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Evaluation of the antibacterial activity of *Coccinia grandis*, against bacteria isolated from chronic suppurative otitis media infection

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ABSTRACT

This present study evaluates the *in vitro* antimicrobial activity of the selected Indian medicinal plant *Coccinia grandis* (*C. grandis*) against this isolated multidrug-resistant bacteria causing chronic suppurative otitis media infection (CSOM) along with their *in vivo* toxicity test using a rat model. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration of the effective leaf extract were measured using the micro broth dilution method. Out of 128 samples, 79 samples were identified as positive for bacterial infection. Out of six solvent extracts of *C. grandis* methanol and ethyl acetate, leaf extracts have shown the best antimicrobial activities compared to the rest of the four solvent extracts. Methanolic extract 1.56 mg/ml and 0.78 mg/ml was the MIC values for the respective plants. *Staphylococcus aureus* and *Pseudomonas aeruginosa* species are the most common bacterial isolates in CSOM infection. Hence, the causative organism and its drug sensitivity pattern should be carried out before treatment. Due to increasing cases of antibiotic resistance among bacteria, an alternative therapy is the need of the day, especially regarding the needier population with lesser toxicity. Hence, plants can be used as a choice of drugs to treat CSOM infection.

1. INTRODUCTION

Chronic suppurative otitis media infection (CSOM) is an inflammation of the opening in the middle ear, which can occur with or without the tympanic membrane intact. The term CSOM refers to a middle-ear infection that lasts longer than 3 months and is accompanied by perforation of the tympanic membrane [1]. In the modern world, Otitis media is one of the most frequent childhood illnesses and a primary cause of antibiotic prescriptions [2]. It is one of the most frequent disorders among people, particularly children. Because of poor diet, poor cleanliness, and a lack of health knowledge, the prevalence of CSOM is higher in developing nations, particularly among the lower socioeconomic strata of society (with an urban-rural ratio of 1:2). The illness is quite common in tropical areas, such as South Asia [3]. In some circumstances, repeated ear infections might lead to a perforation of the tympanic membrane. Pressure from pus under the tympanic membrane can sometimes cause a small hole in the tympanic membrane during a middle ear infection (otitis media). According to the WHO, 65–330 million people worldwide have CSOM, with 60% reporting a hearing loss. Meanwhile, the rate of occurrence is nine instances per 100,000 people [2].

CSOM is caused by infectious agents such as bacteria, fungi, and viruses. *Pseudomonas aeruginosa, Staphylococcus aureus,* and Enterobacteriaceae, such as *Proteus* spp. and *Klebsiella* spp., are the most prevalent causal agents of CSOM. New pathogenic flora might emerge when the external auditory canal's defenses are disrupted. Polymicrobial-mediated infections are common and anaerobic bacteria can be found [1]. Fortunately, with medical diagnosis and antibiotic therapy progress, it is unusual for otitis media to manifest its lethal potential. According to a comprehensive study, antibiotics are not beneficial in preventing the development of acute otitis media (AOM) to CSOM, even among children at high risk for the condition [2]. Fungal infections are seen as secondary infections in CSOM when the discharged ear does not react to local antibiotic ear drops [4].

Antibiotic resistance is a significant public health issue, with rising death rates [4]; as a result, it's critical to look at alternatives to the antibiotics now in use. It might be a promising molecule for halting antibiotic resistance and enhancing medication effectiveness [5]. As a result, the demand for novel antimicrobial drugs is the need of the day from different natural resources with lesser side effects. According

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to the WHO, approximately 80% of underdeveloped countries rely almost totally on traditional medicine for their primary health care. Plants have contributed to preserving human health and improving human living conditions [6].

Coccinia grandis is a several-meter-long perennial vine with a first growth rate. It may develop dense mats that quickly cover plants and small trees on the land. The plant belongs to the Cucurbitaceous family, which has 960 species. The tropics are home to the majority of the family's members. Annual vines make up most of the Cucurbitaceae family [6,7]. The plant has been used as a food crop in Asia, Australia, the Caribbean, the southern United States, and the Pacific Islands. There is a growing interest in correlating phytochemical substances with their biological functions. *C. grandis* is a plant that is native to India, and Indian subcontinents Cambodia, China, Indonesia, Malaysia, Myanmar, Thailand, Vietnam, eastern Papua New Guinea, Australia, and the Northern Territory [8].

The literature survey revealed that *C. grandis* is a significant source of numerous pharmacologically and medicinally relevant compounds, especially since the local tribal population of India use its leaf to treat ear infection. It is evident from the reports that medicinal plants have a critical role in the prevention and treatment of a wide range of diseases, so the plant has been considered for studies of interest in our current therapeutic target [9]. In this work, we have studied the antibacterial effect of *C. grandis* against bacteria isolated from CSOM patients.

2. MATERIALS AND METHODS

This prospective study was carried out at the Institute of Medical Sciences and SUM hospital Bhubaneswar from March to June 2018. The research involved 128 individuals who were seen in the otorhinolaryngology outpatient department. The Institutional ethical committee approved this study vide letter no DMR/IMS/IEC-2018/014.

2.1. Processing of Microbial Sample

The collected ear swabs with pus samples were processed within 4–6 h. The samples were first cultured in blood agar and MacConkey plates. The inoculated plates were incubated at 37°C for 24–48 h. Then, the samples were kept in incubation with 2 mL of Nutrient broth (NB). Then after 24 h, the result of the cultured plate was studied and noted. Colony morphology, biochemical features, and Vitek 2 system were used to identify the isolated bacteria.

2.2. Plant Material Collection and Extraction

Leaf samples of *C. grandis* were collected from Gandhmardhan, Western Odisha (20.8739° N, 82.8428° E). The plant species were identified with the help of regional flora books [10] and the help of a taxonomist. As described, the successive hot extraction method was followed using petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water [11].

2.3. Evaluation of Antibacterial Activity and Determinations of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Plant

Agar well diffusion was used to test antibacterial activity [12]. The agar healthy diffusion technique was used to test the antibacterial potentiality of plant extracts with a 25 mg/ml concentration using isolated bacterial strains that demonstrated resistance to a maximum number of antibiotics. MIC and MBC of the plant extracts were determined based micro broth dilution technique using 96 well microtiter plates. In addition, the MBC value was determined by sub-culturing bacteria from each well of the microplate on a nutrient agar plate at a dilution level. No bacterial growth on the nutrient agar plate was identified. Dimethyl Sulfoxide (10%) was used as negative control while conducting the antibacterial assay [12].

2.4. Toxicity Assay

Both the male and female rats (110–150 g) were obtained at 5–8 weeks from Neelachal tirati, Kolkata, Saha enterprises (1828/PO/Bt/S/15/CPCSEA). The Animal Ethics committee of the Siksha O Anusandhan (Deemed to be University), Bhubaneswar, approved all the experimental procedures (ProtocolIAEC/SPS/SOA/18/2019). Experiments were carried out at a lab with registration number 1171/Po/Re/S/08/CPCSEA. The animals were maintained individually in 10*16 iron cages at room temperature with a 12-h day and light period at the animal house of SOA University. Before beginning the trial, the animals were given a 7-day acclimatization period.

2.5. LD50 Assay

The LD50 (Lethal Dose) is the amount of a drug or extract given all at one time that causes 50% of targeted animals to die. This is one method of determining the drugs or extracts short-term toxicity. A single dosage of methanolic extracts of our plant extract was given orally to selected rats at different doses of 200, 400, 800, 1600, and 2000 mg/kg for LD50 testing. The test animals were then monitored for 72 h [13].

2.6. Subacute and Acute Toxicity Study

Both the male and female rats were divided into five separate experimental groups for acute and sub-acute practical purposes, with each group having six animals. First group of animals was fed a regular diet with distilled water using oral gavage and considered a control group for acute toxicity analysis. Second–Fifth were provided with a steady diet including distilled water containing methanolic extract of the plant, as doses range from 300 to 2000 mg/Kg body weight for acute and sub-acute toxicity analysis. The general behavior bodyweight of the animals was recorded, and clinical toxicity symptoms were evaluated for 14 days. Animals were sacrificed at the end of this study animals were anesthetized by giving ketamine at 20 mg/kg body weight. The heart puncture was conducted after the anesthesia had achieved depth to collect blood for biochemical and hematological assessments [14].

2.7. Hematological Analysis

All surviving animals were hematologically examined at the end of the experiment. A comprehensive blood count was performed using an automated hematology analyzer. Among the hematological tests performed were hemoglobin concentration, red blood cell count, platelet count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration, and white blood cell count [14].

2.8. Blood Serum Biochemistry Analysis

After the experiment, biochemical testing was done on all surviving animals. Each blood sample serum was extracted and kept in cryogenic tubes at a temperature of −80°C. The blood was taken and transferred to tubes without anticoagulant, which was left at room temperature for 60 min before centrifuged for 10 min at 4000 rpm. The following tests were performed: Glucose (G), total cholesterol, triglycerides (TGs),

aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea (Ur), creatinine (Cr), and total protein [14].

2.9. Histopathology

Organs were removed from all surviving animals, cleaned in a 0.9% (w/v) saline solution, weighed, and preserved in a 40% formaldehyde solution. Following that, the organs were prepared for paraffin embedding. Hematoxylin and eosin staining created 5 m thick slices (H and E). The tissues were examined under an optical microscope for the overall structure, indications of inflammation, tubular swelling and edema, degenerative alterations, and necrosis evidence using hematoxylin and eosin staining. The photographs were taken using a microscope (Nikon Eclipse Ts2R-FL) [14].

2.10. Statistical Analysis

The mean, standard means error (SME) of the comparative hematological and biochemical data was provided (SME). The data were subjected to a one-way analysis of variance (one-way ANOVA). The information was given in a mean, SME. $P = 0.05$ was used to determine statistical significance. Samples were analyzed with Student's *t*-test, and it was found that $P > 0.05$, which was not statistically significant.

3. RESULTS

Bacteria mainly cause ear infections, that is, CSOM. In this study, 128 ear infection patients were enrolled to know its causative organism. It was seen that among 128 ear infection patients, 74 was from urban and the rest 54 were from the rural area. With a diagnosis, it was revealed that 79 patients had CSOM infection. All age group patients participated in this study, and it was found that ear infection is expected in all age groups.

3.1. Isolation and Identification of Bacteriological Sample

All ear swab samples were cultured in different agar media to observe the organism's colony and morphology. In 11 samples, there was no growth (8.59%) whereas the single colonies were identified with 105 samples. A total number of 117 samples, colonies are identified with 128 ear discharge samples. Among 117 microbial colonies, 82.03% single colonies were found, whereas eight double colonies and 4 three or more colonies were revealed.

3.2. Antimicrobial Activity of the Different Solvent Extract against Isolated Multidrug-Resistant (MDR) Bacteria

The antibacterial activity of six solvent leaf extracts was assessed using the agar well diffusion technique (1 GP and 4 GNs) on separate lawn cultures of five bacterial isolates. The highest zones of inhibition against *S. aureus* (29 mm) and *Acinetobacter baumannii* were recorded using methanolic leaf extract (27 mm). Similarly, the ethyl acetate leaf extract inhibition zone against *S. aureus* was 27 mm and 26 mm, respectively, against *A. baumannii*. Compared to the other four solvent extracts, the petroleum-ether leaf extract and the aqueous leaf extract had less antibacterial activity. All other solvent extracts were shown to have antibacterial activity [Table 1]. The maximal bactericidal activity of methanolic and ethyl acetate leaf extracts was obtained by determining the MIC and MBC values. *A. baumannii*, *P. aeruginosa*, and *P. mirabilis* had MIC values of 1.56 mg/mL and 3.12 mg/mL, respectively; *Klebsiella* spp. and *S. aureus* had MIC values of 3.12 mg/mL and MBC values of 6.25 mg/mL, respectively. *Klebsiella* spp. and *S. aureus* had MBC values of 12. 5 mg/mL and 12. 5 mg/mL, respectively. MIC and MBC values of ethyl acetate leaf extract were also reported for all microorganisms [Table 2].

3.3. Toxicity

Acute and sub-acute toxicity general symptoms and mortality were not observed in the LD50 experiment within 72 h following administration of the extract. After treatment with 300, 600, 1200, and 2000 mg/kg, there were no symptoms of toxicity in the animal groups. No significant differences in biochemical blood markers or organ functioning were observed [Table 3]. No abnormal behavior in terms of food and drink consumption and body weight was noted during the dose regimen. We tested the liver and kidneys of rats to see if the treatment with plant extracts causes toxicity in normal organs [Figure 1].

3.4. Hematological Parameters

Treatment with the methanolic leaf extract of *C. grandis* with all doses has not produced any remarkable changes in animal hematological parameters.

3.5. Biochemical Parameters

Statistical analysis revealed a non-significant alternation ($P > 0.05$) in TGs and cholesterol values in groups. A non-significant decrease ($P > 0.05$) in ALT and AST is markers for liver injury. This indicated that the extract has no toxic effect on the liver. Urea levels remained unchanged while creatinine levels in blood were slightly reduced after extract administration in all treatment groups. Further, glucose and protein levels were also increased.

There are no detectable pathological abnormalities in the liver or kidney after treatment with plant extract at doses ranging from 200 mg/kg to 800 mg/kg. H and E staining of paraffin-embedded 5 μ-thick sections of the liver and kidney at a magnification of ×200 is shown in the panels [Figure 1]. The hepatic lobular architecture was found to be expected. Normal glomeruli, proximal and distal tubules, interstitium, and blood arteries were seen in the kidneys.

3.6. Statistical Analysis

Samples were analyzed with Student's *t*-test, and it was found that $P > 0.05$, which was not statistically significant [Figures 2 and 3].

Table 1: Antibacterial assay of six hot solvent extracts of *C. grandis* (zone of inhibition in mm).

Strain	Petroleum ether	Chloro-form	Ethyl acetate	Acetone	Methanol	Water	Linezolid/imipenem (30/10 μg/ml)
Acinetobacter spp.	10	21	26	19	27	15	29
Klebsiella spp.	17	15	19	16	26	13	33
P. aeruginosa	11	18	16	19	21	13	26
S. aureus	10	19	27	14	29	14	29
P. mirabilis	09	23	18	19	22	12	29

C. grandis: Coccinia grandis, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, P. mirabilis: Proteus mirabilis

3.7. Histopathological Studies

At the four dosages tested, oral administration of the phytoextracts did not cause substantial dose-dependent histopathological abnormalities; no tissue damage was seen in the rat's kidneys, hearts, livers, or brains.

Table 2: MIC and MBC values of Methanol and ethyl acetate of most bioactive leaf extract of *C. grandis* (in mg/ml).

Strain	Methanol		Ethyl acetate	
	MIC	MBC	MIC	MBC
Acinetobacter spp.	1.56	6.25	6.25	25
Klebsiella spp.	3.12	12.5	3.12	12.5
P. aeruginosa	1.56	6.25	3.12	12.5
S. aureus	3.12	12.5	3.12	12.5
P. mirabilis	1.56	6.25	6.25	12.5

MIC: Minimum inhibitory concentration, MBC: *Minimum bactericidal concentration, C. grandis: Coccinia grandis, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, P. mirabilis: Proteus mirabilis*

Table 3: Different parameters and general behavior assessed during toxicity study.

Behavior type	Treatments				
	Control	300 mg/kg	600 mg/kg	1200 mg/kg	2000 mg/kg
Spontaneous type	N	N	N	N	N
Alertness	N	N	N	N	N
Awareness	N	N	N	N	N
Sound response	N	N	N	N	N
Touch response	N	N	N	N	N
Pain response	N	N	N	N	N
Righting reflex	N	N	N	N	N
Pinna reflex	N	N	N	N	N
Grip strength	N	N	N	N	N
Food intake	N	N	N	N	N
Water intake	N	N	N	N	N
Mortality	N	N	N	N	N

N: Normal, Ab: Abnormal

The liver, kidneys, and lungs histopathology studies with varied dosages (300, 600, 1200, and 2000 mg/kg body weight) revealed no significant differences compared to the control group [Table 4]. The experimental group's liver cells showed no signs of toxicity such as necrosis, infiltration, edema, or conjunction. In the case of LD50, no fatality was reported after 72 h of extract administration.

4. DISCUSSION

CSOM is a common cause of acquired hearing loss in children, particularly in impoverished nations. The irritation and subsequent inflammation of the middle ear mucosa is the first step in the pathogenesis of CSOM. Mucosal edema is caused by an inflammatory reaction. Continuous inflammation eventually leads to mucosal ulceration and epithelial lining disintegration. Most therapeutic options have proven ineffective or prohibitively expensive and difficult to get; for example, parenteral aminoglycosides necessitate prolonged hospitalization and are possibly ototoxic. Topical antibiotics (delivered into the ear) with or without steroids, systemic antibiotics (given by mouth or injection), topical antiseptics, and ear cleansing (aural toileting) are all treatments for CSOM that can be used alone or in combination [2]. Topical quinolones are claimed to have significant efficacy and are relatively easy to give but remain costly. The most common pathogen that causes CSOM is *P. aeruginosa* and *S. aureus* [15]. Although the pathophysiology of acute otitis media is well understood, microscopic study on CSOM has been carried out. With the rise of antibiotic resistance and the possible risk of surgery, a new therapeutic strategy against CSOM is urgently needed. Further, due to the existence and ongoing development of resistant bacteria and new phenotypes, the introduction of novel diseases, and the toxicity of certain present antimicrobials, new sources of antimicrobial agents must be developed. The emergence of bacterial resistance, especially MDR, is inescapable since it is a unique component of the evolution of microbes. New products must be found and developed in response to the rising bacterial resistance to current antimicrobials [16]. Phytochemicals have already been shown to have antibacterial properties when employed. Natural therapeutics have played a significant role in the creation of antibiotics. Natural products have long been relied upon to produce novel molecular entities for all diseases. As a result, complementary medications are developed using 'comparative effectiveness research' concepts, and isolated phytocompounds may be promoted [17].

During the past several years, many traditional formulations from medicinal plants have been used as contemporary medicines, and there has been increasing interest in their use against different ailments. As per available literature, *C. grandis* has been used in traditional medicine

Table 4: Different parameters of hematological studies on tested rats treated for 28 days with different doses (300, 600, 1200, and 2000 mg/kg) of methanolic extracts.

Parameters	Group I	Group II	Group III	Group IV	Group V
HGB (g/dL)	13.02±0.44	11.4±0.26	14.0±0.46	12.9±0.38	13.93±0.73
RBC (10^6/µL)	7.24±0.67	6.30±0.30	6.40±0.17	6.33±0.24	6.34±0.34
PLT (10^3/µL)	791.9±24.1	903.3±17.2	741.3±36.8	936.4±41.0	935.8±25.8
HCT (%)	44.16±2.02	41.2±1.09	34.5±1.07	42.7±1.06	45.06±1.15
MCV (pg)	56.0±0.96	56.0±0.36	53.4±1.56	56.0±0.96	53.0±1.83
MCH (pg)	16.4±0.50	16.2±0.72	22.4±1.06	15.06±0.76	22.03±1.80
MCHC (g/dL))	31.3±1.01	33.3±1.65	34.7±1.61	32.36±0.63	34.26±1.68
WBC (10^3/µL)	5.86±0.20	5.67±0.53	5.69±0.42	5.44±0.63	5.33±0.39

Group I: Control, Group II: 300 mg/kg body weight, Group III: 600 mg/kg body weight, Group IV: 1200 Group V: 2000 mg/kg body weight. HGB: Hemoglobin, RBC: Red blood cell, PLT: Platelet count, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cell

Table 5: Biochemical parameters of tested rat after being treated for 28 days with different doses (300, 600, 1200 and 2000 mg/kg) of methanolic extracts of *C. grandis.*

Parameters	Group-I	Group-II	Group-III	Group-IV	Group V	Normal range
Glucose (mg/dl)	96.01±2.1	93.09±1.98	89.04±1.4	98.03±1.9	92.03±2.1	70-110
Urea (mg/dl)	37.03±3.1	39.03±2.08	43.06±1.78	27.09±2.14	41.04±1.7	15-45
Creatinine (mg/dl)	1.02±1.04	0.03±1.33	0.03±1.02	0.53±1.25	0.49±1.31	0.5-1.5
Total protein (mg/dl)	5.01±2.56	4.18±1.45	4.46±1.76	5.24±1.5	4.03±2.5	6.0-8.0
Total cholesterol (mg/dl)	126.02±1.78	113.02±2.22	126.09±2.3	136.87±2.5	136±1.44	140-250
Tri glycerides (mg/dl)	91.02±2.1	86.01±2.9	96.01±2.8	110.06±2.6	93.02±2.9	25-160
Asparateamino transferase (IU/L)	43.02±2.5	37.99±2.6	43.32±1.9	44.12±3.2	33.01±1.03	Up to 46
Alanineamino transferase (IU/L)	38.89±2.1	26.27±1.6	33.02±1.07	31.39±2.21		Up to 40

Group I: Control, Group II: 300 mg/kg body weight, Group III: 600 mg/kg body weight, Group IV: 1200 Group V: 2000 mg/kg body weight. *C. grandis: Coccinia grandis*

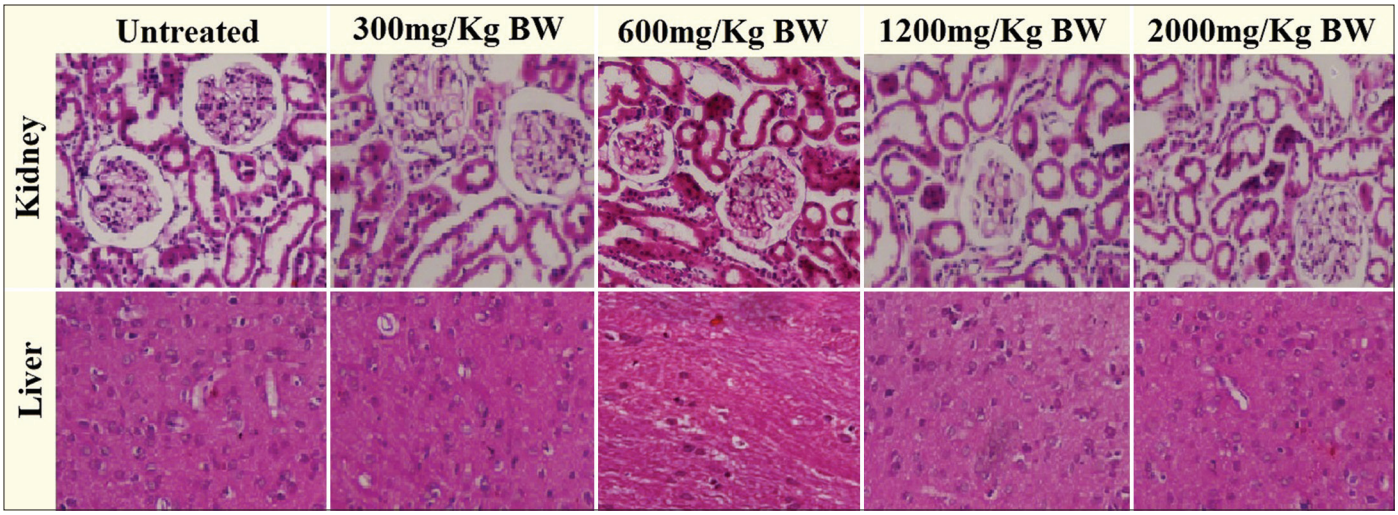

Figure 1: H and E staining of paraffin-embedded 5 µ-thick sections of the liver and kidney at a magnification of ×200.

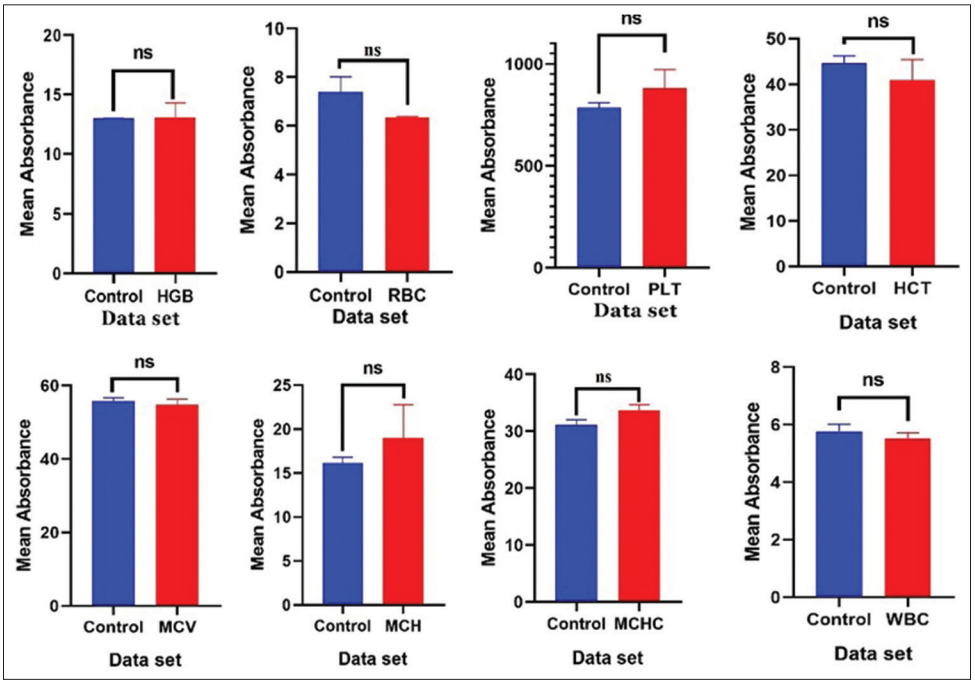

Figure 2: The histogram represents the hematotoxin study for the control and treated group and their significant analysis by *t*-test.

as a household remedy for various diseases [8,9]. Conventionally, the roots and leaves juice is used for diabetes treatment, whereas the stem's juice and fruit extracts are used against cataracts and other eye diseases. Leaf decoction of *C. grandis* can be used as a laxative and is also used

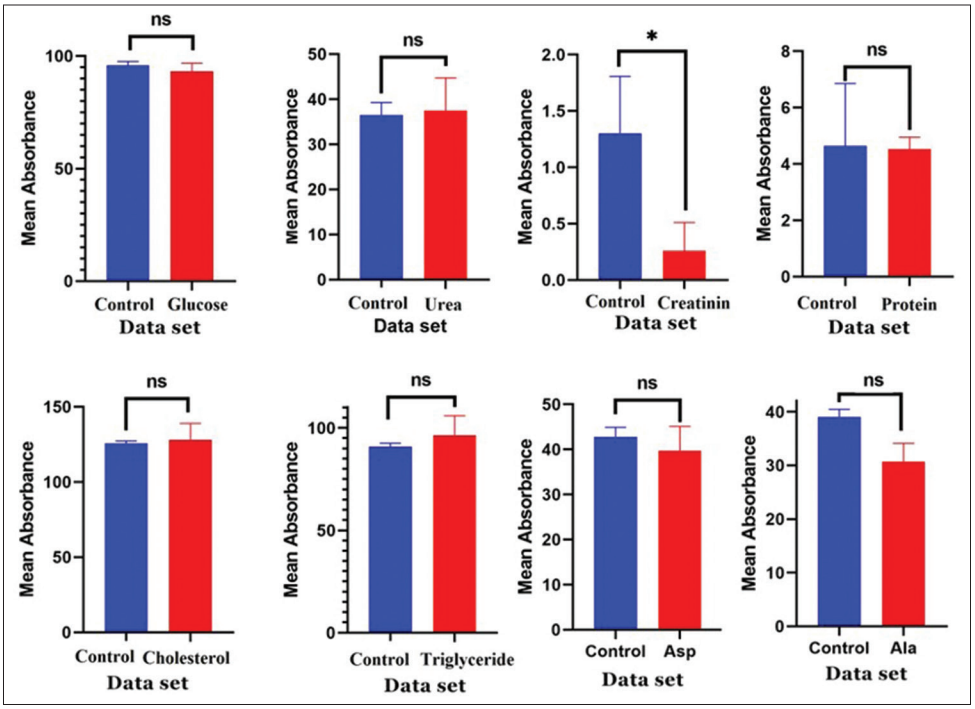

Figure 3: The histogram represents a toxic biochemical study for the control and treated group and their significant analysis by *t*-test.

to treat pregnancy issues, gonorrhea, and skin eruptions. A decoction of this plant is used to treat chest colds. The plant is crushed and applied externally to affected areas to relieve the pain of headache and rheumatism. Aqueous and ethanolic extracts of the plant have shown hypoglycemic principles. This plant has been used to treat diabetics, skin diseases, and jaundice [18,19]. Antioxidant, antimutagenic, antibacterial, antiulcer, hepatoprotective, expectorants, and analgesic properties have been documented, and anthelmintic, cytotoxic, anti-diabetic, mast cell-stabilizing, anti-anaphylactic, and antihistaminic potential, antimitotic, and anti-inflammatory activities also reported [20-23]. All of the tests in this paper are based on crude extract and are valid.

As reported by Deokate *et al*., *C. grandis* contain many pharmaceutical importance phytochemicals such as "Triterpenoid, saponin, and coccidiosis – k(i). $C_{41}H_{66}O_{12}$ Flavonoid glycoside obtain 3-o- arabinofuranoside 3- o-β- (α-l- arabinopyranosyl)-(1→2) –β-d-glucopyranosyl- (1→3)- βhydroxylup – 20(29)- en-28- oic acid. Lupeol, β-amyrin, and β- sitosterol. Stigmast -7- en-3-one," in roots. Further, "Taraxerone, taraxerol, and (24R)-24- ethylcholest- 5- en- 3β- ol glucoside. B- carotene, lycopene, cryptoxanthin, and apo- 6'- lycopenal B- sitosterol and taraxerol" in fruits and "Heptacosane Cephalandrol, C29H58O tritriacontane C33H68 B- sitosterol alkaloids Cephalandrine a and Cephalandrine b" in the aerial part. [24]. These secondary metabolites can be attributed to the major antibacterial properties of this plant. Similarly, *C. cordifolia* has been studied for a variety of pharmacological properties. Aqueous and ethanolic extract of the plant has significant antibacterial activity against *Shigella, Salmonella*, *E. coli,* and *Bacillus subtilis*. The aqueous extract is moderately effective against *Sarcina lutea* and *B. subtilis.* Extracts of ethyl acetate are effective against *S. aureus* [25]. Leaf extracts of the plant have strong antibacterial activities against *E. coli*, *B. cereus*, *K. pneumoniae*, and *S. aureus* [26]. Except for the antibacterial activities the plant has been studied for antifungal [27], anthelmintic [28], antimalarial [29,30], antipyretic [31], antiulcer [32], and anticancer activity [33,34].

5. CONCLUSION

CSOM infection causes a leading role in ear discharging patients. Hence, before initiating the treatment, detection of the causative organism and its drug sensitivity pattern should be carried out. Empirical therapy should be avoided, and the PCR method should be implemented for the early detection of drug sensitivity patterns. It was evident from the traditional healers that. With several ethnomedicinal importances, *C. grandis* has been used in ear infections for decades in the tribal population of Odisha, which validated to have *in vitro* control over a cohort of clinically isolated CSMO bacterial strains significantly. So this plant could be further taken up for synergistic/integrative drug development modules for use in mainstream medicine. A formulation of concoction with herbal medicine can be thought up after isolating and characterizing pure bioactive compounds from this plant. Cost-effective drugs are always sought after in resource-limited areas.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. FUNDING

There is no funding to report.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

The Animal Ethics committee of the Siksha O Anusandhan (Deemed to be University), Bhubaneswar, approved all the experimental procedures (ProtocolIAEC/SPS/SOA/18/2019).

10. DATA AVAILABILITY

All the data found are given in tables.

11. PUBLISHER'S NOTE

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