

Original Research Article

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Evaluation of Antibacterial Activity of some Indian Herbal Extracts

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ABSTRACT

Sixteen traditional medicinal herbs that are used in Indian traditional medicine and which are commonly available in Tamil Nadu were collected for the study. Different medicinal parts of the herbs were selected and dried and grounded under aseptic condition. To determine the *in vitro* antimicrobial effect, aqueous and ethanol extracts were prepared and tested against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella entitidis* and *Clostridium perfringens*. *Allium sativum* bulbs (Garlic) showed significantly higher zone of inhibition against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella entitidis* when compared with *Syzygium aromaticum* bud (Clove). Other extracts evinced no zone of inhibition. In the case of *Clostridium perfringens*, *Syzygium aromaticum* bud (clove) was the only herb showed zone of inhibition. Among the ethanol extracts tested *Syzygium aromaticum* bud (Clove) showed significantly ($p < 0.05$) higher zone of inhibition against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella entitidis*. *Murraya koenigii* leaves (Curry leaves) was found to have highest zone of inhibition against *Clostridium Perfringens*.

Keywords

Antibacterial activity, Indian herbs, Herbal extracts

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Introduction

The past three decades has witnessed the production of various new antibiotics by pharmacological industries for treatment of bacterial diseases. The use of antibiotics at sub therapeutic dose as growth promoters, also was on the increase. The overuse and misuse of antibiotics has led to genetic mutation in bacteria leading to the

development of bacterial resistance to antibiotics. A surge in the development and spread of antibiotic resistance has become a major cause for concern (Aarestrup *et al.*, 2008). The quest for alternative products has clearly intensified in the recent years with the increase in regulations regarding the use of antibiotic growth promoters and the rise in consumer demand for poultry products from “Raised Without Antibiotics” or “No

Antibiotics Ever” flocks (Gadde *et al.*, 2017). Currently there has been an increased awareness on usage of traditional herbal medicine as alternative to antibiotics. These herbal preparations of plant origin are easily available, inexpensive, safe, efficient, and rarely cause any side effects. Plants have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins (Tiwari *et al.*, 2010). These secondary plant metabolites are a source of plant derived antimicrobial substances (Srivastava *et al.*, 2013). Some of these natural plant products are highly efficient in the treatment of bacterial infections (Fernebro *et al.*, 2011). It is with this background the evaluation of antibacterial activity of extracts of some traditional herbs commonly available in Tamil Nadu was undertaken.

Materials and Methods

Collection of herbal samples

Sixteen traditional medicinal herbs *Allium sativum*, *Andrographis paniculata*, *Azadirachta indica*, *Cinnamomum verum*, *Coriandrum sativum*, *Curcuma longa*, *Embllica officinalis*, *Mentha spicata*, *Moringa oleifera*, *Murraya koenigii*, *Ocimum sanctum*, *Phyllanthus niruri*, *Piper nigrum*, *Syzygium aromaticum*, *Trigonella foenum* and *Zingiber officinale* that are used in Indian traditional medicine and which are commonly available in Tamil Nadu were collected for the study.

Different parts of the herbs have different active principles, and hence the part that is traditionally used for medicinal purpose was selected for each particular herb. The herbs and their parts selected for the study are listed in table 1.

Preparation of the herbal extracts

The plant samples (250 gms) were suitably

cleaned from extraneous matter, shade dried for 72 hours and ground to pass through 1 mm sieve using a Willey mill under aseptic conditions (Haniyeh *et al.*, 2010). The ground samples were then stored in air tight containers. To determine the *in vitro* antimicrobial effect of the samples, aqueous and ethanol extracts of the herbal samples were first prepared. To prepare aqueous extract, the ground samples, 100 mg each were taken in a sterile eppendorf tubes to which was added 1 ml of sterile water, vortexed and kept in refrigerator overnight. To prepare ethanol extract, the ground samples, 100 mg each were taken in a sterile eppendorf tubes to which was added 1 ml of 95% ethanol, vortexed and kept in refrigerator overnight. Hence, for each of the herbal sample, aqueous and ethanol extracts were prepared. Each of these extracts (aqueous and ethanol) were tested for their *in vitro* antibacterial activity.

Preparation of microorganisms

The microorganisms under study were *Staphylococcus aureus*, *Escherichia coli*, *Salmonella entitidis* and *Clostridium perfringens*. They were isolated from the clinical samples obtained from Poultry Disease Diagnosis and Surveillance Laboratory, Veterinary College and Research Institute, Namakkal. The organisms were isolated in nutrient agar medium and selectively cultured at 37 °C for 24 hrs. The bacterial strains were identified by gram staining and confirmed by biochemical tests as per the directions from Bergy’s manual for determinative bacteriology.

In vitro antimicrobial assay

To determine the *in vitro* antimicrobial effect of the herbal samples, sterilized circular disc, microbial cultures (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella entitidis* and *Clostridium perfringens*) aqueous and ethanol

extracts of the herbal samples were first prepared and kept ready.

Circular disc of 6 mm diameter were made from the Whatman no 1 filter paper. Discs were impregnated with equal volume (15µl) of the respective plant extracts (aqueous / ethanol). The discs were aseptically placed over plates of Mueller Hinton agar for *Staphylococcus aureus*, Mac Conkey agar for *Escherichia coli* and Nutrient agar for *Salmonella entitidis* and *Clostridium Perfringens* agar for *Clostridium perfringens*, seeded with the respective test pathogens. After drying in the air, the plates were incubated at 37 °C for 24 hours. For the *Clostridium perfringens*, the plates were kept in an anaerobic jar in an upright position at 37 °C for 24 hours. Sterile water and 95 % ethanol were used as control respectively for aqueous and ethanol extracts of samples. Further the inoculums were adjusted to 0.5 Mc Fairland turbidometry (Haniyeh *et al.*, 2010).

The sensitivity of the microorganism species to the plant extracts (aqueous / ethanol) were determined by measuring the size of the inhibitory zones (including the diameter of the disc) on the agar surface around the discs using zone of inhibition scale. Inhibition zones with diameter values 8 mm were considered as not active against microorganisms and zones less than 12 mm were considered as having low antibacterial activity. Diameters between 12 and 16 mm were considered moderately active, and these with >16mm were considered highly active (Indu *et al.*, 2006).

Results and Discussion

The aqueous extracts of sixteen herbs tested for antibacterial activity on four poultry pathogens are presented in Table 2, 3. Aqueous extract of *Allium sativum* bulbs (Garlic) and *Syzygium aromaticum* bud

(Clove) were the only candidates that showed zone of inhibition against *Staphylococcus aureus*. Significantly ($p < 0.05$) higher zone of inhibition was evinced by *Allium sativum* bulbs (Garlic) (13.67 ± 0.21) when compared with *Syzygium aromaticum* bud (Clove) (10.50 ± 0.22). In the case of *Escherichia coli* only *Allium sativum* bulbs (Garlic) (12.50 ± 0.22) had moderate antibacterial activity. The aqueous extracts of other herbs evinced no zone of inhibition. For *Salmonella entitidis*, *Allium sativum* bulbs (Garlic) and *Syzygium aromaticum* bud (Clove) were the only candidates that showed zone of inhibition. *Allium sativum* pods (Garlic) (14.67 ± 0.21) had significantly ($p < 0.05$) higher zone of inhibition (mm) when compared with *Syzygium aromaticum* bud (Clove) (12.50 ± 0.34). The aqueous extracts of other herbs evinced no zone of inhibition. Allicin is the active ingredient of garlic that acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target (Eja *et al.*, 2007). Organo sulfur compounds and phenolic compounds have also been reported to be involved in the garlic antimicrobial activity (Griffiths *et al.*, 2002 and Jombo *et al.*, 2011). Among the aqueous extracts tested *Syzygium aromaticum* (Clove) (15.00 ± 0.00) was the only herbal extract found to have significant moderate antibacterial activity against *Clostridium Perfringens*. The other herbal extracts evinced no zone of inhibition.

Among the ethanol extracts tested *Coriandrum sativum* seeds (Coriander), *Curcuma longa* dry tuber (Turmeric) *Murraya koenigii* leaves (Curry leaves) and *Syzygium aromaticum* bud (Clove) were found to have antibacterial activity against all the four organisms tested. In the case of *Staphylococcus aureus* significantly ($p < 0.05$) higher zone of inhibition was noted by *Syzygium aromaticum* bud (Clove) (21.00 ± 0.00) when compared with other herbal ethanol extracts. *Piper nigrum* seeds (Black

pepper) (19.67 ± 0.21) had the second place. In the case of *Escherichia coli*, *Syzygium aromaticum* bud (Clove) (20.33 ± 0.21) had the significantly ($P < 0.05$) highest anti bacterial activity than other ethanol extracts of other herbs. *Cinnamomum verum* bark (Cinnamon) had significantly ($P < 0.05$) highest antibacterial activity next to *Syzygium aromaticum* bud, as compared to ethanol extracts of other herbs. For *Salmonella entitidis*, *Syzygium aromaticum* bud (Clove) (17.50 ± 0.22) was found to have significantly ($p < 0.05$) higher zone of inhibition when compared ethanol extracts of other herbs. *Andrographis paniculata* leaf with stem (Nilavembu), *Azadirachta indica* leaves (Neem), *Cinnamomum verum* bark (Cinnamon), *Ocimum sanctum* leaves (Tulsi),

Phyllanthus niruri leaves (Keelanelli), *Piper nigrum* seeds (Black pepper) and *Trigonella foenum* seeds (Fenugreek) were found to have no zone of inhibition. Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens (Prabuseenivasan et al., 2006). The high levels of eugenol present in clove essential oil is responsible for its strong antimicrobial activities. It is well known that both eugenol and clove essential oil phenolic compounds can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of gram-negative and gram-positive bacteria as well as different types of yeast (Chaieb et al., 2007, Walsh et al., 2003).

Table.1 List of herbs and their part selected for the study

S. No	Botanical name of the herb	Common name of the herb	Plant part used
1	<i>Allium sativum</i>	Garlic	Bulb
2	<i>Andrographis paniculata</i>	Nilavembu	Leaf with stem
3	<i>Azadirachta indica</i>	Neem	Leaf
4	<i>Cinnamomum verum</i>	Cinnamon	Bark
5	<i>Coriandrum sativum</i>	Coriander	Seed
6	<i>Curcuma longa</i>	Turmeric	Rhizome
7	<i>Embllica officinalis</i>	Amla	Fruit
8	<i>Mentha spicata</i>	Mentha	Leaf
9	<i>Moringa oleifera</i>	Moringa leaves	Leaf
10	<i>Murraya koenigii</i>	Curry leaves	Leaf
11	<i>Ocimum sanctum</i>	Tulsi	Leaf
12	<i>Phyllanthus niruri</i>	Keelanelli	Full plant with root
13	<i>Piper nigrum</i>	Black pepper	Flower bud
14	<i>Syzygium aromaticum</i>	Clove	Flower bud
15	<i>Trigonella foenum</i>	Fenugreek	Seed
16	<i>Zingiber officinale</i>	Dry ginger	Rhizome

Table.2 *In vitro* antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella entitidis* and *Clostridium perfringens* of aqueous extracts of herbs

S.No	Name of the herb	Antimicrobial activity (Zone of inhibition in mm)			
		<i>Staphylococcus aureus</i> ,	<i>Escherichia coli</i>	<i>Salmonella entitidis</i>	<i>Clostridium perfringens</i>
1	<i>Allium sativum</i> (Garlic)	13.67 ^a ± 0.21	12.50 ^a ± 0.22	14.67 ^a ± 0.21	NZI
2	<i>Andrographis paniculata</i> (Nilavembu)	NZI	NZI	NZI	NZI
3	<i>Azadirachta indica</i> (Neem)	NZI	NZI	NZI	NZI
4	<i>Cinnamomum verum</i> (Cinnamon)	NZI	NZI	NZI	NZI
5	<i>Coriandrum sativum</i> (Coriander)	NZI	NZI	NZI	NZI
6	<i>Curcuma longa</i> (Turmeric)	NZI	NZI	NZI	NZI
7	<i>Emblica officinalis</i> (Amla)	NZI	NZI	NZI	NZI
8	<i>Mentha spicata</i> (Mentha)	NZI	NZI	NZI	NZI
9	<i>Moringa oleifera</i> (Moringa leaves)	NZI	NZI	NZI	NZI
10	<i>Murraya koenigii</i> (Curry leaves)	NZI	NZI	NZI	NZI
11	<i>Ocimum sanctum</i> (Tulsi)	NZI	NZI	NZI	NZI
12	<i>Phyllanthus niruri</i> (Keelanelli)	NZI	NZI	NZI	NZI
13	<i>Piper nigrum</i> (Black pepper)	NZI	NZI	NZI	NZI
14	<i>Syzygium aromaticum</i> (Clove)	10.50 ^b ± 0.22	NZI	12.50 ^b ± 0.34	15.00 ^a ± 0.00
15	<i>Trigonella foenum</i> (Fenugreek)	NZI	NZI	NZI	NZI
16	<i>Zingiber officinale</i> (Dry ginger)	NZI	NZI	NZI	NZI

Mean of six observations, values bearing different superscripts within a column differ significantly (p<0.05), NZI- No zone of inhibition.

Table.3 *In vitro* antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella entitidis* and *Clostridium perfringens* of ethanolic extracts of herbs

S.No	Name of the herb	Antimicrobial activity (Zone of inhibition in mm)			
		<i>Staphylococcus aureus</i> ,	<i>Escherichia coli</i>	<i>Salmonella entitidis</i>	<i>Clostridium perfringens</i>
1	<i>Allium sativum</i> (Garlic)	14.67 ^g ± 0.21	NZI	14.67 ^d ± 0.21	NZI
2	<i>Andrographis paniculata</i> (Nilavembu)	17.33 ^e ± 0.21	17.17 ^{cd} ± 0.31	NZI	16.00 ^f ± 0.00
3	<i>Azadirachta indica</i> (Neem)	14.50 ^g ± 0.22	15.83 ^{fg} ± 0.17	NZI	NZI
4	<i>Cinnamomum verum</i> (Cinnamon)	18.83 ^c ± 0.16	18.67 ^b ± 0.21	NZI	NZI
5	<i>Coriandrum sativum</i> (Coriander)	14.83 ^g ± 0.17	16.83 ^{de} ± 0.17	16.17 ^c ± 0.31	18.00 ^b ± 0.00
6	<i>Curcuma longa</i> (Turmeric)	17.83 ^d ± 0.17	16.33 ^{ef} ± 0.21	17.17 ^{ab} ± 0.17	17.83 ^{bc} ± 0.40
7	<i>Emblica officinalis</i> (Amla)	NZI	17.67 ^c ± 0.21	NZI	NZI
8	<i>Mentha spicata</i> (Mentha)	16.50 ^f ± 0.22	15.33 ^g ± 0.42	16.00 ^c ± 0.26	17.00 ^d ± 0.00
9	<i>Moringa oleifera</i> (Moringa leaves)	17.00 ^{ef} ± 0.36	14.67 ^h ± 0.21	15.00 ^d ± 0.26	NZI
10	<i>Murraya koenigii</i> (Curry leaves)	16.50 ^f ± 0.22	17.50 ^c ± 0.22	16.17 ^c ± 0.31	19.00 ^a ± 0.00
11	<i>Ocimum sanctum</i> (Tulsi)	17.17 ^e ± 0.48	15.67 ^g ± 0.76	NZI	17.67 ^c ± 0.21
12	<i>Phyllanthus niruri</i> (Keelanelli)	16.50 ^f ± 0.22	15.50 ^g ± 0.22	NZI	NZI
13	<i>Piper nigrum</i> (Black pepper)	19.67 ^b ± 0.21	17.67 ^c ± 0.21	NZI	16.67 ^e ± 0.21
14	<i>Syzygium aromaticum</i> (Clove)	21.00 ^a ± 0.00	20.33 ^a ± 0.21	17.50 ^a ± 0.22	17.00 ^d ± 0.00
15	<i>Trigonella foenum</i> (Fenugreek)	18.50 ^c ± 0.22	16.67 ^{de} ± 0.21	NZI	15.00 ^g ± 0.00
16	<i>Zingiber officinale</i> (Dry ginger)	16.50 ^f ± 0.22	16.50 ^e ± 0.22	17.00 ^b ± 0.26	NZI

Mean of six observations, values bearing different superscripts within a column differ significantly (P<0.05), NZI- No zone of inhibition

Badei *et al.*, (2002) tested the antimicrobial activities of cardamom, cinnamon and clove essential oils against nine gram-positive bacterial strains, four gram-negative bacterial strains, seven molds, and two yeasts, compared with phenol, using the disc diffusion method. Clove essential oil showed the highest antimicrobial activity, and the antimicrobial spectra (diameter of inhibition zones) of 10 % clove essential oil was 1.48 times as that of 10 % phenol.

Among the ethanol extracts tested *Murraya koenigii* leaves (Curry leaves) (19.00 ± 0.00) was found to have highest zone of inhibition against *Clostridium Perfringens* followed by *Coriandrum sativum* seeds (Coriander) (18.00 ± 0.00). The other ethanol extracts had only low zone of inhibition when compared with these two extracts. Ethanol extracts of *Allium sativum* bulbs (Garlic), *Azadirachta indica* leaves (Neem), *Cinnamomum verum* bark (Cinnamon), *Emblica officinalis* fruit (Amla), *Moringa oleifera* leaves (Moringa leaves), *Phyllanthus niruri* stem with leaves (Keelanelli) and *Zingiber officinale* tuber (Dry ginger) had no activity against *Clostridium perfringens*. The results are comparable to that of previous studies in which the researchers reported that the various organic extracts of *M. koenigii* exhibited significant antimicrobial activity against both gram positive and gram negative bacteria (Panghal *et al.*, 2011; Baskaran *et al.*, 2011). This property was attributed to several carbazole alkaloids present in the organic extracts of *M. Koenigii* (Malwal and Sarin, 2011).

In conclusion, aqueous extracts of herbs behaved differently compared to their ethanol extracts in bringing about antibacterial effect. Among the aqueous extracts of herbs, *Allium sativum* bulbs was the only herb or the herb that had significantly higher antibacterial potency against *Staphylococcus aureus*,

Escherichia coli, *Salmonella entitidis*, however, it had no antibacterial activity against *Clostridium Perfringens*. Ethanol extract of *Syzygium aromaticum* bud showed the significantly highest antibacterial potency against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella entitidis* and ethanol extracts of *Murraya koenigii* leaves showed significantly highest antibacterial potency against *Clostridium Perfringens*.

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