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Determination of Antioxidant Activity and Phytochemical Compounds in Natural Flavor Enhancer

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Abstract

Flavor enhancer plays a major role in many cuisines. Development of flavor enhancer using natural ingredients may bring vast benefits to the humans' nutrition. This study was carried out to examine the potentiality of antioxidant activity, phenolic content and bioactive components of newly developed flavor enhancer. The methanol extracts of three samples of flavor enhancer had significant DPPH activity (68.442 ± 0.20693 mg Gallic Acid Equivalent/100g) and Total phenolic content (137.646 ± 0.577 mg Gallic Acid Equivalent /100g). The bioactive components of newly developed flavor enhancer have been evaluated using GC/MS analysis carried out in three different extracts via Acetone, Methanol and Chloroform which revealed the existence of various phytocomponents. The study also highlighted the presence of some known biologically active phyto components like Antimicrobial compounds, antioxidant compounds, anti-inflammatory compounds, anti cancer and antitumor compound. Hence present study showed the medicinal potentiality of this newly developed natural flavor enhancer.

Keywords: Natural flavor enhancer, Antioxidant activity, Phenolic content, Gas chromatography–Mass Spectroscopy analysis, Bio-active compounds.

Introduction

Flavour enhancer (potentiator) is a substance that enhances the flavours of other substances without giving any characteristic flavour of itself to the food, which is the definition according to Food and Nutrition dictionary by Oxford University. Today nutritionally rich natural flavor enhancers are rarely available in the market. The subjected product for the study is nutritionally rich flavor enhancer which has made using naturally available raw materials.

The raw materials were American oyster mushroom (*Pleurotus ostreatus*), tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum L.*) and garlic (*Allium sativum L.*) Recent studies have shown that, plant contain different types of natural antioxidant compounds, for instance vitamin C, tocopherol, carotenoids, polyphenolics and flavonoids which can prevent damaging to the cell by free radicals[1]. Free radicals are caused to some appalling consequences such as oxidation of protein, damaging to DNA, peroxidation of lipids in living tissues and cells[2,3]. This oxidative stress may possibly lead to cancer, atherosclerosis, diabetes and liver cirrhosis like diseases[4-6].

Therefore, the research was conducted to scrutinize the antioxidant activity, total phenolic content and phytochemical analysis of newly developed natural flavor enhancer using DPPH assay, Folin-Ciocalteu assay and GC-MS analysis respectively.

Materials and Methods

Preparation of flavor enhancer: The raw materials for the preparation of flavor enhancer were american oyster mushroom (*Pleurotus ostreatus*), tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum L.*) and garlic (*Allium sativum L.*) and salt powder. Mushrooms were blanched at 70^{0}C for 15 seconds and blanched mushroom were dried at drying oven at 60^{0}C for 10hours. Tomatoes were blanched at 90^{0}C water for 1minute and dried at drying oven at 60^{0}C for 12hours. Potatoes were blanched in 90^{0}C water for 5minutes and dried at drying oven at 60^{0}C for 10hours. Garlic was dried at drying oven at 60^{0}C for 8hours without prior blanching. Then all the ingredients were ground and sieved (by 200 µm mesh sieve) to obtain powders. Mushroom powder, tomato powder, potato powder, garlic powder and salt were mixed according to the following formula

Ingredient	(W/W %)
Mushroom powder	29.6
Tomato powder	14.8
Potato powder	29.6
Garlic powder	14.8
Salt	11.2

Extraction of flavor enhancer for TPC assay and DPPH assay: About 5g of sample was added to 50 ml of hot 70% methanol and mixed well using a vertex mixture. Then sample was placed in a water bath at 70°C for 10 minutes for the extraction in to methanol. Sample was cooled to the room

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temperature (30°C) and centrifuged at 3500 rpm and decanted the supernatant. Extraction was repeated twice following the same procedure. All extractions were mixed well and dilute up to the required level using 70% methanol.

Determination of TPC: To determine the total phenolic content, Slinkard et al. method[8] was used with some changes. The test was carried out in triplicate for methanol extracts of sample. Initially, Folin–Ciocalteu reagent was diluted 10-fold with deionised water. Then 0.5 ml of 70% methanolic extract was added to 2.5 ml of the prepared Folin–Ciocalteu reagent. Then it was incubated for 3 min at room temperature. Exactly 5.0 ml of 7.5% (w/v) sodium carbonate solution was added to the mixture and mixed well using vertex mixture. Mixture was incubated for 10 minutes in the dark at 45^{0}C water Brand Williams et al. spectrophotometric method[9] was used to examine the antioxidant activity of flavor enhancer. DPPH (2,2 diphenyl-2-picryl hydrazyl hydrate) (Sigma-Aldrich, USA) was freshly prepared for each bath. Finally the absorbance was determined using an UV–Visible spectrophotometer (UV mini 1240, shimadzu) at 765 nm. Deionised water was used as a blank sample. Calibration curve was plotted with a series of gallic acid standards (5, 10, 15 and 20 mg/L) and TPC value was determined using the curve. Results are expressed as mg of gallic acid equivalents/g of flavor enhancer (mg GAE/g).

Determination of antioxidant activity: Experiment. Exactly 2.5 ml of 6.5×10^{-5} M DPPH solution was mixed with 0.5 ml of methanol extract and incubated for 30 minutes in the dark environment. Antioxidant activity was measured at 517 nm using UV–Vis spectrophotometer (UV mini 1240 - SHIMADZU) and standard Gallic acid curve was plotted. The results were expressed as mg Gallic acid equivalent /g of flavor enhancer (mg GA/ g).

Statistical analysis: Each experiment was carried out in triplicate and the average values and standard deviations were calculated. The data were analyzed using MINITAB 14 version, regression analysis.

Extraction of the flavor enhancer for phytochemical analysis: Flavor enhance sample was soaked in 95% methanol for 12 h to extract the phytochemicals in to methanol. After that extract was filtered through anhydrous sodium sulphate bedded Whatmann filter paper No. 42. Then the filtered sample was filled into the vial. The same procedure was followed for Chloroform and Acetone solutions for the extracting procedure.

GC analysis: Gas chromatography technique was used to identify the phytochemical compounds. Gas Chromatograph (GC) (7890 A), Agilent technologies Mass Spectrometer (MS) (5975 C) inert XL EI/CI MSD with triple-axis detector and polar capillary column RTX-5, 0.32 mm internal diameter, 30 mm length and 0.25µm film thickness (Restex Corp., Bellefonte, PA, USA). Carrier gas was Helium (99.999%) under the 1ml/min of constant flow rate. 2µl injection volume (split ratio of 10:1), 80°C of injector temperature and 250°C ion-source temperature was employed for the test. Initial oven temperature was 110°C (it was isothermal for 2 min.) and it was increased with the rate of 10°C/min, to 200°C, then 5°C/min rate to 250°C, finally ends up with 9min isothermal at 280°C. Mass spectra were taken at 0.5 seconds of scan interval and fragments from 45 to 450 Da at 70 eV. The relative retention time and mass spectra was used to identify the compounds.

Identification of components: Identification of compound was carried out according the Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. The interpretation of predicted compound was done by using the spectrum database of NIST library.

Results and Discussion

According to the results, average antioxidant activity was 68.442 ± 0.2 [illegible] mg/100g. Here standard compound was Gallic acid and antioxidant potentials were expressed as mg/100g gallic acid equivalent. Plotted standard curve equation is y = 0.1893543 x + 1.067452 and R^2 = 0.9934. Where: y is absorbance [illegible]

Several [illegible] antioxidants [illegible] Oxidative st[illegible] to cells, cancer cell generating and aging of brain cells[10-12]. Because of the ability of scavenging the free radicals, antioxidant [illegible] health [illegible]

Total phe[illegible] GAE [illegible] total phenol [illegible] Plotted st[illegible] R^2 = 0.99[illegible] phenolic co[illegible] expressed [illegible] nutritional value and quality of food as well as the colour, taste, aroma and flavor. Not only that, it provides several health beneficial effects also[14].

Many diseases like cancer, cardiovascular diseases, cataracts, atherosclerosis, hepatitis, diabetes, asthma, liver injury, immune deficiency diseases arthritis and ageing are arisen because of oxidative damage which can be reduced by phenolic compounds[15-17].

Biologically [illegible] responsible for a variety of activities like antioxidant, anti-inflammatory, antimicrobial, antifungal, and anticancer[18,19]. Gas chromatography-mass spectroscopy analysis was carried out for methanol [illegible] extract and acetone extract of the flavor enhancer. Output of the GC-MS study; retention time, compound and specific bioactivities were summarized in the tables..

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Table-1
GC/MS results and the compounds of the Methanolic extract of product

Retention time	Name of the compound(Prior	Bioactivity
2.972	2-Furanmethanol	Antiviral
7.776	Benzeneacetaldehyde	Antioxidant, Anti-inflammatory
10.278	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	Antimicrobial, Anti-inflammatory
11.910	2-Furancarboxaldehyde, 5-(hydroxymethyl)	Antimicrobial
20.506	Hexadecanoic acid, methyl ester	Flavor, Antioxidant
22.541	9,12,-Octadecanoic acid(Z,Z)	Antioxidant, Anticancer

Table-2
GC/MS results and the compounds of the Acetone extract of product

Retention time	Name of the compound	Bioactivity
15.967	Phenol, 2,4-bis(1,1-dimethylethyl)	Antifungal, Antioxidant
16.890	1-hexadecene	Antibacterial, Antifungal, Antioxidant
19.134	1-Octadecene	Antibacterial, Antioxidant, Anticancer
20.881	n-Hexadecanoic acid	Flavor, Antioxidant
21.155	1-Nonadecene	Antifungal, Anticancer
24.495	dl-alpha-Tocopherol	Antioxidant
27.632	9,17-Octadecadienal,(Z)	Antimicrobial

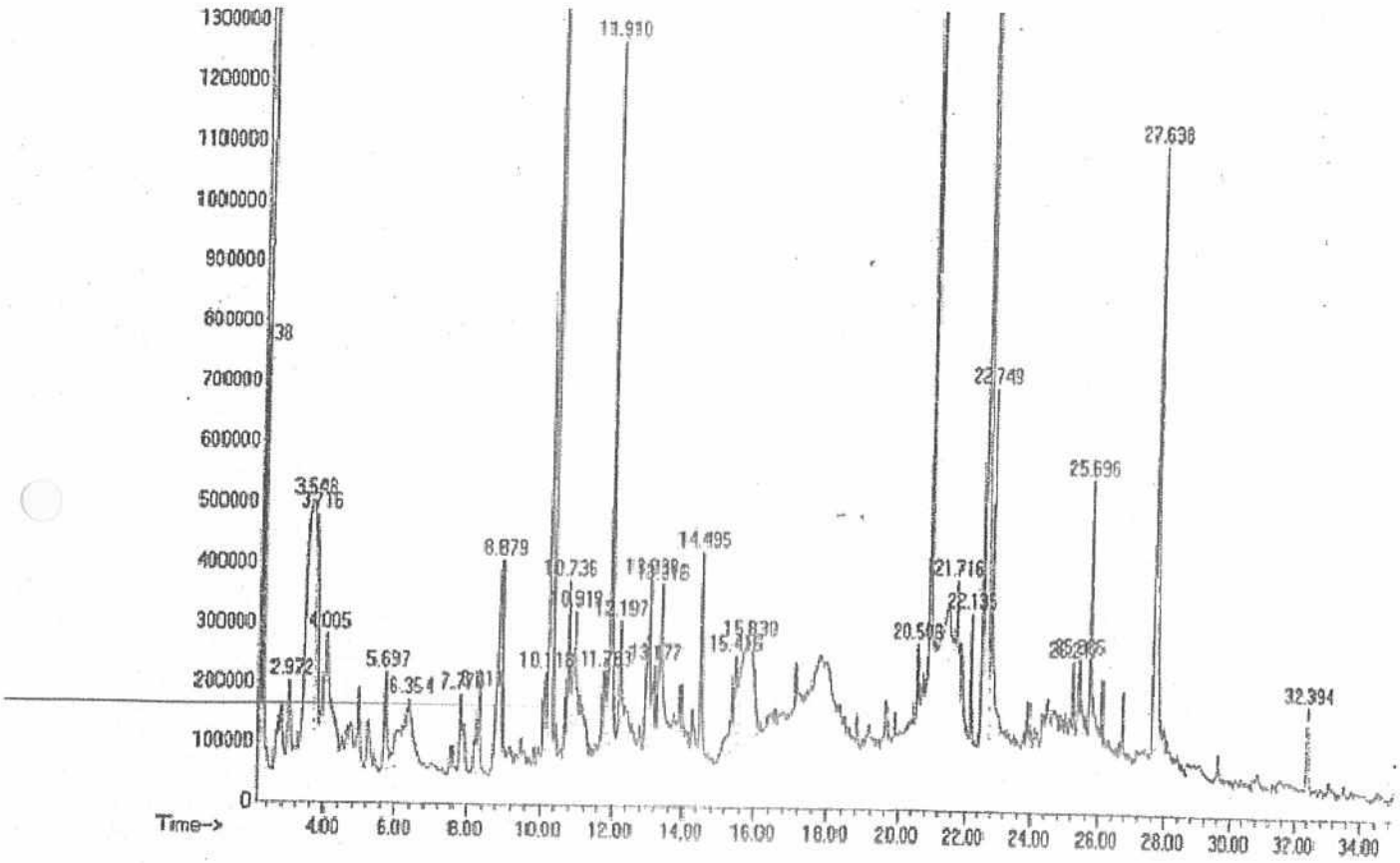

Figure-1
GC/MS results and the compounds of the methanol extract of product

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Table-3
GC/MS results and the compounds of the Chloroform extract of product

Retention time	Name of the compound	Bioactivity
11.110	1-Dodecene	Antibacterial
14.320	1-Tetradecene	Anti-tuberculosis
15.969	Phenol,2,4-bis(1,1-dimethylethyl)	Antifungal, Antioxidant
16.891	1-Hexadecene	Antifungal, Antibacterial, Antioxidant
19.136	1-Octadecene	Antibacterial, Antioxidant, Anticancer
20.879	n-Hexadecanoic acid	Flavor, Antioxidant
22.135	Methyl11,14 octadecadienoate	Anti-inflammatory, Anticancer, Anti-coronary
22.994	5-Eicosene,(E)	Antibacterial, Antifungal, Antitumor
24.493	dl-alpha-Tocopherol	Antioxidant

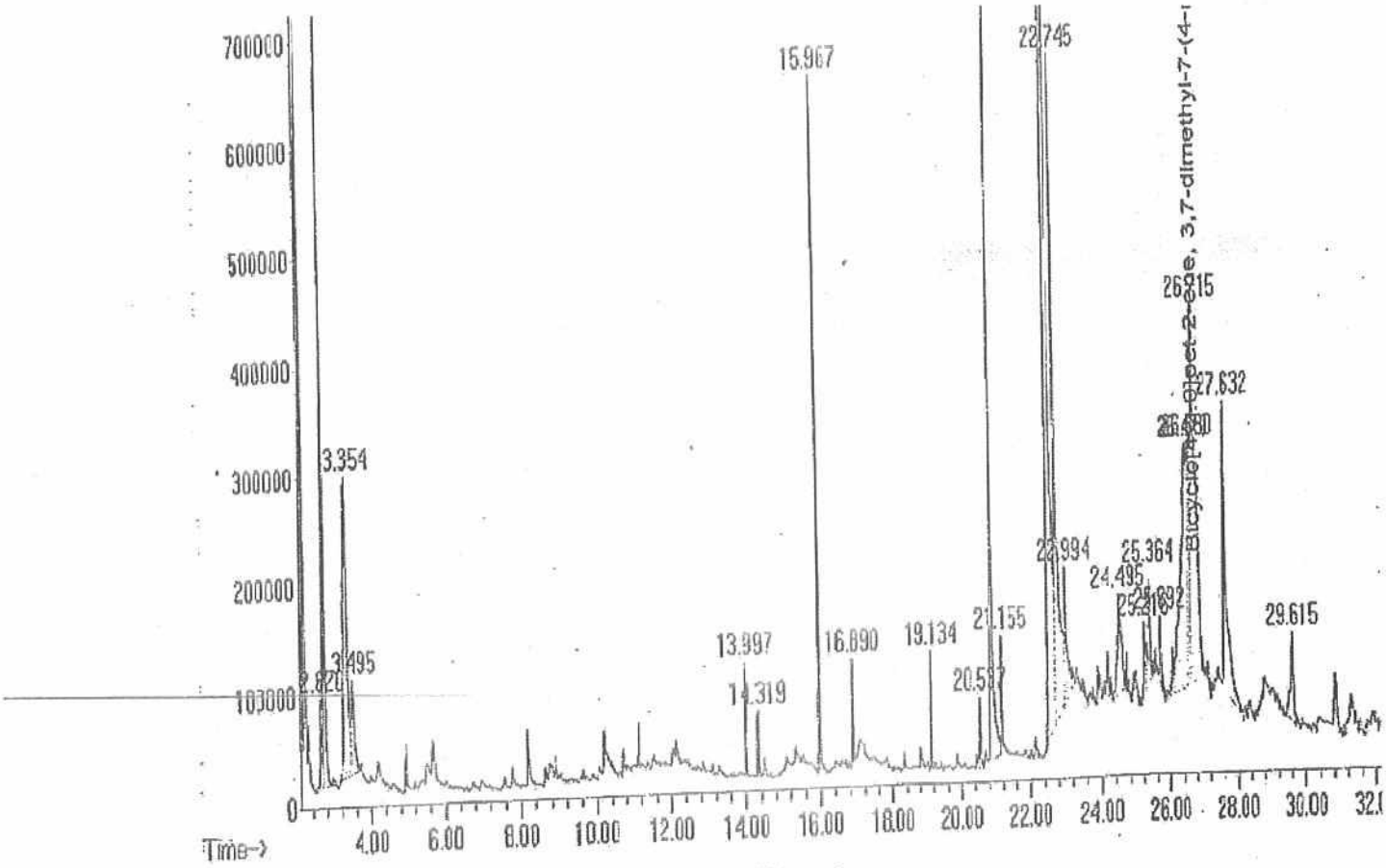

Figure-2
GC/MS results and the compounds of the acetone extract of product

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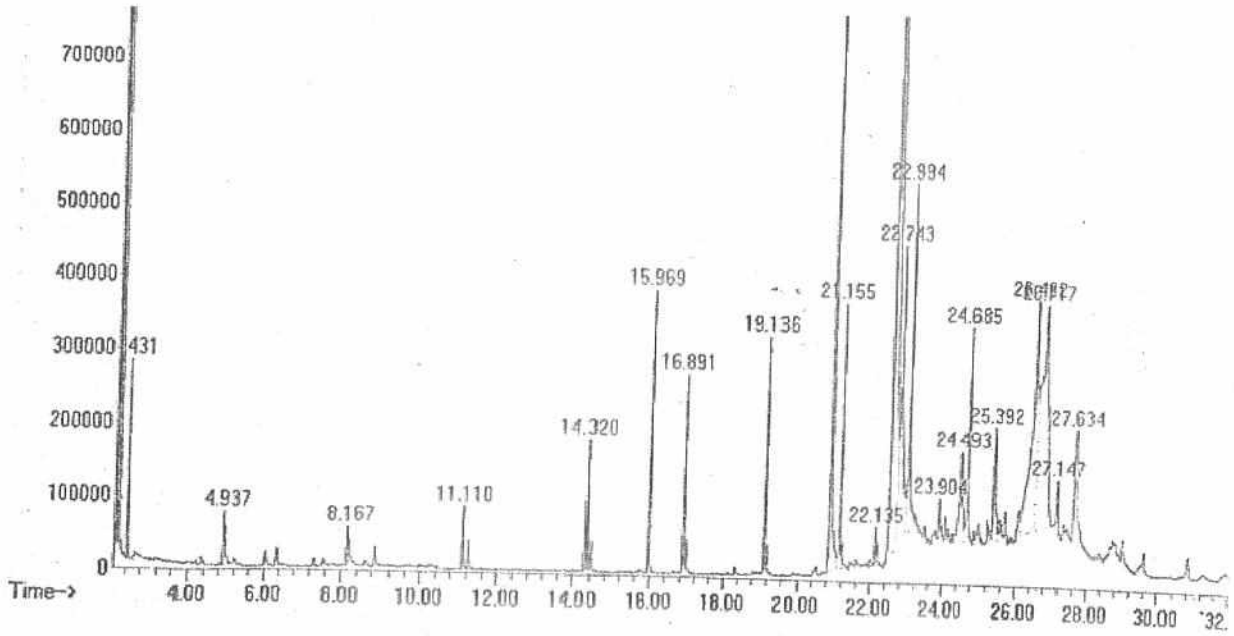

Figure-3
GC/MS results and the compounds of the chloroform extract of product

Antimicrobial compounds including antibacterial compounds, antifungal compound and antiviaral compounds are 2-Furanmethanol, 2-Furancarboxaldehyde, 5-(hydroxymethyl), Phenol, 2,4-bis(1,1-dimethylethyl), 1-hexadecene,1-Octadecene, 1-Nonadecene, 9,17-Octadecadienal,(Z), 1-Dodecene, 1-Tetradecene, 5-Eicosene,(E), Eicosyl trifluoroacetate. Antioxidant compounds are Benzeneacetaldehyde, Hexadecanoic acid, methyl ester, 9,12,-Octadecanoic acid(Z,Z), Phenol, 2,4-bis(1,1-dimethylethyl), 1-hexadecene, 1-Octadecene, n-Hexadecanoic acid, dl-alpha-Tocopherol. Anti-inflammatory compounds include; Benzeneacetaldehyde, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, Methyl11,14 octadecadienoate. Anti cancer and antitumor compounds are 9,12,-Octadecanoic acid(Z,Z), 1-Octadecene,1-Nonadecene, Methyl11,14 octadecadienoate, 5-Eicosene, (E).

According to the results, 18 compounds have been identified from methanol extract, chloroform extract and acetone extract. These identified phytochemicals are responsible for various bioactivities like flavor compounds, antimicrobial, antioxidant, anticancer and anti-inflammation activities. Therefore newly developed flavor enhancer provides valuable antimicrobial, medicinal and physiochemical properties.

Conclusion

Current research described the evaluation of antioxidant activities as well as the total phenolic compound of the flavor enhancer extract. Results revealed that the average antioxidant activity was 68.442 ±0.20693 mg GAE/100g and average total phenolic content of the sample was 137.646 ±0.577 mg GAE/100g. In the current investigation of the three different extracts acetone, Methanol and chloroform have showed the existence of various medicinally important compounds. Thus this research study justifies the newly developed flavor enhancer gives medicinally important properties.

Acknowledgments

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