In Vitro Study of Kampillaka (Mallotus Philippinensis Muell Arg.) As Antimicrobial Activity with Special Reference to Desha

Dr. Tejashree Ganesh Patil
Assistant Professor in Dravyguna vigyana department of vasantdada Patil Ayurvedic medical College and institute of yoga, sangli

***Abstract*—Antibiotic resistance has become a serious and widespread problem in developing countries causing high mortality each year. Antibiotic resistance results in reducing efficacy of antibacterial drugs, making the treatment of patient difficult, costly or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increase mortality. New therapy classes of antibiotics have become a popular choice to reduce antibiotic resistance. One strategy to avoid this is by using alternative therapeutic agents from Ayurvedic medicinal plants that are effective against bacteria, fungi, safe and have low cost. In present study we use kampillaka a herbal drug that have to potential to be used as antimicrobial agent. But according to different regions (Desha) collected samples of kampillaka can have different action on microbes is essential to know for advanced & specific treatment of disease.**

***Index Terms*—kampillaka, antimicrobial activity, invitro**

I. INTRODUCTION

Ayurveda is not just an ancient Indian Medical Science, but a complete guide to healthy living. It has been around for several thousands of years and has stood always with us for the our helthy life.[1] The main aim of Ayurvedic science is to achieve perfect health by creating an equilibrium of perfect harmony between human body and the environment. Man used to live close to nature and whenever he got exposed to some diseases, he used to cure himself using resources and material provide by the Mother Nature.[2] Many herbal medicinal plants are useful to cure various diseases. kampillaka is one of them. Kampillaka (Mallotua philippinensis Muell.Arg) belonging to family Euphorbiaceae as one of the promising drugs with very high medicinal value. Is known for its anthelmintic and purgative action in all classical texts of Ayurveda. this plant is used by local healers for various disease conditions. It has various synonyms like ranjana (colouring agent), rechanaka (purgative), raktachurna (red powder), karkasha (rough) etc. It was used as a medicine from Vedic period.

In ancient period, its leaves were used in yagya and its wood was used to make wooden mixer. It is observed that the drug is used in 44 formulations, indicated in more than 20 disease conditions including krimi, jantu, twacharoga, vibhandha, gulma, vrana, shleshmodara, arsha, shula, jwara, prameha, etc…Therapeutic useful part of the plant are glands and hairs of mature fruit (phala raja) and it should be administered internally after passing through shodhana procedure. It is used in various dosage forms such as churna, vati, varti, kalka, taila, ghrut and malahara.[4] Kampillaka found through the India, occasionally ascending to 1500 m in the outer Himalayas database. Kamala tree is widely distributed in northern, central, western and southern India; it is scarce in the Andaman Island.Kampillaka (Mallotus philippensis Muell. Arg.) is the native of North

West region. In Ayurveda says Himalayas Mountains best in habitat of medicinal plants. Desert among all healthy lands and Marshy lands among all unhealthy lands. The division of areas depending upon climatic conditions appears to be more scientific. We often come across tropical diseases which are challenging the medical science tody. Most of the areas produce different type of disorder, therefore understanding the climatic conditions will be essential in their management. it is said in our classics that one who have the knowledge of drugs and know to use them properly in accordance to Desha, Kala and examines individually, he is to be considered as a best physician.

Ancient Ayurvedic texts mention about three types of areas (Desha) depending upon geographical conditions and natural flora of the respective places. Properties of a plant, chemical constituents and potencyof that medicinal plant may alter with the region in which it grows and the nature of the land, water & air. Ayurvedic physicians used plants to treat Infective disease. Many of these plants inhibits growth of pathogenic microorganisms. Medicinal plants derived therapies have been proven as a quite promising remeday in the treatment of intractable bacterial and fungal infections as a replacement to existing synthetic drug.[6] In Ayurvedic medicine, many Medicinal Plants are useful in strengthening human health care system and the formulations based on such medicinal plants play an important role in modern medicine.

The primary benefits of using plant derived medicine are relatively safer than synthetic drugs and offer profound therapeutic benefits. Recently, it has been Demonstrated that many human pathogen have developed resistance against several synthetic drugs. There are several reports on antimicrobial activity of crude extracts prepared from plants that inhibit various pathogens, but limited numbers of in vitro studies on herbal preparations have been published. It is need of the hour to identify antibacterial potential of herbal products based on diseases for which no medicine or only palliative therapy is available.[7]

Hence an attempt was made to screen the antimicrobial potential of herbal preparation in the control prevention of infective diseases.

AIMS AND OBJECTIVES:

AIMS

In vitro study of Kampillaka (Mallotus philippinensis muell Arg.) as antimicrobial activity with special reference to Desha.

OBJECTIVES

Study of kampillaka from ayurvedic and modern literature.

Pharmacognostic study of kampillaka.

Phytochemical analysis of kampillaka.

Assess the antimicrobial activity of kampillaka.

Compare antimicrobial activity of kampillaka according to desha.

DRUG REVIEW

- In this Nighantu Kampillaka is included in Chandanadi Varga.
- Manya synonyms are mentioned hear.
- It is Virechaka
- Have Katu and Ushna properties
- Use in Vrana, Gulma, Vibandha, Krimi etc.

II. REVIEW OF MICRO ORGANISM

Microorganism- Microorganism or Microbe is a microscopic organism, which may exist in its single-celled form or a colony of cells.

The possible existence of unseen microbial life was suspected from ancient times. Microorganisms include all unicellular organisms and so are extremely diverse.

Antimicrobial Agent- An antimicrobial is an agent that kills microorganisms or stops their growth.

They can be classified according to their function-

Agent that kills microbes are Microbicide

While those that merely inhibit their growth are called bacterioststic

III. METHODOLOGY

The study was designed under four headings-

1) Pharmacognostical study including - a) Microscopic study

b) Macroscopic study

2) Analytical study including-

a) Physicochemical study –

b) Phytochemical study

Thin layer Chromatography (TLC)

Determination of Anti- microbial Activity through- Minimum

Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

ANTI MICROBIAL ACTIVITY:

Antimicrobial activity refers to the process of killing or inhibiting the disease-causing microbes.

A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound.

The most known and basic methods are the disk diffusion and broth or agar dilution mrthods.

MIC AND MBC:

While MIC is the lowest concentration of an antibacterial agent necessary to inhibit visible growth, minimum bactericidal concentration (MBC) is the minimum concentration of an antibacterial agent that results in bacterial death.

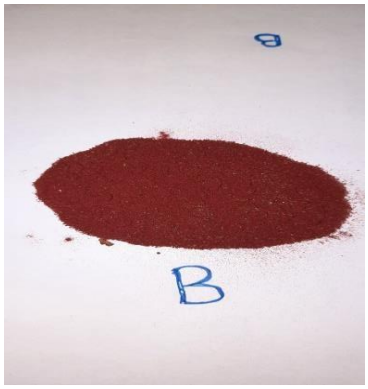
Minimum inhibitory concentration (MIC) is the lowest concentration of a chemical, usually a drug, which prevent visible growth of bacteria.

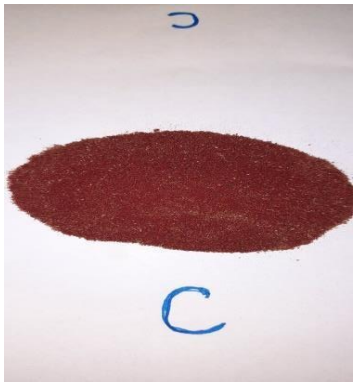
It is often expressed in micrograms per millilitre (ug/mL) or milligrams per litre (mg/L).

Procedure: 1) Serial dilution of the antibiotic (drug sample) is added to growth medium in test tubes. 2) These tubes are then inoculated with the bacteria. 3)Each of these tubes have growth media inoculated with a standard concentration of bacteria and the respective antibiotic concentration. 4) The tubes are allowed to incubate overnight. 5) broth tubes that appear turbid are indicative of bacterial growth while tubes that remain clear indicate no growth.

The minimum bacterial concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours a specific set of condition.

OBSERVATIONS





THIN LAYER CHROMATOGRAPHY REPORT:

TLC- Alcohol extract

Mobile phase- toluene: Ethyl acetate

Ratio- 7:3

Sample-A

Rf values:

Short wave- 0.07, 0.18, 0.25, 0.36, 0.43, 0.66, 0.72, 0.78, 0.85, 0.92

Long wave- 0.09, 0.39, 0.46, 0.65, 0.73, 0.80, 0.96

Sample B

Rf values:

Short wave- 0.09, 0.14, 0.19, 0.25, 0.33, 0.38, 0.42, 0.48, 0.51, 0.57, 0.68, 0.80, 0.89, 0.95

Long wave- 0.09, 0.16, 0.32, 0.40, 0.46, 0.67, 0.80, 0.90, 0.93

Sample-C

Rf values:

Short wave- 0.22, 0.31, 0.42, 0.46, 0.66, 0.75, 0.89, 0.95,

Long wave- 0.10, 0.24, 0.28, 0.43, 0.48, 0.65, 0.72, 0.96,

common Table of Antibacterial activity by MIC for 3 samples

Sample	A	B	C
E-coli	25 micro L	12 micro L	12 micro L
S. aures	50 micro L	25 micro L	25 micro L

common Table of Antibacterial activity by MBC for
3 samples

Sample	A	B	C
E- coli	50 micro L	50 micro L	50 micro L
S. aures	50 micro L	75 micro L	75 micro L

Table No – 31: common Table of Antifungal activity by MIC for 3 samples

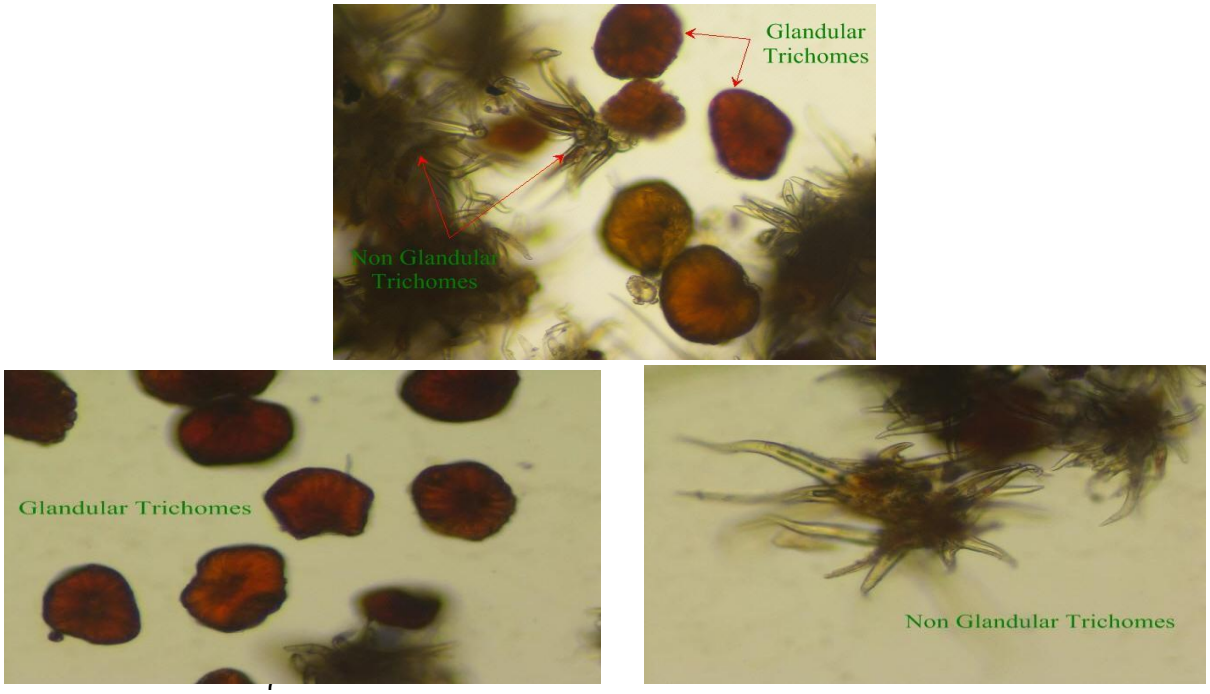
Sample	A	B	C
C.albicans	50 micro L	100 micro L	75 micro L
A.niger	75 micro L	150 micro L	-

Table No – 32: common Table of Antifungal activity by MFC for 3 samples

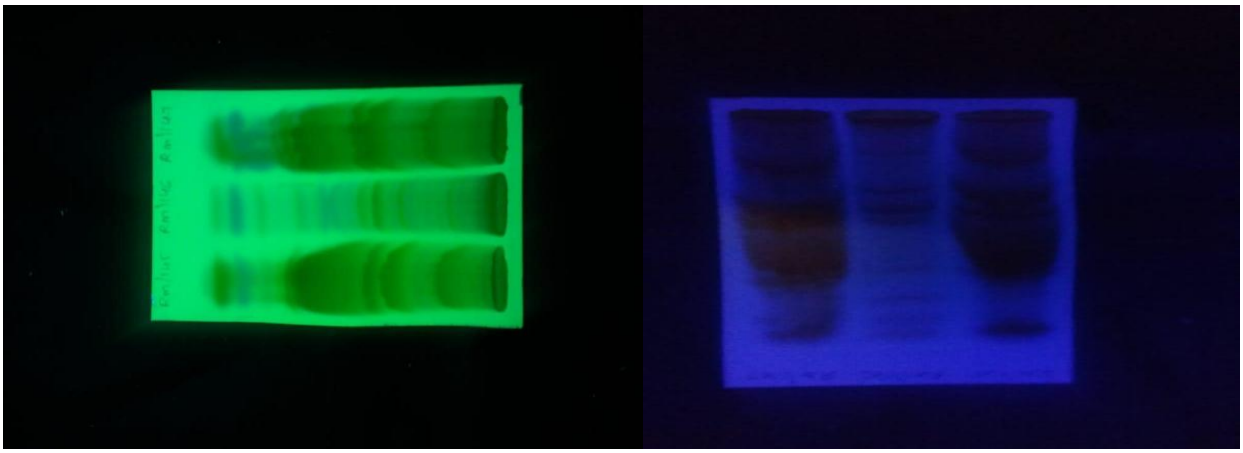
Sample	A	B	C
C.albicans	100 micro L	125 micro L	100 micro L
A.niger	100 micro L	150 micro L	-

IV. RESULTS

Powder Microscopy



Characters observed in the powder microscopy: Stellate Glandular Trichomes (a), Branched thick walled trichomes (NGT) (b), Branched thick walled trichomes (NGT) & Stellate Glandular Trichomes (c).



Here the results of Phytochemical test of 3 Samples is mentioned according to these results, Carbohydrate- Carbohydrate is present in both extracts of sample A. In sample B and C it present only in Water extract. Reducing Sugar- In sample A reducing sugar is present in both extracts, in sample B it present in alcohol extract and in sample C it present in water extract only. Monosaccharides- In sample B monosaccharides is absent in both extract, in sample A and C it is present in water extract only. Pentose sugar- In sample A and B pentose sugar is present in water extract only and in sample C it present in alcohol extract. Non reducing sugar- In all three samples non reducing sugar is absent. Hexose sugar- In all three samples hexose sugar is absent. Proteins- In the sample A protein is absent in both extracts, in sample B it present in water extract and in sample C it present in alcohol extract only. Amino acids- In the sample A it absent in both extracts, in sample B it present in water extract and in sample C it present in alcohol extract. Steroids- In all three samples steroids absent. Flavonoids- In all three samples Flavonoids are present. Alkaloids- In sample A alkaloid is present in both extracts, in sample B it present in water extract and in sample C it present in Alcohol extract. Tannins- It is present in both extract of sample A and C, in sample B it present only in water extract. Cardiac glycosides- It is absent in all 3 samples. Anthraquinone glycosides- It is absent in all 3 samples. Saponin glycosides- It is present in sample B and C in both extracts, but in sample A it present in only water extract.

V. DISCUSSION

Discussion is a process of re-examining oneself. It forms the base for conclusion. In spite of detailed classical study and experimentation in various ways, a theory is accepted only after proper reasoning of the observation. Hence discussion is very much crucial part of any scientific research. Antibiotic resistance results in reducing efficacy of antibacterial drugs, making the treatment of patient difficult, costly or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increase mortality. To minimize the usages of antibiotics and to develop a natural new drug to control pathogenic microorganism. The present research work is, In vitro study of "Kampillaka (Mallotus philippinensis muell Arg.) as Antimicrobial activity with special reference to Desha."" was conducted by collecting samples from 3 different regions of Maharashtra. Self-collection of drug sample is done. These samples were subjected to physical, chemical and pharmacognostic studies. The laboratory analysis of all samples were carried out, result were obtained and data thus generated was copared and concclusions were drawn.

according to the different classical books the Kampillaka is mentioned in the different Varga\Gana due to its various Karmas. In samhita kala it is mentioned virechaka dravya mainly after that Nighantukara revles its other karma and classified it in other Varga like Mustadi, Chandanadi, Amalakyadi, Haritakyadi etc. In the Rasashashtra Kampillaka is mentioned in Sadharana Rasa also.

various synonyms of Kampillka according to there Morphological Appearance, Availability, Form, Sours, structure of Leaves and Fruits and its uses.

synonyms of Kampillaka according to different authors. In sodhal Nighantu and in Dhanvantari Nighantu maximum numbers of synonyms is

mentioned. Raktang is the common name used by many Nighantukaras due to Kampillaka red in colour. Various Karma of Kampillaka according to different acharyas mentioned. In the Shaligram nighantu most of the karma mentioned. Virechana and Krumighna is most important Karma told by all authors. Also Kaphahara and Vranahara are mentioned.

various formulations are mentioned in which Kampillaka is ingredient. In the Charak Samhita most of the formulations are mentioned. It is used in various dosage form such as Churna,Vati, Gutika, Varti, Sidha Ghritas, Taila, Malahara, Avachurna, Kalka and in the formulation of Basti also.

detailed distinguishing features of Jangal, Anupa and Sadharana Desha. This Desha plays an major factor on plant which decides potency and growth of plant. As environment chenges according to Desha plants morphology varies ultimate the chemical constituents also varies. In the Jangam desha few trees are present and rainfall is avrege hence Rasadi porties in plant are more potent because of less moisture contain. In Anupa desha opposite picture is seen and in Sadharana desha balanced condition is present.

Here the number of Krimi is mentioned according to different authors. Almost all the Acharyas mentioned 20 types of Krimi but their classification is different told by them. In Charak Samhita and Ashtang hridya same classification is seen, in Harit Smhita 13 numbers of Krimi told.

the classification of Krimi is mention according to their Function, Location, Cause and Names of Krimi.

all the 3 samples are in powder form, they have same colour and texture. There was not much difference. That is mean all samples are same drug.

All samples have granular, non granular and branched thick walled trichomes, that means all samples from same species of medicinal plant.

Foreign matter was nil in the all 3 sample. So all drug samples are pure.Most of the drugs may be stored safely if the moisture content is reduced to 6% or less. It should be minimum to prevent microbial contamination. Sample A have more moisture than sample B&C. It suggest that it has more hygroscopic nature that leads to higher bacterial growth.Total ash value is used to determine quality and purity of a crude drug. High ash value is the indicative of contamination, substitution, adulteration or carelessness in preparing the crude drugs. Total ash value of Kampillaka should not be more than 6% as per API standards. All 3 sample have very small amount of ash value. So samples were within the prescribed limits. Acid insoluble ash value of a crude drug is always less than total ash value of the same drug. According to API standards it not more than 4%. Sample A has 1.33%, Sample B have 2.46% and Sample C have 1.44%. So it is also within the limit. Alcohol soluble extractive values should not be less than 50% as per API standards. All Samples have more than 50%. sample C have more value than other two samples. Water soluble extractive values is not less than 1% according to API. Sample C have more value it is 21.20%, Sample A have 6.47% and sample B have 4.24%.Sample B and C have nearly same Ph than Sample A. Sample A is more acidic in nature than other

Here the result of Phytochemical test indicated that Reducing sugar, Flavonoids, Alkaloids and Tannins are present in both extracts of drug sample A.

Flavonoids and Saponin glycosides are present in both extracts of drug sample B.

Flavonoids, Tannins and Saponin glycosides are present in both extracts of drug sample C. Two pigments with the same Rf value are likely to be identical molecules. If they have different Rf values, they are definitely different compounds. In the short wave Rf value 0.25 is found in both samples A and B, 0.66, 0.95 found in sample A and C.In the long wave Rf value 0.09, 0.46 & 0.80 are found in both samples A and B, Rf value 0.65 &0.96 are found in sample A and sample C ,Factors which affect Rf value are- The solvent system and its composition, temperature, the quality of paper and distance through which the solvent runs. As per Table no. 15 all three samples have different Rf values after following all above conditions hence it may because of that 3 samples of drug are collected from 3 different climatic conditions regions.

Sample B have more Rf values than sample A & C hence it have more molecules than other two sampes.

comparision of the Antibacterial Activity of 3 samples Kampillaka by MIC. According to this results we understand that the samle B and Sample C have same Antibacterial activity zone and it is better than sample A.

comparision of the Antibacterial Activity of 3 samples of Kampillaka by MBC. Here for the E. coli all Sample have same result and for S.aures sample B and Sample C have same result so sample A is more superior than

other two samples. This table reveals comparison of tha Antifungal Activity of 3 samples of Kampillaka by MIC. Here the sample C shows no Antifungal activity for A.niger. And sample A shows Antifungal Activity for both fungi is for less concentration than other sample hence sample A is better in this activity. This table reveals comparision of the Antifungal Activity of 3 samples of Kampillaka by MFC. Here also Sample C doesn‟t shows any Antifungal activity for A.niger fungi.And Sample A shows more potent Antifungal Activity than other 2 samples by less difference only.

VI. CONCLUSION

In this chapter conclusion of the above study is done by highlighting the outcome of the study. The study was named as “In vitro study of Kampillaka (Mallotus philippinensis muell Arg.) as Antimicrobial activity with special reference to Desha.” From this study it is concluded that Geographical variations are going to influence plants Chemically. Physicochemical study of Kampillaka is done. All test shows result within limit to API Phytochemical analysis carried out, all three samples show different results from one another, that is in all 3 Samples different chemicals are present. As per TLC report sample B that mean Anupa Desha have more Rf values than other two Desha, hence it has more molecules than other two samples.

As per Antimicrobial report Kampillaka shows Antimicrobial Activity in all three Desha drug samples. As per alternative hypotheis there is difference found in antimicrobial activity according to different Desha. As per above study we concluded that kampillaka from Jangal Desha is best for the antimicrobial uses.

REFERENCES

[1] Widén C-J, Puri HS. Natural occurrence and chemical variability of phloroglucinols in Kamala. Planta Medica. 1980;40(3):284–287.

[2] Singh R, Singhal KC, Khan NU. Antifilarial activity of Mallotus philippinensis Lam. on Setaria cervie (Nematoda: Filarioidea) in-vitro. Indian Journal of Physiology and Pharmacology. 1997;41(4):397–403.

[3] Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology. 2006;107(2):182–188. doi: 10.1016/j.jep.2006.03.013.

[4] Shrikumar S, Ravi TK. Approaches towards development and promotion of herbal drugs. Pharmaceutical Reviews. 2007;1(1):180–183.

[5] Orwa C, Mutua A, Kindt R. Agroforestree Database: a tree reference and selection guide. Version 4. 0, 2009.

[6] Usmanghani K, Saeed A, Alam MT. Indusynic Medicine. Karachi. Research Institute of Indusyunic Medicine. 1997; 1997:285–287.

[7] Ahmad F, Hashmi S. Adulteration in commercial Kamila (Mallotus philippinensis Muell.) an anthelmintic drug of repute. Hamdard Medicus. 1995; 38:62–67