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Antimicrobial activity of selected phytobiotics individually and in combination against gram positive and gram negative bacteria

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Abstract
Seven locally available phytobiotics viz., ginger rhizome powder, turmeric rhizome powder, tulsi leaves powder, coriander seeds powder, fenugreek seeds powder, curry leaves powder, moringa leaves powder and its selected combinations were evaluated for *in vitro* antimicrobial activity of aqueous and ethanol extracts against gram positive and gram negative bacterial strains isolated at Poultry Disease Diagnosis and Surveillance Laboratory, Namakkal. The phytobiotics collected from different parts of Tamil Nadu were cleaned, dried and ground to 1 mm size aseptically for obtaining aqueous and ethonalic extracts. The different levels of extracts prepared from herbs starting from 10 µl or 1.0 mg, 15 µl or 1.5 mg, 20 µl or 2.0 mg and 30 µl or 3.0 mg, were tested against *Staphylococcus aureus* and *Escherischia coli*. Antimicrobial activity of selected individual phytobiotics and phytobiotics mixtures were measured as zone of inhibition in mm. Among the phytobiotic extracts, aqueous extracts of the individual herbs did not evince any zone of inhibition at all levels of extracts except at 20 µl or 2.0 mg level. The antimicrobial activity of ethanol extracts of herbs and phytobiotic mixtures gradually increased from lower 10 µl to 20 µl but the activity decreased at 30 µl level. Twenty micro liters of ethanol extracts of ginger rhizome powder, curry leaves powder, turmeric rhizome powder and coriander seeds powder revealed significantly highest antimicrobial activity (P<0.05) against gram positive bacteria over gram negative bacteria. The study also revealed that the aqueous extracts of selected phytobiotic mixtures did not reveal any zone of inhibition. All herbs and herbal mixtures of ethanol extracts possessed antibacterial activity against both gram positive and gram negative bacteria. Among the ethanol extracts of individual herbs, curry leaves powder followed by ginger rhizome powder exerted significantly (P<0.05) highest zone of inhibition at 20 µl level. It could be concluded that ethanol extracts revealed antimicrobial potential in herbs and phytobiotic mixtures compared to aqueous extracts.

Keywords: *In vitro,* herbs, phytobiotics, antimicrobial activity, ethanol extract

Introduction
To improve the growth and productivity of the livestock and poultry, antibiotic growth promoters (AGP) have been widely used for many years as non nutrient feed additives. However the use of AGP in livestock and poultry has increased the cost of production and development of antibiotic resistant microbes in the gut micro flora resulting in residues in milk, meat and eggs (Hosseinzadeh *et al*., 2014) [5]. In order to reduce the residues in animal products without affecting the health status of the consumer, researchers tried using plant extracts and phytochemicals as an alternative to antibiotic growth promoters (Joseph *et al*., 2015) [6]. Some plant extracts influence digestion and secretion of digestive enzymes and besides, also exhibit antibacterial, antiviral and antioxidant (Cross *et al*. 2007) [2] properties. Antimicrobial activity and immune enhancement probably are the two major mechanisms by which phytobiotics exert positive effects on the growth performance and health of animals. Phytogenics are a heterogeneous group of feed additives originating from plants and consist of herbs, spices, fruit and other plant parts with wide range of antimicrobial, anthelmintic, antioxidant, growth enhancer and immune modulating properties. The antimicrobial properties of the herbs and plant extracts are due to the secondary metabolites or active compounds that are synthesized in the secondary metabolism of the plants. Anesini and Perez (1993) [1] from Argentina screened around 122 known plants for their antimicrobial properties and many of the plant extracts used in their study exerted antimicrobial properties against the growth of *Staphylococcous aureus*, *Escherischia coli* etc. and they have been efficiently used for treating

bacterial infections. Keeping this in view, the current study was designed and conducted to screen the antimicrobial properties of some of the selected herbs and herbal mixtures commonly used for treating livestock and poultry as part of Indian traditional folk medicines.

Materials and Methods

This study was conducted at Poultry Disease Diagnosis and Surveillance, Laboratory, Namakkal, Tamil Nadu, India during May, 2019.

Sample collection and preparation of herbal extracts

Seven traditional medicinal herbs *viz*., Ginger rhizome (*Zingiber officinale*), Turmeric rhizome (*Curcuma longa*), Tulsi leaves (*Ocimum sanctum*), Coriander seeds (*Coriandrum sativum*), Fenugreek seeds (*Trigonella foenum*), Moringa leaves (*Moringa oleifera*) and Curry leaves (*Murraya koeningii*) were procured from different parts of Tamil Nadu. The active principle present in each herb varies in different parts of the plants. The selected list of herbs is presented in Table 1. The collected herbs were cleaned from extraneous matter, if any, and were shade dried for 72 hours and ground in a Wiley mill to pass through 1mm sieve to get uniform size and stored in labeled air tight containers for further analysis.

Aqueous and ethanol extracts (10%) of the herbal samples were prepared to determine the *in vitro* antimicrobial effect of the samples. To prepare 10% aqueous extract, the ground samples, 100 mg each, were taken in a sterile eppendorf tube to which 1 ml of sterile water was added, then vortexed and kept in refrigerator overnight. Similarly for 10% ethanol extract, 1ml of 95% ethanol was added to 100 mg ground samples in a sterile eppendorf tube, vortexed and kept in refrigerator overnight.

The selected herbs were subjected to *in vitro* antimicrobial sensitivity studies individually and assessed their potential as phytobiotic. According to their phytobiotic activity, different combinations (Table 2) were prepared and subjected to antimicrobial study in aqueous and ethanol extracts (10%).

Preparation of Microorganism

Staphylococcus aureus and *Escherischia coli* were chosen respectively as gram +ve and gram -ve bacteria for the antibacterial study. Clinical samples obtained from Poultry Disease Diagnosis and Surveillance, Laboratory, Veterinary College and Research Institute, Namakkal, were utilized for isolating the *Staphylococcus aureus* and *Escherischia coli* in nutrient agar medium and selectively cultured at 37^0C for 24 hours. As per the directions from Bergy's manual for determinative bacteriology, the bacterial strains were identified by gram staining and confirmed by biochemical test.

In vitro antimicrobial assay of phytobiotic and their combinations

Antibacterial activity of the aqueous and ethanol extracts of herbal samples and their combinations were carried out by the disc diffusion method (Gulluce *et al*. 2007) [4]. Circular disc of 6 mm diameter were made from the Whatman no 1 filter paper and the discs were impregnated with equal volume (10 µl or 1.0 mg, 15 µl or 1.5 mg, 20 µl or 2.0 mg and 30 µl or 3.0 mg) of the respective plant extracts (aqueous and ethanol, individually). The discs were aseptically placed over plates of Mueller Hinton Agar for *Staphylococcus aureus* and Mac Conkey agar for *Escherischia coli*, and then streaked aseptically with the respective gram +ve and gram –ve bacteria. The plates were then incubated at 37^0C for 24 hours. Sterile water and 95% ethanol were used as control respectively for aqueous and ethanol extracts of samples. Using zone of inhibition scale, the size of the inhibitory zones including the diameter of the disc on the agar surface around the discs were measured, to determine the phytobiotic activity of extracts (aqueous/ethanol) of the herbs and different combinations against the microorganism species studied. The data on antibacterial activity of aqueous and ethanol extracts of selected herbs/phytobiotic mixture were grouped and subjected for statistical analysis of variance (ANOVA) as per the procedure of statistical analysis system (SPSS, version 20.0 for windows) [11].

Results and Discussion

The antibacterial activity of the aqueous extracts of selected herbal and phytobiotic mixtures at different levels (10 µl or 1.0 mg, 15 µl or 1.5 mg, 20 µl or 2.0 mg and 30 µl or 3.0 mg) were assessed and presented in Tables 3 and 4. The results indicated that at 10 µl level of aqueous extracts of herbs, no zone of inhibition (in mm) was observed against both gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Escherischia coli*). However ethanol extracts of ginger rhizome powder, coriander seed powder, turmeric rhizome powder and curry leaves powder revealed significantly (P<0.05) highest antimicrobial activity against *Staphylococcus aureus* compared with control for individual herbs. Among the herbs, 10 µl of ethanol extracts of ginger rhizome powder, turmeric rhizome powder, curry leaves powder and moringa leaves powder revealed (P<0.05) significantly highest antibacterial activity against *Escherischia coli* organism compared to control. Ginger rhizome powder also exerted significantly (p<0.05) highest 17.83±0.17 antibacterial activity against gram negative bacteria. The results of the antibacterial activity of aqueous extracts of selected herbs were comparable with Maher Obeidat *et al.* (2012) [8] and they observed that aqueous extract of medicinal plant A.discoridis revealed highest antimicrobial activity with inhibition zone more than 32 mm.

Aqueous extracts of herbs at 15 µl level did not reveal any zone of inhibition against both gram positive and gram negative bacteria and these results were in accordance with Kavitha *et al.* (2020) [7]. Ethanol extract of herbs at 15 µl level showed antimicrobial activity against gram positive bacteria but the results were not statistically significant (P>0.05) among herbs and control. However among the ethanol extracts of herbs tested, at 15 µl level only turmeric rhizome powder showed significantly (P<0.05) highest antibacterial activity 19.00±0.26 against *Escherischia coli* over other herbs and control. Among the seven herbs selected, at 20 µl level of aqueous extracts only tulsi leaves powder, turmeric rhizome powder, coriander seed powder and Ginger rhizome powder revealed significantly highest (P<0.05) antimicrobial activity against gram positive bacteria over control and other selected herbs. However, aqueous extracts of tulsi leaves powder, coriander seed powder and Ginger rhizome powder revealed antibacterial activity against *Escheischia coli*. The results suggest that aqueous extracts of all the selected herbs have not exhibited any antibacterial activity and the current study indicated that the antibacterial activity vary with the species of the plants, type of extracts used and bacterial species against which it was tested. The result is in accordance with

the findings of Maher Obeidat *et al.* (2012) [8]. The aqueous and ethanol extracts of herbs have shown higher sensitivity against gram positive bacteria, however the gram negative bacteria is much resistant to antibacterial activity of aqueous and ethanol extracts of herbs and herbal mixture which is attributed to presence of resistant antibacterial substances lipopolysaccharides in outer membrane of gram negative bacteria (Gao *et al.*, 1999) [3] and morphological differences and differences in the permeability of the cell wall. Mohamed Senouci Bereksi *et al.* (2018) [9] reported that the *Staphylococcus aureus* was the most sensitive organism compared to the other strains of bacteria to exert antimicrobial activity in herbal extracts.

Among the ethanol extracts of herbs at 20 µl level, ginger rhizome powder (20.17±0.40) and curry leaves powder (20.33±0.33) revealed significantly (P<0.05) highest antibacterial activity against gram positive bacteria. Similarly other herbs like coriander seed powder, turmeric rhizome powder and fenugreek seed powder also revealed significantly (P<0.05) higher antibacterial activity. Ethanol extracts of 20 µl level turmeric rhizome powder, ginger rhizome powder, tulsi leaves powder and coriander seeds revealed significantly highest antibacterial activity 20.33±0.33, 20.00±0.37, 19.50±0.22 and 19.50±0.22, respectively, against gram negative bacteria *E.coli*.

The study also revealed that aqueous extracts of herbs at 30 µl level did not show any zone of inhibition against gram positive bacteria *Staphylococcus aureus*. However, at the same level the aqueous extracts of ginger rhizome powder revealed moderate antimicrobial activity against gram negative bacteria with no significant effect. Similarly, 30 µl level of ethanol extracts did not evince any significant antimicrobial activity against *Escherischia coli*. However among the ethanol extracts of herbs, curry leaves powder revealed significantly (p<0.05) highest antimicrobial activity against *Staphylococcus aureus*.

The antibacterial activity of the ethanol extracts of selected herbs and phytobiotic mixtures at different levels (10 µl or 1.0 mg, 15 µl or 1.5 mg, 20 µl or 2.0 mg and 30 µl or 3.0 mg) were assessed and presented in Tables 5 and 6. Among the phytobiotic mixtures studied, at 10 µl level of aqueous extracts, only two combinations namely turmeric rhizome powder + tulsi leaves powder + ginger rhizome powder + fenugreek seeds powder and the other combination turmeric rhizome powder + ginger rhizome powder + fenugreek seed powder + coriander seed powder exerted antibacterial activity against *Staphylococcus aureus* compared to control and other combinations. However the aqueous extracts of phytobiotic mixtures also did not reveal any zone of inhibition against gram negative bacteria. The results in this study are comparable with the findings of Zaika (1988) [12] and Mohamed Sham Shihabudeen and Hansi Priscilla (2010) [10]. Highest sensitivity of *Staphylococcus aureus* might be due to its cell wall structure and outer membranes compared with gram negative bacteria. The ethanol extracts of phytobiotic mixtures also revealed antibacterial activity against both gram positive and negative bacteria but was not statistically significant (P>0.05).

Among different levels i.e, at 15 µl, 20 µl and 30 µl of aqueous extracts of phytobiotic mixtures, only three combinations viz., turmeric rhizome powder + tulsi leaves powder + ginger rhizome powder + fenugreek seeds powder, turmeric rhizome powder + curry leaves powder + ginger rhizome powder + fenugreek seed powder and the combination turmeric rhizome powder + ginger rhizome powder + fenugreek seeds powder + coriander seed powder revealed antimicrobial activity against gram positive bacteria. However, antimicrobial activity of ethanol extracts of phytobiotic mixtures at 15 µl and 20 µl was not statistically significant (P>0.05) compared with control against both gram positive and negative bacteria.

The study also revealed that among the phytobiotic mixtures at 30 µl of ethanol extracts against gram positive bacteria, only the combination of turmeric rhizome powder + tulsi leaves powder + ginger rhizome powder + fenugreek seeds powder exhibited significantly (P<0.05) highest 19.33±0.62 antimicrobial activity. However no significant differences were observed between phytobiotic mixtures and control in terms of antimicrobial activity against gram negative bacteria. In general, the antibacterial activity of the ethanol extracts of herbs and phytobiotic mixtures against gram positive bacteria was gradually increasing from lower concentration (10 µl) to higher concentration (30 µl) compared to the same concentration of ethanol extracts of herbs and phytobiotic mixtures tested against gram negative bacteria. The result was in accordance with observation of Mohamed Sham Shihabudeen and Hansi Priscilla (2010) [10] that 1mg, 2mg and 4mg levels of methanol extracts of *Eugenia jambolana* and *Cassia auriculata* revealed highest antimicrobial activity against both gram positive and gram negative bacteria and the study also revealed that gram positive bacteria was much sensitive to ethanol extracts compared with gram negative bacteria.

Table 1: List of herbs selected for preparing herbal extracts

S. No.	Name of the herbs	Botanical name	Parts used
1	Tulsi	*Ocimum sanctum*	Leaves
2	Moringa	*Moringa oleifera*	Leaves
3	Curry leaves	*Murraya koenigii*	Leaves
4	Fenugreek seeds	*Trigonella foenum-graceum*	Seeds
5	Turmeric powder	*Curcuma longa*	Rhizome
6	Coriander seeds	*Coriandrum sativum*	Seeds
7	Ginger	*Zingiber officinale*	Rhizome

Table 2: Potential combinations of phytobiotic mixtures

S. No.	Name of the Phytobiotic combinations
1	Turmeric rhizome powder + Tulsi leaves powder + Ginger rhizome powder + Fenugreek seeds powder
2	Turmeric rhizome powder + Moringa leaves powder + Fenugreek seed powder + Coriander seeds powder
3	Turmeric rhizome powder + Curry leaves powder + Ginger rhizome powder + Fenugreek seed powder
4	Turmeric rhizome powder + Tulsi leaves powder + Moringa leaves powder + Curry leaves powder
5	Turmeric rhizome powder + Ginger Rhizome powder + Fenugreek seed powder + Coriander seed powder

Table 3: *In vitro* Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extracts of herbs against *Staphylococcus aureus* and *Escherischia coli*

S. No	Name of the herbs and Herbal mixture	Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extract against *Staphylococcus aureus*				Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extract against *Escherischia coli*			
		10 µl	15 µl	20 µl	30 µl	10 µl	15 µl	20 µl	30 µl
1	Tulsi leaves powder *Ocimum sanctum*)	NZI	NZI	10.00^{b}±0.00	NZI	NZI	NZI	7.00^{b} ±0.00	NZI
2	Moringa leaves powder (*Moringa oleifera*	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI
3	Curry leaves powder (*Murraya koenigii*)	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI
4	Fenugreek seeds powder (*Trigonella foenum-graceum)*	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI
5	Turmeric rhizome powder (*Curcuma longa*)	NZI	NZI	11.00^{b} ±0.00	NZI	NZI	NZI	NZI	NZI
6	Coriander seeds powder (*Coriandrum sativum*)	NZI	NZI	6.00^{b} ±0.00	NZI	NZI	NZI	8.00^{b} ±0.00	NZI
7	Ginger rhizome powder (*Zingiber officinale*)	NZI	NZI	10.00^{b} ±0.00	NZI	NZI	NZI	7.00^{b} ±0.00	1.17±1.17
8	Control for Individual herbs	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI

Means of six observations,
Values bearing different superscripts within a column differ significantly (P<0.05).
NZI- No Zone of Inhibition

Table 4: *In vitro* Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extracts of herbal mixture against *Staphylococcus aureus* and *Escherischia coli*

S. No	Name of the herbs and Herbal mixture	Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extract against *Staphylococcus aureus*				Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extract against *Escherischia coli*			
		10 µl	15 µl	20 µl	30 µl	10 µl	15 µl	20 µl	30 µl
1	Turmeric rhizome powder + Tulsi leaves powder + Ginger rhizome powder + Fenugreek seeds powder	1.33^{a} ±1.33	1.33^{a} ±1.33	1.67^{a} ±1.67	1.67^{a} ±1.67	NZI	NZI	NZI	NZI
2	Turmeric rhizome powder + Moringa leaves powder + Fenugreek seed powder + Coriander seeds powder	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI
3	Turmeric rhizome powder + Curry leaves powder + Ginger rhizome powder + Fenugreek seed powder	NZI	1.17^{a}±1.17	1.50^{a} ±1.50	1.50^{a} ±1.50	NZI	NZI	NZI	NZI
4	Turmeric rhizome powder + Tulsi leaves powder + Moringa leaves powder + Curry leaves powder	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI
5	Turmeric rhizome powder + Ginger Rhizome powder + Fenugreek seed powder + Coriander seed powder	1.00^{a} ±1.00	1.00^{a} ±1.00	1.33^{a} ±1.33	1.33^{a} ±1.33	NZI	NZI	NZI	NZI
6	Control for herbal mixture	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI

Means of six observations,
Values bearing different superscripts within a column differ significantly (P<0.05).
NZI- No Zone of Inhibition

Table 5: *In vitro* Antimicrobial activity (Zone of inhibition in mm) of different levels of ethanol extracts of herbs against *Staphylococcus aureus* and *Escherischia coli*

S. No	Name of the herbs and Herbal mixture	Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extract against *Staphylococcus aureus*				Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extract against *Escherischia coli*			
		10 µl	15 µl	20 µl	30 µl	10 µl	15 µl	20 µl	30 µl
1	Tulsi leaves powder (*Ocimum sanctum*)	14.67^{ab}±0.21	15.33^{b}±0.42	18.67^{de}±0.21	20.33^{bcd}±0.33	15.33^{bc} ±0.21	16.17^{d}±0.79	19.50^{bc}±0.22	18.33^{a}±0.88
2	Moringa leaves powder (*Moringa oleifera*	14.83^{ab} ±0.31	15.17^{b} ±1.47	18.67^{de}±0.21	19.33^{de}±0.33	15.50^{b} ±0.22	18.33^{cd}±0.49	18.50^{cd}±0.22	18.17^{a}±0.87
3	Curry leaves powder (*Murraya koenigii*)	15.50^{a}±0.22	16.50^{b} ±0.67	20.33^{c}±0.33	21.50^{b}±0.34	15.50^{b}±0.22	18.00^{cd}±0.26	18.33^{d}±0.21	18.00^{a}±0.63
4	Fenugreek seeds powder (*Trigonella foenum-graceum)*	14.67^{ab}±0.21	14.33^{b} ±1.02	19.17^{cd}±0.31	18.50^{e}±0.43	13.83^{d}±0.40	16.67^{cd}±0.96	17.83^{d} ±0.17	18.50^{a}±1.31
5	Turmeric rhizome powder (*Curcuma longa*)	15.50^{a}±0.34	15.33^{b} ±0.59	19.67^{cd}±0.21	21.17^{bc}±0.31	16.33^{b}±0.21	19.00^{c}±0.26	20.33^{b}±0.33	20.17^{a}±0.30
6	Coriander seeds powder	15.33^{a}±0.21	15.00^{b} ±0.37	19.33^{cd}±0.33	19.33^{de}±0.33	15.83^{b}±0.31	17.33^{cd}±0.67	19.50^{bc}±0.22	18.33^{a}±1.09

	(*Coriandrum sativum*)								
7	Ginger rhizome powder (*Zingiber officinale*)	15.00^{a}±0.37	15.17^{b} ±0.40	20.17^{c}±0.40	20.83^{bc}±1.68	17.83^{a}±0.17	18.17^{cd}±0.31	20.00^{b}±0.37	19.83^{a}±0.98
8	Control for Individual herbs	13.67^{b} ±0.21	14.67^{b} ±0.59	17.67^{e}±0.21	19.83^{cde}±0.68	14.33^{cd}±0.21	16.33^{d}±0.21	17.50^{d}±0.22	17.33^{a}±0.21

Means of six observations,
Values bearing different superscripts within a column differ significantly (P<0.05).
NZI- No Zone of Inhibition.

Table 6: *In vitro* Antimicrobial activity (Zone of inhibition in mm) of different levels of ethanol extracts of herbal mixture against *Staphylococcus aureus* and *Escherischia coli*

S. No	Name of the herbs and Herbal mixture	Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extract against Staphylococcus aureus				Antimicrobial activity (Zone of inhibition in mm) of different levels of aqueous extract against Escherischia coli			
		10 µl	15 µl	20 µl	30 µl	10 µl	15 µl	20 µl	30 µl
1	Turmeric rhizome powder + Tulsi leaves powder + Ginger rhizome powder + Fenugreek seeds powder	13.17^{b}±0.48	15.00^{b}±0.37	17.33^{b}±0.72	19.33^{a}±0.62	12.50^{a}±0.62	16.67^{b}±0.62	16.33^{a}±0.62	19.33^{b}±1.17
2	Turmeric rhizome powder + Moringa leaves powder + Fenugreek seed powder + Coriander seeds powder	12.17^{b} ±0.60	14.67^{b} ±1.02	17.17^{b} ±0.79	19.00^{ab} ±0.82	12.50^{a} ±0.85	15.50^{b} ±0.56	18.17^{a} ±0.75	18.50^{b} ±0.56
3	Turmeric rhizome powder + Curry leaves powder + Ginger rhizome powder + Fenugreek seed powder	12.50^{b} ±0.62	16.17^{b} ±0.65	18.33^{b} ±0.59	18.00^{ab} ±0.26	12.17^{a} ±0.91	15.17^{b} ±0.48	17.83^{a} ±0.95	19.00^{b} ±0.37
4	Turmeric rhizome powder + Tulsi leaves powder + Moringa leaves powder + Curry leaves powder	12.50^{b} ±0.85	15.50^{b} ±0.85	16.83^{b} ±0.60	18.00^{ab} ±0.52	13.50^{a} ±0.56	15.17^{b} ±1.01	17.17^{a} ±0.95	17.83^{b} ±0.31
5	Turmeric rhizome powder + Ginger Rhizome powder + Fenugreek seed powder + Coriander seed powder	13.00^{b} ±0.86	15.33^{b} ±0.76	17.83^{b} ±0.60	19.17^{ab} ±0.31	13.00^{a} ±0.73	15.00^{b} ±0.48	18.50^{a} ±0.76	17.67^{b} ±0.42
6	Control for herbal mixture	10.17^{b} ±0.75	13.33^{b} ±0.62	15.67^{b} ±0.72	17.00^{b} ±0.26	11.17^{a} ±0.60	14.33^{b} ±0.42	16.50^{a} ±1.06	16.83^{b} ±0.31

Means of six observations,
Values bearing different superscripts within a column differ significantly (P<0.05).
NZI- No Zone of Inhibition.

Conclusion

It could be concluded that that the aqueous and ethanol extracts of herbs and herbal mixtures though exhibited varying degrees of antibacterial activity on the gram positive and gram negative bacteria tested, it was not significant. However, among the ethanol extracts of individual herbs, curry leaves powder followed by ginger rhizome powder exhibited highest zone of inhibition at 20 µl level against *Staphylococcus aureus*.

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