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Acmella Essential Oil: A Potent Antibacterial Agent Against a Clinical Isolate of *Moraxella* sp.

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Abstract: The flowers of *Spillanthes acmella* (Asteraceae) Murr., was subjected to hydrodistillation in a Clevengers apparatus. From the GC-MS data, 16 peaks were observed. The highest peak in the form of percentage (area) was 51.08. Further, the hydrodistillate of *Spilanthes* was studied for its antibacterial efficacy against a clinical bacterial isolate, *Moraxella* sp.. Both the qualitative and quantitative assays were correlated. The hydrodistillate was observed to be cidal in its inhibitory concentrations. The optical microscopic studies more over the Scanning Electron Microscopic (SEM) studies were illustrative about the cellular disruption activity of the herbal hydrodistillate.

Key words: *Acmella* essential oil, antibacterial, *Moraxella sp.*

Introduction

Determination of antimicrobial effectiveness against specific pathogens is essential to proper therapy. Testing can show which agents are most effective against a pathogen and give an estimate of the proper therapeutic dose [23]. *Moraxella sp.* is a gram-negative, aerobic, lactose-non-fermenting, coccoid / coccobacillus bacterium [9,16]. *Moraxella sp.* has been implicated in a variety of infections [2,17,18] and an emerging drug resistance organism [10,22] isolated from immune compromised patients [1]. Further this organism is commensal of mucosal surfaces and sometimes gives rise to opportunistic infection. Authors like [13] and [30] have taken interest to study the susceptibility of *Moraxella* strain towards Antibiotics. But the bacterial strain was reported to be resistant to many- number of therapeutic agents [1,22,27]. The possibility of utilising volatile oils is now being investigated as, although their biological activity has been known for centuries, their mode of action was not fully understood. Browsing over the literature survey depicted here, it may be under-stood that the study for evaluation of essential oil as antibacterial agent is a continuous process. This is pertinent to mention here that, variation in chemical components of essential oils, due to many factors like genetic inconsistency, phenological stages, environmental conditions and pedoclimatic conditions attribute the antimicrobial potency of essential oils [5].

There are reports [20,28] which were descriptive about the ethno medicinal values of a herb, *Spilanthes acmella* Murr. (Asteraceae). Traditionally, this is reported to be tooth ache plant. Western Odisha, India is enriched with the luxurious growth of this plant. Mention may be made here that a strain of *Moraxella* sp. isolated from patients' sample of Veer Surendra Sai Institute for Medical Sciences and Research, Burla was observed to be resistant towards a multitude of marketed drugs. But progress has not been

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made in the direction of evaluating *Acmella* essential oil against the said strain barring a few reports [7,11] so far. Therefore, this is a small attempt to study the antibacterial efficacy of the hydro distillate (essential oil) of this indigenous plant essential oil against this clinically isolated bacterial strain of *Moraxella* sp.

Materials and methods

Collection and hydro distillation of plant material

The plants were collected from the interior of Western Odisha in the month of October and the fresh flowering heads were subjected to hydro distillation by using Clevengers apparatus (Borosil, Product code:3451029) and analysed for its organoleptic properties [15] in the Deptt of Pharmacognosy, Barpalli Pharmacy College, Barpalli, Odisha.

Gas Chromatography analysis of hydrodistillate

The hydro distillate was subjected to Gas Chromatographic study in the Sophisticated Analytical Instrument Facility (SAIF, DST), CSIR-Central Drug Research Institute (CDRI), Lucknow, India.

Isolation and Identification of bacterial strain

The bacterial sample was collected and also provisionally identified in the Deptt. Of Microbiology, Veer Surendra Sai Institute for Medical Sciences and Research, Burla, India. The sputum samples were collected from the patients suffering from lungs related disease and streaked on to General media (Nutrient agar). Further the selected colonies were sub cultured onto Mac Conkey and CLED (Cystine Lactose Electrolyte Defficient) Agar media and the colonies were subjected to colony morphology study, biochemical study and more over microscopic studies by using diagnostic techniques [28].

Qualitative and quantitative assays

The hydro distillate was evaluated for its antibacterial efficacy against the said strain by using methods of Pattnaik, *et. al*., [20]. The qualitative test was carried out by using the "Paper disc diffusion" method and Minimum Inhibitory Concentration (MIC) was followed by using the modified "Agar Cup" method. The drug impregnated antibiotic discs were purchased from Hi-Media, Mumbai were used for qualitative tests. But the marketed solid dosage forms of antibiotics (MACLEODS Pharmaceuticals Pvt. Ltd., Mumbai) were used for quantitative tests. For this purpose, a range of concentrations (5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, 30 µg/ml) of respective drugs Penicillin, Ampicillin and Lincomycin were put in the wells made in bacterial strain swabbed agar plates. DMSO (Dimethyl Sulphonate) was used as solvent for the solid dosage forms. The plates were incubated at 37°C and the observations were taken from the corresponding diameter of zones of inhibitions (in mm). The values were correlated with Kirby Bauer's [4] antibiotic interpretative chart for the interpretation of data. Accordingly, the bacterial strain was categorized as resistant (R), intermediately sensitive (IS) and sensitive (S) towards the said antibiotics.

Bacterial cell topography study

The cells of bacterial strain used in this study was subjected to Optical microscopic study. Further, the Scanning Electron Microscopy (SEM) was carried out in the Materials and Minerals laboratory, (M & M) lab, National Institute of Technology (NIT), Rourkela, Odisha, India.

Results

Characterization of Acmella essential oil

The oil was placed in a transparent bottle over a white background and the color and clarity were observed; the characteristic odor was determined by sniffing; and to determine its characteristic feel to the touch, it was rubbed between fingers [14]. The characteristics are depicted in Table 1. The hydro distillate was found to be positive for the tests like (1) reaction with Sudan IIIrd reagent and (2) reaction with tincture of alkane. There was appearance of red color was indicative of the presence of essential oils. Further, Salkowski test showed appearance of reddish brown precipitate at the interface formed indicated the presence of terpenoids [3].

Table 1 depicting the organoleptic properties of *Acmella* hydrodistillate

Parameters used for the study	Observation
Color	Pale Yellow
Odour	Pleasant
Taste	Tingle
Characteristic feel	Greasy

Gas chromatographic analysis of hydro-distillate

The data obtained from Gas chromatography, is illustrated in Figure 1 and Table 2. From this GC analysis of *Acmella* hydro distillate, it was observed that there were elution of 16 number of peaks. The 4th peak was found to be with higher area percentage (51.08). The 1st eluted peak could be ranked as 2nd major compound with a peak percentage of 18.08. Further the third peak was with a percentage of 16.52. Barring the 10th number of peak, the other peaks so eluted were with minor area percentages values.

Isolation and Identification of bacterial strain

Red dot colonies of isolated bacterial strain were observed on the Mac Conkey agar medium (Figure 2 a) plates. Further Biochemical tests were suggestive about the Catalase positive and Oxidase positive, nonacid fermentation in Glucose, Lactose, Sucrose, Mannitol (GLSM) media characteristics (Table 3). The microscopy and Gram staining revealed presence of Gram negative coccobacillus forms (Figure 2b).

Qualitative and quantitative assays

The results obtained from the qualitative tests and quantitative tests are illustrated in Figure 3(a, b). The bacterial strain was found to be resistant to β-lactam antibiotics Penicillin (30 units/disc), Ampicillin (15 µg/disc and Lincomycin (15 µg/disc). But the strain was observed to be sensitive to *Acmella* essential oil (5 µl/ml, v/v) with zone of

Table 2 depicting the sequence of elution of peaks and the corresponding area percentages

No.	Sequence of eluted peaks	Area in Percentage
1	1st	18.08
2	2nd	2.86
3	3rd	16.52
4	4th	51.08
5	5th	1.22
6	6th	0.06
7	7th	0.40
8	8th	3.04
9	9th	0.92
10	10th	4.33
11	11th	0.09
12	12th	0.11
13	13th	0.03
14	14th	0.08
15	15th	0.05
16	16th	0.23

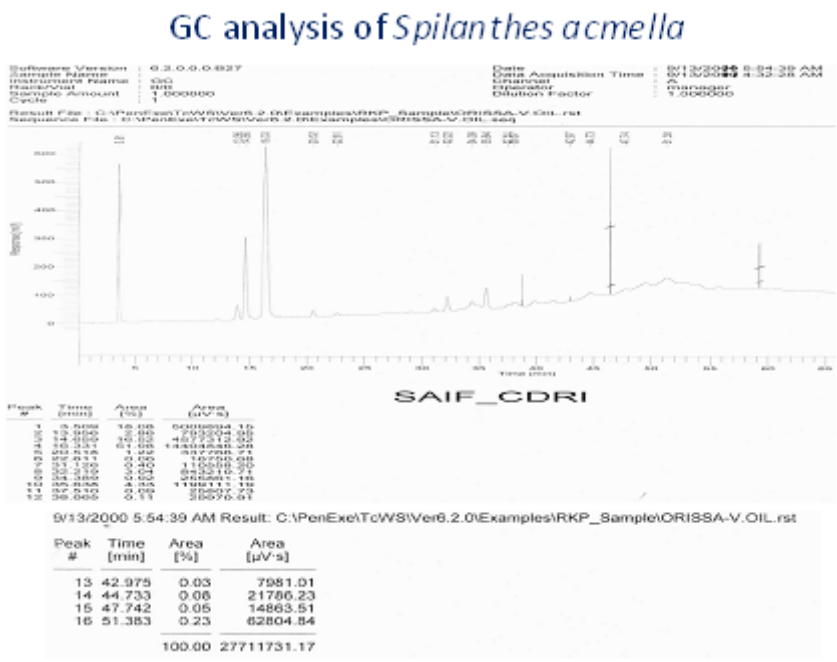

Figure 1. depicting the GC analysis of *Acmella* hydro distillate

Table 3 depicting the data of Bio chemical tests of *Moraxella* sp.

Names of the biochemical tests	Interpretation
GLSM	-ve
Indole	-ve
VP	-ve
Citrate	-ve
Catalase	+ve
Oxidase	+ve

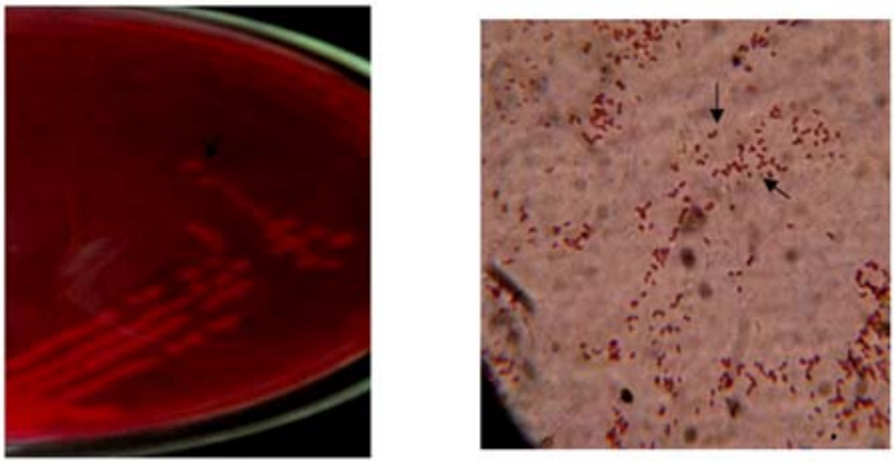

Figure 2 a. depicting the isolated colonies of bacterial strain on Mac Conky media **b:** depicting the Gram negative coccobacilli forms

Figure 3. depicting the susceptibility tests for Antibiotics **(a)** and *Acmella* essential oil **(b)**

inhibition (mean value obtained from triplicates) of 14 mm (diameter). The Minimum Inhibitory Concentration (MIC) of *Acmella* essential oil was observed to be 3.5 µl/ml (v/v). More over the respective MIC values so obtained from "Antibiotic Sensitivity tests" were observed as 25 µg/ml, 30 µg/ml and ≤ 30 µg/ml for Penicillin, Lincomycin and Ampicillin respectively.

Bacterial cell topography study

The Scanning Electron Microscopy study for the topographic analysis of the *Acmella* essential oil sensitive cells revealed presence of disrupted cells (Figure 4,b) in comparison to control cells (Figure 4,a).

Discussion

The strain isolated from the patients samples at V.S.S. Medical and was unique with Gram negative coccobacillary cells. The cultural, biochemical, more over the, microscopic characteristics were in agreement to propose that the strain

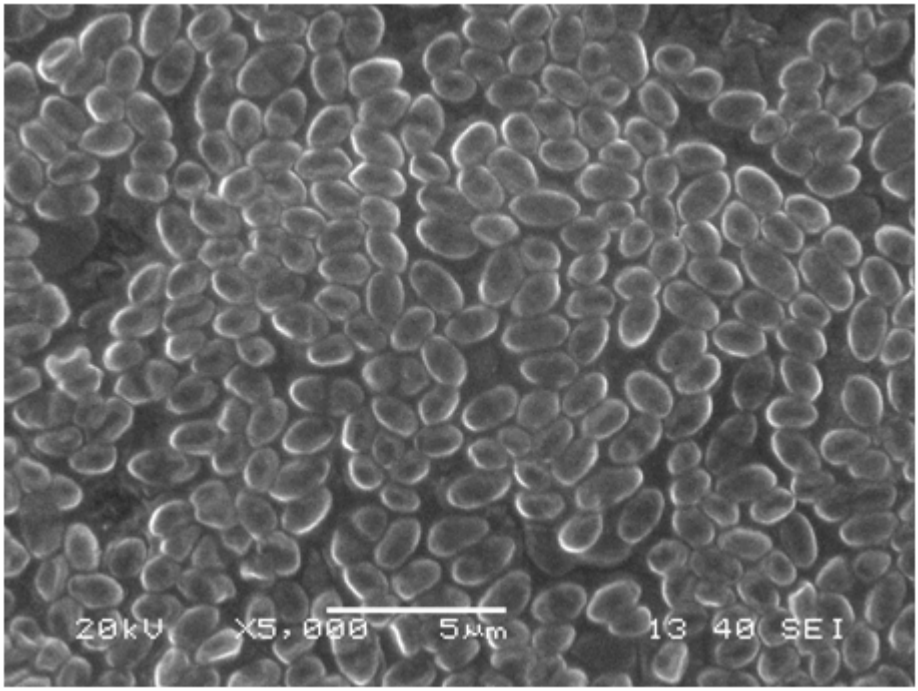

Figure 4,a. shows the coccobacilli shaped *Moraxella* sp. Cell

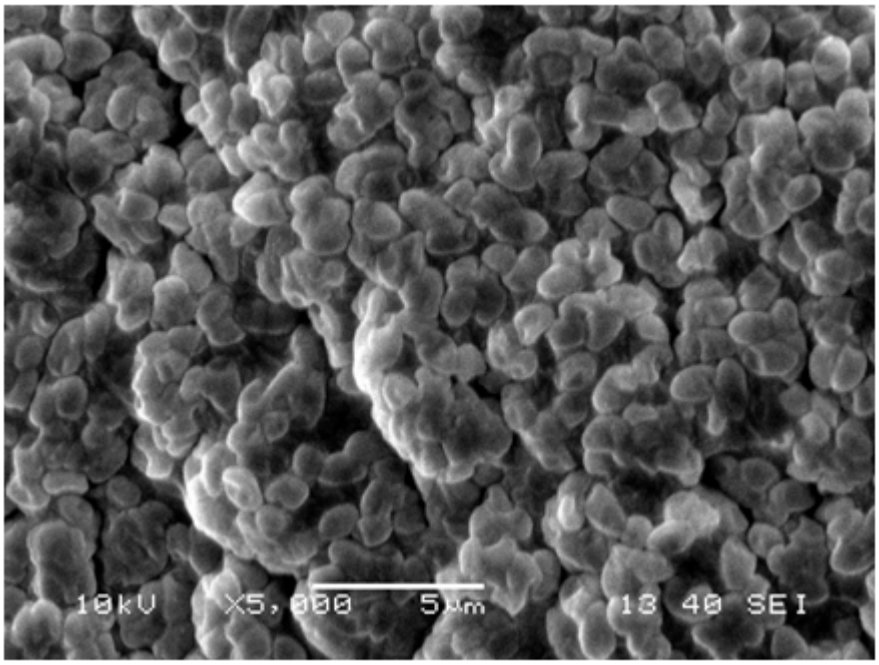

Figure 4,b. shows the cellular disruption of *Moraxella* sp. sensitive Cells (Arrows)

was *Moraxella* sp. Further the Antibiotic susceptibility test had inferred about its resistance towards Ampicillin, Lincomycin and Penicillin. This observation is in accordance with the previous reports [1,6,10,24].

In this context, this study is informative about the antibacterial potency of an indigenous variety of *Acmella* essential oil against a MDR (Multi Drug Resistance) bacterial strain. The hydro distillate was observed to be composited of sixteen no. of compounds including three major compounds (1st:18.08 %, 3rd: 16.52; 4th: 51.08). The compounds were yet to be identified. But the essential oil had absolutely growth inhibitory property against the *Moraxella* strain. The Minimum Inhibitory Concentration was also observed to be low with a cidal activity. More over there was cell disruption activity [21] of the said hydro distillate which could be evidenced from the scanning electron microscopic studies. The SEM study clearly revealed presence of drug free intact cells and mutilated cells in presence of *Acmella* essential oil. Mention may be made here that this oil might be responsible for the cell wall or cell membrane damage may be due to the synergistic effect of all the components. This was earlier reported that resistance conferred by bacterial strains towards Penicillin or β-lactam group of compounds may be due to induction of β- lactamases [8,25]. This is also acknowledged that a combinatorial therapy is prescribed by clinicians to resist the action of beta lactamases [12,15,26]. The oil which is a composited form of a number of compounds as evidenced from GC data could be able to disrupt the cell wall enzyme like Penicillins and also able to resist the action of betalactamses. But a breadth study is obligatory to give a conclusive remark. However this antibacterial efficacy study of an indigenous herbal hydro-distillate against a clinical *Moraxella* strain paved a way to propose a safe, cost effective natural remedy.

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