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Prashant B Bedarkar
Assistant Prof., Dept. of Rasashastra & Bhaishajya Kalpana, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India

Correspondence
Prashant B Bedarkar
Assistant Prof., Dept. of Rasashastra & Bhaishajya Kalpana, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India

Comparative alpha amylase and alpha glucosidase inhibitory activities of *Nishamalaki, Amalaki rasayana*- an ayurvedic formulations

Prashant B Bedarkar

Abstract
Powder of fruit pulp of Indian gooseberry (*Amalaki*) and Turmeric levigated multiple times with juice of *Amalaki* i.e. *Amalaki swarasa bhavita Nisha Amalaki churna* (ASBNAC) is commonly called as *Nishamalaki* (NA) which is recommended in Ayurvedic classics and is widely practiced for treatment and progression of Diabetes (*Madhumeha*) and its complications. Previous studies on *Nishamalaki* also suggest possibility of additive or synergistic effect among 2 drugs Turmeric and *Amalaki* when formulated in to a compound formulation. Although Alpha amylase and alpha glucosidase activities of *Nishamalaki* and *Amalaki rasayana* both prepared by levigation method have been studied, but still comparative study of these activities of *Nishamalaki* and its formulation components, *Amalaki rasayana* and Turmeric powder at different doses have not been evaluated to evaluate synergistic effect in a single study hence, its *in-vitro* study was conducted to evaluate possible synergistic effect due to levigation process. *Nishamalaki yoga* (NA) and *Amalaki Rasayana* were prepared with 16 times lavigation (*Bhavana)* of combination of fine Turmeric powder [*Haridra Churna* (HC)] and fine powder of fruit pulp of goose berry (*Amalaki churna)* in equal quantity with half quantity of fresh fruit pulp juice of *Amalaki (Swarasa)* as that of combined powder for each levigation for preparation of *Nishamalaki* and levigation with equal quantity of Juice to Powder of gooseberry (as that of later) for preparation of *Amalaki Rasayana* followed by drying. Methanolic extracts of *Nishamalaki, Amalaki rasayana,* Turmeric powder and powder of *Emblica officinalis* were tested for Porcine pancreatic α- amylase inhibition and intestinal α- glucosidase activities at 4 concentrations (40, 80, 120 and 160 µg/ml) through starch as base and chromogenic DNSA (3, 5-dinitrosalicylic acid) as colouring agent, in 2 different solutions.
Results and Conclusion: All the tested drugs exhibited comparatively better α- amylase inhibition and α-glucosidase inhibition by acetic acid buffer in the concentration of 40, 80, 120 and 160 µg/ml and inhibition was dose dependent at all concentration thus have potential to reduce post prandial hyperglycemia. *Amalaki rasayana* showed comparatively better inhibition than same concentration of *Nishamalaki* or Turmeric powder or powder of Indian gooseberry.

Keywords: Alpha amylase, Alpha glucosidase, *Nishamalaki, Turmeric, Emblica officinalis*, Diabetes

Introduction
Nishamalaki [(NA), various combination formulations of Turmeric (*Haridra, Curcuma longa* Linn.*)* and Indian goose berry (*Amalaki, Emblica officinalis* Gaertn.]; is recommended in Ayurvedic classics [1-3] proven efficacious and widely practiced in the management [4-8] (treatment, prevention of complications [9]) of *Madhumeha* (Diabetes Mellitus). Among them Powder of fruit pulp of Indian gooseberry (*Amalaki*) and Turmeric levigated multiple times with juice of *Amalaki* i.e. *Amalaki swarasa bhavita Nisha Amalaki churna* (ASBNAC) is commonly used as *Nishamalaki* (NA) traditionally by Ayurvedic physicians.
Indian gooseberry (especially juice) is recommended in Ayurvedic classics, proven efficacious and widely practiced in the management (treatment, prevention of complications) of Diabetes [10-11]. Formulation *Nishamalaki* assures perennial availability of *Amalaki Swarasa*, improves palatability, shelf life and potentiate the classical dosage forms of *Amalaki* (*Churna* and *Swarasa*) and *churna* of Turmeric. Previous studies on *Nishamalaki* also suggest that there is possibility of additive or synergistic effect among 2 drugs Turmeric and *Amalaki* when formulated in to a compound formulation [12, 13]. Studies also suggest synergistic antioxidant effect among these 2 drugs [14, 15]. Process of *Bhavana* to drug in powder form with liquid extract of same drug increases its potency [16].
Although Alpha amylase and alpha glucosidase activities of *Nishamalaki* [17] and *Amalaki rasayana* [18] both prepared by levigation method have been studied, but still comparative study

of these activities of *Nishamalaki* and its formulation components, *Amalaki rasayana* and Turmeric powder at different doses have not been evaluated to evaluate synergistic effect in a single study hence, its *in-vitro* study was conducted to evaluate possible synergistic effect due to levigation process.

Hence, comparative evaluation of *in-vitro* activities of formulation *Nishamalaki* and its composition *Amalaki Rasayana* and Turmeric powder was done on their Porcine pancreatic α-amylase and intestinal α- glucosidase inhibitory activities. Disease *Madhumeha*, a type of *Prameha* described in Ayurvedic classics is equated with diabetes, whose major pathological event is chronic hyperglycemia leading to series of associated complications. Enzymes, pancreatic α- amylase and intestinal α-glucosidase affect glucose degradation and thus its absorption. Rapid degradation of dietary starch by α-amylase leads to elevated postprandial hyperglycemia (PPHG). Human pancreatic α-amylase (HPA) in the small intestine causes an increase in post-prandial glucose levels [19] and Inhibitors of pancreatic α-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels [20-22].

Experimental protocols

Materials and methods

The test drug: *Amalaki churna* was prepared by collecting fresh *Amalaki* followed by separation of fruit pulp, drying in sunlight for 7 days and grinding in pulverizer. *Nisha* (*Haridra*, turmeric) *churna* was prepared by collecting fresh turmeric rhizomes and boiling them in water for 45 minutes, followed by drying for 21 days [23]. Grinding was done in industrial pulverizer. *Nishamalaki* (NA) or. *Amalaki swarasa bhavita Nisha Amalaki Churna* (ASBNAC)] was prepared with 16 times levigation (*Bhavana [24]*) of equal quantity of fine powder of Turmeric and fine powder of fruit pulp of Indian gooseberry (*Amalaki, Emblica officinalis Gaertn.*) with fresh fruit pulp juice of *Amalaki*, half in quantity as that of combined powders for each levigation. For preparation of *Amalaki Rasayana*, powder of *Amalaki* fruit pulp was levigated with equal quantity of its Juice for one *Bhavana*, repeated 16times. The levigated mixtures were dried in oven at bellow 60^{0}C after spreading them in the form of thin sheets in oven at bellow 60 ^{0}C and micronization was done by grinding in pestle and mortar and laboratorial mixer juicer (blender) to fine powder and stored in air tight containers. Thus prepared formulation *Nishamalaki* consists of 53.3% of combined powder and 46.69% of total solid content derived from 426.43% of fresh fruit pulp juice and *Amalaki rasayana* contained 36.33% of powder and 63.66% of total solid content derived from 581.39% of fresh fruit pulp juice of *Emblica officinalis [25]*. Thus upon stichiometric (theoretical) calculations, at same concentration of all 3 drugs, Turmeric powder (NC) is 3.74 times more and *Amalaki Swarasa Bhavit Amalaki churna* (ASBAC) is 1.36 Times more than their content in *Nishamalaki*.

(I) α -Amylase Inhibitory Activity

The following procedure was followed for present study:

- Chemicals and Reagents: Phosphate buffer, Acetic acid buffer, PPA (Porcine pancreatic α-amylase) and Intestinal alpha glucosidase were analytical grade and acquired from Himedia and Difco.
- Test drug total 3-"*Nishamalaki*"(ASBNAC), *Amalaki rasayana* (ASBAC), and *Nisha churna* (NC) (as Inhibitor of alpha amylase enzyme): Four concentrations of Methanolic extract of test drugs prepared by method of Alcohol soluble extractive of Ayurvedic pharmacopoeia further solidified and dehydrated at bellow 40^{0}C with 4 different concentrations (4, 8,12 and 16%) were taken for analysis.

Alpha amylase inhibitory activity

The analysis of ASBAC for PPA inhibition was initially performed qualitatively by starch-iodine colour assay. The lead extracts were further quantified with respect to PPA inhibition using chromogenic DNSA (3, 5-dinitrosalicylic acid) method.

The α-amylase inhibitory activity was determined according to the method described by Miller [26]. Briefly, different solutions were prepared and different concentrations of inhibitor (Methanolic extract of *Nishamalaki*) were incorporated in 4 same concentrations ranging from 4% to 16% (40, 80, 120 and 160 µg/ml) and were incubated at room temperature for 15 min and followed by addition of 1% starch in all test tubes. The reaction was determined the addition of 400 µl of 3,5 di nitro salicylic acid (DNSA) color reagent, placed in boiling water for 5 min, cooling to room temperature and diluted with 15 ml of distilled water. The absorbance measured at 540 nm (Schimadzu UV-VIS spectrophotometer) in triplicate and average values were taken for calculations. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of the plant extracts prepared with 2 different solvents. The results were expressed as % inhibition calculated using the formula:

Inhibitory activity of alpha amylase enzyme =

$$\frac{\text{Abs (control)} - \text{Abs (extract)}}{\text{Abs of (control)}} \times 100$$

(II) α - Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was determined using the standard method [27]. The enzyme solution was prepared by dissolving 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin. It was diluted further to 1:10 with phosphate buffer just before use. Sample solutions were prepared by dissolving 4 mg sample extract (Aqueous extract of *Nishamalaki*) in 400 µl DMSO. Four concentrations: 40, 80, 120 and 160 µg/ml were prepared and 5 µl each of the sample solutions or DMSO (sample blank) was then added to 250 µl of 20 mM p-nitrophenyl-α-D-glucopyranoside and 495 µl of 100 mM phosphate buffer (pH 7.0). It was pre-incubated at 37°C for 5 min and the reaction started by addition of 250 µl of the enzyme solution, after which it was incubated at 37°C for exactly 15 min. 250 µl of phosphate buffer was added instead of enzyme for blank. The reaction was then stopped by addition of 1000 µl of 200 mM Na_2CO_3 solution and the amount of p-nitrophenol released was measured by reading the absorbance of sample against a sample blank (containing DMSO with no sample) at 400 nm using UV visible spectrophotometer in triplicate.

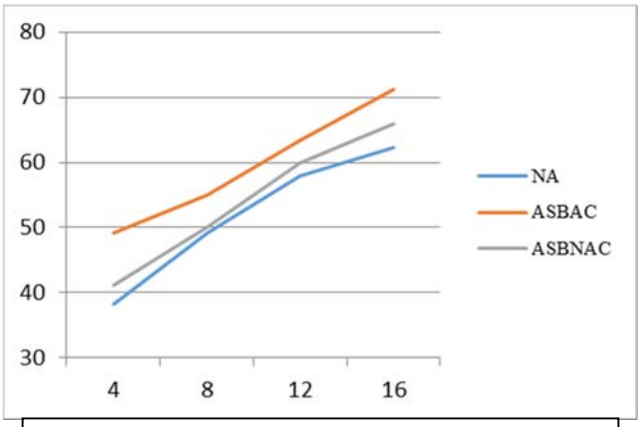
Results and discussion

(I) α -Amylase Inhibitory Activity of Test Drugs

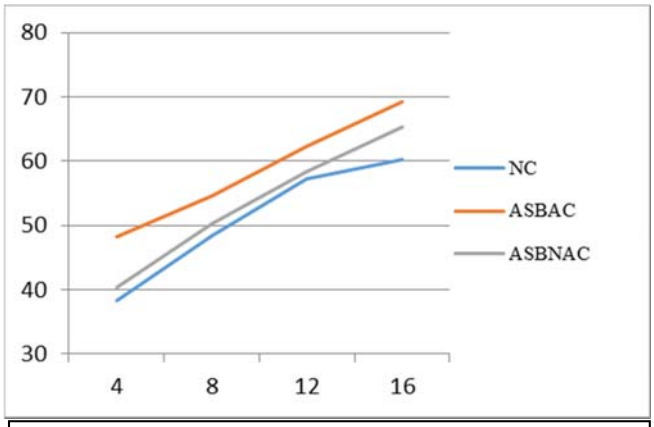
Table 1: α-Amylase inhibitory activity on the basis of concentration of inhibitor and solvent variation.

Samples	Concentration	% Inhibitions with respects to different solutions (Average)	
		Phosphate buffer	Acetic acid buffer
ASBNAC	16%	60.23	62.27
	12%	57.25	57.88
	8%	48.44	49.25
	4%	38.25	38.33
NC	16%	65.33	65.99
	12%	58.33	59.85
	8%	50.25	50
	4%	40.4	41.25
ASBAC	16%	69.25	71.22
	12%	62.33	63.33
	8%	54.55	55
	4%	48.36	49.2

It is evident from Table 1 that, It is evident from Table that comparatively more inhibition of α-amylase enzyme was demonstrated by all test drugs in Acetic buffer at all tested concentrations. Extract of ASBAC had exhibited highest % inhibition at highest as well as lowest concentration followed by % inhibition by ASBNAC and NC respectively.



Comparative Alphaamylase inhibitory activity of test drugs (Acetic Buffer)



Comparative Alphaamylase inhibitory activity of test drugs (Phosphate Buffer)

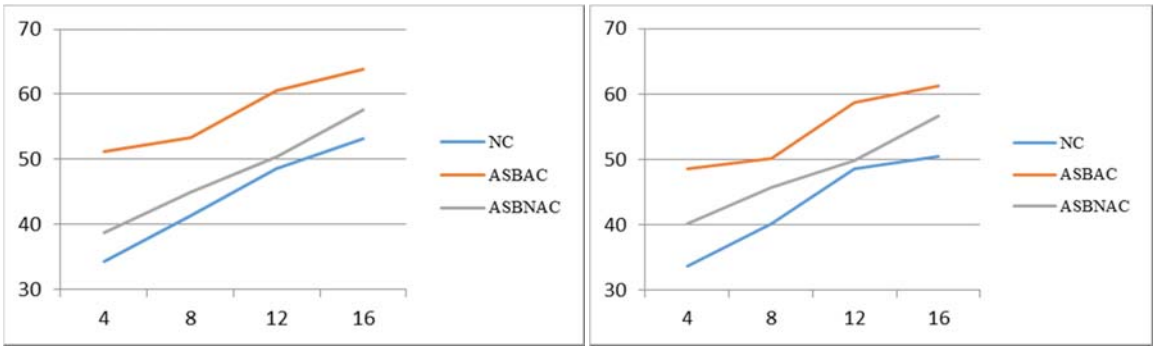
Fig 1: α-Amylase inhibitory activity activity of test drugs at different concentrations with different solvents.

(II) α -glucosidase inhibitory activity of test drugs

Table 2: α-Glucosidase inhibitory activity on the basis of concentration of inhibitor and solvent variation.

Samples	Concentration	Inhibitions with respect to different solutions	
		Phosphate buffer %	Acetic acid buffer %
ASBNAC	16%	50.54	53.25
	12%	48.56	48.55
	8%	40.25	41.33
	4%	33.66	34.28
NC	16%	56.58	57.69
	12%	49.88	50.36
	8%	45.66	45
	4%	40.25	38.66
ASBAC	16%	61.25	63.88
	12%	58.66	60.54
	8%	50.22	53.33
	4%	48.66	51.25

It is evident from Table 2 that, comparatively more inhibition of α-glucosidase enzyme was demonstrated by all test drugs in Acetic buffer and extract of ASBAC had exhibited highest % inhibition at highest as well as lowest concentration followed by % inhibition by ASBNAC and NC respectively at all tested concentrations.



Comparative Alphaglucosidase inhibitory activity of test drugs (Acetic Buffer)

Comparative Alphaglucosidase inhibitory activity of test drugs (Phosphate Buffer)

Fig 2: α-Glucosidase inhibitory activity of test drugs at different concentrations with different solvents.

Results of both the study depicts that, all 3 test drugs exhibited inhibition of Pancreatic Alpha amylase and intestinal alpha glucosidase in dose dependent manner in the concentration of 4,8,12 and 16%.

It is evident from above studies that at all concentrations of test drugs ie 4%, 8%, 12% and 16% combined formulation ASBNAC had produced comparatively better inhibition of alpha glucosidase as well as alpha amylase than that of inhibition by 3.74times higher dose of ingredient NC and had exhibited almost similar inhibition of alpha glucosidase as well as alpha amylase as that of inhibition by 1.36 times higher individually tested quantity of ingredient ASBAC. Hence it is to infer that ingredients NC and ASBAC in combination, formulated in the form of formulation ASBNAC, posses additive effect on alpha glucosidase and alpha amylase inhibitory activities.

Fresh juice (*Swarasa*) is most frequently preferred dosage form of *Amalaki* in Ayurvedic classics, apart from its powder form (*churna*) for the management of Diabetes. *Swarasa*; a crude galanical is most potent dosage form among 5 basic dosage forms of Ayurveda (*Pachavidha kashaya*), due to assurance of all chemical ingredients and thus therapeutic attributes [28]. Researches on significance of *Amalaki Swarasa* as that of dried form suggest that, Ascorbic acid content of fresh fruits (329mg/100gm) was more than that of *Amalaki Churna* (39.5mg/100gm) and *Amalaki swarasa* and *Amalaki swarasa Bhavita Amalaki Churna* had 20 times and 18times more Ascorbic acid content than that of *Amalaki Kwatha* and *Amalaki Kwatha bhavita Amalaki Churna* [29].

As *Amalaki swarasa* is not available throughout the year, therefore it is need of time to modify the formulation. In the present study, comparatively more durable, probably more potent and palatable dosage form of *Amalaki and Turmeric*, "*Nishamalaki"* i.e. "*Amalaki swarasa bhavita Nishamalaki Churna,* (ASBNAC)*"* from their powder and juice dosage forms was formulated and tested.

Although *Nishamalaki* and *Amalaki rasayana* (*Amalaki Swarasa bhavit Amalaki churna*); which is a component of *Nishamalaki* has been proven to posses α- amylase and α-glucosidase inhibitory properties through *in vitro* studies [30-32], still the results may not be equivocal and comparable due to various reasons like different standard of drug, different manufacturing process, different study protocol, different source of standard enzyme etc which have been prove have their impact of variation in study outcomes. Control materials with α-amylase of nonhuman origin were not commutable with the enzyme in human sera and should not be used for inter -method calibration [33].

Bhavana samskara (unique Ayurvedic pharmaceutical levigation process)*;* besides wet trituration process is also a size reduction technology, frequently used in Ayurvedic pharmaceutics is an example of drug combination. It has multi-dimensional pharmaceutical and therapeutic implications. *Bhavana* have its utility in almost all pharmaceutical processing; affecting the physicochemical and biological properties of dosage form. Process of *Bhavana* to drug in powder form with liquid extract of same drug increases its potency [34]. Hence there is need to evaluate status of α- amylase and α- glucosidase properties of levigated product prepared from powder of both the drugs possessing wide range of therapeutics.

Various groups of phytochemicals present in *Emblica officinalis* and *Curcuma longa* are known to posses inhibitory effect on Pancreatic Alpha Amylase and Intestinal Alpha Glucosidase in *in-vitro* studies. Effect of combination of Gallic acid (GA) on inhibitory effect of Acarbose on the enzymes showed that, mixtures of the samples (50% acarbose & 50% GA; 75% acarbose &25% GA; and 25% acarbose & 75% GA) were prepared. The results revealed that the combination of 50% acarbose and 50% GA showed the highest α-glucosidase inhibitory effect, while 75% acarbose & 25% GA showed the highest a-amylase inhibitory effect [35]. Phenolic compounds such as phenolic acids and flavonoids bind covalently to alpha amylase and change its activity due to the ability to form quinones or lactones that react with nucleophilic groups on the enzyme molecule [36].

Previous studies on *Nishamalaki* also suggest that there is possibility of additive or synergistic effect among 2 drugs Turmeric and *Amalaki* when formulated in to a compound formulation [37, 38]. Studies also suggest synergistic antioxidant effect among these 2 drugs [39, 40].

In view of potent α- amylase and intestinal α- glucosidase activity of formulation "*Nishamalaki*, *Amalaki Rasayana;* a component of *Nishamalaki* ", and synergistic inhibitory effect of Gallic Acid with Acarbose, *Nishamalaki* could be a best combination to prevent side effects of α- Amylase and α-glucosidase inhibitors i.e. bloating, belching, fullness of abdomen and diabetic gastropathy as it probably will show synergistic activity of these 2 enzymes and posses mild laxative effect, thus further may reduce gastric emptying time favouring to reduce post prandial hyperglycemia. Hence studies of *Nishamalaki* on Drug-drug interaction with Alpha Amylase and Alpha glucosidae inhibitors is potent area of research, are recommendable, which could started from

retrograde clinical survey studies [41].

Conclusion

All the tested drugs exhibited comparatively better α- amylase inhibition and α-glucosidase inhibition by acetic acid buffer in the concentration of 40, 80, 120 and 160 µg/ml and inhibition was dose dependent at all concentration thus have potential to reduce post prandial hyperglycemia. Although *Amalaki rasayana* showed comparatively better inhibition than same concentration of *Nishamalaki* or Turmeric powder or powder of Indian gooseberry, still in view of tested doses of individual drugs it is evident that ingredients of *Nishamalaki* i.e. turmeric powder and *Amalaki rasayana* in combination, formulated in the form of formulation *Nishamalaki*, posses additive effect on alpha glucosidase and alpha amylase inhibitory activities suggesting effect of *Bhavana samskara*.

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Conflict of Interest-None declared.

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