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ANTIMICROBIAL ACTIVITY OF HERBS AGAINST *YERSINIA ENTEROCOLITICA* AND MIXED MICROFLORA

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The present study aimed at developing herbal medicine against food borne pathogens, therefore the antimicrobial activity of four herbs viz. Arjuna (bark), Ashwagandha (roots), Puthkanda (leaves) and Shalampanja (roots) was checked. Aqueous, ethanolic and petroleum ether extracts of each herb were extracted and their antimicrobial activity against mixed microflora and against *Yersinia enterocolitica* was determined. Tetracycline and gentamicin were used as reference antibiotics. Arjuna extracts showed the highest antimicrobial potential against mixed population and *Yersinia enterocolitica* in comparison to Ashwagandha, Puthkanda and Shalampanja extracts. The antimicrobial activity of Arjuna aqueous extract was lower compared to gentamicin, but comparable to tetracycline. The minimum inhibitory concentration and minimum bactericidal concentration of aqueous extract of Arjuna showed the lowest values indicating that it is more effective in lower concentration of use. The antimicrobial activity of herbs showed the following trend Arjuna > Puthkanda > Shalampanja > Ashwagandha.

Keywords: herbs, antimicrobial activity, Minimum inhibitory concentration

Introduction

Medicinal Plants have antibacterial activity against Pathogens causing Urinary Tract Infections (Sharma *et al.*, 2009). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Cowan, 1999). These herbs and spices are common part of food for flavor, color, aroma, taste and also to enhance shelf life. Some of the common herbs *viz.* Arjuna, Ashwagandha, Puthkanda and Shalampanja were tested for antimicrobial activity. The herbs showing antimicrobial activity may be used for extending shelf life of food.

Herbal plants are a natural source of compounds that can be used against many diseases today. Phytochemicals are natural bioactive compounds found in plants.

These phytochemicals work with nutrients and fibers to form an integrated part of the defense system against various diseases and stress conditions (Pascaline *et al*., 2011). Extracts of plants contain a variety of bioactive compounds which may inhibit the growth of some pathogenic microorganisms (Zarringhalam *et al*., 2013).

Terminalia Arjuna (common name: Arjuna) is a tree and its bark and leaves are used for medicinal treatments in Ayurveda and is traditionally renowned as a cardiac stimulant. Arjuna bark exerted significant hypolipidaemic activity, produced inotropic and hypotensive effects and increased coronary artery flow to protect against heart diseases (Dwivedi, 2007). Antimicrobial efficacy of Arjuna bark extracts was demonstrated on clinical isolates of fifteen bacterial and six fungal species which were multi-drug resistant (Singh *et al*., 2008). Arjuna acetonic extract was able to cause death of *Staphylococcus aureus, however, suppressed the* growth *Escherchia coli*, *Acinetobacter sp*, *Proteus mirabilis*, and *Candida albicans* (Aneja *et al*., 2012).

Withania somnifera (commmonly known as Aswagandha) is one of the most valuable plants in the traditional Indian medicine. The ethnopharmacological properties of the plant include adaptogenic, anti-sedative and anti-convulsion activities, and the plant is used to treat various neurological disorders, geriatric debilities, arthritis, stress and behavior-related problems (Sangwan *et al*., 2004). Methanolic extracts of the whole *Withania somnifera* plant are effective against different pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans* and *Candida albicans*, they have also been found to have significant antibacterial properties (Jovanovic and Simic, 2000). *Withania somnifera* is traditionally used as a therapeutic agent for diarrhea, dyspepsia and gastrointestinal disorders (Acharyya *et al*., 2009)

Achyranthes aspera (commonly known as Puthkanda) is a perennial herb growing up to three meters and belongs to the family *Amaranthaceae*. Puthkanda is an important medicinal herb found throughout India. Almost all parts of the plant are used in traditional systems of medicines (Mukherjee, 2008). It is also useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal, infection, chronic malaria, impotence, fever, asthma, piles and snake bites. The alcoholic extracts have been reported to have wound healing and antioxidant activities. Methanol extracts showed complete inhibition on *Bacillus*, significant inhibition on *citrobacter*, moderate inhibition on *Escherichia coli.* Chloroform extract showed complete inhibition on *Bacillus*, moderate inhibition on *Citrobacter* and *Escherichia coli* (Tahiliani *et al*., 2000).

Dactylorhiza hatagirea (commonly known as Shalampanja) belongs to the family *Orchidaceae*. The plant is native and near endemic to Indian Himalayan region (Badola and Aitken, 2003). Its distribution extends to Pakistan, Afghanistan, Nepal, Tibet and Bhutan. In India, it is reported to be present in Jammu and Kashmir, Sikkim, Arunachal Pradesh, Uttarakhand and Himachal Pradesh (Samant and Joshi, 2005). The population of the species is small due to high anthropogenic pressures (Badola, 2002).

Rhizomatous part of Shalampanja showed antimicrobial potential against all Gram positive and Gram negative bacteria, but its aerial part showed limited resistance against bacteria. This plant can be a potential source for evolving newer antimicrobial compounds for treating dysentery caused by *E. coli* (Ranpal, 2009).

Therefore, the present study was carried out to determine the antimicrobial activity of herb extracts against the mixed population of microorganisms and *Yersinia enterocolitica* for encouragement and development of herbal medicines against food borne pathogens.

Materials and Methods

Materials

Collection of Herbs

Herbs *viz*. Arjuna (bark), Ashwagandha (roots), Puthkanda (leaves) and Shalampanja (roots) were collected from local market, Solan, Himachal Pradesh, India.

Bacterial culture

Pathogenic strain of *Yersinia enterocolitica* (MTCC No. 4858) was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. For mixed microflora, samples were collected from Sewage Water Treatment Plant, Shoolini University, Solan which was predominated by *E. coli*.

Chemicals and Solvents

Yersinia agar media, Yersinia broth, MRS agar, MRS broth, M17 agar and M17 broth were procured from HiMedia Chemicals Limited (Mumbai, India). Ethyl alcohol and petroleum ether were procured from Jiangsu Huaxi International (MO, USA) and Renkem (New Delhi, India), respectively. Dimethylsulphoxide (DMSO) was procured from Fisher Scientific (Mumbai, India). Barium chloride and sulphuric acid required to make McFarland solution was procured from HiMedia Chemicals Limited (Mumbai, India).

Processing of herbs and preparation of herb extracts

All four herbs were cleaned and washed properly with potable water, thoroughly washed with distilled water and kept for drying in sunlight for 2 days and kept in hot air oven at temperature of 45 °C for 6 h. The dried herbs were then powdered in a Falling Number Mill (Model 3100, Sweden) and packed in polyethylene bags and stored in airtight containers at refrigeration temperature (4-7 °C) till further analysis.

Aqueous extraction

The aqueous extracts of four herbs viz. Arjuna, Ashwagandha, Puthkanda and Shalampanja were prepared by dispersing 15 g of herb powders in 150 ml of distilled water in a 250 ml conical glass flask and boiled at 100 °C in water bath for 6 h and then filtered through Whatman no.1 filter paper. The filtrates obtained were then dried in Petri plates at 100 °C on water bath by evaporation and stored in air tight glass vials at refrigeration temperature (4°C) for further study (Sharma *et al*., 2016).

Ethanol and petroleum ether extraction

The ethanolic and petroleum ether extracts of herbs were prepared by dispersing 15 g of herb powders in 150 ml of ethanol and petroleum ether, respectively and kept at 27 °C for 2 days in a shaking water bath (Eppendorf India Limited, Chandigarh, India) and then filtered through Whatman no. 1 filter paper. The filtrates obtained were then allowed to evaporate in refrigerator at 4°C to get dried extract and stored in air tight glass vials at refrigeration temperature (4°C) for further study (Sharma *et al*., 2016).

Percent extract

The percent extract of herb was determined using the method of Sultana *et al.* (2009). The percentage of dried extract obtained after extraction and drying was calculated according to equation 1.

$$\text{Percent extract} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100 \quad (1)$$

Standardization of extracts concentrations used by antimicrobial test

During preliminary analysis, different concentration (5, 10, 15, 20 25 and 30 mg/ml) of dried extracts (aqueous, ethanol and petroleum ether) of herbs *viz.* Arjuna, Ashwagandha, Puthkanda and Shalampanja were dissolved in DMSO and their antimicrobial potential was determined using agar well diffusion assay and disc diffusion assay. Concentrations below 20 mg/ml showed a low zone of inhibition; increasing the concentration over 20 mg/ml resulted in rather similar zones of inhibition. Therefore, 20 mg/ml herb extract was considered for further antimicrobial tests (Sharma *et al*., 2016).

Activity index

The activity index was determined by measuring the inhibition zone of aqueous, ethanol, petroleum ether extract of herbs *viz* Arjuna, Ashwagandha, Puthkanda and Shalampanja and compared to the zone of inhibition of standard antibiotics references, namely tetracycline and gentamicin. The activity index was determined according to equation 2.

$$\text{Activity index} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}} \quad (2)$$

Antimicrobial activity

The effect of herb extracts on *Yersinia enterocolitica* was determined by agar well diffusion assay and disc diffusion assay as described by Kaushik *et al.* (2016).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of different herb extracts for antimicrobial potential was determined by method as described by Singariya *et al.* (2011). MIC values were determined by using various concentrations of the stock 15, 7.5, 3.75, 1.875, 0.938, 0.469, 0.234, 0.117, 0.059 and 0.029 mg/ml was assayed against test pathogens.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration of different herb extracts for antimicrobial potential was determined by method as described by Singariya *et al.* (2011). The

highest dilution that yielded no single bacterial colony was taken as the Minimum Bactericidal Concentration.

Statistical Analysis

The statistical analysis was carried out as per Kaushik *et al*. (2013). Data are presented as mean ± SEM (n=3), linear regression analysis and 95 % confidence intervals were calculated using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA). Data was subjected to a single way analysis of variance (ANOVA) to calculate the Critical Difference value.

Results and discussion

Percent extract

The percent extract obtained from 4 herbs *viz*. Arjuna, Shalampanja, Ashwagandha and Puthkanda in three different media *viz.* aqueous, ethanolic and petroleum ether was determined and values are presented in Table 1. It was observed that the ethanolic extract of Shalampunja showed the highest percentage of extract and the lowest in petroleum ether extract of Ashwagandha.

Table 1. Percentage extracts obtained from herbs

Herbs	Herb extract	Weight of dried plant material (g)	Weight of dried plant extract (g)	Percent extract (%)
Arjuna	Aqueous	15	2.50±0.23	16.67
	Ethanol	15	2.90±0.058	19.33
	Petroleum ether	15	1.70±0.058	11.33
Ashwagandha	Aqueous	15	0.82±0.050	5.47
	Ethanol	15	0.58±0.035	3.87
	Petroleum ether	15	0.33±0.011	2.20
Puthkanda	Aqueous	15	1.82±0.015	12.13
	Ethanol	15	2.26±0.020	15.06
	Petroleum ether	15	0.82±0.042	5.47
Shalampanja	Aqueous	15	3.05±0.060	20.33
	Ethanol	15	1.80±0.089	12.00
	Petroleum ether	15	1.14±0.086	7.60

Data are presented as mean ±SD (n=3)

Sultana *et al.* (2009) reported 34.5% extraction of Arjuna using ethanol which is significantly higher than our results. It may be due to the difference in cultivation location and other factors affecting plant growth. Dhanani *et al*. (2013) reported 9.02 and 9.5 % yield of extracts from Ashwagandha using ethanol and water, respectively.

The trend was the same but the values were higher in comparison to our values. However, no reports were found regarding the percentage yield of Puthkanda and Shalampanja. Hsu *et al*. (2006) reported that the differences in the extract yields from herbs might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants and their solubility in different solvents.

Antimicrobial activity of herb extracts on the mixed population of microorganisms

The antimicrobial activity of Arjuna, Ashwagandha, Puthkanda and Shalampanja extracts was determined by using disc and agar well diffusion assay against the mixed population of microorganisms and the results are presented in Table 2. Antibiotic gentamicin showed a significantly ($p<0.05$) higher zone of inhibition in comparison to tetracycline and aqueous, ethanol, petroleum ether extracts of all herb extracts, respectively. Aqueous extracts of Arjuna, Ashwagandha and Shalampanja showed a significantly higher ($p<0.05$) zone of inhibition than petroleum ether and ethanol extracts; however, there was no significant difference ($p>0.05$) between the petroleum ether and ethanol extracts, whereas petroleum ether extracts of Puthkanda showed the significantly higher zone of inhibition in comparison to aqueous and ethanol extracts. The activity index of gentamicin was lower in comparison to tetracycline in all four herb extracts, which indicate that gentamicin is superior to tetracycline in antimicrobial effects. It was observed that agar well diffusion assay showed a higher zone of inhibition in comparison to disc diffusion assay.

No report was found having antimicrobial activity against the mixed flora of microorganisms. However, there are several studies reported that present herbs as showing antimicrobial activity against coliform bacteria by Arjuna (Samy *et al*., 1998), Ashwagandha (Sundaram *et al*., 2011; Alam *et al*., 2012), Puthkanda (Dash *et al*., 2013; Beaulah *et al*., 2011) and Shatavari (Ranpal, 2009). It was found that the growth of both gram-positive and gram-negative food borne bacteria can be inhibited by the antimicrobial activity of some herbs (Nascimento *et al*., 2000). It was reported that the microbes collected from different geographic areas with different climatic conditions showed different antimicrobial activities (Ejechi and Akpomedaye, 2005). Herbs are commonly used as antimicrobial agents in foods. Some of the natural compounds found in herbs possess antimicrobial activities (Indu *et al*., 2006).

Antimicrobial activity of herb extracts against Yersinia enterocolitica

The antimicrobial activity of Arjuna, Ashwagandha, Puthkanda and Shalampanja extracts was determined by using the disc diffusion and agar well assay against *Yersinia enterocolitica* and the results are presented in Table 3 and Figure 1. Antibiotic gentamicin showed a significantly ($p<0.05$) higher zone of inhibition in comparison to tetracycline and aqueous, ethanol, petroleum ether extracts of all herbs, respectively. The tetracycline showed insignificant difference in the zone of inhibition ($p>0.05$) to aqueous extract of Arjuna, whereas aqueous and ethanol extract showed insignificant difference ($p> 0.05$) between each other; however, they both showed a significantly higher ($p<0.05$) zone of inhibition in comparison to petroleum ether extract. The antimicrobial activity of Ashwagandha was found to be

negative against *Yersinia enterocolitica.* All the extracts of Ashwagandha showed no antimicrobial activity against *Yersinia enterocolitica*. Only petroleum ether extract of Shalampanja showed antimicrobial activity whereas aqueous and ethanolic extract showed no antimicrobial activity against *Yersinia enterocolitica*. The zone of inhibition of petroleum ether extract of Shalampanja was significantly lower ($p<0.05$) than both gentamicin and tetracycline. Both gentamicin and tetracycline showed a significantly higher ($p<0.05$) zone of inhibition against *Yersinia enterocolitica* in comparison to all three extracts of Puthkanda. Both aqueous and ethanolic extracts showed no significant difference ($p>0.05$) in zone of inhibition against *Yersinia enterocolitica.* The petroleum ether extract of Puthkanda showed no zone of inhibition against *Yersinia enterocolitica.* The activity index of gentamicin was lower in comparison to tetracycline in all three herb extracts, which indicate that gentamicin is superior to tetracycline in antimicrobial effects. It was observed that disc diffusion and agar well assay showed the same trend, however agar well diffusion assay showed a higher zone of inhibition in comparison to disc diffusion assay.

The herbs used in the present study were not reported earlier with antimicrobial activity against *Yersinia enterocolictica.* However, they showed antimicrobial activity against other bacteria discussed in earlier sections.

Dwivedi *et al*. (2013) determined that the ethanolic extracts obtained from Arjuna bark inhibited the growth of microbes and zones of inhibition were visible. The total activity of the extracts against each pathogen was also evaluated. The activity of the leaf alkaloid was found to be the highest against *Enterobacter aerogens*. Debnath *et al*. (2013) studied that water and methanolic extracts of Arjuna bark produced a significant zone of inhibition against twenty-two test bacteria including eight uropathogens. Bhattacharyya and Jha (2011) studied the antagonistic activity of the crude extract against five clinically significant anti-dermatophytic fungi *i.e*., *Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans, Microsporum gypseum* and *Microsporum fulvum* using the agar cup diffusion method. The methanol extract was the most effective against *Trichophyton tonsurans* (zone of inhibition 25 mm) and the least effective against *Microsporum. gypseum* (zone of inhibition 3 mm). Ramya *et al.* (2008) determined the antimicrobial potential of aqueous extracts of bark/ stem, root, leaves and fruits of Arjuna against selected Gram positive and Gram negative bacterial species. Bark extracts limited the growth of both Gram-positive and Gram-negative bacteria. Aqueous extracts of leaves and fruits were active towards the Gram negative strains and less active towards the Gram positive stains.

Table 2. Antimicrobial activity of herbs extracts on mixed population of microorganisms using disc and agar well diffusion assay

S.No	Extracts	Disc diffusion Assay			Agar well diffusion Assay		
		Zone of inhibition (mm)	Activity Index (Tetracycline)	Activity Index (Gentamicin)	Zone of inhibition (mm)	Activity Index (Tetracycline)	Activity Index (Gentamicin)
Antibiotics	Tetracycline	21.00±2.00^{c}	-	-	21.60±3.00^{b}	-	-
	Gentamicin	24.00±1.100^{d}	-	-	27.00±1.10^{c}	-	-
Arjuna extracts	Aqueous	14.30±3.00^{b}	0.68	0.59	19.70±2.00^{b}	0.91	0.72
	Petroleum ether	11.30±1.00^{a}	0.54	0.47	15.00±2.00^{a}	0.69	0.55
	Ethanol	13.30±1.00^{ab}	0.63	0.55	15.00±3.00^{a}	0.69	0.55
Antibiotics	Tetracycline	21.00±2.00^{c}	-	-	21.60±3.00^{c}	-	-
	Gentamicin	24.00±1.100^{d}	-	-	27.00±1.10^{d}	-	-
Ashwagandha extracts	Aqueous	13.00±1.00^{b}	0.61	0.54	15.20±1.10^{a}	0.70	0.56
	Petroleum ether	11.00±2.00^{a}	0.52	0.45	14.00±1.10^{a}	0.64	0.52
	Ethanol	10 .00±3.00^{a}	0.47	0.41	16.40±2.20^{b}	0.76	0.61
Antibiotics	Tetracycline	21.00±2.0 0^{c}	-	-	21.60±3.00^{c}	-	-
	Gentamicin	24.00±1.10^{d}	-	-	27.00±1.10^{d}	-	-
Shalampanja extracts	Aqueous	11.30±2.00^{b}	0.53	0.47	16.60±3.20^{b}	0 .77	0.61
	Petroleum ether	8. 30±1.10^{a}	0.39	0.35	11.70±4.00^{a}	0 .54	0.43
	Ethanol	10.00±2.00^{a}	0.47	0.41	14.00±1.00^{a}	0 .65	0.51
Antibiotics	Tetracycline	21.00±2.00^{c}	-	-	21.00±2.00^{c}	-	-
	Gentamicin	24 .00±1.10^{d}	-	-	24 .00±1.10^{d}	-	-
Puthkanda extracts	Aqueous	11.10±1.00^{a}	0.52	0.46	13.00±4.30^{a}	0.60	0.48
	Petroleum ether	13.20±1.00^{b}	0.63	0.55	15.00±2.00^{a}	0.65	0.55
	Ethanol	11.00±3.00^{a}	0.52	0.46	14.90±5.10^{b}	0.83	0.66

Data presented in table is mean of three values ± SD

$^{a-b}$Values with same superscript in column showed non significant difference

Table 3. Antimicrobial activity of herbs extracts on *Yersinia enterocolitica* using disc and agar well diffusion assay

S.No	Extracts	Disc diffusion Assay			Agar well diffusion Assay		
		Zone of inhibition (mm)	Activity Index (Tetracycline)	Activity Index (Gentamicin)	Zone of inhibition (mm)	Activity Index (Tetracycline)	Activity Index (Gentamicin)
Antibiotics	Tetracycline	14.30±1.10c	-	-	15.00±0.90b	-	-
	Gentamicin	23.30±1.10d	-	-	25.00±0.90c	-	-
Arjuna extracts	Aqueous	12.50±3.30bc	0.88	0.54	16.30±1.50b	1.08	0.65
	Petroleum ether	6.90±0.90a	0.48	0.30	12.00±0.00a	0.80	0.48
	Ethanol	11.40±1.10b	0.79	0.49	13.90±1.30ab	0.92	0.56
Antibiotics	Tetracycline	14.30±0.11b	-	-	15.00±0.11b	-	-
	Gentamicin	23.30±0.11c	-	-	25.00±0.11c	-	-
Ashwagandha extracts	Aqueous	0.00±0.00a	0.00	0.00	0.00±0.00a	0.00	0.00
	Petroleum ether	0.00±0.00a	0.00	0.00	0.00±0.00a	0.00	0.00
	Ethanol	0.00±0.00a	0.00	0.00	0.00±0.00a	0.00	0.00
Antibiotics	Tetracycline	14.30±1.10c	-	-	15.00±0.90c	-	-
	Gentamicin	23.30±1.10d	-	-	25.00±0.90d	-	-
Shalampanja extracts	Aqueous	0.00±0.00a	0.00	0.00	0.00±0.00a	0.00	0.00
	Petroleum ether	14.30±1.10c	-	-	15.00±0.90b	-	-
	Ethanol	23.30±1.10d	-	-	25.00±0.90c	-	-
						12.50±3.30bc	
Antibiotics	Tetracycline	6.90±0.90a	0.48	0.30	12.00±0.00a	0.80	0.48
	Gentamicin	11.40±1.10b	0.79	0.49	13.90±1.30ab	0.92	0.56
Puthkanda extracts	Aqueous						
	Petroleum ether	14.30±0.11b	-	-	15.00±0.11b	-	-
	Ethanol	23.30±0.11c	-	-	25.00±0.11c	-	-

Data presented in table is mean of three values ± SD

[a-b]Values with same superscript in column showed unsignificant difference

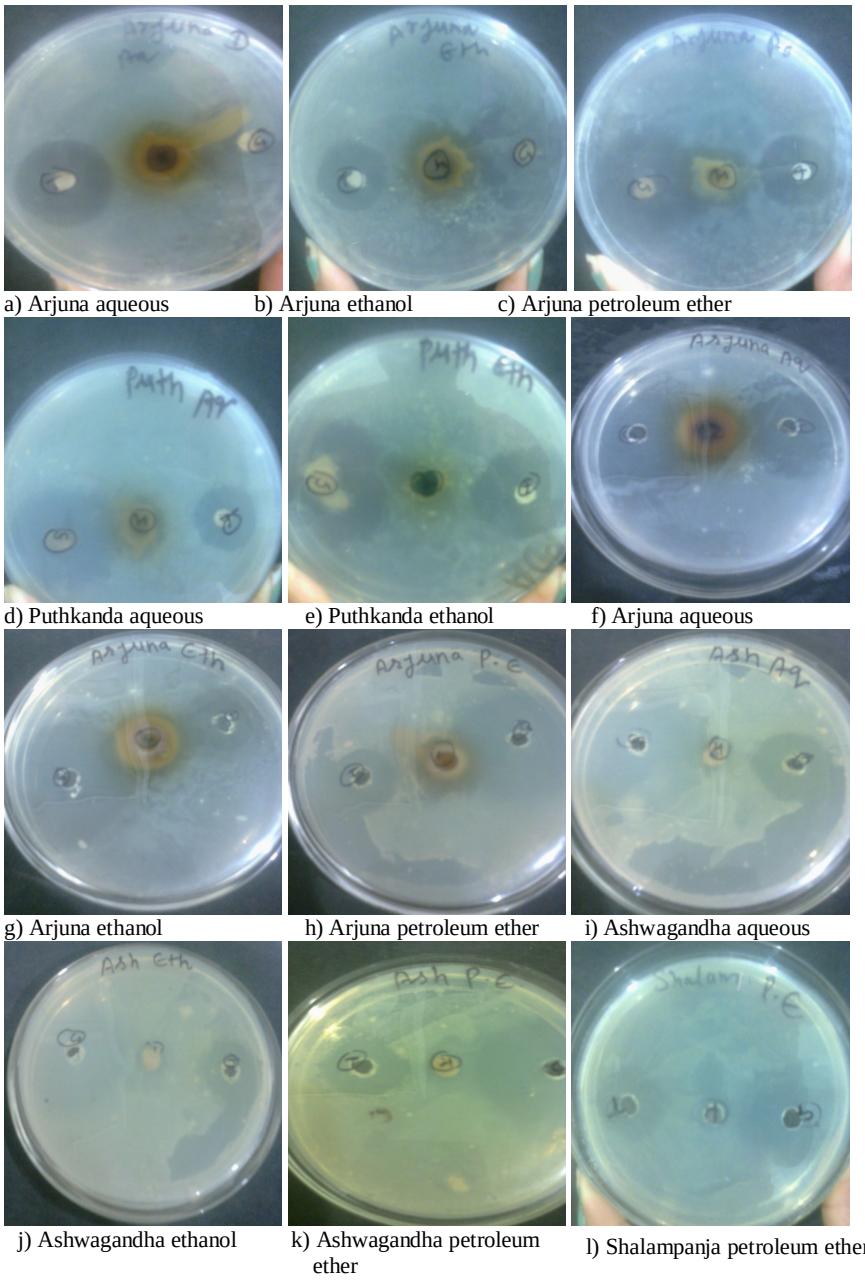

Figure 1. Photographic representation of disc diffusion assay: effect of Arjuna extracts (a, b, c) and Puthkanda extracts (d, e) against *Yersinia enterocolitica;* Agar well diffusion assay showing the effect of Arjuna extracts (f, g, h) Ashwagandha extracts(i, j, k) and Shalampanja extracts (l) against *Yersinia enterocolitica*

Alam *et al*. (2012) determined the antibacterial activity of Ashwagandha using the agar well diffusion method against five pathogenic Gram-negative bacteria *viz. Escherichia coli, Salmonella typhi, Citrobacter freundii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* The leaf extracts displayed the highest activity against *Salmonella typhi*, whereas the lowest activity was against *Klebsiella pneumoniae*. Singariya *et al*. (2011) reported that Chloroform extract of calyx of Ashwagandha showed the highest activity against *Bacillus subtilis.* Srinu *et al*. (2012) tested the methanolic and aqueous extracts of Ashwagandha for antimicrobial activity against *Pseudomonas aeroginosa, Bacillus cereus, Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Klebsiella pneumoneae.* Methonalic extracts of Ashwagandha showed good antibacterial activity when compared to aqueous extracts. Pujari and Gandhi (2012) studied the antibacterial activity of ethanolic, chloroform and aqueous extracts of Ashwagandha roots against three clinically isolated bacterial pathogens *viz. Staphylococcus aureus, Salmonella typhi* and *Shigella dysenteriae.* The crude ethanol root extract was more effective in inhibiting pathogens as compared to chloroform and aqueous root extracts. The results obtained in the study indicates that the ethanol extract of Ashwagandha roots might be exploited as natural drug for the treatment of several infectious diseases caused by these pathogens.

Sinha (2012) reported that methanolic extract better inhibited the growth of *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* than those of aqueous extract. Sundaram *et al*. (2011) reported that the alcoholic and ethyl acetate extracts of Ashwagandha showed antibacterial activity against clinically isolated bacterial pathogens *i.e. Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* causing ear infection in humans.

Ranpal (2009) determined the antibacterial activity of petrol ether, chloroform, methanol and aqueous extracts of Shalampanja‘s rhizome and aerial part against five bacteria.

Dash *et al*. (2013) reported that the methanol and ethyl acetate extracts of Puthkanda showed antimicrobial activity against the pathogenic *Bacillus subtilis, Escherichia coli* and *Klebsiella pneumoniae.* Beaulah *et al*. (2011) determined the antibacterial activity of different extracts of root, stem, and leaf of Puthkanda against *Staphylococcus aureus* a gram positive bacterium and *Escherichia coli* a gram negative bacterium. All of the extracts exhibited different antioxidant and antibacterial activities. The antioxidant and antibacterial activities were compared to the positive control ascorbic acid and gentamycin. Patil and Sharma (2013) reported that methanol extract showed the complete inhibition on Bacillus, negligible on *Escherichia coli* and no inhibition on *Citrobactor klebsiella* and Proteus organism. Methanol extract showed complete inhibition on Bacillus, significant inhibition on citrobactor, moderate inhibition on *Escherichia coli*, negligible inhibition on *Klebsiella* and no inhibition on *Proteus* spp. Chloroform extract showed complete inhibition on bacillus, moderate on citrobactor and *Escherichia coli*, negligible on *Klebsiella* and no on *Proteus* spp.

Geetha *et al.* (2010) reported the antibacterial activity of Puthkanda extract against five bacterial species *viz. Corynebacterium* sp., *Staphyloccocus aureus*, *Klebsiella* sp., *Vibrio* sp. and *Escherichia coli*. It was found that the antibacterial activity of the ethanol extract (75 µg/ml) was shown to be maximum against *Enterobacter aerogens* (19 mm) followed by *Staphyloccocus aureus* (14 mm), *Klebsiella* sp. (11 mm), *Corynebacterium* sp. (9 mm) and *Vibrio* sp. (7 mm). The other solvent (hexane and aqueous) extracts of *A. aspera* exhibited moderate antibacterial activity compared to ethanol extract. Srivastav *et al.* (2011) reported that ethanol and chloroform extracts of seeds of Puthkanda show mild to moderate antibiotic activity against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all the herb extracts (aqueous, ethanol and petroleum ether) of herbs *viz.* arujna, Ashwagandha, Puthkanda and Shalampanja were determined against *Yersinia enterocolitica.* MIC and MBC values were only recorded for those herb extracts which show activity in the disc diffusion assay. Results were shown in Table 4. MIC and MBC for aqueous and ethanol extracts of Arjuna were ranged between 1.875-3.75 mg/ml, whereas the values for petroleum ether extracts were between 3.75-15mg/ml. MIC and MBC values range for Puthkanda aqueous extracts between 1.875-7.5mg/ml and ethanol extracts between 3.75-7.5mg/ml whereas petroleum ether extracts of Puthkanda didn't show MIC and MBC values. MIC and MBC values for petroleum ether extract of Shalampanja ranged between 7.5-15mg/ml, whereas aqueous and ethanolic extract did not show any MIC and MBC. All extracts of Ashwagandha showed no MIC and MBC values. The lowest MIC and MBC values were recorded for aqueous Arjuna extracts.

Dey *et al*. (2010) reported that methanol extracts of Arjuna showed the lowest MIC values against all the tested microorganisms except for *Pseudomonas* as compared to ethanolic extract.

Kumar *et al*. (2014) reported that ethanol extracts of Arjuna showed the lowest MIC values against the selected microorganisms i.e. *Escherichia coli, Klebsiella pneumoniae*, *Enterobacter*, *Pseudomonas aeruginosa* and *Protues vulgaris* compared to aqueous extracts.

Singariya *et al*. (2011) reported excellent antibacterial activities of chloroform extracts of Ashwagandha due to its low MIC and MBC values. MBC values were found higher than the MIC values of the extracts against the tested microorganisms, indicating the bacteriostatic effects of the extracts.

Ranwan and Yadav (2012) reported that hexane extract of root, stem, leaves and seeds of Puthkanda showed the lowest MIC values against the selected strains *i.e*. *Bacillus cereus*, *Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli* compared to ethanol, methanol and aqueous extracts. Geetha *et al.* (2010) reported that minimum inhibitory concentration of Puthkanda against pathogens is lower in aqueous extracts compared to ethanol and hexane extracts.

Table 4. Minimum inhibitory concentration and minimum bactericidal concentration of herb extracts against *Yersinia enterocolitica*

Solvent	Herbs	*Yersinia enterocolitica*		
		MIC	Total activity	MBC
Aqueous	Arjuna	1.875	1.33	3.75
	Ashwagandha	-	-	-
	Shalampanja	-	-	-
	Puthkanda	1.87	0.97	7.5
Petroleum ether	Arjuna	3.75	0.19	15
	Ashwagandha	-	-	-
	Shalampanja	7.5	0.15	15
	Puthkanda	-	-	-
Ethanol	Arjuna	1.875	1.55	3.75
	Ashwagandha	-	-	-
	Shalampanja	-	-	-
	Puthkanda	3.75	0.60	7.5

Data are presented as mean (n=3)

Conclusions

The effect of herb extracts on the growth of mixed microbial population and *Yersinia enterocolitica* was determined by agar well and disc diffusion assays. Results showed that the Arjuna had the most potent inhibitory effect on the growth of mixed microbial population and *Yersinia enterocolitica.* It was observed that aqueous extracts of each herb showed higher antimicrobial potential in comparison with their ethanolic and petroleum ether extracts, respectively. The lowest values of the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration were recorded for Arjuna. The Ashwagandha did not show any MIC and MBC values. It can be concluded that, out of four investigated herbs, Arjuna has the highest antimicrobial potential against *Yersinia enterocolictica.* Therefore it can be recommended that Arjuna may be used for treatment against *Yersinia enterocolictica.*

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