Authentication of antibacterial activity of wound healing Siddha medicinal plants

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Abstract- Several medicinal plants have been used in traditional health care systems to heal wounds. Wound infecting bacteria are one of the major factors that delays or prevent wound healing. In the present study, three medicinal plants those have been widely used in Siddha medicine to treat wounds namely, *Erythrina variegata*, *Tamarindus indica* and *Datura metel* were tested for their antibacterial activity against standard bacterial isolates which isolated from different sources including wounds, viz. *Staphylococcus aureus* subsp. *aureus* (ATCC® 29213™), *Pseudomonas aeruginosa* (ATCC® 25668™), *Escherichia coli* (ATCC® 25922™) and *Enterococcus faecalis* (ATCC® 29212™). Leaf of *E. variegata*, seed coat of *T. indica* and fruit of *D. metel* were extracted with hexane, ethyl acetate and ethanol. Antibacterial activity was tested using two different methods; well diffusion and growth curve analysis in broth culture. Both methods revealed that all the extracts of *T. indica* and fruit of *D. metel* had inhibitory effect on *S. aureus*, a bacterial isolate from wound. Similarly extracts of *E. variegata* had antibacterial activity on *E. faecalis* and *P. aeruginosa*, urine and clinical isolates respectively. The results confirm the antibacterial property of the plant extracts and their possible role in wound healing. Further studies are being carried out to find out the active compounds that responsible for antibacterial activity.

Index Terms- Antibacterial activity, Medicinal plants, Siddha medicine, Wound infection.

I. INTRODUCTION

A huge percentage of human population still depends on traditional health care systems to cure several infectious and non-infectious diseases, especially in developing countries [1]. This is because of less side-effects and low cost of the medicines in traditional health care systems. The conventional health care systems developed through the accumulation of knowledge of ancient people from several hundreds of years. Before the development of modern medical practices human population completely relied on traditional health care systems. Even nowadays, several new drugs are being extracted from various medicinal plants or chemically synthesised based on the structure of natural compounds [2].

The traditional health care systems completely or partly depend on plant sources to formulate various medicines. Naturally, plants produce diverse secondary metabolites through various biosynthetic pathways and these chemicals are important for various bioactivities of plants. Tannins, alkaloids, saponins and terpinoides are some major groups of bioactive compounds present in plants [3]. These secondary metabolites and their derivatives are the key active ingredients in conventional medicine. The type and amount of active ingredients also vary with the diversity and surrounding environmental conditions of the plants. Extraction and identification of active compounds from plants, which have been used in traditional health care systems, make a bridge to modern medicine. Therefore, researchers are very much keen on screening of medicinal plants which have been reported as an ingredient in drug preparations in traditional health care systems.

One of the vital activities possessed by these secondary metabolites is antimicrobial activity. Originally, these active compounds prevent the plants to get infected from various pathogenic microorganisms through various modes of actions. These substances can either inhibit the growth of bacteria or kill them, with no toxicity or minimum toxicity to host cells are considered candidates for developing new antimicrobial drugs [4]. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.
Wound healing is a complex process with many potential factors that can delay healing. Wound infection is detrimental to the wound healing process. In open wounds, the absence of an intact epithelium provides a favorable environment for bacteria. Both acute and chronic wounds are susceptible tocontamination and colonization by a wide variety of aerobic and anaerobic microorganisms. Aerobic or facultative pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and beta-hemolytic *streptococci* are the primary causes of delayed healing and infection in both acute and chronic wounds [5]. Optimal management of wound infection is essential to promote a good healing response. Topical antimicrobials such as silver, iodine and chlorhexidine may reduce bio-burden and improve the wound healing response. Newer topical creams, ointments, gels, and dressings appear to provide adequate, sustained, and apparently nontoxic levels of antiseptics. Unfortunately, there is little information on systemic absorption of the agents and evidence of clinical efficacy is meagre. Nowadays, a broad range of antibiotics are also being used for management of wound infections. But, these have been proved to have undesirable effect on the human body. The pathogens also have been successful in developing resistance against various antibiotics [6]. Investigators and the industry are seeking other ways to deal with chronic wound infections, including various innovative

nonantimicrobial approaches such as Bee honey and *Aloe vera* [7].

Siddha medical practice has flourished in Sri Lanka for thousands of years before the western medical practice became widespread. Even at present, at primary care level a large segment of the population seek siddha treatment [8]. Siddha system of medicine has several treatment methods for wound healing and these methods are widely used in siddha medicine practices. In a text book for siddha medicine, *Pararasaseharam* (part – V), 14 different medicines are listed for wound healing activity [9]. In general, these medicines consists several ingredients; mainly medicinal plants based materials and trace amount of heavy metals. However, there is lack of modern experimental evidences to prove wound healing activity of these medicines. There is gab in knowledge between the wound healing activity of siddha medicine and the exact bioactive compound or compounds present in the medicine. The work reported here was carried out to validate the antibacterial activity of three medicinal plants listed in Siddha medical practice for wound healing activity.

II. MATERIALS AND METHODS

A. Collection of plant samples and extraction

Leaves of *Erythrina variegata*, seed coat of *Tamarindus indica* and fruits of *Datura metel* was collected from healthy plants growing in herbal garden, agriculture farm school, Thirunelveli, Jaffna, Sri Lanka. Shade dried plant parts were ground into fine powder using electric blender. The powder was successively extracted using solvents of increasing polarity. 20 g powder was initially soaked in 100 ml of hexane in air tight conical flask for two days and then it was first filtered through double layered muslin cloth and then filtered through Whatman no 1 filter paper and the filtrate was collected into a sterile air tight bottle. Similar process was repeated twice with fresh hexane and the filtrates were collected together. Later, hexane was removed from the filtrate at 40 °C using oven and the extract was stored at the refrigerator for further studies. The dried residue of each powder was used for sequential extraction with ethyl acetate and ethanol [10].

B. Test bacteria

The standard bacterial isolates, *Staphylococcus aureus* subsp. *aureus* (ATCC® 29213™), *Pseudomonas aeruginosa* (ATCC® 25668™), *Escherichia coli* (ATCC® 25922™) and *Enterococcus faecalis* (ATCC® 29212™) were obtained from Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka and they were stored on nutrient agar slants at 4 °C until used for the assay.

C. Determination of antibacterial activity by agar well diffusion method

20 ml molten nutrient agar media were mixed with 1 ml of (10^6 cfu/ml) each test bacteria and poured insterile Petri dishes separately. After complete solidification, 8mmdiameter wellswere made using sterile cork-borer and filled with 100 µl of (30 mg) hexane extract; (30 mg) ethyl acetate extract, and (30 mg) ethanol extract of *E. variegata*. (3 mg) streptomycin and different solvents (100 µl of hexane, ethyl acetate or ethanol) were used as standard and control respectively. Then the plates were incubated at 37 ºC for 24 hours and antibacterial activity was determined by measuring the diameter of clear zone around the well [10]. Similarly, activities of extracts of *T. indica* and *D. metel* were measured.

D. Determination of antibacterial activity in broth culture

20 ml nutrient broth was taken in 250 ml Nephelo culture flasks and sterilized by autoclaving. 500 µl of each of the test bacteria (10^6 cfu/ml) was added into the above flasks separately. 10 mg of hexane extract of *E. variegata* was dissolved in 500 µl of hexane and added into the above nutrient medium. The optical density (OD) readings were measured at different time points using spectrophotometer. The flask which has nutrient broth and plant extract was used as blank. In similar manner activity of rest of the extracts were tested against all test bacterial isolates. It was assumed that the amount of bacteria in the flask at particular time point was proportional to the absorbance.

Absorbance = OD of test sample – OD of respective blank

III. RESULTS AND DISCUSSION

Plants such as *E. variegate*, *T. indica* and *D. metel* are commonly used in traditional health care systems and in home remedies to treat wounds. In the present study, hexane, ethyl acetate and ethanol extracts of above plants were tested for their antimicrobial activity. Leaf extract of *E. variegate* inhibited the growth of *E. faecalis*, *P. aeruginosa* and *E. coli* in agar well diffusion method (Table 1). Among the three different solvents used for the extraction, ethanol extract of *E. variegate* showed inhibition on all the above three bacterial isolates. However, none of the tested extracts of *E. variegata* had inhibition on the growth of *S. aureus*.All the extracts of *T. indica* and *D. metel* showed significant inhibition on *S. aureus*, but both of them failed to inhibit *E. faecalis* and *E. coli*. Whereas *P. aeruginosa* was inhibited by all the extracts of *E. variegata* and hexane extract of *T. indica* and ethyl acetate extract of *D. metel*.

The antibacterial activity of the extracts was also confirmed by growing the test bacteria in liquid medium with test extracts. The growth pattern of the bacteria in control growth medium (no any test extracts) was compared with the growth pattern of thebacteria in medium having test extracts. *S. aureus* showed differential growth pattern when it grow in hexane, ethyl acetate and ethanol extracts of *D. metel* and *T. indica* (Figure 1and 3). Ethyl acetate and ethanol extracts of *D. metel* had significant inhibition on *S. aureus* compared to hexane extract of same plant (Figure 1). In ethyl acetate extract, the bacteria grow similar to control up to 4 hours from inoculation, but later *S. aureus* grow

slowly and reach stationary phase at about 12 hours, that is 4 hours delay than control.

Table 1: Inhibitory effects of hexane, ethyl acetate and ethanol extracts of *E. variegate*, *T. indica* and *D. metel* against some test bacteria.

Sample		Diameter of inhibition zone (mm)			
Plant	**Solvent**	***E. faecalis***	***P. aeruginosa***	***E. coli***	***S. aureus***
Erythrina variegata	Hexane	9*	11	-	-
	Ethyl acetate	-	10	-	-
	Ethanol	10	15	11	-
Tamarindus indica	Hexane	-	10	-	12
	Ethyl acetate	-	-	-	12
	Ethanol	-	-	-	10
Datura metel	Hexane	-	-	-	12
	Ethyl acetate	-	9	-	13
	Ethanol	-	-	-	12

* Values includes the diameter of well (8mm)

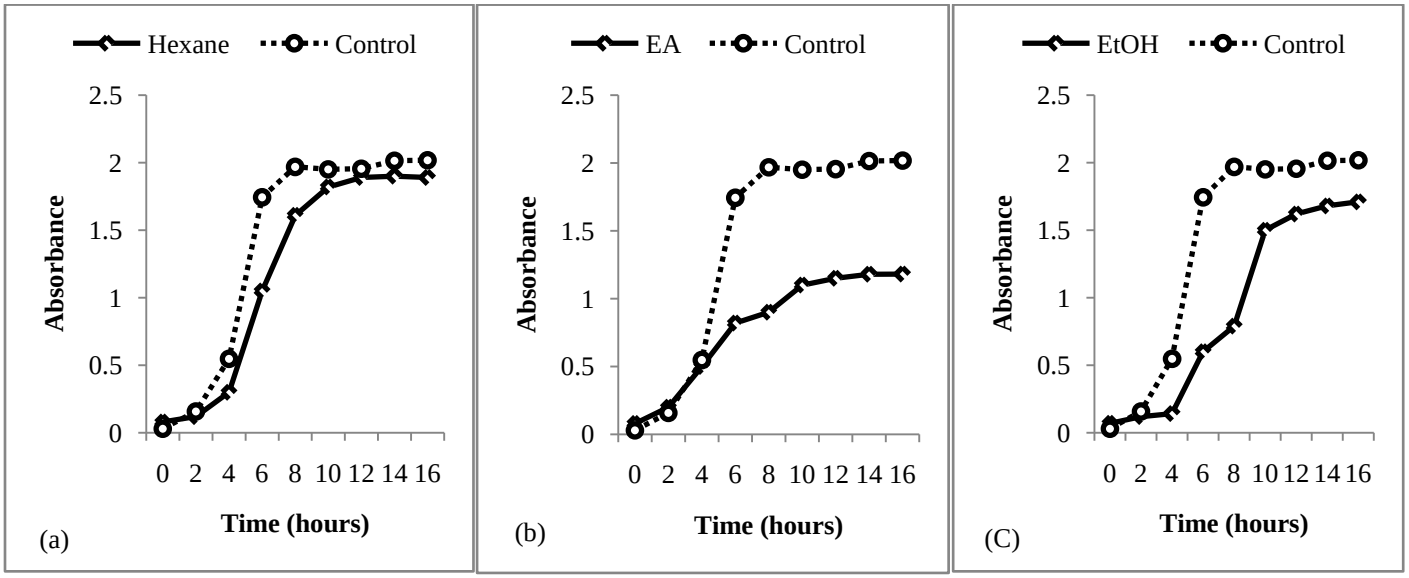

Figure 1: Growth pattern of *S. aureus* in liquid growth medium. In each graph, dot lines represent the reading for control, straight lines shows growth pattern with different extracts of *D. metel*; (a) hexane extract, (b) ethyl acetate extract and (c) ethanol extract.

The amount of absorbance in ethyl acetate extract was reduced to half than control. In ethyl acetate extract of *T. indica*, first 6 hours S. aureus grow in lag phase that is about 2 hours longer than control. Later, the amount of bacteria increased to about half of the amount of bacteria in control, reached stationary phase by 12 hours.

P. aeruginosa showed significant grow retardation when grow with the extracts of *E. variegata* (Figure 2). All the three extracts of *E. variegata* extended the lag phase of bacterial growth further one or two hours compared to control. Hexane and ethanol extracts of the plant reduced the amount of bacteria by 1/3 portion at stationary phase compared to control.*Staphylococcus aureus* subsp. *aureus* (ATCC® 29213™) is a wound isolate. In present study, this isolate was inhibited by the extracts of *T. indica* and *D. metel*. This finding confirms their role in wound healing and their importance in traditional health care system.

IV. CONCLUSION

In conclusion, extracts of *T. indica* and *D. metel* have significant amount of inhibition on *S. aureus*. Similarly, *E. variegata* extracts have inhibition on *P. aeruginosa*. These findings confirm the value of these extracts using in the wound. However, further studies are needed to find out the exact compounds that responsible for this activity.

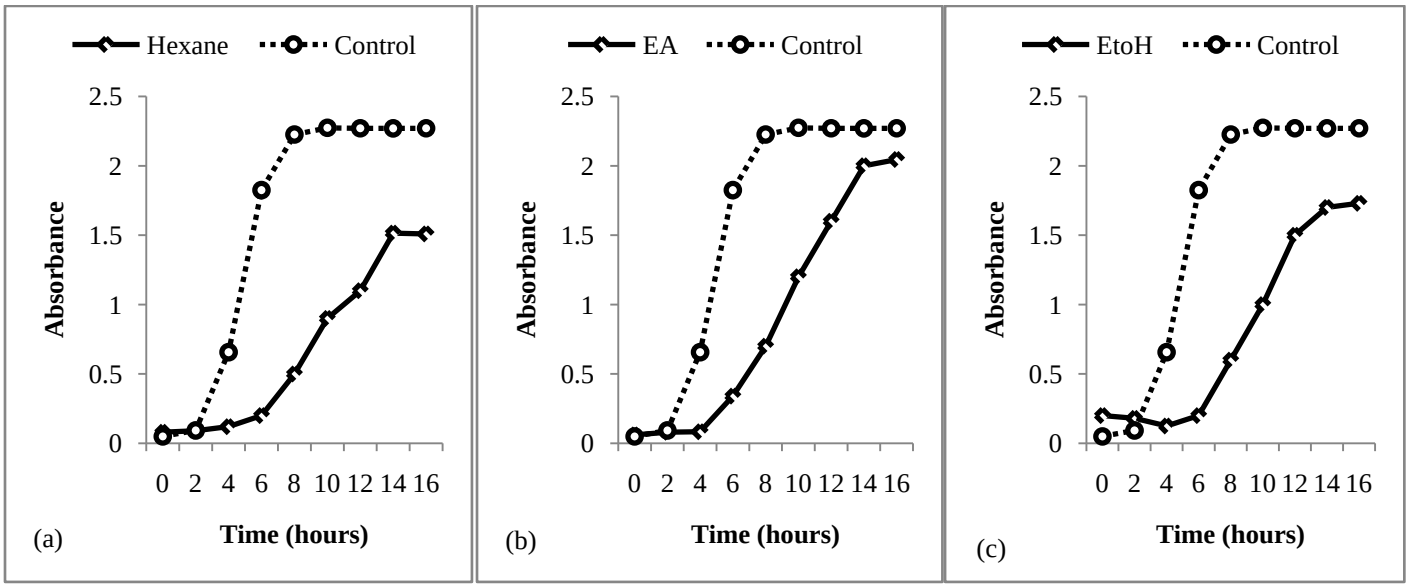

Figure 2: Growth pattern of *P. aeruginosa* in liquid growth medium. In each graph, dot lines represent the reading for control, straight lines shows growth pattern with different extracts of *E. variegata*; (a) hexane extract, (b) ethyl acetate extract and (c) ethanol extract.

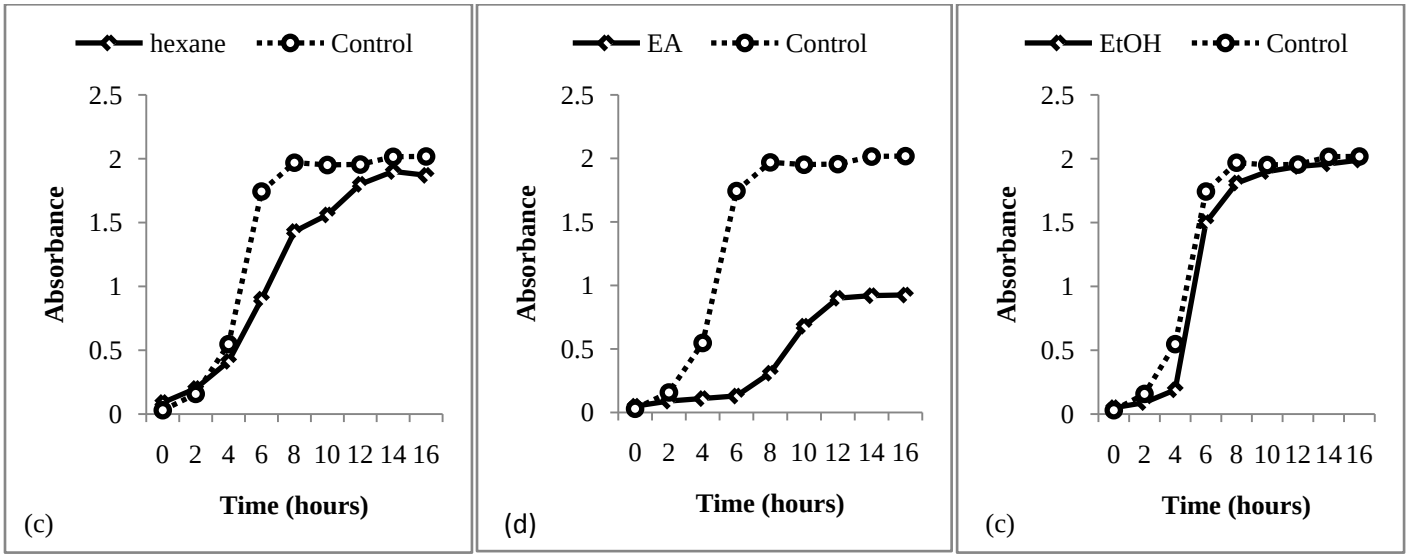

Figure 3: Growth pattern of *S. aureus* in liquid growth medium. In each graph, dot lines represent the reading for control, straight lines shows growth pattern with different extracts of *T. indica*; (a) hexane extract, (b) ethyl acetate extract and (c) ethanol extract.

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