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Short communication

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Study on synergic effect of phytomolecular compounds in terms of anti-amylase activity

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Abstract

Drugs play an essential role in the treatment and prevention of various diseases. The drug derived from the phytocompounds has an impact on a wide variety of ailments. The beneficial effects of plant sources are due to the nature of phytomolecules which serve to heal the human systems. Understanding the interaction of drug-drug compounds has a vital role to increase the overall therapeutic effects and maintain the homeostatic conditions of the system. The plant active compounds with similar activity from different origins and in combinations have been found to enhance the overall activity in synergy. These effects have been used to treat various diseases and disorders. In this research, an essential approach to understand the anti-amylase activity of garlic and ginger extracts, the extracts were analysed individually and in combination as ginger-garlic complex. The synergistic effects on anti-amylase using phytomolecular extractions, analysis of starch concentration, analysis of reducing sugar and FTIR analysis were performed. The results have shown consistency in inhibiting the alpha-amylase activity individually and in combinations. Significant reductions in the starch concentrations of ginger-garlic extract were observed. The study proves that the synergic effect of ginger and garlic extracts has effects on inhibition of alpha-amylase. The outcome of the study indicates the need for future analysis to understand the mechanism of action between the components of ginger-garlic complex. These findings could serve as an important pathway to treat the diabetic disorder effectively.

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Keywords

Anti-amylase, Phytochemicals, Garlic, Ginger, Synergism

Introduction

A drug is the primary source to treat and prevent all kinds of diseases and syndromes.[1] However, irresponsible management of drugs can lead to undesirable or deleterious effects. One common situation is the occurrence of drug-drug interactions where the presence of one drug affects the activity of another when both are administered together. Drug-drug interactions can be classified into synergistic effects, i.e. the activity of the drug is increased or vice versa. There are many concerns of drug-drug interactions and some of them are increased side effects, cessation of the desired therapeutic effect[2] and eliciting secondary reactions.[3] Excluding the above, the drug-drug interactions can also increase the overall therapeutic effect.[4] For example, the presence of clavulanic acid increases the bactericidal effect of amoxicillin.[5] Apart from drug-drug interactions, drug-food and drug-plant compound interactions are also reported.[6]

In drug-drug interaction, for example, analgesics and antipyretics on co-administration delays the absorption rate. In drug-plant compound interaction, the chemotherapeutic drug oxa (oxaliplatina) and mushroom have shown synergic effect. A food and drug interaction occurs when a food or its components interfere with the way the drug functions in the body. The fruit juices (like grapes juice) have found to interact with all types of drugs by affecting the liver's ability to metabolise the drugs. There has been increase awareness about the importance of nutritional and therapeutic values of food. Functional foods and whole foods are considered to be health promoting and contain the right amount of nutritional and energy balance. One of the major targets of functional foods is the gut. Since gut serves as an interface between the diet and metabolic events (following ingestion), the functional foods are utilised to regulate various aspects, including the rate of metabolism,

microflora, macronutrient breakdown, etc. Functional foods that target the colonic microflora are already successful in the market in the form of probiotic drinks.[7] Phytomolecules are micronutrients that are necessary to maintain good health. Phytochemicals from various plant sources are found to act as anti-oxidants,[8] antidiabetic,[9] antihypertensive,[10] antibacterial,[11] anti-inflammatory,[12] anticarcinogenic[13] and anticlotting[14] agents. The industries and researchers have understood the value of food with high phytochemical contents and this can lead to the evolution of nutraceuticals.

The bioactive compounds like dietary fibre, vitamin C, carotenoids, polyphenols, isoflavonoids, glucosinolates, folic acid, etc. are known to have multiple therapeutic potential.[15] In Indian culinary, spices and herbs play a major role in everyday cooking. Some of the commonly used ingredients with therapeutic values include, anise,[16] asafoetida,[17] bay leaf,[18] pepper,[19] ginger,[20] garlic,[21] fennel seeds,[22] fenugreek seeds,[23] onion,[24] turmeric,[25] etc. Since all the spices and herbs are used together in cooking, the presence of highly active compounds in each spice can lead to interactions which can be either synergistic or antagonistic in nature.

However, there is a limited evidence to prove any interaction between the components. Hence, the main aim of the study was directed to investigate the interaction between the two commonly used ingredients, i.e. ginger (*Zingiber officinale*) and garlic (*Allium sativum*).

The effect of their interactions was studied based on the change in the activity of alpha-amylase and the digestive enzyme that catalyses the hydrolysis of carbohydrates whose activity can be correlated to the postprandial glucose levels.[26]

Materials and methods

Materials

All the chemicals used were of laboratory grade and were acquired commercially. 3,5-dintrosalicyclic acid was acquired from SD Fine-Chem Limited, Mumbai. Pure corn starch from LOBA chemicals was used in the following experiments. Alpha-amylase was acquired in the form of diastase from HiMedia, Mumbai. Ginger and garlic were obtained from the local market.

Methods

0.5% starch solution was prepared by adding 100 mg of starch to 20 ml boiling water. The water was left to boil until the starch was gelatinised and evenly distributed.[27] 0.001% alpha-amylase solution was prepared by adding 0.1 mg of alpha-amylase in 10 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM NaCl.

Phytomolecular extraction from ginger (*Zingiber officinale*) and garlic (*Allium sativum*) and understanding the effect of phytomolecules on amylase-mediated starch digestion

5 g of ginger[28] and garlic was macerated and vortexed in 25 ml of chloroform overnight. The mixtures were filtered and the filtrate was evaporated using a rotary vacuum evaporator.

The sediment particles were suspended in buffer. The extracts were stored in vials and refrigerated for future use.[29]

Analysis based on starch concentration: The activity of alpha-amylase was first determined by measuring the rate of disappearance of starch upon enzyme digestion in the presence of extracts. 2 ml of 0.5% starch solution was added to three tubes to which 1 ml of ginger extract, 1 ml of garlic extract and 1 ml of the mixture of ginger-garlic extract were added respectively and incubated at 37°C for 30 min. 1 ml of 0.001% of alpha-amylase solution was added to each tube.

The total volume was made up to 5 ml using buffer. Positive control was prepared by substituting the extract with buffer. The concentration of starch was measured optically at 595 nm at every 20 min for 100 min.[30]

Analysis based on reducing sugar concentration: DNS assay was performed to quantify the amount of reducing sugars produced as a result of digestion of starch by the enzyme.[31]

The alpha-amylase inhibitory activity of the plants extracts was tested individually and in the interacted state. After 100 min, 1 ml of each mixture was taken in a separate tube to which 500 µl of DNS reagent was added. The tubes were incubated at 85°C for 15 min and 1 ml of water was added to each tube. The optical density was measured at 540 nm. Positive and negative controls were prepared accordingly.

Fourier Transform Infrared Spectroscopy (FTIR)

One milligram extract of purified ginger, garlic and ginger-garlic was ground with 100 mg of potassium bromide and pressed with 7500 kg for 30 s to obtain a translucent pellet. The infrared spectra were recorded on Bruker Optics FTIR system within the range of 500-4000 cm^{-1} (wavenumber).

Results and Discussion

The effect of phytomolecules on amylase-mediated starch digestion was determined using the starch concentration, and the rate of disappearance of starch over a period of 100 min was observed (Table 1).

Table 1. Determination of starch concentration

Sample	Starch concentration (%) (SD)					
	Time: 0 min	Time: 20 min	Time: 40 min	Time: 60 min	Time: 80 min	Time: 100 min
Positive control	100±0.0	46.66±2.08	46.66±2.08	46.00±(0)	44.66±2.08	44.00±0.01
Ginger sample	100±0.0	99.77±10.0	99.12±0.005	97.45±(0.005)	95.75±0.005	92.91±0.015
Garlic sample	100±0.0	98.42±12.6	84.44±0.005	80.59±(0.005)	80.24±0.01	79.81±0.01
Ginger-garlic sample	100±0.0	100±0.0	94.51±0.005	93.54±0.005	93.54±0.01	87.09±0.01

The starch concentration was analysed for the sample types, such as positive control, ginger sample, garlic sample and ginger-garlic sample.

The effect of phytomolecules on amylase-mediated starch digestion was determined using the concentration of reducing sugars; the inhibition of the individual and interacted extracts on alpha-amylase was denoted in percentage (Table 2).

Table 2. Determination of reducing sugars

Sample	Optical density at 545 nm			% inhibition
	Control		Sample	
	Positive	Negative		
Ginger	0.244	0.130	0.267	53.13
Garlic	0.546	0.391	0.536	26.55
Ginger-garlic	0.255	0.220	0.376	61.11

The effect of phytomolecules on starch concentration has shown a significant decrease in the rate of reduction of starch concentration in the presence of extracts when compared to the positive control (Fig. 1).

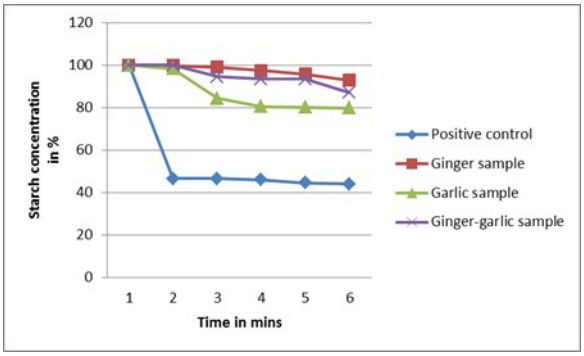

Fig. 1. Phytochemical effect on starch concentration

The reducing sugar concentration was analysed for the sample types, such as positive control, ginger sample, garlic sample and ginger-garlic sample.

The percentage (%) inhibition of ginger-garlic interacted extract was higher than that of individual extracts showing a synergistic interaction between the compounds of ginger and garlic (Fig. 2).

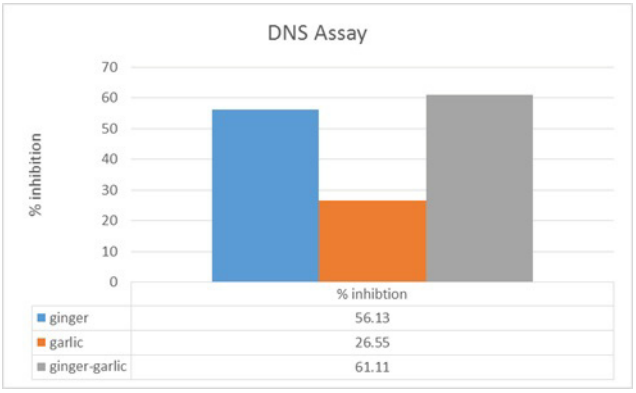

Fig. 2. Phytochemical effect on reducing sugars

Previous studies show that both ginger (*Zingiber officinale*) and garlic (*Allium satvium*) have inhibitory effects on alpha-amylase.[31] The results of our current study (Fig. 1 and Fig. 2) were consistent with the proven studies that both ginger and garlic individually inhibit alpha-amylase. This can be confirmed by the significant decrease in the rate of reduction of starch concentration in the presence of extracts of ginger and garlic. The study also proves that the interaction between the components of ginger and garlic extracts has a synergistic effect on the inhibition of alpha-amylase. Based on the 3,5-dinitrosalicyclic acid assay, the mixture of ginger-garlic extract has shown a significant increase in the percentage inhibition, i.e. 61.11% in comparison to ginger and garlic, i.e. 53.13% and 26.55% respectively (Fig. 2). The outcome of the study indicates the need for future analysis to understand the mechanism of action between the components of ginger and garlic and to further increase our knowledge on the possible interactions between commonly used herbs and their potential use for therapeutic purposes.

Fourier Transform Infrared Spectroscopy (FTIR)

The active compound from garlic extract was absorbed between 500-2000 cm^{-1} (wavenumber) and the transmittance was eleven peaks that were observed in the range of 3900.20-794.49 cm^{-1} and they were 3652.62 cm^{-1}, 3801.59 cm^{-1}, 3738.59 cm^{-1}, 3351.70 cm^{-1}, 2358.58 cm^{-1} and 794.49 cm^{-1} (Fig. 3). All transmittance peak data was measured using standard IR values. The peak 3900.20 cm-1 relates to broad spectrum of O-H stretch of phenolic or alcoholic group.[32] Further, this peak has a strong correlation with C-H stretch of alkynes group. The peaks 3652.62 cm^{-1} and 3801.59 cm^{-1} that correspond to amines produce zero to two N-H absorptins depending on their type. The peak 3738.59 has a strong correlation with the stretch of N-H amide groups. The band peak 3351.70 corresponds to the O-H stretch with H-bonded alcoholic funcational groups with phenols.[31] 2358.58 peak relates with C≡N stretched nitriles. Aromatic C=C bending corresponds to the peak 1653.30 cm^{-1} which can be streched to amide-II band or N-H bend of 1 amines and caroboxylic C=O respectively.[31,32] The peak 1521.07 has a strong asymmetrical stretch of nitro (N-O) compound. The transmittance peak of 1311.03 matches with the C-O stretch and also relates to carboxylic acids, ester and ethers groups.[33,34] The peak 1262.15 cm^{-1} and a lower frequency band at 794.49 cm^{-1} corresponds to C-CI stretch of aliphatic amines and alkyl halides respectively.

The active compound from ginger extract was absorbed between 500-2000 cm^{-1} (wavenumber) and the transmittance was 3928.92 cm^{-1}, 3902.52 cm^{-1}, 3435.26 cm^{-1}, 1652.54 cm^{-1}, 1519.37 cm^{-1}, 1411.88 cm^{-1}, 1311.35 cm^{-1} and 1263.08 cm^{-1} (Fig. 4). All transmittance peak data was measured using standard IR values. The peaks 3928.92 cm^{-1} and 3902.52 cm^{-1} attributed to the amines group. The band peak 3435.26 corresponds to the functional group of NH_2 amides. 1652.54 cm^{-1} peak denotes the alkenes group. Aromatic rings correspond to the peak of 1519.37 cm^{-1}. The peak value of 1411.88 cm^{-1} resembles alkanes group. The peak 1311.35 cm^{-1} relates to ester group and 1263.08 cm^{-1} bending corresponds to ether functional group. The active compound from ginger-garlic extract was absorbed between 500-2000 cm^{-1} (wavenumber) and the transmittance was 3750.62 cm^{-1}, 3647.46 cm^{-1}, 3617.51 cm^{-1}, 3445.99 cm^{-1}, 2356.50 cm^{-1}, 1647.26 cm^{-1}, 1520.70 cm^{-1}, 1456.34 cm^{-1}, 1411.78 cm^{-1}, 1312.36 cm^{-1}, 1262.64 cm^{-1} and 794.43 cm^{-1} respectively (Fig. 5). The peak 3750.62 cm^{-1} relates to the group amide with the strong stretch of N-H bonds. 3646.46 cm^{-1} and 3617.51 cm^{-1} peaks correspond to the group phenolic compounds with alcohols.[32] 3445.99 cm^{-1} peak has strong O-H stretch, H bonded alcohols and phenols groups. 2356.50 cm^{-1} peak has C≡N stretch nitrile groups and 1647.26 cm^{-1} peak relates to the functional group of alkenes. 1520.70 cm^{-1} peak relates to the aromatic rings.[33] 1456.34 cm^{-1} and 1411.78 cm^{-1} peaks correspond to the functional groups of alkanes. The peak 1312.36 cm^{-1} confirms to ester groups. 1262.64 cm^{-1} peak has a strong stretch of C-N with aliphatic amines group and finally the 794.43 cm^{-1} peak relates to the alkyl halides with C-Cl stretches of acid chloride with alkyl halides. The FTIR confirms the presence of phenolic groups in the peaks of 3646.46 cm^{-1} and 3617.51 cm^{-1}.

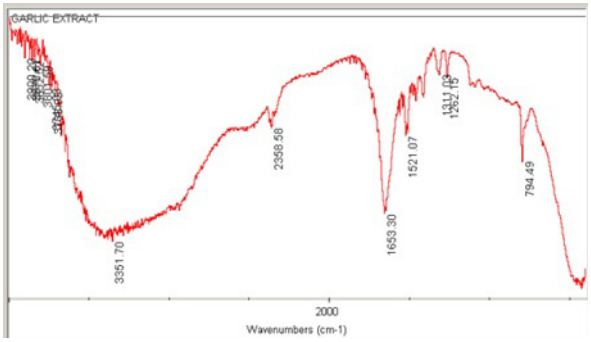

Fig. 3. FTIR peaks of garlic extract

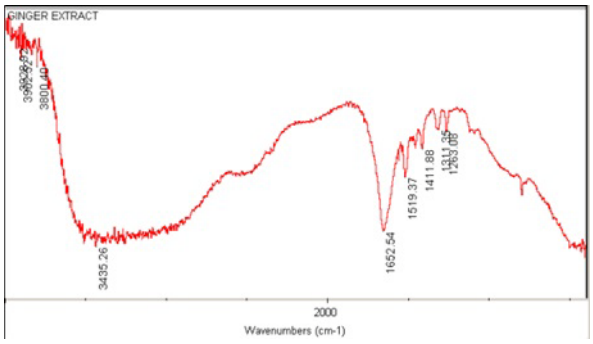

Fig. 4. FTIR peaks of ginger extract

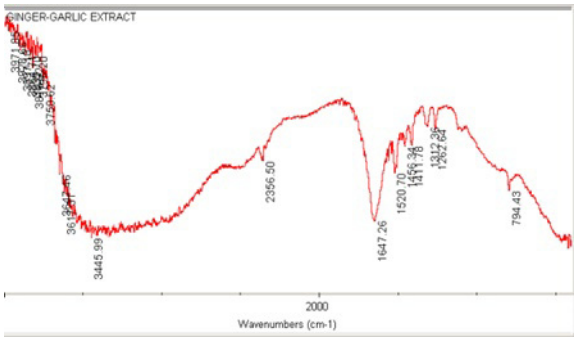

Fig. 5. FTIR peaks of ginger-garlic extract

The phenolic compounds are responsible for the activity of anti-amylase and this was previously reported by Sheikh Julfikar et al., 2008 which supports the results obtained in the ginger-garlic extract, and the possibility for the anti-amylase activity may be due to the presence of phenolic compound.[34]

Conclusion

The current research was carried out to investigate the functionality of ginger and garlic extract independently and together for their anti-amylase activity. The results have identified the synergistic effect between the extracts due to the phytochemicals mixture which has shown the anti-amylase properties. The results indicate that the inhibition rate of the enzymes increased on combination of the extracts instead of individual extract. The FTIR results indicate that the two peaks formed in ginger-garlic extract have shown the presence of phenolic groups in comparison to garlic extract peak, which confirms the presence of synergism in combination of ginger-garlic extracts.

The phenolic group present in the combined extracts has attributed the functional behaviour of anti-amylase activity. The research should be driven on this present investigation in further perspective to treat the diabetic disorder in a better way in future.

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