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Research Article

Evaluation of Enzymatic In-vitro anti diabetic activity of *Butea* monosperma (Lam.) Kuntze

Nilima Dharkar[1], Preetam Pradip Itnar[2*], Arati Dubewar[3], Ramesh Bhonde[4]

1. PhD Guide, Professor & HOD, Ras Shastra Evum Bhaishajya Kalpana, Dr. D. Y. Patil College of Ayurved & Research Centre, Dr. D. Y. Patil Vidyapeeth, (Deemed to be University), Pimpri, Pune. Maharashtra, India.
2. PhD Scholar, Ras Shastra Evum Bhaishajya Kalpana, Dr. D. Y. Patil College of Ayurved & Research Centre, Dr. D. Y. Patil Vidyapeeth, (Deemed to be University), Pimpri, Pune. Maharashtra, India. & Assistant Professor, Sumatibhai Shah Ayurved Mahavidyalaya Hadapsar, Pune, India.
3. Professor, Ras Shastra Evum Bhaishajya Kalpana, Shri.K.R.Pandav Ayurved College, Nagpur, India.
4. Emeritus Scientist, Dr. D.Y.Patil Dental College and Hospital, Pimpri, Pune, Maharashtra, India.

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Abstract

Background: About 830 million people worldwide have diabetes, the majority living in low and middle- income countries. The diabetic population has to consume antidiabetic medicines for a long duration due to its chronic nature. Herbal formulations are preferred due to lesser side effects and low cost. Ancient Ayurvedic texts has mentioned *Palash, Butea monosperma,* (Lam.) Kuntze which belongs to Fabaceae Family, in Diabetes Mellitus. The management of diabetes can be achieved by reducing post-prandial hyperglycemia by delaying the activities of the enzymes α-amylase and α- glucosidase which are responsible for the digestion of carbohydrates and absorption of glucose in the digestive tract, respectively. Objectives: The study aimed to evaluate the in vitro inhibitory potential of water-soluble extracts of *Butea monosperma (Lam.) Kuntze* (Palash Ghana) on α-amylase and α-glucosidase enzymes, which play a crucial role in carbohydrate digestion and glucose absorption. Material and Methods: *Palash Ghana* was prepared by decoction and solidification methods, extracting water- soluble components of the whole plant. α-Amylase inhibitory activity was assessed using the Dinitrosalicylic Acid method with concentrations ranging from 50 to 250 µg/mL. α- Glucosidase inhibition was evaluated using the p-NPG method at concentrations between 100 and 500 µg/mL, with Acarbose as a positive control. Result and Conclusion: *Palash Ghana* exhibited a dose-dependent inhibitory effect on α-amylase, with a maximum inhibition of 83.12% at 250 µg/mL. In contrast, α-glucosidase inhibition was moderate, reaching 22.16% at 500 µg/mL. The findings suggest that Palash Ghana may serve as a potential natural antidiabetic agent.

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Introduction

Diabetes has affected nearly 200 million of population in 1990, which increased to 830 millions in 2022 as per WHO record (1). It is widely recognized that diabetes is one of the major public health challenges in the 21st century. Very few countries have so far been able to halt the rise in diabetes and the rise has been steepest in low- and middle-income countries (LMICs). Diabetes is among the top 10 leading causes of death. More importantly, it is the only major non communicable disease showing an increase in premature deaths between the year 2000 and 2019 (2). As per International Diabetes Federation (IDF), more than 70 million Indians are diagnosed with Diabetes nationwide, including both urban and rural areas (3). Type I and type 2 diabetes are treated with exogenous insulin and oral hypoglycemic agents; respectively, in conventional therapy. Another therapeutic approach is to decrease the post- prandial hyperglycaemia which is achieved with the enzyme inhibitors such as acarbose, voglibose and miglitol which function by retarding the action of the gastrointestinal carbohydrate hydrolysing enzymes α-amylase and α-glucosidase. As a result, these substances delay carbohydrate digestion thereby decreasing the rate of glucose absorption, i.e. they blunt the post-prandial plasma glucose rise .Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva while *α*-glucosidase is found in the mucosal brush border of the small intestine (4). But the numerous synthetic drugs available for the management of diabetes are often found to be associated with side effects. This influence researchers to focus towards natural product and alternative medicines that are effective, inexpensive, affordable and safe to consume.

Diabetes Mellitus is compared with the disease *Prameha/ Madhumeha* in Ayurveda. Ayurveda can be the answer for complete management of Diabetes mellitus as evident from the available treatises. According to WHO, about 70-80% of the World populations rely on non-conventional medicines mainly herbal sources in their healthcare.(5) *Palash, Butea monosperma*, (Lam.) Kuntze is one of the plants mentioned in Ayurvedic

*** Corresponding Author:**
Preetam Pradip Itnar
PhD Scholar in Rasashastra & Bhaishajya Kalpana, Dr. D. Y. Patil College of Ayurved & Research Centre Dr. D. Y. Patil Vidyapeeth, (Deemed to be University), Pimpri, Pune. Maharashtra, India.
Email Id: drpreetamharal@gmail.com

classics (6). It is known for its Anti Helminthic(7), Anti diabetic(8), Anti cancer(9) and Antidiarrhoeal activity(10).

Previous work done to prove anti diabetic activity of plants have used aqueous or alcoholic extracts of individual parts of a plant (11). Ayurveda has emphasized on the importance of *Panchang* or whole plant in therapeutics(12). Also, commonly used Ayurvedic formulations like *Churna*(Powders), *Guti Vati*(Tablets) etc. contains whole plants unless specifically mentioned for their preparations. *Ghana* (Water soluble solid concentrate) is the dosage form exclusively mentioned in Ayurvedic texts, which is a derivative of *Kwath Kalpana* and involves extracting maximum water-soluble and some water-insoluble components via the decoction method, followed by reheating until solidified. To the best of our knowledge there is no scientific evidence on the inhibitory effect of any dosage form or formulation of *Butea monosperma*, (Lam.)Kuntze. on carbohydrate hydrolyzing enzyme. Considering all these, the current study was planned which deals with collection of whole plant(*Palash*), Pharmaceutical preparation of dosage form, physico-chemical analysis and evaluation of its In vitro Anti diabetic effect through Alpha amylase and Alpha glucosidase inhibition assay.

Material and Methods

Collection of Plant

Panchang of *Palash, Butea monosperma (Lam.) Kuntze* i.e ., *Tvak* (Bark), *Phal* (Fruit), *Pushpa* (Flower), *Mula*(Root), *Patra* (Leaves) were collected from field and authenticated from Botanical Survey of India(BSI),Pune. Collected material was shade dried, powdered mechanically and stored in airtight container.

Pharmaceutical Preparation of *Palash Ghana* (Water soluble solid concentrate)

Ghana was prepared in two stages as per Classical reference.

Panchang of drug in coarse powder form(240gm) (passed through sieve no. 20) were soaked with 16 times potable water(3840ml) in a Stainless Steel vessel and kept aside overnight for soaking (12 hrs).Next morning the contents were subjected to mild heat (90°C-95°C) with continuous stirring. Water was evaporated slowly till its reduction to 1/8th(480ml) and was filtered through four fold cotton cloth to obtain Decoction (13).Prepared decoction was further subjected to heat with continuous stirring until it attains honey like consistency. The mixture was spread on stainless steel plate for drying and prepared *Ghana*(12gms) was stored in airtight container.(14)

Physicochemical analysis of *Palash Ghana*

Ghana was standardised at ISO Certified Drug testing Laboratory, Wagholi, Pune as per API norms(15).

Table 1: Results of Physicochemical analysis of Raw *Palash Panchang*

Sr.no.	Tests	Results	API Standards (StemBark)
1	Total Ash	11.84%w/w	Not more than 12%
2	Acid insoluble Ash	1.27%w/w	Not more than 1.5%
3	Water Soluble Extractive	78.70%w/w	Not less than 10%
4	Alcohol Soluble Extractive	5.91%w/w	Not less than 14%

Results

Physicochemical analysis of *Palash Ghana*(PG)

For ***Palash Ghana* (PG)**, the total ash content was 14.84% w/w, Acid-insoluble ash was 1.77% w/w, The water-soluble extractive value was 75.74% w/w, while the alcohol-soluble extractive was 2.91% w/w

In vitro methods employed in antidiabetic studies

Inhibition of alpha-amylase enzyme (16)

α-amylase inhibitory activity of the *Palash ghana* was carried out according to the standard method in accredited laboratory. In the reaction mixture containing 500µl phosphate buffer (100 mM, pH = 6.8), 100µl α–amylase (2 U/ml), and 200µl of varying concentrations of sample (50,100,150,200,250 µg/mL) was pre incubated at 37°C for 20 min. Then, the 200µl of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37°C for 30 min; 1000µl of the DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using spectrophotometer. Acarbose, at various concentrations (50, 100, 150, 200, and 250µg/ml) was used as a standard. Blank (substrate+enzyme without test sample) was set up in parallel as control, and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula, Inhibitory activity

$$(\%) = (Ac - As/Ac) \times 100$$

Where As is the absorbance in the presence of test substance and Ac is the absorbance of control.

α-glucosidase inhibitory activity(17)

α-glucosidase inhibitory activity of *Palash Ghana* was carried out according to the standard method. In reaction mixture containing 500µl phosphate buffer (100 mM, pH = 6. 8), 100µl alpha-glucosidase (1 U/ml), and 200µl of varying concentrations of Test samples (100,200,300,400,500 µg/ml) was pre incubated at 37°C for 15 min. Then, 200µl P-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 500µl Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Spectrophotometer. Acarbose at various concentrations (100,200,300,400,500 µg/ml) was included as a standard. Blank(substrate+enzyme without test sample) was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity } (\%) = (Ac - As/Ac) \times 100$$

Where As is the absorbance in the presence of test substance and Ac is the absorbance of control.

Table 2: Results: Alpha Amylase inhibitory Activity of *Palash Ghana*

Sr.no.	Conc.of *Palash Ghana* (µg/ml)	% Inhibition of *Palash Ghana*	% Inhibition of Acarbose
1	50	62.23	93.40
2	100	72.32	94.55
3	150	75.71	95.08
4	200	81.08	95.56
5	250	83.12	96.98

Figure 1: Alpha amylase inhibition activity of P.G as compared to Acarbose expressed in Percentage

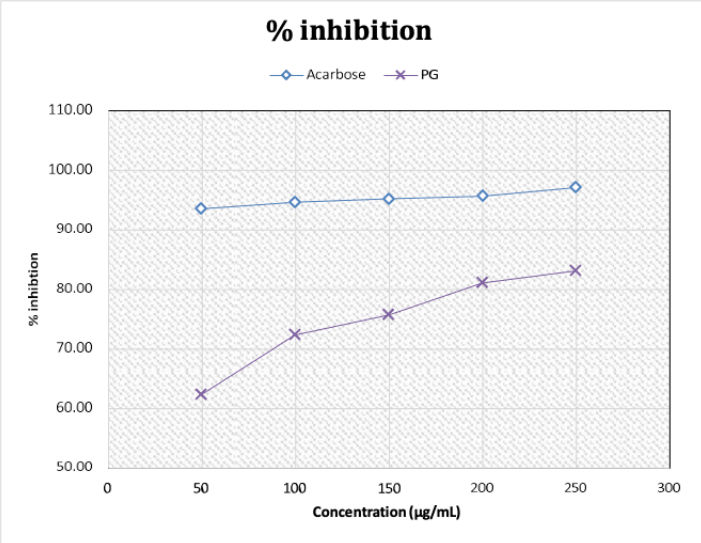
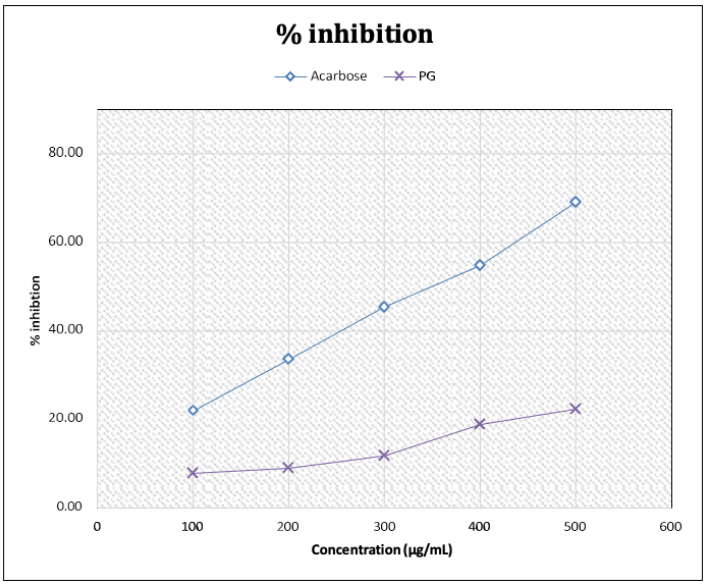


Table 3: Results of Alpha Glucosidase inhibitory Activity of *Palash Ghana*

Sr.no.	Conc.of *Palash Ghana* (µg/ml)	% Inhibition *Palash Ghana*	% Inhibition Acarbose
1	100	7.85	21.89
2	200	9.00	33.51
3	300	11.76	45.38
4	400	18.76	54.79
5	500	22.16	69.04

Figure 2: Alpha Glucosidase inhibition activity of P.G as compared to Acarbose expressed in Percentage



In the α-amylase assay, test sample showed concentration-dependent inhibition, exhibiting the highest inhibitory activity at 250 µg/mL (83.12%) Acarbose demonstrated strong inhibition across all concentrations, with a maximum of 96.98% at 250 µg/mL, establishing its effectiveness as a control. In the α-glucosidase assay, test sample showed the highest inhibition at 500 µg/mL (22.16%).

Discussion

Ayurveda is one of the oldest medical systems, which comprises thousands of medical concepts, drugs and hypotheses. Ayurvedic database comprises of knowledge about the botanical sources that are primarily based on past experiences and present use in India as a living tradition. This gives some distinct advantages to the Drug Discovery process. Ayurvedic medicines use largely herbal and herbo-mineral preparations to treat all the diseases encountered in India which include many found in other parts of the world such as cancer, cardiovascular disease, asthma, viral hepatitis, diabetes. Ayurvedic physicians and hospitals have long histories of drugs used-compositions, formulations, dosage regimens, side effects and therapeutic effects. These records are particularly valuable since effectively these medicines have been tested for many years on people.(18) Reverse Pharmacology refers to reversing the routine clinic practice to the laboratory examination for the proper validation of a traditional medicinal systems. Traditional knowledge-driven drug development can follow a reverse pharmacology path and reduce time and cost of development. Reverse Pharmacology approach can help in reducing failure rates of clinical implications of the herbs or their formulations which are already described in Ayurveda. The mass screening of plants in the search for new drugs is vastly expensive and inefficient. It would be cheaper and perhaps more productive to re-examine plant remedies described in ancient and mediaeval texts(19). *Palash* (*Butea monosperma* Lam.) mentioned in ancient Ayurvedic texts is indicated in *Prameha* (Diabetes mellitus) and is used for the same since times immemorial. *Ghana kalpana* is secondary derivative *kalpana* of *Kwath Kalpana*, Advantages of *Ghana kalpana* is increased shelf life and reduced dose. (20) In present study *Palash Ghana* is prepared from whole plant and yielded 5% of *Ghana,* which was subjected to various physicochemical parameters along with raw materials used(*Panchang*). Physicochemical analysis was carried out as per API norms. Out of the four evaluated parameters of raw material (Table no.1), Total Ash (11.84%), Acid-Insoluble Ash (1.27%), and Water-Soluble Extractive (78.70%) comply with API standards, indicating acceptable inorganic matter levels. However, Alcohol-Soluble Extractive (5.91%) falls below the minimum requirement of 14%, suggesting lower levels of alcohol-soluble phytochemicals, possibly due to plant material variation as whole plant is used in this study, while the API standards referenced pertain specifically to stem bark. For *Palash Ghana* (PG)**,** the total ash content was 14.84% w/w, indicating a moderate level of total inorganic matter. Acid-insoluble ash was 1.77% w/w, suggesting minimal siliceous contamination. The water-soluble extractive value was 75.74% w/w, while the alcohol-soluble extractive was at 2.91% w/w, As no API standards are available for *Palasha Ghana*, these findings represent the first baseline physicochemical profile for PG, contributing valuable reference data for future standardisation.

Various researches has been carried out on *Palash* to prove its Antidiabetic activity where Aqueous and Alcoholic extracts of various parts have been used. A study done by (Harish et al., 2014). (21) said that *B. monosperma* leaves inhibited α-amylase, α-glucosidase and sucrase enzymes in succession to varying degrees, whereas the bark inhibited only α- amylase to a significant extent. Study conducted by KS Chandrashekharaiah said that leaves and bark extract of *Butea monosperma***,** (Lam.) Kuntze showed alpha amylase inhibition by 94% (22). A study was conducted using decoction, extracts and tincture of *Butea monosperma***,** (Lam.) Kuntze flowers, decoction showed alpha glucosidase inhibitory activity which was expressed as IC50

values (mg/mL). Also Salicylic acid and syringic acids are phenolic acids identified from *Butea monosperma***,**(Lam.) Kuntze (23). Syringic acid has shown significant antidiabetic activity in Wistar rats (24). All these studies has proved antidiabetic activity of either one or two parts of *Butea monosperma***,** (Lam.) Kuntze. but no study has been done on whole plant.

Different parts of a plant (roots, leaves, stems, flowers, seeds, bark) contain different types and concentrations of bioactive compounds. For example Roots and Bark often have higher concentrations of alkaloids, glycosides, and tannins while leaves are rich in flavonoids(25). So, use of whole plant might definitely help to achieve higher therapeutic efficacy. A study done on *Ashwagandha* (*Withania somnifera*) to assess the whole plant based phytotherapeutics found that whole plant-based formulations have other metabolites which can nullify the toxicity associated with roots. Extracts made from whole plants, therefore can holistically impart all therapeutic benefits as well as mitigate toxicity(26). Thus use of whole plant may definitely be helpful to achieve synergistic action and also to reduce toxicity if any. Previous studies have demonstrated the antidiabetic activity of individual plant parts; however, this study underscores the benefits of using the whole plant for enhanced therapeutic efficacy.

Conclusion

Data accrued from the present study clearly indicate that the *Ghana(*water soluble solid concentrate) prepared from *Panchang* (whole plant) of *Palash Butea monosperma***,** (Lam.) Kuntze exhibited concentration-dependent inhibition of alpha amylase, exhibiting the highest inhibitory activity at 250 µg/mL (83.12%) however weak inhibition of alpha glucosidase was seen which was concentration dependent and highest inhibitory activity was seen at 500 µg/mL(22.16). Thus the results provided evidence that the studied plant is potential source of natural antidiabetic agents.

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