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COMPARATIVE ANTIMICROBIAL AND SYNERGISTIC POTENTIAL OF EXTRACTS OF LEAVES OF *ZANTHOXYLUM ARMATUM* DC. FROM DIFFERENT GEOGRAPHICAL REGIONS OF HIMACHAL PRADESH, INDIA

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Abstract

Zanthoxylum armatum DC. (syn. *Z. alatum* Roxb) is an important and endangered medicinal plant of Himalayan region commonly called as Timur or Indian prickly ash. The aim of the present study was to evaluate the antimicrobial activity of different solvent extracts (chloroform, methanol and n-butanol) of leaves of *Z. armatum*. All the extracts were tested for the presence of various phytocompounds and were evaluated for their antimicrobial activity against Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*), Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*) bacteria and fungus strain, *Candida albicans* (ATCC90028 and MTCC277) and *Saccharomyces cerevisiae* (H1086). Various phytochemicals are present in different solvent extracts of *Z. armatum* from different geographical locations. n-Butanol extract of leaves from Sirmour district was found to be very effective against *E. coli* (18.3 ± 0.5 mm), *K. pneumoniae* (13.5 ± 0.5 mm) and *B. subtilis* (10.33 ± 0.57 mm), whereas n-butanol extract of leaves of Shimla was more effective against *S. aureus* (13.0 ± 0.5 mm). n-Butanol extract of Bilaspur sample (8.3 ± 0.57 mm), Sirmour (11.3 ± 1.15 mm) and Sirmour (7.6 ± 1.15 mm) have shown maximum inhibition to growth of *C. albicans* (ATCC90028 and MTCC277) and *S. cerevisiae* (H1086) respectively. It was observed that MIC ranged from 0.39-250 µg/ml against bacterial strains (*B. subtilis, S. aureus, E. coli*, and *K. pneumoniae*) and 0.39-0.78 µg/ml against fungal strains {*C. albicans (*ATCC90028 & MTCC277) and *S. cerevisiae* (H1086)}. n-butanol extract of Hamirpur, Bilaspur and Sirmour showed lowest MIC (0.39 µg/ml) against *B. subtilis*, while n-butanol extract of Bilaspur showed lowest MIC (15.63 µg/ml) against *S. aureus*. n-Butanol extract of Bilaspur showed lowest MIC of 7.81µg/ml and 15.63µg/ml against *E. coli and K. pneumoniae,* respectively. MIC value (0.78µg/ml) was found to be similar in n-butanol extract of all the samples against *C. albicans* (ATCC90028); whereas n-butanol extract from Bilaspur, Mandi, Solan and Shimla showed lowest MIC (3.9µg/ml) against *C. albicans* (MTCC277). In case of *S. cerevisiae* (H1086), n-butanol extract of Bilaspur, Mandi, Solan, Shimla and Sirmour showed lowest MIC of 7.81µg/ml. It can be concluded from the present study that *Z. armatum* leaf from Bilaspur region showed comparatively better antimicrobial potential among all other samples. The present study also reported synergistic potential of methanolic and n-butanol extract of *Z. armatum* leaves with both antibacterial as well as antifungal antibiotics.

Key words: *Zanthoxylum armatum,* Phytochemicals, Antimicrobial, Minimum Inhibitory Concentration (MIC), Synergism.

Introduction

From thousands of years, natural products have played an important role throughout the world in treatment and prevention of human diseases. These products have come from various sources including terrestrial plants, marine organisms, terrestrial microorganisms, terrestrial vertebrates and invertebrates. Among these products used, herbal drugs constitute a major part in the traditional system of medical practice. Plants are used as medicines in different countries as source of many powerful and potent drugs (Srivastava *et al*., 1996). More than 25% of the prescribed drugs all over the world are prepared from a variety of plant materials such as rhizomes, leaves, bark, stems etc. (Graham *et al*., 2000). Over 4.3 billion people rely upon such traditional plant systems of medicine to provide primary health care (Owolabi *et al.,* 2007). Due to rapid increase in the rate of infections, side effects of

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synthetic antibiotics and antibiotic resistance in microorganisms, the medicinal plants are in advance popularity over the standard drugs (Babu and Subhasree, 2009).

Improvement of multiple drug resistant (MDR) strains of pathogens to modern in essence antibiotics has generated an inappropriate requirement for new antimicrobial agents from medicinal plants. Antimicrobial resistance is a major threat to animal and human health development, affecting our ability to treat a range of infections. Many medicinal plants have been screened broadly for their antimicrobial potential globally due to the presence of phytochemical bioactive compounds (Mothana *et al*., 2009; Prachayasittikul *et al*., 2008).

Zanthoxylum armatum DC of the Rutaceace family is an important medicinal plant commonly known as Indian Prickly Ash or Toothache tree. It is widely distributed in India from Kashmir to Bhutan at an altitudes up to 2500 m, also occurs throughout North East India. It is a small tree or large spiny shrub. *Zanthoxylum armatum* is a dioecious plant so that male and female plants must be grown if seed is required. Male flowers: stamens 4-6; anthers yellow prior to anthesis; connective apex with oil gland; disk pulvinate; rudimentary carpels lacking. Female flowers: carpels 2 or 3, abaxially often with a visible oil gland; styles recurved; staminodes ligulate or lacking. The fruit follicles are 4-5mm in diameter and usually become reddish in color on ripening. The initial stage of seed development takes place during the mid of June month. Seeds are rounded, 3mm in diameter, shinning black, have bitter taste and aromatically pungent. An aroma is present in the brown fruit wall or pericarp-shell of the dried fruit. It may have an innate capacity to develop an anesthetic feeling on the tongue (Bachwani *et al*., 2012). The plant can be recognized by its shrubby habit, dense foliage, with pungent aromatic taste, prickled trunk and branches and small red, subglobose fruits. The roots of *Z. armatum* are used in toothache, fever, rheumatism and the fruit is used in the treatment of asthma, bronchitis, cholera, fibrosis's, indigestion, skin diseases, stomachache, cough, colic vomiting, diarrhea, and as an aromatic, stimulant and pesticide (Bisht and Chanotiya, 2011; Akhtar *et al*., 2009). *Z. armatum* have antimicrobial and antioxidant activities against pathogenic bacteria as well as fungi (Paridhavi and Agrawal, 2007; Cheng *et al*., 2005) but this plants is slightly neglected to some extent and marginal studies have been done. Thus, the current study was designed to evaluate the *in vitro* antimicrobrial and antioxidant activities, and phytochemicals screening of *Z. armatum*. The compounds associated with this medicinal plant could be used as a potential source of therapeutic agent against many diseases. In the present communication, an attempt has been made to compare the antimicrobial potential of leaf extracts of *Zanthoxylum armatum* collected from different regions of Himachal Pradesh.

Materials and methods

Collection, Processing and Extract Preparation

Leaves of *Z. armatum* were collected from six districts of Himachal Pradesh *i.e.* Hamirpur, Bilaspur, Mandi, Solan, Shimla and Sirmour in the month of June - July 2017 Fig. 1. The collected leaves of *Z. armatum* were washed with running tap water followed by washing with 70% ethanol and finally washed with sterilized distilled water. Then leaves were dried in an incubator at 40ºC and dried leaves were completely ground to fine powder using an electric grinder.

Extracts of different solvents such as chloroform, methanol and n-butanol were prepared using cold maceration method. Prepared extracts were dried at 40ºC and dried extracts were stored at 4°C until further use.

Phytochemical Screening of various solvent extracts of *Z. armatum leaves*

All the leaves extracts of *Z. armatum* from different geographical regions of Himachal Pradesh were tested for the presence of various active phytochemicals such as alkaloids, glycosides, carbohydrates, phenols, flavonoids, tannins, protein, and terpenoids (Khandelwal, 2006; Guleria *et al*., 2016).

Microbial strains and Culture media

The prepared extracts were evaluated against

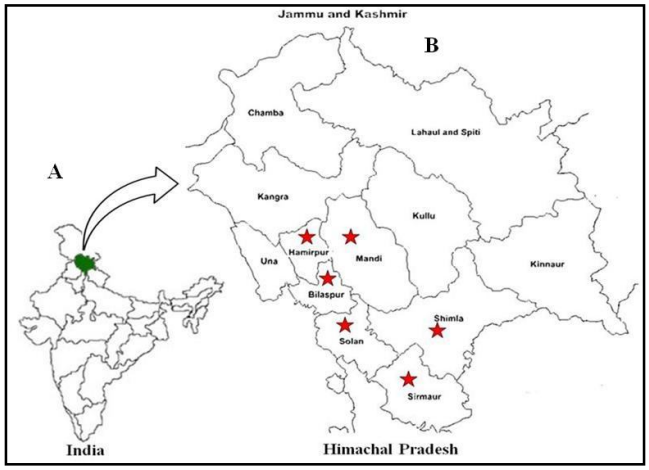

Fig 1: Map of India (A) showing Himachal Pradesh shaded in black. 'B' showed detailed map of Himachal Pradesh. Asterisk (*) sign indicates the sites of collection of leaves of *Z. armatum* from six districts of Himachal Pradesh with different altitudinal variations.

bacterial strains i.e, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis* and fungal strains *i.e, Candida albicans* (ATCC90028 and MTCC277) and *Saccharomyces cerevisiae* (H1086). All the bacterial and fungal strains were obtained from Yeast Biology Lab, Shoolini University, Solan, Himachal Pradesh, India. The bacterial culture were maintained on Nutrient Agar at 37ºC, while, Yeast peptone dextrose agar media was used for growth of fungi at 30ºC.

Antimicrobial activity using agar well diffusion method

The antimicrobial activity of different extracts of *Z. armatum* leaves i.e. methanol, n-butanol, and chloroform were evaluated by agar well diffusion method (Perez *et al*., 1970) and broth dilution method under CLSI, (2012) guidelines using 2, 3, 5-triphenyl tetrazolium chloride (TTC). Ampicillin and Fluconazole were used as a positive control in case of antibacterial and antifungal activities respectively. DMSO (solvent) was used as negative control. After incubation period of 24h to 48 hours, the diameter of zone of inhibition was measured using a HiAntibiotic Zone scale-C (Himedia Biosciences, Mumbai (India)). The experiments were performed in triplicates and results were recorded as mean ± standard deviation.

Checker board method for synergistic activity of various extracts of *Z. armatum* leaves

Synergitic potential of n-butanol and methanolic extracts of leaves of *Z. armatum with* antibacterial (tetracycline and kanamycin) and antifungal (fluconazole and amphotericin B) antibiotics were determined using checkerboard method (Eumkeb *et al.,* 2012; Bonapace *et al.,* 2002; Dev *et al.,* 2017; Rolta *et al.,* 2018a, b). In this method, different combinations of leaf extracts of *Z. armatum* and antibiotics (antibacterial and antifungal) were prepared and rest of the procedure was performed as similar to that for broth dilution method. Synergistic activity of extracts was then calculated on the basis of change in MIC value of both extracts and antibiotics alone and in combination and expressed in terms of FIC index. FIC value for each combination was calculated using the formula:

$$FICI = \Sigma FIC = FIC_{(antibiotic)} + FIC_{(plant\ extract)}$$

Where: $FIC_{(antibiotic)}$ = MIC of antibiotic in combination/MIC of antibiotic alone

$FIC_{(extract)}$ = MIC of extract in combination/MIC of extract alone

Interactions were said to be synergistic if FICI ≤ 0.5, on the other hand, when FICI ≥ 0.5–1, the combination are additive, and when FICI ≥ 1.0– ≤ 4.0, the combination was indifferent. Interaction were said to be antagonistic, when FICI>4.0 (Ahmad *et al.,* 2006; Rolta *et al*., 2020a, b).

Results and Discussion

Qualitative phytochemical screening of different solvent extract of leaves of *Z. armatum*

The medicinal importance of any plant is due to the presence of secondary metabolites such as flavonoids, glycosides, phenolic compounds, proteins, tannins, saponins and carbohydrates. In the current investigation, various solvent extracts of leaves of *Z. armatum* from different geographical locations were screened for the presence of various phytocompounds. It has been observed that the presence of phytocompounds in leaves of *Z. armatum* varies with solvent and geographical location. Chloroform extract of leaves of *Z. armatum* from different geographical locations showed the presence of phenolic compounds, tannins, flavonoids and terpenoids. Carbohydrates were found to be present in all the extracts, except sample from Solan, Shimla and Sirmour. Glycosides were found to be present in samples collected from all regions except from Hamirpur and Bilaspur. No proteins were detected in chloroform extract of all the samples except Mandi. Chloroform extracts of the samples from all region lack saponins, except samples from Shimla and Sirmour districts. Methanol extract from leaves of *Z. armatum* from different geographical locations showed the presence of phenolic compounds, tannins, flavonoids, alkaloids. Proteins were detected in methanolic extract of leaves of *Z. armatum* in all the samples, except from Shimla and Sirmour. Terpenoids were also detected in methanolic extract of leaves of *Z. armatum* in all the samples except Bilaspur and Mandi. Glycosides were found to present in all the samples except from Bilaspur. The n-butanol extract of all the samples showed the presence of phenolic compounds, tannins, flavonoids and alkaloids, however, all other phytocompounds were found to be absent except glycosides and proteins, which were detected in butanol extract of sample collected from Bilaspur and Solan respectively. The result of phytochemical screening was summarized in table 1. Barkatullah *et al.,* (2013) and Alam and Saqib (2015) analyzed phytochemical composition of the leaves, barks and fruits of *Z. armatum* and detected the presence of alkaloids, saponin, tannins and flavonoids in all the parts. In line with present study, Kamalesh *et al*., (2013) and Rynjah *et al*., (2018) also showed the presence of alkaloid, glycoside, carbohydrate,

Table 1: Qualitative screening of phytocompounds in three solvent extracts, viz. chloroform, methanol and n-butanol of leaves of *Z. armatum* from different regions of Himachal Pradesh

Phytocompounds	Places of sample collection from different altitudes																	
	Hamirpur (700-1100 m)			Bilaspur (1100-1500 m)			Mandi (800-1300 m)			Solan (1400-1800 m)			Shimla (1700-2100 m)			Sirmour (1800-2200 m)		
	C	M	B	C	M	B	C	M	B	C	M	B	C	M	B	C	M	B
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
Carbohydrates	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	+	-	-	-	+	+	+	-	+	+	-	+	+	-	+	+	-
Terpenoids	+	+	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-
Proteins	-	+	-	-	+	-	+	+	-	-	+	+	-	-	-	-	-	-
Saponins	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-

'M' indicates methanolic extract; 'B' indicates n-butanol extract; and 'C' indicates chloroform extract; '+' sign indicates the presence of phytoconstituents and '-' sign indicates the absence of the phytocompounds.

tannins, amino acids, sterols and terpenoids in leaf of *Z. armatum*.

Comparison of antimicrobial activity of various solvent extract of leaves of *Z. armatum* collected from different geographical regions of Himachal Pradesh

Antimicrobial activity was compared between various solvent extracts of *Z. armatum* leaves from different geographical regions of Himachal Pradesh using agar well diffusion method and micro dilution method.

With agar well diffusion method, it was observed that n-butanol extract was more effective against both bacterial and fungal strains. Among different extracts, n-butanol extract of leaves of Sirmour showed highest antibacterial activity against *E. coli* (18.3 ± 0.5 mm), *K. pneumoniae* (14.5 ± 0.5 mm) and *B. subtilis* (10.33 ± 0.57 mm), whereas n-butanol extract of leaves collected from Shimla (13.0 ± 0.57 mm) was more effective against *S. aureus*. Similar to antibacterial activity, n-butanol extract also showed comparatively better antifungal

Table 2: Comparison of diameter of zone of inhibition shown by various solvent extracts of *Z. armatum* leaves from different geographical locations of Himachal Pradesh against bacterial strains.

Samples	Zone of inhibition (mm)											
	B. subtilis			*S. aureus*			*E. coli*			*K. pneumonia*		
	C	M	B	C	M	B	C	M	B	C	M	B
HR	8.0± 0.19	9.5± 0.5	9.0± 0.2	6.1± 0.28	8.1± 0.57	8.7± 0.28	9.3± 0.57	13.2± 0.23	14.6± 0.5	8.6± 0.57	9.0± 0	9.6± 0.57
BR	8.0± 0.34	8.83± 0.28	8.83± 0.28	6.0± 0.49	9.0± 0.3	7.0± 0	9.3± 0.57	13.6± 0.5	16.5± 0.5	9.1± 0.28	7.83± 0.78	11.16± 0.28
MN	8.02± 0.49	8.0± 0.28	9.33± 0.57	6.1± 0.57	8.1± 0.5	8.1± 0.28	9.3± 0.57	11.0± 1.0	12.3± 0.57	8.5± 0.50	8.5± 0.50	10.16± 0.28
SN	9.6± 0.57	8.0± 0.23	9.33± 0.57	8.2± 0.28	10.0± 0.9	9.2± 0.5	10.0± 1.0	13.3± 0.57	13.5± 0.5	8.2± 0.57	7.6± 0.57	12.3± 0.57
SH	8.0± 0.23	9.16± 0.28	9.5± 0.50	7.1± 0.29	6.0± 0.37	13.0± 0	9.5± 0.50	12.7± 0.57	14.6± 0.57	8.3± 0.57	9.3± 0.57	9.6± 0.5
SR	9.3± 0.57	9.33± 0.57	10.33± 0.57	6.0± 0.5	8.4± 0.57	11.6± 0.28	9.6± 0.57	14.0± 0	18.3± 0.5	8.16± 0.28	9.6± 0.57	13.5± 0.5
Amp	13.87± 0.70	14.33± 0.7	15.9± 1.4	13.57± 0.7	13.8± 0.71	15.33± 0.7	14.56± 1.41	21.5± 0.7	17.36± 0.7	12.3± 1.31	11.5± 0.7	13.9± 0.7

'M' indicates methanolic extract; 'B' indicates n-butanol extract; and 'C' indicates chloroform extract; 'HR' indicates Hamirpur, 'BR' indicates Bilaspur, 'MN' indicates Mandi, 'SN' indicates Solan, 'SH' indicates Shimla and 'SR' indicates Sirmour district and Amp represents Ampicillin. DMSO was used as negative control, whereas ampicillin was used as positive control.

activity against all the tested fungal strains. n-Butanol extract of Bilaspur (8.3 ± 0.57 mm), Sirmour (11.3 ± 1.15 mm) and Sirmour (7.6 ± 1.15 mm) have showed highest inhibition to growth of *C. albicans* (ATCC90028 and MTCC277), and *S. cerevisiae* (H1086) respectively. The comparison of diameter of zone of inhibition shown by different solvent extracts against bacterial and fungal strains are summarized in table 2, 3 and Fig. 2, 3 respectively.

Antibacterial activity was calculated in terms of zone of inhibition (mm) shown by different solvent extracts of leaves of *Z. armatum* against bacterial strains- *E.coli* (A), *K. pneumonia* (B), *S. aureus* (C) and *B. subtilis* (D) as indicated. DMSO was used as negative control, whereas Ampicillin was used as positive control.

Antifungal activity was calculated in terms of zone of inhibition (mm) shown by different solvent extracts of leaves of *Z. armatum* against fungal strains- *C. albicans* (ATCC90028) (A), *C. albicans* (MTCC277) (B) and *S. cerevisiae* (H1086) (C) as indicated. DMSO was used

Table 3: Comparison of diameter of zone of inhibition shown by various solvent extracts of *Z. armatum* leaves from different geographical locations of Himachal Pradesh against fungal strains.

Samples	Zone of inhibition (mm)								
	***C. albicans* (ATCC90028)**			***C. albicans* (MTCC277)**			***S. cerevisiae* (H1086)**		
	C	**M**	**B**	**C**	**M**	**B**	**C**	**M**	**B**
HR	6.0±0.51	6.0±0.57	7.6±0.57	6.0±0.41	6.0±0.39	9.3±0.57	6.0±0.1	6.0±0.3	6.16±0.3
BR	6.25±0.49	6.5±0.5	8.3±1.15	6.1±0.16	6.1±0.28	9.6±0.57	6.25±0.49	7.33±0.57	6.0±0.57
MN	6.39±0.37	6.0±0.5	8.3±0.57	6.21±0.5	6.5±0.5	10.6±0.57	6.39±0.2	7.0±0.43	6.3±0.57
SN	6.52±0.57	6.0±0.5	7.5±0.5	6.16±0.16	6.39±0.57	10.6±1.15	6.52±0.57	6.07±0.38	7.0±0.39
SH	6.6±0.25	6.0±0.5	8.0±0.2	6.27±0.25	6.07±0.2	10.6±0.57	6.6±0.25	6.2±0.47	7.4±1.04
SR	6.3±0.15	7.0±0.57	7.6±0.57	6.33±0.24	6.67±0.57	11.3±1.15	6.3±0.15	6.4±0.57	7.6±1.15
Flu	14.47±0.29	13.66±0.52	13.3±0.57	17.66±0.57	13.33±0.57	13.5±0.83	14.66±0.5	12.87±0.63	13.65±0.5

'M' indicates methanolic extract; 'B' indicates n-butanol extract; and 'C' indicates chloroform extract; 'HR' indicates Hamirpur, 'BR' indicates Bilaspur, 'MN' indicates Mandi, 'SN' indicates Solan, 'SH' indicates Shimla and 'SR' indicates Sirmour district and Flu represents fluconazole. DMSO was used as negative control, whereas fluconazole was used as positive control.

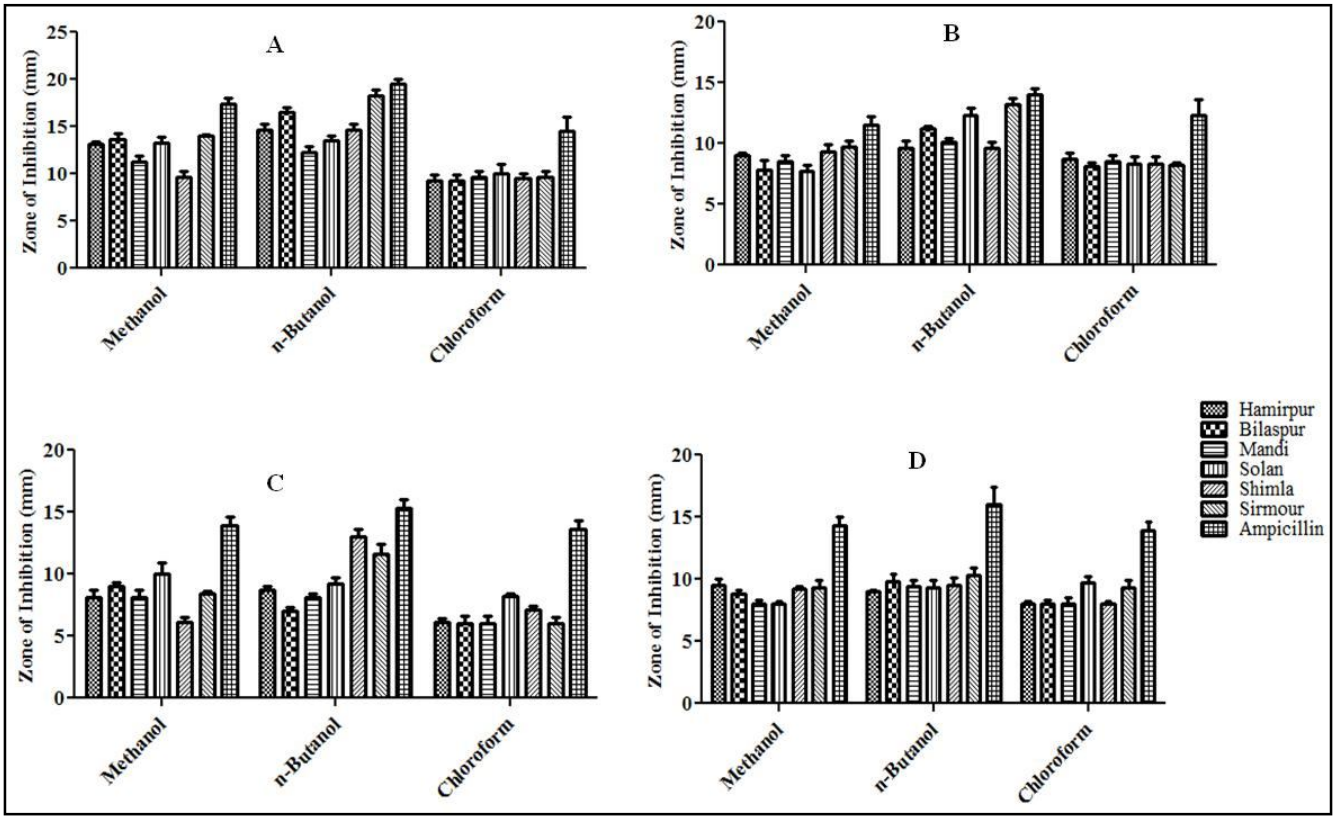

Fig. 2: Qualitative measure of antibacterial activity by agar well diffusion method.

Table 4: Comparison of MIC of various solvent extracts of *Z. armatum* leaves from different geographical locations against bacterial and fungal strains.

Samples	MIC (µg/ml) against bacterial strains												MIC (µg/ml) against fungal strains					
	B. subtilis			*S. aureus*			*E. coli*			*K. pneumoniae*			*C. albicans* (ATCC90028)			*C. albicans* (MTCC277)			*S. cerevisiae* (H1086)		
	C	M	B	C	M	B	C	M	B	C	M	B	C	M	B	C	M	B	C	M	B
HR	62.5	15.63	3.9	500	62.5	31.25	62.5	62.5	31.25	125	125	31.25	ND	ND	7.81	ND	ND	7.81	ND	ND	15.63
BR	7.81	7.81	3.9	31.25	15.63	15.63	15.63	7.81	7.81	62.5	31.25	15.63	ND	ND	7.81	ND	ND	3.9	ND	ND	7.81
MN	15.63	7.81	7.81	62.5	31.25	31.25	31.25	15.63	31.25	62.5	62.5	62.5	ND	ND	7.81	ND	ND	3.9	ND	ND	7.81
SN	15.63	7.81	7.81	62.5	31.25	15.63	31.25	31.25	15.63	125	250	62.5	ND	ND	7.81	ND	ND	3.9	ND	ND	7.81
SH	15.63	7.81	7.81	250	250	250	125	125	125	62.5	62.5	31.25	ND	ND	7.81	ND	ND	3.9	ND	ND	7.81
SR	15.63	15.63	3.9	62.5	15.63	31.25	31.25	15.63	15.63	125	250	62.5	ND	ND	7.81	ND	ND	7.81	ND	ND	7.81
K	62.5	62.5	62.5	250	250	250	7.81	7.81	7.81	15.63	15.63	15.63	ND	ND	ND	ND	ND	ND	ND	ND	ND
T	31.25	31.25	31.25	250	250	250	31.25	31.25	31.25	7.81	7.81	7.81	ND	ND	ND	ND	ND	ND	ND	ND	ND
FL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	15.63	15.63	15.63	62.5	62.5	62.5	31.25	31.25	31.25
AP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5

'M' indicates methanolic extract; 'B' indicates n-butanol extract; and 'C' indicates chloroform extract; 'HR' indicates Hamirpur, 'BR' indicates Bilaspur, 'MN' indicates Mandi, 'SN' indicates Solan, 'SH' indicates Shimla and 'SR' indicates Sirmour district. DMSO was used as negative control, whereas fluconazole was used as positive control. K indicates Kanamycin, T indicates Tetracycline, FL indicates fluconazole and AP indicates Ampicillin.

as negative control, whereas Fluconazole was used as positive control.

Furthermore, all the extracts of different solvents were tested for antimicrobial activity by broth dilution method. The results of broth dilution method were expressed as minimum inhibitory concentration (MIC) and shown in table 4. It was observed that MIC ranged from 0.39-250 µg/ml against bacterial strains (*B. subtilis, S. aureus, E. coli*, and *K. pneumoniae*) and 0.39-0.78 µg/ml against fungal strains [*C. albicans* (ATCC90028 and MTCC27) and *S. cerevisiae* (H1086)]. n-Butanol extract of leaves collected from district Hamirpur, Bilaspur and Sirmour showed lowest MIC (3.90 µg/ml) against *B. subtilis*, while n-butanol extract of Bilaspur showed lowest MIC (15.63 µg/ml) against *S. aureus.* In case of *E. coli and K. pneumoniae,* n-butanol extract of Bilaspur showed lowest MIC of 7.81 µg/ml and 15.63 µg/ml, respectively. MIC value (7.81 µg/ml) was found to be similar in n-butanol extract of all the samples against *C. albicans* (ATCC90028). n-Butanol extract of Bilaspur, Mandi, Solan and Shimla showed lowest MIC (3.90 µg/ml) against *C. albicans* (MTCC277). In case of *S. cerevisiae* (H1086), n-butanol extract of Bilaspur, Mandi, Solan, Shimla and Sirmour showed lowest MIC of 7.81µg/ml. Comparison of MIC of various solvent extracts from different geographical locations is listed in table 5.

Synergistic effect of different extracts of leaves of *Z. armatum* against fungal strains with amphoterecin B and fluconazole is shown in table 6. It was found that methanolic extract of Shimla and Sirmour showed best synergistic potential in combination with amphotericin B against *Candida albicans* (ATCC90028) (260 folds decrease in drug). Similarly, combination of methanolic extract of Mandi, and n-butanolic extract of Bilaspur and Solan with amphotericin B showed higher fold decrease in drug dose (260 folds) against *C. albicans* MTCC277. All the selected extracts of leaves of *Z. armatum* such except n-butanolic extract of Bilaspur showed equal folds (65 folds) decrease of drug (amphotericin B) against *S. cerevisiae* (H1086). Similarly, n-butanolic extract of *Z. armatum* leaves collected from Hamirpur showed higher fold decrease in fluconazole dose against *C. albicans* ATCC90028 (260 folds), *C. albicans* MTCC277 (130 folds) and *S. cerevisiae* H1086 (32 folds). FICI value was found to be less than 0.5 with all the combinations of *Z. armatum* leaf extracts and selected antifungal antibiotics, indicating synergistic nature of all selected samples.

The present study was first to report about synergistic potential exhibited by extracts of *Z. armatum* with antibacterial and antifungal antibiotics. The present study

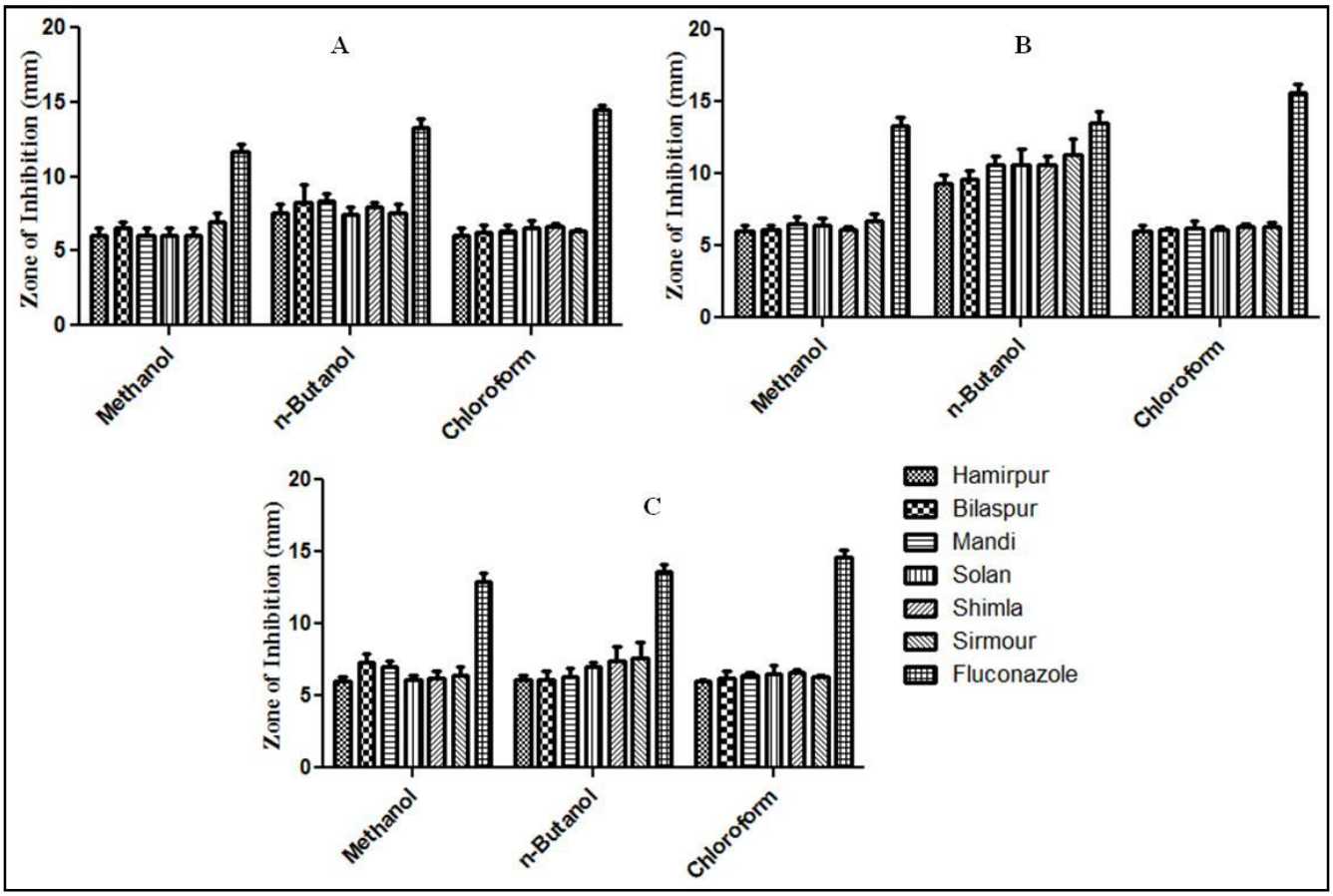

Fig. 3: Qualitative measure of antifungal activity by agar well diffusion method.

Table 5: Synergistic effects of different extracts of leaves of *Z. armatum* in combination with antibacterial antibiotic against bacterial strains.

Bacterial species	Fractional inhibitory concentration Index (FICI)											
	I+K	II+K	III+K	IV+K	V+K	VI+K	I+T	II+T	III+T	IV+T	V+T	VI+T
E. coli	0.25*8	0.25*8	0.25*8	0.25*8	0.25*8	0.25*8	0.125*16	0.125*16	0.25*8	0.046*64	0.5*8	0.03*8
S. aureus	0.06*32	0.06*32	0.12*16	0.17*64	0.12*16	0.003*128	0.125*16	0.09*16	0.125*16	0.007*260	0.12*128	0.06*515

I, III & IV represents n-butanol extract of Hamirpur, Bilaspur and Solan; II,V & VI represents methanolic extract of Mandi, Shimla and Sirmour respectively; K- kanamycin B, T- tetracycline and '*' sign represents the fold decrease in dose of antibiotic.

Table 6: Synergistic effects of different extracts of leaves of *Z. armatum* in combination with antifungal antibiotic against fungal strains.

Fungal species	FIC Index											
	I+A	II+A	III+A	IV+A	V+A	VI+A	I+F	II+F	III+F	IV+F	V+F	VI+F
C. albicans ATCC90028	0.015 *130	0.015 *130	0.015 *130	0.015 *130	0.008 *260	0.008 *260	0.007 *260	0.125 *16	0.125 *16	0.125 *8	0.125 *16	0.125 *16
C. albicans MTCC277	0.015 *130	0.007 *260	0.007 *260	0.007 *260	0.015 *130	0.015 *130	0.015 *130	0.125 *16	0.125 *16	0.125 *16	0.125 *16	0.06 *16
S. cerevisiae H1086	0.031 *65	0.046 *65	0.062 *32	0.031 *65	0.031 *65	0.031 *65	0.062 *32	0.125 *16	0.25 *8	0.25 *8	0.125 *16	0.25 *8

I, III & IV represents n-butanol extract of Hamirpur, Bilaspur and Solan; II,V & VI represents methanolic extract of Mandi, Shimla and Sirmour respectively; A- amphoterecin B, F- fluconazole and '*' sign represents the folds decrease in dose of antibiotic.

used the concept of antimicrobial combination therapy to assess and establish the combination potentials between antimicrobial drugs and plant extract against pathogenic bacteria as well as fungi. The possible mechanism by which combination works was that beta-lactam antibiotics are able to penetrate bacterial cell wall by the interaction

of quinolones with external membrane (Alviano *et al*., 2004). However, some bioactive compounds from plants act on the ribosomal structure and bactericidal enzymes resulted in the synergistic profile observed between inhibitors of cell wall, protein synthesis (tetracycline) and plant extracts. Studies on mechanism of azole drug's action showed that azoles inhibit fungal cytochrome P450 enzymes, which demethylate lanosterol to ergosterol, then block ergosterol formation and cause accumulation of toxic alpha-14-methyl-ester in fungal cells (Avijgan *et al*., 2014).

Conclusions

In the present study, different extracts of *Z. armatum* leaves collected from different regions of Himachal Pradesh were compared for their antimicrobial and synergistic potential. The n-n-butanolic extract was found to be the most effective as compared to methanolic and chloroform extracts. The present study also establishes synergistic potential of extracts of leaves of *Z. armatum* against bacterial and fungal pathogens. Based on antimicrobial data, n-butanol extract of leaves of *Z. armatum* could also be used against various bacterial and fungal infections. However, further investigation needs to be done on mechanism of action of antibacterial and antifungal drugs with phytocompounds of *Z. armatum*.

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