



STUDY OF JAYPALA BEEJ CHURAN FOR IT'S KRIMIGHAN PROPERTY W.S.R TO ANTIBACTERIAL ACTIVITY ON S-AUREUS, AND E-COLI DEALS WITH DETAIL IN-VITRO ANTI BACTERIAL STUDY

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ABSTRACT: -

Visha means poison, visha chikitsa or Agadtantra include the treatment of disease caused by poisons and toxins, such as spoiled food, animal, reptile and insect bites, poisonous minerals, metals and unsuitable food combinations. It is equivalent to toxicology in modern medicine. Medicinal plants are considered the prime and integral part of Ayurvedic system of medicine. A single herb or a drug possesses multiple uses and actions which need to be scientifically re-validated so as to show the potential of the drugs explained by the Acharyas to the present modern world. More than 1700 pharmacological actions have been described in Ayurveda, among which the Krimighna karma is also one. It is synonymous with other activities like Krimisudana, Krimiprashamana and Krimihara. This can be correlated to the antimicrobial activity of the modern pharmacology like antibacterial, antifungal, antiviral etc. In most developing countries, malnutrition and unsanitary living system contribute to massive burden of infectious diseases. Microorganisms such as bacteria, viruses, fungi and parasites, are present everywhere in the soil, water, and atmosphere and on the body surfaces, and are responsible for a large number of infectious diseases in human beings. Despite improved living conditions, wide spread vaccination and availability of effective antibiotics, infectious diseases continue to take very high rank as a cause of death in the world, which is more than ten million persons each year. In present conditions, effective, complete and timely management of these

microbes is a must. Antibiotics provide the main basis for the therapy of infections especially of bacterial origin. Due to the emergence of antibiotic resistant pathogens in hospitals and homes, plants are being looked upon as an excellent alternate to combat the future spread of multidrug resistant microorganisms. The history of the use of medicinal plants for their therapeutic purposes probably dates back to the origin of man. There has also been an increase on the reliance of the use of medicinal plants by the population in the industrialized societies as herbal remedies have become more popular in the treatment of minor ailments and also on account of the increasing costs of personal health maintenance. Plants may contain constituents that can be used to treat diseases such as infections, inflammatory conditions and cardiovascular diseases, but the scientific information on most of these medicinal plants in use are lacking. Therefore as part of the efforts to promote the use of medicinal plants either as an alternative or an adjunct to conventional medicine, it is necessary for scientists to carry out investigations into herbal medicines. This will help bridge the gap between conventional and herbal medicines. Though the available antibacterial drugs have been tried in most of the conditions, their reoccurrence is common, thus making it necessitate the need of an alternative and effective therapy. The drug Jayapala (*Croton tiglium* Linn.), which is an organic irritant poison and available everywhere and is known to have Krimighna activity. Jayapala is one such medicinal plant which is available abundantly and is attributed with krimighna property according to Rasatarangini. In present time there is lack of scientific evidence about antibacterial efficacy of these drugs. In this study try to Evaluation of properties of Jayapala beeja churna for its krimighna property w.s.r. to antibacterial activity on *S. aureus* and *E. coli* - an in vitro study. The strain of bacterias namely *Staphylococcus Aureus*- gram +ve bacteria and *E. coli* gram-ve bacteria were selected for the study as representative microorganisms. In the present study Agar well diffusion /Cup plate method has been followed to detect the Antibacterial property of the drug.

KEYWORDS: - Jaypala Beej Churna, S-Aureus and E-Coli.

INTRODUCTION: -

The origin of Ayurveda can be traced back over 6,000 years. Having the divine origin, this Science of Life, relies heavily on the plants for the therapeutic uses. Toxicology which is the specialty dealing with the poisons, is very rich and elaborated in Ayurveda. It is described under the subject Agad tantra and has in its repertoire different poisonous plants which is also used as medicinal purposes either in alone or in combination. The actions of the drugs have been explained on the basis of several features such as Rasa, Guna, Veerya, and Vipaka. Prabhava.

METHODOLOGY

The current in-vitro and Analytical study has been carried under three headings: Phase I-Collection of raw material.

Phase II-Shodhana and Physico-chemical analysis of Jayapala beeja.

Phase III - In vitro Antibacterial studies.

Locale of the study:

The drug was identified and authenticated by the Deptt. of Dravyaguna V.Y.D.S. Ayurveda Mahavidyalaya Khurja. Phytochemical studies, microscopic studies and In- Vitro Antibacterial activity were carried out at Alcatec research laboratories India Pvt. Ltd., Bahadurgarh, Haryana.

1. Collection of the drugs:

Source of the drugs selected for the study:

Botanically identified jayapala beeja, which is fresh and not infected by any insects were collected from the dept. of R.S. & B.K. V.Y.D.S.A.M. Khurja. This study of

Jayapala Beeja Churna implies to - "powder of seeds of which extractive is used"

PHYSICO-CHEMICAL STUDY: This includes,

1. Shodhana procedures
2. tests.
3. Phyto chemical analysis.

1. Shodhana procedures

SHODHANA

The seeds of a well grown fruit of Jayapala tree are selected. The external covering of the seeds is removed. The testa (greenish tongue like structure inside the seed) of these selected seeds is removed and the cotyledons are tied in a portali. This pottali is hung in dola yantra having go-dugdha as liquid media and subjected to swedana for three hours (one prahara). It should be repeated for three times Each time fresh milk has to be taken. After 3rd swedana procedure, when the drug is cool on its own, it is collected from the pottali and dried under hot sun. Later the dry powder obtained is triturated well and stored in suitable air tight container.

Physico-chemical tests:

- A) Organoleptic tests: include the taste, texture, colour and odour of the samples
- B) Microscopic study-Jayapalabeeja

Equipment's: Compound microscope, eye piece, glass slides, cover slip, watch glass, camel brush mountain brush, filter paper, blades, spirit lamp, pipettes. Chemical: safranin, ethanol, Glycerin, Iodine.

Procedure:

1) Transverse Section Method:

- (a) Put the sample in a test tube and add sufficient water so that sample remains submerged.
- (b) Keep it for half an hour. This will soften the hard sample for getting fine section.
- (c) For section the sample is kept in potato slit (sample which are difficult to hold) and with help of new blade thin transverse section were taken.
- (d) Thick oblique sections were rejected.
- (e) With help of mountain hair brush, selected sections were transferred to watch glass containing water.

2) Staining method:

- (a) Selected the thin Transverse section of the sample was taken and transferred on a slide with the help of mountain hairbrush.
- (b) Drop of water was added.
- (c) Safranin reagent was added & washed with ethanol to remove excess stain & observed.
- (d) Finally, a drop of glycerin was added and the section was covered avoiding air bubble carefully with cover slip.
- (e) With the help of a blotting paper excess water present outsides were wiped off.
- (f) The section was focused under microscope and the arrangements of cells were studied.

OBSERVATION RESULT

The present study has been carried out under following headings:

- ❖ Shodhana and analytical study of the drug
- ❖ In-vitro study-agar diffusion/cup-plate method.

Table No. 1: Brief results of Shodhana of Jayapala beeja in cow milk

Weight of dried Ashuddha seeds	1000gm
Weight of seeds after swedana	1550gm
Weight of seeds after washing in hot water	1300 gm
Weight of seeds after complete drying	900gm

Table No. 2: Organoleptic Observations

1	Sparsha	Smooth and glabrous
2	Rupa	Greyish Brown
3	Rasa	Bitter
4	Gandha	Characteristic
5	Physical appearance	Coarse powder

Photochemical study:**Table NO. 3: Physico-chemical analysis- values in percentage**

Parameter	Ashodhita Jayapala (Churna)	Shodhita Jayapala (Churna)
Total Ash	2.45%	2.22%
Acid Insoluble ash	0.55%	0.66%
Water Soluble ash	2.20%	1.80%
Alochal soluble extractive	22%	24.50%
Water soluble exrtractive	10.6%	12.54%
Chloroform soluble extractive	13.40%	25.50%
Acetone sluble extractive	22.65%	18.40%

Table NO. 3: Result of Preliminary Qualityative Chemical Investigation of Jayapala beeja (Croton tiglium Linn.)

Sl No.	Chemical Tests	Ethanol Extract
1	Test for Sterols	
	a. Salkowaski test	-
	b. Liberman-burchard test.	-
	c. Sulphur test.	-
2	Test for Triterpenoids	-
	a. Liberman-burchard test.	-
3	Test for Flavonoids	
	a. Shinoda test	-
4	Test for Saponins.	
	a. Foam test	+
5	Tests for Carbohydrates.	
	a. Molisch's test	+
	b. Fehling's test	+
	c. Benedict's test	+
6	Test for Alkaloids	
	a. Mayer's test.	+
	b. Wagner's test	+
	c. Hager's test	+
	d. Dragendroff's test	+
7	Test for Tannins	+
8	Test for Proteins	-
	a. Millon's test	-
	b. Biuret test	-

9	Test for Resins	-
10	Test for Starch	-
11	Test for Glycosides	
	a. Legal test	-
	b. Baljet test	-
	c. Borntrager test	-
	d. Keller Killani test	-

Note: “+” Indicates present and “-” Indicates absent.

Observation and Result:

Observations during pilot study:

- Jayapala beeja extract
- Preparation of Standard drug:
- Amoxicillin which was in powder form was dissolved in 0.1N HCL to get uniform solution.
- Selection of Growth Media
- The nutrient agar media was found to be appropriate for selected strain of organism in this study of agar diffusion/cup-plate method
- Cup Plate Method:
- During the pilot study of the antibacterial assay, it was found that:
- Drugs exhibited the zone of inhibition by 24hrs.
- Zone of inhibition was maintained till 48hrs.
- However, by 72hrs there was complete bacterial growth.
- Study proper

Antibacterial activity:

1. Control
2. Jayapala beeja
- 3 Amoxicillin

BETWEEN GROUPS

Sample showed significant antibacterial activity against staphylococci aureus and E.coli. Antibacterial activity against s.aureus with Jayapala beeja aqueous extract at 80µl/ml, with inhibition zone as 0.7, 0.8, and 0.8 mm respectively.

DISCUSSION:

Any form of every research study is based on fundamental principle, scientific logic and reasoning. Discussion is a sequential step of written opinions and its all-possible participants. Discussion and interpretation of the research study becomes an essential and important thing of study for the scientific platform. Only a properly done discussion can fulfil the purpose of research work i.e. to draw some conclusion from the finding and results. Therefore, before concluding this work, it is necessary to discuss about the findings of all sections. Such discussions and scholastic deliberation let the scientific fraternity to know both the theoretical and practical profiles about the work. Hence the present research work has been discussed here thoroughly with all the possible ways and manners.

Infectious diseases have an alarming mortality rate. Statistics clearly show that about 50% of mortality in developing countries and about 20% of mortality in developed countries is due to infectious diseases. Infection control is a great task to field of medicine still today. Even though sufficient data is available about the methodology to treat krimiroga through Ayurvedic principals and a large number of drugs are attributed with krimighna activity by various Samhitas and Nighantus, the present day need is to revalidate the treasure of Krimighna dravyas, precisely demarcating which Krimighna dravya in what dosage form and posology, is active against which pathogen and the significance of its potency has to be zeroed in on, to eventually make it convenient for clinical practice.

The results of the current study are discussed at various levels:

1. Drug selected for the study
2. Selection of Jayapala beeja
3. Selection of Dosage.
4. Selection of microbes.
5. Selection of Standard drug
6. In vitro antimicrobial assay methods
7. Results from the study

1. Drug selected for the study

In Ayurvedic science various drugs have been mentioned under Krimighna Dravyas. It is said to be Jantujit by Priya nighantu, moreover the drug used in many instances of infectious diseases; modern day experimental studies promise for their antimicrobial activity, to be more specific this drug is active against gram + ve and gram bacteria. The drug has been selected for this study based on these factors.

It is having the references in Nighantus because of its Rasapanchaka (Kashaya, Tiktaandkaturasas, Laghu, Rukshagunas and Katuvipaka) for tackling Krimi, Kushtha etc. It is available worldwide and it is cost effective. Seed is the used part, and the references for its Krimighna activity are available in Nighantus.

- The drug Jayapala selected and collected for the study showed physicochemical standards, according to API and was within normal limits.

The macroscopic study confirms the genuinity of the drug Jayapala.

- Physicochemical analysis study of the selected drug Jayapala was carried out and it shows the presence of croton oil, crotonosides, crotonic acid.
- Thus, the genuinity was confirmed of the drug Jayapala selected for the study.
- The sample selected for the study showed that Physicochemical standards were in accordance with API standards

6. In-vitro antimicrobial assay

Phytochemical study: Jayapala beeja churna

Physico-chemical tests:

- **Total ash:** The total ash content of Shodhita Jayapala beeja is 2.22%. This may be due to presence of inorganic salts in the drug
- **Alcohol soluble extractive:** The alcohol soluble extractive in Jayapala beeja (24.50%). This indicates the phyto-constituents which are soluble in alcohol like, tannins, glycosides, etc.
- **Water soluble extractive:** The water soluble extractive in Jayapala beeja (1.80%). This indicates the phyto-constituents which are soluble in water like, Carbohydrates, Saponins etc.

Pilot Study:

- Agar diffusion / cup plate method was preferable than other methods because of the requirement of minimum sample size; as several samples can be tested on one microorganism.
- Pilot study was carried out to determine the dose of the Jayapalabeeja extract, for dose of swarasa 10 ml were tried. But it was not diffused in agar media.

- For dose fixation 10µl/ml to 100µl/ml concentration was tried. If it doesn't has any action further it was planned to try at higher concentration.
- Standard drug -250 mg of Amoxicillin in 0.1N HCl, which was used as (positive control).

Control-distilled water was taken as negative control. It was observed that during the study antimicrobial strain showed encouraging growth in sabouraud dextrose agar media. Hence it was taken for the study proper. The parameter selected for the antibacterial activity was zone of inhibition in microlitre in agar diffusion/ cup plate method. The study proper was carried out in single dose form- with human dose. In agar diffusion / cup plate method, jayapala beeja extract showed good activity against Staphylococcus aureus and E. coli

Understanding the prakruti vighatana brought about by rasapanchaka of

Jayapala beeja

In vitro study for antibacterial activity may be grossly compared to Prakruti Vighata Chikitsa Sutra for Krimi explained by Charaka. The Chikitsa Sutra adopted to bring about Prakruti Vighata is as follows:

"Prakrutivighatastvesham katutiktakashayakshraushanam dravyanamupayogahyatchanyadapi kinchidshelsmapurishapratyaneekabhutam tat syatetiprakrutivighatah" (Charaka Samhita Vimana Sthana 8)

> Role of Katu in bringing about Prakruti Vighata

✓ The Katu rasa of Jaypala Beeja Churna by Guru and Tikshna bring about the Upasoshana of Kleda and shleshma which is appropriate to cause Prakruti Vighata.

> Mode of action of Guna in causing Prakruti Vighatana

The Ruksha & Tikshnaguna of Jaypala Beeja churna is antagonistic to Snigdhe guna of Ama, Shleshma and Kleda, This cause's prakruti vighata of krimi.

Role of Vipaka in causing Prakruti Vighatana

✓ Katu Vipaka is Ruksha and Tikshna, therefore causes Shoshana of Prakruti (shleshma, ama, kleda and mala) thus Krimighna.

CONCLUSION:

Worldwide, food-borne diseases are a major health burden leading to high morbidity and mortality including India. As per Ayurveda, the cause of bacterial infection (krimi) are the ingestion of ksheer (milk), gud, tila, matsya (fish), aamup maans, pistanna (food prepared from flour), ksheer, fetid (puti ahara), putrefied (klina ahara) ill disposed (sankirna ahara) and viruddha ahara. The study was aimed at shodhana study and evaluation of Jayapala beeja (Croton tiglium Linn.) for its krimighna property w.s.r.to antibacterial property on staphylococcus aureus & E.coli-an invitro study. A phytochemical study reveals presence of Croton oil, alkaloids compounds in Jayapala beeja. It is observed from the In-vitro study that the drug Jayapala beeja has shown significant antibacterial activity on staphylococcus aureus and E.coli. Thus it can be concluded that at a lower dose of 80µl/ml Jayapala

beeja extract is having statistically significant antibacterial activity. The Jayapala showed effective antibacterial activity against S.aureus ranging 0.7 to 0.8 diameter zone of inhibition. The swarasa showed less efficacy against Ecoli compared to S.aureus. It is suggested that this medicinal plant may be used to discover new therapy to fight against microbial infection mediated disorders.

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