

1 **Original Research Article**  
2 **Antimicrobial activity of crude extracts of**  
3 ***Oldenlandia auricularia* against some selected**  
4 **human pathogens**

5 **ABSTRACT**  
6

**Aims:** Currently there 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. coli*. 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.

7  
8 **Keywords:** *Oldenlandia auricularia*, Anti-microbial effect, zone of inhibition, minimum  
9 inhibitory concentration

10 **1. INTRODUCTION**  
11

12 Human pathogens are organisms that are capable of producing diseases in human body.  
13 Virtually all microbial groups have some pathogenic members. They are cable of invading  
14 and subsequently multiply within in the host body causing an infection. If the infection causes  
15 damage to the vital functions of the host body it leads to a disease. Different types of micro-  
16 organisms are causing different types of diseases and some microbial infections are also  
17 contribute to some chronic diseases such as cancers, coronary heart disease, etc. [1].  
18

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

25  
26 Therefore, there is a timely need for the discovery of new antimicrobial agent, in order to  
27 replace the drugs which has been developed to be resistant. Thus researchers focus their  
28 interest towards the investigation for new natural sources, which can provide promising anti-  
29 bacterial active chemicals. Plants have been recognized as potential natural sources which  
30 can provide compounds with strong antibacterial activity, as the researches revealed that the  
31 plants contain various chemical compounds with different bioactivities [2].

32  
33 Plants played a major role in traditional medicine systems around the world and in Sri Lanka  
34 there is a rich traditional medicinal system which has been practiced from ancient times. The  
35 traditional medicinal practitioners are using different plants to treat different ailments in  
36 humans. The medicinal herbs, which are prepared in different forms, including decoctions,  
37 ointments, etc. show different curative properties [3]. There are several vegetation which  
38 have been used to treat infectious diseases by Sri Lankan folk in rural areas. However  
39 usage of these folk medicine has been gradually diminished due to emergence of allopathic  
40 medicine which are popular among people due to ease of usages [4].

41  
42 However, due to high cost and emergence of adverse effects by using allopathic drugs,  
43 currently there is a trend in investigation of new agents that can be used to produce low cost  
44 drugs with less side effects. Therefore there is a timely need for scientific validation of the  
45 medicinal properties of the herbs that has been used in folk medicine, in order to prevent  
46 vanishing of traditional knowledge on valuable medicinal plants. The present study was  
47 designed to validate the antimicrobial activity of a vegetation which was commonly used by  
48 the folk of Sri Lanka in rural areas.

49  
50 *Oldenlandia auricularia* is a medicinal plant from rubiaceae family, which is known as  
51 Getakola in Sri Lanka. It is an herbal plant, in which roots, seeds, leaves and also the whole  
52 plant are used to treat the dysentery, diarrhoea, and Azzospermia [5].

53  
54 *Hedyotis* is the previous name used to identify plants belongs to genus *Oldenlandia*. They  
55 are used to treat the dysentery, diarrhea, wounds and snake bite and cancers in traditional  
56 medicinal systems of different countries. Number of phytochemicals such as alkaloids,  
57 anthraquinones, ligands, triterpenes, flavonoids and iridoids have been found out in  
58 plants belong to the genus *Hedyotis*, including *H. chrysotricha*, *H. capitellata*, *H. hedyotideia*,  
59 *H. corymbosa* and *H. lawsonia*. [6].

60  
61 *Oldenlandia diffusa* is a medicinal plant, mainly used to treat against *Heamophilus* influenza.  
62 This plant is used to treat for inflammatory and infectious disease, such as pneumonia,  
63 appendicitis, and urinary tract infections. [7]. Leaves, stem, roots and flowers of *Oldenlandia*  
64 *affinis* showed uterotonic, cytotoxic, and antimicrobial activity, inhibition of trypsin, and  
65 human immunodeficiency virus inhibition to inhibition of neurotensin binding [8].

66  
67 Anti-bacterial activity of the crude extracts from samples of *H. Capitellata* and *H. dichotoma*  
68 indicated strong activity against gram positive *Bacillus substilis* (mutant), *B. substilis* (wild  
69 type) and methicillin resistant *S. aureus* and gram-negative *P. aeruginosa*. Inhibition zones  
70 were observed for four samples of two species [6].

71

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

76

## 77 **2. EXPERIMENTAL DETAILS**

78

### 79 **2.1 Collection of Plant Material**

80 Healthy plant materials including leaves, stem and roots of *Oldenlandia auricularia* were  
81 collected from different areas of Kurunagala district, Sri Lanka during the period between  
82 July 2018 and August 2018. The plant materials were identified by National Herbarium,  
83 Peradeniya, Sri Lanka and the voucher specimen (KDU/FAHS/2018/0101) was deposited in  
84 the herbarium of Faculty of Allied Health Sciences, Kotelawala Defence University.

85

### 86 **2.2 Preparation of Extracts**

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

91

### 92 **2.3 Screening for anti-microbial activity**

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

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

102

#### 103 **2.3.1 Agar well diffusion Method**

104 Each test extract was prepared by re-suspending the powdered sample (250 mg/ml) in  
105 respective solvent. Few colonies of each bacterial species were mixed with 10 ml of saline  
106 within 15 minutes before the start of the experiment. The prepared standardized inocula  
107 were diluted by adding nutrient broth until they contain approximately  $5 \times 10^5$  CFU/ml. Then  
108 each bacterial suspension (50 ul) were spread on the agar plate surface using a sterile  
109 spreader. Four holes with a diameter of 5 mm were punched aseptically on each agar plate.  
110 Gentamycin (0.1 mg/ml) was used as a positive control. The solvent used to prepare each  
111 extract was used as the respective negative control. These wells in each plate were filled  
112 with (100 ul) of test extract (250 mg/ml), positive control and respective solvent. The  
113 inoculated agar plates were kept 2 hours in room temperature and then incubated for 24  
114 hours. After 24 hours, the diameter of the zone of inhibition around each well was measured

115 using a vernier caliper. This procedure was performed for all the selected microbial species.  
116 The procedure was repeated for 3 time for each test extract [8].

117

### 118 **2.3.2 Broth macro dilution Method**

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

122 To standardize the inoculum density for a susceptibility test, a BaSO<sub>4</sub> turbidity standard  
123 equivalent to a 0.5 McFarland standard was used. McFarland standard (0.5) was prepared  
124 by adding a 0.5 ml aliquot of 0.048 mol/l BaCl<sub>2</sub> (1.175% w/v BaCl<sub>2</sub>•2H<sub>2</sub>O) to 99.5 ml of 0.18  
125 mol/l (0.36 N) H<sub>2</sub>SO<sub>4</sub> (1% v/v) with constant stirring to maintain a suspension. The correct  
126 density of the turbidity standard was verified by measuring absorbance using a  
127 spectrophotometer with a 1-cm light path and matched cuvettes. The absorbance at 625 nm  
128 was 0.08 to 0.13 for the 0.5 McFarland standard [10].

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

135 Within 15 minutes, 1 ml of the adjusted inoculum was added to each tube containing 1 ml of  
136 each test extract in the dilution series. Gentamycin (0.1 mg/ml) was used as the positive  
137 control while, a growth control tube was prepared without adding any antimicrobial agent.  
138 The tubes were closed with loose screw-caps, plastic or metal closure caps, or cotton plugs  
139 and incubated at 37<sup>0</sup>C for 24 h. The MIC is the lowest concentration of antimicrobial agent  
140 that completely inhibits growth of the organism in the tubes as detected by the unaided eye.  
141 The turbidity of the suspension of each tube containing the antibiotic dilution series was  
142 compared with the respective growth-control tubes [10].

### 143 **2.4 Statistical analysis**

144

145 The results were given as mean ± SEM. Data analysis was performed by SPSS version  
146 21.0. Statistical comparisons were made using Duncan's new multiple range test.  
147 Significance was set at P=.05.

148

149

## 150 **3. RESULTS AND DISCUSSION**

151

### 152 **3.1 Zone of inhibition for different extracts of *Oldenlandia auricularia***

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

157 When considering the observed values for the diameter of zone of inhibition against *E. coli*  
158 (Table.1), the aqueous extract of roots and stem showed the maximum anti-microbial activity

159 against *E. coli*. Other than that, the hexane root extract, aqueous and acetone extracts of  
 160 leaves and the acetone extract of stem also showed a significant inhibition ( $P = .05$ ) against  
 161 *E. coli*. But when compared the observed zone inhibition values among the test extracts  
 162 against *E. coli*, there was no significant ( $P > .05$ ) difference between the values. When  
 163 compared to the observed values for positive control (Gentamycin), all the extracts showed a  
 164 significant difference ( $P = .05$ ).

165

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

167 ***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 <sup>a</sup>	13.31 ± 0.31*
	Aqueous	5.01 ± 0.02	6.78 ± 0.08 <sup>*a</sup>	14.64 ± 0.37*
	Acetone	5.00 ± 0.03	5.38 ± 0.33 <sup>a</sup>	15.25 ± 0.25*
	Hexane	5.01 ± 0.02	6.71 ± 0.14 <sup>*a</sup>	13.25 ± 0.34*
Leaves	Methanol	5.01 ± 0.01	5.05 ± 0.02 <sup>a</sup>	13.25 ± 0.25*
	Aqueous	5.00 ± 0.03	6.51 ± 0.25 <sup>*a</sup>	15.11 ± 0.16*
	Acetone	5.01 ± 0.01	6.31 ± 0.19 <sup>*a</sup>	13.38 ± 0.26*
	Hexane	5.01 ± 0.01	5.91 ± 0.43 <sup>a</sup>	15.04 ± 0.22*
Stem	Methanol	5.02 ± 0.02	5.02 ± 1.70 <sup>a</sup>	13.31 ± 0.34*
	Aqueous	5.01 ± 0.01	6.78 ± 0.08 <sup>*a</sup>	14.45 ± 0.40*
	Acetone	5.01 ± 0.01	6.71 ± 0.25 <sup>*a</sup>	12.98 ± 0.50*
	Hexane	5.00 ± 0.02	5.65 ± 0.33 <sup>a</sup>	12.98 ± 0.50*

168

\* Significant compared to negative control ( $P = .05$ ), <sup>a</sup> Significant compared to positive control ( $P = .05$ ).

169

170 According to the results (Table 2) the highest diameter of zone of inhibition against *S.*  
 171 *enterica* was shown by methanol extract of roots. The methanol extracts of root and leaves,  
 172 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

173 extracts there was no significant ( $P > .05$ ) difference between them. All the extracts showed  
 174 a significant difference ( $P = .05$ ), compared to the positive control.  
 175 **Table 2. Diameter of zone of inhibition for different extracts of *O. auricularia* against**

176 ***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 <sup>a</sup>	12.77 ± 0.36*
	Aqueous	5.02 ± 0.01	5.97 ± 0.50 <sup>a</sup>	15.04 ± 0.32*
	Acetone	5.01 ± 0.01	6.77 ± 0.29 <sup>a</sup>	14.31 ± 0.42*
	Hexane	5.01 ± 0.01	5.04 ± 0.01 <sup>a</sup>	13.77 ± 0.19*
leaves	Methanol	5.01 ± 0.02	5.97 ± 0.50 <sup>a</sup>	14.97 ± 0.17*
	Aqueous	5.02 ± 0.01	5.04 ± 0.01 <sup>a</sup>	14.70 ± 0.30*
	Acetone	5.00 ± 0.02	5.04 ± 0.01 <sup>a</sup>	13.97 ± 0.07*
	Hexane	5.01 ± 0.01	5.04 ± 0.01 <sup>a</sup>	15.04 ± 0.11*
Stem	Methanol	5.01 ± 0.02	5.04 ± 0.01 <sup>a</sup>	14.97 ± 0.38*
	Aqueous	5.01 ± 0.02	6.04 ± 0.53 <sup>a</sup>	15.04 ± 0.22*
	Acetone	5.01 ± 0.02	5.04 ± 0.01 <sup>a</sup>	15.24 ± 0.13*
	Hexane	5.02 ± 0.01	5.04 ± 0.01 <sup>a</sup>	14.84 ± 0.30*

177 <sup>a</sup> Significant compared to negative control ( $P = .05$ ), <sup>\*</sup> Significant compared to positive control ( $P = .05$ ).

178  
 179 When compared the observed values against ***S. dysenteriae***, only the hexane and acetone  
 180 extracts of stem showed a significant inhibition against the pathogen (Table 3). Out of these  
 181 two active extracts the maximum inhibition was exerted by hexane extract of stem. None of  
 182 the root and leave extracts showed an inhibition against ***S. dysenteriae***. But all the extracts  
 183 showed a significant difference ( $p < 0.05$ ), between the value for respective positive control.  
 184  
 185

186

187

188 **Table 3. Diameter of zone of inhibition for different extracts of *O. auricularia* against**  
 189 ***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 <sup>a</sup>	14.45 ± 0.19*
	Aqueous	5.02 ± 0.02	5.02 ± 0.02 <sup>a</sup>	14.92 ± 0.12*
	Acetone	5.00 ± 0.01	5.03 ± 0.01 <sup>a</sup>	14.85 ± 0.21*
	Hexane	5.01 ± 0.02	5.02 ± 0.02 <sup>a</sup>	14.52 ± 0.39*
Leaves	Methanol	5.02 ± 0.02	5.03 ± 0.01 <sup>a</sup>	14.12 ± 0.37*
	Aqueous	5.01 ± 0.02	5.01 ± 0.02 <sup>a</sup>	14.39 ± 0.14*
	Acetone	5.01 ± 0.03	5.01 ± 0.01 <sup>a</sup>	13.65 ± 0.34*
	Hexane	5.02 ± 0.01	5.00 ± 0.02 <sup>a</sup>	15.19 ± 0.15*
Stem	Methanol	5.01 ± 0.02	5.02 ± 0.02 <sup>a</sup>	13.65 ± 0.30*
	Aqueous	5.01 ± 0.01	5.00 ± 0.02 <sup>a</sup>	14.19 ± 0.46*
	Acetone	5.02 ± 0.02	5.39 ± 0.32 <sup>*a</sup>	15.32 ± 0.43*
	Hexane	5.00 ± 0.02	6.05 ± 0.11 <sup>*a</sup>	14.18 ± 0.23*

190 <sup>a</sup> Significant compared to negative control (P =.05), \* Significant compared to positive control (P =.05).

191

192 All the test extracts exerted a significant inhibition (P =.05) against *C. albicans* except the  
 193 aqueous extract of the stem. Among them the highest activity was shown by methanol  
 194 extract of roots. But there was no significant difference (P > .05) between the values of  
 195 inhibition diameter among the active extracts. However, when compared to the observed  
 196 values for respective positive control, all the extracts showed a significant difference (P  
 197 =.05).

198

199

200

201 **Table 4. Diameter of zone of inhibition for different extracts of *O. auricularia* against**  
 202 ***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 <sup>*a</sup>	12.74 ± 0.26*
	Aqueous	5.01 ± 0.02	5.80 ± 0.40 <sup>*a</sup>	14.54 ± 0.07*
	Acetone	5.02 ± 0.01	6.00 ± 0.48 <sup>*a</sup>	13.94 ± 0.49*
	Hexane	5.02 ± 0.02	5.07 ± 0.03 <sup>*a</sup>	13.94 ± 0.51*
Leaves	Methanol	5.02 ± 0.01	6.41 ± 0.11 <sup>*a</sup>	13.60 ± 0.03*
	Aqueous	5.03 ± 0.02	5.87 ± 0.41 <sup>*a</sup>	15.14 ± 0.70*
	Acetone	5.02 ± 0.02	6.54 ± 0.10 <sup>*a</sup>	12.81 ± 0.50*
	Hexane	5.00 ± 0.01	5.74 ± 0.37 <sup>*a</sup>	14.00 ± 0.37*
Stem	Methanol	5.02 ± 0.01	6.34 ± 0.21 <sup>*a</sup>	14.87 ± 0.24*
	Aqueous	5.02 ± 0.02	5.07 ± 0.03 <sup>a</sup>	13.47 ± 0.31*
	Acetone	5.01 ± 0.02	6.14 ± 0.27 <sup>*a</sup>	12.67 ± 0.36*
	Hexane	5.01 ± 0.03	6.15 ± 0.27 <sup>*a</sup>	14.34 ± 0.28*

203 <sup>a</sup> Significant compared to negative control (P =.05), <sup>a</sup> Significant compared to positive control (P =.05).

204  
 205 According to the obtained results, the diameter of zone of inhibition against *S. aureus* for all  
 206 the test extracts were significantly different (P =.05) from the values obtained for negative  
 207 control as well as the positive control. Similar to the results against the other pathogens, the  
 208 inhibition among different extracts against *S. aureus* also did not showed any significant  
 209 difference (P =.05). The aqueous extract of leaves exerted the highest inhibition against *S.*  
 210 *aureus*.

211  
 212

213

214 **Table 5. Diameter of zone of inhibition for different extracts of *O. auricularia* against**  
 215 ***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 <sup>*a</sup>	11.58 ± 0.41*
	Aqueous	5.03 ± 0.03	5.74 ± 0.36 <sup>*a</sup>	14.68 ± 0.36*
	Acetone	5.01 ± 0.02	6.81 ± 0.09 <sup>*a</sup>	13.28 ± 0.29*
	Hexane	5.02 ± 0.01	6.75 ± 0.16 <sup>*a</sup>	15.01 ± 0.19*
Leaves	Methanol	5.02 ± 0.01	6.01 ± 0.47 <sup>*a</sup>	14.35 ± 0.32*
	Aqueous	5.02 ± 0.01	7.28 ± 2.47 <sup>*a</sup>	14.35 ± 0.23*
	Acetone	5.01 ± 0.02	5.68 ± 0.31 <sup>*a</sup>	13.01 ± 0.17*
	Hexane	5.03 ± 0.02	6.35 ± 0.05 <sup>*a</sup>	14.48 ± 0.25*
Stem	Methanol	5.03 ± 0.01	7.01 ± 0.14 <sup>*a</sup>	14.54 ± 0.14*
	Aqueous	5.03 ± 0.02	5.88 ± 0.41 <sup>*a</sup>	14.88 ± 0.38*
	Acetone	5.02 ± 0.01	6.95 ± 0.19 <sup>*a</sup>	14.28 ± 0.22*
	Hexane	5.02 ± 0.02	6.41 ± 0.15 <sup>*a</sup>	14.68 ± 0.33*

216 <sup>a</sup> Significant compared to negative control (P = .05), \* Significant compared to positive control (P = .05).

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

228 Therefore in order to obtain quantitative information on the anti-microbial activity of each  
 229 extract against the tested pathogens, the second screening was done using broth dilution

230 method. It provided the values for minimum inhibitory concentration for each extract against  
231 the pathogens, which provided a better understanding on the anti-microbial effect of test  
232 extracts.

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

242

### 243 **3.2 Minimum Inhibitory concentration (MIC) for different extracts of** 244 ***O. auricularia***

245

246 The observed MIC Values for different extracts of the plant *O. Auricularia* are presented in  
247 Table 6. The observed lowest MIC value was 31.25 mg/ml and the highest MIC value was  
248 250 mg/ml.

249

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

252

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

255

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

259

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

262

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

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270

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

Extract	Part of the plant	Micro organism				
		<i>E. coli</i> (mg/ml)	<i>S. enterica</i> (mg/ml)	<i>C. albicans</i> (mg/ml)	<i>S. aureus</i> (mg/ml)	<i>S. dysenteriae</i> (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

273

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

279 strain of *S. aureus*. Therefore further investigations are recommended with drug-resistant  
280 strain of *S. aureus*.

281 The results of the quantitative assay confirmed the results obtained from the qualitative  
282 assay. The above results revealed the fact that different parts of the same plant exert the  
283 maximum anti-microbial activity against different pathogens. This suggest that the different  
284 parts of the same plant contain different types of anti-microbial active bio-compounds.  
285 Therefore, the further studies could be carried out using only the specific parts of the plant  
286 which showed the maximum activity, in order to investigate the efficacy of the anti-microbial  
287 activity against each pathogen. This may leads to discovery of new chemicals with potent  
288 activity against them. Thus, further studies can be focus on only towards the extracts which  
289 showed the highest activity during the screening. This confirms the importance of the initial  
290 screening of bioactivities, before starting in-depth studies, which save cost, man-power and  
291 the time of investigators.

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

306

#### 307 **4. CONCLUSION**

308  
309 The results of the present study showed that the different parts of the plant *O. auricularia*  
310 possess anti-microbial activity against different human pathogens. Therefore the present  
311 study revealed the anti-microbial activity of the plant against the pathogens which cause  
312 gastro-intestinal infections. Thus, the present study confirms the usage of vegetation,  
313 *Oldenlandia auricularia* as a medicinal plant which is applied in the treatment of dysentery  
314 and diarrhea by Sri Lankan folk.

315  
316

#### 317 **ETHICAL APPROVAL**

318 Not applicable

319  
320

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#### 365 ABBREVIATIONS

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

#### 371 DEFINITIONS

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

374  
375 **Zone of inhibition:** If an antibiotic stops the bacteria from growing or kills the bacteria,  
376 there will be an area around the medium where the bacteria have not grown enough to  
377 be visible