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ALPHA-AMYLASE INHIBITORY ACTIVITY OF SELECTED INDIAN SPICES AS POTENTIAL ANTIDIABETIC AGENTS

NIKOLAOS MAVRIDIS[1]*, NIAMH KAVANAGH[2], RHYS PENGELLY[3]

Affiliation

[1]Department of Pharmacognosy, Macedonia Plain Institute of Pharmacy, Serres, Greece
[2]Department of Pharmacognosy, Connacht Institute of Pharmacy Practice, Castlebar, Ireland
[3]Department of Phytochemistry, Exmoor Institute of Pharmaceutical Sciences, Taunton, United Kingdom

*Corresponding author: Nikolaos Mavridis, n.mavridis@mpip.gr
ORCID iD: Nikolaos Mavridis 0000-0001-0156-0518; Niamh Kavanagh 0000-0001-0388-0523; Rhys Pengelly 0000-0001-2658-0582

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Abstract

Delaying starch digestion is an accepted way to blunt postprandial glucose, and plant foods have long been explored as sources of milder enzyme inhibitors than acarbose. This narrative review examines published evidence on alpha-amylase inhibition by commonly used Indian spices, including cinnamon, clove, cumin, fenugreek, ginger, turmeric and black pepper. It covers the enzyme and its inhibition by polyphenols, the in vitro assays on which most claims rest, and the limited animal and human data. Spice extracts frequently inhibit the enzyme in the laboratory, but assay conditions vary, tannin effects confound results and clinical translation is weak. This review reports no new experimental data.

1. INTRODUCTION

India has a large and growing burden of type 2 diabetes, and the figures are not subtle. National surveys across Indian states have reported prevalence in the high single digits to double digits, with many more people in the prediabetic range, and global estimates place the country among those with the greatest number of adults with the disease [1, 2]. Diet is an obvious point of intervention. Refined starch dominates many meals, and a spoonful of rice or bread is converted into glucose within minutes of reaching the small intestine. Anything that slows that conversion, even modestly, is of interest to people who look after patients with diabetes.

Pancreatic alpha-amylase carries out the first major step of that conversion, cutting the internal alpha-1,4 linkages of starch into maltose, maltotriose and limit dextrins, which brush-border enzymes then reduce to glucose [3, 4]. Inhibiting the enzyme postpones

digestion and flattens the glucose curve after a meal. Acarbose, a pseudo-tetrasaccharide from *Actinoplanes*, does this and also inhibits alpha-glucosidases, and a Cochrane review found it lowers glycated haemoglobin modestly, though gastrointestinal side effects such as flatulence and diarrhoea limit its use [5, 6]. The wish for milder inhibitors from food sources follows naturally.

Spices fit that wish well. Households in India use them daily, in small quantities and over long periods, and many have been used in traditional medicine for symptoms that resemble diabetes [7, 8]. Their chemistry is also promising, since they are rich in phenolic compounds, essential oils and flavonoids, and these groups have already been shown to interact with digestive enzymes [9, 10, 11]. This review asks what the published evidence says about spices as inhibitors of alpha-amylase, and how far laboratory findings, collected in table 1 and summarised in fig. 1, can be trusted as guides to benefit in people.

Table 1: Selected published findings on spices, polyphenols and alpha-amylase

Spice or topic|feature	Reported finding or feature	Ref.
Acarbose reference inhibitor	Lowers postprandial glucose by delaying starch digestion; gastrointestinal effects limit tolerance.	[5, 6]
Flavonoids structure-activity	Hydroxylation pattern and ring planarity govern inhibition of human amylase.	[12, 13, 14]
Tannins non-specific binding	Bind enzyme and substrate, producing inhibition that is not active-site specific.	[10, 15]
Cinnamon human trial	Lower fasting glucose in a small trial; later review found evidence insufficient.	[16, 17]
Fenugreek clinical trials	Meta-analysis shows modest glucose lowering with viscous fibre as a likely contributor.	[18]
Ginger diabetes review	Reduced glucose and lipids mainly in animal models, with limited human data.	[19]
Clove and phenolic-rich spices extracts	Strong inhibition in vitro that tracks with total phenolic content.	[20, 21]
Indian medicinal plants screening	Wide variation among species; a minority show potent amylase inhibition.	[9, 21]
Turmeric curcumin	Diverse in vitro activity, poor bioavailability and frequent assay interference.	[22]
DNS reagent colorimetric assay	Standard method for amylase activity but responds to reducing compounds in extracts.	[23, 24]

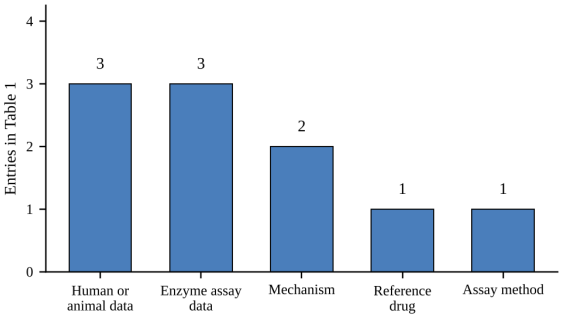


Fig. 1: Number of entries in table 1 by type of evidence

The scale of use makes spices worth studying. A typical Indian household cooks with a combination of turmeric, cumin, coriander, chilli, mustard and cloves, along with fresh ginger and garlic, and a person might consume a few grams of the dry mixture each day. Per serving this is a small dose, but daily exposure over decades is large, and some epidemiological and dietary studies have asked whether regular spice intake is linked to better glucose control, though such studies cannot separate spices from the rest of the traditional diet [7, 11]. Rarely do they measure amylase inhibition, and one should keep the two questions apart. A dietary pattern may help glucose control for many reasons, of which enzyme inhibition is just one candidate [7, 8].

A note on terms is useful. Authors speak of inhibitory activity in different ways: percentage inhibition at a fixed extract concentration, an IC_{50} value, or a comparison against acarbose. These are not interchangeable, and the choice affects how strong a spice appears to be [9, 20, 23]. Papers that quote only percentage inhibition at one high concentration give no way of judging potency, and the number may reflect saturation of the assay or the extract's colour

rather than binding. A full concentration-response curve with at least five points is the minimum that supports a quoted IC_{50} value.

2. ENZYME AND INHIBITION

Human pancreatic alpha-amylase is a calcium-containing, chloride-activated enzyme of about 55 kDa. Its crystal structure shows a catalytic $(beta/alpha)_8$ barrel with a conserved triad of aspartate, glutamate and aspartate residues at the active site cleft, and structures with bound carbohydrate inhibitors have mapped the subsites that hold the substrate chain [25, 26]. Salivary amylase is very similar, which is why most screening laboratories use porcine pancreatic amylase, a cheap and well-characterised substitute. The two enzymes are close but not identical, and inhibitors behave slightly differently against each, a difference seldom discussed in spice papers [14, 26].

The chemistry of the catalytic step explains how inhibitors can work. Amylase cleaves its substrate by a double-displacement mechanism with retention of configuration: an aspartate carboxylate attacks the anomeric carbon, forming a covalent glycosyl-enzyme intermediate, which a water molecule, activated by a second residue, then hydrolyses. Calcium stabilises the fold, and chloride binds near the active site and raises catalytic efficiency [25, 26]. Compounds that mimic the transition state, such as acarbose, bind tightly. Polyphenols are not transition-state mimics, so they are expected to be weaker, and their value, if any, lies in the combination of mild inhibition and long exposure at meal time [4, 26].

Not all inhibitors are small molecules. Proteinaceous amylase inhibitors from cereals and legumes, including the one in white kidney beans, bind the enzyme tightly, and extracts of the bean have been sold as starch blockers; crystal structures of human amylase with such proteins show how they occlude the active-site cleft [9, 26]. Their activity is of a different order from that of crude spice extracts, and it is a useful benchmark when claims of strong inhibition by spices are examined. The comparison also serves as a reminder that complete block of starch digestion is not the aim in diabetes care, since undigested starch passing to the colon causes the same gastrointestinal complaints seen with acarbose [5, 6, 9].

Flavonoids are the best-studied natural inhibitors. Luteolin and several other flavones and flavonols inhibit amylase in vitro, and structure-activity analyses by Tadera and co-workers and by Lo Piparo and colleagues point to the importance of the hydroxyl pattern on the B ring and the planarity of the C ring, with the 3-hydroxyl group sometimes reducing activity [12, 13, 14]. Docking and kinetic data suggest that these compounds bind near the active site, partly by hydrogen bonds to catalytic residues and partly by stacking with aromatic side chains [14, 26]. The patterns explain why polyphenol-rich extracts so often show activity in assays, and they give a starting point for interpreting results on spices [10, 27].

A second group of inhibitors acts less specifically. Tannins and other high-molecular-weight polyphenols bind proteins and starch alike, and precipitate enzymes from solution in a way that has little to do with active-site recognition [10, 15]. When a crude spice extract is added to a reaction, it is impossible to say how much of the observed inhibition arises from precise binding and how much from this kind of nonspecific complexing. The distinction is more than an academic one, since non-specific binders in the gut may be diluted by food proteins and have a different effect from what the test tube indicates [10, 15, 28].

3. SPICES AND REPORTED ACTIVITY

Cinnamon has the longest research history. A randomised trial in primary care reported a small fall in HbA1c in people with type 2 diabetes given 1 g of cinnamon daily for three months [16]. Cinnamon extract and its polyphenols altered the expression of the insulin receptor and glucose transporter 4 in cultured adipocytes [29]. Yet a 2010 review noted that benefits seen in cell and animal work had not been reproduced consistently in people, since trials were small, short and inconsistent in outcome and differed in the preparation and dose of cinnamon used [17]. Reported in vitro amylase inhibition by

cinnamon extracts is plausible given their polyphenol content, but the data do not show that this is the mechanism behind any glycaemic effect seen in patients [9, 17, 20].

Phenolic profiles differ widely between spices. Cinnamon bark is dominated by cinnamaldehyde and procyanidin polymers, clove oil by eugenol and its acetate, ginger by gingerols and shogaols, turmeric by curcuminoids, and black pepper by piperine, while cumin and coriander owe their character mainly to volatile terpenoids and aldehydes rather than to polyphenols [11, 20]. Structure-antioxidant analyses show that the number and arrangement of hydroxyl groups on an aromatic ring determine radical scavenging, and similar features seem to favour enzyme binding, which may explain why antioxidant ranking and amylase ranking correlate imperfectly but significantly in screening studies [12, 20, 28].

Clove, cumin, coriander and cardamom appear repeatedly in screening papers on Indian plants. Clove, with its high phenolic content and the eugenol that dominates its oil, usually ranks among the more active spices in extract-based assays, and the same is true of other phenolic-rich species surveyed in Latin America and India [11, 20, 21]. Cumin and coriander, with lower total phenolics, generally show weaker inhibition. These rank orders fit the idea that total phenolic content, measured by the Folin-Ciocalteu method, predicts amylase inhibition, although the correlation is imperfect and the method also responds to reducing substances other than phenolics [20, 21, 30].

Indian cooking uses spices in combinations, and so a natural question is whether blends behave differently from single spices. Some screening work has examined mixtures and reported that combined extracts can inhibit the enzyme more or less than expected from their parts, which is possible when polyphenols compete for the same binding site or when one component precipitates another [10, 20, 21]. Formal tests of synergy, such as isobologram analysis, are almost never carried out, so such statements are descriptive. The topic matters because garam masala, curry powders and similar blends are what people actually eat, and a single-spice result may not transfer to a pan of dal containing six or seven ingredients [7, 11].

Ginger, turmeric and black pepper offer a different profile. Ginger contains gingerols and shogaols, and a review of its use in diabetes describes reduced fasting glucose and improved lipid indices in animal studies, with smaller human data sets; effects on amylase are reported but not the central feature [19]. Curcumin, the main curcuminoid in turmeric, has diverse in vitro activities, but it is poorly soluble and poorly absorbed, and many reports of its enzyme effects are plagued by aggregation and assay interference [22]. Piperine from black pepper has attracted interest mainly because it raises the bioavailability of other compounds by inhibiting metabolism and efflux [31]. Taken as a group, these spices appear to be more useful as adjuncts than as primary enzyme inhibitors [19, 22, 31].

Fenugreek is a special case. Its seeds contain soluble galactomannan fibre as well as saponins and the amino acid 4-hydroxyisoleucine. A meta-analysis of clinical trials found that fenugreek intake lowered fasting glucose and postprandial glucose modestly, with considerable heterogeneity across studies [18]. The effect is often attributed to viscous fibre, which slows gastric emptying and starch hydrolysis physically, and not to enzyme inhibition by small molecules. Amylase assays with whole seed extracts may therefore capture only part of what the seeds do, while ignoring the fibre effect that is likely to matter in a meal [7, 18, 32].

Cooking changes the picture in ways that laboratory work seldom considers. Spices are fried in oil, simmered in acidic or protein-rich gravies and held at high temperature for long periods, so the chemistry reaching the gut differs from that of a raw powder extracted in methanol. Heat can degrade some polyphenols and release others from the food matrix, and binding to dietary proteins may reduce the free fraction available to the enzyme [10, 11]. Studies on cooked preparations are scarce, although they would describe real exposure much better than a freshly prepared alcoholic extract does [7, 10, 11].

4. ASSAY METHODS AND INTERPRETATION

Most spice studies measure amylase activity through the release of reducing sugars from starch, detected with 3,5-dinitrosalicylic acid. The method derives from Bernfeld's description of amylase assays and from Miller's reagent for reducing sugars, and it is simple, cheap and robust enough to run in teaching laboratories [23, 24]. It also has well-known problems. The reagent responds to reducing compounds in the extract itself, such as phenolics, which can give false readings, and the heat and alkaline conditions of the colour step can degrade some polyphenols. Proper blanks for extract and enzyme are needed, and are not always described [24, 30].

Extract preparation deserves as much care as the enzyme assay. Spices are dried, ground and extracted with methanol, ethanol, acetone or water, often with heating, and the solvent is evaporated and the residue redissolved in buffer or dimethyl sulphoxide. Total phenolic content is then determined with the Folin-Ciocalteu reagent and expressed as gallic acid equivalents [20, 30]. Yield, particle size, extraction time and temperature all alter the composition, and two laboratories using the same spice and nominally the same solvent can obtain extracts with different phenolic content. Reporting phenolic content alongside inhibition lets readers judge whether differences in activity reflect differences in the plant or in the extract [11, 30].

Variables in the assay can shift apparent potency considerably. Pre-incubation time of enzyme and inhibitor, substrate concentration, starch source and pH all influence the result, and so does the solvent used to prepare the extract. Methanol and ethanol extracts differ from aqueous ones in phenolic content, and residual solvent may itself affect the enzyme. A chloride-containing buffer is needed because the enzyme requires chloride for activity [23, 25, 26]. Without these details being reported, a statement that a spice is more potent than acarbose is hard to interpret and nearly impossible to reproduce [9, 20, 33].

Kinetic analysis, when performed, gives more insight than a single percentage. Lineweaver-Burk or Dixon plots can distinguish competitive from non-competitive or mixed inhibition, and work on finger millet seed coat phenolics showed that different fractions of a single plant material act by different modes against alpha-glucosidase and amylase [14, 33]. Such analysis is uncommon in studies of whole spices. Crude extracts contain many components, so the kinetics reflect the mixture and cannot be interpreted as the property of a single molecule [9, 33]. Reporting the inhibition constant and the mode of inhibition for at least the most active extract would add real information.

Choice and reporting of the reference compound are further sources of inconsistency. Acarbose is the usual positive control, and it is potent against pancreatic amylase, with IC_{50} values in the low micromolar range in many reports, though the figure depends on enzyme source, substrate and pre-incubation [4, 5, 9]. Some papers instead express spice activity per milligram of dry extract and acarbose per milligram of pure compound, which makes the comparison look worse for acarbose in molar terms than it really is. Because extracts are mixtures, a mass-based IC_{50} of an extract is not equivalent to a molar IC_{50} of a pure drug, and tables that place the two side by side without comment can mislead [9, 20, 33].

Starch type matters because of digestion in the body. Raw, gelatinised and retrograded starch behave differently, and in a meal the enzyme meets a mixture of starch, protein, fat and fibre. In vitro tests with soluble starch are therefore a long way from physiological digestion, and rapid screening results need confirming in more realistic models, such as simulated digestion with a food matrix or a postprandial glucose response in animals or volunteers [3, 4, 10]. A positive result at the screening stage should be seen as an invitation to continue, not as evidence of a food effect [9, 27].

Alpha-glucosidase assays are usually run alongside amylase assays, and the pairing is sensible because the two enzymes act in sequence. Many spice papers report stronger inhibition of glucosidase than amylase, a

pattern that some authors regard as a favourable one, since excessive amylase inhibition is thought to be responsible for much of the flatulence and abdominal discomfort of acarbose [4, 13, 33]. The conclusion is not secure, because the glucosidase assays commonly use yeast enzyme, which differs from the mammalian brush-border enzymes in its response to inhibitors, so a more selective profile in the test tube may not carry over to people.

5. LIMITATIONS AND FUTURE DIRECTIONS

The evidence base has four recurring weaknesses. First, spices are sourced from markets and rarely authenticated, so variety, origin, storage and processing differ between studies. Second, extraction conditions are not standardised, which makes comparison difficult. Third, tannin and non-specific protein binding are seldom controlled for. Fourth, the link from enzyme inhibition to blood glucose is assumed, not demonstrated [9, 15, 20]. Even the best clinical signals, such as those for cinnamon and fenugreek, come from small trials with variable design, and effect sizes are modest compared with drugs [5, 17, 18].

Safety and regulation need a sentence of their own. Culinary spices have a long record of safe use, but concentrated extracts are a different matter, and capsules sold for glycaemic control are regulated as supplements in most countries without proof of efficacy. Piperine can raise blood levels of co-administered drugs, including some used in diabetes [8, 31]. Patients who take insulin or sulphonylureas should be warned that large amounts of a glucose-lowering supplement may add to hypoglycaemia risk, a point more worth making than the modest benefit claimed [1, 5, 8].

A further issue is dose. People eat spices in grams, and sometimes in fractions of a gram, per meal. At that level, the quantity of phenolic compounds reaching the gut is small, and there is no guarantee that the concentrations needed to inhibit the enzyme in vitro are achieved. Supplements and concentrated extracts can reach higher doses but raise a different question, namely safety with long-term use and interactions with medicines, for example piperine's effect on drug metabolism [8, 10, 31]. Food-based advice needs to take these limits into account and should not promise therapeutic effects from ordinary culinary use [7, 11].

What happens in the intestine and after absorption is the biggest gap of all. Polyphenols are modified by gut microbiota, conjugated in the intestinal wall and liver, and excreted rapidly, so the compounds found in plasma are mostly metabolites that differ from those added in the assay [10, 27]. The luminal fraction is more relevant to amylase, but few studies have measured polyphenol concentration in the small intestine after a spice-containing meal. Without such data it is difficult to say whether a given extract concentration in a test tube is physiologically realistic, which weakens most of the conclusions drawn from enzyme assays alone [10, 27, 28].

Future studies might usefully standardise a few elements. Authenticated plant material with voucher specimens, defined extraction in a small number of solvents, total phenolic content and one or two marker compounds reported for every extract, acarbose included as a positive control with a full concentration-response curve, and kinetic analysis for the most active samples would make results comparable [9, 30, 33]. Beyond that, simulated digestion and small crossover studies with a starch-rich test meal and a measured spice dose would show whether laboratory activity has any meaning for patients [3, 18, 27]. Such work would turn a large body of preliminary data into evidence people can use.

6. CONCLUSION

The published evidence supports a limited but real conclusion. Many spices, particularly those rich in phenolics, inhibit alpha-amylase in vitro, and the likely mechanisms, from flavonoid binding in the active site to nonspecific tannin complexing, are reasonably well understood [10, 12, 14, 20]. Clinical support is weaker. Small trials of cinnamon and fenugreek show modest benefit, but they do not show that enzyme inhibition is the reason, and meta-analysis has

found inconsistent effects for cinnamon [16, 17, 18].

For readers who work in this area, the most useful steps are methodological. Standardised extracts, proper blanks, kinetic analysis, tannin controls and a realistic starch model would make laboratory claims more credible, and carefully designed meal studies could establish whether any effect reaches the clinic [15, 23, 24, 33]. Until then, spices are best regarded as flavourful parts of a balanced diet that may help in a small way, and they should not be offered as substitutes for proven treatment of diabetes [1, 5, 7].

7. ABBREVIATIONS

DNS-3,5-Dinitrosalicylic acid, IC_{50}-Half-maximal inhibitory concentration, HbA_{1c}-Glycated haemoglobin, kDa-Kilodalton, T2DM-Type 2 diabetes mellitus, ICMR-Indian Council of Medical Research, IDF-International Diabetes Federation

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9. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

10. CONFLICT OF INTERESTS

Declared none

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