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Original Article

Antibacterial activity of crude extracts of *Camponotus compressus* (Fabricius, 1787) (Hymenoptera: Formicidae)

Atividade antibacteriana de extratos brutos de *Camponotus compressus* (Fabricius, 1787) (Hymenoptera: Formicidae)

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Abstract

The current study evaluates the antibacterial activity of *Camponotus compressus* (Hymenoptera: Formicidae) body crude extracts. The increasing antibiotic resistance of bacteria has prompted the world to turn its attention towards insects in the search for new sources of antibacterial compounds. The body crude extract obtained with different solvents were tested against both Gram positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram negative bacteria *(Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*). Standard disc diffusion method was used to perform the activity. The extracts of *C. compressus* were investigated for their effectiveness against all resistant pathogenic bacteria. *Staphylococcus aureus* was found to be the most susceptible, exhibiting a high average growth inhibition, while *Bacillus subtilis* showed a lower average growth inhibition zone. Our findings regarding the inhibitory effect of *C. compressus* extracts show the presence of a broad-spectrum antibacterial compound. This will be helpful in the search for novel natural antibiotics against robust pathogenic bacterial strains.

Keywords: *Camponotus compressus*, antibacterial compounds, crude extracts, antibiotic, pathogenic.

Resumo

O presente estudo avalia a atividade antibacteriana de extratos brutos corporais de *Camponotus compressus* (Hymenoptera: Formicidae). A crescente resistência das bactérias aos antibióticos levou o mundo a voltar a sua atenção para os insetos na procura de novas fontes de compostos antibacterianos. O extrato corporal bruto obtido com diferentes solventes foi testado contra bactérias Gram-positivas (*Staphylococcus aureus*, *Bacillus subtilis*) e Gram-negativas (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*). O método padrão de difusão em disco foi utilizado para realizar a atividade. Os extratos de *C. compressus* foram investigados quanto à sua eficácia contra todas as bactérias patogênicas resistentes. *Staphylococcus aureus* foi considerado o mais suscetível, exibindo uma inibição média de crescimento elevada, enquanto *Bacillus subtilis* apresentou uma zona média de inibição de crescimento mais baixa. Nossos resultados quanto ao efeito inibitório dos extratos de *C. compressus* mostram a presença de um composto antibacteriano de amplo espectro. Isto será útil na busca de novos antibióticos naturais contra cepas bacterianas patogênicas resistentes.

Palavras-chave: Camponotus compressus, compostos antibacterianos, extratos brutos, antibiótico, patogênico.

1. Introduction

Nowadays, the immense resistance in pathogenic bacterial strains towards antibiotics has made it necessary to search for novel antibiotic sources. The search for natural antioxidants is also being considered due to their negligible side effects compared to synthetic products. In comparison to plants, a little study has conducted on medicinal value of animals and especially insects (Wilsanand et al., 2007). Mostly insects live in moist habitat which favors the growth of pathogenic microbes. They protect their colonies by developing specific defense strategies such as the production of antimicrobial compounds (Stow and Beattie, 2008), which marks insects as a successful mean of novel antibiotics (Mendonca et al., 2009; Zeng et al., 2016). The evolution of specific defense mechanism made them able to resist pathogenic microbes in their surrounding habitat (Rich et al., 2008). Chemical compound produced by some species were also used as defensive tool by other organisms (Roode et al., 2013; Mason and Singer, 2015). For instance, the secondary metabolites originated from plants were utilized for protection by different herbivorous insects against their enemies (Lefèvre et al., 2010; Nishida, 2002). The use of crude extracts of insect as local medicine

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for treatment purpose against robust pathogenic strains of bacteria grabs scientific attention towards the generation of new antibiotic (Wu et al., 2018).

Being a social, ants exist in colonies and have the ability to defend their self and also colony member against microbes contaminated environment (Mekhlif, 2021). The development of immune proteins in social arthropods which also includes ants is the results of continuous exposition to microbes (Viljakainen et al., 2009). Previously study conducted by Bot et al. (2002) and Marin et al. (2006) recognized metapleural gland as major and preliminary source of antimicrobial chemicals in different ants taxa. Ants have metapleural and mandibular glands which release antimicrobial agent to defend their self against microbial attack (Rich et al., 2008). Blum et al. (1958) first described the antimicrobial agent, piperidine alkaloids in fire ant and studied later proved it as potent inhibitory effect on Gram-positive in comparison to Gram-negative bacteria (Jouvenaz et al., 1972). The secretions of Dufour's glands in Argentine ant contain pyrazines, which has antibacterial nature (Premkumar and Govindarajan, 2005). The Glandular discharges of Attines ants have Phenols and carboxylic acids which has antimicrobial activity (Yek and Mueller, 2011).

The *Camponotus* ant lack metaplural gland (Ayre and Blum, 1971; Holldobler and Engel Siegel, 1984), which is substituted by the mandibular gland (Mohana et al., 2018) a paired sacular structure filled chemicals such alcohol, aldehydes and ketones (Blum and Hermann, 1978). Despite active metapleural gland; however the secretion of mandibular gland function as antibiotic (Voegtle et al., 2008). Mandibular gland extracts has strong inhibitory effect on resistant bacterial strains such as *Escherichia coli* and *Staphylococcus aeureus* (Mohana et al., 2018). Ants of subfamily Formicinae have formic acid a major constituent (60%) of their secreted venom as defense with cytotoxic in nature (Blum, 1984). Carpenter ants do not sting directly but they pierce the skin of their enemy with their strong mandible and then inject formic acid by rotating their abdomen. They can manage the rate and direction of venom discharge. Study reports by Hermann and Blum (1981) described formic acid as antibacterial agent and has role in regulation of soil microbes in the vicinity of colony. It's the need of time to search novel antibiotic from natural means due to the enormous emergence in resistance to synthetic antibiotic in pathogenic bacteria (Čeřovský et al., 2008).

2. Material and Methods

2.1. Ants collection

Camponotus compressus (Figure 1) was selected to study its antimicrobial activity. The collection of ants was done by hands from their natural habitat in district Peshawar. The collected specimens were freeze killed and kept in the refrigerator for further analysis.

2.2. Solvents used for extraction

Six solvents of different polarity were used in the process of extraction such as methanol, acetone, ethyl acetate, chloroform, ether and hexane.

2.3. Preparation of crude extracts

For the extraction of crude extract, 10 gram *C. compressus* ants were collected and dried in shade at room temperature. The dried ants were then crushed with mortar and pestle. After crushing, the ants were added to flask containing 200 ml of solvent and were put on automatic shaker at 200 rpm overnight, after stirring process they were filtered and the crude extract is obtained. The crude extracts obtained were then subjected to rotary to evaporate the solvents completely and the dried extracts were transferred to labeled vials and preserved at 4ºC for experimental treatments.

2.4. Test organism

Both Gram positive i,e *S. aureus, B. subtilis* and Gram negative i,e *E. coli, K. pneumoniae* and *P. aeruginosa* isolates of pathogenic bacteria were selected as test organism. The bacterial strains were provided by the Department of Soil Microbiology and Plant Nutrition (DSPN), Agriculture Research Institute (ARI) Tarnab Farm Peshawar.

Figure 1. a) dorsal, **b)** lateral, **c)** frontal view of *Camponotus compressus*.

2.5. Control groups

For antibacterial activity we used two control groups in order to compare it with the activity of experimental group (crude extracts). Dimethyl sulfoxide (DMSO) was used as negative while two standard antibiotic drugs Clarithromycin and Amoxicillin were used as positive control groups.

2.6. Growth media preparation

For the bacterial culture, a nutrient agar (peptone, beef or yeast extract, NaCl and agar) was used as nutrient media for bacterial growth. For preparation nutrient agar media, 28 gram of nutrient agar powder were dissolved in 1000 ml of distilled water in flask. The flask containing media is then placed in autoclave for 30 minute at temperature 121°C to sterilize it completely. Once the nutrient agar was autoclaved, it is then poured in to petri dish plates with in the laminar flow hood and left the plates on sterile surface to become completely solidified. The plates were sealed and stored in the refrigerator for future use.

2.7. Bioassay of antibacterial activity

The antibacterial activity of sequentially obtained crude extracts of *C. compressus* was analyzed by using standard agar disc diffusion method (Mekhlif, 2021) with little modification. Nutrient ager media plates were properly labeled. Then each plate was inoculated from each bacterial stock plate with sterilized loop to properly spread the bacterium on surface of nutrient agar plate. Sterilized whatmann filter paper discs (4mm) were placed at different position in the plate and 30ul crude extract (20 and 40mg/ml) of each solvent was poured on discs. A 30ul of both control, Clarithromycin and Amoxicillin with a concentration of 0.05 mg/ml and DMSO solvent was poured on the test disc. The plates were properly sealed with parafilm in order to prevent contamination and were incubated at 37°C for 24 hrs. The activity was carried out in triplicate. The zone of growth inhibition was measured with graduated scale in millimeters. The data obtained was further statistically analyzed by applying ANOVA test. The whole process of extraction and antimicrobial activity of ant was carried out at Department of Soil Microbiology (DSPN), Agriculture Research Institute (ARI), Tarnab Farm Peshawar.

3. Results

The antibacterial activity of *C. compressus* body crude extracts was analyzed against pathogenic bacteria. At concentration of 20 mg/ml of *C. compressus* crude extract shows antibacterial activity (Figure 2). All the crude extracts were detected effective and shows growth inhibitory effect against tested bacteria such as *P. aeruginosa* (11.5±1.0, 0±0.0, 12±1.0, 10.5±1.5, 15±0.8 and 9.5±0.8 mm inhibition zone), *E. coli* (12.5±0.3, 10.5±0.5, 9±0.8, 9±1.0, 10±1.4 and 8.5±1.0 mm inhibition zone), *K. pneumoniae* (8±0.5, 7±1.4, 10±1.0, 8±0.7, 16±0.6 and 9±0.4 mm inhibition zone),

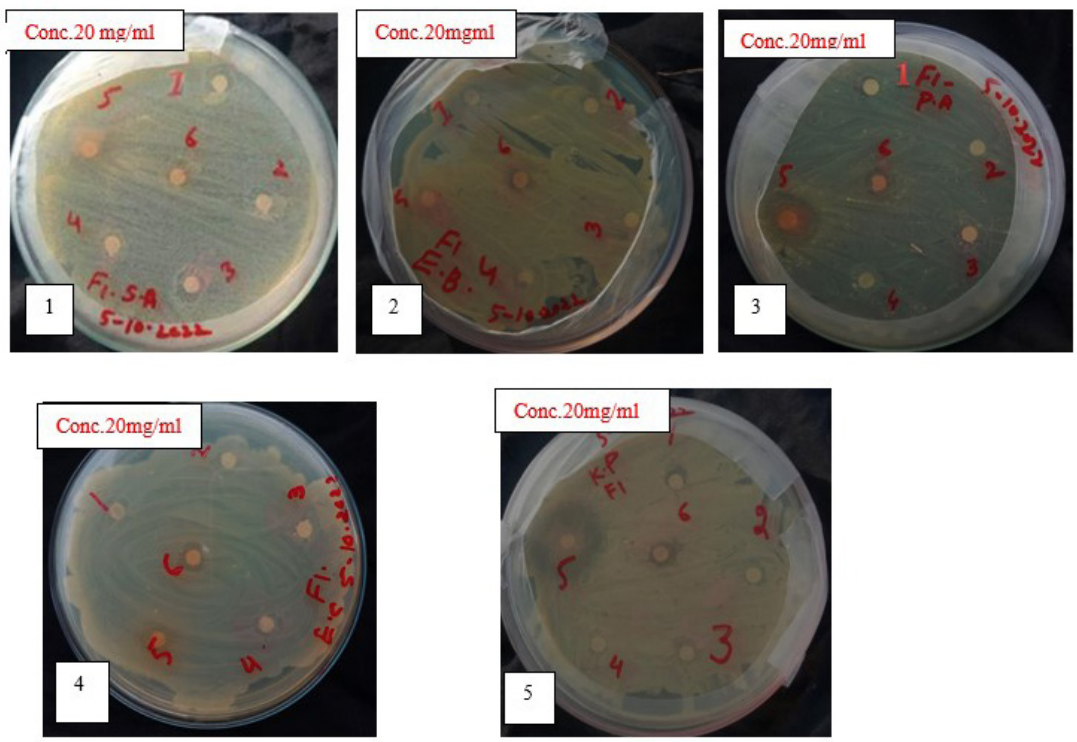

Figure 2. Photographic evidence of *Camponotus compressus* (**F1**) antimicrobial activity at concentration of 20 mg/ml. **1**) *Staphylococcus aureus* (**S.A**), **b**) *Bacillus subtilis* (**E.B**), **3**) *Pseudomonas aeruginosa* (**P.A**), **4**) *Escherichia coli* (**E.C**), **5**) *Klebsiella pneumonia* (**K.P**). **Note**. Disc number shows different solvents extract activity. (**1**) Ethyl acetate, (**2**) Hexane, (**3**) Acetone, (**4**) Ether, (**5**) Methanol, (**6**) Chloroform.

S. aureus (11.5±0.2, 11.5±0.8, 11.5±1.4, 10.5±1.4, 12±1.0 and 12.7±1.7 mm inhibition zone) and *B. subtilis* (5±0.5, 7.5±1.0, 0±0.0, 9.5±0.8, 0±0.0 and 5±0.5 mm inhibition zone) (see Table 1). At concentration of 40 mg/ml crude extracts of *C. compressus* have shown greater potential of antibacterial activity during in vitro application against the pathogenic strains (Figure 3). Each bacterium response was different in susceptibility to crude extracts obtain with different solvents such as *P. aeruginosa* (15.6±1.2, 12.5±1.0, 15.5±0.7, 13.6±0.4, 15.5±1.1 and 19±0.8 mm inhibition zone), *E. coli* (20.3±0.8, 13.6±0.4, 13.3±1.2, 17±0.9, 13.6±0.8 and 15.3±1.3 mm zone inhibition), *K. pneumoniae* (12.6±1.0, 16±1.2, 17.5±0.5, 17±1.3, 18±1.0 and 16±1.6 mm inhibition zone), *S. aureus* (12.6±0.6, 14.6±0.8, 11.6±1.1, 17.5±1.5, 14±0.5 and 20±1.2 mm inhibition zone) and *B. subtilis* (11.5±1.1, 8±0.7, 12.5±1.0, 13.6±1.4, 13.2±0.3 and 11.2±0.5 mm inhibition zone) (see Table 2). At concentration of 40 mg/ml the antibacterial activity of crude extracts was observed high as compared to 20mg/ml. A large difference was detected in activity of crude extracts of both concentrations, in effect on different tested bacteria as well as between the extracts of different concentration (see Table 3). So variation in concentration shows fluctuation in the antibacterial activity of the extracts against pathogenic bacterial strains. Table 4 elaborate and compare solvent wise overall activity of crude extracts of *C. compressus*. The extracts of *C. compressus* obtained with methanol was detected with high average zone of inhibition (12.88 mm), followed by

Table 1. Antibacterial activity of *C. compressus* crude extracts (20 mg/ml) against different tested bacteria.

Bacteria	Extraction solvents growth inhibition zone (mm)						Control growth inhibition zone (mm)		
							Positive		Negative
	Ethyl Acetate	Hexane	Acetone	Ether	Methanol	Chloroform	Clarithromycin	Amoxicillin	DMSO
P. aeruginosa	11.5±1.0	0±0.0	12±1.0	10.5±1.5	15±0.8	9.5±0.8	26.5±1.2	25±1.1	0±0.0
E. coli	12.5±0.3	10.5±0.5	9±0.8	9±1.0	10±1.4	8.5±1.0	23±0.8	21±1.0	0±0.0
K. pneumoniae	8±0.5	7±1.4	10±1.0	8±0.7	16±0.6	9±0.4	28±1.0	19±0.8	0±0.0
S. aureus	11.5±0.2	11.5±0.8	11.5±1.4	10.5±1.4	12±1.0	12.5±1.7	32±1.3	29.5±1.4	0±0.0
B. subtilis	5±0.5	7.5±1.0	0±0.0	9.5±0.8	0±0.0	5±0.5	19±0.5	22.5±1.0	0±0.0
Average growth inhibition zone (mm)	9.7	7.3	8.5	9.5	10.6	8.9	25.7	23.4	0

Data is represented as mean and standard deviation from three replicates. Different letters in a column (P-value = 0.02) shows a significant difference (ANOVA test).

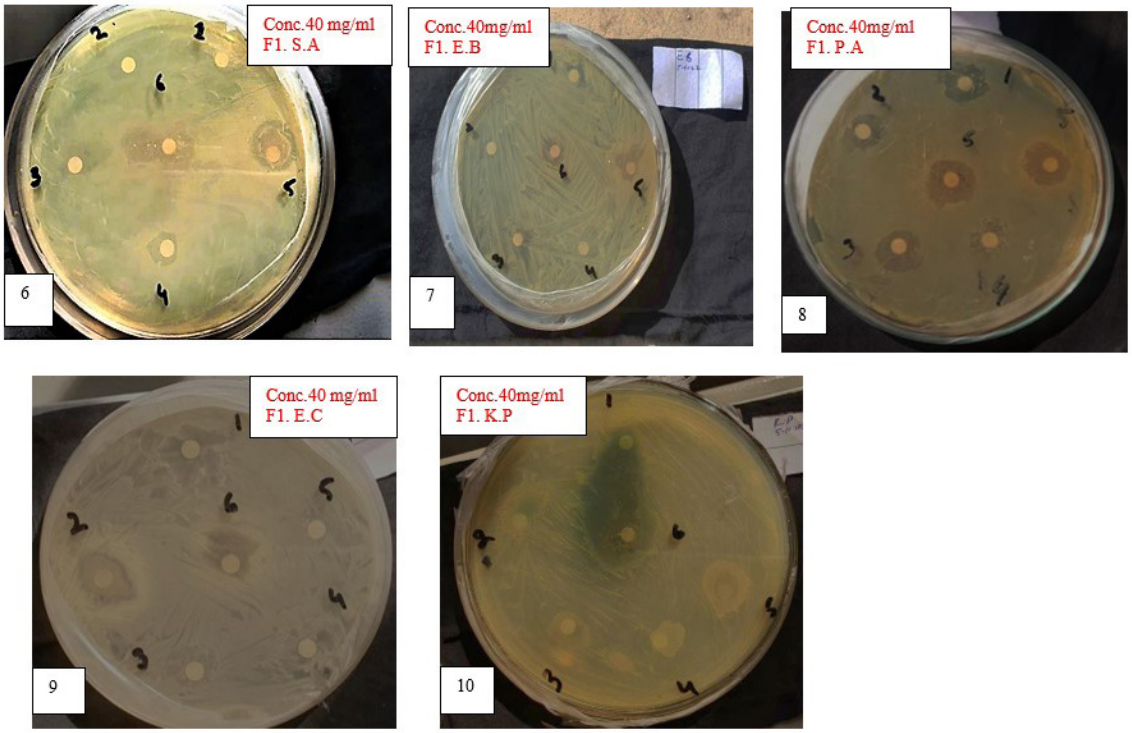

Figure 3. Photographic evidence of *Camponotus compressus* (**F1**)antimicrobial activity at concentration of 40 mg/ml. **6**) *Staphylococcus aureus* (S.A), **7)** *Bacillus subtilis* (**E.B**), **8**) *Pseudomonas aeruginosa* (**P.A**), **9**) *Escherichia coli* (**E.C**), **10**) *Klebsiella pneumoniae* (**K.P**). **Note**. Disc number shows different solvents extract activity. (**1**) Ethyl acetate, (**2**) Hexane, (**3**) Acetone, (**4**) Ether, (**5**) Methanol, (**6**) Chloroform.

Table 2. Antibacterial activity of *C. compressus* crude extract (40 mg/ml) against different tested bacteria.

Bacteria	Extraction solvents growth inhibition zone (mm)						Control growth inhibition zone (mm)		
							Positive		Negative
	Ethyl acetate	Hexane	Acetone	Ether	Methanol	Chloroform	Clarithromycin	Amoxicillin	DMSO
P. aeruginosa	15.6±1.2	12.5±1.0	15.5±0.7	13.6±0.4	15.5±1.1	19±0.8	26.5±1.2	25±1.1	0±0.0
E. coli	20.3±0.8	13.6±0.4	13.3±1.2	17±0.9	13.6±0.8	15.3±1.3	23±0.8	21±1.0	0±0.0
K. pneumoniae	12.6±1.0	16±1.2	17.5±0.5	17±1.3	18±1.0	16±1.6	28±1.0	19±0.8	0±0.0
S. aureus	12.6±0.6	14.6±0.8	11.6±1.1	17.5±1.5	14±0.5	20±1.2	32±1.3	29.5±1.4	0±0.0
B. subtilis	11.5±1.1	8±0.7	12.5±1.0	13.6±1.4	13.2±0.3	11.2±0.5	19±0.5	22.5±1.0	0±0.0
Average growth inhibition zone (mm)	14.52	12.94	14.08	15.74	14.86	16.3	25.7	23.4	0±0.0

Data is represented as mean and standard deviation from three replicates. Different letters in a column (P-value = 0.03) shows a significant difference (ANOVA test).

Table 3. Comparison of antibacterial activity of two different concentrations of *C. compressus* extracts on tested bacteria.

Bacteria	Average growth inhibition zone (mm)		Average growth inhibition zone (mm)
	20 mg/ml extract	40 mg/ml extract	
P. aeruginosa	9.7	15.37	12.5
E. coli	9.92	15.52	12.7
K. pneumoniae	9.66	16.18	12.9
S. aureus	11.58	15.05	**13.3**
B. subtilis	4.5	11.66	**8.08**
Average growth inhibition zone (mm)	9.078	14.756	

Bold letter shows *S. aureus* most (13.3) and *B. subtilis* (8.08) a least susceptible bacteria. Different letters in a column (P ≤0.0008) and rows (P ≤0.02) shows a significant difference (ANOVA test).

Table 4. Overall solvents wise comparison in antibacterial activity of *C. compressus* crude extracts at different concentration.

Solvents	Average growth inhibition zone values of crude extracts at different concentration (mm)		Average growth inhibition zone (mm)
	20 mg/ml	40 mg/ml	
Ethyl Acetate	9.7	14.52	12.11
Hexane	7.3	12.94	10.12
Acetone	8.5	14.08	11.29
Ether	9.5	15.74	12.62
Methanol	10.9	14.86	**12.88**
Chloroform	8.9	16.3	12.6
Average growth inhibition zone (mm)	9.13	14.74	

ether (12.62 mm), chloroform (12.6 mm), ethyl acetate (2.11 mm), acetone (11.29 mm) and hexane (10.12 mm). Clarithromycin and Amoxicillin (0.05 mg/ml) were used as positive control against the tested bacteria. Both positive controls were detected effective against the tested bacteria such as *P. aeruginosa* (26.5±1.2 and 25±1.1 mm growth inhibition zone), *E. coli* (23±0.8 and 21±1.0 mm growth inhibition zone), *K. pneumoniae* (28±1.0 19±0.8 mm growth inhibition zone), *S. aureus* (32±1.3 and 29.5±1.4 mm growth inhibition zone) and *B. subtilis* (19±0.5 and 22.5±1.0 mm growth inhibition zone)(see Table 1).

4. Discussion

The purpose of current study was to evaluate the antibacterial activity of *Camponotus compressus* crude extracts. As ants are social insect and such way of life have rapid chances of infection transmission. Hypothetically, mostly social insect are supposed to be a good source of antimicrobial compound to defend their self, broods and nest mate in densely populated habitat with better chances of microbes transmission. During sampling *C. compressus* was collected mostly from such habitats with high moisture such as agriculture fields, along bank of canals, from roots of trees and rotten wood logs. Such habitat favors the growth of pathogens as compared to dry areas. Environmental condition of ants colony such as soil moisture and moderate temperature favors microbial growth due to its effects on substrate and oxygen transportation (Christe et al., 2003). So we can assume that *C. compressus* have some sort of defense mechanism or production of antimicrobial compounds against infectious microbes. So in order to

know whether they show resistance to pathogenic bacteria or not, the current study was conducted.

Our findings on antibacterial activity of *C. compressus* crude extract of different solvents at two different concentrations 20 and 40 mg/ml was quite satisfactory against all tested pathogenic strains of bacteria. At 20 mg/ml *S. aureus* (Gram positive) and *P. aeruginosa* (Gram negative) was detected the most susceptible and *B. subtilis* was detected less susceptible to the effect of crude extract. Statistically a significant difference was detected antibacterial activity of *C. Compressus* crude extracts (20 mg/ml) of different solvent against different bacteria (P-value = 0.02). At 40 mg/ml *K. pneumoniae* (Gram negative) was detected most sensitive while *B. subtilis* was less susceptible to the effects of different extracts (see Table 2). There is significant difference (P-value = 0.03) in the antibacterial activity of the *C. compressus* crude extract (40 mg/ml) against experimental strains of pathogenic bacteria. Table 3 discusses the overall comparison of antibacterial activities *C. compressus* crude extracts of both (20 and 40 mg/ml) concentrations. There was a large difference in the activity of crude extracts of two different concentrations from both angles i.e effect on different tested bacteria (P-value = 0.02) as well as between the extracts of different concentration (P-value = 0.0008), so variation in concentration shows fluctuation in the antibacterial activity of the extract against pathogenic bacterial strains. *S. aureus* (average inhibition zone 13.3mm) were investigated most and *B. subtilis* (average inhibition zone 8.08 mm) least sensitive to the effect of crude extracts (see Table 3). The crude extract shows satisfactory results in comparison to Clarithromycin and Amoxilline. The extract of *C. compressus* obtained with methanol was detected most effective against tested bacterial strains.

Ants are thought to be a reservoir for the existence of peptide molecule with antimicrobial activity (Cazander et al., 2013). Our findings were also supported by previous analysis by Aboya et al. (2020) studied the antibacterial activity of *Camponotus maculatus* extracts obtain with three different solvents (water, ethanol, dichloromethane) are found effective against *E. coli, S. aureus* and fungus *Candida albican.* Yamuna and Raja (2019) described the antibacterial of *C. compressus* crude extracts obtained with four solvents (70% ethanol, methanol, petroleum ether and water) *S. aureus* was observed mostly with higher susceptibility. In our study *S. aureus* was detected most susceptible to crude extracts of *C. compressus*. A slight difference was also observed in our result in comparison to previous studies but the reason may be due to difference in concentration of extracts used, types of solvents used for extraction of extracts, volume of extracts used for activity and type method used for antibacterial activity. According to Yamuna and Raja (2019) extraction methods, solvents system used and concentration of an extract plays a significant role in determination of antimicrobial role of an extract or drugs.

5. Conclusion

The current study evaluates antimicrobial activity of *C. compressus* ants. The search of novel antibiotics is the demand of time due to extreme resistance shown by microbes against the antibiotics on repeated exposure. So our study provide new gateway for search of new antimicrobial compound from insects. The crude extracts obtained with different solvents from *C. compressus* shows growth inhibition of both Gram positive and negative bacteria which shows the existence of antimicrobial compound in crude extract. So our study provides a baseline in future for exploring new antimicrobial compound.

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