GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF SPICES

INTRODUCTION

Gas chromatography-mass spectrometry refers to the interfacing of gas chromatography and mass spectrophotometry (Handley AJ, Adlard ER 2001) methods of analysis. It is a hyphenated technique whose main applications of the methods are detection and identification. From the broad advantages, there is profiling of samples which helps in identification of impurities to ensure environmental wellness, analytical research,and development (Handley AJ, Adlard ER 2001). In addition, operators learn about the composition of a sample hence are able to conclude on the purity and identify unknown substances.

GC-MS relies on the techniques of electron impact and chemical ionization(R.P 2002). It denotes the ration of the mass of a sample particle, denoted as Da, to the number of electrostatic charges present in the particle. Some of the advantages of the technique are the applicability in the analysis of esters, alcohols, fatty acids, aldehydes, and terpenes among others(Willard H. Hobart, Meritt L.Lyme et al 2004). Apart from this, it is efficient, fast, and ensures reproducibility and effectiveness of results. This advancement in technology offers the merits depending on the routine of the operator.

Food authenticity is a topic that many have taken with seriousness. Acceptance of foods by consumers depends on the physical and aromatic characteristics. Insufficient demonstration of any of the aspects leads to the adoption of poor dietary habits and eventually health complications. As a result, nutritionists and health practitioners have combined efforts to combat general unwell.

Today, with the invention of advanced sensor systems and adequate training of staff, food security has become a trending matter. The extensive assessment has saved the lives of millions that would otherwise be lost if the spices were below the recommended standards. The assessment provides information about the origin because of the differences in quantities of volatile organic compounds between species.

GOALS OF WORK

Extraction of essential oils from spices, turmeric, cinnamon, clove and red pepper

Spice profiling using GC-MS

LITERATURE REVIEW

GC-MS

GC-MS of spices helps in the profiling of substances to classify, identify and conclude on their applicability because each has a different composition as you move from one region to another (McCaleb R, Leigh E, Morien K. Turmeric, Curcuma 2000). For example, by analyzing, the operator learns the quantity of each component hence ceases to use for wrong purposes. There are multiple studies about spices since they are important in daily life.

The GC–MS conditions were optimized to ensure accuracy. The enzymes used in the extraction of clove were either glucoamylase or alpha-amylase or both,and the solvent was t-butanol. This

was an improvement since the extraction time decreased drastically allowing for voluminous processing and the yield of the oleoresin increased.

SPICES

They add significant nutritional value, stimulate appetite and enhance the visual appeals of food, which makes consumers adopt healthy dietary habits. Some of the areas where spices have been highly appreciated are in the management and prevention of cancer and lifestyle conditions like diabetes and cardiovascular complications (RP 2004). That nutritional and health aspects are intricately linked is an unforgettable manner which has drawn the attention of nutritionists and health practitioners. The correlation is the reason for the development of diets for different patients since nutritionist's advice after drawing conclusions from facts(Iranian Herbal Pharmacopoeia 2002).

Looking at the past studies, researchers concluded that antioxidants from spices, such as turmeric, clove, red pepper and cinnamon, control the oxidative stress in cells. It is particularly important in the prevention of the accumulation of radicals in aerobic metabolism since they interfere with signal transduction pathways (G. 1999).In the analysis of spices, the techniques fall into four main categories as, extraction of natural colours, natural flavours and oils, bioactive compounds from plants, and saturated fatty acids. All processes produce maximally in the presence of some enzymes.

From the reports presented, clove is an interesting plant,and the proven biological activities pave the way for development in the manufacture of pharmaceutical and veterinary products. It

confirms why this the suitability of the plant in the management of a wide range of illnesses while ensuring cellular safety and extension of life.

Unfortunately, there are no reported forms of curcumin or analogues that appear to possess the features necessary for the good drug such as high potency and selectivity and high therapeutic effect, chemical stability, solubility in water, high bioavailability, stable metabolism, broad tissue distribution, and minimal adverse effects. Evaluation reports are erroneous due to impurities appearing in the sample when preparing samples or running tests.

In spite of the discoveries, eulogizing may be a premature move since the results are an extrapolation from animals, investigation in humans remains a poorly investigated area. Although there are significant similarities between research animals and humans, the minimal differences that exist cannot be disregarded.

Generally, spices are used in small quantities and determining the quantities that will give a pharmacological effect is a challenge. Determining the mode of action and the pharmacokinetics when administered in small doses is a challenge. The results obtained from the investigation are inadequate to conclude that spices should take the place of some pharmaceutical products.

Aromatherapy is the new method of preventing and treating health conditions since millions of people have embraced the continuous use of spices in meals. The aroma leads to adoption and sustenance of proper dietary habits hence quickening the healing process and reinforcing the

immune system. From previous studies, the effects of essential oils include antibacterial, antioxidants, antivirals, antifungals, and insecticidal.

The suitability of any technique depends on the level of compliance with the guidelines. Lack or inadequate compliance is the root of errors (P.S 2004). For example, analytes used in the GC ought to range between 30°C and 300°C. In the gas chromatograph, the dictating features are phase and column properties.

In the column, variations in the length, diameter and film thickness cause changes in the method. Molecules are eluted at different times depending on the similarities with substance in the mobile phase. A combination of the methods reduces the margin of error significantly by overcoming the errors produced by each (Kitson FG, Larsen BS, McEwen CN 1996).

For example, mass spectrometry requires the purest form of samples and gas chromatograph uses traditional detectors. The outdated machines had low specificity and oftentimes could not delineate two molecules that have similar retention durations. Today, mass spectrophotometers are the universal companions of gas chromatographs. The high specificity is the main reason why operators have departed from the use of traditional detectors.

Each has an important role because the gas chromatograph performs excellently in separation while the mass spectrophotometer detects. Study the extent of compatibility. The main hindrance is the pressure requirements. On its own, GC presents insufficient details about a sample. Identification using the technique alone depends on retention time, which in most cases is inaccurate. When combined, they allow analysis of a wide range of products that are both volatile and sensitive to heat, advanced sensitivity, and quick sample identification.

GC-MS relies on the techniques of electron impact and chemical ionization (R.P 2002). Some of the advantages of the technique are the applicability in the analysis of esters, alcohols, fatty acids, aldehydes, and terpenes among others (Willard H. Hobart, Meritt L.Lyme et al 2004). Apart from this, it is efficient, fast, and ensures reproducibility and effectiveness of results. This advancement in technology offers the merits depending on the routine of the operator.

Even though targeted techniques focus on specific groups of known compounds, the sophisticated approaches allow simultaneous detection of compounds in a sample. It is particularly useful when operators lack prior knowledge about the identity of the metabolites. Diversity opens channels for investigation and draws many researchers into the laboratory.

The high-throughput methods generate large volumes of data,but on special analysis and visualization techniques, operators can extract the required information. Oftentimes, the number of metabolites outweighs the samples, which makes statistical analysis an important branch of the techniques. However, for optimal operation and acquisition of relevant and accurate data, operators should not exceed the number of samples indicated.

The aim of this study is to review previouslyon the nutritional value and the possible ways of combining to enhance the value. With the increase in demand for nutraceuticals, the information is the basis of the manufacturing to aid in the management of chronic illnesses. Pharmaceutical products have numerous side effects and sometimes pave the way for secondary infections.

RESEARCH ANALYSIS

The aim of the study was the identification of components in four commonly used spices. Hexanol was used in the extraction of turmeric and clove while methanol was useful for cinnamon and red pepper. 0.5 µl of each spice was injected during the investigation. The machine of analyzing was Perkin Elmer USA model. To excite electrons and aid detection, 80eV were used. The carrier gas was helium flowing at a rate of 2ml per minute,and the temperature ranged between 70°C and 120°C.

Identification was confirmed after comparison with the spectrum of standard samples as documented in the library of phytocompounds. Compounds whose spectrums did not match with the standard remained unidentified. There is a low possibility of error because guidelines of sample analysis and preparation were closely observed. The percentage of similarity, retraction time and the area were automatically generated by the machine. To calculate manually, compare the peak area with the total area occupied by the sample.

Experimental analysis

Sample preparation of turmeric

Materials: MRS Medium (Yeast Extract, Glucose, Meat Extract, Sodium Acetate, Dihydrogen Potassium Phosphate, Magnesium Sulphate, Triammonium Citrate, Manganese Sulphate, Agar)

Chromatographic System- HPLC Pump, HPLC Detector, Injector

Instrument Conditions- Mobile Phase has a combination of alcohol and methanol are mixed in ratio 60:40 and the mixture degassed and filtered. The injection size was 30ml, flowing at a rate of 2ml per minute and analyzed at 254nm.

Instrument: Mixer Grinder, Spectrophotometer, Siever, Dhona pan Balance, water bath, shaker, Bidwell-Sterling, conical flasks, funnel.

500 g of dried turmeric were ground in a mixer grinder. The powder was then subjected to a separating chamber and separation facilitated through vibration. The powders of uniform sizes were accumulated and labelled for further analysis. The resulting powder was extracted with methanol and hexanol at different times. The ratio of the solvent and the solid was 5 volumes on the dry weight of powder. The procedure was repeated 5 times and the individual extracts combined. The concentration of filtrates was the same and the absorbance measured. The extracts were concentrated by filtering then subjected to vacuum at a temperature of 40°C to obtain turmeric oleioresin.

20 g of turmeric oleioresin and 20ml of solvent were added, mixed well and stored overnight to allow for precipitation. The curcuminoid crystals were purified by washing with the initial solvent severally.Standard Curcumin*:* 25 mg of standard curcumin is weighed into a 25 ml volumetric flask and dissolved in hexanol and ethanol at different times and warmed in the waterbath. The solvents were added continuously while stirring.

Cinnamon

The bark of Cinnamomum zeylanicumwas obtained from the local market and the active ingredient extracted using Soxhlet extraction method. 80g of cinnamon the stick were mashed into smaller pieces and loaded into the Soxhlet extractor. The extraction solvents were methanol and hexanol at intervals and heated. The products were collected and purified. The samples were

placed in the fume hood to allow for evaporation of the solvents. All chemicals and reagents met the recommended standards. Cinnamaldehyde had 99% purity.

Red Pepper

Instruments used were electric pulverizer, silica gel plates PolygranSilG/UV254, CAMAG Linomat 5 sample applicator, rotary evaporator, water bath, Soxhlet extractor.

Dried red chilli peppers, Capsicum frutescens, were purchased from the shop. They were ground using a blender and sieved using a siever of the dimension of 25 μm to ensure uniformity. The fine powder was stored in airtight containers. Grinding and sieving were repeated three times to acquire enough quantity of the sample. They were analysed using methanol and hexanol. The reagents were according to the documented analytical grade.

Methanol extracts:a solution of one gram of sample and 20ml of methanol was extracted by heating at 80oC for 10 minutes, cooling then filtering the solution. The filtrate was placed in a water bath and evaporated to dryness. It was later diluted in 10ml methanol

Ethanol extracts: Soxhlet extraction: 10g of the sample was injected in the separatory chamber of the instrument and extracted with 100 ml hexanol for three hours from the start of reflux. The filtrate was filtered and concentrated to 20 ml at 60oC.

Standard preparation:standard capsaicin was available in the working station. 10mg of capsaicin were dissolved in hexanol and methanol respectively and topped to the 10 ml mark.

Isolation of Clove Oil

Materials: waterbath, hexanol and ethanol, Anhydrous Na2SO4 and flasks.

10 g of dry cloves was weighed upto 10g as the quantity of the working sample. The sample was transferred to a 500ml flask and dissolved in water. The method of extraction was distillation by placing in the heating mantle for 2hrs. Anhydrous Na2SO4 was added and the quantity of oil calculated. In the water–eugenol emulsion, because the oils are insoluble in water, 2 mL of CH2Cl2 were added and shaken to allow the layers to separate. The solution was transferred to a dry 5-mL conical vial while taking extra precaution to avoid transferring water. The process was repeated severally to acquire enough quantity. The solvent was evaporated in the chamber.

Parameters

Avoid column bleed since it leads to a significant decrease of detection limits and an increase in the spectral noise.

Fit with high-capacity oxygen and hydrocarbon

Keep the mass range as narrow as possible

The transfer device, ion source, and mass analyzer should be at the temperatures specified by the manufacturer

RESULTS

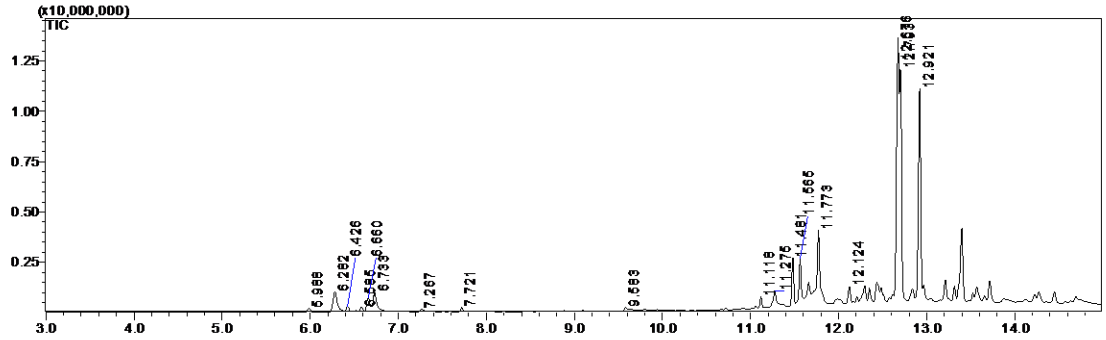
H1 :

The chemical composition of Turmeric sample extracted in Hexanol

	compound	formula	Retraction time	Similarity %	Area
1	.alpha.-Phellandrene		5.983	90	421639
2	2-Propanol, 1,1'-oxybis-		6.275	97	2698167
3	Benzene, 1-methyl 4-(1-methylethyl)-		6.425	87	467397
4	Eucalyptol		6.583	91	388971
5	1-Propanol, 2-(2-hydroxypropoxy)-		6.658	97	1247534
6	1-Propanol, 2-(2-hydroxypropoxy)-		6.733	97	1726352
7	1-Propanol, 2-(2-hydroxypropoxy)-		7.275	93	337108
8	1,6-Octadien-3-ol, 3,7-dimethyl-		7.717	91	369798

9	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate		9.583	88	243840
10	Caryophyllene		11.117	87	660530
11	Dimethyl phthalate		11.275	81	506314
12	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-		11.483	95	3179530
13	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)		11.567	89	2903113
14	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-		11.775	87	4000318
15	Tridecane, 2-methyl-2-phenyl-		12.125	83	1409902

16	Ar-tumerone		12.675	91	7581506
17	Tumerone		12.700	93	258217
18	Curlone		12.925	95	18143260



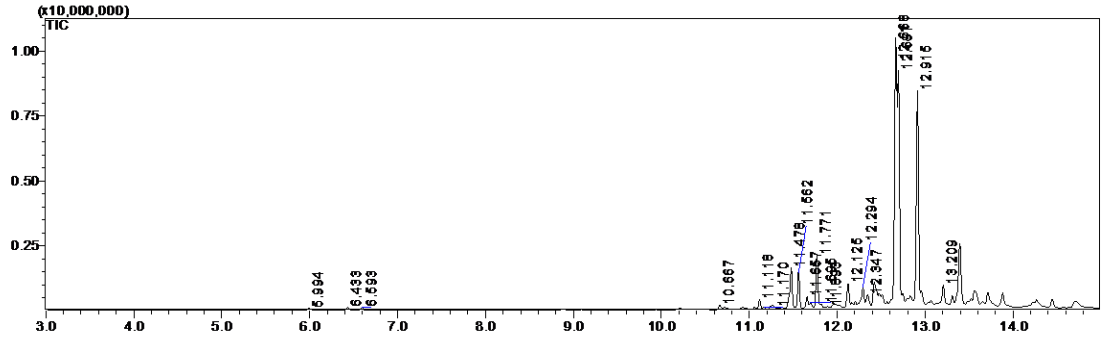
M1 :

The chemical composition of Turmeric sample extracted in Methanol

	compound	formula	Retraction time	Similarity %	Area
1	.alpha.-Phellandrene		6.000	84	233718
2	Benzene, 1-methyl-3-(1-methylethyl)-		6.442	89	237139
3	Eucalyptol		6.592	88	228148

4	Dodecane, 2,6,11-trimethyl-		10.667	86	255295
5	Caryophyllene		11.117	91	503181
6	1-Chloroeicosane		11.175	83	82484
7	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-		11.475	94	2642443
8	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-		11.558	92	1969742
9	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-		11.658	92	283175
10	Urea, N'-cyclooctyl-N,N-dimethyl-		11.700	81	87922
11	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-		11.775	94	2995212
12	1-Chloroeicosane		11.892	83	46986
13	Pentadecane, 2-methyl-2-phenyl-		12.117	80	1138292
14	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-		12.292	80	621582
15	4-(2,2-Dimethyl-6-		12.350	84	603602

	methylenecyclohexyl)butanal				
16	Ar-tumerone		12.667	92	8411442
17	Tumerone		12.700	93	1255195
18	Curlone		12.908	96	13273011
19	Cyclopentanecarboxylic acid, 3-isopropylidene-, bornyl ester		13.208	81	1135027

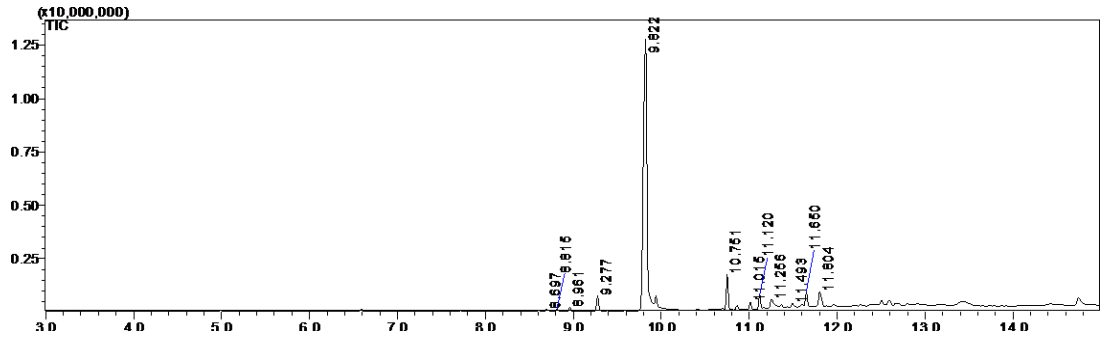


H2:

The chemical composition of cinnamon sample extracted in Hexanol

	compound	formula	Retraction	Similarity	Area

			time	%	
1	Borneol		8.700		233718
2	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-		8.817		237139
3	3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-		8.958		228148
4	2-Propenal, 3-phenyl-		9.275		255295
5	2-Propenal, 3-phenyl-		9.825		503181
6	Copaene		10.750		82484
7	trans-.alpha.-Bergamotene		11.008		2642443
8	Caryophyllene		11.125		1969742
9	2H-1-Benzopyran-2-one		11.258		283175
10	Tricyclo[4.1.0.0(2,4)]heptane, 3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)-		11.492		87922
11	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-		11.658		2995212
12	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-		11.808		46986

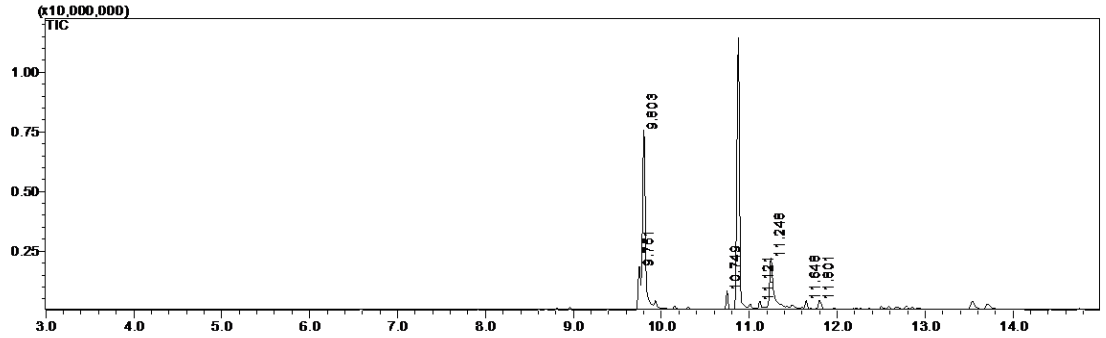


M2:

The chemical composition of cinnamon sample extracted in Methanol

	compound	formula	Retraction time	Similarity %	Area
1	Benzene, (1-methoxyethyl)-		9.758	83	1758820
2	Cinnamaldehyde, (E)-		9.800	97	12794325
3	Copaene		10.750	94	1063892
4	Caryophyllene		11.117	87	397542
5	2H-1-Benzopyran-2-one		11.258	94	4454170
6	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-		11.642	88	463159
7	Naphthalene, 1,2,3,5,6,8a-		11.800	84	299226

	hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-				

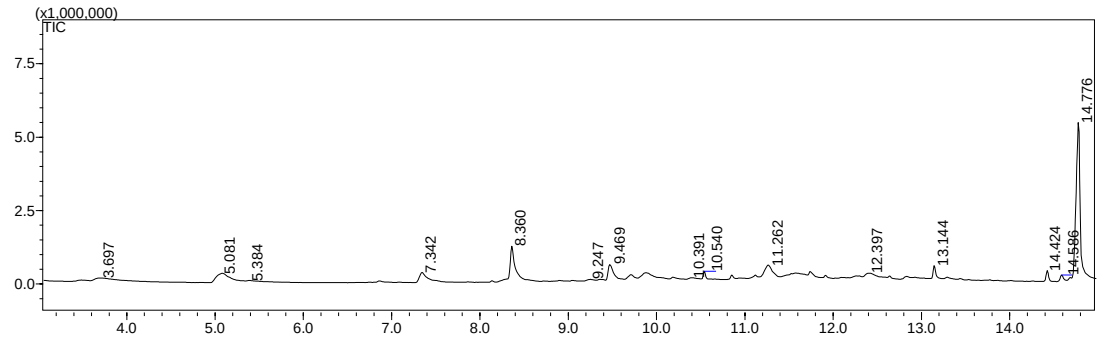


M4:

The chemical composition of Pepper sample extracted in Methanol

	compound	formula	Retraction time	Similarity %	Area
1	2-Propanone, 1,3-dihydroxy-		3.717	90	804958
2	Glycerin		5.075	95	2582601
3	Glycerin		5.400	80	21067
4	1,3,5-Triazine-2,4,6-triamine		7.358	81	1465764
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-		8.358	89	3254132

	methyl-				
6	2-Deoxy-D-galactose		9.233	82	57867
7	1,2,3-Propanetriol, 1-acetate		9.475	88	1403590
8	Isosorbide Dinitrate		10.392	84	76608
9	Phenol, 2-methoxy-3-(2-propenyl)-		10.542	81	390371
10	Sucrose		11.275	85	1890799
11	2-Deoxy-D-galactose		12.417	80	452964
12	Tetradecanoic acid		13.150	88	753329
13	Hexadecanoic acid, methyl ester		14.425	91	625925
14	9-Hexadecenoic acid		14.592	88	422215
15	l-(+)-Ascorbic acid 2,6-dihexadecanoate		14.758	92	16155599

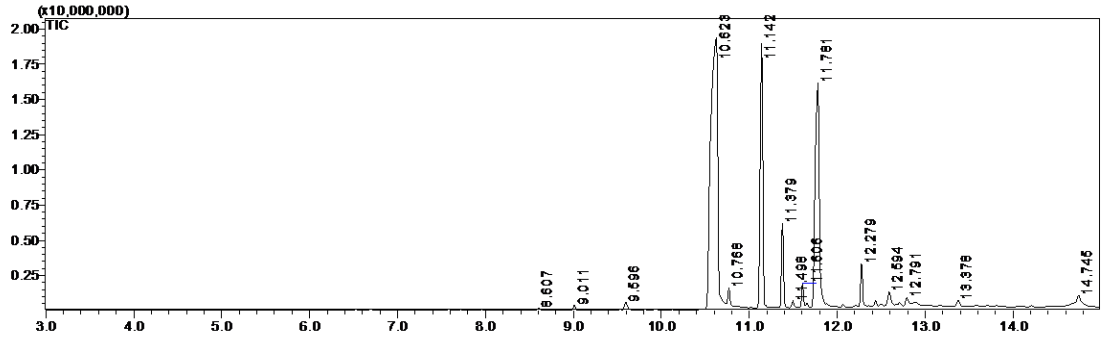


H3:

The chemical composition of Clove sample extracted in Hexanol

	compound	formula	Retraction time	Similarity %	Area
1	Acetic acid, phenylmethyl ester		8.608	91	307595
2	Benzoic acid, 2-hydroxy-, methyl ester		9.025	90	409918
3	Phenol, 4-(2-propenyl)-		9.592	92	1061906
4	Phenol, 2-methoxy-3-(2-propenyl)-		10.608	95	101109834
5	Copaene		10.775	89	1688419
6	Caryophyllene		11.133	96	38418380
7	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-		11.375	96	9525952
8	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-		11.500	88	701918
9	.alpha.-Farnesene		11.608	93	1325920
10	Phenol, 2-methoxy-4-(2-propenyl)-, acetate		11.783	94	46417369

11	Caryophyllene oxide		12.283	93	3872811
12	Longipinocarveol, trans-		12.592	81	1730947
13	10-12-Pentacosadiynoic acid		12.792	82	795533
14	Benzyl Benzoate		13.383	81	816847
15	n-Hexadecanoic acid		14.750	80	-7573028

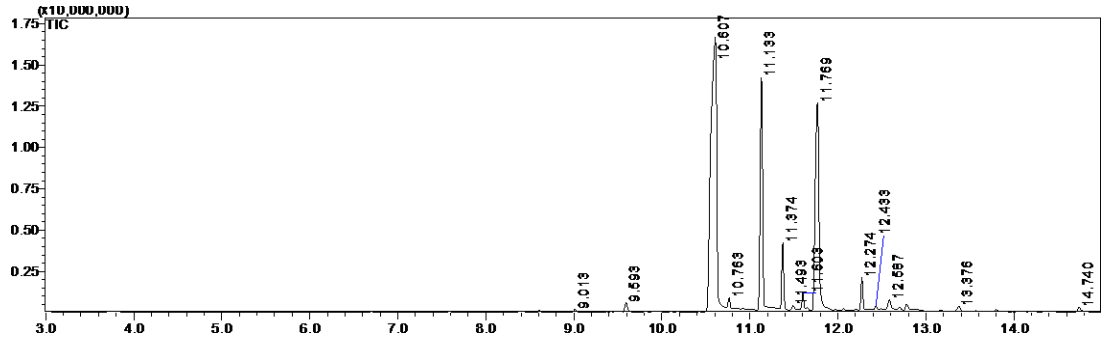


M3:

The chemical composition of Clove sample extracted in Methanol

	compound	formula	Retraction time	Similarity %	Area
1	Benzoic acid, 2-hydroxy-,		9.008	92	116148

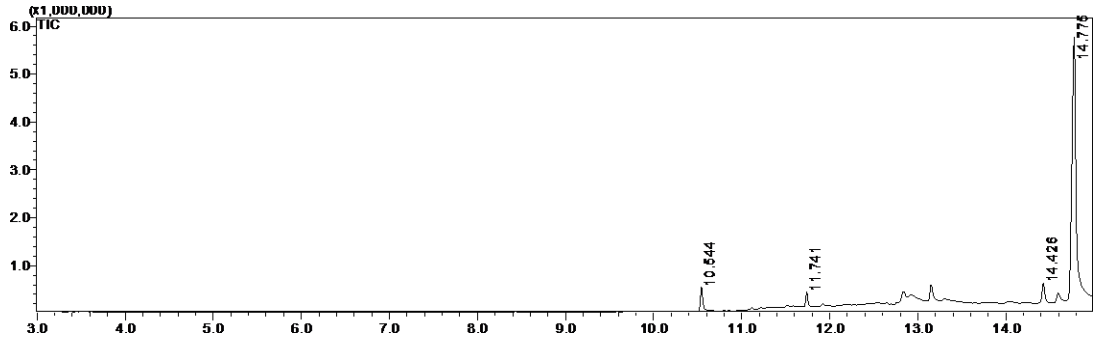
	methyl ester				
2	Phenol, 4-(2-propenyl)-		9.592	92	960092
3	Eugenol		10.592	96	74494919
4	Copaene		10.767	89	900102
5	Caryophyllene		11.133	96	25658183
6	.alpha.-Caryophyllene		11.375	95	6083229
7	Tricyclo[4.1.0.0(2,4)]heptane, 3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)-		11.500	84	265929
8	.alpha.-Farnesene		11.608	93	1271885
9	Phenol, 2-methoxy-4-(2-propenyl)-, acetate		11.758	94	35783479
10	Caryophyllene oxide		12.267	92	2692998
11	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-		12.433	82	309862
12	Isoaromadendrene epoxide		12.583	82	1106353
13	Benzyl Benzoate		13.383	85	429212
14	n-Hexadecanoic acid		14.758	87	371984



H4:

The chemical composition of Pepper sample extracted in Hexanol

	compound	formula	Retraction time	Similarity %	Area
1	Phenol, 2-methoxy-3-(2-propenyl)-		876823	94	116148
2	Phenol, 2-methoxy-4-(2-propenyl)-, acetate		441177	86	960092
3	Hexadecanoic acid, methyl ester		709062	85	74494919
4	l-(+)-Ascorbic acid 2,6-dihexadecanoate		16055202	92	900102



DISCUSSION

The retraction times vary because of the differences in solubility in the extraction solvents. In hexanol, which has a long hydrophobic chain, the miscibility is high which in term decreases the retention time. Fragrance components in spices are contained in different parts of the plant. For example, clove comes from the bud and cinnamon from the bark. Nowadays, due to the rise in the need for spices are increasingly useful in healthcare. This is a new discovery apart from the primary use as organoleptic enhancers in culinary art.

The nature of a botanical substance that contains the essential oils dictates the method of extracting. There is a range of techniques and the common are an expression, which is basically a cold press, use of solvents, distillation, supercritical fluid extraction among others. In most cases, the methods of extraction are direct or indirect steam distillation.

One of the most employed methods in the extraction of essential oils from spices is solvent extraction. As is expected, Soxhelt has some hindrances such as the inclusion of other soluble

residues. due to the long duration of heating, thermal degradation is a common tragedy. There are several modifications in the conventional solvent extraction process as technological and analytical processes change.

GC–MS is an advanced method useful in the identification and quantification of chemical components in different substances. Some traditional methods such as steam distillation, hydrodistillation, reflux extraction and Soxlet extraction are gradually becoming extinct due to the problems of lengthy protocols, unreliability and disposition of toxic residues in the products. These shortcomings led and have continuously inspired the development of new techniques, which are cost friendly and time saving such as GC–MS.

The current method has a higher efficiency as compared to the conventional. Soxhlet extraction employs the use of reactor equipped with an agitator that has four blades and motor1200 rpm speed. In solvent extraction, choice of solvent is important to obtain large quantities which contain all the volatile components. The solvents used to give an insight into the polarity of botanical components. The extraction is usually carried out for a long period.

Steam distillation, as the name suggests, uses steam to separate constituents in the botanical materials. The method is only effective for the substances that have high boiling points. For an uninterrupted process, the flow of vapour should be smooth. A large number of essential oils have a boiling pointy close to 100°C, which leads to the preservation of the structure. Components that have low boiling temperatures can also be separated.

In the indirect steam distillation, the water level is below the operating plant. Co-distillation is the distillation of components that are insoluble in water. Steam from the plant vaporizes the

essential oils that have high boiling points. As the hot vapoursget in contact with the cold surfaces in the cooling system, it condenses back into a liquid.

The oils that are immiscible in water come out in two layers as oil floating on the water. The layer of water can be syphoned off using a funnel. In the case of an emulsion, which is common when dealing with small quantities, allow the components to separate. The immiscibility of the polar solvent in the oil makes extraction and isolation effective and free of error.

For acquisition of accurate, relevant and reproducible data, validation of the method is essential. The main areas of concern were linearity, stability, repeatability, sensitivity, accuracy,and reproducibility. Stability was enhanced by injecting standards at hourly intervals. Repeatability is a pillar of accuracy and the average of the result.

Linearity is the concept of plotting concentration against the average peak area. The machines detected minute amounts of components even after repeated tests. Care and maintenance of machines such as flushing after each measurement maintained sensitivity at optimum levels.

Clove

Clove oil comes from dried buds. Traditionally clove oil was used to flavour food and as an antimicrobial agent(Shan B, Cai YZ, Sun M, Corke H. 2005). They are comprised of various classes of chemical compounds such as sesquiterpenes, monoterpenes, phenolics,and hydrocarbons. The main components are eugenol and caryophyllene,and the high concentration leads to adoption in the manufacture of herbal and modern medicine. They exhibit a high

therapeutic effect as antibacterial, antifungal, and antioxidants(Shan B, Cai YZ, Sun M, Corke H. 2005).

Clove is a rich source of phenolic compounds hence fits in the cosmetic, pharmaceutical, and food industry (Sofia PK, Prasad R, Vijay VK, Srivastava AK 2007). The boiling point of eugenol is 248 °C. It can also be isolated at lower temperatures using steam distillation.The distillate has both water and eugenol,but the two do not form a homogeneous solution. The oil must be extracted using an organic solvent. Upon extraction using the organic solvent, hexanol and methanol differently, the organic layer is separated from the oil through evaporation.

Eugenol is a phytochemical used to relieve a toothache temporarily. It is applied to a cavity remaining after extraction or caused by dental problems. The mechanism of action is the activation of chloride and calcium ion channels in ganglionic cells(Shan B, Cai YZ, Sun M, Corke H. 2005). The action of clove as capsaicin agonist helps in pain management. Daniel *et al.* reported the antinociceptive activity of eugenol.

Clove is the characteristic odour in a dentist's room(Hekimoğlu MA, Ergun M 2012). To fill tooth cavities, dentists combine with zinc oxide. Methoxyophenol is used in perfumes,andflavouring activitieshence qualifyas a chief ingredient in the formulation ofdifferent classes of chemicals such as analgesics, insect attractants,antiseptics, and UV absorbers. They are also used as stabilizers and antioxidants in plastics and rubbers.

Turmeric

The contact time between solvent and the powdered component was minimized to avoidthe addition of components that can cause the formation of extra of minimal peaks on the spectrum. Foreign components interfere with the matrix. After extraction, the solvent is evaporated and the dried material placed in the solvent to precipitate.

To separate the active substance from impurities that may be present in the plant or placed by the operator when operating, preparative column chromatography is used. In this, the glass column with a length of 900 mm, a diameter of 50 mm and a PTFE stopcock USA model was utilized. The column is packed with silica gel and fine granular quartz.

For sufficient separation, a mobile phase was established for all extracts. Hexanol and methanol were the extraction components. The extract was removed, evaporated to dryness dissolved in an ample volume of the mobile phase and .Fractions of eluting samples are collected in appropriate time periods.

Turmeric, commonly called a golden spice in some regions, is a rhizomatous plant with innumerable species. The flavour is due to their flavour due to oleoresins and essential oils. Apart from the global role in the culinary culture, it has ancient and innumerable medical properties. In folklore medicine,when the preparations were applied to fresh wounds and bruises, they prevented infection and irritation on the injured parts.

The paste is useful in the cases of scabbing in chickenpox and smallpox,diseases affecting the urologic and hepatobiliary systems and as an anthelminthic(GS Rajorhia, R Macrae, RK

Robinson, MJ Sadler 1993). Members of some communities describeit as a cancer remedy in some communities. As a food additive, turmeric improves the aesthetic appeal, deliciousness and shelf life of some products. The powder is extensively used as a preservative and colourant.

Recently, turmeric has received considerable attention for its therapeutic importance. It contains biologically active substances which if properly harnessed can change the narrative of medicine. The potential is due to the presence of curcumin. Curcumin is used for multiple sclerosis, rheumatoid arthritis, and Alzheimer's disease. It also facilitates biliary secretion, protects from liver injury, and prevents the formation of cataract (GS Rajorhia, R Macrae, RK Robinson, MJ Sadler 1993)..

Curcumin disrupts signal transduction by inhibiting the functionality of protein kinase C, cyclooxygenases and other enzymes produced in the onset of inflammation. In another aspect, it inhibits tumour cell proliferation and suppresseschemical-induced carcinogenesis(RP. 2004). The mechanism is the reasons behind its use as an anticancer. Curcumin has shown anti-angiogenic properties and angioinhibitoryproperties. It downregulates angiopoietin and invades endothelial cells. They induce apoptosis, which is a necessary survival requirement for cancer patients.

Resistance is a common tragedy that leads to a massive loss of life. In the management of cancers, certain apoptotic inducers are used together with curcumin and radiation to enhance susceptibility and initiate apoptosis(RP. 2004). Additionally, it can also act as a chemopreventive agent. The mechanism of action is the suppression of colonic aberrant crypt foci formation(RP. 2004).

It demonstrates partial inhibition against protein kinase. Despite being consumed regularly, there are no complaints about toxicity or intestinal disturbances. As stated by Rakhunde*et al.*, the contents of essential oils in turmeric rhizomes and curcuminoids vary with genotypes, geographical locations, varieties, environmental conditions, sources, cultivation conditions, harvest methods and seasons, drying process, and storage conditions.

It is an essential laboratory substance for differentiation between acidic and alkaline substances. If added to an acidic solution, it retains the usual yellow colour but changes to red in an alkaline solution and its chemical structure, an alternating sequence ofsingle and double bonds, is the reason behind the changes McCaleb R, Leigh E, Morien K.

The length of the sequence affects the wavelengths of light absorbed. When added to an alkaline solution, the change in structure alters the wavelengths of light absorbed hence the colour change from yellow to red.

Cinnamon

To extract cinnamon from the bark easily, steam distillation is the simplest method since it is cheaper than other methods, does not damage the components and does not require any solvent. Apart from the economic status, it is faster than other methods. Soxhlet extraction used methanol and hexanol as the solvents because they dissolve the oils,unlike water.

Cinnamon contains a range of resinous compounds such as cinnamate, cinnamaldehyde, cinnamic acid, and essential oils. Each component has a specific role and cinnamaldehyde is

linked to the spiciness due to absorption of oxygen. As cinnamon ages, the colour becomes dark and improves the resinous compounds.

Cinnamaldehyde, which is the main ingredient, has a high antibacterial activity while other substances have mild activity. Today, the prevalence of infectious diseases and extensivity of antibiotic resistance has become a problem that modern medicine is unable to resolve. Plant-based products areamong the alternative agents examined in order to replace conventional antibiotics.

The technique showed seven components with cinnamaldehyde at 97%. Although there are multiple documented reports about the analysis, note that the results differ as per operational conditions, climate, duration of harvest, immediate and post-treatment, and maturity at the onset of harvest. Several types of research state that the antibacterial effect of cinnamon oil is more profound against Gram-positive bacteria than Gram-negative bacteria.

The mechanism of action depends on the type of microorganisms under attack and morphological properties. For example,in Gram-negative strain, the hydrophilic surface creates a barrier against inhibition of growth by antibiotic molecules. Consequently, they develop resistance towards hydrophobic compounds present in the essential oil compared to Gram-positive bacteria, which have a thin layer(P. Hili, C. S. Evans, and R. G. Veness 1997). Gram-positive bacteria, has a single peptidoglycan layer making it easy for antibacterial compounds to destroy the cytoplasmic membrane and cell wall.

Cinnamaldehyde interferes with the integrity of the plasma membrane causing changes in the morphology hence disrupt the permeability, cause leakage, and eventually death. The

hydrophobic bonds of phenols damage the cell membranes and the dissolution of components that bind hydrophobic. These circumstances cause low cell permeability (P. Hili, C. S. Evans, and R. G. Veness 1997).

Phenolic compounds are the damage of plasma membrane ergosterol;othersaffect the synthesis of nucleic acids or the main elements of the fungal cell wall such as chitin, mannoprotein and β glucans(P. Hili, C. S. Evans, and R. G. Veness 1997). Cinnamon extracts inhibit the activity of vascular endothelial growth factor subtype 2 kinasesand in the process angiogenesis involved in cancer. Several researchers such as Jeong et al. reported that CB403, a chemical synthesized from 2′-hydroxycinnamaldehyde derived from cinnamaldehyde, inhibit tumour growth. Obesity and related problem is a global issue,but many people have adopted the use of pharmaceutical products for weight management.

Red pepper

The flavour of peppers is perceived duringconsumption and is the product of taste, odour, mouthfeel, and sight.Many consumers enjoy and look forward to the burning effect of peppers brought by the harmonic interplay of senses. The eyes capture the form and colour, ears record the consistency of biting patterns, the tongues get the burning effect,and the noses capture the scent.

According to Luning et al., the composition of the aroma is affected by the composition of volatile compounds while the non-volatile compounds influence the sensory perceivedsuch as the taste. Studies about these aspects are inadequate,but there have been developments since the

last decade. This gives assurance that future conclusions will be based on facts and minimally influenced by assumptions.

As they age andripen, the number of chlorophyll pigments decreases through decomposition, while other pigments increase, until it is red. Thecolour is due to the production of capsanthin, carotenoids,and capsorubin. Biosynthesis of carotenoids includes incorporation of eight isoprene units. B-carotene is formed first,and it is then subsequently oxidized to alcohols or epoxides.

The spiciness of chillies is due to capsaicinoids such as capsaicin and dihydrocapsaicin. They are the reason behind the burning effect of pepper once they get in touch with the mucous membrane due to interaction with pain and heat sensing neurons. Capsaicin is used in the manufacture of pepper spray commonly used as a security gadget. In addition, there are some studies linking the compound to the killing of cancer cells in the lungs and prostate(Bartley G.E., Scolnik P.A 1995).

The capsaicinoids bind to a receptor in the mucous membrane of the to perceive the burning sensation(Bartley G.E., Scolnik P.A 1995). There is no evidence that pepper cause any physical or tissue damage. Repeated ingestionallows the buildup of tolerance. The heat present in chillies can be measured using several methods. First, Scoville scale measuresthe extract of dried pepper diluted with a sugar solution until the heat is no longer detected with a panel of five testers. There are several tests linking the concentration of capsaicin to approximately 18µmol dm^{-3}.High power chromatographic test is another method although less common(Cunningham F.X., Gantt E

1998). To facilitate separation, thesolvent sample is pumped through a thin column under high pressure.

The synthesis of paprika ketones, capsanthin and capsorubin, takes place through a chemical reaction called a pinacol rearrangement. The main requirement is a concentrated acid catalyst,butpeppers react in a near-neutral condition and at room temperature. It also involves some enzymes although they have not been identified(Cunningham F.X., Gantt E 1998). Pepper ripening is manifested through changes in colour,but it also exhibits changes in the compounds that give peppers their aroma.

The concentration of these volatile compounds decreases as the pepper ripens,but there are a few notable exceptions. There are improvements in breeding activities to enhance production, disease resistivity, firmness and shelf life. However, the flavour is a complex multifactorial trait hence determining a common denominator is a challenge. The flavour is affected by multiple factors such as environmental factors during growth and treatment before and after processing. For this, there is no definite standard measure on how best to measure the flavour.

GC-MS can distinguish more than 254 different volatile compounds when incorporated with other complex analysis techniques. Differences between genotypes arecaused by qualitative and quantitative differences in the metabolic clusters. High concentration of alkanes, phenolic derivatives, sesquiterpenes,andlipid-derivedvolatiles are the major determinants as per the accumulated results.

Changes in the expression of genes through methods such as deletion, or addition can alter the composition of compoundsand in the process individual attributes or overallflavor. Luning et al. also reported the correlation between the concentrations of citric and ascorbic acids with some attributes. Citricacid is responsible for the sourness. Organic acids, on the other hand, have no effect on the sourness in pepper.

SUMMARY

Summary

With the rise in cases of morbidity and mortality due to infections, there is a need for the development of new methods of approaching the matter. The resistance of bacterial strains has become a big problem. Drug development is a lengthy, costly and hectic process but the use of natural substances ensures environmental friendliness, balancing of the supply and demand chains and cost-effectiveness to the producer and consumer.

There is a minimal argument about the suitability in the maintenance of optimal human health and their role in changing the dynamics of the development of pharmaceutical products. The remarkable results give hope that they are the next set of medicines.

Botanical components have an abundance of phytogenic components. The selection of extraction method depends on the nature of the component. The right method should not introduce impurities in the essential oils or damage some. The most common components include sterols, phenols, tocopherols and organic acids. Today, they are used in pharmaceutical companies in the manufacture of nutraceuticals.

CONCLUSION

There is a need for research into the composition of active ingredients at different times during growth and those growing in different parts of the world. The information is useful for guidance to end users about the best time of harvesting and storage conditions which ensure minimal loss of active ingredient and maintain safety.

Little investigations have been conducted on the manner of measuring actual doses. Also, factors that can affect the pharmacokinetics such as interactions are minimally explained. It is only from proven findings that the Food and Drugs Administration agency will have confidence that it is not putting health at risk.

REFERENCES

Handley AJ, Adlard ER (2001) Gas chromatographic techniques and Applications. Sheffield Academic, London.

Kitson FG, Larsen BS, McEwen CN (1996) Gas chromatography and massSpectrometry: Academic Press, Boston.

Niessen WMA (2001) Current practice of gas chromatography-massSpectrometry. Marcel Dekker, New York, NY.

Kalsi P.S. "Spectroscopy of Organic Compounds" sixth edition, 2004 Published by New Age International Pvt. Ltd. P.NO. - 512-513

Kira, K.; Rendell, L.A. A Practical Approach to Feature Selection. In Proceedings of the Ninth International Workshop on Machine Learning, 1992; pp. 249–256.

Bosland PW, Votava EJ. Peppers: vegetable and spice capsicums. New York: CABI Publishing; 2000.

Baker EA. Chemistry and morphology of plant epicuticular waxes. In: Cutler DF, Alvin KL, Price CE, editors. The plant cuticle. London: Academic; 1982.

Schönherr J. Resistance of plant surfaces to water loss: transport properties of cutin, suberin and associated lipids. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, editors. Physiological plant ecology. Berlin: Springer; 1982.

McCaleb R, Leigh E, Morien K. Turmeric, *Curcumalonga*, Zingiberaceae. In: The Encyclopedia ofPopular herbs

Martín-Cordero C, López-Lázaro M, Gálvez M, Jesús Ayuso M. Curcumin as a DNA topoisomerase II poison. J Enzyme Inhib Med Chem. 2003;18:505- 509.

Adams RP. Identification of essential oil components by Gas Chromatography-MassSpectrometry. Illinois: Allured Publishing; 2004.

Iranian Herbal Pharmacopoeia. Vol 1. Tehran: Food and Drug Deputy of Health Ministry; 2002. p. 365.

Samuelsson G. Drugs of Natural Origin, A Textbook of Pharmacognosy. Stockholm: Swedish Pharmaceutical Press;1999.

Chung G, Oh SB (2013). Eugenol as localanesthetic. Springer-Verlag Berlin; In: Natural Products - Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes; Part XIV. pp. 4001–4015.

GS Rajorhia; R Macrae;RK Robinson;MJ Sadler. *Encyclopedia of Food Science. London*: AcademicPress,1993**,**4, 2186–99.

T. Tanaka, Y. Matsuo, Y. Yamada, and I. Kouno, "Structure of polymeric polyphenols of cinnamon bark deduced from condensation products of cinnamaldehyde with catechin and procyanidins," Journal of Agricultural and Food Chemistry, vol. 56, no. 14, pp. 5864–5870, 2008.

Harumi Kaga , Masakatsu Miura , Kazuhiko Orito, *J. Org. Chem***.**1989**,***54*, 3477–3478

Andrews, *The Pepper Trail: History and Recipes from Around the World*, The University of North Texas Press, Denton, TX, USA.

Shan B, Cai YZ, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J Agric Food Chem. 2005;53(20):7749–7759

Gülçin I, Elmastaş M, Aboul-Enein HY. Antioxidant activity of clove oil-A powerful antioxidant source. Arab J Chem. 2012;5(4):489–499.

Sofia PK, Prasad R, Vijay VK, Srivastava AK. Evaluation of antibacterial activity of Indian spices against common foodborne pathogens. Int J Food Sci Technol. 2007;42(8):910–915.

Hekimoğlu MA, Ergun M. Evaluation of clove oil as anaesthetic agent in fresh water angelfish, *Pterophyllum scalare*. Pak J Zool. 2012;44(5):1297–1300.

A. Sangal, "Role of cinnamon as beneficial antidiabetic food adjunct: a review," Advances in Applied Science Research, vol. 2, no. 4, pp. 440–450, 2011.

P. Hili, C. S. Evans, and R. G. Veness, "Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil," Letters in Applied Microbiology, vol. 24, no. 4, pp. 269–275, 1997.

Cunningham F.X., Gantt E. Genes and enzymes of carotenoid biosynthesis in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1998;49:557–583.

Bartley G.E., Scolnik P.A. Plant carotenoids: Pigments for photoprotection, visual attraction, and human health. Plant Cell. 1995;7:1027–1038