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Seasonal Studies on Antimicrobial Activities of Crude Hexane and Ethanolic Leaf Extracts of *Acacia nilotica* L. against *E. coli*, *Staphylococcus aureus* and *Candida albicans*

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ABSTRACT: *Acacia nilotica* has been used by locals over the years to treat several microbial infections. The fresh plant parts of *Acacia nilotica* is considered as astringent, demulcent, aphrodisiac, anthelmintic, antimicrobial, antidiarrhoeal, with good nutritional value in Indian traditional medicine system. In the present study antimicrobial activity of the crude extracts of six samples of *Acacia nilotica* were screened against two bacterial strains, *E. coli* (gram –ve), *Staphylococcus aureus* (gram +ve) and one fungal strain, *Candida albicans*. The leaves were collected in different seasons from six regions of Himachal Pradesh. Antimicrobial activity was determined by well diffusion method. The concentration of plant extract (75mg/ml) was mixed with DMSO and added into the well. The inhibitory effect of ethanolic extract of *Acacia nilotica* was relatively higher than that of hexane extract. Combined effect of plant extract and drug was also determined. Leaves extract exhibited considerable bacteriostatic activity against selected bacteria and fungus. This study encourages the cultivations of nutrient rich valuable plants in large scale and support to use medicinal plants in traditional medicines.

Key words: Herbal extracts, fungicide, *Acacia nilotica*, zone of inhibition.

INTRODUCTION

The genus Acacia includes about 1350 species and is second largest in leguminosae family. Out of these *Acacia nilotica* is well known species that has been used to treat various ailments like tuberculosis, dysentery, smallpox, leprosy, cough, toothache and skin cancer by the people. It occurs from sea level to 2000 m and can has high temperature tolerating capacity (> 50° C), but it is sensitive to frost at young age (Kiran and Bargali 2009). Leaves of *Acacia nilotica* are found to possess a significant level of antimicrobial activity against certain pathogens. The potential of antimicrobial activity also depend upon type of extract used (Mustafa *et al*., 1999). Many herbal plants are reported to treat certain diseases like respiration diseases, cutaneous infections, urinary tract infection etc (Somchit *et al*., 2003). According to WHO medicinal plants would be the best sources for obtaining the drugs (Nair and Chanda, 2006).There are several studies on antimicrobial activity of herbal extract (Bonjar, 2004). Studies showed that various extracts like aqueous, ethanol, methanol, n-hexane, petroleum ether and chloroform showed variable antimicrobial activity against gram negative, gram positive (Solomon-Wisdom and Shittu, 2010) and fungal pathogens (Mariita *et al.*, 2011). The aim of present study was to determine the antibacterial and antifungal activity of hexane and ethanolic extracts of *Acacia nilotica* in different seasons.

MATERIAL AND METHODS

A. Plant material and extract preparation

Young leaves of *Acacia nilotica* were collected from six different regions of Himachal Pradesh in summer (March-June), monsoon (July-October) and winter (November-February). Leaves of *Acacia nilotica* were rinsed with distilled water and dried at room temperature. Dried leaves were suspended in 98% ethanol and 98% n-hexane for 3-7 days at 60°C in extraction bottle. After one week, mixture was filtered using whatman filter paper. The solvents were completely evaporated at 40°C by using water bath to obtain the extract. The extracts so obtained were stored at 4°C for screening of antimicrobial activity.

Preparation of samples. The extract (75 mg) was taken and dissolved in 1 ml of DMSO hence this stock solution (75mg/ml) was used to determine antibacterial activity. Along with this, solutions of standard antibiotics (amoxicillin, 50mg/ml and ciprofloxacin, 5mg/ml) were also prepared. Standard antibiotics and DMSO were used as positive and negative control.

B. Antibacterial activity of Acacia nilotica (leaves) extract

Antibacterial activity of plant extracts was determined by well diffusion method using nutrient agar media (Lino and Degracious, 2006). The presence of zone of inhibition (mm) was indicated as the presence of antibacterial activity while no zone represented zero antibacterial activity. Each extract of *Acacia nilotica* leaves was tested against the test organisms in triplicates along with media control and organism control plates.

Bacterial strains used. Antibacterial activity of leaves of *Acacia nilotica* was tested against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). The organisms were maintained on nutrient agar plates at 4°C.

Preparation of agar plates. 2.3 g of nutrients agar was dissolved in 100ml of distilled water and pH was adjusted 7. Media sterilization was done by autoclaving at 121° C for 15 minutes. Now let it cool up to 45° C. After pouring the media it was allowed to solidify. Sufficient inoculum (10^8CFU/ml) was added to the surface of medium. 5 wells (for ethanolic extract plates) and 4 wells (for hexane extract plates) were made with sterilized cork borer (6mm), because ethanolic extract was dissolved in DMSO while n-hexane was not soluble in it.

Pouring of test solutions: incubation and measurements of zone of inhibition. Using micropipette, 50 µl of plant extract (75mg/ml), and 20 µl of antibiotics were added into the wells and same quantity of both plant extract and antibiotics were added in next well to check the combined effect (synergestic/antagonistic). The plates were incubated at 37° C for 24 hrs. After incubation period, the diameter of zone of inhibition was measured by a ruler.

Antifungal activity by Agar well Diffusion Method. Potato dextrose agar (PDA) plates were inoculated by spreading the fungal culture on the media. For analyzing ethanolic extract activity, the PDA plates were punched with 5 wells (6mm) while for n-hexane activity analysis; plates were punched with 4 wells (6mm). 50 µl of Plant extracts (75mg/ml) was added into the well. The susceptibilities of the microbial strains to different antibiotics were tested using fluconazole and PCNB. 20 µl of fluconazole (50mg/ml), 80µl of PCNB (100mg/ml) were used as positive control. Well containing DMSO alone act as a negative control. Combined effect (drug + plant extract) was also examined by adding equal quantity of the two. The plates were incubated at room temperature for 2 hours for proper diffusion of extract and antibiotics in the wells. After that plates were kept in incubator at 25° C for two days. The antifungal activity was assessed by measuring the diameter of the zone of inhibition (in mm)

Fungal strain used. Antifungal activity of leaves of *Acacia nilotica* was tested against *Candida albicans* (ATCC-10231). The organisms were maintained on PDA plates at 4°C.

C. Determination of extract yield

For determining the extract yield from leaves of selected plant, we weighed 7 g of dry sample and suspended in 70 ml of solvent (ethanol/hexane). By using water bath solvent was evaporated to get thick paste at the end. The percentage yield of the leaves extract of *Acacia nilotica* in different seasons was determined by following formula

Percentage yield = x/y × 100

x = dry weight of extract
y = soaked samples material

RESULTS

A. Quantity of crude extracts of leaves of Acacia nilotica in different season

It is clear from the figure 1 that yield of ethanolic plant extract is more in all samples than hexane solvent. Sample from Una district gave maximum yield (30%) in winter season.

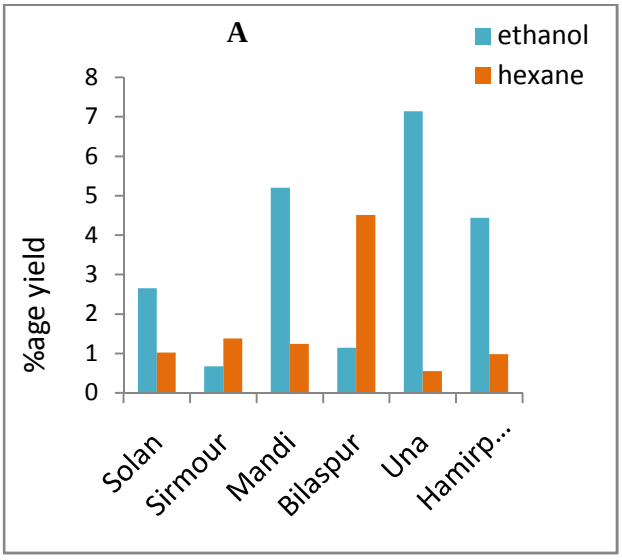


Fig. 1. Percentage yield of ethanolic and n-hexane extract of *Acacia nilotica* (leaves) in (A) summer.

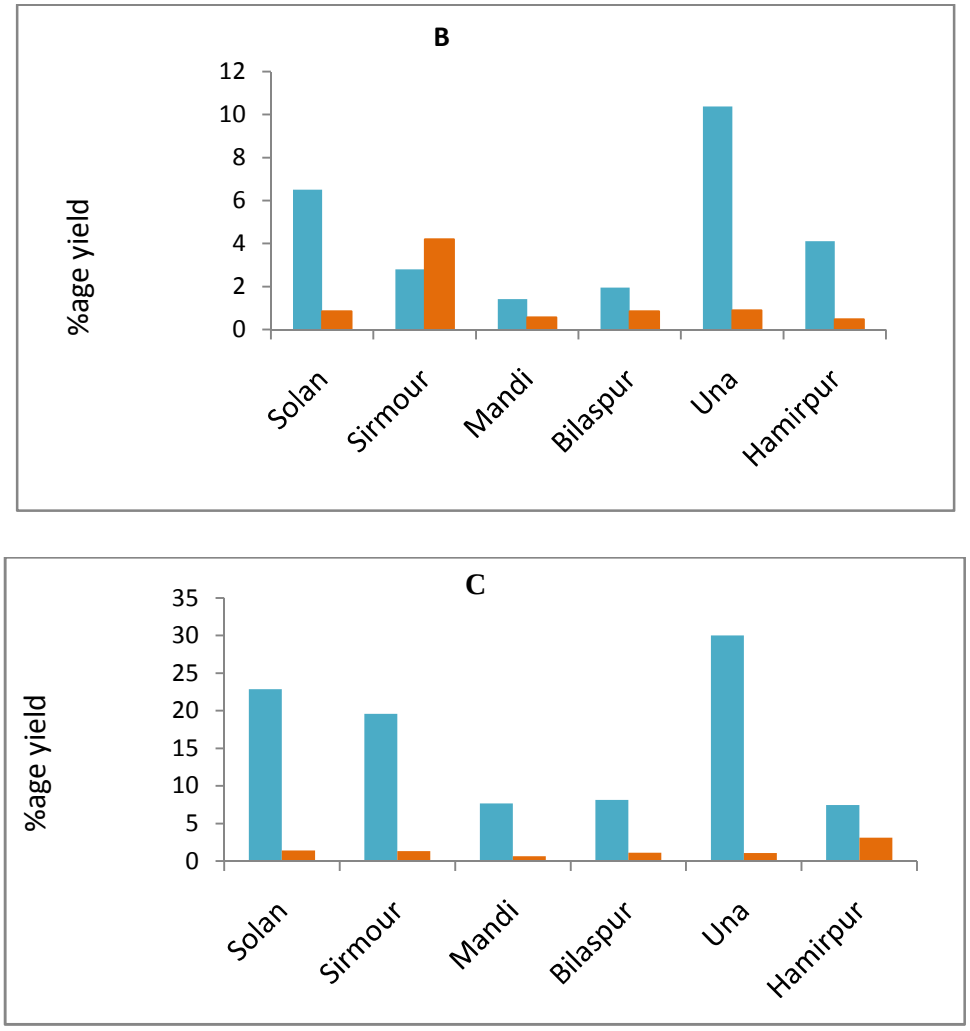


Fig. 1. Percentage yield of ethanolic and n-hexane extract of *Acacia nilotica* (leaves) in (B) monsoon and (C) winter season.

Therefore, to obtain maximum yield of plant extract from *Acacia nilotica*, winter season could be best season to collect the samples.

Antimicrobial activity. The extracts prepared from *Acacia Arabica* leaves using different solvents showed varying degree of antimicrobial activity against both Gram positive (*S. aureus*) and Gram negative *(E. coli*) bacteria selected for the study. Among the extracts prepared using different solvents, ethanolic extract was found to be more effective than hexane extract. Ethanolic extracts showed higher efficacy against *E. coli* and *S. aureus* at 75mg/ml concentration, while this concentration was not as effective for *Candida albicans* strain (Table 1).

From table 1 it is clear that ethanolic extract (75mg/ml) of *Acacia nilotica* has more potential against *E. coli* and *S. aureus* but not against *C. albicans*. Hexane extract gave almost zero activity in comparison to ethanolic extract. Highest activity was given by extract from Bilaspur district against *E. coli* and *S. aureus* (22 mm) in monsoon season. The diameter of zone of inhibition ranged from 10 mm to 22 mm. The extract from Hamirpur district did not show any activity against any of selected microrganisms. The extract from Bilaspur district only showed activity against *C. albicans* in monsoon (16 mm) and winter (15 mm) season. Lowest activity was given by extract from Una district (10 mm) against *S. aureus* in summer season.

Table 1: Antibacterial activity of ethanolic and hexane extracts of *Acacia nilotica* (leaves) against selected microorganisms in three seasons (summer monsoon and winter).

		Zone of inhibition (mm)								
		Summer			Monsoon			Winter		
Districts	**Plant extracts (PE)**	**O1**	**O2**	**O3**	**O1**	**O2**	**O3**	**O1**	**O2**	**O3**
Solan	Ethanolic	14	10	0	12	14	0	14	11	0
	Hexane	0	0	0	0	0	0	0	0	0
Sirmour	Ethanolic	14	10	0	12	13	0	16	11	0
	Hexane	0	0	0	11	0	0	0	0	0
Mandi	Ethanolic	13	11	0	12	13	0	0	0	0
	Hexane	0	0	0	0	0	0	0	0	0
Bilaspur	Ethanolic	14	11	0	22	22	16	21	13	15
	Hexane	0	0	0	0	0	0	0	0	0
Una	Ethanolic	11	10	0	12	16	0	16	12	0
	Hexane	0	0	0	0	0	0	0	0	0
Hamirpur	Ethanolic	0	0	0	0	0	0	0	0	0
	Hexane	0	0	0	0	0	0	0	0	0

O1 (Organism 1) = *E. coli*, O2 = *S. aureus*, O3 = *Candida albicans*

Table 2: Combined effect (PE+ antibacterial drug) study of *Acacia nilotica* against O1 and O2 in three seasons.

			Zone of inhibition (mm)					
			Summer		Monsoon		Winter	
Districts	**Extracts**	**(PE+drug)**	**O1**	**O2**	**O1**	**O2**	**O1**	**O2**
Solan	Ethanolic	PE+Am	17	24	20	28	17	26
		PE+C	15	22	13	13	14	24
	Hexane	PE+Am	14	22	19	26	16	27
		PE+C	17	11	20	12	16	26
Sirmour	Ethanolic	PE+Am	16	25	18	25	19	28
		PE+C	15	18	12	20	14	18
	Hexane	PE+Am	19	25	22	30	18	27
		PE+C	17	12	13	17	16	25
Mandi	Ethanolic	PE+Am	15	25	19	22	17	27
		PE+C	16	22	18	12	17	15
	Hexane	PE+Am	19	26	20	27	17	27
		PE+C	17	12	21	12	18	26
Bilaspur	Ethanolic	PE+Am	11	24	20	21	22	28
		PE+C	15	21	23	20	22	27
	Hexane	PE+Am	17	25	22	27	18	26
		PE+C	17	12	20	15	19	26
Una	Ethanolic	PE+Am	13	24	16	16	24	26
		PE+C	12	23	14	12	14	19
	Hexane	PE+Am	17	25	19	27	17	28
		PE+C	14	12	21	12	15	22
Hamirpur	Ethanolic	PE+Am	17	24	18	19	16	28
		PE+C	14	20	17	12	11	21
	Hexane	PE+Am	21	26	21	20	18	27
		PE+C	16	17	21	22	16	25

+ve control reference value: FLU = Fluconazole; PCNB = Pentachloronitrobenzene.

Table 3: Combined effect (PE+ antifungal drug) study of *Acacia nilotica* against O3 in three seasons.

			Zone of inhibition (mm)		
			Summer	Monsoon	Winter
Districts	**Extracts**	**(PE+drug)**	**O3**	**O3**	**O3**
Solan	Ethanolic	PE+FLU	24	18	22
		PE+PCNB	12	11	10
	Hexane	PE+FLU	22	13	16
		PE+PCNB	11	12	0
Sirmour	Ethanolic	PE+FLU	22	18	18
		PE+PCNB	12	10	0
	Hexane	PE+FLU	24	17	20
		PE+PCNB	10	11	0
Mandi	Ethanolic	PE+FLU	26	17	20
		PE+PCNB	11	10	10
	Hexane	PE+FLU	17	19	21
		PE+PCNB	10	10	11
Bilaspur	Ethanolic	PE+FLU	24	25	22
		PE+PCNB	10	14	15
	Hexane	PE+FLU	19	15	22
		PE+PCNB	10	10	0
Una	Ethanolic	PE+FLU	25	16	18
		PE+PCNB	11	14	13
	Hexane	PE+FLU	25	21	21
		PE+PCNB	10	10	0
Hamirpur	Ethanolic	PE+FLU	22	19	23
		PE+PCNB	11	11	0
	Hexane	PE+FLU	16	21	18
		PE+PCNB	11	10	0

+ve control reference value: FLU = Fluconazole; PCNB = Pentachloronitrobenzene.

From table 2 we concluded that ethanolic plant extract plus antibiotic showed synergistic activity against *E. coli* and *S. aureus*. Hexane extracts showed antagonistic activity when combined with drugs. Fig. 2 is showing synergistic effect of plant extract (Sirmour sample) with drugs in different seasons.

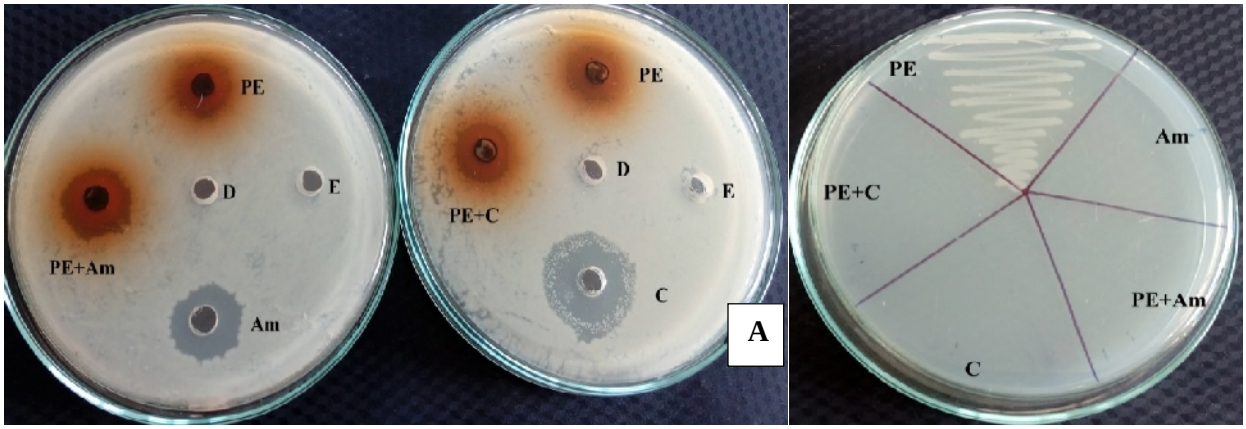
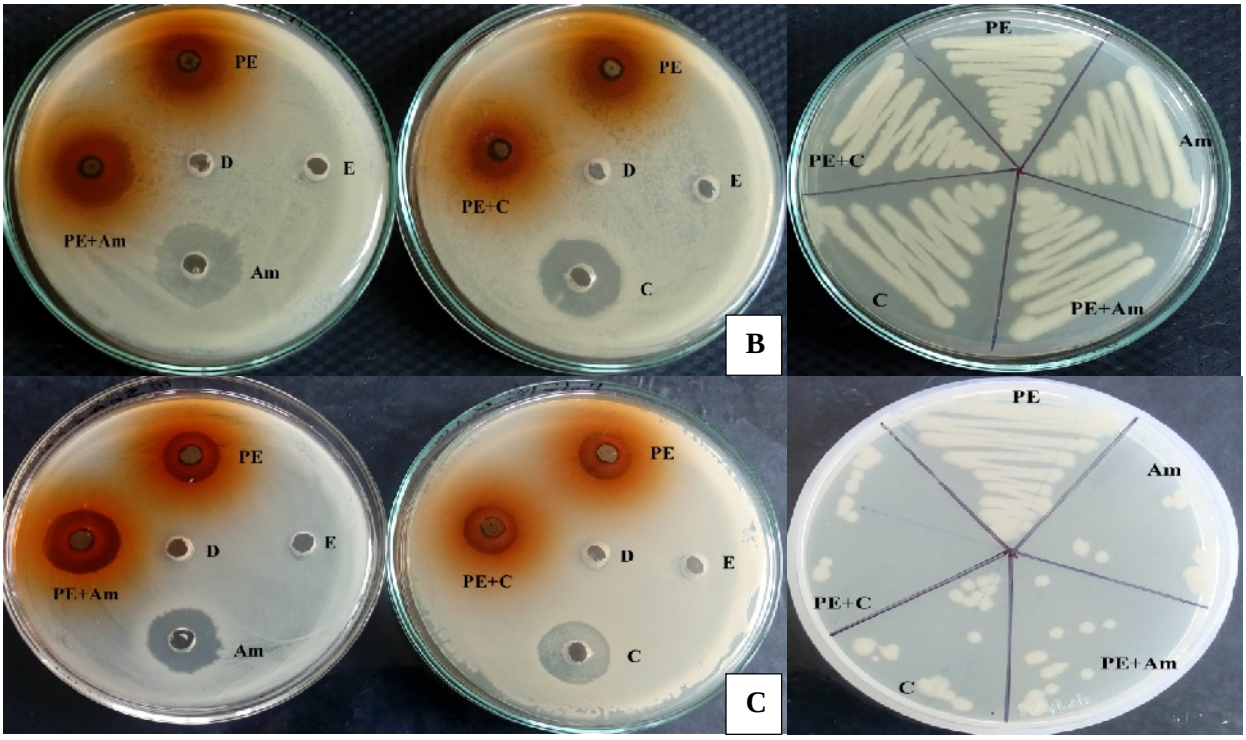


Fig. 2. Antibacterial activity of ethanolic extract of *Acacia nilotica* from sirmour district against *E. coli* in different seasons: (A) summer season.



PE=plant extract; E= ethanol; D= DMSO; Am= amoxicillin; C= ciprofloxacin.

Fig. 2. Antibacterial activity of ethanolic extract of *Acacia nilotica* from sirmour district against *E. coli* in different seasons: (B) monsoon season (C) winter season.

DISCUSSION

The antimicrobial activities of *Acacia nilotica* plant extracts have been studied for years. The use of medicinal plants for treating various ailments play a vital role covering basic health needs. Plants extracts have great potential (as it contains antimicrobial compounds) against certain microorganisms. The climatic conditions and phenological cycle are factors that have a strong influence on the composition and content of the extract of plants. The seasonality of the constituents in the plants influences the antimicrobial activity of its extracts. In present study, zone of inhibition of ethanolic plant extract from Simour district against *E. coli* (14 mm) in summer, (12 mm) in monsoon and (16 mm) in winter was observed (Table 1). While synergistic effect was shown when extract and drug were used in combination e.g. zone of inhibition of ethanolic plant extract from Simour district against *E. coli* (16 mm) in summer, (18 mm) in monsoon and (19 mm) in winter was observed. According to a study by Ranwan and Yadav (2012), maximum activity of *Achyranthus aspera* hexane extract was observed in January month against all the test organisms, which support present investigation. According to Morais (2009), the chemical composition and content of the essential oil may undergo changes during the seasons of the year. As temperature and luminosity play important roles in photosynthesis, they influence the vegetative physiological process; as a consequence, interfere with the content and composition of the EOs in the producing plants (Souza *et al*., 2008).

CONCLUSION

The present investigation on antimicrobial activity of herbal plant extracts of *Acacia nilotica* showed that ethanol extract showed promising antibacterial activity against *E. coli* and *S. aureus* in comparison to hexane extract. Ethanolic leaf extract of *A. nilotica* at 75mg/ml concentration was found effective for *E. coli* and *S. aureus.* However, this concentration was found ineffective for *C. albicans*. This study justifies the traditional use of *Acacia nilotica* in medicine for treatment various infectious diseases caused by the microbes.

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