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Research Article

Extraction time is a critical factor to prepare poly herbal extract “Nilavembu Kudineer” and antibacterial activity against human pathogens

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Abstract

Medicinal plants serve as specific targets for effective antibacterial agents. In the present study the aqueous extract (hot water) of the “Nilavembu Kudineer” (a poly herbal formula) (mixture of *Andrographis paniculata*, *Vetiveria zizanioides*, *Santalum album*, *Trichosanthes cucumerina*, *Cyperus rotandus*, *Zingiber officinale*, *Piper nigrum* and *Mollugo cerviana*) was prepared and evaluated for antimicrobial activity against five bacterial strains by determining zone of inhibition by agar well diffusion method. The antibacterial activity of the aqueous extract was found to be high after 40 min of extraction at 100 °C. Water extract of these plants exhibited very strong *in vitro* antibacterial activity against various bacteria including, *Staphylococcus aureus*, *E. coli*,

Enterococcus sp., *Pseudomonas aeruginosa* and *Staphylococcus epidermis*.

Keywords: Antibacterial activity, nilavembu kudineer, poly herb

Introduction

Medicinal plants represent a rich source of many potent and novel drugs. A wide range of medicinal plants are used as raw drugs and they possess numerous medicinal properties. These plants are important source of novel chemical substances with good therapeutic effects. Plants contain various secondary metabolites and are directly used as intermediates for the production of new drugs. Traditional medicine play important role in the modern primary healthcare system of the developing and under developing countries (Kar, 2007). The

natural medicines are more acceptable to the human body than synthetic drugs. So, it is very important to derive the maximum benefit from the traditional system of medicine for providing adequate healthcare service to rural people. The micro-organisms induced various diseases are responsible for more than 50% deaths and are still a key concern to public health (WHO, 2002). Synthetic antimicrobial drugs are highly expensive and unavailable in developing and under-developed countries (Okele et al., 2005). Again, the safety aspect of synthetic drugs is an important concern since they may be responsible for causing serious side effects.

Emergence of anti-microbial resistance in bacteria against antimicrobial drugs is another important concern. Infectious diseases cause mortality and morbidity among the general population, particularly in developing and under developing countries. Hence, pharmaceutical companies have been initiated to develop novel antimicrobial drugs in recent years, especially due to the constant emergence of antimicrobial resistance among pathogenic bacteria. These bacterial species have the ability to transmit and acquire resistance against currently available antibacterial drugs since there are frequent reports on the isolation of bacteria that are known to be

sensitive to routinely used drugs and became multidrug resistant to other drugs available on the market (Sakagami and Kajimura, 2002). Plants contain varieties of phytochemicals including terpenoids, tannins, flavonoids and alkaloids, and which have been found *in vitro* to have antimicrobial properties (Santhosh et al., 2016). Global prevalence of infectious diseases caused by various bacteria is an important public health problem (Paterson, 2008). The bacterial species such as, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* cause various human infections (Peirano, 2008). Emergence of various antibiotic resistance and related toxicity problems limit the use of many currently available antimicrobial agents (Eggleston et al., 2010) and is prompting a revival in research of the antimicrobial role of various medicinal plants against resistant strains due to comparable efficacy and safety (Dorman and Deans, 2000).

Andrographis paniculata (Acanthaceae) is an annual herb. This plant is mainly found in Sri Lanka and in India, especially Tamilnadu, Karnataka, Maharashtra, Orissa and Uttar Pradesh. This plant has the medicinal properties like antidiarrhoeal, antiinflammatory and immunostimulant effect have been attributed in the traditional system of Indian medicine

(Mukherjee, 2002; Kokate et al., 2002). Further reported activities are antimalarial, antihypertensive, antipyretic, hepatoprotective, and antithrombotic activities (Mishra et al., 2009). In the present study, a poly herbal formula, mixture of [Nilavempu \(*Andrographis paniculata*\)](#), Vettiver (*Vetiveria zizanioides*), Vilamiccam ver (*Vetiveria zizanioides*), Cantanam (*Santalum album*), Peyputtal (*Trichosanthes cucumerina*), Koraik kilanku (*Cyperus rotandus*), Chukku (*Zingiber officinale*), Milaku (*Piper nigrum*) and Parpatakam (*Mollugo cerviana*) were used to prepare poly herbal formula for antibacterial screening. Nilavembu kudineer (poly herb) was suggested by government of Tamilnadu to treat and prevent Dengue fever. However, the information related to the extraction time and the efficacy of the poly herbal extract is not clearly addressed previously.

Materials and methods

The dried medicinal plants ([Andrographis paniculata](#), *Vetiveria zizanioides*, *Vetiveria zizanioides*, *Santalum album*, *Trichosanthes cucumerina*, *Cyperus rotandus*, *Zingiber officinale*, *Piper nigrum* and *Mollugo cerviana*) were collected from herbal shop, Nagercoil, Kanyakumari District, Tamilnadu, India.

Test microorganisms

The pathogenic bacteria such as, *Staphylococcus aureus*, *E. coli*, *Enterococcus* sp., *Pseudomonas aeruginosa* and *Staphylococcus epidermis* were used.

Preparation of inoculum

A loop full culture of these bacterial isolates were transferred into the nutrient broth medium consisted of (g/l) (yeast extract 1.5; peptic digest of animal tissue, 5.0; beef extract, 1.5 and sodium chloride, 5.0). These Erlenmeyer flasks were incubated at 37 °C for 24 h and were used as the inoculum.

Extraction procedure and antibacterial activity of plant extracts

The medicinal plants were directly used for extraction. For hot water extraction, 10 g dried powder of these plants was soaked in 1000 ml double distilled water and boiled for 40 min. After every 10 min interval, about 5 ml of extract was withdrawn and the extract was sieved through a muslin cloth. The collected supernatant was used for antibacterial assay. Amikacin (30 µg) was used as the positive control. The antibacterial activity of poly herb against *Staphylococcus aureus*, *E. coli*, *Enterococcus* sp., *Pseudomonas aeruginosa* and *Staphylococcus epidermis* was determined.

Results and Discussion

Global burden of infectious diseases caused by various pathogenic bacteria is a major threat to public health. Antibiotic treatment is a best choice to treat these bacterial infections; however, emergence of multiple drug resistance and toxicity issues subside the administration of antibacterial agents (Malini et al., 2013). Efficacy and

safety related limitations to antibiotics augment biological research on the antimicrobial role of plants due to comparable efficacy and toxicity (Alviano and Alviano, 2009). In the present study, the water extract obtained was tested for the antimicrobial activity against the selected five bacterial strains. The water extract from poly herb exhibited varying degree of antibacterial activity against the tested bacterial isolates. Among the tested bacterial species, the phytochemicals were highly active against *Pseudomonas* sp., and showed less activity against *S. aerues*. *A. paniculata* is the important ingredient in poly herbal formula and is extensively used in Unani, Ayurveda and Siddha medicine as home remedy for various infectious diseases in Indian traditional system. Aromatic and medicinal plants are widely used as medicine against various drug resistant bacteria. This result was supported by various researchers reported, *A. paniculata* as potent antimicrobial agent. Mishra *et al.* (2013) stated that 75% methanol extract of *A. paniculata* leaves was found to be highly active against pathogens such as, *M. tuberculosis*, *S. aureus* and *E. faecalis*. The water extracts of *A. paniculata* showed potential antibacterial activity against both Gram positive and Gram negative bacteria (Zaidan *et al.*, 2005). Humnabadkar and Kareppa (2012) reported that the aqueous extracts of *A. paniculata* showed maximum antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Alcohol extracts of *A. paniculata* with higher inhibitory activity against *Bacillus subtilis* and

Staphylococcus aureus (Hosamani *et al.*, 2011). The alcoholic and aqueous extracts of *Stachys annua*, *Nuphar lutea*, *Genista lydia*, *Vinca minor* and *Nymphaea alb* showed potent activity. *Fragaria* herbs of Turkey showed antibacterial activity against *Lactococcus garvieae*, *Enterococcus faecalis* and *A. hydrophila* (Turker *et al.*, 2009).

In *A. paniculata*, the presence of flavonoids in the leaves enables them to be used as potential antioxidant agent. In plants, alkaloids function in the defence of plants against pathogens and herbivores, and are widely exploited as stimulants, narcotics, poisons and pharmaceuticals due to their potent bioactivities (Kar, 2007). The important function of phenolic compound is plant defence against various pathogens and thus they are widely applied in the control of various human pathogenic infections. The phytochemical such as, tannins act as antiseptic agents because of the presence of the phenol group. In Ayurvedic medicine many tannin rich formulations are used for the treatment of various infections. The presence of compounds such as, phenolics, alkaloids, and tannins may responsible for antifungal and antibacterial activity exhibited by the leaves of *A. paniculata*. *A. paniculata* has various water soluble lactone andrographoloidic properties (Kar, 2007). Medicinal plants are very important in

pharmaceutical industries for the development of novel drug.

The present investigation mainly focused on the influence of extraction time against bacteria. The antibacterial activity of the herbal mixture was extracted and assayed. It was reported that the methanolic extracts of *A. paniculata* at the highest concentration showed the potent bacterial inhibitory activity than other solvent extracts (Kaushik and Goyal, 2011). *A. paniculata* has also been widely used clinically for symptomatic treatment of the uncomplicated sinusitis and common cold, pneumonia, pharyngotonsillitis, and bronchitis (Hancke et al., 1995). In this study hot water extraction was carried out upto 40 min. At 10 min of extraction, inhibitory activity of secondary metabolite was found to be less and after 40 min of extraction phytochemical activity was found to be high (Fig. 1-6). In the present study, Gram-positive bacteria were the most susceptible to growth inhibition by water extract of poly herb. Susceptibility differences between Gram-negative and Gram-positive bacteria may be due to structural differences of cell wall between these Gram's negative and Gram-positive bacteria. The antimicrobial activity may be due to the presence of secondary metabolites such as flavonoids, saponins and phenolic compounds present in the

crude extracts. The present study explored the antimicrobial potential of the poly herb against the clinical bacterial isolates in relation with extraction time. "Nilavembu kudineer" plays a significant role to treat and prevent bacterial and viral fever including, "Dengue fever". Dengue outbreak was effectively control in Tamilnadu, India by "Nilavembu kudineer" in 2017.



Figure

1. Antibacterial activity of water extract of poly herb against *Enterococcus* sp.



Figure 2. Antibacterial activity of water extract of poly herb against *Pseudomonas aeruginosa*.



Figure 3. Antibacterial activity of water extract of poly herb against *S. aerues*.

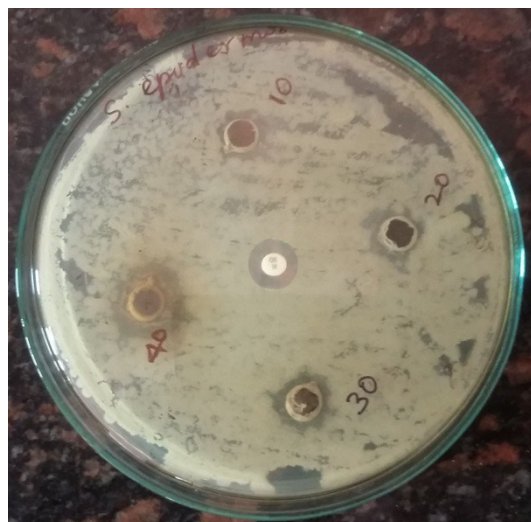


Figure 4. Antibacterial activity of water extract of poly herb against *S. epidermis*.

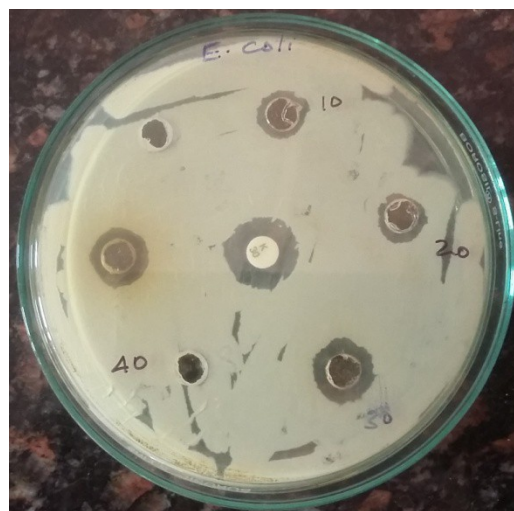


Figure 4. Antibacterial activity of water extract of poly herb against *E. coli*.

Figure 5. Effect of extraction time and the activity of poly herb against selected human pathogens.

Conclusion

The poly herb (*A. Paniculata*, *Vetiveria zizanioides*, *Vetiveria zizanioides*, *Santalum album*, *Trichosanthes cucumerina*, *Cyperus rotandus*, *Zingiber officinale*, *Piper nigrum* and *Mollugo cerviana*) were widely used in Unani, Siddha and Ayurveda medicines from ancient times. This poly herbal formula was widely suggested to treat almost all types of bacterial and viral fever, fever associated inflammation and fever associated shivering. Extraction time is a critical factor for the activity of traditional medicine. Extraction with hot water (30 min to 40 min) showed good activity. Extraction time is a key

to extract active ingredients from this poly herb.

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