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Original article

In-vitro antimicrobial activities of organic solvent extracts obtained from *Dipcadi viride* (L.) Moench

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ABSTRACT

In a pharmacological screening methanol, ethanol and chloroform extracts of *Dipcadi viride* (L.) Moench, were assayed for antibacterial and antifungal activities. Pathogenic bacteria and fugai were used as test strain to validate their anti-microbial potential. Disc diffusion and agar tube dilution methods were used to assess antibacterial and antifungal activities, respectively. Crude plant extracts exhibited inhibitory effect against maximum of pathogenic microbes. Ethanol and methanol proved better solvents compared with the chloroform. Results of crude plant extracts and standard antibiotic doxycycline (DOX) showed comparable effect against pathogenic bacteria and fungi (*A. niger, 124 mm; A. fumigates, 109 mm)*. Results of the current study were interesting as inhibition zones were observed even, when extracts were used in lower concentrations. There should be detailed pharmacological screening of the crude extracts of this plant for exploration of effective and natural drug.

1. Introduction

Plants produce secondary metabolites, which constitute a main source of biologically active substances (Antonisamy et al., 2015; Balamurugan, 2015; Al-Dhabi et al., 2015; Valsalam et al., 2019a; Rajkumari et al., 2019). Today, the increasing resistance pattern of pathogenic microbes to commonly used antimicrobial drugs has increased the interest of scientists in secondary metabolites for discovery of new therapeutic agents from plants (Mahmood et al., 2013; Glorybai et al., 2015; Barathikannan et al., 2016; Al-Dhabi and Arasu, 2016; Haritha et al., 2016; Cuong et al., 2017; Park et al., 2016a,b; Elango et al., 2017; Gurusamy et al., 2019). Antibiotic resistance is one of the major issues of WHO for present era (Mahmood et al., 2012; Arokiyaraj et al., 2015; Elango et al., 2016a;). However, in last few years, an increase in screening of plants as a source of disease management has been observed (Prashanth et al., 2001; Elango et al., 2016b; Fowsiya et al., 2016). More ever, it is estimated that two-third population of the world dependent on traditional plants in healthcare system due to expensive pharmaceutical products (Tagboto and Townson, 2001; Helan et al., 2016; Ilavenil et al., 2017; Park et al., 2016a,b; Park et al., 2017)

Dipcadi is a diverse genus of family Hyacinthaceae, having a number of medicinally important species, bearing essential oil and secondary metabolites over 400 plant species (Rathi et al., 2015; Valsalam et al., 2019b). Secondary metabolites and essential oils of this genus have been used since many years for different

Abbreviations: SDA, Sabouraud dextrose agars; DMSO, dimethyl sulfoxide; MIC, minimum inhibitory concentration; DOX, doxycycline.

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medicinal purposes. *Dipcadi viride* is a commonly known plant with wide distributions. *D. viride* has been reported ethnomedicinally as effective in jaundice, ulcer, diuretic and purgative; plant extract also exhibit effect for cardiac diseases along with the insecticidal properties (Mahmood et al., 2011b; Akhtar et al., 2013; Surendra et al., 2016a; Surendra et al., 2016b). A number of species of Dipcadi have been studied for their biological activities. This genus is considered to have the majority of medicinally valued metabolites (Akhtar et al., 2013). A variety of secondary metabolites such as flavonoids, alkaloids and steroids etc. from different species of Dipcadi have been detected by HPLC, GC–MS and NMR (Kutacek et al., 1981; Araghiniknam et al., 1996; Surendra et al., 2016c). The screening of latent antimicrobial activities of *D. viride* extracts could be useful to treat diseases as natural antimicrobial agents.

Presenting research work was aimed to screen the antimicrobial activities of bio-active compounds against infectious and resistant pathogens. *D. viride* was investigated for antibacterial and antifungal activities against different pathogens, food poisoning bacteria and fungi. *D. viride* is glabrous plant, varied size, one to several leaves per shoot, lenear-lanceolate and some time crinkly or spirally twisted margins.

2. Materials and methods

2.1. Plant materials

Fresh plants *D. viride* was collected in May 2018 from Taif region, which is located at main foothills of western mountains at 2500 m a.s.l. (above sea level) and identified as *Dipcadi viride* (L.) Moench. Herbarium specimen was prepared and research work was done at Botany and Microbiology Department, College of Sciences, King Saud University, Saudi Arabia.

2.2. Test organisms

Five bacteria i.e. two Gram-positive *Bacillus subtilis*, *Staphylococcus aureus* and three Gram negative, *Enterobacter aerogenes*, *Klebsiella pneumonia* and *Escherichia coli* and two fungus i.e. *Aspergillus niger* and *Aspergillus fumigates* were used as test organisms. These test organism's cultures were obtained from the Botany and Microbiology Department, College of Sciences, King Saud University, Saudi Arabia. Bacterial cultures were sub-cultured on nutrient agar (MERCK; Germany) and fungus was sub-cultured on SDA (MERCK; Germany).

2.3. Preparation of plant extract

Whole plant of *D. viride* was shade dried and pulverized by grinder into powder form. A 100 g of powdered plant material was soacked in 1L of methanol, ethanol and chloroform solvents for 7 days at room temperature. This mixture was filtered by Whatman 41 filter paper and then concentrated under reduced pressure by using rotary evaporator at 40 °C. Before performing the antimicrobial activity stock solution of each extract were made by dissolving 15 mg of plant extract in 10 ml of DMSO. Furthermore, eight dilutions of 15 mg/ml, 12.50 mg/ml, 10 mg/ml, 7.5 mg/ml, 5 mg/ml and 3 mg/ml, 2 mg/ml and 1 mg/ml concentration were made by this stock solution.

2.4. Antibacterial activities

Antimicrobial activities of methanol, ethanol and chloroform extracts was determined by using Agar well diffusion method followed by Kivack et al. (2001). Culture plates were allowed to solidify and then seeded with bacterial strains. After that plates were punched with sterile cork borer and these open wells were inoculated by 80 µl test solution. Standard antibiotic doxycycline was used as positive control. Finally, these plates were incubated at 37 °C for 24 h.

2.5. Antifungal activities

Antifungal activity was performed by agar tube dilution method (Fatima et al., 2009). Test plant material was prepared by dissolving 20 mg/ml in DMSO of each extract i.e. methanol, ethanol and chloroform. 100 µl Test plant material was added to the autoclaved media. Terbinafin was used as positive control and DMSO was used as negative control. 83 µl of terbinafin and 100 µl of pure DMSO was added to media. Test tubes were allowed to solidify in slanting positions. Each tube was inoculated with 3 mm piece of fungus from one week old culture. Linear growth of fungus was measured in mm and percentage inhibition was calculated by formula:

$$\%\ \textit{inhibition of fungal growth} = 100 - (\textit{Linear growth in test} / \textit{Linear growth in control}) \times 100.$$

3. Results

3.1. Antibacterial activities

Results showed that crude methanolic, ethanolic and chloroform extract of *D. viride* has potential against all the bacterial strains screened (Figs. 1, 2 & 3). Crude extract of *D. viride* in ethanol was more effective compared with the methanol and chloroform extract. Maximum activity of ethanolic extract was observed against *Escherichia coli* and *Bacillus subtilis*. The rank of antibacterial activity of ethanolic extract against each bacterial strain is; *Escherichia coli* > *Bacillus subtilis* > *Enterobacter aerogenes* > *Klebsiella pneumonia* > *Staphylococcus aureus*.

Methanolic extract of *D. viride* showed considerable activity. The rank of its activity against each strain is; *Bacillus subtilis* > *Staphylococcus aureus* > *Escherichia coli* = *Enterobacter aerogenes* = *Klebsiella pneumonia*. MIC of methanolic plant extract was found 5 mg/ml. Chloroform extract also showed moderate activity against all the bacterial strains. But its activity was low compared with the other two extracts. It showed maximum activity against *Enterobacter aerogenes* i.e. 13 mm inhibition zone at the concentration of

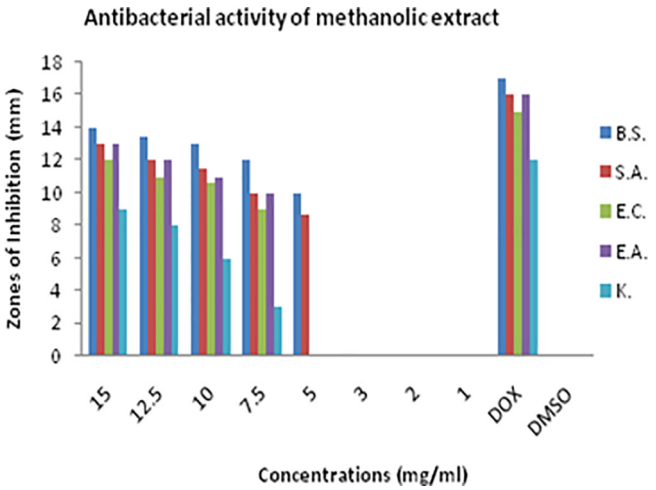

Fig. 1. Zones of inhibitions (mm) showing antimicrobial activity of methanolic extract of *D. viride* against *Bacillus subtilis* (B.S.), *Staphylococcus aureus* (S.A.), *Escherichia coli* (E.C.), *Enterobacter aerogenes* (E.A.) and *Klebsiella pneumonia* (K).

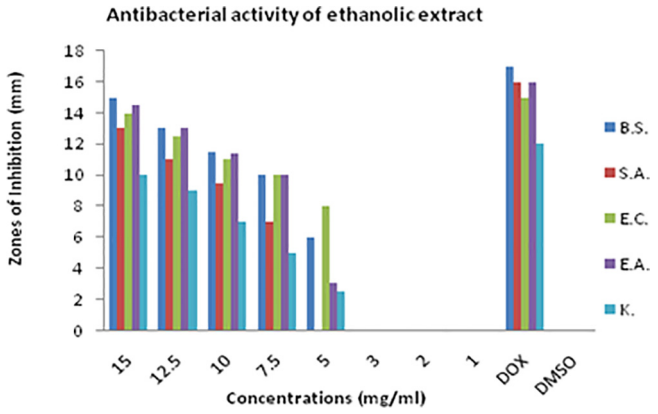

Fig. 2. Zones of inhibitions (mm) showing antimicrobial activity of ethanolic extract of *D. viride* against *Bacillus subtilis* (B.S.), *Staphylococcus aureus* (S.A.), *Escherichia coli* (E.C.), *Enterobacter aerogenes* (E.A.) and *Klebsiella pneumonia* (K).

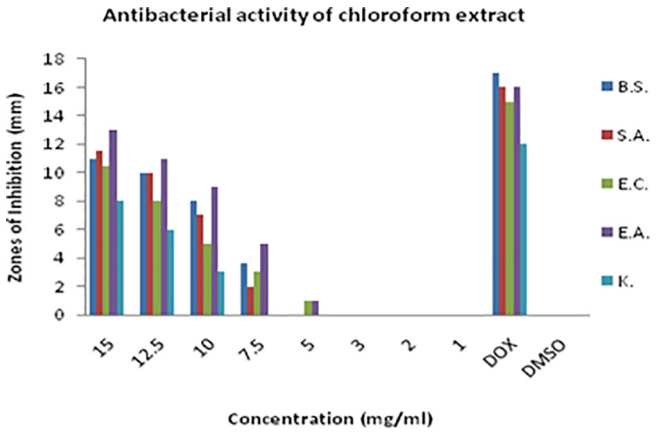

Fig. 3. Zones of inhibitions (mm) showing antimicrobial activity of chloroform extract of *D. viride* against *Bacillus subtilis* (B.S.), *Staphylococcus aureus* (S.A.), *Escherichia coli* (E.C.), *Enterobacter aerogenes* (E.A.) and *Klebsiella pneumonia* (K).

15 mg/ml. MIC was found 5 mg/ml. The rank of crude chloroform extract of *D. viride* is; *Enterobacter aerogenes* > *Klebsiella pneumonia* > *Escherichia coli* = *Staphylococcus aureus* > *Bacillus subtilis*.

The results showed that ethanol is better solvent for the extraction of antibacterial agents compared with the methanol and chloroform. Antibacterial activity of methanolic extract was also prominent and was almost equal or little bit less than ethanolic extract. Rank of solvents based on antibacterial activity of *D. viride* is; ethanol > methanol > chloroform.

3.2. Antifungal activities

Results showed that the methanolic and ethanolic extract of *D. viride* exhibited best antifungal activities against *Aspergillus niger* and *Aspergillus fumigates*. Maximum activity was shown by methanolic extract against *A. fumigates* i.e. 77.1% and ethanolic extract against *A. niger* i.e. 76.8%. Crude extract of chloroform also showed average antifungal activity against both the tested strains. Results are presented in Table 1.

4. Discussion

D. viride is indigenous medicinal plant which is being used to treat various diseases for many years such as bronchitis, cough, fever, jaundice, cardiac problems and skin problems. It is diuretic, emetic, antidote and purgative (Mahmood et al., 2011b). Present study reported the antimicrobial activity of different extracts of *D. viride* in an attempt to verify the reported beneficial uses. Antibacterial and antifungal activities were investigated in this research work as antibiotic resistance is a burning issue of ongoing time.

Pathogenic microorganisms are being resistance to the available antibiotics resulting, a number of clinical infections (Priscila et al., 2007). Regarding antibiotic resistance, Asia Pacific is at the top, compared with the whole world. This infection is the result of frequent use of antibiotics even against a minor health problem. Thus, it is required to establish the understanding of this budding issue to reduce this problem in healthcare (Anderson and Keye, 2009). This issue forced the pharmacists and botanists to focus on discovering new plant originated drugs (Mahmood et al., 2011a). Now, pharmaceutical companies are also paying their attentions to discover plant based drugs (Kannan and Agastian, 2015).

D. viride is an effective plant against pathogenic microbes. Antibacterial and antifungal activity of *D. viride* was strongly dependent on the solvent, which was used for the extraction. Agar well diffusion method is most convenient to evaluate the maximum number of crude extract concentration tested against bacterial strains. Ethanol was the best solvent for extraction, because it extracts active compounds that exhibit maximum antimicrobial activities. Methanolic extract also showed better results. It is confirmed through this research work that all the solvents used for extraction of bio-active compounds for biological activities of *D. viride*, were best. Our results showed that there was a consistent behavior of antibacterial and anti-fungal activities. In antibacterial activity MIC was found 5 mg/ml against 90% of the tested strains. Antifungal activity of each crude extract was above than 50%. Maximum antifungal activity was exhibited by the methanolic and ethanolic extract that was 77.1% and 76.8% against *A. fumigates* and *A. niger* respectively.

5. Conclusion

It is concluded that all extracted compound has potential to treat superficial fungal and bacterial infections, when used properly after modifying in crude drug. Actually, these finding justifies the use of *D. viride* in traditional healthcare system for the treatment of various ailments, whose symptoms might engage fungal and bacterial infections. This study also proves that ethno-

Table 1
Antifungal activity of methanolic, ethanolic and chloroform extract of *D. viride* against *Aspergillus niger* and *Aspergillus flavus*.

Test plant extract	*Fungal strains used	*L.G.C. (mm)	*L.G.T. (mm)	% Inhibition
Methanolic extract (*D. viride*)	*A.niger*	124	35	71.2%
	A. fumigates	109	25	77.1%
Ethanolic extract (*D. viride*)	*A.niger*	125	29	76.8%
	A. fumigates	109	30	72.5%
Chloroform extract (*D. viride*)	*A.niger*	125	51	59.2%
	A. fumigates	109	49	55.1%

Aspergillus niger – *A. niger* and *Aspergillus fumigates* – *A. fumigates*.
L.G.C. (mm) = Linear Growth in Control (millimeter), L.G.T. (mm) = Linear Growth in Test (millimeter).

medicinal approaches are useful for the discovery of new biological active agents to synthesize new drugs. Further, detail research is required to isolate the bio-active agents responsible for antibacterial and antifungal activity of *D. viride*.

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Conflict of Interest statement

The authors of the manuscript entitled "In-vitro antimicrobial activities of Dipcadiviride (L.) Moench" declared no conflict in this manuscript and publications.

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