COMPARATIVE STUDY OF PHARMACOLOGICAL & THROMBOLYTIC ACTIVITY OF PLANT LEAVES EXTRACT (*Garcinia indica, Psidium guajava, Artocarpus heterophyllus)*

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Abstract : India has a long tradition of practicing ethnobotany. The bioactive secondary metabolites in plants have diverse pharmacological activities and hence always been used as traditional medicine to treat various ailments. There is dire need of drugs that are cost effective, has less or no side effects, synthesized sustainably, do not pose drug resistance issue and has same effectiveness as synthetic medicine available in the market. The study carried out on leaf extract of various plants puts some lights on potent secondary metabolites and their analysis as pharmacological agent.

The anti-amylase drug helps in prevention of prolonged hyperglycemic condition in diabetic patients, thrombolytic drug is used in the treatment of thrombosis & antiplaque drug is used for treating dental caries by inhibiting plaque formation. The study was aimed to check & compare the anti-amylase, thrombolytic & antiplaque activity of methanolic leaf extract of *Garcinia indica*, *Psidium guajava & Artocarpus heterophyllus.* Soxhlet extraction of these plant leaves was done using a polar solvent, methanol & the powder obtained after drying the extracts in hot air oven were used as sample for phytochemical screening, anti-amylase activity detection using DNSA method, thrombolytic assay & antimicrobial susceptibility testing (AST). *G. indica*, *P. guajava & A. heterophyllus* showed 66.87%,50.38% & 48.83% inhibition of amylase enzyme (anti-amylase activity) while 53.67%,39.24% & 36.25% clot lysis (thrombolytic activity) were found respectively. Streptokinase was used as positive control for thrombolysis. Anti-plaque activity was found only in *G. indica* extract.

Keywords : Anti-amylase, Hyperglycemic, Thrombosis, Dental caries, Antimicrobial susceptibility testing.

I.INTRODUCTION

Diabetes mellitus is a disease that prevents our body from properly using the energy from the food we eat. It is characterized by postprandial and fasting hyperglycemia with disturbances in carbohydrates, fat and protein metabolism. There are two types of diabetes mellitus, Type I (caused by deficiency of insulin secretion) and Type II (occur due to insulin resistance). Prolonged hyperglycemic condition in diabetes patients induces non-enzymatic glycation reaction which leads to formation of AGEs (advanced glycation end products) which induces cellular dysfunction and in turn leads to long term diabetes complications & age related diseases like Alzheimer's disease, etc. [12]. So, to prevent the postprandial hyperglycemic condition the inhibition of α-amylase & α-glycosidase enzyme need to be done to delay digestion and absorption of carbohydrates in gastrointestinal tract. Thus, these inhibitors lowers blood glucose levels [13].

Thrombosis is a process of formation of blood clot in blood vessel leading to blockage [15]. Thrombolysis is a process in which thrombolytic drugs are used to dissolve dangerous clots in blood vessels, which improve blood flow & prevent damage to tissues & organs. These drugs either directly or indirectly activate plasminogen which is the precursor for plasmin & destroys the fibrin surrounding the clot. Thus, preventing from life threatening disorders like myocardial infarction, vein thrombosis, ischemic stroke, etc. The thrombolytic drugs available in the market shows various side effects like bronchospasm, uncontrolled hypertension, internal bleeding, nausea & anaphylaxis, etc. [15].

Dental caries is a localized, transmissible pathological infectious disease which results in destruction of enamel of teeth. It is caused by demineralization (loss of calcium & phosphate) of teeth due to acid produced by fermentation of sugars (sucrose) by microorganisms residing in dental plaque (biofilm). *Streptococcus mutans* is the main etiological agent of dental caries. It is a gram positive cocci which colonize the tooth surface & forms plaque by forming exopolysaccharide (EPS) using sucrose. Dental plaque is a biofilm that grows in the oral cavity. Biofilm is an extracellular matrix that surrounds microbial cells & is made up of exopolysaccharide, protein & DNA. It is formed due to unhygienic oral condition. These biofilms are resistant to many antibiotics/antimicrobial agents. The drugs available in market for example; Penicillin, Cephalosporins, etc. have side-effects like vomiting, tooth staining, etc. [19].

According to WHO, 80% population of some Asian & African countries depends on traditional herbal medicine. Also, 25-40% of pharmaceutical drugs available are derived from plant origin [11]. The medicinal plants have various pharmacological activities like anti-diabetic, antiviral, antioxidant, antimicrobial, etc. which helps in treatment of various diseases or conditions due to the presence of different secondary metabolites. Secondary metabolites are chemical compounds not required for the growth & maintenance of the cellular functions & are the end products of primary metabolism [7]. Various parts of plant like leaf, bark, stem, fruit & seed, etc. can be used to obtain plant extract possessing different secondary metabolites responsible for treating different diseases. The phytochemical screening is carried out to determine various bioactive compounds like flavonoid, phenols, esters, etc. present in plant extract by using detection tests.

Garcinia indica is commonly known as 'Kokum', belonging to Clusiaceae family & is found in Western Ghats of India & South Konkan region of Maharashtra. The fruit rinds and leaves are used to treat various inflammatory ailments, rheumatic pain and bowel complaints [8].

Psidium guajava, commonly known as 'Guava', belonging to Myrtaceae family & grows widely in tropic areas as it can be grown on a big range of soils. It is used for the treatment of diarrhea, dysentery, gastroenteritis, hypertension, diabetes, caries and pain relief and for improvement in locomotors coordination [9].

Artocarpus heterophyllus is a plant belonging to Moraceae family & is commonly referred as 'Jackfruit' in English & 'Katahar' in Nepali [11]. The infusion of mature leaves and bark is supposed to be effective in the treatment of diabetes, gall stones and relieve asthma. Leaves are believed to possess wound healing effects, reduce pain and relieve ear problems [10].

MATERIALS AND METHODS

2.1 Collection of sample

Healthy fresh leaves of *Garcinia indica* was collected from Devgad village (Maharashtra), *Psidium guajava* from Mahindra Company, Kandivali (E) & *Artocarpus heterophyllus* from Patkar Varde College Campus, Goregaon (W).

2.2 Extract preparation

The collected plant leaves were washed thoroughly with water, air dried for a week and then dried in hot air oven at 80ºC for few days. The dried leaves were grinded to obtain coarse powder for Soxhlet extraction.10g of leaf powder was extracted in 150mL methanol using Soxhlet apparatus at 65-70ºC. The liquid extract obtained was dried in oven at 60ºC for 1-2 days followed by scrapping and storing in eppendorf tubes sealed with paraffin at 4ºC for subsequent use.

2.3 Phytochemical screening

The screening and identification of bioactive compounds of these three extracts were carried out using the standard procedure as described [1–6].

Table No.1: Phytochemical screening tests

Phytochemical constituent	Name of the Test
Carbohydrates	Molisch's test
Alkaloids	Dragendorff's test
Steroids	Salkowski's test
Glycosides	Keller-Kiliani test
Amino acids & proteins	Ninhydrin test
Saponins	Foam test
Flavones	Alkaline reagent test
Phenolic compound & tannins	$FeCl_3$ test
Anthocyanin	NaOH test
Oils & fats	Spot test
Triterpenoids	Salkowski's test

2.4 Anti-amylase activity

The inhibition of alpha amylase was determined by using a method described by Prabhakar et al.[13]
Formula: % inhibition = [(O.D. of control – O.D. of extract)]/ [O.D. of control] x100

2.5 Thrombolytic activity

• Sample preparation :
The plant extract was used to prepare sample solution of concentration (10mg/mL) using sterile distilled water & mixed properly by vortexing.

• Streptokinase solution preparation :
Icikinase vial (15,00,000 IU) bought from Vijay Life Care [Bhandup (W)] was used as positive control. 5mL distilled water was added in this vial & mixed properly to prepare stock solution.

• Thrombolytic assay :
1.5mL of blood sample was collected from healthy individual & 500 µL blood was transferred in a clean pre-weighed Eppendorf tubes & incubated at 37ºC for 45 min.
2. After clot formation, the tubes were centrifuged using cooling centrifuge at 2000 RPM for 5 mins.
3. The serum was removed completely & the tubes were weighed to determine clot weight.
4.100 µL of plant extract was added to each tube, 100 µL of streptokinase & distilled water were used as positive & negative

control respectively.
5. All the tubes were incubated at 37ºC for 90 mins.
6. After incubation, the excess fluid was decanted & all the tubes were again weighed to determine the difference in weight
.

Formula: % clot lysis = (Weight of lysis clot/Weight of clot before lysis) x 100

2.6 Antiplaque activity

• Isolation of *Streptococcus mutans* :
Dental plaque sample was collected from one of the group member by the assistance of Diagno Lounge (Goregaon) using sterile swab method. The sample suspension was prepared using sterile saline. This sample was swabbed on TYCS (Tryptone Yeast Extract Cystine) agar medium supplemented with 5% & 20% sucrose using sterile cotton swab & incubated at room temperature for 48 hours (anaerobic condition). White colonies with crystalline appearance & exopolysaccharide surrounding to them were selected & subcultured on fresh TYCS (5% & 20%S) agar plates using T-streaking method & further subcultured on TYCS (5% & 20%S) & Luria Bertani agar slants [22-24].

• Identification of isolate :
Identification was done using Gram staining & KOH test [20]. Various biochemical tests such as sugar fermentation, salt tolerance (6.5% NaCl), catalase, oxidase, urease, Hughleifson's & Voges-Proskauer (VP) test [22, 25, 27] were carried out.

• Antimicrobial Susceptibility Testing (AST):
This test was carried out to check whether the isolated *Streptococcus spp* is susceptible against the plant leaf extract. To perform AST, agar well diffusion method was used, in which sterile MH (Mueller-Hinton) agar molten butt (15mL) was seeded with 1mL of isolated *Streptococcus spp* (5% & 20% sucrose tolerant)[O.D = 0.5-0.6] & plates were prepared. Using sterile cork borer 4 wells were made. 0.1 mL of each plant leaf extract of different concentration (10mg/mL, 20mg/mL & 30mg/mL) prepared in sterile distilled water was added in wells & sterile distilled water was used as a control. The plates were incubated at 4ºC for pre-diffusion for 30 mins. After pre-diffusion, the plates were incubated at 37ºC for 48 hours & zone of inhibition was observed & measured.

RESULTS AND DISCUSSION

3.1 Phytochemical screening

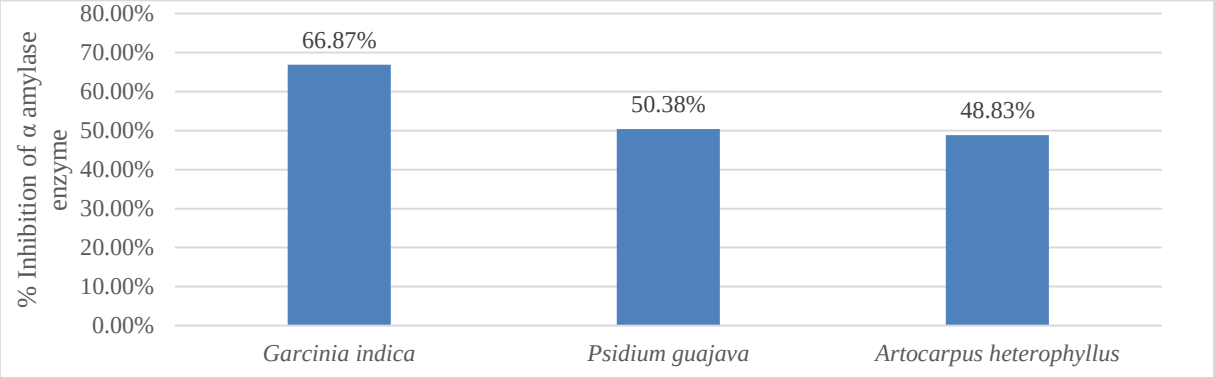
Table No.2: Phytochemical screening results

Phytochemical constituent	*Garcinia indica*	*Psidium guajava*	*Artocarpus heterophyllus*
Carbohydrates	+	+	+
Alkaloids	+	+	+
Steroids	-	-	+
Glycosides	-	+	+
Amino acids & proteins	-	-	-
Saponins	+	+	-
Flavones	-	-	-
Phenolic compound	+	+	-
Anthocyanins	-	-	-
Oils & fats	-	-	-
Triterpenoids	+	-	+

KEY: + : Positive test , - : Negative test

Phytochemical screening test of methanolic extract of *G. indica, P. guajava & A. heterophyllus* was done using different tests. All the three extract showed presence of carbohydrates & alkaloids while they showed absence of amino acids, flavones, anthocyanins & oil. Glycosides was present in *P. guajava* & *A. heterophyllus.* Saponins & Phenolic compound were present in both *G. indica* & *P. guajava. G. indica* & *A. heterophyllus* showed presence of Triterpenoids. Steroids was present only in *A. heterophyllus.*

3.2 Anti-amylase activity



Graph No.1: Anti-amylase activity of plant leaves extract

The inhibition of α-amylase enzyme was found highest in methanolic extract of *G. indica* (66.87%), followed by *P. guajava* (50.38%) & *A. heterophyllus* (48.83%).

3.3 Thrombolytic activity

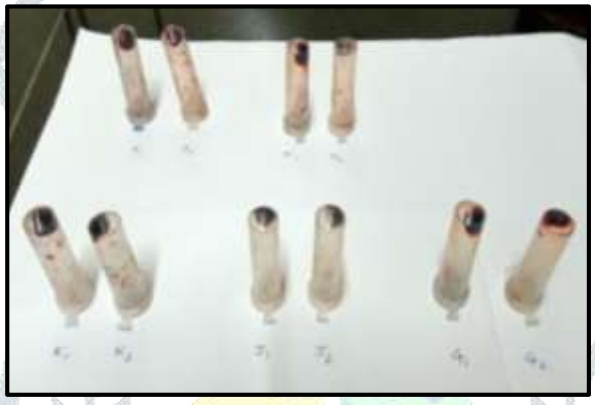
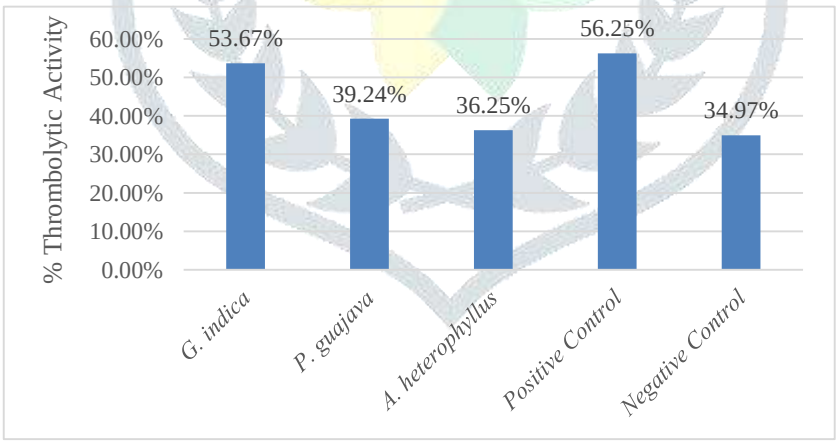


Fig No.1: Thrombolytic assay (after clot lysis tubes)



Graph No.2: Thrombolytic activity of plant leaves extract

Thrombolytic activity of methanolic extract of *G. indica*, *P. guajava* & *A. heterophyllus* was estimated using thrombolytic assay. The percentage of clot lysis was shown to be highest by *G. indica* (53.67%), followed by *P. guajava* (39.24%) and *A. heterophyllus* (36.25%). The positive and negative control showed 56.25% and 34.97% clot lysis respectively.

3.4 Antiplaque activity

• Isolation of *Streptococcus mutans* :

(a)

(b)

Fig No.2: (a) Isolated colony subcultured on sterile TYC agar plate with 5% sucrose

(b) Isolated colony subcultured on sterile TYC agar plate with 20% sucrose

The white colonies with exopolysaccharide surrounding to them were obtained on fresh TYCS agar plates with 5% and 20% sucrose after subculturing.

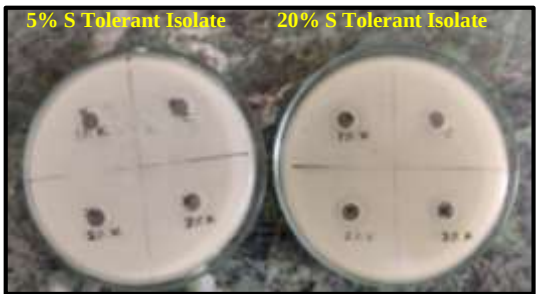
• Identification of Isolate :

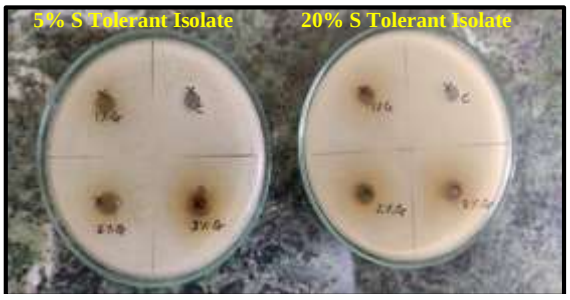
Table No.3: Biochemical tests results

TEST	5%S tolerant isolate	20%S tolerant isolate
Gram nature	Gram positive	Gram positive
Arabinose	-	-
Erythritol	-	-
Glucose	+	+
Inulin	+	+
Lactose	-	-
Maltose	+	+
Mannitol	+	+
Ribose	-	-
Sorbitol	+	+
Starch	-	-
Sucrose	+	+
Xylose	-	-
Salt tolerance (6.5% NaCl)	+	+
Catalase	+	-
Oxidase	+	-
Hugh-Leifson	O and F	-
Voges Proskauer (VP)	-	-
Urease	-	-

Both, the 5% and 20% sucrose tolerant isolate showed the presence of gram positive cocci , fermented the sugars (glucose, inulin, maltose,mannitol,sorbitol and starch). The tolerance to 6.5% Sodium Chloride can be seen due to growth of both isolates on plate. The isolate showed negative result for VP and urease tests. Only 5% sucrose tolerant isolate showed the presence of catalase and oxidase enzyme.

• Antimicrobial Susceptibility Testing:





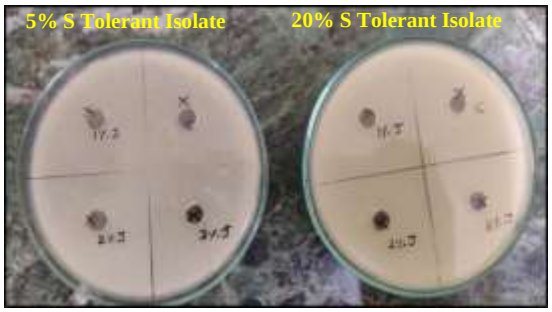


Fig No.3: AST of *Streptococcus spp* against *G. indica*, *P. guajava* & *A. heterophyllus* extract.

Antimicrobial susceptibility testing of both the isolates (*Streptococcus spp)* were determined against different concentrations (1%, 2% and 3%) of methanolic extract of *G. indica*, *P. guajava* and *A. heterophyllus.* Only *P. guajava* extract showed the small inhibition zone around the wells (1mm).

4. CONCLUSION

The study carried out mainly focuses on Anti-amylase activity, Thrombolytic activity and Anti-plaque assay of selected plant extracts. The methanolic leaves extract of *Garcinia indica*, *Psidium guajava* and *Artocarpus heterophyllus* have shown presence of different secondary metabolites which is indicative of potent pharmacological activities and can be explored for treatment of various diseases. The inhibition of amylase enzyme was predominantly high in *G. indica* (66.87%) while moderate activity were shown by *P. guajava* and *A. heterophyllus* extracts indicating helpful in prevention of postprandial hyperglycemic condition and promote low blood glucose level.

G. indica exhibited comparatively good thrombolytic activity i.e. 53.67% whereas *P. guajava* and *A. heterophyllus* have shown weak thrombolytic activity i.e. 39.24% and 36.25% respectively when compared to the standard streptokinase result (56.25%). Thus, these extracts pose as possible therapeutic agent to lyse blood clot and can prevent from life threatening disorders caused by thrombosis. The biochemical tests shows that the isolates obtained from dental plaque sample may be of *Streptococcus spp.* as per reference to Bergey's manual. However, 16S rRNA sequencing would help in confirming the isolate at species level. AST result of three extracts of different concentrations against 5% and 20% Sucrose tolerant *Streptococcus* isolates showed no zone of inhibition around wells except *P. guajava* which showed small inhibition zone (1mm). This is indicative of extract lacking any anti-plaque activity though increasing the concentration further of extract might help in determining the efficiency.With the help of advanced techniques such as HPLC, FTIR further characterization of bioactive component present in plant extract is needed to be done to formulate an effective drug.

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