showing the highest zone on lower concentration measuring approximately 10 mm with 1 mg/ml extract. *M. canis* is a fungus that also belongs to the phylum Ascomycota, known to cause infection on the dried skin of humans and animals [24]. Zone of inhibition with *Epidermophyton floccosum* is reported 4 mm on 5 mg/ml, 6 mm on 2.5 mg/ml concentrations, and 8 mm on 1 mg/ml concentration. *Epidermophyton floccosum* is known to cause an infection like onychomycosis, tinea corporis, and tinea pedis on skin and nails [25]. *P. chrysogeum* is commonly found on the wet surface, the fungi are showing highest zone of inhibition with 1 mg/ml concentration [26]. *Candida albicans* cause hospital-acquired infections, infection in gastrointestinal tract and mouth of immunocompromised individuals. Acetone crude extract with *C. albicans* is showing zone of 6 mm with 5 mg/ml, 8 mm with 2.5 mg/ml, and 10 mm with 1mg/ml. [27]. *B. subtilis* Gram-positive found in soil showing highest zone of inhibition with 1mg/ml concentration (Table 2).

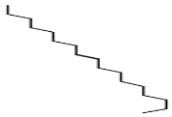
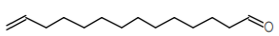
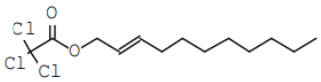
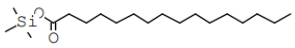
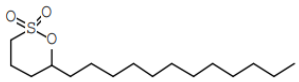
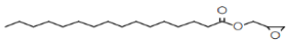
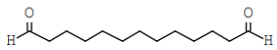
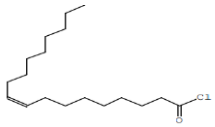
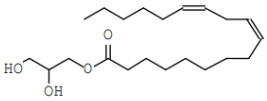
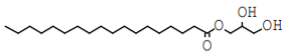
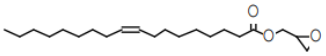
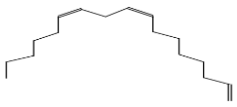
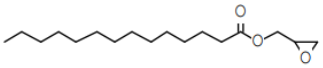
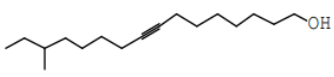
Table 2 Antibacterial activity with acetone crude extract

	Bacillus subtilis			*Pseudomonas aeruginosa*		
Concentrations (mg/ml)	5	2.5	1	5	2.5	1
Zone of inhibition size is in millimetre	4	6	8	00	00	00

00- No zone of inhibition

Table 3 GC-MS assay

S. No.	RT	Compound name	Peak area (%)	Molecular formula	Molecular Weight g/mole	Structures
1	9.244	p-Cresol	0.86	C7H8O	108	
2	12.464	2-Ethylhexyl acrylate	0.13	C11H20O2	184	
3	12.708	Dodecane, 4,6-dimethyl-	0.51	C14H30	198	
4	13.266	2-Decenal, (E)-	0.27	C10H18O	154	
5	13.546	Cyclohexasiloxane, dodecamethyl-	0.90	C12H36O6Si6	444	
6	13.920	2,4-Decadienal, (E,E)-	0.50	C10H16O	152	
7	15.213	2-Undecenal	0.40	C11H20O	168	
8	15.515	cis-4,5-Epoxy-(E)-2-decenal	0.15	C10H16O2	168	
9	16.991	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	0.14	C14H20O2	220	
10	17.079	Dodecane, 1-chloro	0.09	C12H25Cl	204	
11	17.321	Dodecane, 4,6-dimethyl-	0.10	C14H30	198	
12	17.528	Decane, 3,7-dimethyl-	0.09	C12H26	170	
13	20.221	5-Heptadecene, 1-bromo-	0.16	C17H33Br	316	

14	20.332	8-Heptadecene	0.24	C17H34	238	
15	23.339	13-Tetradecenal	0.11	C14H26O	270	
16	24.531	Trichloroacetic acid, undec-2-enyl ester	0.09	C13H21Cl3O2	314	
17	24.990	Palmitic Acid, TMS derivative	0.18	C19H40O2Si	328	
18	25.358	1-Nonadecene	0.21	C19H38	266	
19	27.152	1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide	35.54	C16H32O3S	304	
20	27.601	Glycidyl palmitate	14.97	C19H36O3	312	
21	28.574	Tridecanedial	6.74	C13H24O2	212	
22	28.792	Oleoyl chloride	6.67	C18H33ClO	300	
23	28.866	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	4.37	C21H38O4	354	
24	29.007	Octadecanoic acid, 2,3-dihydroxypropyl ester	4.33	C21H42O4	358	
25	29.242	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	8.83	C21H38O3	338	
26	29.318	1,8,11-Heptadecatriene, (Z,Z)-	4.28	C17H30	243	
27	29.463	Myristic acid glycidyl ester	3.70	C17H32O3	284	
28	29.907	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	2.38	C17H32O	252	

Identification of phytochemicals by GC MS analysis: Total 28 phytochemicals were identified in plant extract the particular are p-Cresol (0.86%), 2-Ethylhexyl acrylate (0.13%), Dodecane, 4,6-dimethyl- (0.51%), 2-Decenal, (E)- (0.27%), Cyclohexasiloxane, dodecamethyl- (0.90%), 2,4-Decadienal, (E,E)- (0.50%), 2-Undecenal (0.40%), cis-4,5-Epoxy-(E)-2-decenal (0.15%), 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)- (0.14%), Dodecane, 1-chloro (0.09%), Dodecane, 4,6-dimethyl- (0.10%), Decane, 3,7-dimethyl- (0.09%), 5-Heptadecene, 1-bromo- (0.16%), 8-Heptadecene

(0.24%), 13-Tetradecenal (0.11%), Trichloroacetic acid, undec-2-enyl ester (0.09%), Palmitic acid, TMS derivative (0.18%), 1-Nonadecene (0.21%), 1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide (35.54%), Glycidyl palmitate (14.97%), Tridecanedial (6.74%), Oleoyl chloride (6.67%), 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester (4.37%), Octadecanoic acid, 2,3-dihydroxypropyl ester (4.33%), 9-Octadecenoic acid (Z)-, oxiranylmethyl ester (8.83%), 1,8,11-Heptadecatriene, (Z,Z)- (4.28%), Myristic acid glycidyl ester (3.70%), (R)-(-)-14-Methyl-8-hexadecyn-1-ol (2.38%). Molecular formula, RT, molecular weight, and structures is given in (Table 3).

Characterization of phytochemicals: p-Cresol is an antiseptic it can kill bacteria and fungi [28-29]. 2-Decenal, (E)- can act like an alarm compound for insects and use as a pheromone [30-31]. Cyclohexasiloxane, dodecamethyl is antimicrobial, antifungal, antinflammatory, and antioxidan in nature [32]. 2,4-Decadienal, (E,E)- is nematicidal in nature [33]. 2-Undecenal is reported as antimicrobial compound [34-35]. 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)- is antibacterial, antioxidant in nature and it may use as an antifungal [36-37]. (*Z*)-8-Heptadecene is reported as a parasitic mites reducer on honey-bee [38]. cis-4,5-Epoxy-(E)-2-decenal is present in black tea [39-40]. 13-Tetradecenal may have anti-inflammatory activity [41]. 1-Nonadecene is an antioxidant by nature it also stops the growth of microbes, antituberculosis, and gastric cancer in rats [42-44]. Glycidyl palmitate is a fatty acid, reported as an inhibitor of apoptosis [45]. Oleoyl chloride is antimicrobial by nature. Oleoyl chloride is lipophilic and can able to couple covalently with lysozyme. It can change the antimicrobial behavior of lysozyme to kill both gram-positive and gram-negative microbes like *E. coli* and *S.* aureus [46]. 5-Alpha reductase inhibitor, antiandrogenic, anti-inflammatory, antiarthritic, anticoronary, antihistaminic antieczemic, hypocholesterolemic cancer preventive, hepatoprotective, nematicide insectifuge in nature [47]. Octadecanoic acid, 2,3-dihydroxypropyl ester is anticancer, and antimicrobial in nature [48].

CONCLUSION

The present study concludes that the crude acetone extract of *S. lanata* is showing antimicrobial activity against *T. rubrum, M. canis, E. floccosum, P. chrysogeum, C. albicans*, and *B. subtilis* comparing with other primary metabolites like carbohydrate and protein, the amount of lipids is higher in *S. lanata*. GC-MS analysis conclude total four anticancer, apoptosis inhibitor, cancer preventing compound in the plant extract (1-Nonadecene, Glycidyl palmitate, 5-Alpha reductase, and Octadecanoic acid, 2,3-dihydroxypropyl ester). Presence of antimicrobial compound is also reported by GC-MS analysis (2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-, 2-Undecenal, Oleoyl chloride). Anti-inflammatory and antioxidant compounds like 13-Tetradecenal, 5-Alpha reductase, 1-Nonadecene are also reported for the first time.

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