Original Research Article

Antimicrobial activity of crude extracts of *Oldenlandia auricularia* against some selected human pathogens

ABSTRACT

Aims: Currently **t**here is a high demand on novel anti-microbial agents derived from natural sources due to low cost and less adverse effects. The present study was designed to screen the anti-microbial activity of different extracts of *Oldenlandia auricularia* against common pathogenic bacteria and fungi.

Study design: Experimental study

Place and Duration of Study: Department of Basic Sciences at Faculty of Allied Health Sciences and Research Laboratory at Faculty of Medicine, General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka, between July 2018 and November 2018.

Methodology: The aqueous, methanol, acetone and hexane extracts were prepared with the leaves, roots and stem of the plant *Oldenlandia auricularia* separately. The agar well diffusion method and broth macro dilution method were applied in order to screen the anti - microbial activity of each test extract against the *Escherichia coli, Salmonella enterica, Shigella dysenteriae, Candida albicans and Staphylococcus aureus.*

Results: The zone of inhibition of most of the test extracts showed a significant (P =.05) difference, when compared with the negative control. The lowest MIC value for test extracts was 31.25 mg/ml, while the highest was 250 mg/ml. The acetone extract of the stem showed the lowest MIC value against *E. coil.* The highest anti-bacterial activity against *S. enterica* exerted by the root of the plant. All three tested parts of the plant were active against *S. aureus* and the maximum activity against *C. albicans* was shown by the leave extracts.The lowest MIC value against *S. dysenteriae* was 62.5 mg/ml, which indicated that the plants materials are less sensitive to the *S. dysenteriae* than the other tested pathogens. The results of the quantitative assay confirmed the results obtained from the qualitative assay.

Conclusion: The different parts of *Oldenlandia auricularia* plant displayed potential antimicrobial activity against different pathogens.

Keywords: Oldenlandia auricularia, Anti-microbial effect, zone of inhibition, minimum inhibitory concentration

1. INTRODUCTION

Human pathogens are organisms that are capable of producing diseases in human body. Virtually all microbial groups have some pathogenic members. They are cable of invading and subsequently multiply within in the host body causing an infection. If the infection causes damage to the vital functions of the host body it leads to a disease. Different types of micro-organisms are causing different types of diseases and some microbial infections are also contribute to some chronic diseases such as cancers, coronary heart disease, etc. [1].

Infections caused by microbial pathogens are controlled with antimicrobial drugs called antibiotics, which act via various mechanisms within the human body. However, due to indiscriminant usage of antimicrobial drug, there is a continuous evolution of drug resistant strains of pathogens all around the world. Consequently, antibiotic resistance has become a global health threat as well as an economic burden as it led to the reemergence of several disease during past decade [2].

Therefore, there is a timely need for the discovery of new antimicrobial agent, in order to replace the drugs which has been developed to be resistant. Thus researchers focus their interest towards the investigation for new natural sources, which can provide promising anti-bacterial active chemicals. Plants have been recognized as potential natural sources which can provide compounds with strong antibacterial activity, as the researches revealed that the plants contain various chemical compounds with different bioactivities [2].

Plants played a major role in traditional medicine systems around the world and in Sri Lanka there is a rich traditional medicinal system which has been practiced from ancient times. The traditional medicinal practitioners are using different plants to treat different aliments in humans. The medicinal herbs, which are prepared in different forms, including decoctions, ointments, etc. show different curative properties [3]. There are several vegetation which have been used to treat infectious diseases by Sri Lankan folk in rural areas. However usage of these folk medicine has been gradually diminished due to emergence of allopathic medicine which are popular among people due to ease of usages [4].

However, due to high cost and emergence of adverse effects by using allopathic drugs, currently there is a trend in investigation of new agents that can be used to produce low cost drugs with less side effects. Therefore there is a timely need for scientific validation of the medicinal properties of the herbs that has been used in folk medicine, in order to prevent vanishing of traditional knowledge on valuable medicinal plants. The present study was designed to validate the antimicrobial activity of a vegetation which was commonly used by the folk of Sri Lanka in rural areas.

Oldenlandia auricularia is a medicinal plant from rubiaceae family, which is known as Getakola in Sri Lanka. It is an herbal plant, in which roots, seeds, leaves and also the whole plant are used to treat the dysentery, diarrhoea, and Azzospermia [5].

Hedyotis is the previous name used to identify plants belongs to genus *Oldenlandia.* They are used to treat the dysentery, diarrhea, wounds and snake bite and cancers in traditional medicinal systems of different countries. Number of phytochemicals such as alkaloids, anthraquinones, ligands, triterpenes, flavonoids and iridoids have been were found out in plants belong to the genus *Hedyotis,* including *H. chrysotricha, H. capitellata, H. hedyotidea, H. corymbosa and H. lawsonia.* [6].

Oldenlandia diffusa is a medicinal plant, mainly used to treat against *Heamophilus* influenza. This plant is used to treat for inflammatory and infectious disease, such as pneumonia, appendicitis, and urinary tract infections. [7]. Leaves, stem, roots and flowers of *Oldenlandia affinis* showed uterotonic, cytotoxic, and antimicrobial activity, inhibition of trypsin, and human immunodeficiency virus inhibition to inhibition of neurotensin binding [8].

Anti-bacterial activity of the crude extracts from samples of H*. Capitellata and H. dichotoma* indicated strong activity against gram positive *Bacillus substilis* (mutant), *B. substilis* (wild type) and methicillin resistant *S. aureus* and gram-negative *P. aeruginosa*. Inhibition zones were observed for four samples of two species [6].

Although the other plants belong to the genera *Oldenlandia* were extensively studied, the species *Oldenlandia auricularia* was ignored. Therefore the present study was designed to screen the anti-microbial activity of different extracts of *Oldenlandia auricularia* against common microbial pathogens causing gastro-intestinal diseases.

2. EXPERIMENTAL DETAILS

2.1 Collection of Plant Material

Healthy plant materials including leaves, stem and roots of *Oldenlandia auricularia* were collected from different areas of Kurunagala district, Sri Lanka during the period between July 2018 and August 2018. The plant materials were identified by National Herbarium, Peradeniya, Sri Lanka.

2.2 Preparation of Extracts

The collected plant materials were washed with distilled water. They were dried in open air and ground into powder separately. Each powered sample were soaked in distilled water, methanol, acetone and hexane for 7 days separately and then filtered. The prepared plant materials were freeze dried and stored under 8 ^{0}C until using for experiments [9].

2.3 Screening for anti-microbial activity

Each extract was screened for anti-bacterial sensitivity against different bacterial strains including *Salmonella enterica* (ATCC 14028), *Shigella dysenteriae* (ATCC 11835), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 10231).

The first screening was performed using Agar well diffusion method, a qualitative method which provided the information on the inhibitory zone of each test extract compared to negative and positive controls. Further screening was done using broth macro dilution method, a quantitative assay which determined the Minimum inhibitory concentration of each test extract.

2.3.1 Agar well diffusion Method

Each test extract was prepared by re-suspending the powdered sample (250 mg/ml) in respective solvent. Few colonies of each bacterial species were mixed with 10 ml of saline within 15 minutes before start the experiment. The prepared standardized inocula were diluted by adding nutrient broth until they contain approximately 5 x 10^5 CFU/ml. Then each bacterial suspension (50 ul) were spread on the agar plate surface using a sterile spreader. Four holes with a diameter of 5 mm were punched aseptically on each agar plate. Gentamycin was used as a positive control. The solvent used to prepare each extract was used as the respective negative control. These wells in each plate were filled with (100 ul) of test extract (250 mg/ml), positive control and respective solvent. The inoculated agar plates were kept 2 hours in room temperature and then incubated for 24 hours. After 24 hours, the diameter of the zone of inhibition around each well was measured using a vernier caliper. This procedure was performed for all the selected microbial species. The procedure was repeated for 3 time for each test extract [8].

2.3.2 Broth macro dilution Method

A two-fold dilution series of each test extract was prepared (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg /ml) using freeze dried samples. Five sets of dilution series of each test extract were prepared one for each microbial species.

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard equivalent to a 0.5 McFarland standard was used. McFarland standard (0.5) was prepared by adding a 0.5 ml aliquot of 0.048 mol/l $BaCl_2$ (1.175% w/v $BaCl_2 \cdot 2H_2O$) to 99.5 ml of 0.18 mol/l (0.36 N) H_2SO_4 (1% v/v) with constant stirring to maintain a suspension. The correct density of the turbidity standard was verified by measuring absorbance using a spectrophotometer with a 1-cm light path and matched cuvettes. The absorbance at 625 nm was 0.08 to 0.13 for the 0.5 McFarland standard [10].

The inoculum of each test pathogen was prepared by making a direct broth suspension of isolated colonies selected from an 18- to 24-hour agar plate. Few colonies of each bacterial species were mixed with 10 ml of saline within 15 minutes before start the experiment. The prepared inoculum was diluted by adding nutrient broth until each tube contains approximately 5×10^5 CFU/ml. Then the bacterial inoculum was diluted using nutrient broth until it is comparable to the turbidity of the prepared 0.5 McFarland suspension [10].

Within 15 minutes, 1 ml of the adjusted inoculum was added to each tube containing 1 ml of each test extract in the dilution series (and a positive control tube containing only broth), and mixed. Growth control tube, which contained only inoculated broth without antimicrobial agent was prepared for each organism. The tubes were closed with loose screw-caps, plastic or metal closure caps, or cotton plugs and incubated at 37^0C for 24 h. The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the tubes as detected by the unaided eye. The turbidity of the suspension of each tube containing the antibiotic dilution series was compared with the respective growth-control tubes [10].

3. RESULTS AND DISCUSSION

3.1 Zone of inhibition for different extracts of *Oldenlandia auricularia*

Results of Zone of inhibition for different parts of *Oldenlandia auricularia* are presented in Table 1,2,3,4 and 5. When compared to the negative control, some of the test extracts showed a significant inhibition (P = .05) against the tested microbial species, while others do not showed a significant inhibition (P > .05).

When considering the observed values for the diameter of zone of inhibition against *E. coli* (Table.1), the aqueous extract of roots and stem showed the maximum anti-microbial activity against *E. coli.* Other than that, the hexane root extract, aqueous and acetone extracts of leaves and the acetone extract of stem also showed a significant inhibition (P = .05) against *E. coli.* But when compared the observed zone inhibition values among the test extracts against *E. coli*, there was no significant (P > .05) difference between the values. When compared to the observed values for positive control (Gentamycin), all the extracts showed a significant difference (P = .05).

Table 1. Diameter of zone of inhibition for different extracts of *O. auricularia* against *E. coli*

Part of the plant	Extraction	Negative control (mm)	Test extract (mm)	Positive control (mm)
Root	Methanol	5.02 ± 0.01	5.98 ± 0.47[a]	13.31 ± 0.31*
	Aqueous	5.01 ± 0.02	6.78 ±0.08*[a]	14.64 ± 0.37*
	Acetone	5.00 ± 0.03	5.38 ± 0.33[a]	15.25 ±0.25*
	Hexane	5.01 ± 0.02	6.71 ± 0.14*[a]	13.25 ± 0.34*
Leaves	Methanol	5.01 ± 0.01	5.05 ± 0.02[a]	13.25 ± 0.25*
	Aqueous	5.00 ± 0.03	6.51 ± 0.25*[a]	15.11 ± 0.16*
	Acetone	5.01 ± 0.01	6.31 ± 0.19*[a]	13.38 ± 0.26*
	Hexane	5.01 ± 0.01	5.91 ± 0.43[a]	15.04 ± 0.22*
Stem	Methanol	5.02 ± 0.02	5.02 ± 1.70[a]	13.31 ± 0.34*
	Aqueous	5.01 ± 0.01	6.78 ± 0.08*[a]	14.45 ± 0.40*
	Acetone	5.01 ± 0.01	6.71 ±0.25*[a]	12.98 ± 0.50*
	Hexane	5.00 ± 0.02	5.65 ±0.33[a]	12.98 ± 0.50*

[*] Significant compared to negative control (P =.05), [a] Significant compared to positive control (P =.05).

According to the results (Table 2) the highest diameter of zone of inhibition against *S. enterica* was shown by methanol extract of roots. The methanol extracts of root and leaves, aqueous extracts of root and stem and acetone extract of root showed a significant inhibition (P =.05), compared to the negative control. But when compared the values among test extracts there was no significant (P > .05) difference between them. All the extracts showed a significant difference (P = .05), compared to the positive control.

Table 2. Diameter of zone of inhibition for different extracts of *O. auricularia* against *S. enterica.*

Part of the plant	Extraction	Negative control (mm)	Test extract (mm)	Positive control (mm)
Root	Methanol	5.00 ±0.02	6.91 ± 0.19*[a]	12.77 ± 0.36*
	Aqueous	5.02 ± 0.01	5.97 ± 0.50*[a]	15.04 ± 0.32*
	Acetone	5.01 ± 0.01	6.77 ± 0.29*[a]	14.31 ± 0.42*
	Hexane	5.01 ± 0.01	5.04 ± 0.01[a]	13.77 ± 0.19*
leaves	Methanol	5.01 ± 0.02	5.97 ± 0.50*[a]	14.97 ± 0.17*
	Aqueous	5.02 ± 0.01	5.04 ± 0.01[a]	14.70 ± 0.30*
	Acetone	5.00 ± 0.02	5.04 ± 0.01[a]	13.97 ± 0.07*
	Hexane	5.01 ± 0.01	5.04 ± 0.01[a]	15.04 ± 0.11*
Stem	Methanol	5.01 ± 0.02	5.04 ± 0.01[a]	14.97 ± 0.38*
	Aqueous	5.01 ± 0.02	6.04 ± 0.53*[a]	15.04 ± 0.22*
	Acetone	5.01 ± 0.02	5.04 ±0.01[a]	15.24 ±0.13*
	Hexane	5.02 ±0.01	5.04 ± 0.01[a]	14.84 ± 0.30*

171 * Significant compared to negative control (P =.05), [a] Significant compared to positive control (P =.05).

172
173 When compared the observed values against ***S. dysenteriae***, only the hexane and acetone
174 extracts of stem showed a significant inhibition against the pathogen (Table 3). Out of these
175 two active extracts the maximum inhibition was exerted by hexane extract of stem. None of
176 the root and leave extracts showed an inhibition against ***S. dysenteriae***. But all the extracts
177 showed a significant difference ($p < 0.05$), between the value for respective positive control.
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183 **Table 3. Diameter of zone of inhibition for different extracts of *O. auricularia* against**

184 ***S. dysenteriae***

Part of the plant	Extraction	Negative control (mm)	Test extract (mm)	Positive control (mm)
Root	Methanol	5.01 ± 0.03	5.05 ± 0.03[a]	14.45 ± 0.19*
	Aqueous	5.02 ± 0.02	5.02 ± 0.02[a]	14.92 ± 0.12*
	Acetone	5.00 ± 0.01	5.03 ± 0.01[a]	14.85 ± 0.21*
	Hexane	5.01 ± 0.02	5.02 ± 0.02[a]	14.52 ± 0.39*
Leaves	Methanol	5.02 ± 0.02	5.03 ± 0.01[a]	14.12 ±0.37*
	Aqueous	5.01 ± 0.02	5.01 ± 0.02 [a]	14.39 ± 0.14*
	Acetone	5.01 ± 0.03	5.01 ± 0.01[a]	13.65 ± 0.34*
	Hexane	5.02 ±- 0.01	5.00 ± 0.02[a]	15.19 ±0.15*
Stem	Methanol	5.01 ± 0.02	5.02± 0.02[a]	13.65 ± 0.30*
	Aqueous	5.01 ± 0.01	5.00 ± 0.02[a]	14.19 ± 0.46*
	Acetone	5.02 ± 0.02	5.39 ± 0.32*[a]	15.32 ± 0.43*
	Hexane	5.00 ± 0.02	6.05 ± 0.11*[a]	14.18 ± 0.23*

185 * Significant compared to negative control (P =.05), [a] Significant compared to positive control (P =.05).

186

187 All the test extracts exerted a significant inhibition (P =.05) against *C. albicans* except the
188 aqueous extract of the stem. Among them the highest activity was shown by methanol
189 extract of roots. But there was no significant difference (P > .05) between the values of
190 inhibition diameter among the active extracts. However, when compared to the observed
191 values for respective positive control, all the extracts showed a significant difference (P
192 =.05).
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196 **Table 4. Diameter of zone of inhibition for different extracts of *O. auricularia* against**

197 ***C. albicans***

Part of the plant	Extraction	Negative control (mm)	Test extract (mm)	Positive control (mm)
Root	Methanol	5.02 ± 0.01	6.81 ± 0.39*[a]	12.74 ± 0.26*
	Aqueous	5.01 ± 0.02	5.80 ± 0.40*[a]	14.54 ± 0.07*
	Acetone	5.02 ±- 0.01	6.00 ± 0.48*[a]	13.94 ± 0.49*
	Hexane	5.02 ±- 0.02	5.07 ± 0.03*[a]	13.94 ± 0.51*
Leaves	Methanol	5.02 ± 0.01	6.41 ± 0.11*[a]	13.60 ± 0.03*
	Aqueous	5.03 ± 0.02	5.87 ± 0.41*[a]	15.14 ± 0.70*
	Acetone	5.02 ± 0.02	6.54 ± 0.10*[a]	12.81 ± 0.50*
	Hexane	5.00 ± 0.01	5.74 ± 0.37*[a]	14.00 ± 0.37*
Stem	Methanol	5.02 ± 0.01	6.34 ± 0.21*[a]	14.87 ± 0.24*
	Aqueous	5.02 ±0.02	5.07 ± 0.03[a]	13.47 ± 0.31*
	Acetone	5.01 ± 0.02	6.14 ± 0.27*[a]	12.67 ± 0.36*
	Hexane	5.01 ± 0.03	6.15 ± 0.27*[a]	14.34 ± 0.28*

198 * Significant compared to negative control (P =.05), [a] Significant compared to positive control (P =.05).

199
200 According to the obtained results, the diameter of zone of inhibition against *S. aureus* for all
201 the test extracts were significantly different (P =.05) from the values obtained for negative
202 control as well as the positive control. Similar to the results against the other pathogens, the
203 inhibition among different extracts against *S. aureus* also did not showed any significant
204 difference (P =.05). The aqueous extract of leaves exerted the highest inhibition against *S.*
205 *aureus.*
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209 **Table 5. Diameter of zone of inhibition for different extracts of *O. auricularia* against**

210 ***S. aureus***

Part of the plant	Extraction	Negative control (mm)	Test extract (mm)	Positive control (mm)
Root	Methanol	5.01 ± 0.02	6.95 ± 0.12*[a]	11.58 ± 0.41*
	Aqueous	5.03 ± 0.03	5.74 ± 0.36*[a]	14.68 ± 0.36*
	Acetone	5.01 ± 0.02	6.81 ± 0.09*[a]	13.28 ± 0.29*
	Hexane	5.02 ± 0.01	6.75 ± 0.16*[a]	15.01 ± 0.19*
Leaves	Methanol	5.02 ± 0.01	6.01 ± 0.47*[a]	14.35 ± 0.32*
	Aqueous	5.02 ± 0.01	7.28 ± 2.47*[a]	14.35 ± 0.23*
	Acetone	5.01 ± 0.02	5.68 ± 0.31*[a]	13.01 ± 0.17*
	Hexane	5.03 ± 0.02	6.35 ± 0.05*[a]	14.48 ± 0.25*
Stem	Methanol	5.03 ± 0.01	7.01 ± 0.14*[a]	14.54 ± 0.14*
	Aqueous	5.03 ± 0.02	5.88 ± 0.41*[a]	14.88 ± 0.38*
	Acetone	5.02 ± 0.01	6.95 ± 0.19*[a]	14.28 ± 0.22*
	Hexane	5.02 ± 0.02	6.41 ± 0.15*[a]	14.68 ± 0.33*

211 * Significant compared to negative control (P =.05), [a] Significant compared to positive control (P =.05).

212
213 The agar well method was performed for the first screening of the antimicrobial activity of the
214 test extracts. It is a qualitative method which provides only a relative idea about the anti-
215 microbial activity compared to the negative and positive controls. When considering overall
216 results obtained, most of the test extracts showed a significant (P = .05) inhibition against
217 the test pathogens when compared with the negative control. It suggests that majority of the
218 extracts of the selected plant material possess antimicrobial activity against the tested
219 pathogens. But when compared the observed zone inhibition values among the test extracts
220 against each microbial species, there was no significant (P > .05) difference between the
221 values. Hence, the results only provided a quantitative measurement on the anti-microbial
222 activity of the each test extract.

223 Therefore in order to obtain quantitative information on the anti-microbial activity of each
224 extract against the tested pathogens, the second screening was done using broth dilution
225 method. It provided the values for minimum inhibitory concentration for each extract against
226 the pathogens, which provided a better understanding on the anti-microbial effect of test
227 extracts.

When compared to the observed values for respective positive control, all the extracts showed a significant difference (P = .05), which indicated that the activity of the test extracts was not potent compared to the standard drug gentamicin. This may be, because the test extracts are the crude extracts which contain plenty of chemicals and therefore the antimicrobial activity of a particular active compound may diluted. But as the gentamicin is a pure compound, it may show a potent activity. Therefore the higher concentrations of the test extracts may show more activity than the activity observed in present study. Also if the bioactive compound are identified and purified, they may also show a potent activity than the crude extracts.

3.2 Minimum Inhibitory concentration (MIC) for different extracts of *O. auricularia*

The observed MIC Values for different extracts of the plant *O. Auricularia* are presented in Table 6. The observed lowest MIC value was 31.25 mg/ml and the highest MIC value was 250 mg/ml.

The acetone extract of the stem showed the lowest MIC value of 31.25 mg/ml against *E. coil* while majority of the test extracts obtained the MIC value of 62.5 mg/ml.

The lowest MIC value (31.25 mg/ml) against *S. enterica* was shown by the methanol and acetone extracts of the root. This suggest that the highest anti-bacterial activity against *S. enterica* exerts by the root of the plant.

The lowest MIC value (31.25 mg/ml) against *S. aureus* was shown by several test extracts including aqueous leaves extract, methanolic root extract, methanolic stem extract, acetone root extract and acetone stem extract. The results shows that, all three tested parts of the plant are active against *S. aureus.*

The aqueous and methanolic leaves extracts showed the lowest (31.25 mg/ml) MIC value against *C. albicans*, indicating the leaves contain the bio-compounds which are highly active against *C. albicans.*

The lowest MIC value against *S. dysenteriae* was 62.5 mg/ml, which indicated that the plants materials are less sensitive to the *S. dysenteriae* than the other tested pathogens. It was exerted by hexane and acetone extracts of stem, suggesting that mainly the stem contain the active chemicals against *S. dysenteriae*.

Table 6. Observed MIC values for different test extracts of *O. auricularia* against tested pathogens

Extract	Part of the plant	Micro organism				
		E. coli (mg/ml)	*S. enterica* (mg/ml)	*C. albicans* (mg/ml)	*S. aureus* (mg/ml)	*S. dysenteriae* (mg/ml)
Aqueous	Leaves	62.5	62.5	31.25	31.25	125
	Roots	62.5	62.5	62.5	62.5	125
	Stem	125	62.5	125	62.5	125
Hexane	Leaves	250	125	62.5	62.5	125
	Roots	62.5	125	125	62.5	125
	Stem	125	125	62.5	62.5	62.5
Methanol	Leaves	125	62.5	31.25	62.5	125
	Roots	62.5	31.25	62.5	31.25	125
	Stem	62.5	62.5	62.5	31.25	125
Acetone	Leaves	62.5	125	125	62.5	125
	Roots	62.5	31.25	125	31.25	125
	Stem	31.25	62.5	62.5	31.25	62.5

According to the observed results highest number of test extracts showed maximum activity against *S. aureus.* This is an interesting finding as there are drug-resistant strain of *S. aureus*, which are more virulent than the wild type. They are responsible for the morbidity and mortality of majority of hospitalized patients. Therefore the plant materials of *O. Auricularia* may contain secondary metabolites which are highly active against drug-resistant strain of *S. aureus*. Therefore further investigations are recommended with drug-resistant strain of *S. aureus.*

The results of the quantitative assay confirmed the results obtained from the qualitative assay. The above results revealed the fact that different parts of the same plant exert the

maximum anti-microbial activity against different pathogens. This suggest that the different parts of the same plant contain different types of anti-microbial active bio-compounds. Therefore, the further studies could be carried out using only the specific parts of the plant which showed the maximum activity, in order to investigate the efficacy of the anti-microbial activity against each pathogen. This may leads to discovery of new chemicals with potent activity against them. Thus, further studies can be focus on only towards the extracts which showed the highest activity during the screening. This confirms the importance of the initial screening of bioactivities, before starting in-depth studies, which save cost, man-power and the time of investigators.

The previous studies were investigated the the anti-microbial effect of the plant materials of the other species of the same genus. Wajima *et al.,* [7] observed that *Oldenlandia diffusa* extracts showed positive results against *S. pneumoniae*. *Hedyotis* is the previous name used to identify plants belongs to genus *Oldenlandia.* A study conducted by Ahamad *et al.,*[6] reported, that the roots and the stems of *H. canitellata* showed weak to moderate activities against both gram positive and gram-negative bacteria. The root extraction of *H. dichotoma* showed moderate anti -bacterial activity towards gram positive *B. substilis* and gram-negative *P. aeruginosa* and it surpassed other extracts in exhibiting strong activity against *B. subtilis* compared to the control. The present study reported that the majority of tested extracts of *O. auricularia* are also active against tested gram negative and gram positive bacteria as well as *C. albicans.* Further studies should conduct in order to evaluate the efficacy of anti-microbial activity shown by different extracts using higher concentrations. Further, identification and purification of active compounds may leads to discovery of new chemical agents with promising anti-microbial potential.

4. CONCLUSION

The results of the present study observed that the different parts of the plant *O. auricularia* possess anti-microbial activity against different human pathogens. Therefore the present study revealed the anti-microbial activity of the plant against the pathogens which cause gastro-intestinal infections. Thus, the present study confirms the usage of vegetation, *Oldenlandia auricularia* as a medicinal plant which is applied to treat the dysentery and diarrhea by Sri Lankan folk.

ETHICAL APPROVAL

Not applicable

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ABBREVIATIONS

ATCC - American Type Culture Collection
CFU – Colony Forming Units
MIC – Minimum Inhibitory Concentration

DEFINITIONS

Minimum inhibitory concentration (MIC): the lowest concentration of a chemical, usually a drug, which prevents visible growth of a bacterium

Zone of inhibition: If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the medium where the bacteria have not grown enough to be visible