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RESEARCH ARTICLE
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ANALYSIS OF LAKSHADIGANA TAILA: A TRIAL AYURVEDIC FORMULATION TO HEAL INFECTED WOUNDS (DUSHTA VRANA)

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Abstract

Background

Due to awareness and general acceptability for the use of herbal drug in today's medical practice. Standardization of herbal formulation is essential in order to assess the quality, purity, Safety and efficacy of drug which is based on amount of their active constituents. Newer guidelines for standardization, manufacture, quality control and scientifically rigorous research are essential for traditional treatments for its global acceptance. Herbal drugs induce healing by a number of modes of action. Because of their traditional applicability, affordability and safety plants gained a reputed position in the world of Infected wound (Dushta Vrana) management but scientific evidence for their wound healing potentials is less.

Aim

To Analysis of trial drug Lakshadigana Taila and its ingredients.

Materials and methods

Four most available plants in local area of Banaras Hindu University plants were selected to make a Ayurvedic formulation in the form of Taila for Shodhana and Ropana of Dushta Vrana (Infected Wound). These plants were Laksha, Nimba, Haridra and Amaltas from Lakshadigana of Sushruta Samhita and after discussion it named as Lakshadigana Taila. This was used to treat patients of Dushta Vrana (Infected chronic wounds).

Investigation performed: Macro-Microscopy, pH, Methylation & GC-MS identification of Medicated Oil (Base – Til oil) Sesamum indicum

Sample details: Medicated oil (Base – Til oil) Sesamum indicum, Laksha, Nimba, Haridra and Aragvadha.

Results

By Observing analysis of Lakshadigana Taila and its ingredients it is clear that this trial drug can be used as in Dushta Vrana (Infected chronic wound) due to its antimicrobial activity (Vranashodhana Activity). Many supportive finding like analysis and antimicrobial activity of Lakshadigana Taila and its four drugs (Laksha, Nimba, Haridra and Amaltas with Til Taila (Sesame oil) indicate that it having Vranaropaka activity too.

Key Words: Lakshadigana Taila, Analysis, Infected Wound, Dushta Vrana, Antimicrobial activity.

Introduction

India is rich heritage of traditional medicines. Ayurveda, Siddha, Unani, Homeopathy and Naturopathy are various branches of medicines. According to WHO, about 70% of world population extensively use traditional and alternative medicines for the healthcare. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will definitely help not only to preserve the traditional heritage but also to rationalize the use of natural product in healthcare. It is very important to establish a system of standardization for every plant medicine in the market, science the scope for variation in different batches of medicine is enormous.

Plant material when used in bulk quantity may vary in its chemical content and therefore, in its therapeutic effect according to different batches of collection with respect to different geographical and environmental conditions. So as to assess the uniformity in manufacture there is a need for ensuring the quality, efficacy and standard of Ayurvedic formulations. Quality assurance of herbal product may be ensured by proper quality control of herbal ingredients and by means of good manufacturing practice. As per American Herbal Product Association "standardization refers to the body of information and control necessary to product material of reasonable consistency".1

Breaching of skin or underlying tissue due to any accident, act of violence or surgery is called as wound.2 A rough prevalence rate for chronic nonhealing wounds in developed countries is 1% to 2% of the general population3

Certain factors that influence wound healing include bacterial infection, nutritional deficiency, drugs, site of wound etc., All chronic wounds intrinsically contain bacteria, and the process of wound healing can still occur in their presence. It is, therefore not the presence of bacteria but their interaction with the host that determines the organisms' influence on chronic wound healing. However, the relative number of micro-organisms and their pathogenicity, in combination with host response and factors, such as immunodeficiency, dictate whether a chronic wound becomes infected or shows signs of delayed healing.

Wound infection is defined as the presence of replicating microorganisms within a wound with a subsequent host response that leads to delayed healing. Because of this it is important that infection is recognized as early as possible. The signs and symptoms of local infection are redness (erythema), warmth, swelling, pain, and loss of function. Foul odor and pus may accompany this. Eventually, the local bacterial burden will increase further and become systemically disseminated resulting in sepsis, which if not actively treated could progress to septicemia and multi-organ failure.

There are several factors known to affect the bacterial burden of chronic wounds and increase the risk of infection. These include the number of microorganisms present in the wound, their virulence, and host factors. Experimental studies have demonstrated that regardless of the type of microorganism, impairment of wound repair may occur when there are more than 1×105 organisms per gram of tissue. Hence, it becomes essential that a drug used to control the wound infection must necessarily antimicrobial activities.

Wound in Ayurveda is Vrana which has been stated as " Vranagatravichurne" i.e of tissue distrucion and discoloration of viable tissue to various etiology. In Ayurveda Infected wound or nonhealing ulcer may be correlated with Dushta Vrana and lakshana of it well explained by Maharshi Sushruta, Charaka , Vagbhata and Madhavakar are clearly mentioned as foul smelling, continuously flowing pus, along with blood, with cavity, since long time etc. Maharshi Sushruta has scientifically classified in a systemic manner a wealth of clinical material and the principles of management which are valid even today. The present analysis is an attempt to highlight one of the Dhusta Vrana Shodaka (Wound debridement) and Vrana Ropaka (healing of wound) drug i.e, Lakshadigana which has been described in Sushruta Samhita Sutra Sthana. From Lakshadigana of Maharshi Sushruta's description four drug (Laksha, Nimba, Haridra, Amaltas) selected and formed a medicated Taila for local application to Dushta Vrana (infected wound).

Table No.1. Ingredients of Lakshadigana Taila and their general Information:

Sr. No.	Name	English name	Scientific Name	Family	Used Part
1.	Laksha	lac	Laccifera Indica	Lacciferidae	Resin of Insect origin
2.	Nimba	Neem	Azadirachta indica	Meliaceae	Leaves
3.	Haridra	Turmeric	Curcuma longa	Zinziberaceae	Rhizome
4.	Amaltas	Purging Cassia	Cassia fistula	Leguminosae	Fruit Pulp
	Til (Base oil)	Sesame	Sesamum indicum	Pedaliaceae	Tail

Materials and Methods:

The study employed for analysis of trial medicated oil (Lakshadigana Taila) so that its antimicrobial and wound healing properties can be established. The approval from the Institutional Ethical Committee (IEC) was taken and informed consent from each patient All the formulations were standardized on the basis of organoleptic, microscopical, physical characteristic and physico chemical properties.

Trial Drug: Lakshadigana Tail

Collection and identification of plant materials

The ingredients of Trial drug were fresh pods of plants Aragvadha, leaves of Nimba and Rhizome of Haridra were collected from various region of Banaras Hindu University Campus. Laksha was purchased from local market of Varanasi, U.P. India. The plant specimens were identified and deposited in the Dravyaguna Department, Faculty of Ayurveda, IMS, BHU, Varanasi.

For further analysis trial drug and its ingredients were sent for analysis to Karnataka, S.D.M. Centre for Research in Ayurveda and Allied Sciences (AYUSH Centre for Excellence and Recognized Scientific and Industrial Research Organizations (SIROS) by the Department of Scientific and Industrial Research (DSIR). ANALYSIS REPORT FOR 711/16011302-06

Preparation of Trial Drug (Lakshadigana Taila):

Fresh Nimba leaves, Haridra rhizome was cleaned with normal water and then dried in shade. Before releasing pulp of Aragvadha from its Pods, Aragvadha kept under sand for 7 days. Laksha cleaned with normal water and then dried in shade.

Figure 1. IMAGES OF INGREDIENT OF LAKSHADIGANA TAILA.

Laccifera lacca (Resin of insect origin)	Azadirachta indica (Leaves of Nimba)	Curcuma longa (Rhizome of Haridra)	Cassia fistula (Pulp of Amaltas)

Identified ingredients Laksha (resin of insect origin (Laccifera lacca), Nimba patra (Azadirachta indica, Rhizome of Haridra (Curcuma longa) and Amalatas Pulp (Cassia fistula) of Lakshadigana was prepared to make kwath (decoction) and after then Lakshadigana Taila was made according description as classical method Sneha pak kalpana (Kalka, Sneha ,Til Tail (Sesamum indicum) and Kwath-Ratio 1:6:24) according to Sharangadhar Samhita Madhyam Khand 9/6 and madhyam pak lakshana used according to Sushruta Chikitsasthan 31/ 8-12. Then, Lakshadigana Tail was kept in air tight container.

Part A: Particulars of sample submitted

Investigation performed: Macro-microscopy, pH, Methylation & Gas chromatography-mass spectrometry (GC-MS).
Sample coded as: 16011302-06
Sample details: Medicated oil, Laksha, Nimba, Haridra and Aragvadha.

Part B: Methodology

Organoleptic characters
Organoleptic characters of the test sample were documented by means of examination using sensory organs

Macroscopy

The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication.

Microscopy

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Determination of pH

Preparation of buffer solutions
Standard buffer solution: Dissolved one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water.
Determination of pH: 1 ml of sample was taken and make up to 10 ml with distilled water, stirred well and filtered. The filtrate was used for the experiment. Instrument was switched on. 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading were taken as result.

Methylation:

0.2 g of medicated oil sample is taken in 50 ml F.B Flask. Then added 2.4 ml of Methanolic-HCl & 12ml of Methanol. Reflux it for 1 Hour at 100°C. Then check the TLC for the completion of the reaction & reaction mixture was extracted with Hexane, Hexane layer was taken, dried it at room temp & sample Gas chromatography-mass spectrometry (GC-MS) was given for LCMS Analysis.

GC – MS

Instrument : GC-MS-5975C [AGILENT]
GC CONDITION
Column Oven Temperature : 70˚C
Injector Temperature : 250˚C

Injection Mode : Split
Split Ratio : 10
Flow Control Mode : Linear Velocity
Column Flow : 1.5 ml/min
Carrier Gas : Helium 99.9995% purity
Injection volume : 1 microlitre

Column Oven Temperature Program

Rate	Temperature(˚C)	Hold Time(min)
-	70.0	3.0
10	300	9.0 [35.0 mts total]

COLUMN :DB-5ms Agilent
Length :30.0m
Diameter:0.25mm
Film Thickness :0.25um
MS CONDITION
MS
Ion source temp : 230 °C
Interface temp: 240°C
Scan range : 40 – 700 m/z
Solvent cut time: 3 mins
MS start time : 3 (min)
MS end time : 35 (min)
Ionization : EI (-70ev)
Scan speed : 2000
MS LIBRARY
NIST- 11.L
Part C: Results

Table 2: Organoleptic characteristics of medicated oil

Parameters	**Medicated oil**
Color	Greenish yellow
Odor	Characteristic
Taste	Acrid

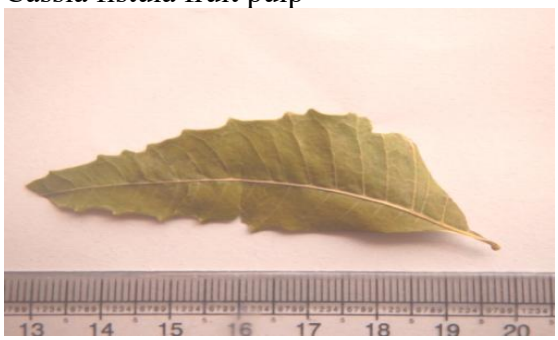
Table 3. pH of Medicated oil

Parameters	**Medicated oil**
pH	6.0

Figure 2: Macroscopy of Cassia fistula, Azadirachta indica, Curcuma longa and Laksha

Cassia fistula fruit pulp

Curcuma longa rhizome



Azadirachta indica leaf

Laksha-Laccifera lacca-Resin

Figure 3: Microscopy of leaf of Azadirachta indica

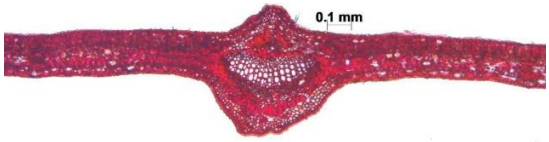


Fig 3a: TS of lamina passing through midrib

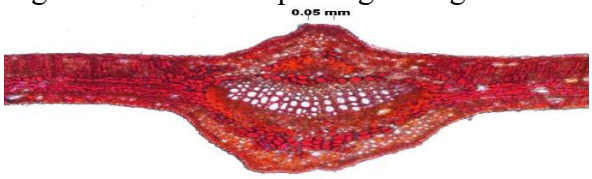


Fig 3b: Midrib and Lamina enlarged

Col – Collenchyma; LE – Lower epidermis; Me – Mesophyll; Pa – Parenchyma; Pal – Palisade; PF – Pericyclic fibres; Ph – phloem; SP – Spongy parenchyma; UE – Upper epidermis; T – Trichome; Xy – Xylem.

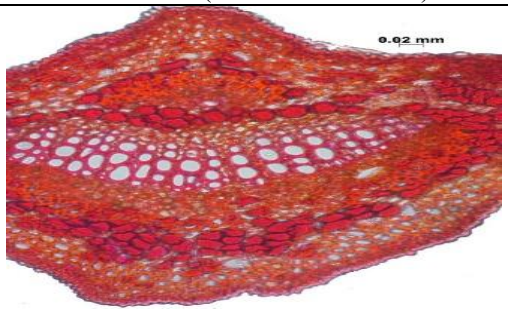


Fig 3c: Midrib enlarged

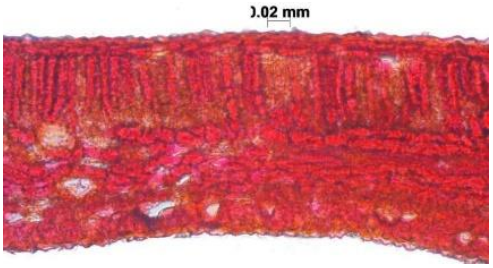


Fig 3d: Lamina enlarged

Col – Collenchyma; LE – Lower epidermis; Me – Mesophyll; Pa – Parenchyma; Pal – Palisade; Pap – Papilla; PF – Pericyclic fibres; Ph – Phloem; SP – Spongy parenchyma; UE – Upper epidermis; Xy – Xylem.

Figure 4: Microscopy of rhizome of Curcuma longa

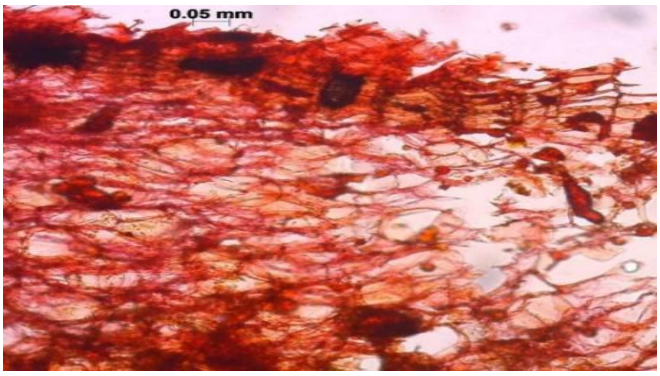


Fig 4a: Outer region – cork and cortex

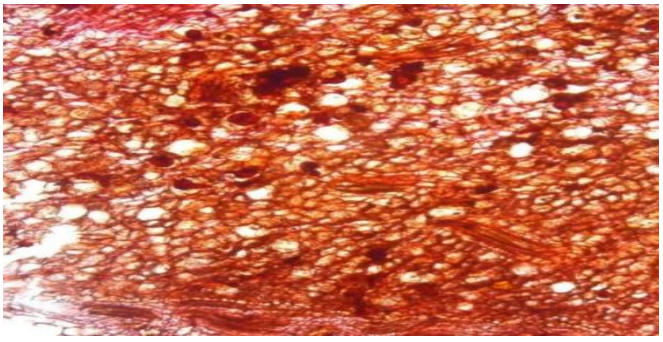


Fig 4b: Inner region- inner cortex and Endodermis

Ck – Cork; Ct – Cortex; End – Endodermis; OD – Oil drop; Pa – Parenchyma; Ve – Vessel; VOC – Volatile oil cell.

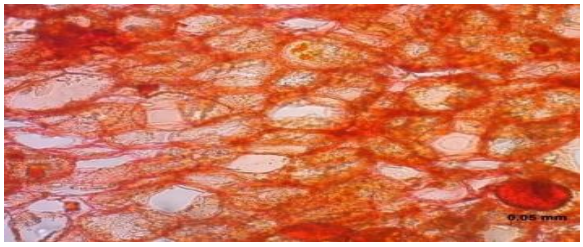


Fig 4c: Cortex

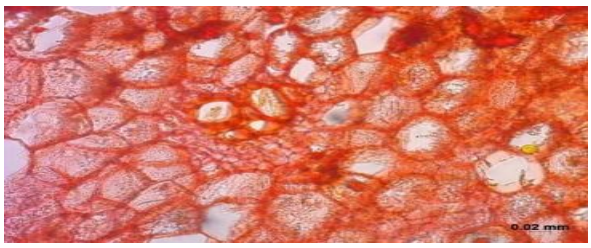


Fig 4d: Vascular bundle

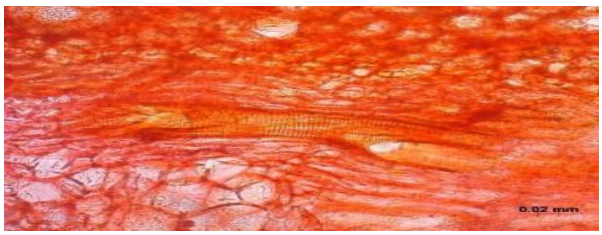
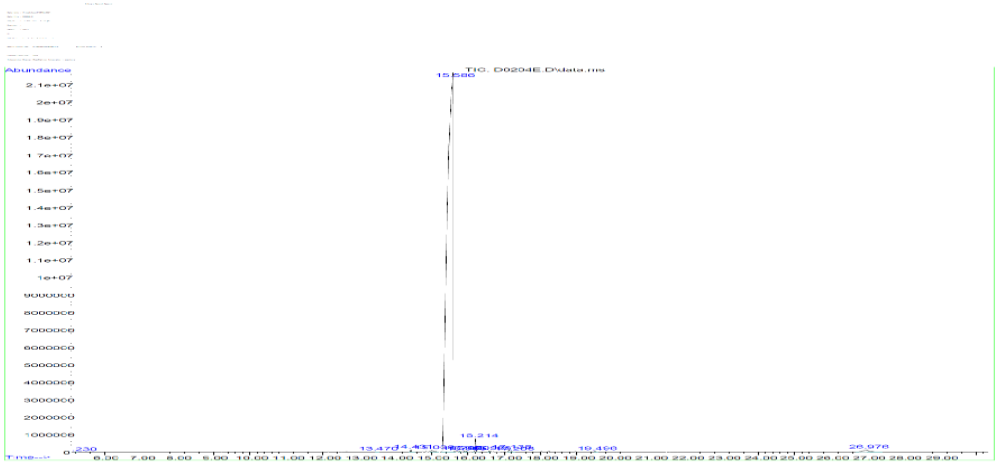


Fig 4e: Endodermis and xylem parenchyma

Ck – Cork; Ct – Cortex; End – Endodermis; Pa – Parenchyma; SG – Starch grains; Ve – Vessel; VOC – Volatile oil cell.

Figure 5. GLC chromatogram of Medicated oil



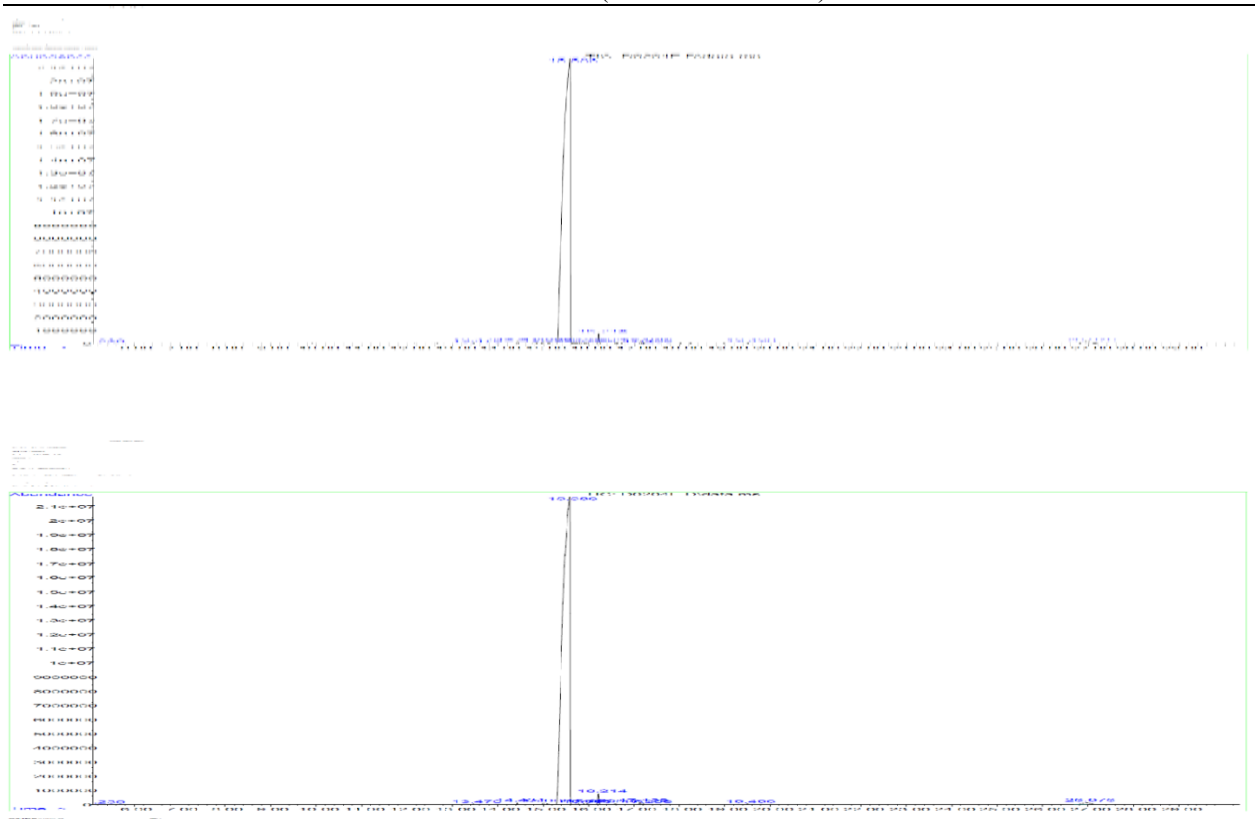
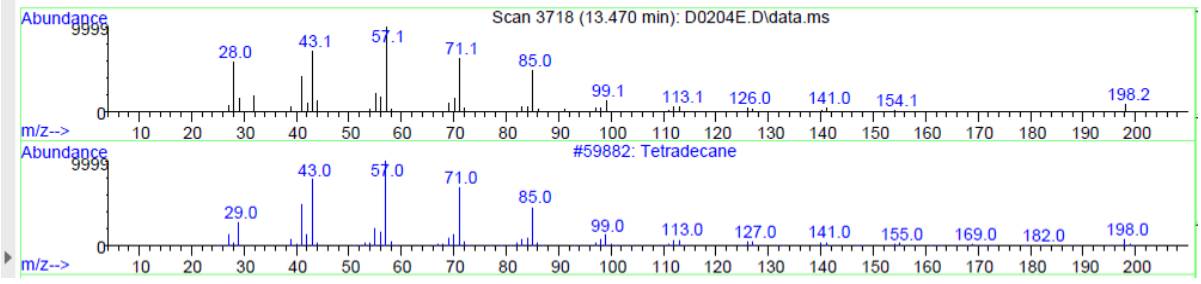


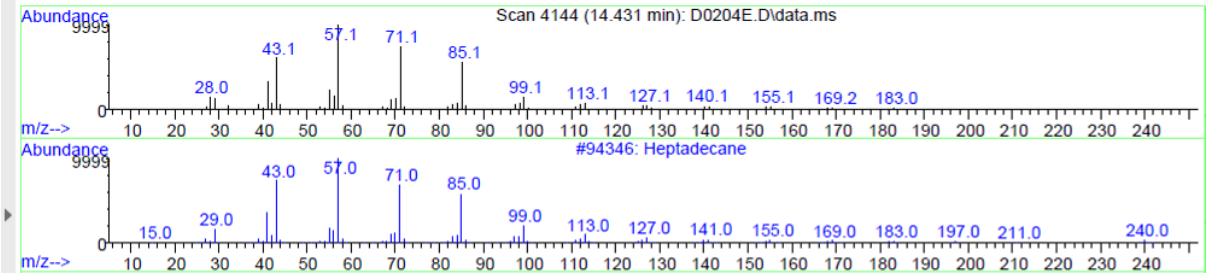
Table 4. GC-MS identification of Medicated Oil (Base – Til oil) Sesamum indicum

Peak	RT	% Area	Name
1	5.231	0.00	-
2	13.470	0.02	Tetradecane
3	14.431	0.09	Heptadecane
4	15.008	0.21	Pentadecane, 2-methyl-
5	15.585	98.25	Hexadecane
6	15.779	0.04	Dodecane, 2,6,10-trimethyl-
7	15.906	0.03	-
8	15.971	0.03	-
9	16.215	0.50	Heneicosane
10	16.587	0.03	-
11	17.139	0.09	Octadecane
12	17.209	0.03	-
13	19.496	0.04	Octacosane
14	26.975	0.63	Squalene

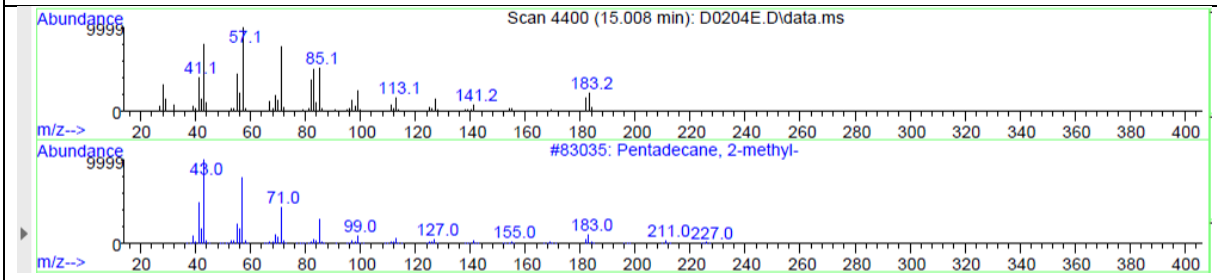
Figure 6. Mass spectrum of compounds identified from Medicated oil



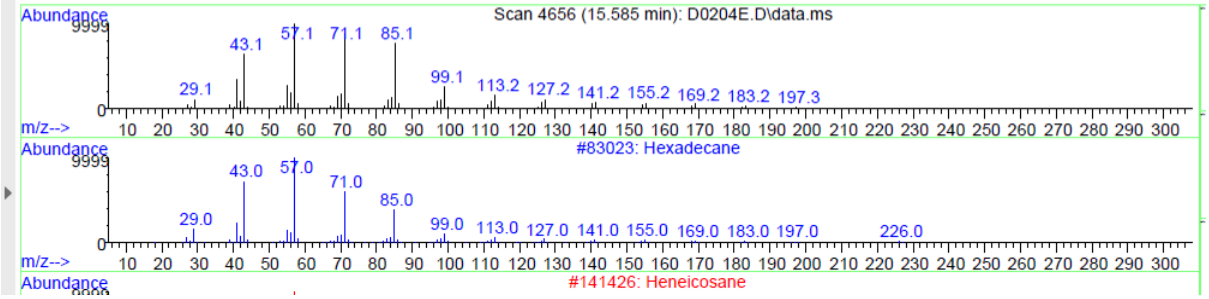
Tetradecane



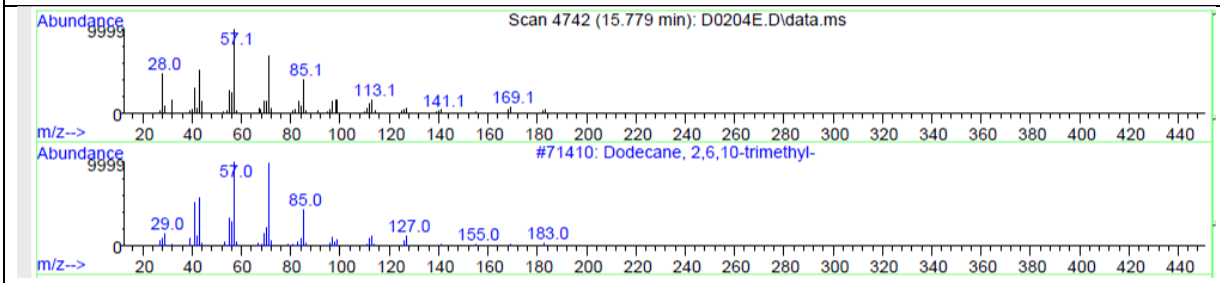
Heptadecane



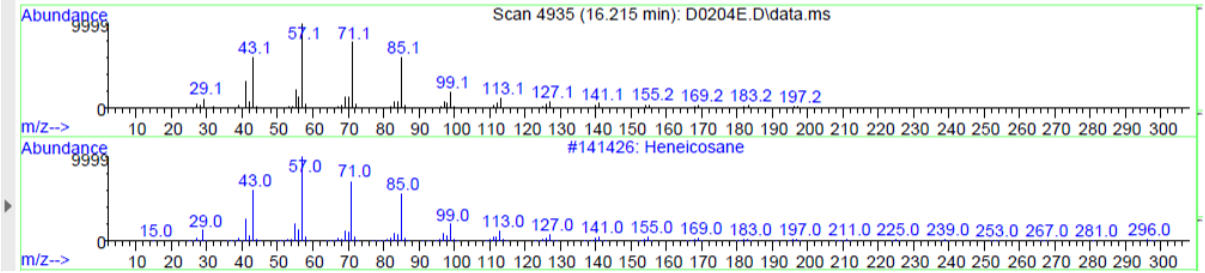
Pentadecane, 2-methyl-



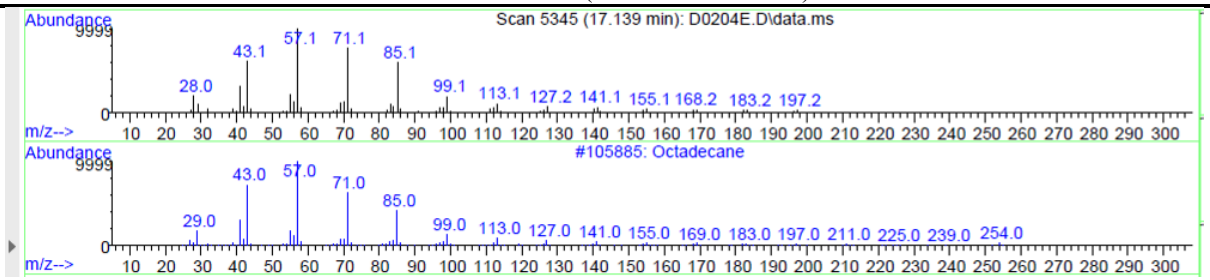
Hexadecane



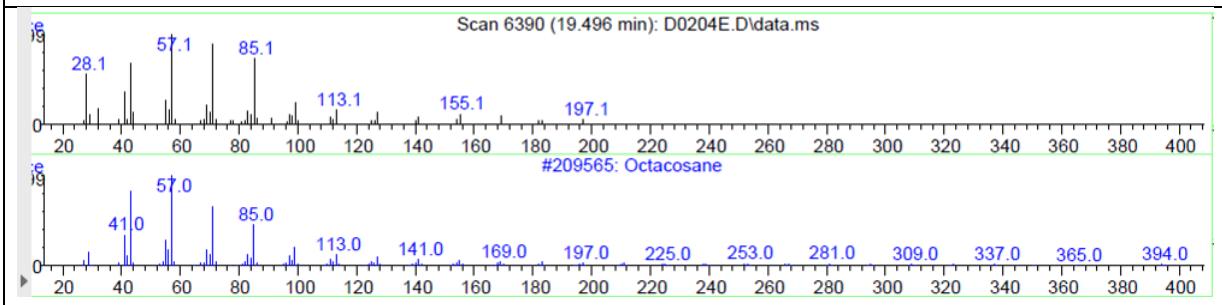
Dodecane, 2,6,10-trimethyl-



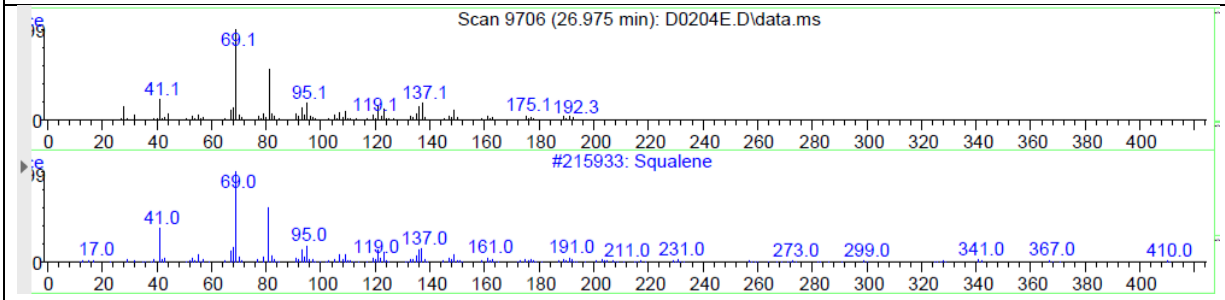
Heneicosane



Octadecane



Octacosane



Squalene

Part D: Remarks

Macroscopy characters of Aargavdha pulp, Haridra rhizome, Nimba leaf and Laksha were documented. Microscopies of only organized drugs such as Haridra rhizome, Nimba leaf were performed. The authenticity of the raw drugs is confirmed by the macro-microscopic examination.

Medicated oil was examined for pH and compositional analysis by methylation of fatty acids. Fourteen constituents were eluted by GCMS. Out of fourteen constituents 5 could not be identified as the mass fragmentation showed similarity below 70%. The chromatogram is shown in Figure 5. Mass spectrum matching of identified compounds with that of reference mass spectrum of the compounds are shown in Figure 6. Peak number, RT, % area and name of the compounds detected from the sample is given in Table 4. These botanical chemical fingerprints can be used for authentication of the medicated oil.

Culture and Sensitivity of Trial Drug & Extract of Ingredients of Trial Drug:

Method of preparation of ethanolic extract of Laksha, Nimba, Haridra and Amaltas

Fresh Nimba leaves, Haridra rhizome was collected from Banaras Hindu University campus cleaned with normal water and then dried in shade. The pods of Aragvadha were collected from Banaras Hindu university campus. Before releasing pulp of Aragvadha from its Pods, Aragvadha kept under sand for 7 days. Laksha collected from local market of Varanasi and cleaned with normal water and then dried in shade. Coarse powder was made of all part of plants. This powder was filled in a thimble of blotting paper and put in Soxhlet apparatus for extraction 16 hrs. It was filtered with Whatman no. 1 filter paper and concentrated in vacuum by rotary evaporation. Thus, types of solvent free extracts were obtained and their % yield (w/w) was calculated according to the amount of coarse drug powder used for extraction.

Table No.5: Yield of Extracts

Drug	Part used	Yield (w/w)
Laksha	Resins	16%
Nimba	Leaves	18%
Haridra	Rhizomes	22%
Amaltas	Pulp	27%

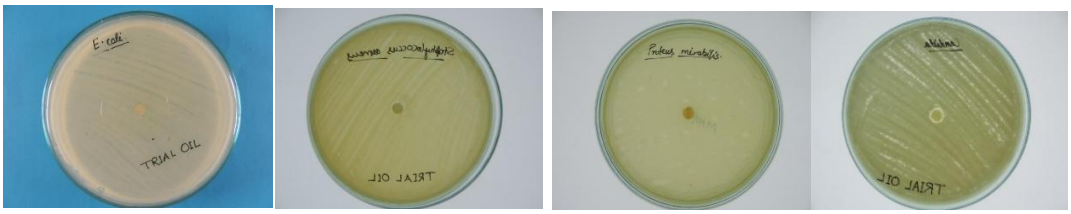
Test microorganisms and growth media

The newly formed trial oil was tested for its activity against different bacteria. The test was performed in the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi. The following organism strains Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis and Candida krusi were chosen based on their clinical and pharmacological importance. Mueller Hinton agar (Himedia, Mumbai) without glucose was used for antibacterial activity and fungus was grown in potato dextrose agar (PDA) media at 280C. The stock cultures were maintained at 40c.

Disk diffusion method

The assay was performed for primary screening of antimicrobial activity of trial oil, suspension of fresh grown bacterial isolates was made in normal saline (0.9%) to achieve concentration of (1-2) × 108 CFU/ml. This suspension was spread with sterile cotton swab to get a uniform microbial growth on test plates and allowed to dry for 5 minutes. The essential oil was dissolved in 10% aqueous Dimethyl sulfoxide (DMSO) and Tween 80 (0.5% v/v for easy diffusion) and sterilized by filtration through 0.45µm membrane filter.4 Prepared sterile discs (Whatman filter paper No.1) of 5 mm diameter was placed on the medium surface and 10 µl of trial oil and 5 extract of plant was dispensed and allowed to diffuse for 10 min and the plates were kept for incubation at 370C for 24 hrs following refrigeration storage at 40c and 5 µl of extract of plant part (Resin of Laksha, leaves of Nimba, Rhizome of Pulp of Aragvadha) were taken with repetition of above same procedure.5,6 After incubation, inhibitions zones were examined around the disc which if present were measured in millimeters. The sensitivity of the microorganism species to the plant extract and oil were determined by measuring the sizes of inhibition zones (including the diameter of disc) on the agar surface around the discs, and value < 8 mm were considered as not active against microorganisms.

Figure No.7: Culture and Sensitivity of Trial Drug



Disc images showing

1.Inhibition Zone of Extract of Curcuma longa

2.Inhibition Zone of Extract of Azadirachta indica

3.Inhibition Zone of Extract of Laccifer lacca

4.Inhibition Zone of Extract of Cassia fistula

Figure No.8: Each plate showing of extract of trial drug ingredients culture and sensitivity

Disc images showing

1. Inhibition Zone of Extract of *Curcuma longa*

2. Inhibition Zone of Extract of *Azadirachta indica*
3. Inhibition Zone of Extract of *Laccifer lacca*
4. Inhibition Zone of Extract of *Cassia fistula*

Figure No.8: Each plate showing of extract of trial drug ingredients culture and sensitivity

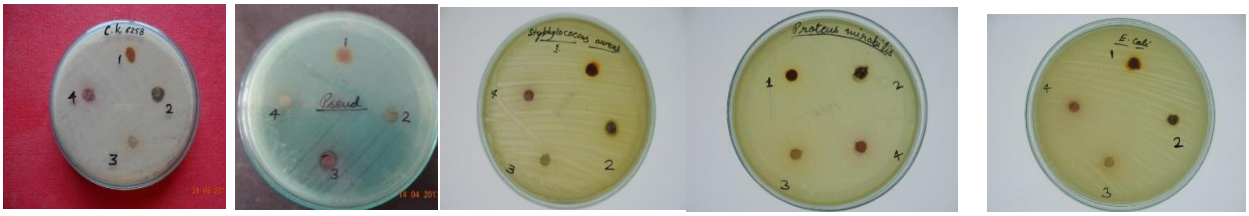


Table No.6: Result of Antimicrobial Activity of Ethanolic Extract of Trial Drug Ingredients and Trial Drug

Microorganism	Zone of inhibition (mm)				
	Ethanolic Exract of Trial oil Ingredients (5µl)				Trial oil (10µl)
	Azadirachta indica	Cassia fistula	Curcuma longa	Laccifera lacca	
Escherichia coli	11	10	-	13	8
Pseudomonas aeruginosa	12	-	9	14	-
Staphylococcus aureus	12	11	-	12	8
Proteus mirabilis	13	12	8	-	-
Candia krusei	9	8	-	-	12

Culture and sensitivity study of all ingredients was performed. In this study, Lakshadi Gana Taila shows activity against Candida krusei, Staphylococcus aureus and Escherichia coli. Extract of Nimba leaves (Azadirachta indica) shows activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis and Candida krusei. Extract of Amaltas pulp (Cassia fistula) shows activity against Escherichia coli, Staphylococcus aureus, Proteus mirabilis & Candida krusei. Extract of Haridra rhizome (Curcuma longa) showed activity against Pseudomonas aeruginosa and Proteus mirabilis. Extract of Laccifer lacca showed activity against Escherichia coli, Pseudomonas aerugenosa, and Staphylococcus aureus.

Discussion:

The authenticity of the raw drugs is confirmed by the macro-microscopic examination. Medicated oil was examined for pH and compositional analysis by methylation of fatty acids. Gas chromatography mass spectrometry identification of was performed. GCMS showed that there is 14 compounds were present out of which 5 new compounds detected in Lakshadi Gana Taila. These 9 compounds are – Tetradecane, Heptadecane, 2-Methy Pentadecane, Hexadecane, 2, 6,10- Trimethyl Dodecane, Heneicosane, Octadecane, Octacosane and Squalene.

Result:

The main objective of treating a wound is to either shorten the time required for healing process or to minimize the undue effects. Plants due to presence of various valuable active phytoconstituents have immense potential for management and treatment of wounds over the years. I may be concluded that Lakshadigana anad its parts analysis shows that it having antimicrobial and wound healing factors and it may be used to treat infected wound (Dushta Vrana).

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