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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF MEDICINALLY IMPORTANT *ACHILLEA MILLEFOLIUM* L AND *CHAEROPHYLLUM VILLOSUM* WALL EXDC

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Abstract

This study was carried out to explore the phytochemical analysis and antimicrobial activity of different extracts of *Achillea millefolium* and *Chaerophyllum villosum.* Crude methanolic and chloroform extracted samples of *Achillea millefolium* and *Chaerophyllum villosum* showed good antibacterial activity against *Staphylococcus aureus*, *Shigella flexneri*, *Salmonella typhi* and *Staphylococcus epidermidis*. The extracts of both plants showed potent activity against Gram positive bacteria as compared to Gram negative bacteria. The antifungal activity revealed that methanolic and chloroform extracts of *Achillea millefolium* and *Chaerophyllum villosum* showed maximum growth inhibition of *Fusarium solanum, Penicilium notatum* and *Aspergillus flavus*.

Key words: *Achillea millefolium, Chaerophyllum villosum,* Antimicrobial, Phytochemical screening.

Introduction

Plants contain large number of bioactive constituents such as alkaloids, tannins, flavonoids, terpenoids, glycosides etc. The antibacterial properties of plants are due to these bio-molecules (Wajid *et al*., 2017; Madiha *et al*., 2018). Antimicrobial drugs are mostly used in the treatment of infectious diseases. However, these synthetic antibiotics have many side affects due to their misuse and the developing resistance of pathogens against it. In recent years antibiotic resistance has increased and this has given rise to many serious health problems. Plants contain antibiotic resistance inhibitors and they can be used to lessen resistance to antibiotics. Plants produce several bioactive constituents to cope with variety of pathogens (Sen & Batra, 2012). Different medicinal plants have antimicrobial substances that can be used against food borne pathogens and spoilage bacteria and other infectious diseases (Gupta *et al.,* 2016). Generally, different skin diseases are caused by fungal infections in developing countries. Fungal infections have increased in the last two decades, affecting millions of people worldwide. Use of synthetic chemicals for controlling these skin diseases are un-safe and have many side effects (Akhtar *et al*., 2017). Bioactive constituents of plants are eco-friendly and are safe for living organisms. Several plants contain natural substances that are lethal to several fungi causing plant diseases (Tabassum & Hamdani, 2014).

Achillea millefolium L. (Asteraceae) is known as Baranjasif (Shinwari, 2010). It occurs in swat, Azad Kashmir, Kaghan and Hazara (Fazal *et al*., 2013). *A. millefolium* has seen in use as traditional medicine due to its astringent effects. It contains isovaleric acid, salicylic acid, asparagin, sterols, and flavonoids. *Achillea millefolium* can be used as anti-hypertensitive, anti-inflammatory, anti-oxidant, anti-diabetic, antimicrobial, analgesic, anticancer, anti-diarrheal and antimicrobial. Tea made from flowers is prescribed to treat chronic catarrh, fever and to stimulate the appetite (Lakshmi *et al*., 2011). *Chaerophyllum villosum* Wall. ExDC (Apiaceae) is commonly known as Kinjari (Rawat *et al*., 2013) or Jangligajar (Mehta & Bhatt, 2007). It is extensively distributed in China, Nepal, India and Bhutan and usually develops in moist and cold environment at 2100-3500 m (Joshi & Mathela, 2013). In Pakistan *Chaerophyllum villosum* is very common in Azad Kashmir, Chitral and Kurram agency. The seeds and leaves are used to treat several ailments like cough, cold and stomach pain (Ikram *et al*., 2015). Tea prepared from dried leaves and roots are used as herbal remedy to soothe sore throat, and allergies (Shafaghat, 2013).The present study investigates various bioactive compounds and antimicrobial activity of different extracts from medicinally important *Achillea millefolium* and *Chaerophyllum villosum.*

Materials and Methods

Plants of *Achillea millefolium* and *Chaerophyllum villosum* were collected from Miranjani top (2,992 m), Nathia Gali, Khyber Pakhtunkhwa, Pakistan. These plants were identified by plant taxonomist at University of Peshawar, Pakistan and deposited via voucher numbers Muhammad AdilBot. 2244 (PUP) and Muhammad AdilBot. 2245 (PUP) in the Herbarium of the Department of Botany, University of Peshawar.

Preparation of the crude extract: Crude extract in different solvents was prepared by the methods described by Madiha *et al*., (2018). These extracts were tested for antimicrobial activity against different species of Gram positive bacteria (*Staphylococcus epidermidis* and *Staphylococcus aureus*), Gram negative bacterial strains (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Salmonella typhi* and *Shigella flexneri*) and fungal species (*Aspergillus flavus, Aspergillus fumigatus, Fusarium solani, Aspergillus niger* and *Penicillium notatum*).

Phytochemical screening and antimicrobial activity: Phytochemical screening was determined by Madiha *et al*., (2018). The crude methanolic and chloroform extracts of *Achillea millefolium* and *Chaerophyllum villosum* were evaluated for antibacterial activity by agar well diffusion procedure (Ahmad *et al*., 2011) and antifungal activity carried out by agar tube dilution method (Hussain *et al*., 2010).

Statistical analysis: The data is presented as mean of original triplicate data and LSD (Least Significant Difference) test was used upon obtaining significant difference at p<0.05 (Steel *et al*., 1997).

Results

The results of the phytochemical screening revealed the alkaloids, amino acids, reducing sugar, fats, oils, phytosterol, saponin, glycosides and tannin were present (Table 1). Crude methanolic extracts of *Achillea millefolium* were found rich in amino acids, reducing sugar, fats, oil, tannins and saponins. Chloroform extract of the same plant contained alkaloids, fats, oil, glycosides and tannins. Similarly, aqueous extract of *Achillea millefolium* indicated the presence of alkaloids, amino acids, reducing sugar, saponins and glycosides. Phystochemical analysis of *Chaerophyllum villosum* revealed that crude methanolic extract contained alkaloids, amino acids, reducing sugar, saponins and glycosides. Chloroform and aqueous extract of the same plant species was rich in alkaloids, amino acids, reducing sugar, saponins, glycosides, fats and oil (Fig. 1; Table 1).

The antibacterial activity of methanolic extract of *Achillea millefolium* showed that the highest zone of inhibition (24mm) was measured against *Staphylococcus aureus* followed by *Staphylococcus epidermidis, Shigella flexneri* (22 mm) and the lowest zone of inhibition (12 mm) by *E. coli* (Fig. 1; Table 2). Similarly, the chloroform extract of *Achillea millefolium* showed the maximum growth inhibition (22 mm) was noted against *Staphylococcus aureus* and *Shigella flexneri* followed by *E. coli* and *Klebsiella pneumonia* (20 mm) and minimum (16 mm) against *Salmonella typhi*. The methanolic and chloroform extract of *Achillea millefolium* had significant MIC values (2 mg/mL each) against *Staphylococcus aureus* and *Salmonella typhi* and *Klebsiella pneumonia*. The antibacterial activity of methanolic extract of *Chaerophyllum villosum* showed the highest zone of inhibition (28 mm) against *Staphylococcus epidermidis* followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* (26 mm) and the lowest zone of inhibition (20 mm) was shown by *Klebsiella pneumonia*. Similarly, the chloroform extract of *Chaerophyllum villosum* showed maximum inhibition (26 mm) against *Salmonella typhi* followed by *Staphylococcus aureus* (24 mm) and the lowest zone of inhibition (14 mm) was shown against *E. coli*. The methanolic and chloroform extract of *Chaerophyllum villosum* has significant MIC values 2 mg/ml against *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus aureus* (Fig. 1; Table 1).

The antifungal activity revealed that the methanolic extract of *Achillea millefolium* showed significant antifungal activity (14 mm) against *Fusarium solani* followed by *Aspergillus fumigatus* (13 mm) and lowest antifungal activity against *Aspergillus flavus* (6 mm) (Table 3). Similarly, the chloroform extract of *Achillea millefolium* showed highest antifungal activity (15 mm) against *Aspergillus flavus* followed by *Penicillium notatum* (12 mm) and the lowest antifungal activity (7 mm) against *Aspergillus niger* at the maximum extract concentration (40 mg/ml). The MIC values showed that methanolic extract of *A. millefolium* measured lowest MIC value against *A. fumigatus* whereas the chloroform extract of *A. millefolium* has lowest MIC value against *A. niger* (Fig. 2). The methanolic extract of *Chaerophyllum villosum* showed profound antifungal activity (16 mm) against *Penicillium notatum* followed by *Aspergillus flavus* (12 mm) and lowest antifungal activity against *Aspergillus niger* (4 mm). Similarly, the chloroform extract of *Chaerophyllum villosum* showed highest antifungal activity (19 mm) against *Aspergillus flavus* followed by *Penicillium notatum* (13 mm) and the lowest antifungal activity (5 mm) against *Aspergillus niger* at the maximum (40 mg/ml) extract concentration (Table 3). The MIC values showed that methanolic extract of *C. villosum* revealed lowest MIC value against *A. fumigatus* whereas the chloroform extract of *C. villosum* had lowest MIC value against *P. notatum* (Fig. 2).

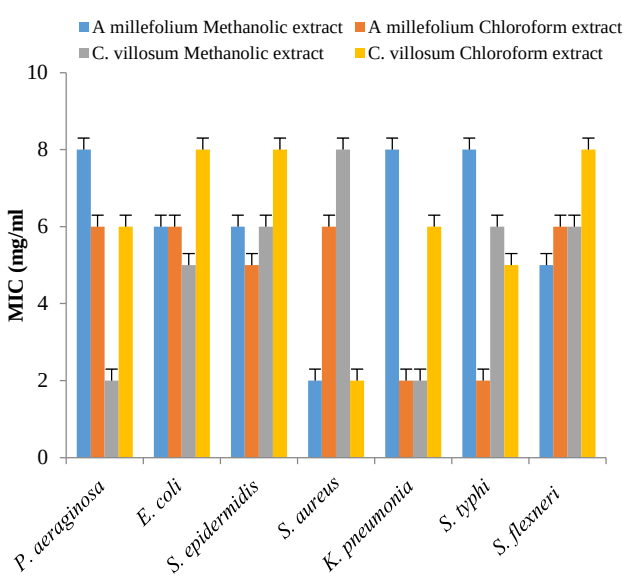

Fig. 1. MIC value of crudemethanolic and chloroform extracts of ***Achillea*** *millefolium* L and *Chaerophyllum villosum* against different bacteria (Bar shows LSD at p<0.05).

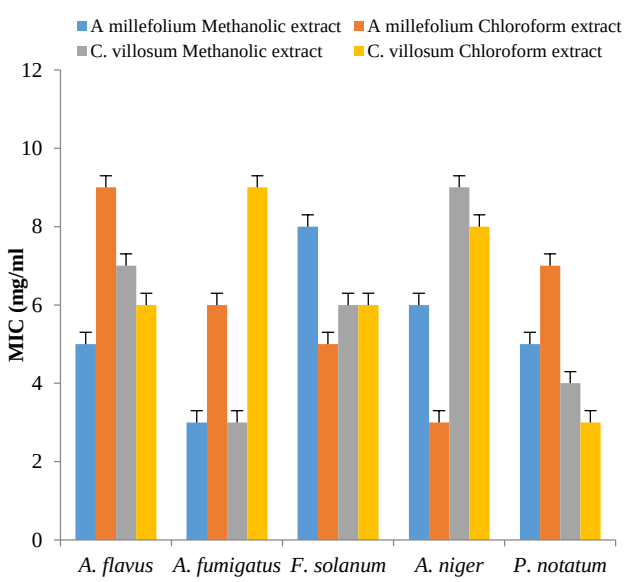

Fig. 2. MIC value of crude methanolic and chloroform extracts of ***Achillea*** *millefolium* and *Chaerophyllum villosum* against different fungi (Bar shows LSD at p<0.05).

Table 1. Phytochemical screening of *Achillea millefolium* L. and *Chaerophyllum villosum* Wall.ex DC.

Chemical constituents	*Achillea millefolium* L.			*Chaerophyllum villosum* Wall. ex DC.		
	Methanolic extract	Chloroform extract	Aqueous extract	Methanolic extract	Chloroform extract	Aqueous extract
Alkaloids	-	+	+	+	+	+
Amino acids	+	-	+	+	+	-
Reducing sugar	+	-	+	-	+	-
Fats	+	+	-	-	-	+
Oils	+	+	-	-	-	+
Phytosterol	-	-	-	+	-	-
Saponins	+	-	+	+	+	-
Glycosides	-	+	+	-	+	+
Tannins	+	+	-	+	-	+

Table 2. Antibacterial activity of *Achillea millefolium* L. and *Chaerophyllum villosum* Wall.ex DC.

Bacteria	ZI (mm)	*Achillea millefolium* L.								*Chaerophyllum villosum* Wall. ex DC							
		Zone of inhibition (mm)															
Test organisms Concentration	+ ve CO	-ve CO	Methanolic extract (mg/ml)				Chloroform extract (mg/ml)				Methanolic extract (mg/ml)				Chloroform extract (mg/ml)			
			10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40
Pseudomonas aeruginosa	30	0	6	12	16	20	6	6	14	18	8	12	18	26	6	10	16	22
Staphylococcus epidermidis	30	0	6	10	18	22	4	8	14	18	6	12	18	28	6	8	16	22
Staphylococcus aureus	27	0	6	10	16	24	6	6	12	22	8	8	18	24	6	8	16	24
Klebsiella pneumonia	25	0	8	16	16	18	8	14	18	20	6	14	18	20	4	12	16	18
Salmonella typhi	29	0	4	10	16	20	4	8	16	16	6	8	16	24	4	6	16	26
Shigella flexneri	27	0	6	12	16	22	6	10	16	22	8	18	22	22	8	16	18	20

Table 3. Antifungal activity of*Achillea millefolium* L. and *Chaerophyllum villosum* Wall.ex DC.

Fungi	*Achillea millefolium* L.										*Chaerophyllum villosum* Wall. ex DC.							
	+VE CO	-VE CO	Methanolic extract (mg/ml)				Chloroformic extract extract (mg/ml)				Methanolic extract (mg/ml)				Chloroformic extract (mg/ml)			
			10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40
Aspergillus flavus	21	0	0	0	2	6	0	3	6	15	2	4	7	12	3	6	8	19
Aspergillus fumigatus	16	0	4	6	10	13	2	2	4	10	0	0	0	6	0	0	5	9
Fusarium solani	19	0	2	5	8	14	0	2	5	9	4	6	8	8	4	6	10	10
Aspergillus niger	18	0	0	8	10	11	2	4	4	7	0	0	3	4	2	2	4	5
Penicilium notatum	20	0	4	4	6	8	6	6	11	12	5	6	14	16	6	8	15	13

Discussion

Phytochemical screening revealed that both plants showed the presence of alkaloids, amino acids, reducing sugar, fats, oils, phytosterols, saponin, glycosides and tannins (Table 1). The antibacterial activity revealed that extracts of both plants inhibited the growth of bacteria in a dose dependent manner. *S. aureus* were the most sensitive bacteria to methanolic extract of *Achillea millefolium* as compared to the chloroform extract (Bakht *et al.,* 2017). The MIC values revealed that *S. typhi* and *K. pneumonia* were the most susceptible bacteria to chloroform extract of *A. millefolium*. Similar results are also reported by Dahiya and Purkayastha, (2012) who revealed lowest MIC value of methanolic extract of *Azadirachta indica* against *S. aureus. S. epidermidis.* These bacteria were the most sensitive bacteria to methanolic extract of *Chaerophyllum villosum* (Sanjesh *et al*., 2011). Likewise, *S. typhi* was the most sensitive to the chloroform extract of *Chaerophyllum villosum.* The MIC values showed that *P. aeruginosa* and *K. pneumonia* were the most susceptible bacteria to the methanolic extract of *Chaerophyllum villosum* whereas *Staphylococcus aureus* were most susceptible to chloroform extract of *C. villosum.* These results agree with Ali *et al*., (2012) who reported the lowest MIC value for the methanolic extract of *Cannabis sativa* against *P. aeruginosa.* These results showed that extracts from both the tested plants showed profound antibacterial activity against Gram positive bacteria as compared to Gram negative bacteria. These results are supported by Darah *et al*., (2013) who reported that Gram positive bacteria were more susceptible to the extracts of *Wedelia chinensis* as compared to gram negative bacteria. The resistance of Gram negative bacteria towards the plant extracts may due to the fact that outer membrane of Gram-negative bacterium is composed of phospholipids and lipo-polysaccharides which act as a barrier to the entrance of antibiotics and other antimicrobial agents (Dahiya & Purkayastha, 2012).

The methanolic extract of *A. millefolium* revealed maximum antifungal activity against *F. solani.* Similarly, the chloroform extract of *A. millefolium* revealed highest antifungal activity against *A. flavus*. These findings agree with Mostafa *et al*., (2011) who reported highest antifungal activity against *A. flavus* and *F. solani.* The MIC values showed that

A. fumigatus was susceptible to the methanolic extract of *A. millefolium* and *A. niger* to the chloroform extract of *A. millefolium*. The methanolic extract of *Chaerophyllum villosum* revealed maximum antifungal activity against *P. notatum* and chloroform extract of *Chaerophyllum villosum* measured highest antifungal activity against *A. flavus*. These results agree with Rastegar & Gozari (2016) who reported profound antifungal activity against *A. flavus* and *P. notatum*. The MIC values showed that *A. fumigates* was susceptible to the methanolic extract of *C. villosum* and *P. notatum* was susceptible to the chloroform extract of *C. villosum*. The antibacterial activity of both the tested plants may be due to the presence of phytochemicals such as alkaloids, amino acids, saponins, tannins, glycosides and reducing sugar. These results agree with Anwar *et al*., (2017) who reported that alkaloids, tannins, saponins, glycosides showed therapeutic activity against numerous pathogens. Tannins interfere in the protein synthesis by binding to proline rich proteins (Vedhanarayanan *et al*., 2013). Saponins alter the permeability of cell walls and cause harmful effects on all organized tissues. They combine with cell membranes to stimulate changes in cell morphology leading to cell lysis and hence exert some antibacterial activity (Chandra *et al*., 2017).

Conclusion

It can be concluded from these results that the methanolic and chloroform extract of *A. millefolium* and *C. villosum* possessed different phytochemical substances that can be of pharmacological significance. The methanoilc extract of *A. millefolium* demonstrated highest antibacterial property against the *S. aureus* as compared to chloroform extract. The methanolic extract of *C. villosum* causes greater inhibition of *S. epidermidis* as compared to chloroform extract. The chloroform extract of *A. millefolium* and *C. villosum* measured highest inhibition of *A. flavus* as compared to methanolic extract.

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