

Short Communication

Phytochemical analysis and antimicrobial activity of *Persea duthiei*

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Persea duthiei plant crude extracts were subjected to evaluation for antibacterial and antifungal activities. For antibacterial activities, seven bacterial strains; *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa*, *Klebsiella pneumonia* (ATCC 700603), *Staphylococcus epidermidis* (NCTC 11047), *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA: NCTC 13143) and *Salmonella* were used. All fractions of the plant were completely inactive on three bacterial strains: *E. coli*, *P. aeruginosa*, and *S. epidermidis*, which were used in the antibacterial assays. Water fraction showed promising activity against MRSA. Ethyl acetate, chloroform, ethanol, and n-Hexane extracts showed activity against *Salmonella*, MRSA and *Staphylococcus aureus*. n-Hexane extract also showed activity against *Klebsiella* up to some extent. For antifungal activities, four fungal strains: *Aspergillus flavus*, *Fusarium solani*, *Aspergillus fumigatus* and *Aspergillus niger* were subjected to crude extract. Water fraction was found highly active against *F. solani* and *A. niger*, while it showed good activity against other strains. Ethyl acetate fraction was prominent against *F. solani* and *A. niger*. Chloroform fraction showed significant activity against *F. solani*. Phytochemical analysis was also performed using the literature methods.

Key words: *Persea duthiei*, antimicrobial activity, crude extracts.

INTRODUCTION

Phyto-remedies have also shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections (Loiy et al., 2011). Lack of development of new antimicrobial agents in the last decades associated with their misuse led to the emergence of multiresistant microorganisms (Lai et al., 2012). Screening of medicinal plants may result in the discovery of novel effective compounds against pathogenic

microorganisms. The compounds that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs (Maher, 2011).

Persea duthiei is a small or medium-sized evergreen tree widely distributed around Nainital, at altitude of 2500 m. The fruit is a globular black drupe. The root stocks are acrid, bitter, pungent, heating, and astringent and are generally used in inflammation, asthma, pain, foul breath, bronchitis, vomiting, and in blood disease (Padalia et al., 2009). Keeping in mind the importance of *P. duthiei*, it was selected for the present study.

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MATERIALS AND METHODS

Plant

P. duthiei plant weight of 3 kg was collected from district Buner Khyber Pukhtoon Khwa in June 2010, in flowering season. The plant was identified by plant taxonomist Mehboob Ur Rehman, Professor in Department of Plant Sciences Post Graduate Jehanzeb College, Saidu Sharif Swat, Pakistan. All studies were carried out at the Department of Chemistry and Department of Pharmacy, Kohat University of Science and Technology, KUST Khyber Pukhton Khwa, Pakistan. A small part was also performed in Pakistan Council of Scientific and Industrial Research (PCSIR) laboratory, Peshawar.

Experimental

The air dried plant material (1000 g) was crushed and soaked in methanol (500 ml) for one week. The methanolic extract was combined and evaporated under reduced pressures to afford a gummy residue (200 g). The concentrated residue was suspended in a mixture of water and methanol and defatted with *n*-Hexane (400 ml) to afford fraction weighing (0.25 g). It was again fractionated with chloroform (500 ml) to afford fraction weighing (2.00 g). Similarly, it was fractionated with ethyl acetate (500 ml) to afford fraction weighing (1.25 g). After that, it was fractionated with methanol (500 ml) to obtain the fraction weighing (3.25 g). At last, water fraction was obtained weighing 50 g.

Preparation of crude extract

100 g of each of the coarsely powdered plant material was taken and extracted with methanol. Then, this crude of methanol is further fractionated into *n*-hexane, chloroform, ethyl acetate and water. The extracts were filtered and sodium chloride solution was then added to the filtered extract to form precipitates. The precipitates were then separated, air dried and transferred to air tight amber glass container. The crude extract was dissolved in chloroform and water to make the final concentration, which was kept in refrigerator till it was used (Harborne, 1973).

Phytochemical and antimicrobial bioassay

Phytochemical analysis and antimicrobial activities assay were conducted as described by Hussain et al. (2011).

RESULTS AND DISCUSSION

The anti-bacterial study was performed against seven bacterial strains, that is, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella*, methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Klebsiella pneumonia* and *S. aureus*. Results obtained are tabulated in Table 1.

All fractions of plant were completely inactive on three bacterial strains out of the seven pathogenic bacteria, which were *E. coli*, *P. aeruginosa*, and *S. epidermidis*, used in the antibacterial assays. Chloroform fraction showed promising activity (18 mm zone of inhibition),

followed by *n*-Hexane extract of 14 mm zone of inhibition against MRSA.

Ethyl acetate, chloroform, ethanol and *n*-Hexane extracts showed 16, 13, 10 and 14 mm zone of inhibition, respectively against *Salmonella*. Ethyl acetate, chloroform, ethanol and *n*-Hexane fraction were also found active against MRSA and *S. aureus*. *n*-Hexane extract showed very low activity against *Klebsiella* with 2 mm zone of inhibition. Generally, the activity of the extracts was found to be higher on Gram-positive bacteria than the Gram-negative strains. The observed low activity of the extracts against the Gram-negative bacteria is in agreement with other studies (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999; Velazquez et al., 2007; Ruiz-Bustos et al., 2009). Moreover, according to Suffredini et al. (2006), Gram-negative bacteria are hardly susceptible to plant extracts in doses as low as 200 µg/ml. This study was initiated because of the increasing resistance to antibiotics of many skin pathogens including bacteria and fungi. Plant extracts and compounds are of new interest as antiseptics and antimicrobial agents in dermatology (Maher, 2011).

This is a preliminary study on the *P. duthiei* for the first time. The results obtained were different from the *Persea americana*, member of the same genus (Idris et al., 2009).

Four different types of fungus: *Aspergillus flavus*, *Fusarium solani*, *Aspergillus fumigatus* and *Aspergillus niger* were used. Water fraction was found highly active against *F. solani* and *A. niger*, while it showed good activity against other strains. Ethyl acetate fraction was prominent against *F. solani* and *A. niger*, but almost inactive against other strains. Similarly, chloroform fraction completely inhibited *F. solani*, but was inactive for other strains. While *n*-Hexane fraction was inactive against all the strains except *A. niger* (Table 2).

From Table 3, it is clear that *P. duthiei* give positive result for flavonoids, saponins, terpenoids, tannins, reducing sugar and cardiac glycosides. This plant species can be used as a source for isolation of different classes of natural product including flavonoids, saponins, anthraquinone, terpenoids, tannins, reducing sugar and cardiac glycosides. Flavonoids are a group of phenolics that are found in varying amounts in foods and medicinal plants which have been shown to exert anti-allergic, anti-inflammatory (Yamamoto and Gaynor, 2006), antimicrobial and antihepatotoxic activities (Robert et al., 2001).

Conclusion

The results suggest that each fraction has variable effects in different bioassays. It is recommended that *P. duthiei* is an important plant from medicinal point of view and can be a potential candidate for further bio-assays

Table 1. Antibacterial activity extract and fractions of *P. duthiei*.

Microorganism	Zone of inhibition (mm)				
	Ethyl-acetate fraction	Chloroform fraction	Aqueous fraction	Ethanol fraction	n-Hexane fraction
<i>E. coli</i>	0	0	0	0	0
<i>P. aeruginosa</i>	0	0	0	0	0
<i>Salmonella</i>	16	13	0	10	14
MRSA	7	18	12	5	14
<i>S. epidermidis</i>	0	0	0	0	0
<i>Klebsiella</i>	5	0	0	0	2
<i>S. aureus</i>	10	11	0	9	0

Table 2. Anti-fungal activities of different fractions of *Persea duthiei*.

Fungal strain	Ethyl-acetate fraction	Chloroform fraction	Aqueous fraction	Ethanol fraction	n-Hexane fraction
<i>A. flavus</i>	+ + +	+ +	+ +	+ +	+ + + +
<i>A. niger</i>	+	+ + + +	-	+	+
<i>A. fumigatus</i>	+ + +	+ +	+ + +	+	+ +
<i>F. solani</i>	+	-	-	+	+ + +

(-) No growth, (+) 25% growth, (+, +) 50% growth, (+, +, +) 75% growth, (+, +, +, +) 100% growth.

Table 3. Phytochemical screening of powder of *P. duthiei*.

S/n	Test	<i>Persea duthiei</i>
1	Flavonoids	Positive
2	Saponins	Positive
3	Terpenoids	Positive
4	Tannins	Positive
5	Reducing sugar	Positive
6	Cardiac glycosides	Positive

which would lead to synthesis of safe herbal drugs of global interests.

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