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Comparative functional potential of some plant materials in India

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Soluble dietary fibers are known to play an important role in maintaining health. These may have prebiotic activity and additionally their basic physico-chemical properties may contribute to their multifunctional health potential. Present study was planned with 30 plant materials grouped as fruits, vegetables, and other tuber crops using gum acacia as standard, being a known dietary fiber having prebiotic potential. The materials were studied for water holding capacity, cholesterol and bile salt binding activity, α-amylase inhibition and trypsin inhibition activity. Fifteen plant materials showed cholesterol binding capacity significantly higher than gum acacia by 0.7 to 86 %. Nineteen plant materials were found to have bile salt binding capacity (27-99 %) higher than gum acacia (24 %). Water holding capacity was observed in the range of 2.71 to 35.4 g/ g dry material for all the materials, while gum acacia had the least value of 0.82 g/ g dry weight. Sixteen materials showed α-amylase inhibition in the range of 9.99-95.0 % as compared to 9.65 % of gum acacia. Trypsin inhibition activity showed in the range of 49-98 %. This is the first report of multifunctional activities of plant materials with prebiotic potential. The results may help to develop prebiotic formulations having multifunctional potential for treatment of gastrointestinal disorders.

Keywords: Bile salt binding, Cholesterol binding, Trypsin inhibition, Water holding capacity, α-Amylase inhibition.

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Introduction

In ancient times, physicians treated their patients with herbal and food supplement having medicinal properties, which led to linking the diet with the health benefits[1]. Consumption of fruits and vegetables is believed to be beneficial for human health[2]. Apart from being good sources of vitamins, minerals, and fiber, fruits and vegetables are also rich sources of potentially bioactive compounds known as phytochemicals conferring the status of functional foods[3].

In recent years, attempts of scientific investigations are taken forward to identify novel plant based ingredients from fruit and vegetables that convey health benefits. Evidence also points to the effect of whole fruit and vegetable consumption on health, which indicates additive influence of the individual components which acts synergistically[4].

Several epidemiological clinical trials indicate the link between intake of fruits and vegetables rich diets and a lower risk of non-communicable diseases including stroke[5,6], cancer[1,7], and heart disease[8,9]. Such plant based diets are also adding benefits in terms of controlling diabetes as well as reduction in the threat of obesity[1].

Dietary fiber is the indigestible cell wall component that has an important role in human diet and health[3]. Dietary fibers as a group of compounds contain a mixture of oligosaccharides and polysaccharides such as resistant gums, inulin, and hemicelluloses, which are associated with lignin and other non-carbohydrate components[10].

According to Phillips *et al*., dietary fibers may have prebiotic activity[11]. Prebiotic dietary fibers such as gum acacia are non-digestible, low molecular weight oligosaccharides and are believed to have prebiotic activity[11]. In addition to their effect on probiotics and the concomitant health effects, they may also directly influence the health of the host due to their innate physico-chemical properties. In this way, they may act to promote additional beneficial effects. These include laxation, lowering of total serum, and low density lipoprotein cholesterol through the cholesterol and the bile acid binding, lowering the glycemic and insulinemic response and water holding capacity with

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effect on gut transit time. These properties could be useful to improve gut health in order to reduce the risk of diseases like constipation infections, inflammatory, and malignant colonic diseases. In light of these facts, present study was planned to assess various indigenous plant materials for their *in vitro* functional activities like water holding capacity, cholesterol and bile salt binding activity, α-amylase inhibition and trypsin inhibition activity.

Materials and Methods

Selection of plant materials

Indigenous plant materials available in Pune (India) were selected (Table 1) and brought from local market. Plant materials were selected depending on their hydrocolloidal and mucilaginous properties. Materials were grouped as fruits, vegetables, and others materials which included roots, plant exudates like gums and flowers. All the materials were washed with water to remove soil or dirt and blotted on filter paper. Plant materials were identified and authenticated by Prof. S.S. Deokule, Department of Botany, Savitribai Phule University (Formerly University of Pune), Pune, India.

Preservation of plant materials

All the materials were chopped into fine pieces and oven dried at 55 °C till complete drying and was processed to make fine powder. The time required for drying depended on the moisture content of the respective materials. Fine powders of all the plant materials were preserved at -20 °C. Moisture content of all materials was determined simultaneously using oven drying till constant weight method as mentioned by Nielsen[12]. Dry powdered material was used for assessment of functional activities like cholesterol binding, bile salt binding, and water holding capacity, α-amylase inhibition and trypsin inhibition potential.

Table 1 — Plant materials used in this study

Code	Common Name	Scientific Name	Family
M1	*Ber*	*Zizipus jujuba* Lam.	Rhamnaceae
M2	Fig (*Anjir*)	*Ficus carica* L.	Moraceae
M3	Gooseberry	*Emblica officinalis* Gertan.	Euphorbiaceae
M4	Guava	*Psidum guajava* L.	Myrtaceae
M5	Grapes	*Vitis venifera* L.	Vitaceae
M6	Okra/Lady finger	*Hibiscus esculantus* L.	Malvaceae
M7	Papaya	*Carica papaya* L.	Caricaceae
M8	Pomegranate	*Punica granatum* L.	Punicaceae
M9	Red tamarind	*Tamarindus indica* L.	Leguminosae
M10	Mint	*Mentha spicata* L.	Labiatae
M11	Safflower leaves	*Carthamus tinctorius* L.	Compositae
M12	Spinach	*Spinacia oleracea* L.	Chenopodiaceae
M13	Custard apple	*Annona squamosa* L.	Annonaceae
M14	*Chikoo*	*Achras sapota* L.	Sapotaceae
M15	*Arvi* (Colocasia)	*Arum curvatum* Roxb.	Araceae
M16	Sweet potato	*Ipomoea batatas* Lam.	Convolvulaceae
M17	Yam (*Suran*)	*Amorphophalus companulatus* Roxb.	Araceae
M18	*Bhatkanda* (edible tuber)	*Manihot esculanta* Crantz.	Euphorbiaceae
M19	Purple yam	*Dioscorea bulbifera* L.	Dioscoreaceae
M20	Wood apple	*Limonia acidissima* W. & A.	Rutaceae
M21	Wild Sweetsop (*Ramphal*)	*Annona reticulata* L.	Annonaceae
M22	Drumstick gum	*Moringa olifera* L.	Moringaceae
M23	*Kathilya* gum	*Acacia senegal* L.	Fabaceae
M24	*Bael* fruit	*Aegel marmelos* Corr.	Rutaceae
M25	Cluster fig	*Ficus racimosa* L.	Moraceae
M26	Ivy gourd	*Coccinia indica* W. & A.	Cucurbitaceae
M27	Water-chestnut	*Trapa bispinosa* Roxb.	Onagraceae
M28	Apple fruit	*Malus sylvestris* Mill.	Rosaceae
M29	*Idlimbu* (Citron)	*Citrus aurantium*	Rutaceae
M31	*Mahua* flowers	*Madhuca latifolia* L.	Sapotaceae

Chemicals

Bile salts and iodine were purchased from HiMedia laboratories. HCl, H_2SO_4, and furfural were purchased from Qualigens. Chemicals for functional activities like soluble starch, amylase (Diastase), cholesterol, bile salts (deoxycholic acid, cholic acid, and lithocholic acid), and trypsin were purchased from SRL and Benzoyl DL-arginine paranitroanilide hydrochloride (BAPNA) was bought from Sigma. Cholesterol kit was purchased from Ranbaxy, India.

Analysis of functional properties

For analysis of functional activities, gum acacia was used as standard dietary fiber. All the plant materials were analyzed for functional activities and results were expressed in comparison with gum acacia.

Water holding capacity

The experiment was done according to the method of Takahashi *et al*[13] with some modifications. Sample (1 %) was incubated overnight in distilled water and then centrifuged. Weight of water added, removed, and water held was used to calculate water holding capacity as g/ g of sample.

Cholesterol binding capacity

Cholesterol binding activity was evaluated according to the method of Gorecka *et al.*[14], in which 1 % sample was incubated overnight in distilled water and centrifuged. The hydrated material obtained was incubated for 2 h on water bath shaker at 37 °C with 5 mL emulsion (1.375 % deoxycholic acid, 0.225 % cholesterol, and 1 % lecithin). Supernatant was estimated for cholesterol content using biochemical kit. Cholesterol binding activity was calculated using the formula:

% cholesterol binding = (Initial cholesterol level-Supernatant cholesterol level) x 100/ Initial cholesterol level

Bile salt binding capacity

This assay was done according to the method of Gorecka *et al*[14]. Sample (1 %) was incubated overnight in distilled water and then centrifuged. The precipitate obtained was incubated for 2 h on water bath shaker at 37 °C with 4 mL of 1 mM bile salt emulsion of deoxycholic acid, lithocholic acid, and cholic acid. After centrifugation supernatant was used to estimate bile salt concentration using Pettonkoffer reaction. 1 mL 70 % H_2SO_4 was added in 1 mL supernatant and incubated for 2 min. After incubation, 0.4 mL of 0.25 % furfural was added and color was read at 510 nm after 5 min incubation. Results were expressed as % bile salt bound / g sample.

α-Amylase inhibition potential

α-Amylase inhibition potential was studied using the method of Raghuramulu *et al*[15]. 1 % sample was extracted in 0.2 M phosphate buffer of pH 5.9 and supernatant was used to study inhibition of α-amylase activity as compared to control activity using starch as substrate. After 10 min of incubation, reaction was terminated using 5 % H_2SO_4 and then, iodine solution was added and color was measured at 640 nm. α-Amylase inhibition activity was expressed as % inhibition of starch hydrolyzed. Further, IC_{50} values were determined for promising materials.

Trypsin inhibition activity

Trypsin inhibition was studied according to the method of Kumar *et al*.[16], in which defatted and dried powdered flour (0.2 g) was extracted with 10 mL of 0.01 N of NaOH for 3 h with constant stirring at 125 rpm in an orbital shaker so as to keep samples in suspension. Suspension so obtained was appropriately diluted so that 2 mL of the sample extract inhibited 40-60 % of the trypsin used as standard in analysis. Sample (2 mL) was incubated with 2 mL trypsin solution on water bath at 37 °C for 10 min and then, 5 mL BAPNA was added immediately. Incubation was continued for 10 min at 37 °C in water bath. Reaction was terminated by addition of 1 mL of 30 % glacial acetic acid and absorbance was taken at 410 nm. Trypsin inhibition was calculated as % trypsin inhibited/ mg of defatted sample.

Statistical analysis

All the observations were done in triplicates. The data was summarized as mean values and standard deviations. One-way and Two-way ANOVA, critical differences, confidence interval, % coefficient of variation, student t test and paired t test were applied to the data for statistical analysis using Microsoft Office Excel 2007. The p values p <0.05 were considered as significant.

Results

Gum acacia was used as standard dietary fiber for assessment of functional activities.

Water holding capacity

All the test plant materials showed variable water holding capacity (Fig. 1) in the range of 2.71 to

35.4 g/ g dry material. The standard dietary fiber gum acacia had the least value of 0.82 g/ g dry material. M6 and M22 showed maximum water holding capacity as 35.4 and 35.3 g of H_2O hold/ g dry material, respectively. As indicated by Fig.1, papaya, ivy gourd, citron, spinach, mint, gooseberry were also promising water holder candidates followed by *ber*, custard apple, *chikoo*, cluster fig and *mahua* flowers (p <0.01); purple yam (0.02) and sweet potato, yam, wood apple, water-chestnut (p <0.05) as compared with gum acacia. Whereas M9 and M18 plant materials didn't show significant increase in water holding capacity when compared with gum acacia.

Cholesterol binding capacity

Cholesterol binding capacity was found to range in between 0.7 to 86 %, which was significantly higher than gum acacia (p <0.01) (Fig. 2). Gooseberry showed highest cholesterol binding capacity (86 %) followed by safflower leaves (62 %), mint leaves (57 %), okra (48 %), citron (43 %), *ber* (30 %), and guava (29 %).

Bile salt binding capacity

Nineteen plant materials were found to have bile salt binding capacity (27 to 99 %) higher than gum acacia (24 %). Bile salt binding (Fig. 3) was maximum for M24 (*bael*) (98 %) followed by M22 (drumstick gum) (99 %), M12 (spinach) (88 %), M29 (citron) (74 %), M21 (*ramphal*) (65 %).

α- Amylase inhibition activity

The present study identified 16 materials with inhibition in the range of 9.99 to 95.0 %. Gum acacia showed 9.65 % α- amylase inhibition (Fig. 4). IC_{50} values per mg of materials were determined for 6 promising materials in the sequence of M6 (okra, 1.12) < M24 (*bael* fruit, 2.12) < M22 (drumstick gum, 2.20) < M4 (guava, 2.26) < M1 (*ber*, 2.28) < M16 (sweet potato, 2.34), respectively.

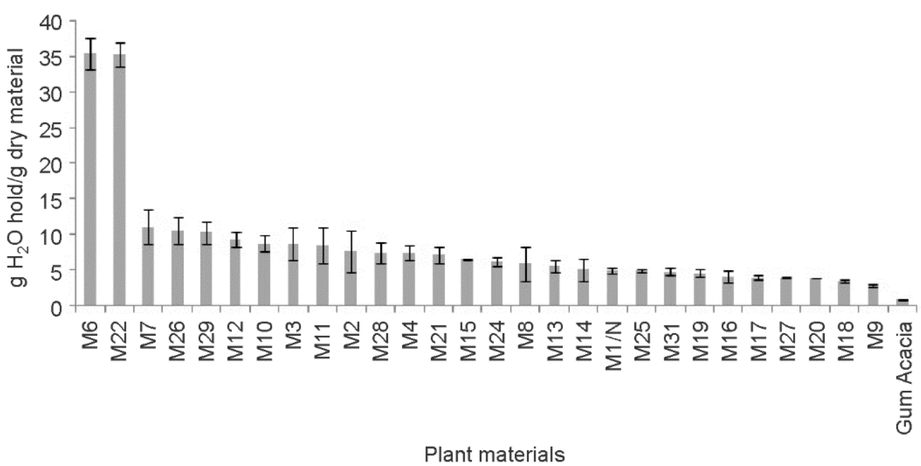

Fig. 1 — Water Holding Capacity of the materials as compared to gum acacia

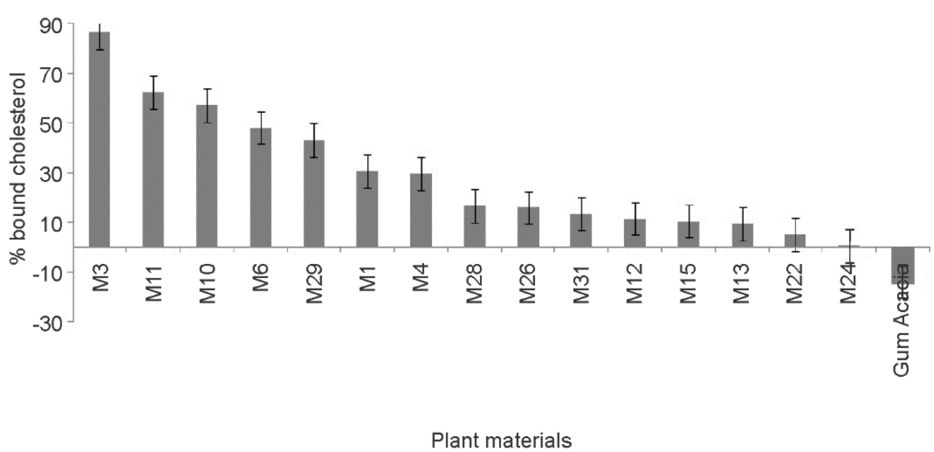

Fig. 2 — Cholesterol Binding Capacity of the materials as compared to gum acacia

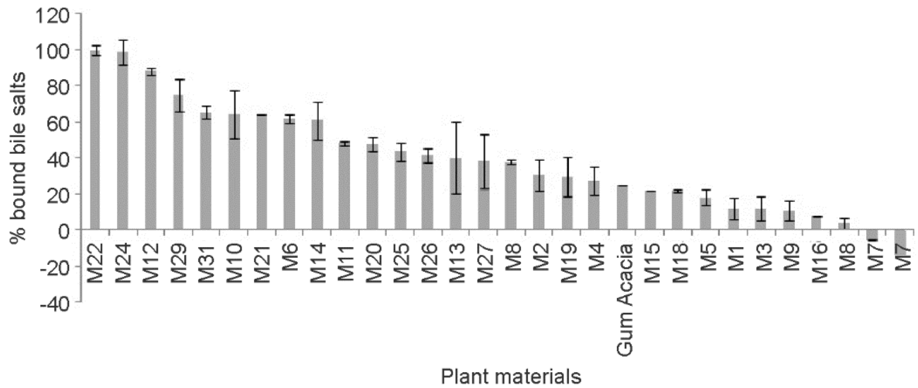

Fig. 3 — Bile Acid Binding Potential of the materials as compared to gum acacia

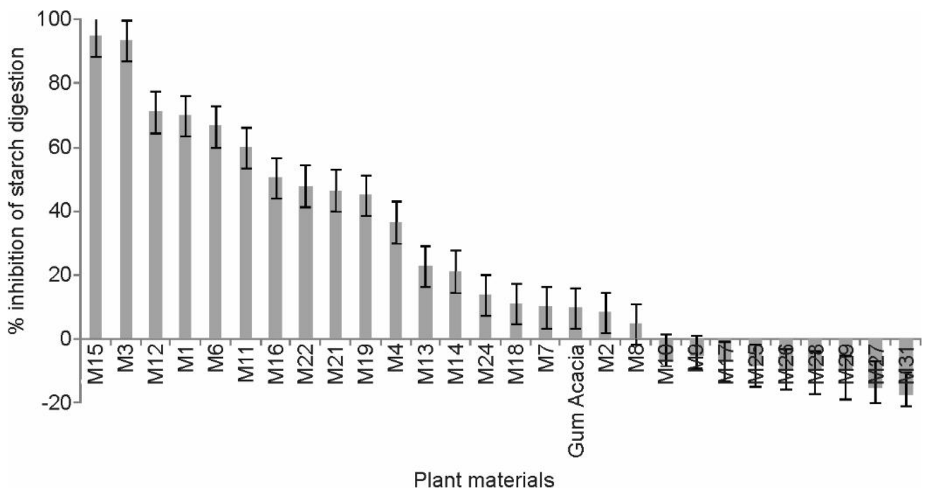

Fig. 4 — α-Amylase Inhibition Potential of the materials as compared to gum acacia

Trypsin inhibition activity

As trypsin inhibitors are the proteinase inhibitors that are responsible for inhibition of biologically active trypsin, this property is considered as an anti-nutritional property of a material. Out of 30 materials, trypsin inhibitor range was negligible for 25 materials in the present study indicating no significant differences within trypsin inhibition. However, materials like drumstick gum, *mahua* flowers, apple, cluster fig, citron showed trypsin inhibition activity ranging from 49-98 % (Table 2).

To achieve multifunctionality of the materials, pooled statistical analysis was done for ranking and critical difference was calculated considering all functional activities. Scoring was done and the materials with first 5 ranks were selected for formulation development with multifunctional potential. Further, M6 (okra), M2 (fig), M24 (*bael* fruit), M4 (guava), M8 (pomegranate), M7 (papaya), M5 (grapes), M11 (safflower leaves), M19 (purple yam), M9 (tamarind), M14 (chikoo), M18 (*bhatkand*), M31 (*mahua* flowers), M26 (ivy gourd), M27 (water-chestnut), M25 (cluster fig) and M28 (apple) were selected for multifunctional properties (Fig. 5).

Table 2 — % Trypsin inhibition by plant materials

Sample	% trypsin inhibition (mean±S.D.)
M15	2.2±1.7
M12	9.2±2.4
M1	11.1±1.4
M16	21.7±0.9
M3	22.1±3.5
M21	25.2±0.4
M10	31.8±1.8
M22	49.7±7.4
M31	85.0±1.1
M28	88.8±2.3
M25	95.2±2.5
M29	98.3±6.4

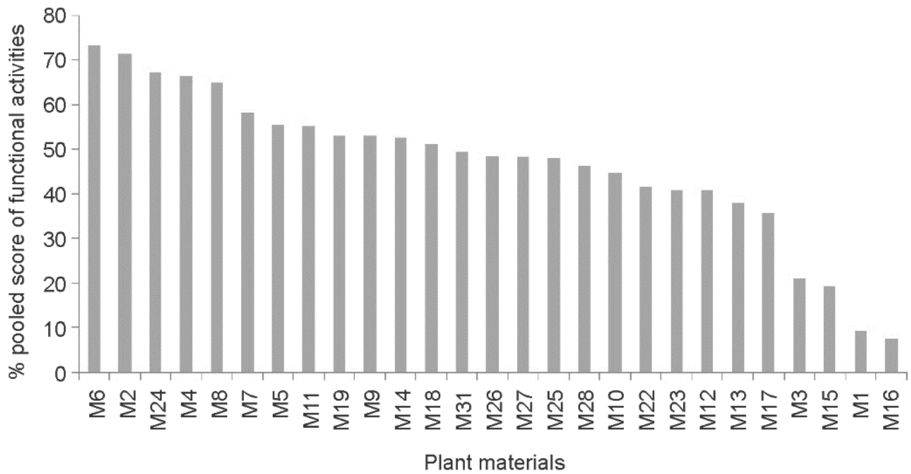

Fig. 5 — Ranking of mterials based on functional activities of the materials as compared to gum acacia

Discussion

Human diet supplementation with fruits, legumes, cereals, and vegetables are found as major sources of the dietary fiber[14]. These fibers show different physiological properties in terms of increase in fecal weight, reduction of transit time, decrease of glucose and cholesterol level in blood, bile acids binding, water holding capacity, and functional properties such as cations exchange capacity, fat adsorption[17-20]. Recently, a review has summarised prospects of the dietary fibers and their application in health attributes[21]. Plant materials under present study have indicated significant cholesterol, bile salt binding, and water holding capacity. Dietary fibers upon water retention tend to become viscous. Such viscous fiber forms reticulation, resulting into increase in their resilience and strength. Such reticulated viscous dietary fibers help in more absorption of the cholesterol[22]. Water holding capacity of these fibers, help to increase faecal moisture and weight with increased excretion. A study discussed that high water holding capacity results into increased faecal moisture and weight with low faecal pH[23]. Dietary fibers resulting increased faecal excretion and the defecation frequency, may be useful in weight management, arthritis and coronary heart diseases[24]. Also deconjugated bile acids are less soluble and thus, not absorbed in intestinal lumen[25,26]. Bile acid forms complex with dietary fibers and are excreted through faeces[14] and therefore, deconjugation of bile acids leads to reduction of serum cholesterol either by increasing demand of cholesterol for *de novo* synthesis of bile acids or by reducing cholesterol solubility and thereby reducing absorption of cholesterol through intestinal lumen[27]. The results of cholesterol and bile salt binding will be helpful in development of formulations for disease like hypercholesterolemia and also for increasing the stool bulk in the patients of bowel diseases, constipation, etc. α-Amylase inhibition property will be helpful in decreasing the glycemic index of food for diabetes patients. This result of α-Amylase inhibition can be explained based on lowering glycemic index which was first reported[28] indicating hypoglycemic effects of the dietary fibers. Hence, the property of α-amylase inhibition used in the present study could be one of the parameter to design the foods for diabetics. The inhibitory effect of dietary fibers would result in lower absorption of digestible carbohydrates and hence, slower release of free glucose resulting into lower blood sugar levels for the diabetic patients. Therefore, these results would be useful in terms of diet regulation of diabetic patients with reduced insulin demands resulting into minimizing the problems associated with the diabetes[22]. Furthermore, the present study analyzed trypsin inhibition potential of the fibers. Trypsin is a biologically active enzyme that is involved in digestion and breaking down of many proteins in humans and animals. Trypsin inhibitors are a type of proteinase inhibitors that reduce its activity and leads to anti-nutritional effect to the host. Hence, the study was designed so as to eliminate anti-nutritional factor from the diet. Absence of trypsin inhibitor is a good indicator and such materials can easily be supplemented in any type of food materials. Hence, the results for the materials such as drumstick gum, *mahua* flowers, apple, cluster fig, citron indicating trypsin inhibition cannot be considered for their use in the supplementation. However, this finding needs to be confirmed in more details.

Conclusion

Statistical ranking led to 10 ranks of test plant materials, out of which 17 materials like M6 (okra), M2 (fig), M24 (*bael* fruit), M4 (guava), M8 (pomogranate), M7 (papaya), M5 (grapes), M11 (safflower leaves), M19 (purple yam), M9 (tamarind), M14 (*chikoo*), M18 (*bhatkand*), M31 (*mahua* flowers), M26 (ivy gourd), M27 (water-chestnut), M25 (cluster fig), and M28 (apple) were ranked in top five numbers based on their functional activities. These results have further importance in development of functional food formulations with multifunctional properties like cholesterol binding, bile salt binding, and water holding capacity. Such multifunctional plant materials with the properties of dietary fiber may find importance in reducing the risk of coronary heart diseases as detected by lowering of cholesterol levels. The plant materials screened under the present study for their functional attributes towards prebiotic activity will provide insight to the researchers in the development of prebiotic functional foods. The promising top three plant materials viz., okra, fig, *bael* fruit may serve as a readily available source of dietary fibers that can be used in the treatment of diarrhoea, constipation, and normalising the disturbed bowel functions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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