



ISSN 2456-3110

Vol 8 · Issue 9

September 2023

Journal of
**Ayurveda and Integrated
Medical Sciences**

www.jaims.in

JAIMS

An International Journal for Researches in Ayurveda and Allied Sciences



Maharshi Charaka
Ayurveda

Indexed

Comparative antimicrobial study of bark extract (Aqueous, Chloroform, Ethanol) of *Pum Kutaja* (*Holarrhena antidysenterica* [Linn] Wall ex.G.Don) & *Stri Kutaja* (*Wrightia tinctoria* R.Br)

Anupa Rani¹, Ankita², Renu Bansal³

¹Final Year Post Graduate Scholar, PG Dept. of Dravyaguna, Post Graduate Training and Research Institute, Govt. Ayurved College, Patiala, Punjab, India.

²Lecturer, PG Dept. of Dravyaguna, Post Graduate Training and Research Institute, Govt. Ayurved College, Patiala, Punjab, India.

³Professor, Department of Microbiology, Govt. Medical College & Hospital, Patiala, Punjab, India.

ABSTRACT

Antimicrobial resistance has become a global health crisis, leading to increase morbidity, mortality and health care costs. Antibiotic resistance is a growing global health concern. Overuse and misuse of antibiotics have led to the emergence of bacteria that are resistant to many commonly used drugs. Natural plant-based medicines offer an alternative approach to combat infections, as they contain various bioactive compounds that can effectively inhibit the growth of bacteria, fungi, viruses, and other microorganisms. This study describes a procedure for retrieving the ethanol, chloroform, and aqueous extracts from the bark samples of Sample A - *Pum Kutaja* (*Holarrhena antidysenterica* [Linn] Wall ex.G.Don) and Sample B - *Stri Kutaja* (*Wrightia tinctoria* R.Br) testing them against a variety of microorganisms, both Gram-negative bacteria *Vibrio cholerae* (MTCC 3906), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC2592), and Gram-positive bacteria *Streptococcus pneumoniae* (MTCC 655), *Staphylococcus aureus* (ATCC 29213) and fungus *Candida albicans* (MTCC 227) for antimicrobial effect.

Key words: *Stri Kutaja*, *Pum Kutaja*, *Holarrhena antidysenterica*, *Wrightia tinctoria*, Antimicrobial Study, Bark Extracts

INTRODUCTION

A drug is defined as any substance used for the purpose of diagnosis, prevention, relief or cure of a diseases. According to W.H.O. the drug is a substance used in the diagnosis, treatment, or prevention of a disease or as a component of a medication. Microbial infections have been point of serious concern. Moreover the problem

of drug resistance has multiplied its magnitude many fold. In the present study an effort was made to screen potential herb against diseases caused by microbes and to highlight their potential against resistant microbes. Ayurvedic classics deal different procedures and medication to treat the *Atisaar* (Diarrhea) and *Krimi*. *Kutaja* is one such medicinal herb which has the potential to treat diarrhea caused by *Krimi* and so was selected for the study. *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall Ex G.Don) belongs to Apocyanacea family. It is tree with simple and opposite leaves. Phytochemically the bark contains *Holarrifine*, *Kurchamide*, *Kurcholessine*. Pharmacogonostically have anti-amnesic, Neuroprotective activity, Acetylcholinesterase inhibitory, Antidiabetic activity. Propagation is done by Air layering, root sucker, seed. *Stri Kutaja* (*Wrightia tinctoria* R.Br) belongs to Apocyanacea family. It is tree with simple and opposite leaves. Phytochemically the bark have *Lupeol*, *Stigmasterol*, *Compestrol*. Pharmacogonostically have

Address for correspondence:

Dr. Anupa Rani

Final Year Post Graduate Scholar, PG Dept. of Dravyaguna, Post Graduate Training and Research Institute, Govt. Ayurved College, Patiala, Punjab, India.

E-mail: anupasaini1302@gmail.com

Submission Date: 00/07/2023 Accepted Date: 00/08/2023

Access this article online

Quick Response Code



Website: www.jaims.in

DOI: 10.21760/jaims.8.9.8

antipsoriatic, antihelminthic, antidiabetic, wound healing property. Due to its *Krmighan*, *Grahi* properties, easy availability, cost effectiveness and low dose required for its therapeutic effect the drug was evaluated for its therapeutic use. *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall Ex G.Don) have *Katu*, *Tikat Rasa* and *Sita Virya* while in case of *Stri Kutaja* (*Wrightia tinctoria* R.Br) have *Katu*, *Kashya*, *Tikat rasa* and *Ushna Virya* both have *Atisarghan* property as mentioned in *Raja Nighantu*.^[1] In *Susruta Samhita*, *Utar Tantra Krimi* is mentioned as *Nidana* of *Atisara* (Diarrhea).^[2] In *Kaiydev Nighantu* the *Kutaja* and the other variety of *Kutaja* has been told which has *Jantuhar* and *Atisaarhar* property. *Jantu* is one of the synonyms for *Krimi* and so the *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall Ex G.Don) and other variety i.e., *Stri Kutaja* (*Wrightia tinctoria* R.Br) can be considered here to have *Krimihar* i.e., antimicrobial property. As both the variety of *Kutaja* have *Atisaarghan* property so study was undertaken to see and compare the antimicrobial efficacy of both the drugs and to conclude that which drug has better action.

In Indian Materia Medica, *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall Ex G.Don) whole plant is used for Acute, Chronic dropsy, menorrhagia and Dyspepsia^[3]. In case of other variety *Wrightia tinctoria* flowers have fragrance. Bark is of reddish brown colour and smooth appearance as compared to (*Holarrhena antidysenterica*[Linn] Wall Ex G.Don). In *Wrightia tinctoria* seeds, the tuft is on the end next to the foot stalk.^[4]

In Indian Medicinal Plant, *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall Ex G.Don) bark is bitter, dry, pungent and as antihelminthic, antidiarrheal properties and alleviates dysentery, fever, piles, leprosy, skin diseases.^[5] In other variety *Stri Kutaja* (*Wrightia tinctoria*) bark and seed have the same properties as those of (*Holarrhena antidysenterica*[Linn] Wall Ex G.Don). They also mentioned that bark is especially useful in piles, skin disease and biliousness.^[6]

The Wealth of India quotes *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall Ex G.Don) bark (*Kurchi*) has astringent, antidiysenteric, anthelmintic, stomachic,

febrifugal and tonic properties.^[7] It is used in the treatment of amoebic dysentery and diarrhea. The total value of *Kurchi*- the common word used for bark is due to presence of alkaloids which occur as tannates. In case of *Stri Kutaja* (*Wrightia tinctoria*) the bark is commonly used as an adulterant of the well known drug Conessi, Tellicherry or *Kurchi* bark which is obtained from (*Holarrhena antidysenterica*[Linn] Wall Ex G.Don).^[8]

The microbe taken for the study i.e., *Vibrio cholera*, *Escherichia coli*, *Staphylococcus aureus*, *Psuedomonas aeruginosa*, *Candida albicans*, *Streptococcus pneumonia* causes diarrhea indicating *Sukshmata* (minuteness), *Adrishyata* (not visible by naked eye), *Krimija Nidana* (microbes causing diarrhea) and *Vaikarikta* (pathogenicity of microbes) of *Krimi*. Hence their antimicrobial study was done.

Vibrio Cholerae - A Gram negative bacteria, 1.5 x 0.2-0.4 µm in size, motile in nature causes the infections in human beings by ingestion of contaminated foods and drink. The ingested organisms pass through the acid barrier of the stomach and multiply in the alkaline medium of the small intestine and responsible for diarrhea.^[9]

E.coli - A Gram negative bacteria 1-3 x 0.4- 0.7µm in size, motile in nature is transmitted by the fecal- oral route. Pili - a structure in the intestine allow the bacteria to colonize the ileal mucosa. Cytotoxic enterotoxins induce watery diarrhea. Plasmid - encoded invasion factors permit invasion of the mucosa and induce tissue damage, the presence of either of these factors induce a host inflammatory reaction with an influx of lymphocytes and resulting diarrhea.^[10]

Staphylococcus aureus - A Gram positive bacteria which ferment on sugar media and causes food poisoning which is an intoxication that result from consumption of food containing sufficient amount of enterotoxins which lead to nausea vomiting and diarrhea.^[11]

Pseudomonas aeruginosa - A Gram negative bacteria 1.5 x 0.5µm motile bacteria also produce enterotoxin which is responsible for diarrhea.^[12]

Candida albicans - 3-5µm diameter oval in shape, has motility causes diarrhea.^[13]

Streptococcus pneumoniae - A Gram Positive, 1 μ m in diameter causes diarrhea.^[14]

As stated above that *Krimi* are found in abdomen have motility, one of the causative factor for the *Atisaar* so all these microbes were taken for study.

MATERIALS AND METHODS

Stem bark of Sample A & Sample B was collected and washed and shade dried and fine powder was done and stored in air tight container.

Preparation crude extract

Different solvents ethanol, chloroform, Aqueous were chosen for successive solvent extraction based on polarity using Soxhlet extraction apparatus and the extract of Sample A - *Pum Kutaja (Holarrhena antidysenterica*[Linn] Wall ex.G.Don) & Sample B - *Stri Kutaja (Wrightia tinctoria* R.Br) were concentrated under reduced pressure under rotator evaporator. The procedure were done HHrc, Amritsar (Fig no. 1,2,3,4).

Dilution of extract

DMSO was used for dilution of ethanol, chloroform and aqueous extract of bark samples of Sample A - *Pum Kutaja (Holarrhena antidysenterica*[Linn] Wall ex.G.Don) and Sample B - *Stri Kutaja (Wrightia tinctoria* R.Br).

Test Microorganism

The Microbial Type Culture Collection and Gene Bank (MTCC) stains of *Vibrio cholerae* (strain no 3906), *Streptococcus pneumoniae* (strain no 655), and *Candida albicans* (strain no 227), were procured from CSIR- IMTEC, Section 39, A, Chandigarh.(Fig No.5).American Type Culture Collection (ATCC) strains of *Staphylococcus aureus*(ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 2592) were procured from Department of Microbiology, Governmental Medical College, Patiala and were used for present study.

Preparation of Media: Mueller-Hinton Agar (MHA), which is the best medium for routine susceptibility tests was used for the antimicrobial study in the department of microbiology GMC, Patiala.

Disc Diffusion Method

The Antimicrobial activity was studied using disc diffusion method. The following steps were taken:

Antimicrobial Disks Preparation

The disk diameter was approximately 6 mm were punched out of Whatmann paper no 1 following which were sterilized in the hot air oven at 160° C for 2 hrs. The disks used for the study were impregnated with leaf extracts of ethanol, chloroform and aqueous extract diluted with DMSO₄ of bark samples of Sample A - *Pum Kutaja (Holarrhena antidysenterica*[Linn] Wall ex.G.Don) and Sample B - *Stri Kutaja (Wrightia tinctoria*R.Br).

Inoculation of Plates

1. A sterile cotton swab was dipped into the standardized bacterial suspension.
2. The excess inoculum was removed by lightly pressing the swab against the tube wall at a level above that of the liquid.
3. The agar was inoculated by streaking with the swab containing the inoculum.
4. The plate was rotated by 60° and the rubbing procedure was repeated two times. This ensured an even distribution of the inoculum.
5. The surface of the medium was allowed to dry for 3-5 minutes but not longer than 15 minutes to allow for absorption of excess moisture.

Application

1. Using sterile forceps or disk dispenser (disc impregnated with extract of different disc of Sample A & Sample B) were placed on the surface of the plate inoculated with different bacterial and fungal strains.
2. It was ensured that the disks were not moved once they had come into contact with the agar surface since some diffusion of the drug occurs instantaneously.
3. A maximum of six disks may be placed in a 9-cm petri dish.
4. The plates were incubated in an inverted position at 37°C.
5. Different zone of inhibition was observed and recorded after 16 to 18 hours using graduated ruler.

RESULTS

Zone of inhibition *Vibrio cholera*, *Escherichia coli*, *Staphylococcus Aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Candida albicans* against different concentration are listed in Table No. 1,2,3,4,5,6 & Fig No. 5,6,7,8,9,10 respectively.

Table 1: Aqueous extract of bark of *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don) : antimicrobial activity against the different microbes.

SN	Strains	Zone of Inhibition (mm)		
		10µl	20µl	30µl
1.	<i>Vibrio cholera</i>	9	12	15
2.	<i>Escherichia coli</i>	8	11	13
3.	<i>Staphylococcus aureus</i>	10	12	13
4.	<i>Pseudomonas aeruginosa</i>	9	11	13
5.	<i>Streptococcus pneumoniae</i>	NA	NA	NA
6.	<i>Candida albicans</i>	8	9	11

Table 2: Aqueous extract of bark of *Stri Kutaja* (*Wrightia tinctoria* R.Br): antimicrobial activity against the different microbes.

SN	Strains	Zone of Inhibition (mm)		
		10µl	20µl	30µl
1.	<i>Vibrio cholera</i>	8	9	10
2.	<i>Escherichia coli</i>	8	10	11
3.	<i>Staphylococcus aureus</i>	8	9	12
4.	<i>Pseudomonas aeruginosa</i>	7	9	11
5.	<i>Streptococcus pneumoniae</i>	NA	NA	NA
6.	<i>Candida albicans</i>	8	10	13

Table 3: Ethanol extract of bark of *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don) : antimicrobial activity against the different microbes.

SN	Strains	Zone of Inhibition (mm)		
		10µl	20µl	30µl
1.	<i>Vibrio cholerae</i>	16	19	24
2.	<i>Escherichia coli</i>	14	18	21
3.	<i>Staphylococcus aureus</i>	16	18	20
4.	<i>Pseudomonas aeruginosa</i>	10	13	17
5.	<i>Streptococcus pneumoniae</i>	NA	11	14
6.	<i>Candida albicans</i>	10	13	16

Table 4: Ethanol extract of bark of *Stri Kutaja* (*Wrightia tinctoria* R.Br): antimicrobial activity against the different microbes.

SN	Strains	Zone of Inhibition (mm)		
		10µl	20µl	30µl
1.	<i>Vibrio cholerae</i>	13	17	22
2.	<i>Escherichia coli</i>	12	15	18
3.	<i>Staphylococcus aureus</i>	14	16	18
4.	<i>Pseudomonas aeruginosa</i>	11	13	16
5.	<i>Streptococcus pneumoniae</i>	NA	10	13
6.	<i>Candida albicans</i>	12	15	19

Table 5: Chloroform extract of bark of *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don) : antimicrobial activity against the different microbes.

SN	Strains	Zone of Inhibition (mm)		
		10µl	20µl	30µl
1.	<i>Vibrio cholerae</i>	14	16	19

2.	<i>Escherichia coli</i>	12	14	16
3.	<i>Staphylococcus aureus</i>	14	16	18
4.	<i>Pseudomonas aeruginosa</i>	9	12	15
5.	<i>Streptococcus pneumoniae</i>	NA	8	10
6.	<i>Candida albicans</i>	8	10	13

Table 6: Chloroform extract of bark of *Stri Kutaja* (*Wrightia tinctoria* R.Br): antimicrobial activity against the different microbes.

SN	Strains	Zone of Inhibition (mm)		
		10µl	20µl	30µl
1.	<i>Vibrio cholerae</i>	12	14	17
2.	<i>Escherichia coli</i>	8	10	12
3.	<i>Staphylococcus aureus</i>	10	12	14
4.	<i>Pseudomonas aeruginosa</i>	10	11	13
5.	<i>Streptococcus pneumoniae</i>	NA	7	9
6.	<i>Candida albicans</i>	11	14	17

DISCUSSION

Among all three extracts of *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don) i.e., Aqueous, ethanol and chloroform, ethanol extract shows maximum sensitivity against all the tested microbes, then Chloroform extract and then aqueous extract. The antimicrobial activity was maximum in case of *Vibrio cholera* and rest of the organism shown the order like - *Vibrio cholera*> *Escherichia coli*> *Staphylococcus aureus*> *Pseudomonas aeruginosa*> *Candida albicans*> *Streptococcus pneumoniae*

The similarly results were found in all three extracts of *Stri Kutaja* (*Wrightia tinctoria* R.Br). The antimicrobial activity was maximum in case of *Vibrio cholera* and rest of the organism shown the order like - *Vibrio cholera*>

Escherichia coli> *Staphylococcus aureus*> *Candida albicans*> *Pseudomonas aeruginosa*> *Streptococcus pneumoniae*

Although *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don) showed markedly increasingly sensitivity for all the tested microbes in 10 to 30µl as shown in Table no. (6, 8,10). Similarly in case of Sample B - *Stri Kutaja* (*Wrightia tinctoria* R.Br) results are shown in Table no. (7, 9, 11).

On comparing zones of inhibition of both the samples of bark i.e. Sample A - *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don) and Sample B - *Stri Kutaja* (*Wrightia tinctoria* R.Br) three different extracts i.e., aqueous, ethanol and chloroform extracts, Sample A - *Pum Kutaja* (*Holarrhena antidysenterica* [Linn] Wall ex.G.Don) showed more antimicrobial sensitivity against all tested pathogens i.e. *Vibrio cholera*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas areuginosa*, *Streptococcus pneumoniae* than Sample B - *Stri Kutaja* (*Wrightia tinctoria* R.Br). But in case of *Candida albicans*, Sample B - *Stri Kutaja* (*Wrightia tinctoria* R.Br) showed more sensitivity than Sample A - *Pum Kutaja*- (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don). There is no activity seen in 10, 20,30µl of Sample A & Sample B for aqueous extract in case of *Streptococcus pneumoniae*. Similarly in case of Ethanol, Chloroform extract there is no activity seen in 10µl of Sample A& B for *Streptococcus pneumoniae*.

CONCLUSION

Results showed that both the plants *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don) and *Stri Kutaja* (*Wrightia tinctoria* R.Br) were found to have antimicrobial activity against tested microbes. *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don) and *Stri Kutaja* (*Wrightia tinctoria* R.Br) can be used as an antimicrobial and antifungal agent against different pathogens. They can be used against Gastrointestinal and skin infections. Both the plants could effectively be used as best natural alternative to synthetic antimicrobial agents. On comparing both the plants *Pum Kutaja* (*Holarrhena antidysenterica*[Linn] Wall ex.G.Don) was found to be more potent action against microbes causing diarrhea than *Stri Kutaja* (*Wrightia tinctoria* R.Br).

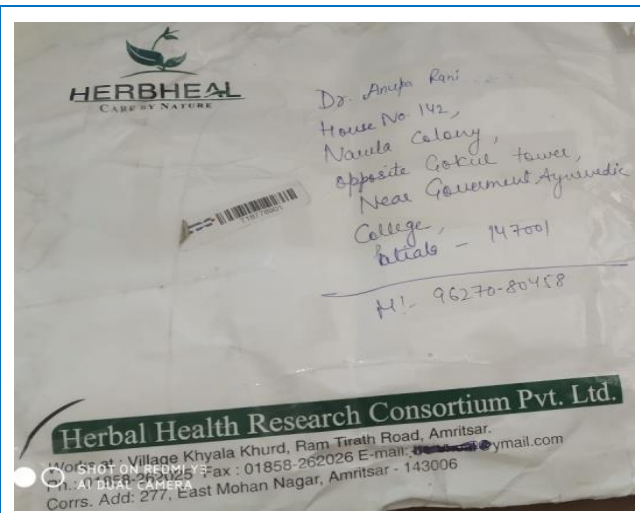


Fig. 1: Extract received from HHRC Amritsar



Fig. 2: Extracts of Sample A - Pum Kutaja (A,E,C) & Sample B - Stri Kutaja (A,E,C)



Fig. 3: Extracts & Powder of Sample A - Pum Kutaja (A,E,C)



Fig. 4: Extracts & Powder of Sample B - Stri Kutaja (A,E,C)

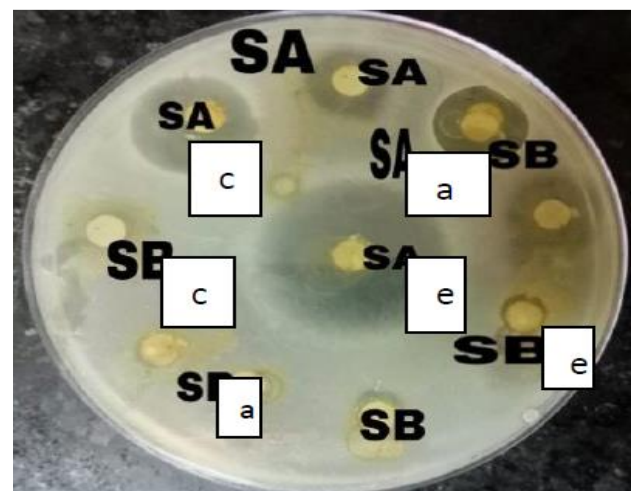


Fig. 5: Zone of Inhibition of *Vibrio Cholerae*

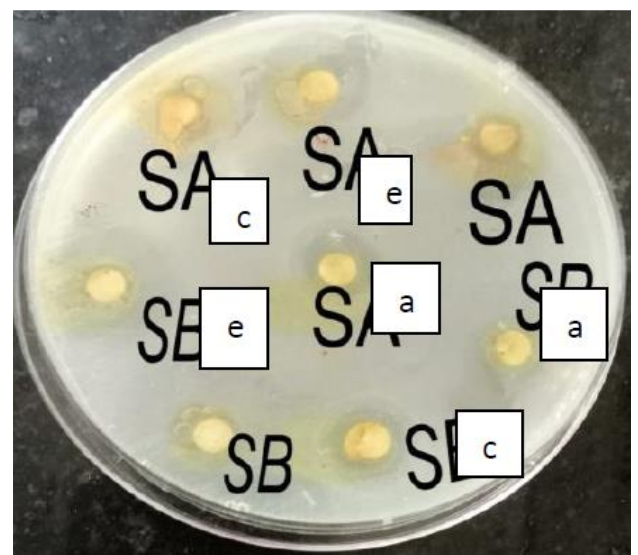


Fig. 6: Zone of inhibition of *Escherichia coli*

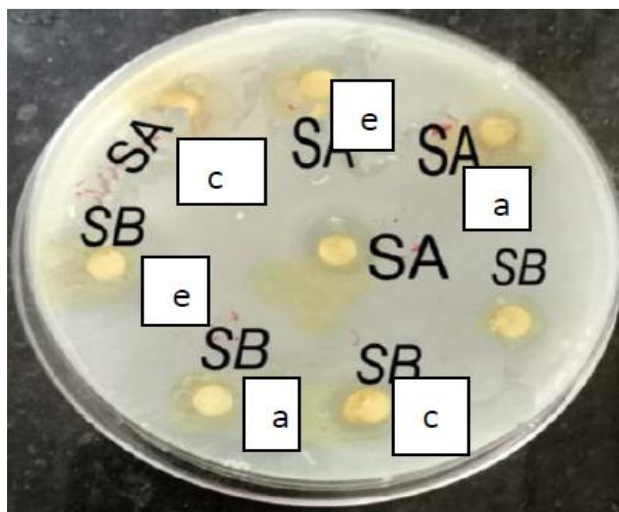


Fig. 7: Zone of inhibition of *Staphylococcus aureus*

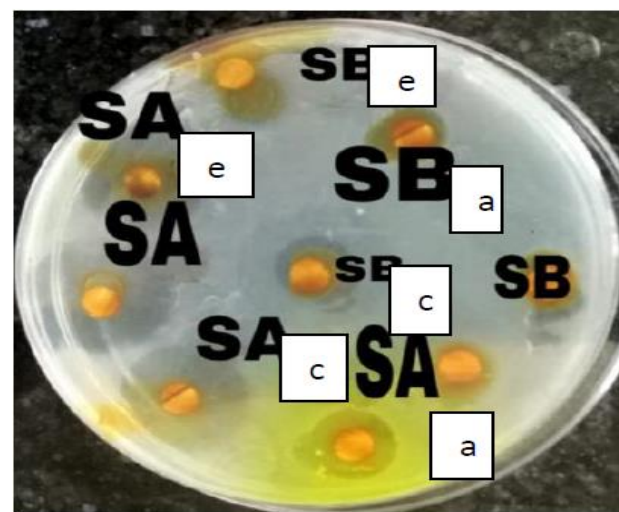


Fig. 10: Zone of inhibition of *Candida albicans*

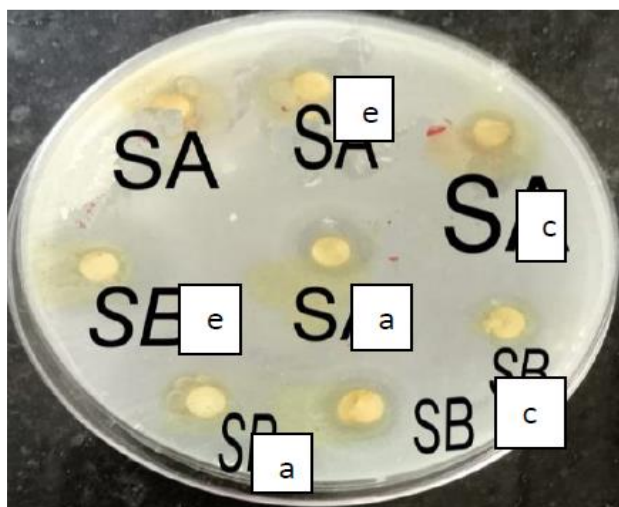


Fig. 8: Zone of inhibition of *Pseudomonas aeruginosa*

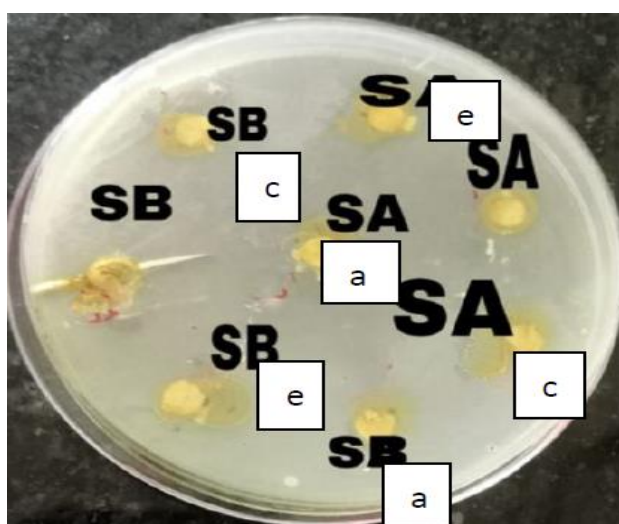


Fig. 9: Zone of inhibition of *Streptococcus pneumoniae*

REFERENCES

1. Narhart, Raj Nighantu. (e Nighantu). Developed by National Institute of Indian Medical Heritage, Hyderabad for Central Council for Research in Ayurvedic Science New Delhi: 2012 (Available from:<http://www.nimh.nic.in/ebooks/eNighantu>).
2. Acharya Susruta, Susruta Samhita, commentary by Shastri Dutt Ambika, Vol1, Uttartantra 40/3-5; Published by Chaukhamba Sanskrit Sansthan Varanasi;2015,272p.
3. Nadkarni AK, Indian Materia Medica, Bombay: Popular Prakashan Pvt. Lad 1995. Volume-I p. 634-651.
4. Nadkarni AK, Indian Materia Medica, Bombay: Popular Prakashan Pvt. Lad 1995. Volume-II p.
5. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2 ed. Delhi: Periodical Book Agency, 1993. Vol-2 p.1569-1573
6. Kirtikar KR, Basu BD, Indian Medicinal Plants. 2 ed. Delhi: Periodical Book Agency, 1993. Vol-2 p.1581-1583
7. Anonymous, The Wealth of India, New Delhi: Council of Scientific & Industrial Research, 2003; Vol V: A p. 103-107
8. Anonymous. The Wealth of India, New Delhi: Council of Scientific & Industrial Research. 2003; Vol X: A p. 588-590.
9. Textbook of Microbiology(4th) edition 2012, Published by Arya Publication, Delhi, Ch No 34, Pg.No.281-287.
10. Textbook of Microbiology(4th) edition 2012, Published by Arya Publication, Delhi, Ch No.31, Pg.No.247-253.

11. Textbook of Microbiology(4th) edition 2012, Published by Arya Publication, Delhi, Ch No 23, Pg.No.178-184.
12. Textbook of Microbiology(4th) edition 2012, Published by Arya Publication, Delhi, Ch.No.36, Pg.No.297-300.
13. Textbook of Microbiology(4th) edition 2012, Published by Arya Publication, Delhi, Ch 25, Pg.No.200-203.
14. Textbook of Microbiology(4th) edition 2012, Published by Arya Publication, Delhi, Ch.No.66, Pg.No.563-565.

How to cite this article: Anupa Rani, Ankita, Renu Bansal. Comparative antimicrobial study of bark extract (Aqueous, Chloroform, Ethanol) of *Pum Kutaja* (*Holarrhena antidysenterica* [Linn] Wall ex.G.Don) & *Stri Kutaja* (*Wrightia tinctoria* R.Br). *J Ayurveda Integr Med Sci* 2023;09:46-53.

<http://dx.doi.org/10.21760/jaims.8.9.8>

Source of Support: Nil, **Conflict of Interest:** None declared.

Copyright © 2023 The Author(s); Published by Maharshi Charaka Ayurveda Organization, Vijayapur (Regd). This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by-nc-sa/4.0>), which permits unrestricted use, distribution, and perform the work and make derivative works based on it only for non-commercial purposes, provided the original work is properly cited.