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Pus culture and sensitivity of *Staphylococcus aureus* with *Nimba Patra* (*Azadirachta indica* A.Juss) in *Dushta Vrana* (non healing diabetic ulcer)

Malakiya Chintankumar.G[1], Ajantha[2], MShashirekha.K.S[3]*

1 *PG Scholar, Department of Roganidana, Sri Dharmasthala Manjunatheshwara (SDM) College of Ayurveda & Hospital, Hassan, Karnataka-573201 India*

2 *Associate Professor & Head, Department of Roganidana, Sri Dharmasthala Manjunatheshwara (SDM) College of Ayurveda & Hospital, Hassan, Karnataka-573201 India*

3 *Microbiologist, Department of Roganidana, Sri Dharmasthala Manjunatheshwara (SDM) College of Ayurveda & Hospital, Hassan, Karnataka-573201 India*

ABSTRACT

Prevalence of diabetes mellitus worldwide is 285 million people and India is 50.8 million among whom diabetic foot ulcers is 3%. Non healing diabetic ulcer causes an increasing burden to healthcare and also antibiotic resistance to bacteria is noted. *Nimba Patra* is described as *Krimighna* in Ayurveda and researches show its antimicrobial effects against microorganisms.Therefore, Present study was planned to evaluate and compare sensitivity of alcoholic extract of *Nimba Patra* and aqueous extract of *Nimba Patra* on *Staphylococcus aureus* from pus of non healing diabetic ulcer (*Dushta Vrana*) patient by culture and sensitivity *in vitro*. On comparing the mean values of zone of inhibition observed against different concentrations of alcoholic and aqueous extracts of *Nimba Patra*, the mean value of zone of inhibition of alcoholic extract of *Nimba Patra* (in mm) was higher than that of aqueous extract of *Nimba Patra* which is statistically highly significant. Therefore it is concluded that alcoholic extract of *Nimba Patra* has better antimicrobial action than aqueous extract of *Nimba Patra* against *Staphylococcus aureus* derived from pus of *Dushta Vrana* (non healing diabetic ulcer) patient.

Keywords: Non healing diabetic ulcer, *Dushta Vrana*, *Pus culture and sensitivity*, *Staphylococcus aureus*, *Nimba Patra*, Agar well diffusion method.

INTRODUCTION

The prevalence of diabetes mellitus and its complications is increasing worldwide. There are 50.8 million diabetics in India. Currently 285 million people, i.e. 6.4% of the world's adult population lives with diabetes. In India prevalence of foot ulcers in diabetic patients in clinical population is 3%.[1,2,3]Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action.[4,5]Plants are rich in a wide variety of secondary metabolites, which have also been found in vitro to have antimicrobial properties.[6,7]

In an effort to expand the spectrum of antibacterial agents from natural sources on non-healing ulcer due to diabetes mellitus, a very promising drug *Nimba* (*Azadirachta indica* A.Juss*)* is selected for in vitro study. If *Nimba* is proved to have potent bactericidal effect on organism *Staphylococcus aureus*; then sufficient preliminary scientific evidence is generated in vitro study to use *Nimba* on patients of non healing diabetic ulcer. *Nimba* is described to be useful against ulcers, wounds, skin diseases etc. in folklore practice. *Nimba* leaf paste is applied to boil, ulcer, abscess, inflammation and other similar ailing conditions.[8]Even though *Nimba* is generally used, its efficacy on *Staphylococcus aureus* from diabetic non-healing ulcer needs to be scientifically investigated. Present study was planned to evaluate and compare sensitivity of alcoholic extract of *Nimba Patra* and aqueous extract of *Nimba Patra* on *Staphylococcus aureus* from pus of non healing diabetic ulcer patient by culture and sensitivity *in vitro*.

AIMS & OBJECTIVE

To evaluate and compare sensitivity of alcoholic and aqueous extract of *Nimba Patra (Azadirachta indica* A.Juss) on *Staphylococcus aureus* from pus of *Dushta Vrana* (non healing diabetic ulcer) patient by culture and sensitivity *in vitro*.

***Corresponding author:**
Dr. Malakiya Chintankumar.G
PG Scholar, Department of Roganidana, Sri Dharmasthala Manjunatheshwara (SDM) College of Ayurveda & Hospital, Hassan, Karnataka-573201 India
Email:
malakiyachintan[at]gmail.com

MATERIALS AND METHODS

Source of data

32 patients fulfilling diagnostic and inclusion criteria was included in present study from OPD & IPD of Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, other referrals, special camps etc.

Diagonostic and inclusion criteria

Patients aged between 18-70 years of either sex with FBS ≥126mg/dl and PPBS ≥200mg/dl along with non healing ulcer of at least more than six weeks with or without one or more *Dushta Vrana Lakshanas* like *Kandu, Amanoghnagandha, Atisavrruta, Atimrudu*, *Atiavasanna, Rakta*, *Krushna, Panduvarna*, Covered with *Putimansa, Shotha*, *Paka, Unmargi Vrana*, Excessive *Dushtashonita* discharge, *Puyasrava*.

Exclusion criteria

Patient with any other complication which may interfere in course of the study like varicose vein ulcer, tubercular or malignant ulcer and with ulcer of less than six weeks duration was excluded from this study.

Research design

An observational experimental study.

METHODOLOGY

Pus Specimen was collected from *DushtaVrana*(non healing diabetic ulcer)with sterile swab and subjected to microscopic examination and streak culture by Bloodagar plate and placed in incubator at 37°C and cultured for 24 hours. Identification of bacteria has done by differential staining method, biochemical and serological test were performed to confirm*Staphylococcus aureus*. Aqueous extract of *Nimba Patra* was prepared by soaking method and alcoholic extract by Soxhlet apparatus.[10,11]Mueller Hinton agar plates was swab inoculated with a suspension inoculum, with the turbidity of 0.5 Mac Farland standard.In vitro study was conducted by agar well diffusion method[12,13]. Five equidistant wells were made by sterile cork borer,100µl of different concentrations (2µg, 1µg, 0.5µg, 0.25µg& 0.125µg) ofalcoholic and aqueous *Nimba Patra* extractwere filled separately in different media platesand incubated at 37°C for 24 hrs. Zone of inhibition was measured in mm.

Assessment criteria

If the drug is sensitive a clear circular " halo" (technically known as Zone of Inhibition) will appear around the well, indicating an absence of bacteria, which shows that particular drug is effective against *Staphylococcus aureus*.

Based on in-vitro study susceptibility of *Staphylococcus aureus* against Alcoholic extract of *Nimba Patra* is fairly evident between 20 – 18 mm zone of inhibition. Hence it is considered sensitive, 16 – 14 mm is intermediate, hence moderately sensitive and below 14 mm zone of inhibition as resistant. Zone of inhibition inAqueous extract of *Nimba Patra* is fairly evident between 14 - 12 mm is considered as sensitive, zone of inhibition from 10 - 08 mm is taken as moderately sensitive and below 08 mm is considered as resistant.

Here S - Sensitive, M - Moderately sensitive, R - Resistant

OBSERVATION AND RESULT

In vitro antibacterial activity of alcoholic and aqueous extract of *Nimba Patra* was evaluated by agar well diffusion methodand zone of inhibition is measured as follows.

Alcoholic extract of *Nimba Patra*

Table 1: Mean values of zone of inhibition at different concentrations of alcoholic extract of *Nimba Patra* against *Staphylococcuss aureus*

Different concentrations of alcoholic extract of *Nimba Patra*	2µg	1µg	0.5µg	0.25µg	0.125µg
Total number of patients N	32	32	32	32	32
Mean of zone of inhibition in mm	18.25	15.31	12.12	00	00

Here 18.25 mm is sensitive, 15.31 mm is moderately sensitive, and 12.12 isconsidered as resistant to *Staphylococcus aureus* in spite of possessing weak antibacterial action. Here the alcoholic extract of *Nimba Patra* with the concentration of 0.25µg & 0.125µg has not shown any zone of inhibition.

Aqueous extract of *Nimba Patra*

Table 2: Mean values of different concentrations of aqueous extract of *Nimba Patra* against *Staphylococcus aureus*

Different concentrations of aqueous extract of *Nimba Patra*	2µg	1µg	0.5µg	0.25µg	0.125µg
Total number of patients N	32	32	32	32	32
Mean of zone of inhibition in mm	11.28	9.18	6.0	00	00

Here 11.28 mm and 9.18 mm are moderately sensitive and 6.00 mm is considered as resistant to *Staphylococcus aureus* in spite of possessing weak antibacterial action. Here the aqueous extract of *Nimba Patra* with the concentration of 0.25µg & 0.125µg has not shown any zone of inhibition.

Table 3: Comparing antibacterial action of alcoholic and aqueous extracts of *Nimba Patra* against *Staphylococcus aureus*

Different concentrations of alcoholic and aqueous extract of *Nimba Patra*	2µg	1µg	0.5µg	0.25µg	0.125µg
N (Total number of samples)	32	32	32	32	32
Mean of zone of inhibition of different concentration alcoholic extract of *Nimba Patra* (mm)	18.25	15.31	12.12	00	00
Mean of zone of inhibition of different concentration aqueous extract of *Nimba Patra* (mm)	11.28	9.18	6.0	00	00
Difference of mean in mm	6.97	6.13	6.12	00	00

Statistical analysis of the data was performed using SPSS 20.0 (IBM corp). The means were compared using unpaired- t test.

Table 4: Showing the statistical values by unpaired t test

Concentration	Total number of patient N	Alcohol extract mean	Aqueous extract mean	Mean difference	t value	p value	Significance
2µg	32	18.25	11.28	6.97	18.26	0.000	HS
1µg	32	15.31	9.18	6.13	18.93	0.000	HS
0.5µg	32	12.12	6.00	6.12	9.37	0.000	HS
0.25µg	32	00	00	00	-	-	-
0.125µg	32	00	00	00	-	-	-

On comparison of mean of zone of inhibition (in mm) between groups by unpaired t test at concentration 2µg, 1µg, 0.5µg shows that there is statistically highly significant difference with $p < 0.001$.

On comparing the same concentrations of alcoholic and aqueous extracts of *Nimba Patra* by unpaired- t test, alcoholic extract of *Nimba Patra* is having better antibacterial action than aqueous extract of *Nimba Patra* against *Staphylococcus aureus.*

DISCUSSION

In the present study 47 patients presenting with *Dushta Vrana* (non healing diabetic ulcer) were screened, among them 32 patients fulfilled diagnostic and inclusion criteria and remaining 15 patients were excluded. Among excluded 15 patients, 4 were below 18 years of age, 3 patients samples were infected with microorganism other than *Staphylococcus aureus*, 5 patients were non diabetic, 2 Patients were with varicose vein ulcer and 1 patient had ulcer of less than six weeks duration. This shows the higher prevalence of *Staphylococcus aureus* bacteria in causation of *Dushta Vrana* (non healing diabetic ulcer).

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as fungi, bacteria etc. *Nimba* (*Azardiracta indica* A.Juss) has antibacterial, antifungal, antiparasitic, anti-viral and insecticidal properties. *Nimba* has *tikta rasa, laghuguna, sheetavirya*, *katuvipaka,* it pacifies *kapha* and *pitta dosha*, prevents the formation and growth of *krimi.* In *Susruta Samhita Nimba* is mentioned in *Aragwadhadi, Guduchyadi* and *Lakshadigana*. In *Charaka Samhita* it is mentioned in *Kandugna* and *Tiktaskanda*. Therefore considering the merits *Nimba Patra* was selected for present study. Alcoholic extract of *Nimba Patra* was obtained by soxhalation process in Soxhlet apparatus. Aqueous extract of *Nimba Patra* was prepared by soaking method. *Nimba Patra (Azadirachta indica* A.Juss) alcoholic and aqueous extract was dissolved in Dimethyl Sulfoxide (DMSO) and further diluted to required concentration like 2µg, 1µg, 0.5µg, 0.25µg & 0.125µg.

The phyto-constituents like alkaloids, glycosides, saponinsetc are active principles of the plant having defensive mechanism against different pathogens.[14] Inthe present study Soxhlet extraction method was chosen as it is very easy to extract and potent among all dosage forms and is very commonly used in day to day practice. The Soxhlet method is very simple and cheap. The advantages of conventional Soxhlet extraction include the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix, maintaining a relatively high extraction temperature with heat from the distillation flask and does not require filtration after leaching. Alcohol provides a particularly effective way of maximizing the bioavailability of the actives extracted from the plant. Ethanol is a molecule with both the polar and non-polar ends.The soaking method aqueousextraction is very easy to extract and potent in dosage forms. It is commonly used and cheap.

From *Dushta Vrana* pus specimen was collectedwith sterile swab and streak culture by Blood agar plate and placed in incubator at 37°C and cultured. Next, Gram staining was carried out and *Staphylococcus aureus* bacteria was identified. Then Mueller Hinton agar plate was swabbed with *Staphylococcus aureus* from 24 hours old culture. Study was conducted by agar well diffusion methodwith different concentrations of alcoholic and aqueous extract of *Nimba Patra* separately in different media platesand incubated at 37°C for 24 hrs. Zone of inhibition was measured in mm after incubation.

On consideration of mean value of zone of inhibition derived by alcoholic extract and aqueous extract of *Nimba Patra (Azadirachta indica* A.Juss) against *Staphylococcus aureus* shows that, Mean value of zone of inhibitions at 2µg concentration of alcoholic extract (mean=18.25 mm) is greater than 2µg concentration of aqueous extract of *Nimba Patra* (mean=11.28 mm) with a mean difference of 6.97mm. Mean value of zone of inhibitions at 1µg, concentration of alcoholic extract of *Nimba Patra* (mean=15.31 mm) is greater than 1µg, concentration of aqueous extract of *Nimba Patra* (mean=9.18 mm) with a mean difference of 6.13 mm. Mean value of zone of inhibitions at 0.5µg concentration of alcoholic extract (mean=12.12 mm) is greater than0.5µg concentration of aqueous extract (mean=6.0 mm) with a mean difference of 6.12 mm. In present study alcoholic extract and aqueous extract of *Nimba Patra* with concentration of 0.25µg & 0.125µg did not show any zone of inhibition. Therefore current study shows that lower concentration (0.25µg & 0.125µg) of alcoholic extract and aqueous extract of *Nimba Patra* does not have any antibacterial activity against *Staphylococcus aureus*.

CONCLUSION

Alcoholic and aqueous extract of *Nimba Patra* (*Azadirachta indica* A.Juss) possesses antimicrobial (*Krimighna*) action against *Staphylococcus aureus* derived from pus of *Dushta Vrana* (non healing diabetic ulcer) patient. On comparing the mean values of zone of inhibition observed against different concentrations of alcoholic and aqueous extracts of *Nimba Patra,* the mean value of zone of inhibition of alcoholic extract of *Nimba Patra* (in mm) is higher than that of aqueous extract of *Nimba Patra*and statistically highly significant. Therefore itis concluded that Alcoholic extract of *Nimba Patra* has better antimicrobial action than Aqueous extract of *Nimba Patra* against *Staphylococcus aureus* derived from pus of *Dushta Vrana* (non healing diabetic ulcer) patient. Further it is evident that as the concentration of the alcoholic and aqueous extract of *Nimba Patra* increases progressively, zone of inhibition also increases respectively. Hence concluded that as the concentration increases the antimicrobial activity also increases against *Staphylococcus aureus*.

REFERENCE

1. Shah S N. API Text Book of Medicine. 7th ed. New Delhi: association of Physicians of India; 2003. p.1624.
2. Singh N,Armstrong DG, Lipsky BA.Preventing foot ulcer in patients with diabetes. JAMA2005;293:217-228.

3. Pendsey SP, Epidemiological aspects of Diabetic Foot. Int. J Diab. Dev Countries 1994;:37-38.
4. Runyoro D,Matee M, Olipa N , Joseph C, Mbwambo H. Screening of *Tanzanian medicina* plants for anti –Candida activity .BMC Complement Altern Med.2006;6:11.
5. Shahidi BH. Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus*, *Serratia marcescens*, *Klebsiella pneumonia* and *Bordetella bronchoseptica*. Asian JPl ant Sci.2004;3:82–6.
6. DahanukarSA , Kulkarni RA, Rege N N.Pharmacology of medicinal plants and natural products. Indian J Pharmacol.2000;32:S81–118.
7. Cowan MM.Plant products as anti-microbial agents.ClinMicrobiol Rev.1999;12:564–82.
8. Pandey.G. Dravyagunavijnana(material medica vegetable drugs)part 2-. 1st ed. Varanasi: Chowkhambhakrishnadas Academy;2001.p.693
9. B.S.Nagoba,AshaPichare,Medical Microbiology.2nded.new Delhi; a division of Reed Elsevier India private Limited;2014.Pg.16
10. Wikipedia.accesed on 2nd February.2016.
11. William Charles Evans,Trease and Evans Pharmacognosy.15th ed. New Delhi; a division of Reed Elsevier India private Limited;2005.Pg.98
12. Wikipedia. Agar diffusion test. http://en.wikipedia.org/wiki/Agar_diffusion (accessed 04/03/16).
13. Saokar M. Reshma, Sarashetti R.S, Kanthi, SavkarMadhav, Nagthan C.V. Screening of Antibacterial and Antifungal activity of GandhakaRasayana – An Ayurvedic Formulation. International Journal of Recent trends in Science and Technology. 2013;8(2):134-37.
14. Maragathavalli, S. Brindha, S., Kaviyarasi, N.S., B. Annadurai, B. &Gangwar, S.K. Antimicrobial activity in leaf extract of neem (*Azadirachta indica* Linn.) I.J.S.N., VOL. 3(1) 2012: 110-113.

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