

Study on efficacy of herbal and copper nanoparticle ointment against wound pathogen isolated from diabetic foot ulcer

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ABSTRACT

Aim: This study aims to check the antibacterial activity of ointment produced with herbs such as Siriyanangai, Kandankathiri, and naval seed, and copper nanoparticles against diabetic foot ulcer pathogens such as *Staphylococcus aureus* and *Escherichia coli*. **Materials and Methods:** The foot ulcer sample was collected from diabetic patients by swab method. In this current research, screening of herbal plant extractions which are supposed to be having antimicrobial property has been analyzed. The screened herbal extract was incorporated into prepared copper nanoparticles by the method of nanoencapsulation. The herbal composite was optimized by varying the concentration of herb and nanoparticles. The characterizations of the prepared composite were achieved by instrumentation analysis. Further, the composite was prepared into an ointment using petroleum jelly as a base and its antibacterial activity was analyzed against diabetic foot pathogens. **Result and Discussion:** About 10 μ L of the ointment showed a zone of inhibition of 14 mm and 15 mm for *S. aureus* and *E. coli*, respectively. This shows that the prepared herbal ointment has antibacterial activity against foot ulcer pathogens. **Conclusion:** The findings of this research may provide an economically viable method for the treatment of diabetic foot ulcer.

KEYWORDS: Copper nanoparticles, *Escherichia coli*, herbal extraction, ointment, *Staphylococcus aureus*, yellow petroleum jelly

INTRODUCTION

The world has been facing a most important epidemic of diabetes mellitus. The foot ulcer is one of the most major and devastating complications of diabetes. The report of the diabetes statistics revealed the fact that the foot was affected by ulceration that is associated with peripheral neuropathy and peripheral arterial disease in the lower limb in a patient with diabetes. The prevalence of diabetes in the medically developed may be one-third higher than previously estimated. In India, with more than 62 million diabetic individuals were currently diagnosed with this disease. India stands first in diabetes followed by China (20.8 million) and the United States of America (17.7 million), respectively. It is anticipated that by 2030 diabetes mellitus may

harrow up to 79.4 million people in India, while China (42.3 million) and the United States (30.3 million) will likewise observe critical increments in those influenced by the ailment. Approximately 20% of diabetic patients with the foot ulcer will primarily display inadequate peripheral arterial supply. About 30% will display a combination of both conditions “peripheral arterial and peripheral neuropathy.^{[1,2]”}

In ancient period, natural honey dressings have been used to treat the wounds.^[1] A wound evaluation was carried out daily and used to decide on the continuation of treatment with the natural honey.^[3] The natural honey possesses antibacterial activity well in excess of that needed to inhibit the growth of microorganisms. It was found to be effective against most common clinical pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella*, and *Pseudomonas* sp. The main bacteria are β -hemolytic streptococci.^[1,4,5] There are too many herbs used in the treatment of diabetic foot ulcer throughout the ages. The *Andrographis*

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paniculata is an annual herbaceous plant and commonly used to treat infections. It belongs to the family Acanthaceae, native to India. Some of the herbs are termed as insulin plant, playing the main role in curing diabetes. The literature study of *A. paniculata* already confirmed to be an effective antibiotic, antiparasitic, antiviral, and immune system stimulant.

The plant has been found to treat the diabetic conditions. The whole plant has been used for preparing various formulations in treating diabetic patients as an antihyperglycemic and as an antioxidant agent.^[6] *Syzygium cumini* is known as Jambul or Jamun is an evergreen tropical tree in the following plant family "Myrtaceae." It is native to India. The parts of Jamun trees such as whole seeds, seed coat, and kernel are used in the traditional medicines. In previous reports, the phytochemical studies of seeds show the presence of glycoside, jambosine, gallic acid, ellagic acid, corilagin, quercetin, and β -sitosterol. The Jamun seeds and kernel have the following unique properties such as astringents, antidiabetes, antihyperglycemic, and mild digestive. It indicates that the kernels of Jamun fruit might be used to treat diabetes mellitus type-2, peptic ulcers, foot ulcers, and burning feet syndrome.^[7,8]

MATERIALS AND METHODS

Herb Collection

The herbs such as Siriyangai (*A. paniculata*), Kandankathiri (*Solanum xanthocarpum*), and Naval seed (*S. cumini*) were procured from the Paripoorna herbal stores, Coimbatore, Tamil Nadu, India.

Sample Collection

Samples were collected from diabetic patients wound. Swab sticks were used to collect the samples. The Pus samples present in the wound were collected.

Processing of Herbs

Fresh leaves were shadow dried at 37°C. Drying was done to reduce the moisture content of leaves to <20%. The shade dried herbs were powdered and then passed through a microsieve to get a fine powder. The particle size of the powder was found to be 20–25 μ m. The microscopic characters of dried powder were noted. The powder was stored in an airtight container for further experiments.

Isolation of Pathogens from Wound Sample

The collected samples were swabbed on sterile nutrient agar plates. This process was repeated for all the collected samples. The inoculated plates were incubated at 37°C for 24 h.

Herbal Extraction

The finely powdered material was extracted to obtain the active substances with a suitable solvent (methanol

and aqueous). 10 g of powdered leaves was extracted in 100 mL of 80% methanol for 18 h in shaker.

Phytochemical Analysis of Prepared Herbal Extract

The tests for alkaloids, flavonoids, saponins, phenols, glycosides, reducing sugar, tannins, phytosterols, terpenoids, proteins, anthraquinone, coumarin, anthocyanin, phlobatannins, and acids were performed and the results were noted.^[9-11]

Copper Nanoparticles Preparation

Copper oxide nanoparticles were prepared by the wet chemical method. 6.9 g of copper sulfate pentahydrate was dissolved in 100 mL distilled water (solution-A). 34.6 g of sodium potassium tartrate and 12 g of sodium hydroxide were dissolved in 100 mL distilled water (solution-B). 50 mL of each solution were mixed and kept for stirring. 5 g of glucose was added dropwise to the stirring mixture and allowed to stir for 10 min. The obtained mixture was centrifuged and washed thoroughly without impurities using distilled water. The obtained copper was air dried, and the resulting powder was collected and stored for further use. The solution is then kept undisturbed overnight to allow the precipitation of copper oxide.^[12]

Ointment Preparation

Two concentrations of the ointments were prepared for the study. The concentrations 1 and 2 containing different concentrations of the Siriyangai aqueous extract (1 mL and 1.5 mL) in the ointment base were correspondingly prepared. The ointment was prepared with the following formulation, which includes the soft paraffin 5% and the active components of 2% and 3% were gently used. The fat particles 0.5% were used for to carry the wound from the water substance. Gently the 0.001 ppm copper particles and the sample were mixed homogeneously. The 10 g of blank yellowish petroleum jelly and the pain reliever oil gaultheria were used for the diffusion. Finally, 70% of carboxymethylcellulose was added.

Antibacterial Activity by Well Diffusion Method

The antibacterial activity of the selected herbs, prepared nanoparticles, and the developed product was evaluated by agar well diffusion method. 0.1% inoculum suspension of *S. aureus* and *E. coli* were swabbed uniformly over the surface of the agar. 100 μ L of each herbal extract was loaded into the well and the plates were kept for incubation at 37°C for 24 h. The zone of inhibition was measured and recorded in millimeters. The same process is repeated for evaluating the antibacterial activity of the prepared ointment.

RESULTS AND DISCUSSION

Phytochemical Analysis

The phytochemical analysis was carried out for all the extracts and the results are given in Table 1.

Copper Nanoparticles Preparation

About 1.5 g of copper nanoparticles was obtained from 100 mL of the solutions [Figure 1].



Figure 1: Purified copper nanoparticles

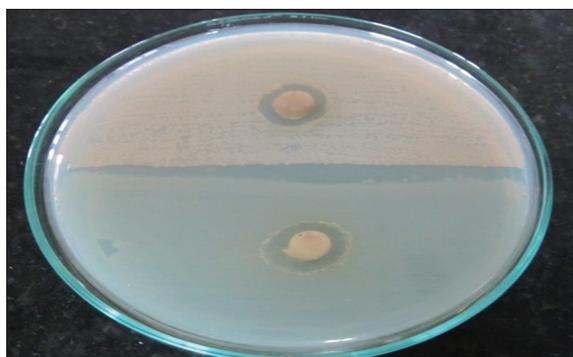


Figure 2: Antibacterial activity of prepared ointment against *Staphylococcus aureus* and *Escherichia coli*

Antibacterial Activity (Kirby-Bauer Method)

The antibacterial tests of copper nanoparticle and the herbal extracts show a zone of inhibition against pathogens [Tables 2 and 3].

Antibacterial Activity of Prepared Ointment

The result of the antibacterial activity of prepared ointment indicates that the ointment sample has the ability to prevent the growth of tested organisms [Table 4 and Figure 2].

CONCLUSION

Wound healing is a complex process that involves the expression of growth factors which is delayed in diabetic patients due to vascular insufficiency and decreased blood flow. Various methods have been used for promotion of wound healing. Recent clinical trials proved that herbal extracts are also safe and effective for the treatment of diabetic foot ulcers. The current research reveals that a total of two species of organisms were isolated from the diabetic foot ulcer patient using sterile swabs. The organisms include *S. aureus* and *E. coli*. It was found that in three types of wound diabetic patient in the highest percentage of the mild grade wound frequency of 42.3% was caused by *S. aureus*. The results of the present study showed that diabetic wound care ointment prepared using herbal formulation was found to be effective against diabetic wound pathogens. The antibacterial activity of herbal extracts was measured and also the zone of incubation was observed. The results were showed that the herbal extraction was effective at the minimum concentration. There were a few limitations in the present study. The prolonged use of the herbal cream which may have beneficial or probable side effects was not assessed. Finally, this study was not a randomized clinical trial. In conclusion, daily application of prepared herbal

Table 1: Phytochemical analysis of Siriyangai leaf extracts

Phytochemical tests	Siriyangai		Kandankathiri	Naval seed	
	Aqueous extract	Methanol extract	Aqueous extract	Aqueous extract	Methanol extract
Alkaloid	Absent	Absent	Absent	Absent	Absent
Flavonoids	Present	Absent	Absent	Absent	Absent
Saponins	Present	Absent	Absent	Present	Absent
Phenols	Present	Present	Present	Absent	Absent
Glycosides	Present	Present	Present	Present	Present ++
Reducing sugar	Absent	Absent	Absent	Present++	Present
Tannins	Absent	Absent	Absent	Presence of catecholic tannins++	Presence of catecholic tannins+
Phytosterols	Absent	Absent	Absent	Absent	Absent
Terpenoids	Absent	Absent	Absent	Absent	Absent
Protein	Present	Absent	Absent	Absent	present
Antraquinone	Present	Present	Absent	Absent	Absent
Coumarin	Present	Present	Present	Absent	Absent
Anthocyanin	Absent	Absent	Absent	Absent	Absent
Phlobatannin	Absent	Absent	Absent	Absent	Absent
Acids	Present	Absent	Present	Absent	Absent

Table 2: Antibacterial activity of copper nanoparticles

Sample	Tested organisms	Zone of the incubation
Copper nanoparticle	<i>E. coli</i>	12±1
Copper nanoparticle	<i>S. aureus</i>	11±1

S. aureus: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*

Table 3: Antibacterial activity of herbal extracts

Test organisms	Siriyanangai		Kandankathiri	Naval seed	
	Aqueous	Methanol	Aqueous	Aqueous	Methanol
<i>S. aureus</i>	14±1	13±1	-	10±1	10±1
<i>E. coli</i>	15±1	12±1	-	10±1	11±1

S. aureus: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*

Table 4: Antibacterial activity of prepared ointment

Tested organisms	Herbal ointment	Zone of the incubation
<i>S. aureus</i>	10 µL	14±1
<i>E. coli</i>	10 µL	15±1

S. aureus: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*

ointment could reduce the wound size significantly approximately in a mean period of 6 weeks without any adverse side effects. The herbal ointment may be effective in treating diabetic foot ulcers along with current standard care.

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