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Sprouts as functional food- an approach towards the identification of natural antibiotic resistance breakers

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ABSTRACT

Antibiotics are medicines used to prevent and treat bacterial infections. Antibiotic resistance occurs when bacteria change in response to the use of these medicines. Investigation studies related to discovery of novel antibiotics to deal with antibacterial resistance from natural edible food products have been one of the significant research interests in recent years. The main objective of the study is to identify the bioactive compounds having the natural antibiotic resistance breaking property, by giving scientific validation to the existing bioactive compounds present in the sprouts and recommending the horse gram and mixed sprouts as a natural dietary supplement, a measure for the management of the disease, Shigellosis. Qualitative screening of the phytoconstituents (using different solvent extracts) and quantitative analysis of the primary and secondary phytoconstituents were carried out in methanol and aqueous extracts of the horse gram and mixed sprouts (fresh and dried) using standard protocols in two different samples- horse gram sprouts (*Macrotyloma uniflorum* (Lam.) Verdc.) and mixed sprouts of combination (*Cicer arietinum* L. (Chick pea), *Macrotyloma uniflorum* (Lam.) Verdc. (horse gram) and *Vigna radiata* (L.) R. Wilczek (Green gram). Phytochemical characterization was done through FTIR and GC-MS analysis. Antibacterial activity of both the samples against human pathogens namely *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*e and *Shigella flexneri* were studied. In horse gram and mixed sprouts, maximum zone of inhibitions were shown by *Shigella flexneri*, a food and water borne pathogen leading to outbreaks of Shigellosis, a major public health concern. Ciprofloxacin is a broad spectrum of antimicrobial carboxyfluoroquinolones. The bactericidal action of Ciprofloxacin is by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV, which are required for bacterial DNA replication. Phytochemical characterization (FTIR and GC-MS) and antibacterial studies proved the presence of essential phytoconstituents like terpenoids, fatty acids, proteins, carbohydrates and vitamins. Several bioactive compounds obtained from GC-MS analysis were screened for Ciprofloxacin antibiotic resistance. The specific phytoconstituents, DL-Proline from horse gram sprouts and Geranyl geraniol from mixed sprouts was tend to act as novel antibiotic resistance breakers which was proved through *in silico* docking. Thus, the horse gram sprouts and mixed sprouts enriched with the phytoconstituents are the natural source of antibiotic resistance breakers; one of the most economical and easily available sources for consumption can be recommended as a preventive measure for the disease, Shigellosis.

Keywords: Horse gram sprouts, mixed sprouts, FTIR, GC-MS, antibiotic resistance, Ciprofloxacin, *in silico* docking.

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INTRODUCTION

Traveler's Diarrhea (TD) is an important public health concern. It is mainly caused by eating contaminated food or drinking contaminated water. Various human pathogens including *Salmonella* sp. and *Campylobacter* were also been identified as the pathological agents of this disease, with *Shigella* sp. being one of the most common etiological agents[1]. *Shigella* species are host adapted organisms which mainly infect humans and other primates. Identification of shigelllae in foods is not as easy as in other sources. The factors such as composition of fat content of the food, physical parameters such as pH and salt and natural microbial flora of the food in which other microbes in a sample may overgrow shigellae in broth media may also affect the successful recovery of shigellae. Shigellosis is distinguished from the diseases caused by most of the other food-borne pathogens in the production of bloody diarrhea or dysentery whereas the low infectious dose causes various other clinical symptoms. The dysentery stage of disease correlates with the extensive bacterial colonization of the colonic mucosa. The bacteria invade the epithelial cells of the colon and spread from one cell to the other. The incubation period for Shigellosis is 1-7 days. Complications arising from

this disease include severe dehydration, intestinal perforation, toxic mega-colon, septicemia, seizures, Reiter's syndrome (a form of reactive arthritis). *Shigella* virulence is multigenic, involving both chromosomal and plasmid encoded genes[2]. Antibiotics such as quinolones (ciprofloxacin, norfloxacin), rifaximin and azithromycin were reported to be effective and safe to use against TD. But it has been found that *Shigella* sp. acquired resistance to these clinically important antibiotics[3].

Fluoroquinolones are one of the most effective antibacterial compounds and second-line drugs which are used against bacterial infections[4,5]. Fluoroquinolones like ciprofloxacin and norfloxacin have a broad spectrum of antibacterial activity, used for the treatment of large number of infectious diseases. However, due to widespread use of these antibiotics, the bacterial pathogens develop resistance to it. These mainly target DNA gyrase and Topoisomerase IV[6]. DNA gyrase plays an important role in the regulation of DNA topology especially DNA super coiling activity and it helps in the survival of bacteria inside the host cells. The topological stress which arises from the translocation of transcription and replication complexes along DNA is relieved by DNA gyrase. Topoisomerase IV is a decatenating enzyme which resolves interlinked daughter chromosomes following DNA replication[7,8].

The resistance to quinolones in DNA gyrase occurs through mutations in the Quinolone Resistance-Determining Region (QR-DR)[9]. These DNA gyrase and topoisomerase IV acts as the target for fluoroquinolones[10]. Emerging resistance to quinolones such as ciprofloxacin has been studied in several bacteria, such as in *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*[11]. In last few years, several studies related to resistance mechanism have been reported, but studies related to the mutations occurring in the QR-DR of *Shigella flexneri* is yet to be explored.

Functional foods are potentially beneficial components found natural in foods or added in foods as functional ingredients namely phenolic acids, carotenoids, dietary fiber, fatty acids, flavonoids, isothiocyanates, plant sterols, polyols, soy protein, vitamins and minerals etc which acts as health-giving additives[12]. The sprouts are known to be an excellent source of proteins, vitamins and minerals which act as a functional food. Legumes are one of the most important sources of food products in terms of food energy and nutrients available naturally, especially in developing countries which are economical dietary source of protein than most other plant foods[13]. Legumes are cost effective and widely available source of proteins for human consumption.

Cereals, millets and legumes are usually pre-processed by fermentation, germination (sprouting), cooking, milling in order to enhance the functionality and the nutritional value. Germination or sprouting is a biochemical process which mainly involves transition of a seed from dormant state to vital active state. Sprouting is one of the simplest techniques which have been reported to improve the nutritive value of foods. Studies on the effect of germination on legumes have found that sprouting can increase the protein content and dietary fibre, increases mineral bioavailability and reduces the phytic acid content[14-16]. Sprouting is the most environment friendly and cost effective method in improving the quality and the nutritional value of the seeds which acts as functional foods.

Horse gram sprouts occupies an important place in human nutrition and has a rich source of proteins, vitamins and minerals and helps in eliminating kidney stones, reduces cholesterol levels and also with antioxidant properties[17]. Chick pea sprouts have aphrodisiac, estrogenic, antioxidant, antidiabetic, anti-inflammatory, hypocholesterolaemic, anti-diarrhoeal, anticonvulsant, hepatoprotective, anticancer, diuretic, anti-nephrolithiasis and many other pharmacological effects[18]. Green gram is a widely consumed pulse which is an excellent source of protein, high in dietary fibre, vitamins and minerals. It's high folate content and low glycemic index reduce blood glucose level and neural tube defects in newborn babies[19]. Regular consumption of green gram sprouts decrease the toxic substances, regulate the Enterobacteria and reduce the levels of hypercholesterolemia, also used in cancer treatment[20]. The energy from green gram sprouts, is highly beneficial for obesity and diabetes[21].

Several studies related to usage of different combinations of cereals, millets, pulses, seeds, sprouts like green gram, horse gram, lentils, mung bean, chick pea satisfies the nutritional requirements of different classes of people. These multi-nutrient food components help to prevent various harmful diseases. Due to the synergistic effect of the bioactive compounds found in these functional foods, they act as a natural preventive measure against several disease causing organisms and the occurrence of diseases. The main advantage of using these mixed combinations is, they are enriched with essential nutrients, easily available, cost effective and free from artificial flavouring agents[22].

The leguminous sprouts are used in the present study using horse gram sprouts as Sample I and mixed sprouts as Sample II. The main objective of the study was to identify the bioactive compounds having the antibiotic resistance breaking property in addition to several qualitative and quantitative analysis of the phytoconstituents, characterization of the phytochemicals by FTIR and GC-MS analysis. The bioassays for antibacterial, anti-inflammatory and antioxidant activity of the sprouts were also performed. *In silico* docking studies were carried out to identify and prove that the bioactive compounds from these sprouts can act as potent natural antibiotic resistance breakers.

MATERIALS AND METHODS

Collection of samples, sprouting and preparation of crude extracts

Macrotyloma uniflorum (Lam.) Verdc. (Horse gram), *Cicer arietinum* L. (Chick pea) and *Vigna radiata* (L.) R. Wilczek (Green gram) seeds were purchased from horticultural society, Chennai, Tamil Nadu. Sample I was horse gram sprouts and sample II was mixed sprouts (chick pea, horse gram and green gram in equal proportion). The experimental seeds were germinated using standard procedures[23], these fresh sprouts were used for further studies. Dried samples were prepared using 200gms of each of the sprouts after shade drying for three weeks which was then ground using a blender and stored in air tight containers for further analysis.

The crude extract preparation of the fresh and dried form of both the samples were carried out using 10gms of the sprouts ground with 100ml (1:10 ratio) of each of the solvents namely butanol, acetone, methanol and water (aqueous) separately using cold percolation method[24].

Qualitative phytochemical screening and Quantification of the phytoconstituents

Different solvents like butanol, acetone, methanol and water (aqueous) were used for the study. The fresh horse gram sprouts (HB, HAc, HM, HA), dried horse gram sprouts (HBD, HAcD, HMD, HAD) and fresh mixed sprouts (MB, MAc, MM, MA), dried mixed sprouts (MBD, MAcD, MMD, MAD) were

used for the qualitative phytochemical analysis using standard protocols[25,26].

The methanol and aqueous extracts of the fresh and dried sprout samples which showed good results were taken for further analysis. The quantification of the phytoconstituents such as total soluble sugars[27,28], proteins[29], flavonoids[30], terpenoids[31] were carried out using UV Spectrophotometer (UV 1650PC Shimadzu) and the amount of phytic acid was also quantified [32].

Fourier Transform Infrared Spectrophotometer (FTIR) analysis

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

Antibacterial assay

Different concentrations of the extracts of the samples (50µg/ml, 75µg/ml, 100µg/ml) was assayed against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Shigella flexneri* (Bacterial cultures obtained from Department of Microbiology, Ethiraj College for Women, Chennai) were used. Antibacterial assay was carried out by well diffusion method using Mueller-Hinton agar media. Streptomycin was used as positive control. Triplicates were maintained for all the samples. Zone of inhibition around the well was observed after 24 hours.

***In vitro* anti-inflammatory and antioxidant assay**

In vitro anti-inflammatory assay was carried out using the method of inhibition of the protein (albumin) denaturation using UV Spectrophotometer (UV 1650PC Shimadzu)[33].

In vitro antioxidant assay, 1, 1- diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity[34] of the methanol and aqueous extracts of the fresh and dried sprout samples were analysed through standard method (using UV Spectrophotometer- UV 1650PC Shimadzu). The experiments were conducted in triplicates and values were expressed as equivalents of ascorbic acid in µg/mg of the extract.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis of the methanol extract of fresh horse gram and mixed sprouts were carried out by the standard method[35]. 100µl methanol extract of fresh horse gram sprouts and mixed sprouts were used for GC-MS analysis. A Shimadzu GC-2010 plus gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used for the sprout sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35°C, hold for 2minutes, then ramp at 20°C per minute to 450°C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode). The mass spectrum had a direct connection with capillary column metal quadupole mass filter pre-rod mass spectrometer operating in electron ionization (EI) mode with software GC-MS 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 compound 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.

Identification of natural antibiotic resistance breakers through *in silico* docking studies

In antibacterial assay, since the maximum zone of inhibition was shown by *Shigella flexneri*, it was taken for further study. The bioactive compounds identified in GC-MS studies were screened against the target protein *Shigella flexneri*, to study the antibiotic resistance breaking property. The target molecule (normal *Shigella flexneri* and Ciprofloxacin resistance (QR-DR of *Shigella flexneri*) were retrieved from Protein Data Bank (PDB). The details of the bioactive compounds were retrieved from the Pubchem database. By using standard protocol[36], docking was carried out to identify the bioactive compounds which can act as natural antibiotic resistance breakers.

Statistical analysis

For each experiment, data presented are the means of three replicates. Values are expressed as mean ±SE of three replicates.

RESULTS AND DISCUSSION

Qualitative phytochemical screening

The fresh and dried horse gram and mixed sprouts indicated the presence of alkaloids, saponins, terpenoids, glycosides, steroids, triterpenoids, resin, quinone, proteins, amino acids, carbohydrates, flavonoids, cardiac glycosides, tannins, phenols, fixed oils, fats and fatty acids. Among the samples analysed for phytoconstituents in four different solvents, methanol and aqueous solvents showed prominent results both in fresh and dried samples. Hence further work was carried out only in methanol and aqueous extracts.

Similar study carried out in common south Indian legumes such as *Mucuna pruriens* (velvet bean), *Macrotyloma uniflorum* (horse gram), *Phaseolus lunatus* (lima bean) and *Canavalia ensiformis* (jack bean) indicated the presence of glycosides, tannins, phenols, flavonoids, alkaloids, saponins, steroids and terpenoids[37]. Our results found to be more superficial as it also indicated the presence of cardiac glycosides, triterpenoids, resin, quinone, proteins, amino acids, carbohydrates, fixed oils, fats and fatty acids apart from other phytoconstituents reported.

Phytoconstituents are the dependable sources for the treatment of several health disorders. Phytochemical techniques play a significant role in the discovery of raw materials and resources for pharmaceutical industry. Legumes are an economical source of proteins with desirable characteristics such as presence of carbohydrates, ability to lower the serum cholesterol, high fibre content, low fat content (except oilseeds), high concentration of polyunsaturated fatty acids and a long shelf life period. In addition to phytochemicals, these are also an excellent source of B complex vitamins, minerals and fibre[38]. These biologically active metabolites present in the sprout samples are essential chemical substances which are involved in several anti-inflammatory, antioxidant, anti-diuretic, anti-microbial, anticancer and hepaticidal properties. Thus the

results indicating a wide variety of phytoconstituents are promising evidence which makes the horse gram sprouts and mixed sprouts as a natural healthy edible product from plants with antibiotic resistance breaking property.

Quantification of the phytoconstituents

Among the fresh and dried horse gram sprouts and mixed sprouts, methanol extract of fresh sprouts of both the samples showed maximum total soluble sugars (0.58 ± 0.2mg/g of glucose and 0.57 ± 0.1mg/g of glucose), protein content (37 ± 0.9mg/ml of protein and 38 ± 0.6mg/ml of protein), flavonoid content (0.26 ± 0.11mg QE/g and 0.25 ± 0.009mg QE/g) and terpenoid content (88 ± 1.4mg/g and 89 ± 1.3mg/g) when compared to the dried sprouts of both the samples with reduced levels of phytic acid (0.15 ± 0.005mg/g and 0.16 ± 0.006mg/g). The phytic acid content in the fresh and dried sprouts were found to be less when compared to the control seeds used for the samples where horse gram seeds had 1.4 ± 0.3mg/g and mixed seeds had 1.5 ± 0.2mg/g of phytic acid (Fig. 1-3).

Similar studies were carried out in different samples of *Glycine max* which showed a prominent amount of phytoconstituents[39]. Our results were found to be superficial as it indicated more amount of phytoconstituents which were already reported.

Pulses and legumes are a vital source of plant-based proteins and free amino acids which are highly essential for human beings and should be consumed as a part of healthy diet to prevent obesity as well as to prevent and manage chronic diseases such as diabetes, coronary diseases and cancer. These are also an important source of plant-based proteins, fibres, as well as a significant source of vitamins and minerals, such as iron, zinc, folate and magnesium with less amount of phytic acid (anti-nutritional factor). Consumption of legumes enhance the quality of diet by increasing the intake of these nutrients. Because of the high nutrient content, legumes may be considered both as a vegetable and as a protein food. Pulses do not include fresh beans or peas.

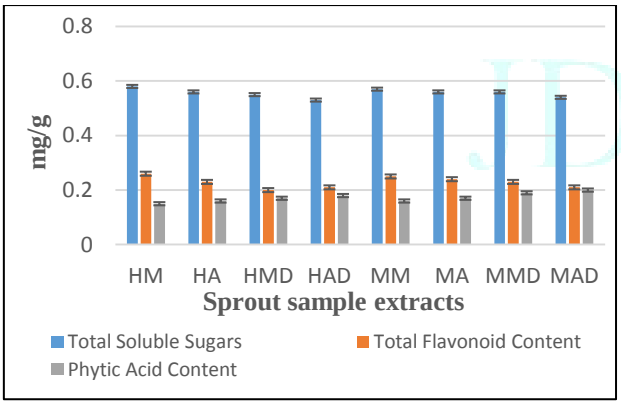

Figure 1: Total soluble sugars, Total flavonoid and Phytic acid content of sprout samples

They are just related to pulses because they are also considered as edible seeds of podded plants as they have a much higher fat content, whereas pulses contain virtually no fat which is of great advantage of lowering the cholesterol levels[40,41]. Thus the results clearly indicated that the sprout samples contain essential phytoconstituents fulfilling the nutrient requirements of human body and it showed, apart from primary phytoconstituents like carbohydrates and proteins, the sprouts are also enriched with secondary phytoconstituents like flavonoids, terpenoids with reduced levels of phytic acid. Quantification studies helps us to know how much amount of bioconstituents are present in the sprout samples. Thus, these are clear evidence indicating that these phytoconstituents might be responsible for the natural antibiotic resistance property of the sprouts.

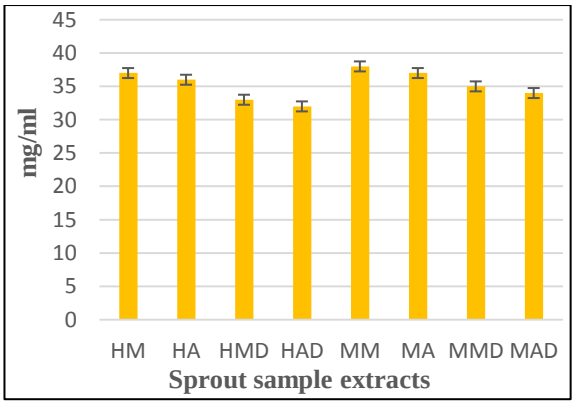

Figure 2: Total protein content of horse gram sprouts and mixed sprouts

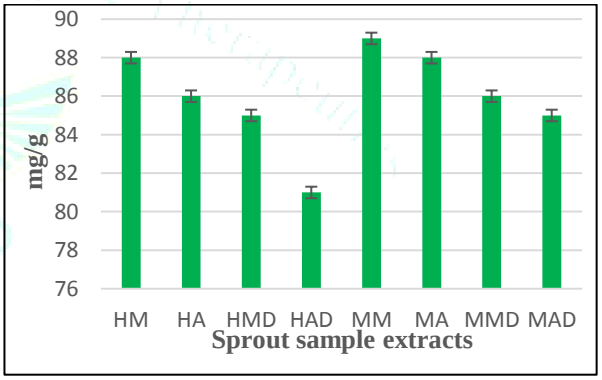

Figure 3: Total terpenoid content of horse gram sprouts and mixed sprouts

Fourier Transform Infrared Spectrophotometer (FTIR) analysis

FTIR analysis of the methanol and aqueous extracts of fresh horse gram sprouts and mixed sprouts showed the presence of essential functional groups (Tables 1-4). The results revealed terpenoids were found in significant amount in fresh and dried horse gram sprouts because of the presence of C-H stretch at 2928.07 cm^{-1} in methanol extract and 2927.1 cm^{-1} in aqueous extract of fresh horse gram sprouts. In the dried horse gram sprouts methanol extract had terpenoids with C-H stretch at 2930 cm^{-1} and in aqueous extract at 2928.07 cm^{-1}. In fresh and dried mixed sprouts, terpenoids were found in significant amount due to 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}.

Table 1: FTIR analysis of methanol extract of fresh horse gram sprouts

Peak Frequencies	Chemical bond	Functional group
434, 530.45, 580.6	C-Br Stretch	Alkyl halides
666.43	C-H Bend	Alkynes
759.02	C-Cl Stretch	Alkyl halides
855.47	C-H out of plane	Aromatics
1033.89, 1156.37, 1244.14	C-F Stretch	Alkyl halides
1410.02, 1414.85	S=O Sulfate ester	Esters
1451.5	CH_2 and CH_3	Alkanes
1540.23	NH out of plane	Amides
1650.17	C=C Stretch	Alkenes
2061.99, 2356.15	P-H Phosphine	Phosphines
2928.07	CH Stretch	Alkanes
3429.58	O-H Stretch	Alcohols

Table 2: FTIR analysis of aqueous extract of fresh horse gram sprouts

Peak Frequencies	Chemical bond	Functional group
422.43, 522.73, 575.78	C-Br Stretch	Alkyl halides
705.01, 993.38	= CH out of plane	Alkenes
761.91	C-Cl Stretch	Alkyl halides
854.5	C-H out of plane	Aromatics
931.66	P-OR Esters	Esters
1161.2	C-F Stretch	Alkyl halides
1375.3, 1455.35	CH_2 and CH_3	Alkanes
1524.79	N-O Stretch	Aromatic nitro compounds
1543.12	NH out of plane	Amides
1654.03	C=C Stretch	Alkanes
2074.53	N=C Stretch	Nitro compounds
2402.44	P-H phosphine sharp	Phosphines
2565.44	S-H (sharp)	Thiols
2927.1	CH Stretch	Alkanes
3040.91, 3065.98	= C-H Stretch	Alkenes
3409.33	O-H Stretch	Alcohols

Table 3: FTIR analysis of methanol extract of fresh mixed sprouts

Peak Frequencies	Chemical bond	Functional group
436.9, 520.8, 531.41, 575.78	C-Br Stretch	Alkyl halides
695.37	= CH out of plane	Alkenes
855.47	C-H out of plane	Aromatics
1007.85, 1147.69, 1245.1	C-F Stretch	Alkyl halides
1448.6	S=O Sulfate ester	Esters
1534.44	NH out of plane	Amides
1662.71	C=C Stretch	Alkenes
2000.27	N=C	Nitro compounds
2272.24	Si-H silane	Silane compounds
2414.98	P-H Phosphine sharp	Phosphines
2928.07	CH Stretch	Alkanes
3436.33, 3452.73	O-H Stretch	Alcohols

Table 4: FTIR analysis of aqueous extract of fresh mixed sprouts

Peak Frequencies	Chemical bond	Functional group
440.75, 582.53	C-Br Stretch	Alkyl halides
694.4	= CH out of plane	Alkenes
852.57	C-H out of plane	Aromatics
1004.96, 1152.52	C-F Stretch	Alkyl halides
1443.78	S=O Sulfate ester	Esters
1531.55	NH out of plane	Amides
1656.92	C=C Stretch	Alkenes
1933.72, 2008.95, 2053.31	N=C	Nitro compounds
2414.02	P-H Phosphine sharp	Phosphines
2924.21	CH Stretch	Alkanes
3436.33, 3499.99	O-H Stretch	Alcohols

In comparison, mixed sprouts had a variety of functional groups than horse gram sprouts. However, terpenoids were found in higher amounts in both the fresh sprout samples. The term 'infrared' refers to the range of the electromagnetic spectrum between 0.78- 1000 mm. It is useful to divide the infrared region into three sections namely, near, mid and far infrared. The most useful infrared region lies between 4000-670 cm^{-1}. FTIR is the measurement of the wavelength and intensity of the absorption of mid-infrared light by the particular sample. Mid-infrared light (2.5-50 µm, 4000- 200 cm^{-1}) is energetic to excite molecular vibrations to higher energy levels. For any molecule to absorb infrared, the vibrations or rotations within a molecule should cause a net change in the dipole moment of the molecule. The wavelength of many IR absorption bands are the characteristic of specific types of chemical bonds and FTIR finds its greatest utility for the qualitative analysis of both organic and organometallic molecules. It is mainly used in the identification of a particular bioactive compound by indicating its functional groups[42]. Thus based on the results, it is proved that the terpenoids were found in larger amounts in both the sprout samples which make the sprouts to possess the natural antibiotic resistance breaking property. The study clearly proved the presence of essential functional groups (stretches) of phytoconstituents in the sprouts which are mainly involved in the metabolic pathways resulting in the antibiotic resistance breaking property.

Antibacterial assay

Methanol extract of fresh horse gram sprouts (HM) showed maximum zone of inhibition of (30 ± 0.4mm) against *Shigella flexneri* followed by *Klebsiella pneumoniae* (28 ± 0.2mm) and *Salmonella typhi* (26 ± 0.3 mm) at 100µg/ml concentration (Fig. 4-6). Methanol extract of fresh mixed sprouts (MM) at 100µg/ml indicated maximum zone of inhibition (30 ± 0.6mm) against *Shigella flexneri* followed by *Klebsiella pneumoniae* (29 ± 0.3mm) and *Salmonella typhi* (25 ± 0.4 mm) (Fig. 7-9). The results revealed that the antibacterial activity of methanol and aqueous extracts of fresh sprout samples showed maximum zone of inhibition at 100 µg/ml concentration against different food borne pathogenic bacteria. In fresh methanol extracts of both the sprout samples, the per cent over control was found to be 10 to 25 per cent (Fig. 10). Similar study was carried out in *Vigna radiata* L. against bacterial pathogens involved in food spoilage and food borne diseases[43]. Our results were found to be superficial as it showed increased zone of inhibition compared to other studies against several human pathogens.

In the natural environment, during germination of the seeds, the enhancement of their defensive responses through phenolics biosynthesis including modified vitamins, enzymes, and receptors takes place[44]. Among the enhanced defensive mechanisms during germination (sprouting), the antimicrobial defenses might be highly involved. However, these defenses were not covered adequately in germinated sprouts in most of medicinal plants yielding seeds. Plant phenolic metabolites are gaining interest due to their potential role in human disease prevention and treatment. In recent years, the use of phytoconstituents as natural antimicrobial agent is commonly called as 'biocides' are gaining popularity[45]. Legumes are considered as staple foods for different classes of people throughout the world. From the present study, the results are clear evidence for the sprouts having a strong natural antibacterial property as the fresh sprouts showed prominent zone of inhibition against pathogenic bacteria and also showed minimum zone of inhibition against *Escherchia coli* which indicated that when these sprouts are consumed it will not affect our intestinal natural microbial flora. These biological properties and the presence of potential secondary metabolites like terpenoids and flavonoids might also be responsible for the natural antibacterial activity of the sprouts.

Figure 4: Zone of inhibition of methanol extract of fresh horse gram sprouts against *Shigella flexneri*

Figure 5: Zone of inhibition of methanol extract of fresh horse gram sprouts against *Klebsiella pneumoniae*

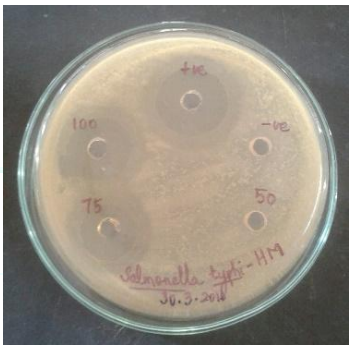

Figure 6: Zone of inhibition of methanol extract of fresh horse gram sprouts against *Salmonella typhi*

Figure 7: Zone of inhibition of methanol extract of fresh mixed sprouts against *Shigella flexneri*

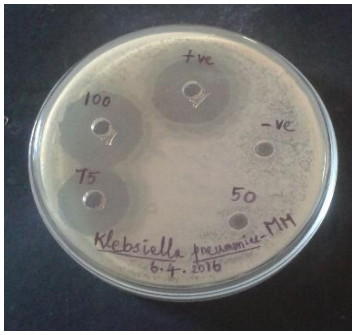

Figure 8: Zone of inhibition of methanol extract of fresh mixed sprouts against *Klebsiella pneumoniae*

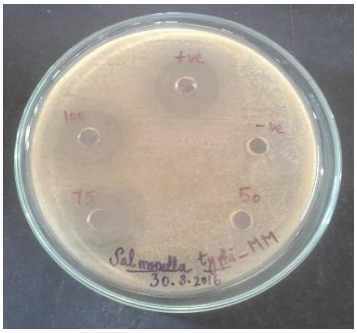

Figure 9: Zone of inhibition of methanol extract of fresh mixed sprouts against *Salmonella typhi*

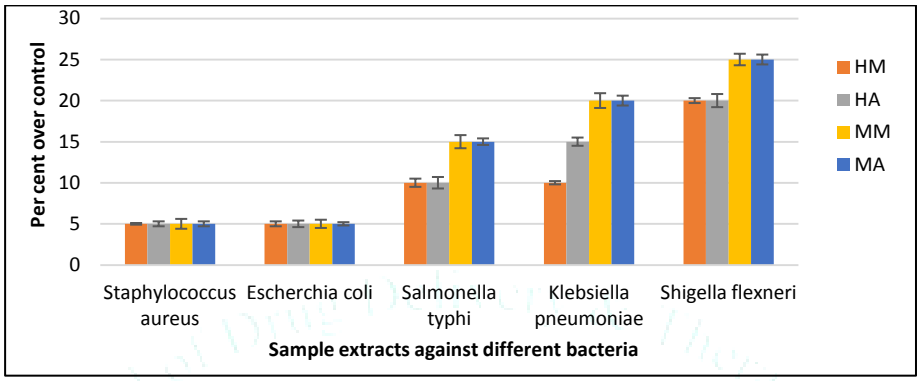

Figure 10: Per cent over control of methanol and aqueous extracts of fresh horse gram sprouts and mixed sprouts at 100 µg/ml concentration against food borne pathogenic bacteria

***In vitro* anti-inflammatory assay**

The anti-inflammatory assay (at different concentrations of 100, 200, 300, 400, 500µg/ml) of methanol and aqueous extracts of both the sprouts, revealed inhibition of thermally induced protein (albumin) denaturation in dose dependant manner. The methanol extract (HM) and aqueous extract (HA) of fresh horse gram sprouts showed per cent maximum inhibition of 83 ± 1.1 and 80 ± 1.2 respectively at 500µg/ml concentration with IC_{50} value of 225.3µg/ml and 247.8µg/ml. The methanol extract (HMD) and aqueous extract (HAD) of dried horse gram sprouts showed per cent maximum inhibition of 79 ± 1.4 and 78 ± 1.6 respectively at 500µg/ml concentration with IC_{50} value of 250.3µg/ml and 269.7µg/ml. The methanol extract (MM) and aqueous extract (MA) of fresh mixed sprouts showed per cent maximum inhibition of 81 ± 1.3 and 80 ± 1.4 respectively at 500µg/ml concentration with IC_{50} value of 239.3µg/ml and 258.8µg/ml. 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 500µg/ml concentration with IC_{50} value of 253.4µg/ml and 272.4µg/ml (Fig. 11). The anti-inflammatory activity of standard diclofenac sodium showed per cent maximum inhibition 90 ± 1.5 at 500µg/ml concentration with IC_{50} value of 125.8µg/ml.

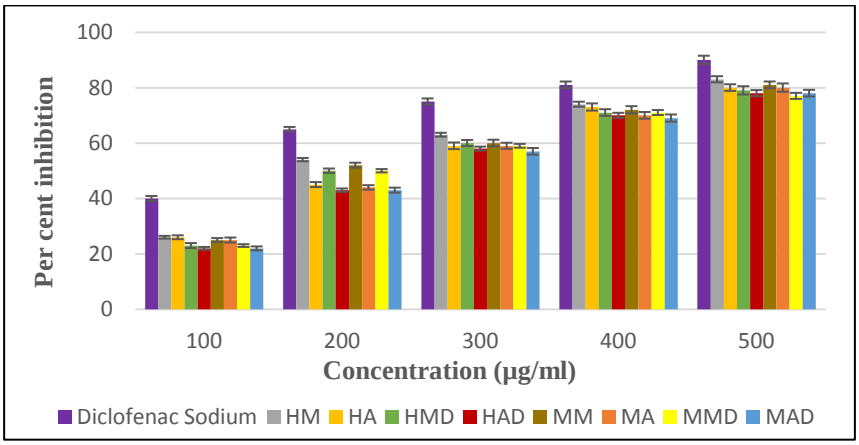

Figure 11: Anti-inflammatory activity of methanol and aqueous extracts of fresh and dried horse gram sprouts and mixed sprouts

Denaturation of proteins is the main cause of inflammation. During denaturation, the proteins loses its complex structure to its simpler form. The results obtained in dose dependant manner are the clear evidence for consumption of the horse gram sprouts and mixed sprouts which could be a potent anti-inflammatory agent which may be due to the presence

of terpenoids and flavonoids. Terpenoids are the hydrocarbons and their originated derivatives which are found in several plants and plant products[46]. Terpenes are natural products whose structure is divided into isoprene units. These are branched chain, 5 carbon units containing 2 unsaturated bonds. The isoprene units arise from acetate via mevalonic acid[47]. Flavonoids include flavone, isoflavone, flavonoids and isoflavonoids which are mostly water-soluble compounds, possess several biological activities, also involved in cell signaling[48-50]. The basic structure of flavonoids is derived from C_{15} body of flavone. They differ from other phenolic substances in the degree of oxidation of their central pyran ring. The variability of the flavonoids is largely based on the hydroxylation or methylation pattern of the three ring systems.

In Asia, legumes especially mung beans have been used in various folk remedies and cuisines to treat toxic poisoning, heat stroke associated with thirst, irritability and fever. These beneficial effects of legumes might be related to the inflammatory response[51]. Apart from the phytoconstituents (terpenoids and flavonoids) in the sprouts, it is also clear that this anti-inflammatory property might also be responsible for making the sprouts one of the potent natural antibiotic resistance breakers.

***In vitro* antioxidant assay**

Methanol extract (HM) and aqueous extract (HA) of fresh horse gram sprouts showed per cent maximum inhibition of 86 ± 1.3 and 84 ± 1.4 respectively at 500µg/ml concentration with IC_{50} value of 180.9µg/ml and 193.3µg/ml whereas the methanol extract (HMD) and aqueous extract (HAD) of dried horse gram sprouts showed per cent maximum inhibition of 84 ± 1.3 and 82 ± 1.4 respectively at 500µg/ml concentration with IC_{50} value of 206.3µg/ml and 219.3µg/ml.

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 500µg/ml concentration with IC_{50} value of 187.8µg/ml and 196.6µg/ml. 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 500µg/ml concentration with IC_{50} value of 211.4µg/ml and 224.3µg/ml (Fig. 12). The scavenging activity of standard ascorbic acid showed per cent maximum inhibition of 90 ± 1.5 at 500µg/ml concentration with IC_{50} value of 149.1µg/ml. Similar study on antioxidant activity of four different pulses namely white bean (*Phaseolus vulgaris*), Common vetch (*Vicia sativa*), Lentil (*Lens culinaris*) and Chickpea (*Cicer arietinum*) seeds before and after germination were investigated. Our results coincide and found to be superficial as all the extracts indicated antioxidant activity in a dose dependant manner.

Various epidemiological studies suggest a correlation between the consumption of foods with a high content of phenolics with decreasing incidence of cardiovascular diseases and cancer[52]. In recent past, pulses have also been studied for their antioxidant properties, because of the increasing interest in the health benefits associated with antioxidants. Moreover, the studies on changes in their antioxidant compounds and antioxidant capacity during processes have also gained interest. Seed sprouts have long been used in the diet as nutrient rich food and recent research shows that, in addition of being a good source of basic nutrients, they also contain important phytochemicals with disease preventive and health promoting properties which are mainly involved in antioxidation[53]. Sprouting causes important changes in the biochemical, nutritional and sensory characteristics of pulse seeds. As a consequence of germination, some anti-nutritional factors decrease or even disappear and bioactive compounds with antioxidant effects increase[54]. Because of increasing interest in the health benefits associated with antioxidants, pulses are studied for their antioxidant properties.

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals which start the chain reactions that cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit the other oxidation reaction by being oxidized themselves. Antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Although oxidation reactions are essential for life, they can also be damaging the cells. Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Insufficient or reduced levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress which damages or kills the cells. As oxidative stress appears to be an important part of many human diseases, the use of antioxidants is intensively studied, particularly as treatments for stroke and neurodegenerative diseases[55,56].

Moreover, the oxidative stress is both the cause and the consequence of the particular disease. The importance and health benefits of legume consumption in the prevention of chronic diseases such as cancer and heart disease have been well documented. This is only because they contain phytochemicals that combat oxidative stress in the human body by helping to maintain a balance between oxidants and antioxidants. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as asthma, arthritis, ischemia, anaemia, inflammation, neuro-degeneration, Parkinson's disease, mongolism, ageing and dementias. Antioxidants are widely used in dietary supplements and also have been investigated for the prevention of diseases such as cancer or coronary heart disease[57-60].

In natural plant derived food products, the electron donation ability can be determined by DPPH bleaching where scavenging of DPPH occurs through the addition of a radical antioxidant which results in the purple decolourization of the DPPH solution. The results obtained in dose dependant manner are promising evidence which indicated that the sprouts possess prominent antioxidant property due to the phytoconstituents like terpenoids and flavonoids which may also be responsible for the antibiotic resistance breaking property. Especially in malnourished populations, these horse gram sprouts and mixed sprouts can be used as an important dietary supplement since it can act as an natural antioxidant and also this antioxidant property might make the sprouts to possess a natural antibiotic resistance breaking property. This protects from several harmful diseases.

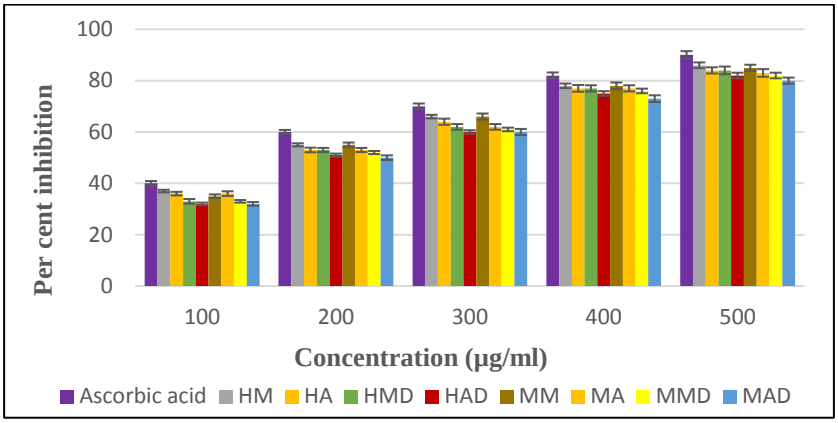

Figure 12: DPPH radical scavenging activity of methanol and aqueous extracts of fresh and dried horse gram sprouts and mixed sprouts

Gas Chromatography Mass Spectrometry (GC-MS) analysis

GC-MS analysis of the methanol and aqueous extracts of fresh horse gram sprouts and mixed sprouts showed the presence of bioactive compounds (Tables 5-6). These bioactive compounds in the extracts were identified using NIST database on comparison with actual mass spectral obtained. The bioactive compounds analysed were mostly of terpenoids, fatty acids, carbohydrates, amino acids and other small functional groups. These compounds are mainly involved in several metabolic pathways resulting in antibacterial, anti-inflammatory and antioxidant properties. Similar study was carried out in *Vicia faba* seeds which showed several compounds which were mostly of fatty acids and few other phytochemicals[61]. Our results were found to coincide and also superficial as it showed both primary and secondary phytoconstituents apart from fatty acids which are of potent use to human health.

GC-MS is a combination of two different analytical techniques, Gas Chromatography and and Mass Spectrometry which is mainly used to analyze the complex organic and biochemical mixtures. It is particularly specific that GC-MS is often referred to as the 'molecular fingerprint'. GC can separate the volatile and semi-volatile compounds with great resolution, but it cannot identify them whereas MS can provide detailed structural information on most of the bioactive compounds such that they can be exactly identified, but it cannot readily separate them. Thus GC-MS is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify the different substances within a test sample. The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility by flowing an inert gas (mobile phase), which carries the sample, through a particular stationary phase fixed in the column. Spectra of the compounds are collected as they exit through chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed[62].

Table 5: GC-MS analysis of methanol extract of fresh horse gram sprouts

Compound Name	Retention time	Area per cent	Height per cent
Diglycerol	5.558	1.72	1.05
DL-Proline	6.892	0.37	0.70
DL-Phenylalanine	7.442	1.82	2.30
1,3-Propanediol	7.705	1.89	2.04
Quinoline	10.877	0.38	0.71
Vinyl caprylate	11.479	11.69	7.48
Beta-D-mannofuranoside	11.583	6.05	7.57
Myo-inositol	14.250	0.54	1.09
Isopropyl myristate	14.880	0.18	0.46
Oxirane	15.368	0.18	0.42
Ascorbic acid	18.424	21.62	27.56
n-Nonadecanol-1	19.186	0.80	1.62
Cis-Vaccenic acid	23.172	13.26	12.94
1-Heptacosanol	24.600	0.18	0.40
Gamma-Linolenic acid, methyl ester	26.830	0.37	0.67
Glycerol tricaprylate	42.775	0.26	0.49
Stigmasterol	43.183	2.07	2.17
Geranyl geraniol	43.656	0.91	2.63
Gamma-sitosterol	44.666	10.48	11.35
Fumaric acid	45.225	7.14	4.41
Gamma-tocopherol	46.046	18.08	11.94
		100.00	100.00

GC-MS has a wide range of applications which include petrochemical and hydrocarbons analysis, geochemical research, forensics, environmental and pesticide analysis, food safety, pharmaceutical and drug analysis, clinical toxicology[63]. GC-MS in the food sector are among the fastest developing fields in science and industry. The need for accurate molecular characterization of the food, demanded both by consumers and regulatory agencies, is leading the food industry to apply advanced techniques like GC-MS for detailed analytical assessment of several food commodities. These also shifts the food industry towards the direction of the pharmaceutical sector, with respect to analytical chemistry and also several other aspects. Foods are complex mixtures of different bioactive components in varying amounts, making analysis a challenging task. GC-MS is in the forefront of this analytical challenge being a unique technique for reliable characterization of complex mixtures. Its excellent feature of the combination of the separative power of gas chromatography to the power of mass spectrometry to identify molecular structure, it became possible to characterize any food substance at the molecular level. Desirable and undesirable molecules are often routinely identified and quantified in various foods. GC–MS is useful analytical method which allows simultaneous assessment of a variety of bioactive components in complex mixtures such as foods[64].

Table 6: GC-MS analysis of methanol extract of fresh mixed sprouts

Compound Name	Retention time	Area per cent	Height per cent
Dodecanoic acid	9.425	1.24	2.34
Alpha-D-Glucopyranoside	10.551	4.75	8.48
Myo-inositol	11.283	12.28	5.67
Vinyl caprylate	11.475	13.41	11.73
Myo-inositol	11.561	16.80	12.09
Methyl mannose	14.093	2.41	5.26
Cis-vaccenic acid	22.993	10.15	13.09
Geranyl geraniol	23.746	2.56	4.34
Gamma-sitosterol	44.658	8.72	9.72
Gamma-tocopherol	45.290	27.68	27.28
		100.00	100.00

Thus the present study is strong evidence and it is well documented that the horse gram sprouts and mixed sprouts have a wide variety of bioactive compounds with antibacterial, anti-inflammatory and antioxidant activities. These bioactive compounds which include terpenoids, fatty acids, carbohydrates, amino acids and other small functional groups are involved in the metabolic pathways of breaking down of the antibiotic resistance. Thus these sprouts enriched with these potent bioactive compounds can act as natural dietary supplements against the prevention of Shigellosis.

Identification of natural antibiotic resistance breakers through *in silico* docking studies

Maximum zone of inhibition in antibacterial assay was shown by *Shigella flexneri*, the same was taken for *in silico* analysis for the identification of natural antibiotic resistance breakers. *Shigella flexneri* is a food and water borne pathogen leading to outbreaks of Shigellosis, a major public health concern by causing diarrhea and dysentery. Ciprofloxacin is a broad spectrum of antimicrobial carboxyfluoroquinolones used to treat diarrhea. The bactericidal action of Ciprofloxacin is by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV, which are required for bacterial DNA replication. In DNA gyrase, the antibiotic resistance occurs through mutations in the Quinolone Resistance-Determining Region.

The bioactive compounds from the GC-MS analysis were screened against the target molecules (normal *Shigella flexneri* and Ciprofloxacin resistance (Quinolone Resistance Determining Region of *Shigella flexneri*). DL-Proline, a free amino acid from fresh horse gram sprouts and Geranyl geraniol, a diterpene from fresh mixed sprouts showed prominent binding affinity against normal *Shigella flexneri* and with Ciprofloxacin resistant *Shigella flexneri* (QR-DR). More negative values are indication of higher binding affinity which clearly proves the natural antibiotic resistance property. Docking analysis of DL-Proline from HM showed docking scores of -4.7, -4.5, -4.2, -4.1 against normal *Shigella flexneri* and -6.9, -6.6, -5.9, -5.8 against Ciprofloxacin resistant *Shigella flexneri* (QR-DR) (Fig. 13-14). Docking analysis of Geranyl geraniol from MM showed docking scores of -4.6, -4.4, -4.3, -4.1 against *Shigella flexneri* and -6.9, -6.8, -5.8, -5.7 against Ciprofloxacin resistant *Shigella flexneri* (QR-DR) (Fig. 15-16).

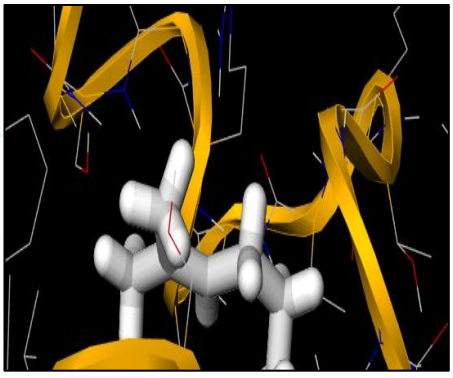

Figure 13: DL-Proline from methanol extract of fresh horse gram sprouts docked with normal *Shigella flexneri*

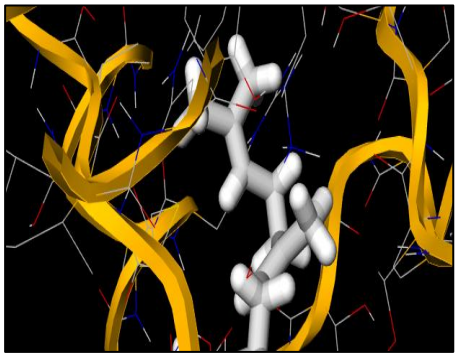

Figure 14: DL-Proline from methanol extract of fresh horse gram sprouts docked with Ciprofloxacin resistant *Shigella flexneri* (QR-DR)

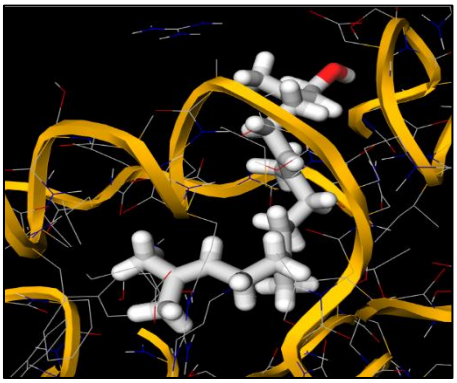

Figure 15: Geranyl geraniol from methanol extract of fresh mixed sprouts docked with normal *Shigella flexneri*

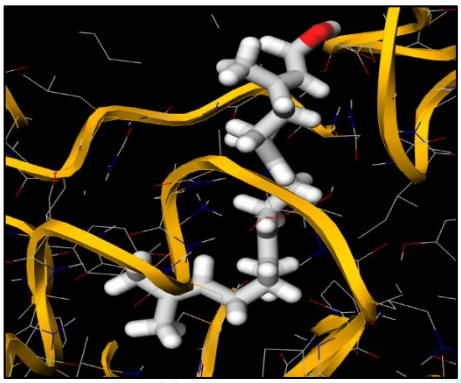

Figure 16: Geranyl geraniol from methanol extract of fresh mixed sprouts docked with Ciprofloxacin resistant *Shigella flexneri* (QR-DR)

The emergence of antibiotic resistance among the most important bacterial pathogens is recognized as a major public health threat affecting humans worldwide. Understanding the genetic and biochemical basis of resistance is of great importance to design the strategies to inhibit the emergence and spread of resistance; to devise several innovative therapeutic approaches against multidrug-resistant organisms. It is highly important to recognize that the concept of antimicrobial resistance or susceptibility in clinical practice is a relative phenomenon with many layers of complexity. The establishment of the clinical susceptibility breakpoints (susceptible, intermediate and resistant) mainly relies on the *in vitro* activity of an antibiotic against a sizeable bacterial sample combined with some pharmacological parameters (e.g., blood and infection site concentrations of the antimicrobial). Thus, during the treatment of antibiotic-resistant bacteria, the interpretation of susceptibility patterns may vary according to the clinical scenario and the availability of treatment options[65]. Therefore *in silico* methods can be carried out initially to study the antibiotic resistance and also to overcome the complexity. After attaining the complete information about the organism, *in vitro* activites can be performed which helps to get accurate results.

Molecular docking is the computational modeling of the structure of various complexes formed by two or more interacting molecules. Docking is a method which predicts the preferred orientation of one molecule to another when bound to each other to form a stable complex[66]. Knowledge of the preferred orientation is used to predict the binding affinity between two molecules using the scoring functions. The affinity between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, lipids or any other potent molecules plays an important role in signal transduction. Furthermore, the relative orientation of the two interacting molecules may affect the type of signal produced (e.g. agonism or antagonism). Therefore *in silico* docking is beneficial for predicting both the strength and type of the signal produced. *In silico* docking is mainly used to predict the binding orientation of the ligand (drug) to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays a key role in the designing of drugs[67].

In the last few years, a vast amount of work has been directed for developing efficient docking methods and scoring functions as tools for the identification of ligands. Considerable progress has been made in the computational prediction of ligand and target binding modes. There are two approaches which are particularly popular within the molecular docking. One approach uses the matching technique that describes the target and the ligand as complementary surfaces[68,69]. The second approach simulates the docking process in which the ligand-target pair wise interaction energies are calculated[70]. Molecular docking is divided into rigid docking, flexible docking and full flexible docking strategies. In the rigid docking, the interacting molecules are considered as rigid bodies without any conformational changes as they interact with each other, however, in the flexible docking, the conformational flexibility of the interacting molecules upon the protein association is considered as an essential factor. In the rigid docking, the total conformational space is mainly represented by six variables, which consists of relative translational vectors (x, y, z) and rotational angles (φ, θ, ψ) of the target protein components. Whereas in the flexible docking, torsional angle changes of each protein molecule are added to the particular conformational space. In the full flexible docking, the ligand is flexed via its torsion angles as well as the side chains of active sites residues[71].

The bioactive lead compounds, DL-Proline and geranyl geraniol from the sprout samples docked against the target molecule using rigid docking method showed high binding affinity. Thus the docking results have proved that the horse gram sprouts and mixed sprouts with the potential of primary and secondary phytoconstituents possess natural antibiotic resistance breaking property. Thus these sprouts with enriched therapeutic phytoconstituents can be recommended as a natural edible product for the management of Shigellosis.

CONCLUSION

Based on the bioassays and *in silico* docking, the horse gram sprouts (HM) and mixed sprouts (MM) with phytoconstituents such as proteins, carbohydrates, terpenoids and flavonoids possess strong antibacterial, anti-inflammatory and antioxidant activities which might be responsible for acting as a natural antibiotic resistance breakers. GC-MS and docking studies confirmed the presence of lead compounds, DL-Proline, a free amino acid from fresh horse gram sprouts and Geranyl geraniol, a diterpene from fresh mixed sprouts (showing a strong specific binding affinity against *Shigella flexneri* and Ciprofloxacin resistant *Shigella flexneri*- QR-DR). Thus, the functional foods like horse gram sprouts and mixed sprouts enriched with the phytoconstituents are the natural source of antibiotic resistance breakers, one of the most economical and easily available source for consumption can be recommended as a value based dietary supplement for the prevention of Shigellosis.

CONFLICTS OF INTERESTS

The authors declared that they had no conflicts of interests.

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REFERENCES

1. Diemert DJ, Prevention and self-treatment of traveler's diarrhea, Clinical Microbiology Reviews, 2006; 19(3):583-594.
2. Vijaya Ramesh K. Food microbiology. MJP Publishers; 2007. P. 438-441.
3. Mensa L, Marco F, Vila J, Gascon J, Ruiz J, Quinolone resistance among *Shigella* spp. isolated from travellers returning from India, Clinical Microbiology and Infection, 2008; 14(3):279-281.
4. Vashist J, Vishvanath, Kapoor R, Kapil A, Yennamalli R, Subbarao N, MR R, Interaction of nalidixic acid and ciprofloxacin with wild type and mutated quinoloneresistance-determining region of DNA gyrase A, Indian Journal of Biochemistry and Biophysics 2009; 46(2):147-153.
5. Jeon YL, Nam Y, Lim G, Cho SY, Kim YT, Jang JH, Kim J, Park M, Lee HJ, Quinolone-resistant *Shigella flexneri* isolated in a patient who travelled to India, Annals of laboratory medicine, 2012; 32(5):366-369.
6. Kampranis SC, Maxwell A, The DNA gyrase-quinolone Complex: ATP hydrolysis and the mechanism of DNA cleavage. Journal of Biological Chemistry, 1998; 273(35):22615-26.
7. Piton J, Petrella S, Delarue M, Andre-Leroux G, Jarlier V, Aubry A, Mayer C, Structural insights into the quinolone resistance mechanism of *Mycobacterium tuberculosis* DNA gyrase, PLoS ONE, 2010; 5(8):122-145.
8. Reece RJ, Maxwell A, Wang JC, DNA Gyrase: Structure and function, Critical Reviews in Biochemistry and Molecular Biology, 1991; 26(3-4):335-375.
9. Heddle J, Maxwell A, Quinolone-binding pocket of DNA gyrase: Role of GyrB. Antimicrobial Agents and Chemotherapy, 2002; 46(6):1805-1815.
10. Ruiz J, Mechanisms of resistance to quinolones: Target alterations, decreased accumulation and DNA gyrase protection, Journal of Antimicrobial Chemotherapy 2003; 51(5):1109-1117.
11. Lowy FD, Antimicrobial resistance: The example of *Staphylococcus aureus*, The Journal of Clinical Investigation, 2003; 111(9):1265-1273.
12. Raquel PF Guine, Maria Joao R Lima, Maria Joao C Barroca, Role and health benefits of different functional food components, International Journal of Medical and Biological Frontiers, 2010; 17(9):02-31.
13. Antigone Kouris-Blazos, Regina Belski, Health benefits of legumes and pulses with a focus on australian sweet lupin, Asia Pacific Journal of Clinical Nutrition, 2016; 25(1).
14. Rao BSN, Prabhavathi T, Tannin content of foods commonly consumed in India and its influence on ionisable iron, Journal of the Science of Food and Agriculture, 1982; 33(1):89-96.
15. Hussein L, Ghanem KZ, Calcium bioavailability from selected Egyptian foods with emphasis on the impact of germination and fermentation, International Journal of Food Sciences and Nutrition, 1999; 50(5):351-356.
16. Ghavidel RA, Prakash J, The impact of germination and dehulling on nutrients, antinutrients *in vitro* iron and calcium bioavailability and *in vitro* starch and protein digestibility of some legume seeds, LWT, 2007; 40(7):1292-1299.
17. Reddy NR, Salunkhe DK, Sathe SK, Biochemistry of black gram, CRC Critical Reviews in Food Science and Nutrition, 2005; 16:49-114.
18. Ali Esmail Al-Snafi, The medical importance of *Cicer arietinum* - A review. IOSR Journal of Pharmacy, 2016; 6(3):29-40.
19. Paul T, Rubel-Mazumdar NHM, Sayed MA, Akhataruzamman M, Proximate composition, mineral content and determination of protease activity from green gram, Bangladesh Research Publication Journal, 2011; 5(3):207-213.
20. Kruawan K, Tongyonk L, Kangsadalampai K, Antimutagenic and comutagenic activities of some legume seeds and their seed coats. Journal of Medicinal Plants Research, 2012; 6(22):3845-3851.
21. Lin LH, Li WZ, The research of mung bean SOD oral liquid, Food Science, 1997; 18:25-26.
22. Murugkar DA, Gulati P, Gupta C, Effect of sprouting on physical properties and functional and nutritional components of multi-nutrient mixes, International Journal of Food and Nutritional Sciences, 2013; 2(2):8-15.
23. Khyade VB, Jagtap SG, Sprouting exert significant influence on the antioxidant activity in selected pulses (Black gram, cowpea, desi chickpea and yellow mustard), World Scientific News, 2016; 35:73-86.
24. Eloff JN, Which extractant should be used for the screening and isolation of antimicrobial components from plants?, Journal of Ethnopharmacology, 1998; 60:1-8.
25. Sofowora AO. Medicinal plants and traditional medicine in Africa. Sunshine house, Nigeria: Spectrum books Ltd., Screening plants for Bioactive agents. 1993. P. 134-156.
26. Harborne AJ. Phytochemical Methods- A Guide to Modern Techniques of Plant Analysis. Thomas Science Publications; 1998.
27. Miller GL. Use of dinitosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 1972; 31:426-428.
28. Nelson N, A photometric adaptation of the Somogyi method for the determination of glucose, The Journal of Biological Chemistry, 1944; 153:375-380.
29. Lowry OH, Rosebrough NJ, Fan AL, Randall RJ, Protein measurement with the Folin-phenol reagent. The Journal of Biological Chemistry, 1951; 193:265-275.
30. Patel S, Patel J, Patel RK, To study proximate analysis and biological evaluation of Triphala Guggulu formulation. International Journal of PharmTech Research 2012; 4(4):1520-1526.
31. Ferguson N. A Textbook of Pharmacognosy. Max Millam Company; 1956. P. 191.
32. Wheeler EL, Ferrel RE, A method for phytic acid determination in wheat and wheat fractions. Cereal Chemistry 1971; 48:312-320.
33. Mizushima Y, Kobayashi M, Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. Journal of Pharmacy and Pharmacology, 1968; 20:169-173.
34. Mansoor Ahmad, Farah Saeed, Mehjabeen, Noor Jahan, Evaluation of insecticidal and antioxidant activity of selected medicinal plants, Journal of Pharmacognosy and Phytochemistry, 2013; 2(3):153-158.
35. Adams RP. Identification of essential oils by ion trap mass spectroscopy. Academic Press: New York; 1989.
36. Syed A, Fatima N, *In silico* analysis and molecular docking studies of potential angiotensin converting enzyme inhibitor using quercetin glycosides, Pharmacognosy, 2015; 11(1):123-126.
37. Marimuthu M, Gurumoorthi P, Phytochemical screening and FT-IR studies on wild and common south Indian legumes, Asian Journal of Pharmaceutical and Clinical Research 2013; 6(2):141-144.
38. Louise RB, Susan NK, Tropical grain legumes. In: Inglett GE, Charalambous G (Eds), Proceedings of an International Conference on Tropical Foods: Chemistry and Nutrition held in Honolulu, Hawaii. 1979; P. 547-574.
39. Arora M, Singh S, Kaur R, Phytochemical analysis, protein content and antimicrobial activities of selected samples of *Glycine max* Linn., International Journal of Research in Engineering and Technology, 2013; 2(11):570-574.
40. Amal BE, Aurang Z, Nizakat B, Impact of germination time and type of illumination on carotenoid content, protein solubility and *in vitro* protein digestibility of chickpea (*Cicer arietinum* L.) sprouts, Food Chemistry, 2008; 109:797-801.
41. Agte V, Tarwadi K, The importance of nutrition in the prevention of ocular disease with special reference to cataract, Ophthalmic Research, 2010; 44:166-172.
42. Raaman N. Phytochemical Analysis. New India Publishing Agency; 2006.

43. Priya AJP, Sudhalakshmi GY, Banu F, Gopalakrishnan S, Dhanalakshmi P, Sagadevan E, Manimaran A, Arumugam P, Phytochemical screening and antibacterial activity of *Vigna radiata* L. against bacterial pathogens involved in food spoilage and food borne diseases. Journal of Academia and Industrial Research, 2012; 1(6):355-359.
44. Randhir R, Shetty K, Mung bean processed by solid-state bioconversion improves phenolic content and functionality relevant for diabetes and ulcer management, Innovative Food Science and Emerging Technologies, 2007; 8:197-204.
45. Smid EJ, Gorris LGM. Natural antimicrobials for food preservation. In: Rahman, M.S. (Ed.), Handbook of Food Preservation. Marcel Dekker, New York; 1999. P. 285-308.
46. Raje VN. Pharmacognosy. CBS Publishers and Distributors; 2010.
47. Arumugam KR, Murugesh N. Text book of Pharmacognosy. Sathya Publishers; 1998.
48. Prokudina E, Havlicek L, Al-Maharik N, Lapcik O, Strnad M, Gruz J, Rapid UPLC-ESI-MS/MS method for the analysis of isoflavonoids and other phenylpropanoids. Journal of Food Composition and Analysis, 2012; 26:36-42.
49. Wang M, Gillaspie A, Morris J, Pittman R, Davis J, Pederson G, Flavonoid content in different legume germplasm seeds quantified by HPLC, Plant Genetic Resources: Characterisation and Utilisation, 2008; 6:62-69.
50. Koes RE, Quattrocchio F, Mol JNM, The flavonoid biosynthetic pathway in plants: Function and evolution, BioEssays, 1994; 16(2):123-32.
51. Lee SJ, Lee JH, Lee HH, Lee S, Kim SH, Chun T, Imm JY, Effect of mung bean ethanol extract on pro-inflammatory cytokines in LPS stimulated macrophages. The Food Science and Biotechnology, 2011; 20(2):519-24.
52. Fernandez-Orozco R, Frias J, Zielinski H, Munoz M, Piskula MK, Kozlowska H, Vidal-Valverde C, Evaluation of bioprocesses to improve the antioxidant properties of chickpeas. Food Research Technology, 2009; 42:885-892.
53. Fernandez-Orozco R, Piskula MK, Zielinski H, Kozlowska H, Frias J, Vidal-Valverde C. Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var Zapaton, European Food Research Technology, 2006; 223:495-502.
54. Doblado R, Frias J, Vidal-Valverde C, Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea (*Vigna sinensis* var. Carilla) seeds induced by high pressure treatment, Food Chemistry, 2007; 101:918-923.
55. Carlo R, Riccardo A, Sridhar D, Petriziz P, Carla M, Raffaela T, Florence B, Bilal C, Giovanni, Metabolic engineering of β-carotene and lycopene content in tomato fruit, The Plant Journal, 2000; 24(3):413-419.
56. Bibi N, Aurang Z, Amal BK, Mohammad SK, Effect of germination time and type of illumination on proximate composition of chickpea seed (*Cicer arietinum* L.). American Journal of Food Technology, 2008; 3:24-32.
57. Mangels AR, Holden JM, Beecher GR, Forman M, Lanza E, Carotenoid content of fruits and vegetables: An evaluation of analytic data, Journal of the American Dietetic Association, 1993; 93:284-296.
58. Nishino H, Cancer prevention by carotenoids, Mutation Research, 1998; 402:159-163.
59. Mao JJ, Dong JF, Zhu MY. Effect of germination conditions on ascorbic acid level and yield of soybean sprout, Journal of the Science of Food and Agriculture, 2005; 85:943-947.
60. Jian Zhao, Nutraceuticals, nutritional therapy, phytonutrients and phytotherapy for improvement of human health: A perspective on plant biotechnology application. Recent Patents on Biotechnology, 2007; 1:75-97.
61. Pasricha V, Satpathy G, Gupta RK, Phytochemical and antioxidant activity of underutilized legume *Vicia faba* seeds and formulation of its fortified biscuits, Journal of Pharmacognosy and Phytochemistry, 2014; 3(2):75-80.
62. Skoog A, Holler EJ, Crouch SR. Principles of instrumental analysis; 2007.
63. Sahil K, Prashant B, Akanksha M, Premjeet S, Devashish R, Gas Chromatography-Mass Spectrometry: Applications. International Journal of Pharmaceutical and Biological Archives, 2011; 2(6):1544-1560.
64. Hussain SZ, Maqbool K, GC-MS: Principle, technique and its application in food science. International Journal of Current Science, 2014; 13:116-126.
65. Munita JM, Arias CA, Mechanisms of antibiotic resistance, Microbiol Spectrum, 2016; 4(2):1-40.
66. Lengauer T, Rarey M, Computational methods for biomolecular docking, Current Opinion in Structural Biology, 1996; 6(3):402-406.
67. Kitchen DB, Decornez H, Furr JR, Bajorath J, Docking and scoring in virtual screening for drug discovery: Methods and applications, Nature reviews drug discovery, 2004; 3(11): 935-949.
68. Meng EC, Shoichet BK, Kuntz ID, Automated docking with grid-based energy evaluation, Journal of Computational Chemistry, 2004; 13(4):505-524.
69. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, Journal of Computational Chemistry, 1998; 19(14):1639-1662.
70. Feig M, Onufriev A, Lee MS, Im W, Case DA, Brooks CL, Performance comparison of generalized born and poisson methods in the calculation of electrostatic solvation energies for protein structures, Journal of Computational Chemistry, 2004; 25(2):265-284.
71. Mukesh B, Rakesh K, Molecular docking, International Journal of Research in Ayurveda and Pharmacy, 2011; 2:1746-1751.