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Synergistic Effect of Phytoconstituents in Mixed Sprouts- An Approach Towards Therapeutic Applications

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ABSTRACT

Intake of healthy food is highly essential to prevent various diseases. The natural product derived drugs seems to be less toxic and more effective. Identification and investigation of antimicrobial, antioxidant and anti-inflammatory agents from natural substances have been one of the research interests in recent years. Sprouts are highly nutritious as it contains enormous amount of phytoconstituents, vitamins, minerals, enzymes and amino acids which are of great importance as these are most useful in maintaining human health with less amount of anti-nutritional factor especially the phytic acid. Phytic acid present in the seeds gets degraded due to the phytase enzyme produced during sprouting. Individually each sprout is highly nutritious. The present study was carried out to analyse the bioactive compounds present in the mixed leguminous sprouts. Screening the phytoconstituents by preliminary qualitative phytochemical tests and quantification of the primary and secondary constituents were carried out in fresh aqueous and methanol extracts of the sprouts. The characterization of phytochemicals were analysed through FTIR. Identification of the specific phytochemicals was done through TLC analysis and the confirmation of the specific bioactive compounds was carried out through GC-MS analysis. Antibacterial activity of the mixed sprouts against several human pathogens like Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae and Shigella flexneri were studied. Maximum zones of inhibition were shown by Shigella flexneri, Salmonella typhi and Klebsiella pneumoniae. Antioxidant and anti-inflammatory studies proved the presence of therapeutic potential of the phytochemicals such as terpenoids, fatty acids, proteins, carbohydrates and vitamins. Further through in silico analysis, docking studies were performed to confirm the functional role of the specific therapeutic phytochemicals. Thus, the fresh and dried mixed sprouts enriched with the phytoconstituents can be recommended as a good source of natural therapeutic agents.

Keywords: Sprouts; Phytochemical characterization; Antibacterial; Antioxidant; Anti-inflammatory; *In silico* analysis

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INTRODUCTION

Phytochemicals (from the Greek word ‘phyto’, meaning plant) are biologically active, naturally occurring chemical compounds found in plants providing health benefits for humans, by attributing several macronutrients and micronutrients [1]. Phytoconstituents protect plants from diseases and damages which also contributes colour, aroma and flavor to the plants. Phytoconstituents protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack [2,3]. Recently, it is clearly known that phytoconstituents has an important role in the protection of human health, when their dietary intake is significant. Currently there are more than 4,000 phytochemicals which have been classified on the basis of protective function, physical and chemical characteristics [4]. Phytoconstituents like common sugars, amino acids, proteins, chlorophyll’s are said to be primary phytoconstituents. Secondary phytoconstituents includes alkaloids, terpenes, flavonoids, steroids, phenols, glycosides [5]. Phytochemicals are mostly accumulated in different parts of the plants, such as in the roots, stems,

leaves, flowers, fruits or seeds [6]. Currently the attention of nutritionists focusses on the determination of the biological value of the nutritional sprouts [7]. During sprouting, the amount of anti-nutritive materials (e.g.: Trypsin inhibitor, phytic acid, pentosan) decreases and after germination, the compounds with health-benefiting phytochemicals (natural antioxidants) could be detected which is of great importance to human health. Thus, sprouting can lead to the development of several beneficial foods that have a positive effects on human health [8]. Effective phytoconstituents can be found in the sprouts in a much higher concentration than in the developed plant [9,10]. The application of sprouts containing bioactive compounds can lead to the improvement of the food technologies and to healthy nutrition supplements [11,12]. The sprouts consumed are found to be nutritious because of some of the biological properties or effects such as anti-cancer effect, its sulforaphane and isothiocyanate content, glucosinolate content, flavonoid and other curative effects [13]. Essential natural products produced from several plant sources are consumed directly as a food. The constituents of chick pea sprouts possessed aphrodisiac, estrogenic, antioxidant, antidiabetic, anti-inflammatory, hypocholesterolaemic, anti-diarrhoeal, anticonvulsant, hepatoprotective, anticancer, diuretic, anti-nephrolithiasis and many other pharmacological effects [14].

Overall, regular consumption of green gram sprouts regulate the Enterobacteria, thereby decreasing the toxic substances, reducing the risk of hypercholesterolemia and coronary heart disease, also used in cancer treatment [15]. It is highly beneficial for obesity and diabetes [16]. It is reported to have significant biological functions, promoting digestion. In addition to high protein content, it also contains various enzymes and microelements [17].

Horse gram sprouts helps in eliminating kidney stones. It also helps in lowering cholesterol levels and could play a prominent role in anti-oxidation [18]. B-Sitosterol and Stigmasterol were investigated [19] and recently reported the cytotoxicity assessment of the horse gram [20]. Mixed sprouts (*Cicer arietinum* L. (Chick pea), *Vigna radiata* (L.) R. Wilczek (Green gram) and *Macrotyloma uniflorum* (Lam.) Verdc. (Horse gram), with enormous nutrients can be used as a natural edible product as it provides the necessary nutrition and improves human health. Individually each sprout is highly nutritious. The main objective of the present study was to analyse the synergistic effect of bioactive compounds present in the mixed leguminous sprouts by giving scientific validation to the existing phytoconstituents present in the mixed sprouts (chick pea, green gram and horse gram) and thereby recommending the mixed sprouts as a natural source for the prevention of bacterial infections leading to inflammations, enhancing the anti-diabetic, antioxidant, anticancer properties. The essential phytoconstituents makes the mixed sprouts to be rich nutritive source and when consumed in fresh or dried form can be one of the most economical sources for the betterment of human health and can also be used as a source of nutrients and will be beneficial for improving the health of mal nutrition children.

MATERIALS AND METHODS

Sample Collection and Sprouting

The experimental seeds, (*Cicer arietinum* L. (Chick pea), *Vigna radiata* (L) R. Wilczek (Green gram) and *Macrotyloma uniflorum* (Lam.) Verdc. (Horse gram) were purchased from horticultural society, Chennai, Tamil Nadu. Mixed sprouts seeds were selected and washed with water. A sterilized cotton cloth was used to germinate the seeds. 200 gms of each seeds were spread on the sterilized cotton cloth at room temperature. Water was sprinkled as and when required to keep the cloth wet. Sprouts with 1.5-2.0 cm length were used for analysis. It took about 72 hours for the seeds to germinate up to the desired length. The fresh mixed sprouts were used for further study. The analyses of dried sprouts were carried out using shade dry method for three weeks (200 gms of each leguminous sprout). Then they were ground using a blender and stored in air tight containers for further analysis and were subjected to cold percolation with different solvents (Figures 1 and 2).

Figure 1: Fresh mixed sprouts

Microbial Content Screening

Screening for microbial contamination is essential to analyze the purity of the samples and checking whether it can be used for further tests [21]. Using serial dilution method, the fresh and dried mixed sprouts were checked for bacterial contamination with Nutrient Agar plates (dilutions 10^{-6} and 10^{-7}) and fungal contamination with Potato Dextrose Agar plates (dilutions 10^{-3} and 10^{-4}) through pour plate technique. Nutrient agar plates were incubated at 37°C and PDA plates were kept in room temperature. Further the plates were observed after 24 hours for any bacterial growth and 48 hours for fungal growth.

Figure 2: Dried mixed sprouts powder

Preparation of Crude Extracts and Qualitative Phytochemical Screening

10 gms of the fresh mixed sprouts was ground with 100 ml of each of the solvents such as butanol, acetone, methanol and water (aqueous) separately [22] which was then filtered using Whatmann No.1 filter paper and was centrifuged at 5000 rpm for 15 minutes. The supernatant was used for qualitative phytochemical tests. Similarly, 10 gms of the dried powder was dissolved in 100 ml of several organic solvents namely (butanol, acetone, methanol and water) and filtered after 48 hours for the dried sprouts analysis. Using a flash evaporator, these filtrates were then concentrated at 40 to 50°C which resulted in a paste form. This was then freeze dried and stored in a refrigerator in air tight containers for further analysis. The fresh and dried mixed sprouts butanol, acetone, methanol and aqueous extracts (MB, MAc, MM, MA and MBD, MAcD, MMD, MAD) were used for the preliminary qualitative phytochemical analysis. Phytochemical tests for the identification of several primary and secondary phytoconstituents were carried out for all the four extracts (fresh and dried) using standard protocols [23-25].

Quantitative Estimation of the Phytoconstituents

The methanol and aqueous extracts of the fresh and dried mixed sprouts showed prominent results enriched with essential phytoconstituents. Thus these two extracts alone were taken for further study. Using UV Spectrophotometer (UV 1650PC Shimadzu), estimation of the primary and secondary phytoconstituents such as proteins, terpenoids, total soluble sugars and flavonoids were carried out and the amount of anti-nutritional factor, phytic acid was also quantified.

Estimation of Carbohydrates (Total Soluble Sugars)

Using Dinitrosalicylic acid (DNS) method, the total soluble sugars were estimated. 1 ml of the methanol and aqueous extracts (fresh and dried sprouts) were taken in the test tubes, to which 1ml of DNS reagent was added, then the test tubes were placed in a boiling water bath for 5 minutes and were cooled to room temperature. Reagent blank was prepared similarly without the sample extract. The absorbance of the reddish coloured solution was measured at 575 nm using a UV Spectrophotometer (UV 1650PC Shimadzu). The amount of sugars in the extracts were calculated with the standard curve prepared from glucose [26,27].

Estimation of Proteins

Using Lowry's method, the protein samples (fresh and dried) of both methanol and aqueous extracts (1 gm ground in 10 ml of the solvent) were placed in 1 ml of 1 N sodium hydroxide at 100°C for 4 to 5 minutes and 5ml of alkaline copper reagent was added, the mixture was allowed to stand at room temperature for 10 minutes. Immediately, 0.5 ml of Folin-Ciocalteu reagent was added and mixed well. After 30 minutes, the absorbance was measured at 750 nm using UV Spectrophotometer (UV 1650PC Shimadzu) and the amount of protein in the samples were calculated with the standard curve prepared using Bovine Serum Albumin (BSA) [28].

Estimation of Flavonoids

Using aluminium chloride method, 1 ml of the methanol and aqueous extracts of the fresh and dried mixed sprouts were taken in the test tubes to which 0.3 ml of 5% of sodium nitrite solution was added. After 5 minutes, 0.3 ml of 10% aluminium chloride solution, 2 ml of 1M sodium hydroxide solution was added. The absorbance of the yellowish coloured solution was measured at 510 nm using a UV Spectrophotometer (UV 1650PC Shimadzu). The amount of flavonoids were calculated using quercetin as standard [29,30].

Estimation of Terpenoids

Using Ferguson's method, the fresh and dried mixed sprouts of 500 mg was soaked in methanol and distilled water (aqueous) respectively for 24 hours and filtered separately and each filtrate was extracted with petroleum ether. The resulting ether extract was treated as total terpenoids. The residue obtained was dried and weighed [31]. The terpenoid content was calculated using the formula:

$$\text{Terpenoid content (\%)} = \frac{\text{Weight of terpenoid extract (gms)}}{\text{Weight of the sample (0.5gm)}} \times 100$$

Estimation of Phytic Acid

1 gm of each sample (fresh and dried mixed sprouts) was homogenized with 20 ml of 3% TCA and the homogenate was kept in the shaker for 30 minutes, centrifuged at 5000 rpm for 10 minutes. Later the pellet was discarded and the supernatant was collected to which 2 ml of 1 N ferric chloride solution was added and mixed well. The obtained mixture was incubated in a hot water bath for 45 minutes and then centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the precipitate obtained was dissolved in 10 ml of 3% TCA. The attained mixture was heated in a hot water bath for 5 minutes followed by centrifugation at 5000 rpm for 10 minutes. 2 ml of water and 1.5 ml of 1.5 N sodium hydroxide solution was added to the precipitate obtained and the volume was made up to 15 ml using distilled water. The mixture was kept in a hot water bath for 30 minutes, cooled and filtered using Whatman No. 1 filter paper. The resultant precipitate was washed several times with hot water and further dissolved in 10 ml hot 3.2 N hydrochloric acid. The contents were cooled to room temperature and diluted with 10 ml of water. Absorbance was measured at 480 nm using UV Spectrophotometer (UV 1650PC Shimadzu) and iron content from $Fe(NO_3)_3$ standard was calculated. Phytate phosphorous from the iron results were calculated [32].

Fourier Transform Infrared Spectrophotometer (FT-IR) Analysis

In FT-IR analysis, Spectrum FT-IR system (Shimadzu, IR Affinity 1, Japan), equipped with a DLATGS detector with a mirror speed of 2.8 mm/sec. scan range: from 400-4000 cm^{-1} with a resolution of 4 cm^{-1} was used. Firstly, the methanol and aqueous extracts of the fresh and dried mixed sprouts were prepared after which these extracts were evaporated using flash evaporator, and then mixed with KBr salt, using a mortar and pestle and then compressed into a thin pellet. Infrared spectra were recorded on KBr pellet on a Shimadzu FTIR spectrometer 4000 – 500 cm^{-1}.

Antibacterial Activity

Using well diffusion method with Mueler-Hinton agar media the antibacterial assay was carried out. Different concentrations of the methanol and aqueous extracts of the fresh mixed sprouts (50 µg, 75 µg, 100 µg) was assayed against several human pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*e and *Shigella flexneri* (Bacterial cultures were obtained from Department of Microbiology, Ethiraj College for Women, Chennai). Positive control was Streptomycin. The plates were incubated at 37°C for bacterial growth. Zone of inhibition around the well was observed after 24 hrs for antibacterial assay. Triplicates were maintained for all the samples.

***In-vitro* Anti-Inflammatory Assay (Inhibition of Protein Denaturation)**

Methanol and aqueous extracts (fresh and dried) of different concentrations (100 –500 µg) and 1% aqueous solution of bovine albumin fraction were prepared. 10 mg of Diclofenac sodium was used as a standard drug. Both the sample extracts and the standard were incubated at 37°C for 20 minutes which was then heated to 51°C for 20 minutes. After cooling, using UV Spectrophotometer (UV 1650PC Shimadzu) the turbidity was measured at 660 nm. The experiment was performed in triplicates [33].

Per cent inhibition of protein denaturation was calculated using the formula:

$$Per\ cent\ Inhibition = \frac{Control\ OD - Test\ OD}{Control\ OD} \times 100$$

Where, Control OD is the absorbance without sample, Test OD is the absorbance of sample extract or standard.

***In-vitro* Antioxidant Activity**

Hydrogen peroxide scavenging assay:

Using the standard method [34], the methanol and aqueous extracts of the fresh and dried samples of concentrations from 100-500 µg were prepared. The extracts of 0.1 ml of each concentration were dissolved in 3.4 ml of 0.1 M phosphate buffer (pH- 7.4) and mixed well with 0.6 ml of 43 mM hydrogen peroxide solution. Using a UV Spectrophotometer (UV 1650PC Shimadzu), the absorbance of the reaction mixture was read at 230 nm. Blank solution used was phosphate buffer without hydrogen peroxide solution. Ascorbic acid was used as standard. The hydrogen peroxide scavenging activity was calculated using the following formula:

$$\text{Hydrogen peroxide scavenging effect } (\%) = \text{Ac} - \text{Ao}/\text{Ac} \times 100$$

Where, A_c is the absorbance of control, A_o is the absorbance of sprout extract.

Reducing Power Assay

Using standard method [35], different concentrations of the sprout samples (100- 500 µg) were prepared to which 2.5 ml of phosphate buffer (pH- 6.6) was added. 2.5 ml of 1% potassium ferricyanide solution was added and mixed well. This mixture was kept at 50°C in a water bath for 20 minutes. After cooling the test tubes, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride solution and the mixture was kept undisturbed for 10 minutes. The absorbance of the resulting solution was measured at 700 nm using a UV Spectrophotometer (UV 1650PC Shimadzu). Potent reducing power is noted with increase in absorbance of the reaction mixture. The assay was conducted in triplicates and values were expressed as equivalents of ascorbic acid in µg/mg of the extract.

DPPH Radical Scavenging Assay

Using 0.1 mM 1, 1- diphenyl-2-picrylhydrazyl (DPPH) solution, free radical scavenging activity of methanol and aqueous extracts of the fresh and dried mixed sprouts were measured. 1 ml of DPPH was added to 3 ml of methanol and aqueous extracts of different concentrations ranging from 100- 500 µg/ml, which was shaken well and allowed to stand at room temperature for 30 minutes. Using a UV Spectrophotometer (UV 1650PC Shimadzu), the absorbance was measured at 517 nm. Ascorbic acid was reference standard [36]. The per cent inhibition was calculated using the following equation:

$$\text{DPPH scavenging effect} = \text{A0} - \text{A1}/\text{A0} \times 100$$

Where, A_0 was the absorbance of control and A_1 was the absorbance of the standard or test sample.

Thin Layer Chromatography (TLC) Analysis

TLC Sheets of 10 × 10 cm pre coated silica gel (Kieselgel 60 F254 DC- Aluofolein) which was prepared commercially was used. TLC studies were carried out using fresh mixed sprouts methanol extract which showed potent results. The crude extracts of the fresh mixed sprouts were spotted onto the TLC sheet (about 1cm from base) which was then placed in a developing tank containing the chosen solvent system [37]. The solvent system used for TLC were, Proteins- n-butanol: Pyridine: Water (1:1:1), Terpenoids- Chloroform: Methanol: Water (30:4:1 v/v), Flavonoids- Chloroform: Methanol (96:4). The chromatogram sheet was placed inside the solvent chamber. Then it was allowed to run until it reached ¾ positions. For proteins, Ninhydrin solution (0.2%) was used as spraying reagent, terpenoids and flavonoids were detected by exposing the chromatogram to UV light. After spraying, the sheet was allowed to dry. The spots were noted and Rf values were calculated using the formula for different phytoconstituents:

$$\text{Rf value} = \text{Distance travelled by the solute}/\text{Distance travelled by the solvent}$$

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

A Shimadzu GC-2010 Plus gas chromatograph equipped with a straight deactivated 2 mm direct injector liner and a 15 m Alltech EC-5 column (250 µ I.D., 0.25 µ film thickness) was used. 100 µl methanol extract of fresh mixed sprouts (MM) was loaded using a split injection and the split ratio was set to 10:1. Further, the oven temperature was

programmed to start at 35°C, then holded for 2 minutes and then ramped at 20°C per minute to 450°C and holded for 5 minutes. Then the helium carrier gas was set to 2 ml/minute flow rate which was a constant flow mode.
A direct connection with capillary column metal quadupole mass filter pre-rod mass spectrometer operating in electron ionization (EI) mode with software GCMS solution ver. 2.6 was used for all analysis. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 1000 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 1000 at 1 second per scan.
Identification of the components of the various bioactive compounds was matched with their recorded spectra from the data bank mass spectra of NIST library V 11 provided by the instruments software. GC/MS metabolomics database was used for the similarity search with retention index.

***In silico* Analysis (Docking Studies)**
The compounds identified by GC-MS analysis in the mixed sprouts were screened against the target protein (*Helicobacter pylori*) to study the anti-ulcer property. The target molecule retrieved from (PDB) Protein Data Bank was used. The bioactive compound details were obtained from the Pubchem. The several bioactive compounds were docked against the target protein in Mcule database. Docking results in interactions between the target and ligand molecules which prove the anti-inflammatory property of the bioconstituents from the mixed sprouts.
Standard anti-inflammatory compound used was Diclofenac sodium [38].

Statistical Analysis
For the above assays and tests, the data presented are the means of triplicates. All the values are expressed as mean ±SD of triplicates.

RESULTS AND DISCUSSION

The leguminous sprouts are known to be excellent sources of phytoconstituents such as proteins, vitamins, minerals and several secondary metabolites. Individually the sprouts have been analysed but not much work is carried out in mixed sprouts having the combination (chick pea, green gram and horse gram) and not proven scientifically. The study of various medicinal properties of mixed sprouts by analysing their antimicrobial, anti-inflammatory, antioxidant and anti-ulcer properties leads to the choice of cost effective nutritive food for the betterment of human health aspects.

Microbial Content Screening
In order to check the purity of the samples, the mixed sprouts were examined for microbial contamination after 24 hours and 48 hours, where after 24 hours, microbial population was found to be nil and following are the results recorded after 48 hours. Results revealed that mixed sprouts were found to be pure with very minimal microbial contamination (1×10^{-7}, 1×10^{-3} and 1×10^{-4} CFU) (Table 1).

Table 1: Microbial content screening of fresh and dried mixed sprouts

S.No.	Sample	Bacteria			Fungi		
		Control	10-Jun	10-Jul	Control	10-Mar	10-Apr
1	Fresh Mixed sprouts	-	-	1	-	-	1
2	Dried Mixed sprouts	-	-	1	-	1	-

High levels of aerobic bacteria and coliforms were enumerated in fresh sprouts of red radish, alfalfa and broccoli, use of electron beam and gamma irradiation at appropriate doses is a promising approach for producing safe and pathogen-free sprouts for consumers [39]. Thus the study indicated that the lowered level of microbial content, is due to the sterilization during sprouting, drying and powdering of mixed sprouts. This is of great significant value in maintaining the quality of the sample used since the mixed sprouts are recommended for consumption either in fresh or dried form.

Qualitative Phytochemical Screening
The fresh and dried mixed sprouts showed the presence of essential primary and secondary phytoconstituents like alkaloids, saponins, terpenoids, glycosides, steroids, triterpenoids, resin, quinone, proteins, amino acids, carbohydrates, flavonoids, phenols, tannins, fixed oils and fats. The presence of cardiac glycosides are revealed only by the fresh mixed sprouts (Table 2).

Table 2: Qualitative phytochemical screening of different solvent extracts of fresh and dried mixed sprouts

S.No.	Phytoconstituents	Butanol (MB)		Acetone (MAc)		Methanol (MM)		Aqueous (MA)	
		F	D	F	D	F	D	F	D
1	Alkaloids	-	-	-	-	-	-	+	+
2	Saponins	+	+	+	+	+	+	+	+
3	Terpenoids	+	+	+	+	+	+	+	+
4	Glycosides	-	+	+	+	+	+	+	+
5	Steroids and Triterpenoids	+	+	+	+	+	+	+	+
6	Resin	+	+	+	+	+	+	+	+
7	Quinone	+	+	+	+	+	+	+	+
8	Gum and Mucilage	-	-	-	-	-	-	-	-
9	Coumarin	-	-	-	-	-	-	-	-
10	Anthroquinone	-	-	-	-	-	-	-	-
11	Protein and Amino acids	+	+	+	+	+	+	+	+
12	Anthocyanin and Betacyanin	-	-	-	-	-	-	-	-
13	Carbohydrates	-	+	-	+	+	+	+	+
14	Phlobatannin	-	-	-	-	-	-	-	-
15	Flavonoids	-	-	+	-	+	-	+	+
16	Cardiac glycosides	+	-	+	-	+	-	+	-
17	Phenols	-	-	+	-	+	-	+	-
18	Tannins	-	-	+	-	-	-	+	+
19	Phytosterols	-	-	-	-	-	-	-	-
20	Polyphenols	-	-	-	-	-	-	-	-
21	Fixed oils and fats	-	-	-	-	-	-	+	+
22	Fatty acids	-	-	-	-	-	-	+	-

(F) - Fresh; (D) - Dried; (+) presence; (-) absence

Among the fresh and dried mixed sprouts analysed for primary and secondary phytoconstituents in four different solvents, methanol and aqueous solvents showed prominent results both in fresh and dry samples. Hence further work was carried out only in methanol and aqueous extracts. The results coincides with the phytochemical analysis of seed extracts of *Macrotyloma uniflorum* using various solvents such as hexane, diethyl ether, butanol, chloroform, methanol, acetone, and water which showed the presence of terpenoids, flavonoids, tannins, steroids, cardiac glycosides, carbohydrates and saponins [40]. Individually, the essential food nutrients such as proteins, free amino acids and carbohydrates present in the sprouts enhance the nutritional quality. Whereas the phytoconstituents in mixed sprouts such as terpenoids have prominent medicinal properties such as anti-carcinogenic, antimalarial, anti-ulcer, hepaticidal, antimicrobial and diuretic activities [41]. Saponins possess antimicrobial and antimalarial properties [42]. Alkaloids are secondary phytoconstituents which are of great biological value having anti-inflammatory, antioxidant, antibacterial, antifungal properties which aid in benefitting human health [43]. Cardiac glycosides are a potent cardioprotective agent. The various other phytochemicals such as glycosides, steroids resin, quinone, flavonoids, phenols, tannins, fixed oils and fats also makes the mixed sprouts as a healthy edible product by acting against several human pathogens. The study clearly revealed the advantage of consuming mixed sprouts than consuming individually because of the increase in the levels of novel phytoconstituents in mixed form of leguminous sprouts which might be responsible for the synergistic effects.

Quantitative Estimation of the Phytoconstituents

The total soluble sugars were quantified in the fresh and dried mixed sprouts with glucose as standard (Standard curve equation y = 0.0078x + 0.0577). The results revealed that methanol and aqueous extract of fresh mixed sprouts had 0.57 ± 0.1 mg/g and 0.56 ± 0.2 mg/g of glucose and the dried mixed sprouts had 0.56 ± 0.15 mg/g and 0.54 ± 0.1 mg/g of glucose. Protein content in fresh and dried mixed sprouts samples were quantified using BSA as standard (Standard curve equation y = 0.0008x + 0.0614). The results indicated that methanol and aqueous extract of fresh mixed sprouts had 38 ± 0.6 mg/ml and 37 ± 1.3 mg/ml of protein and the dried mixed sprouts had 35± 0.5 mg/ml and 34 ± 1.2 mg/ml of protein. Investigation studies on total soluble sugars and proteins carried out in methanol extracts of chick pea, green gram and horse gram indicated 0.21 mg/g, 0.23 mg/g and 0.25 mg/g of carbohydrates whereas the protein content was 25 mg/ml, 27 mg/ml and 28 mg/ml in the individual sprout analysis.
Flavonoids were quantified in fresh and dried mixed sprouts using quercetin as standard (Standard curve equation y = 0.0008x + 0.0495). The results revealed that methanol and aqueous extract of fresh mixed sprouts had 0.25 ± 0.01 mg QE/g and 0.24 ± 0.13 mg QE/g and the dried mixed sprouts had 0.23 ± 0.1 mg QE/g and 0.21 ± 0.01 mg QE/g.

In quantification studies on terpenoids, methanol and aqueous extract of fresh mixed sprouts had 89 ± 1.3 mg/g and 88 ± 1.2 mg/g of terpenoids and the dried mixed sprouts had 86 ± 1.3 mg/g and 85 ± 1.1 mg/g of terpenoids.
In a study on individual sprouts like chick pea, green gram and horse gram, the flavonoids was reported to be in less amounts about 0.2 mg/QEg whereas the terpenoid content in the chick pea, green gram and horse gram was 50 mg/g, 52 mg/g and 55 mg/g. Thus the results are a clear evidence that when compared to the individual sprouts, consumption of mixed sprouts are of great significance as there is an enormous increase in the levels of phytoconstituents. The results also proved that sprouting enhanced the amount of carbohydrates, protein content and various other bioconstituents like flavonoids, terpenoids making the sprouts highly nutritious than the seeds. The prominent amount of phytoconstituents present in the mixed sprouts might be responsible for the therapeutic synergistic effects. Phytic acid content in the fresh and dry sprouts were estimated which showed that methanol and aqueous extract of fresh mixed sprouts had 0.16 ± 0.006 mg/g and 0.17 ± 0.005 mg/g of phytic acid and the dried mixed sprouts had 0.19 ± 0.003 mg/g and 0.20 ± 0.009 mg/g of phytic acid. The phytic acid content in the fresh and dried mixed sprouts was found to be less when compared to the control seeds used for the samples where fresh mixed sprouts seeds had 1.5 ± 0.2 mg/g and dried mixed sprouts seeds had 1.6 ± 0.4 mg/g of phytic acid. Phytic acid chemically known as myoinositolhexakis-dihydrogenphosphate is an anti-nutritional factor, which is a major storage form of organic phosphorous in several cereals, oilseeds, legumes and nuts. Due to its chelation of various cations in human beings, this results in the reduction in the digestibility of proteins, starch and lipids. Phytase enzyme chemically known as myoinositol-hexaphosphatephosphohydrolase which belongs to acid phosphatases group has the capacity to hydrolyze phytic acid to lower phosphate esters of myoinositol and phosphate. Especially during sprouting, the synthesis of phytases occurs in higher quantity which results in the reduction of phytic acid [44]. For vegetarians as well as diets of inhabitants of rural areas of developing countries, the daily consumption of phytic acid was estimated to be 2000–2600 mg and 150–1400 mg for several mixed diets. Overall the routine intake of phytate can be upto 4500 mg [45]. The results indicated the reduced levels of phytic acid in mixed sprouts when compared to the individual seeds proving its biological significance.

Fourier Transform Infrared Spectrophotometer (FT-IR) Analysis

FT-IR studies on fresh and dried mixed sprouts showed the distribution of functional groups within the organic fractions and provides a basis for a comparison of compositional differences between the sprout samples (fresh and dried). Various functional groups such as alkyl halides, alkenes, aromatics, esters, amides, nitro compounds, phosphines, alkanes and alcohols were found in both methanol and aqueous extracts of fresh mixed sprouts whereas silane compounds was restricted only to fresh mixed sprouts (Figures 3 and 4). Several functional groups like alkyl halides, alkenes, esters, phosphines, alkanes and alcohols were observed both in methanol and aqueous extracts of dried mixed sprouts but aldehydes was restricted to methanol extract of dried mixed sprouts and alkynes was restricted to aqueous extract of dried mixed sprouts (Figures 5 and 6). The study showed that the terpenoids were found in significant amount in fresh and dried mixed sprouts because of the presence of C-H stretch at 2928.9 cm^{-1} in methanol extract and 2924.21 cm^{-1} in aqueous extract of fresh mixed sprouts. The dried mixed sprouts had terpenoids with C-H stretch at 2926.14 cm^{-1}. The presence of different functional groups may be recognized to the existence of variety of potential primary and secondary phytoconstituents and the FT-IR analysis clearly showed the presence of terpenoids in enormous amount apart from carbohydrates, proteins and amino acids which might be responsible for the synergistic effect of mixed sprouts having several therapeutic properties.

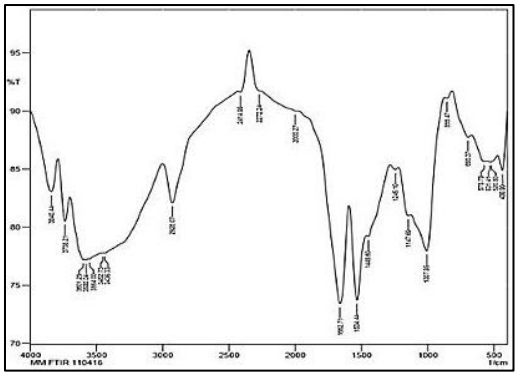

Figure 3: FT-IR spectrum of methanol extract of fresh mixed sprouts (MM)

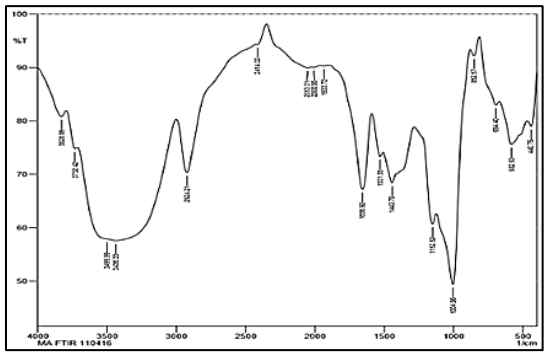

Figure 4: FT-IR spectrum of aqueous extract of fresh mixed sprouts (MA)

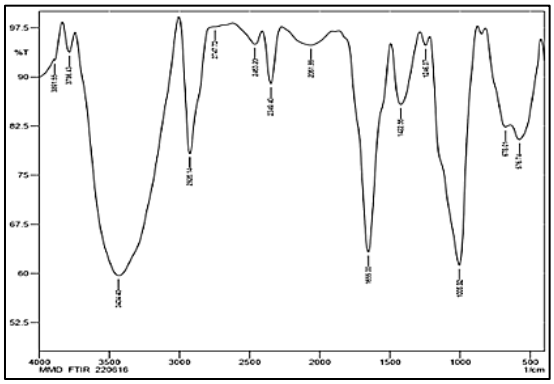

Figure 5: FT-IR spectrum of methanol extract of dried mixed sprouts (MMD)

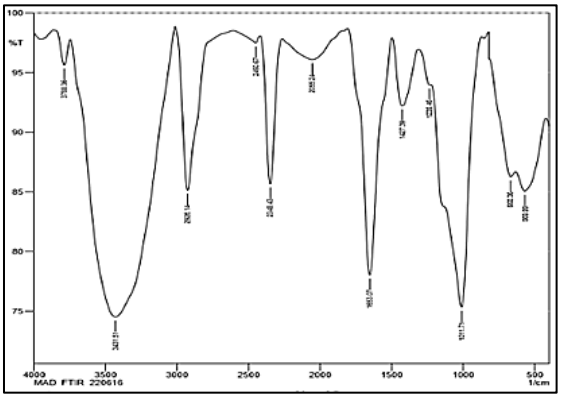

Figure 6: FT-IR Spectrum of dried mixed sprouts aqueous extract (MAD)

Antibacterial Activity

Methanol extract of fresh mixed sprouts (MM) at 100 µg showed maximum zone of inhibition (30 ± 0.6 mm) against *Shigella flexneri* and minimum zone of inhibition (3 ± 0.3 mm) against *Escherichia coli* (Figures 7 and 8). Aqueous extract of fresh mixed sprouts (MA) showed maximum zone of inhibition (25 ± 0.8 mm) against *Shigella flexneri* and minimum zone of inhibition (3 ± 0.2 mm) against *Escherichia coli* (Figures 9 and 10). Against *E.coli*, the minimum zone of inhibition was observed, revealed that the intake of mixed sprouts in fresh form will not bring down the natural microbial flora found in the intestine. The two extracts of dried mixed sprouts were also tested against the human pathogens and only minimum zone of inhibition was observed whereas fresh mixed sprouts showed maximum antibacterial activity. Fresh mixed sprouts showing prominent antibacterial activity might be due to the presence of potent phytoconstituents like terpenoids and flavonoids.

Figure 7: Methanol extract of fresh mixed sprouts (MM) showing maximum zone of inhibition against *Shigella flexneri*

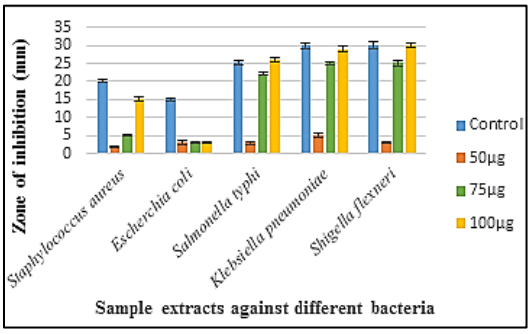

Figure 8: Antibacterial activity of methanol extract of fresh mixed sprouts (MM) of different concentrations against food borne pathogenic bacteria

Figure 9: Aqueous extract of fresh mixed sprouts (MA) showing maximum zone of inhibition against *Shigella flexneri*

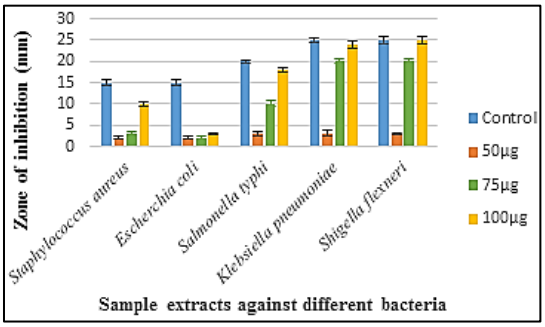

Figure 10: Antibacterial activity of aqueous extract of fresh mixed sprouts (MA) of different concentrations against food borne pathogenic bacteria

One of the major concern for both consumers and industrial producers despite the use of several preservation methods is food poisoning. Food borne illness has a major economic impact on society which cause disorders of the digestive tract [46]. The results clearly showed that the mixed sprouts can act as a potent antibacterial agent against several food borne pathogenic bacteria. The synergistic effect of phytoconstituents present in the mixed sprouts (carbohydrates, proteins, terpenoids, flavonoids) are mainly responsible for promoting the zone of inhibition against the human food borne pathogens. The mixed sprouts produce synergistic effect involving enhanced defense mechanisms and modified phytochemical activities which might be the synthesis of competent antimicrobial phytochemicals that might share its antimicrobial effect with human and animal pathogens. This proves that the mixed sprouts are a potent antibacterial agent. Thus consuming mixed sprouts as food will be highly beneficial in resistance towards any foodborne diseases.

***In-vitro* Anti-Inflammatory Assay (Inhibition of Protein Denaturation)**

The anti-inflammatory assay carried out at different concentrations (100-500 µg) of methanol and aqueous extracts of fresh and dried mixed sprouts revealed inhibition of thermally induced protein (albumin) denaturation in dose dependent manner. The anti-inflammatory potential is mainly determined based on IC_{50} value. The IC_{50} value is the measure of the extract concentration that is required for 50% inhibition. Lesser IC_{50} value denotes the higher anti-inflammatory potential of the mixed sprouts. The methanol extract (MM) and aqueous extract (MA) of fresh mixed sprouts showed per cent maximum inhibition 81 ± 1.3 and 80 ± 1.4 respectively at 500µg concentration with IC_{50} value of 239.3 µg/ml and 258.8 µg/ml (Figure 11). The methanol extract (MMD) and aqueous extract (MAD) of dried mixed sprouts showed per cent maximum inhibition of 77 ± 1.1 and 78 ± 1.2 respectively at 500 µg concentration with IC_{50} value of 253.4 µg/ml and 272.4 µg/ml (Figure 12). The anti-inflammatory activity of standard diclofenac sodium showed per cent maximum inhibition 90 ± 1.5 at 500 µg concentration with IC_{50} value of 125.8 µg/ml.

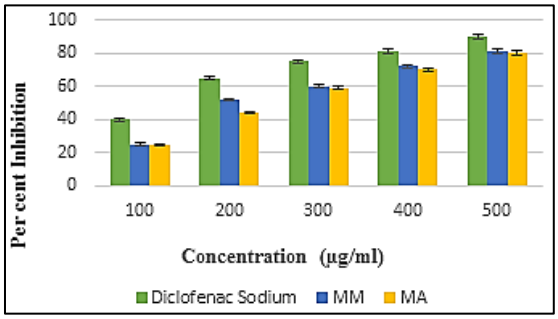

Figure 11: Methanol extract (MM) and aqueous extract (MA) of fresh mixed sprouts showing anti-inflammatory activity

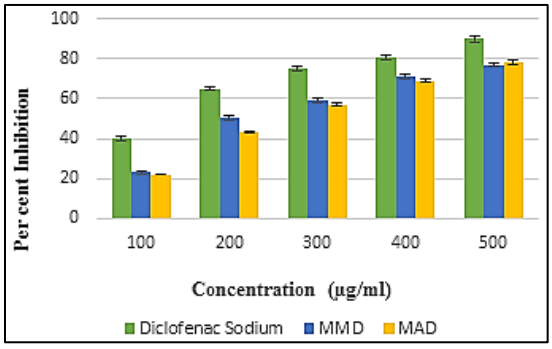

Figure 12: Methanol extract (MMD) and aqueous extract (MAD) of dried mixed sprouts showing anti-inflammatory activity

Methanol extract of fresh mixed sprouts have shown maximum anti-inflammatory activity than the dried sprouts. The results revealed that the fresh mixed sprouts have higher anti-inflammatory potential and also have shown inhibition in a dose dependant manner. Protein denaturation- where the protein loses its secondary and tertiary structure by application of external stress or compounds which are acidic or basic in nature. Denaturation of proteins is one of the causes of inflammation. The results revealed potent anti-inflammatory activity in a dose dependent manner. The results obtained are the clear evidence for consumption of the mixed sprouts which may induce anti-inflammation by being a strong natural anti-inflammatory agent due to the presence of potential phytoconstituents such as terpenoids and flavonoids which has a potent synergistic effect of stabilization of lysosomal membranes.

The stabilization is important in limiting the inflammatory response thereby preventing the release of lysosomal constituents of specific activated neutrophil, such as proteases and bacterial enzymes which specifically cause tissue inflammation and damage upon extra cellular release. The extra cellular activities of these enzymes are found to be related to acute or chronic inflammation [47]. The natural mixed sprout extract act either by inhibiting these lysosomal enzymes or by stabilization of lysosomal membrane. Thus the leguminous sprouts consumed in this combination resulted in its synergistic effect thereby being a strong anti-inflammatory agent.

In-vitro Antioxidant Activity

Antioxidant assay was carried out at different concentrations (100-500 µg) of methanol and aqueous extracts of fresh and dried mixed sprouts. The obtained results revealed inhibition in a dose dependent manner. The antioxidant potential is also determined based on IC_{50} value.

Hydrogen Peroxide Scavenging Assay

The methanol extract (MM) and aqueous extract (MA) of fresh mixed sprouts showed per cent maximum inhibition of 84 ± 1.1 and 78 ± 1.3 respectively at 500µg concentration with IC_{50} value of 236.6 µg/ml and 252.6 µg/ml (Figure 13). The methanol extract (MMD) and aqueous extract (MAD) of dried mixed sprouts showed per cent maximum inhibition of 82 ±1.3 and 76 ± 1.4 respectively at 500 µg concentration with IC_{50} value of 250.7 µg/ml and 267.9 µg/ml (Figure 14). The hydrogen peroxide scavenging activity of standard ascorbic acid showed maximum per cent inhibition of 90 ± 1.5% at 500 µg concentration with IC_{50} value of 149.1 µg/ml. The principle behind hydrogen peroxide scavenging assay is, few enzymes are inactivated by hydrogen peroxide, through oxidation of thiol (-SH) groups. When H_2O_2 reacts with Fe^{2+} and Cu^{2+} ions results in the formation of hydroxyl radical which is the origin of oxidative damage [48]. Thus the results are with clear evidence having prominent antioxidant potential irrespective of the sprouts fresh and dried form using different solvents. Terpenoids and flavonoids present in the mixed sprouts are mainly responsible for the synergistic effects and making the mixed sprouts a potent antioxidant.

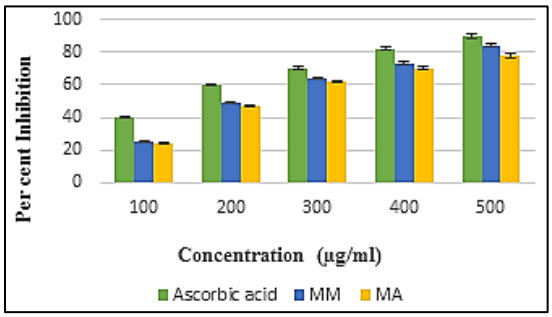

Figure 13: Methanol extract (MM) and aqueous extract (MA) of fresh mixed sprouts showing hydrogen peroxide scavenging activity

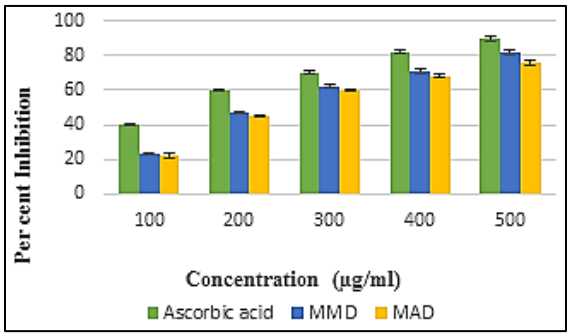

Figure 14: Methanol extract (MMD) and aqueous extract (MAD) of dried mixed sprouts showing hydrogen peroxide scavenging activity

Reducing Power Assay

Methanol extract (MM) and aqueous extract (MA) of fresh mixed sprouts showed IC_{50} value of 242.1 µg/ml and 278.6 µg/ml (Figure 15). The methanol extracts (MMD) and aqueous extract (MAD) of dried mixed sprouts showed IC_{50} value of 283.4 µg/ml and 296.5 µg/ml (Figure 16). The reducing power activity of standard ascorbic acid showed IC_{50} value of 149.1 µg/ml. Antioxidant potential is determined through the reducing capacity of the compound. The principle behind reducing power assay is, usually the reducing ability is determined in terms of the transformation of Fe^{3+} to Fe^{2+} in the presence of different concentrations of the extract. The results revealed that

assayed fresh and dried mixed sprouts was able to reduce the ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in concentration dependent manner [49].

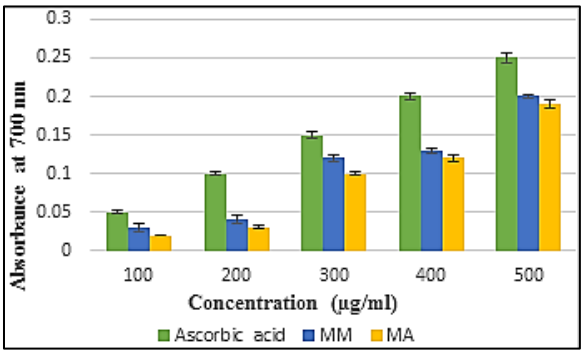

Figure 15: Methanol extract (MM) and aqueous extract (MA) of fresh mixed sprouts showing reducing power activity

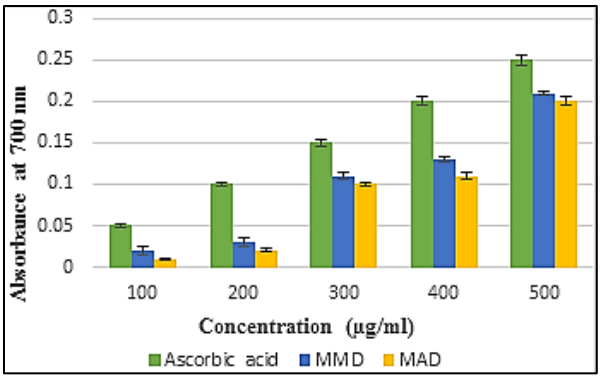

Figure 16: Methanol extract (MMD) and aqueous extract (MAD) of dried mixed sprouts showing reducing power activity

DPPH Radical Scavenging Assay

Methanol extract (MM) and aqueous extract (MA) of fresh mixed sprouts showed per cent maximum inhibition of 85 ± 1.1 and 83 ± 1.4 respectively at 500 µg concentration with IC_{50} value of 187.8 µg/ml and 196.6 µg/ml (Figure 17). The methanol extract (MMD) and aqueous extract (MAD) of dried mixed sprouts showed per cent maximum inhibition of 82 ± 1.3 and 80 ± 1.4 respectively at 500 µg concentration with IC_{50} value of 211.4 µg/ml and 224.3 µg/ml (Figure 18). The DPPH scavenging activity of standard ascorbic acid showed per cent maximum inhibition of 90 ± 1.5 at 500 µg concentration with IC_{50} value of 149.1 µg/ml. Stable free radical is produced by DPPH and it accepts an electron or hydrogen radical for becoming a stable diamagnetic molecule. In natural products, the electron donation ability can be determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) purple- colored solution bleaching where the method is based on scavenging of DPPH through the addition of a radical antioxidant which results in the decolourization of the DPPH solution. The degree of the color change is proportional to the concentration and potency of the antioxidants [50]. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers the electrons from a substance to a particular oxidizing agent. Oxidation may produce free radicals which in turn produce chain reactions that damage the cells. Antioxidants terminate these chain reactions by removing the free radical intermediates thereby inhibiting other oxidation reactions. Oxidative damage has a significant role in several human diseases like cancer, atherosclerosis and arthritis [51]. Plant antioxidants are composed of different substances like ascorbic acid, tocopherols, terpenoids. Among the three antioxidant assays carried out in fresh and dried mixed sprouts, DPPH scavenging assay indicated a prominent antioxidant activity when compared to hydrogen peroxide scavenging assay and reducing power assay. Thus it clearly shows that the mixed sprouts can be a potent natural antioxidant agents due to the presence of rich phytoconstituents specifically terpenoids and flavonoids.

Significant mechanism of terpenoids being a natural antioxidant, support other antioxidant molecules especially α-tocopherol, which clearly indicated that the terpenoids not only being a strong antioxidant but also help in functioning of other antioxidants revealing its synergistic effects. Flavonoids, a large class of benzo-pyrone deriatives, exhibit significant antioxidant activity. The antiradical activity of flavonoids is directed mostly towards hydroxyl, superoxide as well as peroxyl and alkoxyl radicals. Flavonoids aid in free radical scavenging activity, in addition have multiple activities like antibacterial, anti-inflammatory, immune-stimulating, anti-allergic,

vasodilatory and estrogenic effects. These biological properties are said to be related to their antioxidative properties which are the synergistic effects of the mixed sprouts. The fresh and dried methanolic and aqueous extracts of mixed sprouts showed a prominent free radical scavenging activity on par with the standard ascorbic acid used. Thus, the consumption of the mixed sprouts can be highly beneficial in preventing oxidative damage.

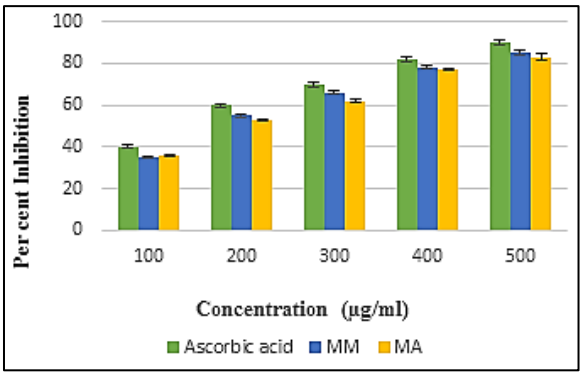

Figure 17: Methanol extract (MM) and aqueous extract (MA) of fresh mixed sprouts showing DPPH free radical scavenging activity

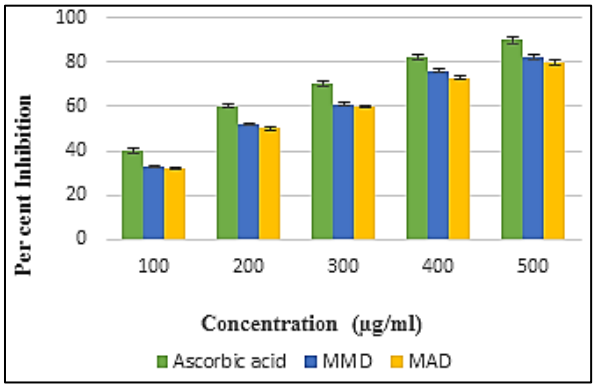

Figure 18: Methanol extract (MMD) and aqueous extract (MAD) of dried mixed sprouts showing DPPH free radical scavenging activity

Thin Layer Chromatography (TLC) Analysis

Methanol extract of the fresh mixed sprouts showed prominent results for antibacterial, anti-inflammatory and antioxidant assays, hence TLC analysis was carried out only in fresh sprouts samples of methanol extract. Methanol extract of fresh mixed sprouts (MM) using the solvent system n-butanol: pyridine: water (1:1:1), showed yellow colour with a Rf value of 0.7 indicating the presence of proteins, with the solvent system Chloroform: Methanol: Water (30:4:1) greenish yellow colour with Rf value of 0.26 indicated the presence of terpenoids and using the solvent system, Chloroform: Methanol (96:4) orange colour with Rf value of 0.83 indicated the presence of flavonoids (Figure 19). Plant proteins in human diet vary in amino acid content and its digestibility. Consumption of protein rich diets helps in prevention of hypertension, cardiovascular disease and osteoporosis. Individually, the chromatogram developed with methanol and chloroform extracts of mung bean sprouts in the ratio of 1:9 revealed the presence of major compounds, glycosides, steroids, phenols, saponins, alkaloids and flavonoids with Rf values of 0.42, 0.60, 0.69, 0.75, 0.84 and 0.87 as visualized under iodine vapour and UV illumination. The results clearly indicated the strong synergistic effect of mixed sprouts where terpenoids possess significant therapeutic activities, like anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities [52]. Flavonoids contribute a lot in the protection of biological systems against the several harmful effects of oxidative processes on macromolecules which include carbohydrates, proteins, lipids and DNA [51]. Hence the results confirmed the presence of various phytoconstituents such as proteins, terpenoids and flavonoids in the methanolic fresh mixed sprout extracts.

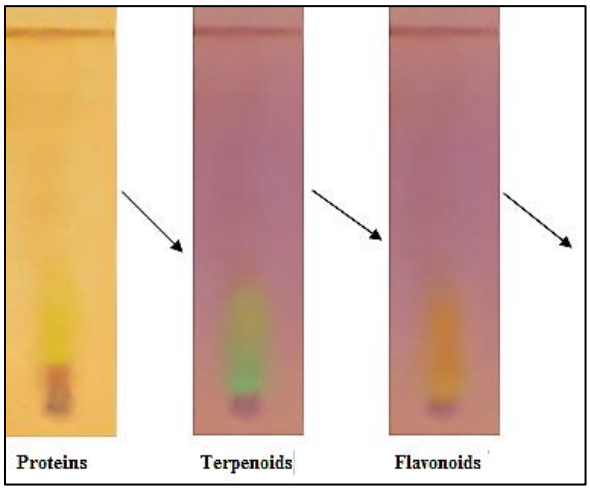

Figure 19: TLC studies on methanol extract (MM) of fresh mixed sprouts

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

GC-MS, a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS) is of great significant value in analyzing the complex organic and biochemical mixtures [53]. Since the methanol extract of fresh mixed sprouts had higher amount of phytoconstituents and showed prominent results for antibacterial, anti-inflammatory and antioxidant assays, GC-MS analysis was carried out in fresh mixed sprout samples of methanolic extract to analyse the presence of specific therapeutic bioactive compounds.

The GC-MS spectrum of methanol extract of fresh mixed sprouts (MM), indicated the presence of bioactive compounds like dodecanoic acid, alpha-D-glucopyranoside, myo-inositol, vinyl caprylate, methyl mannose, cis-vaccenic acid, geranyl geraniol, gamma sitosterol and gamma tocopherol (Figure 20 and Table 3). The compounds are mostly of terpenoids, fatty acids, carbohydrates, amino acids and other small functional groups. These compounds can be attributed for the presence of antimicrobial, anti-oxidant, anti-ulcer, anti-cancer properties.

Consumption of natural foods like sprouts has attracted huge attention in recent decades, since many biochemical studies have demonstrated a clear and significant studies regarding regular intake of these natural edible food products which has reduced the rates of cardiovascular diseases, aging and other degenerative diseases. The synergistic effects of mixed sprouts are of great biological significance because of the presence of several antioxidants. Sprouts are a part of regular diet in appropriate amounts; they provide long-term health benefitting effects. Thus, the GC-MS studies are further taken for *in silico* analysis with the therapeutic compounds identified for several biological activities especially with anti-inflammatory and antiulcer properties.

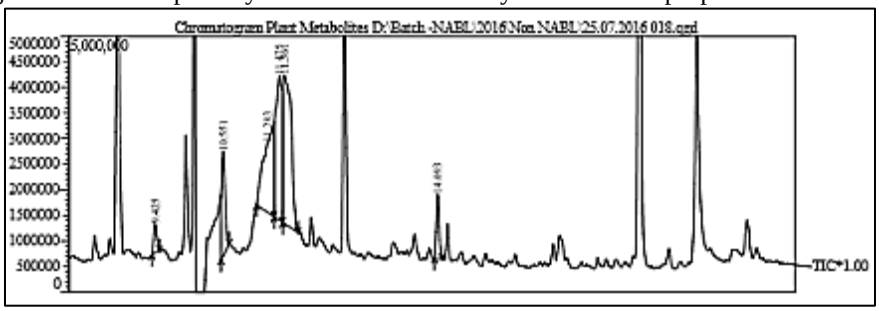

Figure 20: GC-MS spectrum of methanol extract of fresh mixed sprouts (MM)

In silico Analysis (Docking Studies)

GC-MS studies confirmed the presence of terpenoids, fatty acids, carbohydrates, amino acids and other small functional groups. Thus from the GC-MS analysis, screening was carried out for the therapeutic compounds related to anti-ulcer property. Among the several compounds screened, Geranyl geraniol from fresh mixed sprouts was found to be related to the present study having anti-ulcer activitiy. Geranyl geraniol, a diterpene is an anti-inflammatory agent used to treat peptic ulcers. Diclofenac sodium was used as a standard. Diclofenac is a non-steroidal antiinflammatory agent (NSAID) which possesses antipyretic and analgesic actions. It is primarily available as the sodium salt.

Table 3: GC-MS analysis of methanol extract of fresh mixed sprouts (MM)

Peak	R. Time	Name	Area	Area %	Height	Height %
1	9.425	Dodecanoic acid	1951683	1.24	561043	2.34
2	10.551	Alpha-D-Glucopyranoside	7449936	4.75	2034230	8.48
3	11.283	Myo-Inositol	19271612	12.28	1360713	5.67
4	11.475	Vinyl caprylate	21042142	13.41	2815716	11.73
5	11.561	Myo-Inositol	26363814	16.8	2902657	12.09
6	14.093	Methyl mannose	3779364	2.41	1261234	5.26
7	22.993	Cis-Vaccenic acid	15918877	10.15	3142537	13.09
8	23.746	Geranyl geraniol	4023713	2.56	1040529	4.34
9	44.658	Gamma-Sitosterol	13674967	8.72	2333550	9.72
10	45.29	Gamma-Tocopherol	43422579	27.68	6547322	27.28
			156898687	100	23999531	100

Peptic ulcer is caused by a bacteria, *Helicobacter pylori* or severe allergic reactions to non-steroidal anti-inflammatory drugs found in the digestive tract in the stomach or the duodenum [54]. *H. pylori* is a gram-negative bacillus, micro-aerophilic, motile, flagellate bacteria. The pathogenic activity of causing peptic ulcer is found in the Type-I strains of the bacteria which encodes the effector protein cytotoxin-associated gene (cagA). When the bacteria enters the host cell, cagA effects shape of the cell, increases the cell motility, intrupt the cell junctional activity which results in gastric carcinomas and ulcers [55]. The antibiotics at appropriate doses may result in minimal chance for recurrence of ulcers. Peptic or stomach ulcers result in abdominal pain and several discomforts. Some of the other symptoms are nausea, weight loss, poor appetite, bloating and blood in stool and vomiting, black stools which indicates the gastrointestinal bleeding [56]. The target protein of *Helicobacter pylori*, was obtained from Protein Data Bank (PDB ID: 1G60). The bioactive compound details (Geranyl geraniol) were retrieved from Pubchem database. The bioactive compound docked against the target protein in mcule database showed the anti-ulcer property of the compound through docking scores. More negative values are indication of higher binding affinity which clearly indicates the strong anti-ulcer property. Docking analysis of geranyl geraniol from fresh mixed sprouts methanol extract (MM) showed docking scores of -6.9, -6.6, -5.9 and -5.8 (Figure 21). Docking analysis of standard diclofenac sodium showed docking scores of -7.5, -7.3, -7.1 and -6.8 (Figure 22). Mixed sprouts showed prominent binding affinity against *Helicobacter pylori* on par with the standard drug compound diclofenac sodium. The docking results are a clear evidence for mixed sprouts possessing anti-ulcer properties. Thus the mixed sprouts with enriched therapeutic phytoconstituents can be recommended as a natural edible product for ulcer problems. The maximum binding affinity was reported with fresh methanolic extracts of mixed sprouts with Geranyl geraniol- an antiulcer agent, when compared with the standard diclofenac sodium.

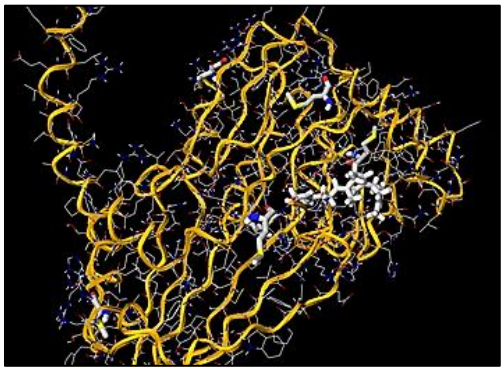

Figure 21: Illustration of geranyl geraniol from methanol extract (MM) of fresh mixed sprouts docked against target protein 1G60-*Helicobacter pylori*

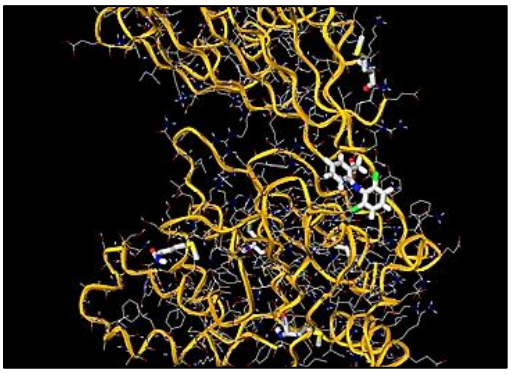

Figure 22: Illustration of standard Diclofenac sodium docked against target protein 1G60- *Helicobacter pylori*

CONCLUSION

The present study revealed that the mixed sprouts are enriched with increased levels of therapeutic phytoconstituents such as terpenoids and flavonoids along with the presence of carbohydrates and proteins which have prominent antibacterial, anti-inflammatory and antioxidant activities. Further, GC-MS and docking studies confirmed the presence of Geranyl geraniol, a diterpenoid which showed a strong specific antiulcer affinity against the target protein *Helicobacter pylori*, an ulcer causing bacteria. Currently there is an increasing need for the consumption of nutrient rich diet. Even though individually the leguminous sprouts have strong biological value, the synergistic effect of fresh mixed sprouts makes the sprouts as a strong natural therapeutic agent, economical potent food source for human health and can be a nutrient supplement with cost effective approach. Further, the dried sprouts can also be recommended to food industry for large-scale production of nutrient-based foods with a quality check.

CONFLICT OF INTERESTS

The authors declared that they had no conflicts of interests.

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