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Research Article

Fortification of Common salt with locally available Indian Herbs

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Abstract

Aim of the present work is to fortify common salt with the extracts of locally available Indian herbs thereby enhancing the nutritional value and medicinal properties of salt. Common salt is fortified with herbal extracts to improve the levels of carbohydrates, proteins and lipids further with antimicrobial and antidiabetic properties. Ten methanolic extracts from leaves of *Coriandrum sativum, Solanum trilobatum, Ocimum tenuiflorum, Piper betle, Mentha* species*, Triticum aestivum, Murraya koenigii, Plectranthus amboinicus, Spinacea oleracea* and fruits of *Ribes uva-crispa* were collected from local markets were assayed for in-vitro antibacterial activity against 2 Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and 2 Gram-negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) using agar dilution methods. In addition, their possible toxicity to *Candida albicans* and *Aspergillus niger* was determined, using agar dilution method and *Bacillus subtilis* were the most sensitive bacterial strains to *Piper betle, Ribes uva-crispa* and *Ocimum tenuiflorum* extracts among both Gram positive and Gram negative bacteria tested. The plant extracts also showed high inhibitory activity against the yeast *Candida albicans*. Further the plant extracts were also screened for the presence of phyto constituents and their antidiabetic activity was studied by alpha amylase inhibition assay. As well the proximate analysis was performed to the mixture of salt and plant powders to determine the amount of carbohydrates, proteins and lipids after experiment.

Keywords: Herbal extracts; Fortified salt; Indian herbs; Nutritive salts.

Introduction

The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing modern pharmaceuticals. The bioactive compounds such as polyphenols including flavonoids, tannins, catechins, vitamins C and E, β-carotene, etc., and several others confer these health protective benefits [1]. The mixture of these compounds may provide better protection than single phytochemical due to their synergistic effects. Essential oils extracted from the leaves of *Ocimum sanctum L.* has been found to inhibit in-vitro growth of *E. coli, B. anthracis* and *P. aeruginosa* showing its antibacterial activity [2]. All these compounds have been ascribed to the scavenging of free radicals, reducing oxidative stress and preventing the oxidation of biomolecules that can break reaction chains of pathogens in the deterioration of physiological functions. Specifically, free radicals produce cell damages, tissue injuries and increased levels of reactive oxygen species. In recent diabetic treatments, α-amylase and α-glucosidase inhibitors are most warranted because they increase post-prandial hyperglycemic conditions. Therefore, natural antioxidants can also inhibit the key enzymes α-amylase, α-glucosidase and control the post-prandial hyperglycemic conditions which are a potential approach to cure the type 2 diabetes mellitus. Nowadays the therapeutic properties of plant polyphenols have also demonstrated antimicrobial effects by causing structural or functional damage to the bacterial cell membrane.

Food fortification is the practice of adding essential vitamins and minerals (e.g. iron, vitamin A, folic acid, iodine) to staple foods to improve their nutritional content. Fortification is a safe, effective way to improve public health that has been used around the world since the 1920s. Commonly fortified foods include staple products such as maize flour, wheat flour, sugar, vegetable oil, and rice. Many diets, especially those of the poor, contain insufficient amounts of

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vitamins and minerals due to lack of variation and / or consumption of predominantly processed foods. Adequate amounts of vitamins and minerals in the diet, also called micronutrients since they are only needed in small amounts, are critical to an individual's health and development. Since most populations in resource-poor settings do not have access to adequate quantities of fruits, vegetables, and meats where micronutrients are abundant, and because providing vitamin tablets poses logistical and economic constraints, food fortification is a practical and inexpensive alternative [3].

Common salt is a mineral composed primarily of sodium chloride (NaCl), a chemical compound belonging to the larger class of salts. Salt is essential to the health of people and animals and is used universally as a seasoning. It is used in cooking, is added to cooked foodstuffs and is often present on the table at mealtimes for individuals to sprinkle on their own food. Table salt is a refined salt containing about 97 to 99 percent sodium chloride. Some table salts sold for consumption contain additives which address a variety of health concerns, especially in the developing world. Iodine is an important micronutrient for humans, and a deficiency of the element can cause lowered production of thyroxine (hypothyroidism) and enlargement of the thyroid gland (endemic goitre) in adults or cretinism in children. Likewise, iodized salt is further fortified with herbal extracts of the following medicinal herbs. Since herbs are rich in minerals, vitamins, flavouring agents and natural antioxidants. Roots, stems, leaves or seeds of herbal plants have long been used in cooking and in naturopathy all over the world [4].

Materials and methods

Plant collection and extraction

The leaves *of Coriandrum sativum, Solanum trilobatum, Ocimum tenuiflorum, Piper betle,, Triticum aestivum, Mentha* species, *Plectranthus amboinicus, Spinacea oleracea, Murraya koenigii,* and fruits of *Ribes uvacrispa* were collected. The leaves of these plants were washed with distilled water to remove any impurities and finally dried under shade. The dried samples were then ground into fine powder with warring commercial laboratory blender and further milled (mesh size 850 um). 10 g of plant powder was mixed with 100 mL of methanol solvent. The mixture was kept on the rotary shaker for 72 h. Extract was filtered through Whatmann No. 1 filter paper and centrifuged at 5000g for 15 min. and the supernatant was collected and concentrated using rotary evaporator. The concentrated plant extract was stored at 0-4°C for further use.

Chemicals and equipments

Nutrient agar media, Sabouraud Dextrose Agar, petridishes, sterile cork borer, Gentamycin, Clotrimizole. 5% Ferric Chloride, 2 N NaOH, Conc. H2SO4, Glacial acetic acid, Chloroform, Sodium carbonate, Folin–Ciocalteu reagent, Mayer's reagent, 0.02 M sodium phosphate buffer (pH 6.9), 0.02 M alpha-amylase, 1% starch solution, DNSA.

Microorganisms

Bacterial strains - *Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis,* and *Staphylococcus aureus,* Fungal strains - *Candida albicans and Asperigillus niger* were used in this study.

In-vitro Antibacterial activity: Well diffusion method

Standardization of microorganisms

Anti-microbial substances derived from plants have received considerable attention in recent years [5]. One loop full of micro-organisms (*Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus*) were inoculated into 50ml of sterile medium and incubated for 24 h at 37ºC for bacterial culture and for 48 h at 27ºC for bacterial culture.

Antibacterial activity (cup plate method)

Ten petri dish containing agar medium was inoculated with one bacterial strain culture by swabbing the suspension of the organism with a sterile cotton swabs. In each plate wells of 6 mm diameter were made at equal distances using sterile cork borer. Gentamycin (standard) solution was prepared at a concentration of 25 µg/ml. The extracts of ten plants were tested. The Petri dishes were incubated at 37 ^{0}C for 24 hrs. Diameter of the zone of inhibition was measured [6].

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***In-vitro* Antifungal Activity**

Principle and interpretation

Sabouraud Dextrose Agar is Carliers modification of the formulation described by Sabouraud for the cultivation of fungi (yeasts, moulds), particularly useful for the fungi associated with skin infections [7]. Mycological peptone provides nitrogenous compounds. Dextrose provides an energy source. High dextrose concentration and low pH favours fungal growth and inhibits contaminating bacteria from test samples. Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet. For heavily contaminated samples, the plate must be supplemented with inhibitory agents for inhibiting bacterial growth with lower pH.

Procedure for antifungal activity (cup-plate method)

The agar well diffusion method was performed to determine the antifungal activity of ten plant extracts. Fungal strains of *Candida albicans* and *Asperigillus niger* was used and maintained on Sabouraud dextrose agar media in plates maintained at 27 °Cfor 48h. Sterilised Sabouraud dextrose agar media was poured into sterile petri dishes. The respective clinical strain was spread separately on the agar medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The methanolic extracts of ten plants were tested at a concentration of (50 µL). Clotrimizole was used as standard at a concentration of 20 µL. The Plates were incubated at room temperature for 48 h and zones of inhibition were measured.

***Invitro* Antidiabetic activity**

Assay for α-amylase inhibition

The antidiabetic properties of plants can be evaluated in vitro by several methods such as study of glucose uptake, effect on glycosylation of the haemoglobin and inhibition of alpha glucosidase and alpha amylase enzymes [8]. The determination of α-amylase inhibition was carried out by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated. A modified dinitrosalicylic acid (DNS) method was adopted to estimate the maltose equivalent. 100 UL from each methanol extract of the ten plant extracts were pre-incubated with α-amylase 100 UL for 30 min and thereafter 200UL (1% w/v) starch solution was added. The mixture was further incubated at 37°C for 15 min. Then 100 UL from the incubated mixture was added with 1900 UL of water and the reaction was stopped by adding 1 mL DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3, 5- dinitrosalicylic acid solution) and the contents were heated in a boiling water bath for 15 min. A blank was prepared without plant extracts and another without the amylase enzyme, replaced by equal quantities of buffer (0.02 M Sodium phosphate buffer with pH 6.9). The absorbance was measured at 540 nm. The reducing sugar released from starch was estimated as maltose equivalent from a standard graph. Distilled water was used as positive control [9] The anti-diabetic activity was determined through the inhibition of α-amylase which was expressed as a percentage of inhibition and calculated by the following equations:

% reaction=(maltose) test /(maltose) control × 100

% inhibition = 100% reaction

Phytochemical Analysis

Identification of sterols and terpenoids

Salwoski's Test: 1 ml of chloroform was added to 1mL of ten methanolic leaf extracts each and 2 to 3 drops of concentrated sulphuric acid was added to form a lower layer. Reddish-brown colour at the inter phase indicates the presence of steroidal ring.

Lieberman-Burchard's reaction: 1 ml of chloroform was added to 1 mL of ten methanolic leaf extracts each and 1ml drops of concentrated sulphuric acid was added to form a lower layer. Reddish-brown colour at the inter phase indicates the presence of terpenoids

Identification of Alkaloids

Mayer's Test: To the methanolic leaf extracts in test tubes, 1 ml of mayer's reagent was added drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids.

Identification of Saponins

Frothing test: The powdered leaves (0.5 g) was placed in a test tube and 10 ml of distilled water was added and shaken vigorously for 30 s. It was then allowed to stand for 30 min and

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observed. Formation of honey comb froth indicates the presence of saponins [10].

Identification of Tannins

Ferric chloride test: Three drops of diluted solution of $FeCl_3$ was added to the leaf extracts in the test tubes, production of a blue or greenish-black colour that changes to olive green as more ferric chloride is added indicates the presence of tannins [11].

Identification of Phenol

One ml of leaf extract was added with 1 ml of sodium carbonate solution and 1 ml of Folin–Ciocalteu reagent. Formation of blue-green colour indicates the presence of phenol.

Identification of Flavanoids

Sodium hydroxide test: To the test tubes containing 1 ml of extract, 2 ml of 10% NaOH solution was added, yellow solution indicates the presence of flavonoids which on adding dilute hydrochloric acid becomes colourless.

Identification of Quinones

To 1 ml of leaf extract, 1 ml of conc. H2SO4 was added. A pink, violet or red colour in the ammonical layer indicates the presence of quinones [12].

Identification of Cardioglycosides

To 1ml of leaf extract, 1ml of glacial acetic acid is added to which few drops of 15% Ferric chloride was added. Formation of brown ring on addition of 1 ml of conc. H_2SO_4 along the sides of the test tube indicates the presence of cardioglycosides.

Identification of Anthrocyanin and Betacyanin

To 1 ml of leaf extract, 1 ml of 2 N NaOH was added and heated at 100°C for a minute. Formation of blue colour indicates the presence of anthrocyanin whereas formation of yellow colour indicates the presence of betacyanin.

Identification of Coumarins

To 1 ml of leaf extract, 1 ml of 10% NaOH was added. Formation of yellow colour indicates the presence of Coumarins.

Protein Estimation

A standard protein solution of Bovine serum albumen (1 mg) was dissolved in 10 ml of distilled water. Various aliquots of the standard solution (0.2, 0.4, 0.6, 0.8 & 1 ml) were added in test tubes. 100 µl of sample dissolved in 10% DMSO (containing 100 µg sample) were added in test tubes. A test tube containing 1 ml of distilled water served as blank solution. All tubes were made up to 1 mL witl distilled water. The test tubes were added with 5 mL lf Reagent I (2% Sodium Carbonate in 0.1 N sodium hydroxide, 1% Sodium potassium Tartrate in distilled water & 0.5% copper sulphate in distilled water; 48:2:2) and incubated at room temperature for 10 minutes.0.5 mL of Folin – Phenol: Water (1:1) was added and the contents were mixed well. The tubes were incubated at room temperature for 30 minutes and the colour developed was read at 660 nm. A standard calibration curve was constructed by plotting the absorbance of standard solution (200-1000 µg) and the concentration of the protein in the unknown sample was determined using the graph.

Carbohydrate estimation

Anthrone reagent was prepared by dissolving 20 mg of Anthrone in 10 ml of concentrated sulphuric acid followed by 90 ml of distilled water. Glucose solution prepared by dissolving 10 mg of D-Glucose in 100 mL of distilled water was used as a standard carbohydrate solution. Various concentration of the standard glucose solution (0.2, 0.4, 0.6, 0.8 & 1 ml containing 20, 40, 60, 80 & 100 µg glucose) were added in five test tubes. Sample was powdered and dissolved in 10% DMSO. 100 µl of the solution containing 100 µg of each sample were added in test tubes. A test tube containing 0.5 mL distilled water was taken as control. All the tubes were made upto 1 ml with distilled water. 4 ml of Anthrone reagent was added into each tube and the contents were mixed well and placed in boiling water bath for 10 min. It was allowed to cool to room temperature and the optical density was measured at 620 nm. A calibration curve was constructed by plotting the absorbance of standard glucose (20 to 100 µg). The concentrations of carbohydrates in the samples were calculated from the graph [13].

Lipid estimation

One gram of the crushed sample powder was added with 10 ml of concentrated sulphuric acid for the digestion of fats into lipids and kept for 15 min. Chloroform and methanol (3:1) was added to the mixture and it was mixed well. The

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contents were centrifuged at 5000 rpm for 10 min. maintained at 20°C. The aqueous layer was transferred into a fresh tube and this step was repeated again. Hexane: Isopropyl alcohol (2:1) was added to the tubes and centrifuged at 4000 rpm for 5 min. The organic layer was transferred to a fresh tube. 5 ml of distilled water was added to separate the lipids. The organic layer was pipetted out carefully and the tubes were placed on a hot lid maintained at 40°C for 20 min to evaporate the residual hexane and alcohol. The lipids extracted were weighed and the yield was calculated [13].

Results and discussion

Antimicrobial Activity

As a result of in-vitro antibacterial activity against 2 Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and 2 Gram-negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) using agar dilution methods, *B. subtilis* and *S. aureus* were the most sensitive bacterial strains to *Piper betle, Ribes uva-crispa, Coriandrum sativum* and *Ocimum tenuiflorum* extracts among both Gram-positive and Gram-negative bacteria tested. In addition, their possible toxicity to *Candida albicans* and *Aspergillus niger* was determined using agar dilution method. The *Piper betle* extract showed high inhibitory activity against the yeast *C. albicans* (Table 1; Fig. 1 and Fig. 2).

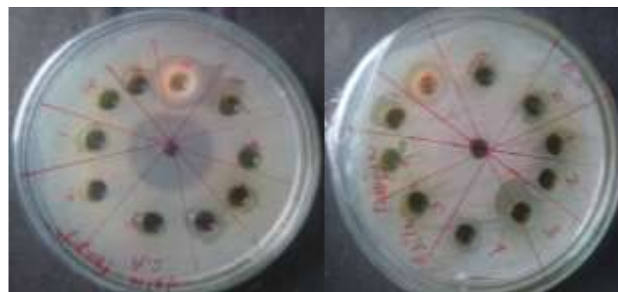

Fig. 1. Antibacterial inhibition zone against *S. aureus* and *B. subtilis*

Fig. 2. Antifungal inhibition zone against *C. albicans*

Table 1. Antimicrobial activity of methanolic extracts of ten plants

S. No.	Compounds	Diameter of zone of inhibition(mm)		
		S. aureus	*B. subtilis*	*C. albicans*
1	*Coriandrum sativum*	13	9	-
2	*Triticum aestivum*	7	10	-
3	*Piper betle*	15	14	17
4	*Murraya koenigii*	12	1	-
5	*Plectranthus amboinicus*	10	13	-
6	*Ribes uvacrispa*	18	15	-
7	*Mentha* species	2	10	-
8	*Ocimum tenuiflorum*	10	20	-
9	*Spinacia olracea*	1	12	-
10	*Solanum trilobatum*	2	1	-
11	Clotrimizole	-	-	2
12	Gentamycin	21	19	-

Phytochemical Analysis

The present finding of phytochemical screening of the plant extracts confirmed the presence of several bioactive compounds like alkaloids, flavonoids, tannins, phenols, saponins, quinones, cardioglycosides, terpenoids, cocumarins, steroids, anthrocyanin and betacyanin which could be responsible for the versatile medicinal properties of these plants.

Antidiabetic Activity

The inhibition of the activity of alpha amylase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation. In this present study, it was evaluated that the in-vitro alpha amylase activity of methanol extracts of the plant showed significant inhibition activity (Table 2 and Fig. 3).

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Table 2. In-vitro antidiabetic activity of alpha amylase method

S. No.	Methanolic extracts	Absorbance (540 nm)	Percentage of inhibition (%)
1	*Coriandrum sativum*	0.047	74
2	*Triticum aestivum*	0.049	73
3	*Piper betle*	0.162	12.5
4	*Murraya koenigii*	0.161	12
5	*Plectranthus*	0.066	64
6	*Ribes uvacrispa*	0.18	1.6
7	*Mentha* species	0.082	55
8	*Ocimum tenuiflorum*	0.077	58
9	*Spinacia olracea*	0.066	64
10	*Solanum trilobatum*	0.072	60
11	Control	0.183	0

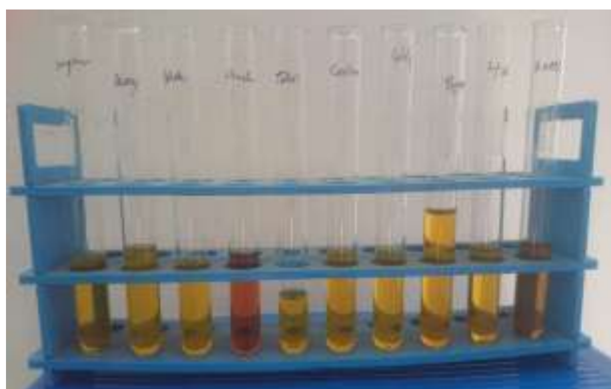

Fig. 3. State of mixture at reading absorbance

Proximate Analysis

The protein content in the sample was found to be 18.05 %. The amount of carbohydrates in Sample was found to be 11.61 %. The amount of lipids extracted from sample was 13.47 % (Fig. 4).

Fig. 4. Digested sample and extracted lipids

Conclusions

Fortification of food has been reported so far with fruit juices, maize flour, wheat flour, sugar, vegetable oil, and rice. Salt has only been iodized and improved with the iron content. Hence this would be the first attempt to fortify salt with protein, fats and lipids. Here, the common salt is fortified with the extracts of locally available Indian herbs. The analyses prove that the quantities of proteins, carbohydrates and lipids are enhanced along with the anti-microbial and ant diabetic properties of common salt. Thus the common salt which basically lacks carbohydrates, proteins and lipids is fortified with the plant powders to have the above mentioned quantities of proteins, carbohydrates and lipids.

Conflicts of Interest

Authors declare no conflict of interest.

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