ORIGINAL RESEARCH PAPER

Ayurveda

ANTIBACTERIAL ACTION OF BUTEA MONOSPERMA SEED

KEY WORDS: Butea Monosperma Seed, Antibacterial, MIC,MBC

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ABSTRACT

Butea monosperma i.e. Palash tree is native to India. This plant is used to treat various diseases like Kasa,Grahani,Arsha,Udarroga,Kushta,Gulma etc. Leaf, stem bark, flowers, seeds and gum are the parts used in different yogas..Seeds are indicated in many skin diseases. Internally mainly for worms and externally for ringworm, boils, pimples, bubbles, tumors and also in various infective diseases. In ayurveda prime property of Palasha is told as Krimghna .To validate this property against microbes, palash seed water and alcohol extract for S.aurus (gram+ve) and E.coli (gram-ve)bacteria as test organisms were selected.To evaluate antibacterial efficacy minimum inhibitory concentration(MIC) and minimum bactericidal concentration(MBC) methods were selected.The antibacterial is compared with standard antibiotics Gentamicin (10µg) and Doxycycline (30 µg).

INTRODUCTION

'Palash' is Sanskritized Ayurvedic name of a medicinal tree botanically equated to *Butea monosperma Linn* belongs to family *Fabaceae.* It is medium sized deciduous tree, with a somewhat crooked trunk,10-15'in height and 5-6'in girth. The bark is bluish grey or light brown and yields a gum. Its bright orange red flowers (1.5-2"long)bloom in great profusion at the beginning of the hot season before the appearance of new leaves. The pod contains a single seed(1"x3/4") at its apex. Palash tree is common throughout India, Burma and Ceylon,except in very arid parts.

Vernacular Names-

Hindi-Dhaka; English-Flame of the forest tree;Telugu-Moduga chettu;Tamil-Paras;Kannad-Muttuga;Malayalam-Palisin samat.

The literature review of Palash in Ayurveda was found in Vedas, Upavedas, Caraksamhita, Susrut Samhita , Astang sangraha, Ashtang hrudaya and in Nighantus. Susruta and Vagbhata mentioned Palasha in Rodradi, Muskadi, Ambasthadi, Nyagrodhadi gana.It has katu ,tikta, kashay rasa, katu vipaka, Ushna virya and vatakapha hara properties.

Palasha fruit and seed are hot, dry. digestible, anti-helmintic; aperient; used in urinary problems, piles; cures Vata and Kapha vitiation. skin diseases. tumors, abdominal troubles; given for scorpion- sting.The bark and the seeds are given For snake bite. Susrut prescribes pulverized seeds of Palash mixed with rice- water as a very effective remedy in eliminating intestinal worms and chronic diarrhoea.. However, clinical studies have not unequivocally demonstrated the antibacterial activity of the drug when used against the bacterial pathogens of clinical importance. So, study reveals antibacterial efficacy of palash seed for S.aurus (gram+ve) and E.coli (gram-ve)bacteria as test organisms. To evaluate antibacterial efficacy minimum inhibitory concentration(MIC) and minimum bactericidal concentration(MBC) methods were selected.

Materials:- Palash beej were self collected and authenticated by experts. S.aureus ATCC29213 Bacteria (gram+ve) and E.coli ATCC25922 Bacteria (gram-ve) were used as test organisms.The antibacterial potential is compared with Gentamicin (10µg) and Doxycycline (30 µg).

Methods:-Collection of Material-Palash beej were self collected in the vicinity of Pune and identified by the teacher of Dravyaguna department in the faculty of Ayurveda. Seeds were dried at room temperature in shady area until they were free from the moisture after that pulverized in the mechanical grinder to a moderate fine powder and stored in closed airtight vessel for further analysis.Physicochemical parameters such as pH,foreign matter,Ash value, Acid –insoluble ash, Water soluble extractive, Alcohol soluble extractive values were determined as per the standard Ayurvedic Pharmacopoeial methods and recorded as-

Physicochemical Study

Tests	Results
PH	Alkaline
Foreign Matter	Nil
Ash Value	5.90%
Acid –insoluble ash	0.236%
Water soluble extractive	35.13%
Alcohol soluble extractive	10.04%

Preliminary Phytochemical analysis carried out according to standard procedures and recorded as

Preliminary Phytochemical tests-

Tests	Water	Alcohol
Alkaloids	Positive	Negative
Carbohydrates	Positive	Positive
Flavonoids	Positive	Positive
Terpenoids	Positive	Positive
Protiens	Positive	Negative
Saponin	Positive	Negative
Steroids	Positive	Positive
Tannins	Positive	Negative
Starch	Positive	Positive

Macroscopic Characters of Butea monosperma seed-

Seed flat, kidney shaped.2.5 to4cm long.1to 3cm wide, dark reddish-brown, thin, glossy; hilum clear, situated near middle of concave edge of seed; odour, faint; taste, slightly acrid and bitter.

Microscopic Characters of Butea monosperma seed-

The seed taken for the study were very hard.So they were soaked in brine till they set softened and enable for easy sectioning. The transverse section as required are taken and viewed under microscope.

It shows a wide zone of testa , consisting of a layer of palisade

cells, a row of bearer cells and many layers of parenchy matous cells; palisade cells compactly arranged, columnar shaped and covered with thick cuticle, followed by single row of bearer cells; parenchymatous layers consisting of many rows of cells, filled with reddish-brown contents; a number of vascular bundles occur in a row,in middle region of parenchymatous zone; cotyledon consists of a single layered epidermis, composed of square to oval cells, covered with cuticle; mesophyll cells bear hyaline walls, oval to irregular shaped with small intercellular spaces; simple, oval to round, starch grains with concentric striations, and cetric hilum, compound grains having 2 to 4 components measuring 8 to 16µ in dia., present in cotyledons.

Antibacterial activity-

The evaluation of the antibacterial activity of palash seed extracts against test organisms *Eschrichia coli* ATCC 29213 and *Staphylococcus aureus* ATCC 25922 (Sahu and Padhy 2013) was performed .The antibacterial activity was evaluated by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. The clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) recommendation methods were followed for minimum inhibitory concentration(CLSI,M07-A10,2015) and minimum bactericidal concentration(CLSI,M26-A10,2015).

Media used for Antibacterial activity:

Muller Hinton agar (Hi Media M173-500G) and Muller Hinton broth (Hi Media M391-500G)
Were used as per CLSI guidelines.

Minimum inhibitory concentration(MIC)-

The determination of antibacterial activity of seed extracts against the microorganisms was performed by broth dilution method for minimum inhibitory concentration(MIC)(Sahu and Padhy 2013). The sterile Muller Hinton broth (1ml) tube was added with 0.1ml of microorganism with density adjusted to a 0.5 McFarland turbidity standard (10^6cfu/ml).The 10,12.5,25,50,100,150,and 200µl of each seed extracts was added. The inoculated Muller Hinton broths were incubation was done at 37°Cfor 24 hours. Minimum inhibitory concentration(MIC)were determined for water and alcohol extracts of Palash beej against *Eschrichia coli* ATCC 29213 and *Staphylococcus aureus* ATCC 25922 .

Name of the extract:-Palash beeja water soluble extract(WSE)

Minimum inhibitory concentration (MIC)

	1	2	3	4	5	6	7	PC	NC
	200µl	150	100	50	25	12.5	10	No.drug	No organism
S.aureus	NT	NT	NT	NT	NT	T	T	T	NT
E.coli	NT	NT	NT	NT	NT	T	T	T	NT

NT-No turbidity T-Turbidity

Name of the extract:-Palash beeja Acid soluble extract(ASE)

Minimum inhibitory concentration (MIC) -

	1	2	3	4	5	6	7		
	200µl	150	100	50	25	12.5	10	No.drug	No organism
S.aureus	NT	NT	NT	NT	NT	NT	T	T	NT
E.coli	NT	NT	NT	NT	NT	NT	T	T	NT

Minimum bactericidal concentration (MBC)-

The evaluation of antibacterial activity of seed extracts against the microorganisms was performed by agar plate method for minimum bactericidal concentration (Sahu and Padhy 2013) .The sterile Muller Hinton agar plates were streaked with one loop full form the incubated tubes of minimum inhibitory concentration(MIC)determination . The Muller Hinton agar plates were incubated at 37°Cfor 24 hours and then checked for the growth of microorganisms. The minimum bactericidal concentration(MBC) were determined for water and alcohol extracts The minimum bactericidal concentration(MBC) were determined for water and alcohol extracts of Palash seed against *Eschrichia coli* ATCC 29213 and *Staphylococcus aureus* ATCC 25922 .

Minimum bactericidal concentration (MBC) of Palash beeja water soluble extract(WSE)

	1	2	3	4	5	6	7		
	200µl	150	100	50	25	12.5	10	No.drug	No organism
S.aureus	NG	NG	NG	NG	G	G	G	G	NG
E.coli	NG	NG	NG	NG	NG	G	G	G	NG

NG-No growth, G-Growth

Minimum bactericidal concentration (MBC)of Palash beeja Alcohol soluble extract(ASE)

	1	2	3	4	5	6	7		
	200µl	150	100	50	25	12.5	10	No.drug	No organism
S.aureus	NG	NG	NG	NG	G	G	G	G	NG
E.coli	NG	NG	NG	NG	NG	G	G	G	NG

NG-No growth, G-Growth

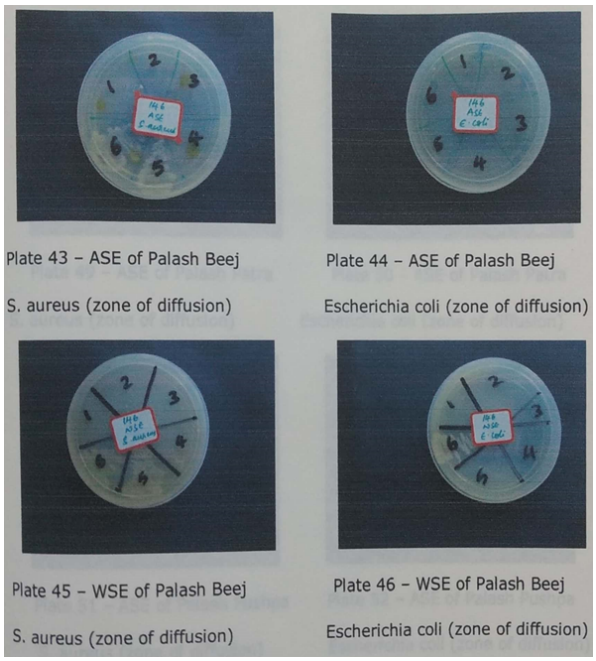

Plate 43 – ASE of Palash Beej S. aureus (zone of diffusion)

Plate 44 – ASE of Palash Beej Escherichia coli (zone of diffusion)

Plate 45 – WSE of Palash Beej S. aureus (zone of diffusion)

Plate 46 – WSE of Palash Beej Escherichia coli (zone of diffusion)

Observations :-

To explore the antimicrobial activity of palash beeja study was conducted. For this purpose both gram positive(S.aureus) and gram negative (E.coli) bacteria were used as test organisms.
Observations were as follows:-

- Palash beej sample tested positive for alkaloid, proteins, saponin and tannins in water soluble extract. Carbohydrates, Terpenoids, starch, flavonoids and steroids are present in both water and alcohol soluble extracts
- Palash beeja water soluble extract for Minimum inhibitory concentration (MIC) shows no turbidity for S.aureus on 25 µl concentration and for E.coli on 25 µl.This means at the above mentioned concentration bacterial growth is inhibited
- Palash beeja water soluble extract for Minimum bactericidal concentration (MBC) Shows no growth for S.aureus on 50µl concentration and for E.coli on 25 µl. It means the above mentioned concentration is bactericidal.
- Palash beeja acid soluble extract for MIC shows no turbidity for S.aureus on 12.5 µl concentration and for E.coli on 12.5 µl.This means at the above mentioned at

same concentration bacterial growth is inhibited.

- Palash beeja acid soluble extract for MBC shows no growth for S.aureus on 50µl concentration and for E.coli on 25 µl.This means at the above mentioned concentration bactericidal action is approved.

CONCLUSION

In this study, antimicrobial activity of Palash beeja was assessed in water and alcohol extract for S.aurus (gram+ve) and E.coli (gram-ve)bacteria .Study showed both extract have significant effect of antibacterial activity on test organisms so study supports Krimighna property of Palasha.This is preliminary report further investigations are necessary to evaluate antibacterial activity. Moreover, other parts of the plants need to be studied to evaluate the antibacterial activity.

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