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ANTIMICROBIAL ACTIVITY OF LODRA VATA KASHAYA ON MICRO-ORGANISMS OF VAGINAL INFECTIONS

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ABSTRACT

Background: The increased occurrence of the antibiotic resistance has added up to the misery of mankind. There is an immediate need to search alternatives and *Symplocos racemosa* and *Ficus benghalensis* one such potent medicinal plants which is helpful in treating Shweta pradara. The plant may exhibit tremendous activity for its potential phytoconstituents. An action of drugs depends on the phytochemical constituents present in it, which make the drug effective enough to cure the disease. The aim of the study was to explore various antimicrobial activities of the active components. **Methods:** *Lodra Vata kashya* is mentioned in *Astanga sangarha* for treatment of Shweta pradara. Hence the same reference is used here. Cultures were prepared using well diffusion method. **Formatted:** Line spacing: single **Formatted:** Line spacing: single **Formatted:** Left **Formatted:** Line spacing: single. **Results:** Antibacterial effect was seen during 24 hrs of incubation at higher volumes of Lodra vata kashaya used against *Staphylococcus aureus*. The activity of these plants against different bacteria, fungi and parasites might be due to the presence of a wide variety of active secondary metabolites such as flavonoids, phenolic acids, coumarins, terpenoids and sterols. **Conclusions:** These findings suggest that *Symplocos racemosa* and *Ficus benghalensis* bark could be a potential source of antimicrobial

metabolites, which could substantially contribute to the arsenal of existing natural drugs for combating the antimicrobial resistance.

KEYWORDS: Anti-microbial resistance, *Symplocos racemosa*, *Ficus benghalensis*, *Staphylococcus aureus*.

INTRODUCTION

Shwetapradara is mentioned as a symptom in various *yoni vyapada* and various other gynaecological diseases but not a disease. *Shweta pradara* occurs due to vitiation of *dosha.* Similarities can be seen in colour, consistency of discharge described in the classics for various *yonivyapada* with presence of various microorganisms. Treatment of Shweta pradara is mainly based on the use of drugs which are having predominance of Kashaya rasa and and Ruksha properties. Both the drugs *Lodra* and *Vata* are easily available, cheap and has shown the inhibitory actions in antimicrobial assay in various studies.

Antimicrobial resistance (AMR), a phenomenon which owes its emergence to overuse of antibiotics in humans and food- producing animals, is a global threat now a days and its spread is facilitated by suboptimal infection control measures. There is a surge in infectious diseases that are difficult and sometimes impossible to treat successfully due to multidrug resistant nature of the causal organisms.[1] Existing studies on the pharmacological functions have revealed that these drugs possessed a broad range of biological properties, including antioxidants, antidiabetic, anti-inflammatory, anticancer, antitumor and antiproliferative, antimutagenic, antimicrobial, anti-helminthic, hepatoprotective, wound healing, anticoagulant, immunomodulatory activities, antistress, toxicity studies.

Lodhra[2]

Botanical name- *Symplocos racemosa*

Symplocos – connection i.e. stamen united at the base, having an inflorescence with a long undivided axis.

- *Racemosa*- In form of cluster of grapes.
- Family- Symplocaceae
- Vernacular names
- Hindi – Lodh, Lodhra
- Kannada- Balaloddi
- Tamil- Velli lethi, vellilothiram

- Telugu- Lodduga
- Malyalam- Velli lothiram
- English- Lodh, Symplocos bark.

Morphology

- It is a small tree with stems of 6 meter height and 15cms in diameter. Leaves 9- 18 cm long and 3-5 cm wide, and oval.
- Bark- Dark grey, rough blaze 7-13 mm thick shortly fibrous, pale yellow in color and finely mottled with pale orange brown.
- Flower- white fading yellow and aromatic. Fruit- purplish black, drupe. Seeds- oblong, reddish brown 1-3 seeds in 1 fruit.

Chemical composition-[3] The plant may exhibit tremendous activity for its potential phytoconstituents, that mainly consist of flavonoids, phenolics, terpenes, and terpenoids. Apart from that, bioactivity-based fractionation and bioactive compound isolation should be validated for their abundance, accuracy, reproducibility, and cost-effectiveness. Bark – Flavanol glucosides like symplocoside, symposide, leucopelargonidin 3- glucoside, ellagic acid, tritrepenoids sampnin, β sitosterol, botulin, oleanolic acid, α – amyrin, alkaloids loturine, isoloturine, oleanolic acid, betulinic and harmane. 19 a-hydroxyarjunolic acid-3 α – amyrin- anti-inflammatory. Bark is astringent, expectorant, anti-inflammatory, febrifuge, haemostatic, etc.

Research work

Devmurari (2010) evaluated the antibacterial activity spectrum of petroleum ether and ethanolic bark extract against 3 gram positive bacteria, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus* and three gram negative bacteria's *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*. Ethanolic extract of *S. racemosa* Roxb. shows better antibacterial activity as compared to petroleum ether, but it has poor antibacterial activity against gram negative microorganisms like *P. aeruginosa* and *E. coli*.[4]

- Traditionally, bark is given in menorrhagia and other uterine disorders. It is a potent remedy for inflammation and cleaning uterus.[5]
- The bark of *Symplocos racemosa* contain antimicrobial activity showed inhibitory effect on the growth of *Micrococcus pyogens* var. aureus, *E. coli* and other enteric and dysenteric groups of organisms.[6]

- Kumar GS et al. (2007) assessed the antimicrobial activities of *Symplocos racemosa* (Barks) against *Propionibacterium acnes* and *Staphylococcus epidermidis*. The outstanding antimicrobial properties of *Symplocos racemosa* against *Propionibacterium acnes* evaluated based on the disc diffusion assay and dilution method.[7]

Vata

Botanical name- *Ficus benghalensis*

Family- Moracecae

Vernacular names[8]

- Hindi – Bada
- Kannada- Aladamara, Ala
- Tamil- Alamaram
- Telugu- Marrichettu
- Malyalam- Ala
- English- Banyan tree

Morphology

It is a very big tree possessing supporting roots & therefore may spread into miles sometimes.

- Leaves simple, alternate, orbicular to ovate, subcordate below up to 25 x 15 cm stipules coriaceous.
- Male, Female & gall flowers borne in the same receptacle. Gall flowers perianth same as in male, style short (in female flowers style elongate perianth short)
- Receptacles solitary or paired, globose ovoid, minutely pubescent, red at age.
- Flowering & fruiting time- April, June

Chemical constituents[9]

Tannins, leucocyanidin-3-O-β-D-glucopyrancoside, leucopelargonidin-3-O-β-D-glucopyrancoside, leucopelargonidin-3-O-α-L-rhamnopyranoside, 20-tetratriaconthene-2-one, pentatriacontan-5-one, β-sitosterol, meso-inositol, alpha-D-glucose, beta glucoside, saponin, leucoanthocyanidin, leucoanthocyanin, meso-inositol, bengalensisteroic acid acetate, heneicosanyl oleate, 6-heptatriacontene-10-one, etc.

Research works

- In a study by Murti and Kumar they reported *Ficus benghalensis*, root extract showed growth inhibition with increased concentration (25,50,75 mg/mL) in three strains of bacteria (*S.aureus, E.coli*, and *K. pneumonia*).

- The extract has effectively inhibited the *S. aureus, E. coli*, and *K. pneumonia* with the diameter of inhibition zones 30, 24, and 22 mm respectively, at its highest concentration compared to the standard drug (Ampicillin) used, with 40, 35, and 35 mm inhibition zones. Previous studies using alcohol extract of *F. bengalensis* plant parts (leaf, root, and fruit) against *S. aureus*, *E. coli*, *Pseudomonas protobacteria*, and *Bacillus cereus* showed moderate activity.[10]

- Antifungal effect Fernando K.A.B, Bandara H.M.W.R in a study reported *Ficus bengalensis* has antifungal activity against *Candida albicans* infection. Wilfred C supported the study by explaining the release of proteins from fungal cells and the movement of cytosolic enzyme along with glucose-6- phosphate dehydrogenase, in the plant extracts are assayed which determines the efficacy of anti-microbial activity.[11,12]

METHODS AND MATERIALS

1. **Test to be performed:** Antibacterial activity Sample name: Lodra vata kashaya

Sample presentation: Plastic bottle

Preparation of Casein Soya bean Digest Agar Medium (CSDAM): Dissolve casein peptone (15 g), soya peptone (5 g), Sodium Chloride (5 g) were taken and dissolved in 990 ml distilled water and pH was adjusted to 7.3±0.2 and make up the volume to 1000 ml. Finally add 15 g of agar to the media and autoclaved at 121°C for 20 minutes.

Preparation of the inoculum

Staphylococcus aureus (MTCC 3160) was procured from culture collection centre, IMTECH, Chandigarh. Loopful of 24 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Well diffusion method

The media was cooled to around 45-55°C, around 20 ml each was poured into sterile Petri plates. One ml of the inoculum was immediately added to the plate, swirled for uniform

distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 37°C and observed after 24 h.

Table 1: In vitro antibacterial activity test for *Lodra vata* kashaya against *S. aureus*.

Sample	Volume	Zone of inhibition - (Radius in mm)	
Lodra vata kashaya	25 µl	0	0
	50 µl	06	06
	100 µl	07	07
Control (Distilled water)	50 µl	0	0
Standard (Ampicillin) (1 mg/ml)	30 µl	15mm	15mm

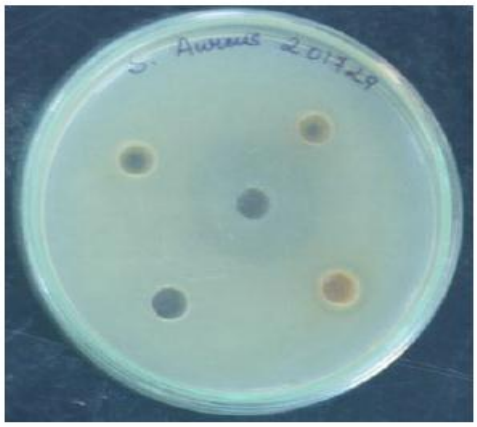

Fig. 1: In vitro antibacterial activity test for *Lodra vata* kashaya against *S. aureus.*

2. **Test to be performed:** Antibacterial activity

Sample name: Lodra vata kashaya

Sample presentation: Plastic bottle

Preparation of Nutrient agar media

Protocol

Beef extract (1 g), yeast extract (2 g), peptone (5 g) and Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121°C for 20 minutes.

Preparation of the inoculum

Klebsiella pneumonae (MTCC 7407) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 24 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Well diffusion method

The media was cooled to around 45-55°C, around 20 ml each was poured into sterile Petri plates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 35°C and observed after 24 h.

Table 2: *In vitro* antibacterial activity test for *Lodra vata* kashaya against *K. pneumonae*.

Sample	Volume	Zone of inhibition - (Radius in mm)	
Lodra vata kashaya	25 µl	0	0
	50 µl	0	0
	100 µl	0	0
Control (Distilled water)	50 µl	0	0
Standard (Ampicillin) 1 mg/ml	100 µl	09	09

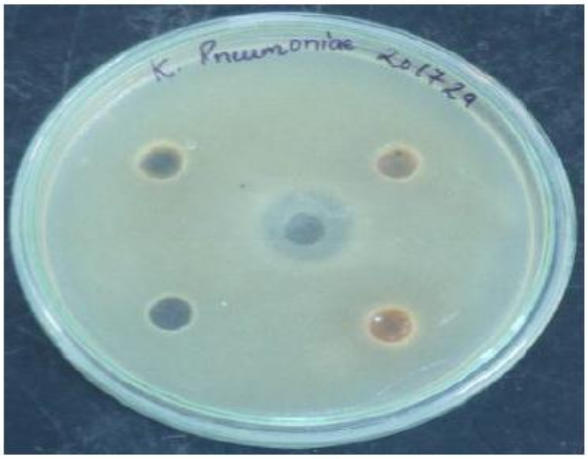

Fig. 2: *In vitro* antibacterial activity test for *Lodra vata* kashaya against *Klebsiella pneumonae*.

3. Test to be performed: Antifungal activity

Sample name: Lodra vata kashaya

Sample Code: 210729

Sample presentation: Plastic bottle

Protocol

Preparations of yeast extract dextrose agar media

Yeast extract (3 g), peptone (10 g) and dextrose (20 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.4 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121°C for 20 minutes.

Preparation of the inoculum

Candida albicans MTCC 183 was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 48 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Well diffusion method

The media was cooled to around 45-55°C, around 20 ml each was poured into sterile Petri plates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 30°C and observed after 48 h.

Table 3: *In vitro* antifungal activity test for *Lodra vata* kashaya against *C. albicans*.

Sample	Volume	Zone of inhibition - (Radius in mm)	
Lodra vata kashaya	25 µl	0	0
	50 µl	0	0
	100 µl	0	0
Control (Distilled water)	50 µl	0	0
Standard (Ampicillin) 1 mg/ml	100 µl	09	09

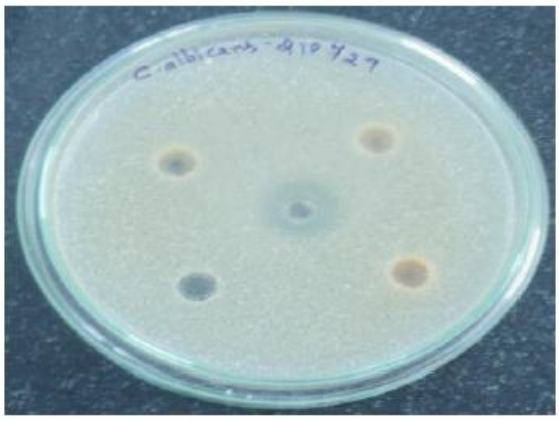

Fig. 3: *In vitro* Antifungal activity test for *Lodra vata* kashaya against *Candida albicans.*

RESULTS AND DISCUSSION

Antibacterial effect was seen during 24 hrs of incubation at higher volumes of Lodra vata kashaya used against *S. aureus*. Antibacterial effect was not seen during 24 hrs of incubation at the different volumes used against *K. pneumonaie*. Antifungal effect was not seen during 48 hrs of incubation at the different volumes used against *C. albicans*.

CONCLUSION

Medicinal properties of the plants is known to the mankind since generations and use of plant extracts for medicinal purpose in all civilizations and cultures has been playing a major role in health care systems all over the world. The plant may exhibit tremendous activity for its potential phytoconstituents. An action of drugs depends on the phytochemical constituents present in it, which make the drug effective enough to cure the disease.

Lodra and Vata having Astringent property which is attributed to tannins and flavonoids. Flavonoids have anti-inflammatory, anti-oxidant, immunomodulatory, antibacterial, antiparasitic, and antiviral properties. Anti- Bacterial and Anti-fungal properties of the drugs have been proved by analytical studies done previously on raw drugs. In a study by Garg, V.K., Paliwal, S.K. reported Ficus benghalensis and the Anti-inflammatory activity by root extract exhibited wound healing properties by enhancing the epithelization. Wilfred c quote in a study about, the Anti-fungal activity as, the release of proteins from fungal cells and the movement of cytosolic enzyme along with glucose-6- phosphate dehydrogenase was observed.

In this study we observed that Lodra Vata Kashya showed potential effect as antibacterial, hence we can conclude that drugs have antibacterial activity specifically on *S. aureus*.

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