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Analysis of consortium of spices: ginger, cinnamon and ajwain on their phytochemical content and anticancerous effect on lung Cancer cell lines

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Abstract
Ingestion of spices and herbs with active phytochemicals protects from chronic diseases. In the present study, the phytochemical components present in the consortium of three selected spices viz., ginger, cinnamon and ajwain were assessed to find out the synergistic effect on cancer cell lines. Equal proportions of dried and powdered spices were combined together, extracted and tested both quantitatively and qualitatively. The synergistic effect of the extract on human lung cancer cell line was assessed using cell viability and cytotoxic assays. The phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, steroids, terpenoids, tannins, coumarins, phlobatannins and anthraquinones. The total phenolic, tannin and total antioxidant content were 0.00693g%, 0.0072g% and 2.42% respectively. Cell viability test indicated that a concentration ranging from 25-50 µg/ml is effective in inhibiting cancer cell proliferation. Increasing the concentration above 50 µg/ml did not show a concomitant rise in inhibition.

Keywords: Phytochemicals, antioxidant, tannin, cell viability

1. Introduction

Spices and herbs exhibit various physiological and pharmacological properties (Haseena *et al.,* 2015) [9]. Phytochemicals in plants are responsible for the colour; taste and aroma of foods also protect us from environmental and ingested carcinogens by arming our antioxidant enzymes, enhancing DNA repair pathways and have direct effects on the fundamental hall markers of cancer progression and metastasis (Thomas *et al.,* 2015) [16]. In different spices and herbs, a wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulphides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins and phthalides have been identified. Cancer is a disease of gene disorder that occurs in the normal processes of cell division (Zaahkouk *et al.,* 2015) [22]. An extremely promising strategy for cancer prevention today is chemoprevention. Modern researchers have developed many synthetic chemo-preventive agents to cure various types of cancer (Harsharan *et al.,* 2014) [8]. Yet, those synthetic drugs are expensive and also cause genetic and metabolic alterations. However, safe and sound mode of treatment is needed to control the disease development and progression (Rahmani *et al.,* 2014) [3]. Therefore, the focus has now shifted toward natural products, such as spices and plants, among many others, to save the future of cancer treatment (Kumar *et al.,* 2014) [16]. Sasikumar and Eagappan (2014) [23] have quoted that traditional medicines, functional foods and phytonutrients are generally free of the deleterious side effects and usually inexpensive. Hence plant derived agents are being used for the healing of cancer (Nema *et al.,* 2012) [18]. Herbal drug treatment can be given to poor people in the rural areas to treat different cancers effectively at an affordable cost (Reddy *et al.,* 2015) [14].

2. Materials and Methods: Collection, Processing and Extraction of Plants

The well-known commonly used Indian spices namely ginger, cinnamon and ajwain were procured from the local markets of Coimbatore. The samples were dried for a week and powdered using a grinder. 15 g of each spice powder was weighed accurately, mixed well and continuously refluxed with ethanol at 120 °C using soxhlet apparatus. The mixture was filtered and evaporated in a water bath at 45 °C. The dried extracts were transferred to separated glass jars and stored at 4 °C in a refrigerator. It was further used for phytochemical screening, quantitative analysis of total phenols, tannins and antioxidant activity and to find the synergistic effect on cancer cell lines.

2.1 Qualitative Analysis of Phytochemicals

The spice extracts were tested for several phytochemicals using standard procedures as quoted by Mostafavi and Pezhhanfar (2015) [11] to identify the components.

2.2 Alkaloids

(i) Hager's Test: To 0.5 ml of spice extract, a few drops of Hager's reagent was added. Formation of yellow precipitate confirms the presence of alkaloids. (ii) *Wagener's Test:* To 0.5 ml of spice extract, the Wagener's reagent was added (Solution of Iodine in Potassium Iodide). A reddish brown precipitate confirms that the test is positive.

2.3 Carbohydrates

Benedict's test: To 0.5 ml of spice extract, 1.25 ml of Benedict's solution was added and boiled for 5 minutes. Formation of brick red precipitate indicates the presence of carbohydrates. (ii) *Molisch's Test:* To 0.5 ml of spice extract, few drops of alcoholic α-naphthol in alcohol were added followed by 0.2 ml of concentrated sulphuric acid along the sides of test tubes. Reddish-violet ring at the junction of two layers indicates the presence of carbohydrates.

2.4 Flavonoids (i) *Ferric Chloride Test:* To 0.5 ml of spice extract, a few drops of ferric chloride were added. The presence of green coloration indicated the presence of flavonoids. (ii) *Lead Acetate Test:* To 0.5 ml of spice extract, few drops of 10% lead acetate solution were added. Formation of a yellow precipitate showed the presence of flavonoids. (iii) *Alkaline Reagent Test:* To 0.5 ml of spice extract, few drops of sodium hydroxide solution were added. A yellow colouration which turns colourless by addition of few drops of dilute acetic acid indicates the presence of flavonoids.

2.5 Cardiac Glycosides

(i) Chloroform Test: 0.5 ml of spice extract was dissolved in 2ml of chloroform and concentrated sulphuric acid was carefully added to form a layer. Deep reddish brown colour at the interface of steroid ring indicates the presence of cardiac glycosides. (ii) *Keller Killaini's Test:* 0.4 ml of glacial acetic acid containing traces of ferric chloride and 0.5 ml of concentrated sulphuric acid were added to the spice extracts carefully. A reddish-brown colour formed at the junction of two layers and the upper layer turned bluish green indicates the presence of glycosides.

2.6 Saponins

(i) Froth Test: A pinch of dried powdered sample was added to 3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicates the presence of saponins. (ii) *Benedict's test:* 0.5 ml of extract was dissolved with 2ml of Benedict's reagent. Blue black precipitate indicates the presence of saponins.

2.7 Steroids and Terpenoids

(i) Salkowski Test: 0.5 ml of extract was treated with chloroform with few drops of concentrated sulphuric acid, shaken well and allowed to stand for some time. Red colour at the lower layer indicates the presence of sterols and the formation of yellow coloured lower layer indicates the presence of terpenoids. (ii) *Chloroform Test:* 0.5 ml of extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown indicates the presence of terpenoids.

2.8 Tannins

(i) Lead Acetate Test: To 0.5 ml of spice extract, few drops of 10% lead acetate solution were added. Formation of a yellow precipitate showed the presence of flavonoids.

(ii) Ferric Chloride Test: To 0.5 ml of spice extract, a few drops of ferric chloride were added. The presence of green coloration indicated the presence of flavonoids.

2.9 Starch

Iodine Test: To 0.5 ml of extract, iodine as a reagent was added. Appearance of dark blue colour which disappeared on heating and reappears on cooling indicates the presence of starch.

3.10 Phenolic Compounds

(i) Lead Acetate Test: To 0.5 ml of spice extract, few drops of 10% lead acetate solution were added. Formation of a yellow precipitate showed the presence of flavonoids.

(ii) Ferric Chloride Test: To 0.5 ml of spice extract, a few drops of ferric chloride were added. The presence of green coloration indicated the presence of flavonoids.

3.11 Amino Acids

(i) Millon's Test: To 0.5 ml of extracts 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) was added. White precipitate appears which turns red when gentle heating. (ii) *Ninhydrin Test:* To 0.5 ml of extract few drops of 5% Ninhydrin was added and then boiled. Appearance of violet colour indicated the presence of amino acids.

3.12 Proteins

(i) Biuret Test: To 0.5 ml of extracts, 4% NaOH solution and few drops of 1% $CuSO_4$ solution were added. Violet colour appears, indicated the presence of protein. (ii) *Millon's Test:* To 0.5 ml of extracts 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) was added. White precipitate appears which turns red when gentle heating.

3.14 Coumarins

To 0.5 ml of spice extract, 10% sodium hydroxide solution was added. The appearance of yellow colour indicates the presence of Coumarins.

3.15 Phlobatannins: To 0.5 ml of spice extract, 10% ammonia solution was added. Appearance of pink colour indicates the presence of phlobatannins.

3.16 Anthraquinones

To 0.5 ml of spice extract, few drops of 2% Hydrochloric acid were added. Red colour precipitate indicates the presence of anthraquinones.

3.17 Quinones

To 0.5 ml of spice extract, few drops of concentrated hydrochloric acid were added carefully along the sides of the test tube. Formation of yellow precipitate or colouration indicates the presence of quinones.

3.18 Oxalates

To 2 ml of extracts, few drops of glacial acetic acid were added. A greenish black coloration indicates the presence of oxalates.

3.19 Quantitative Analysis
3.19.1 Estimation of Total Phenols
Different aliquots of 0.2-1.0 ml of gallic acid working standard of concentration 10-20 µg/ml were pipetted into different test tubes. Volume was made to 1 ml with distilled water. All the above test samples were made up with 1.5 ml of Folin Ciocalteau Reagent and 1.2 ml of sodium carbonate. Blank was prepared by mixing 1 ml of distilled water, 1.5 ml of Folin reagent and 1.5 ml of sodium carbonate. The tubes were incubated in room temperature for 4 minutes. OD was read at 765 nm for the above standard and samples. A graph of optical density versus concentration of gallic acid (µg/ml) was plotted and the unknown concentration of samples was determined from the standard graph.

3.19.2 Estimation of Tannins
The tannins were determined by Folin Ciocalteau Method. About 0.5 ml of sample was added to a volumetric flask containing 7.5 ml of distilled water and 0.5 ml of Folin reagent. 1 ml of 35% sodium carbonate solution was added and made to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solution of gallic acid (10, 20, 30, 40 and 50 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/g of extract.

3.19.3 Analysis of Total Antioxidants
Different aliquots of 0.4-2.0 ml of ascorbic acid working standard of concentration 0.228-1.14 mM were pipetted into different test tubes. 4 ml of reagent solution was added and the volume was made up to 2.0 ml with distilled water. The test tubes were incubated in boiling water bath for 90 minutes. OD was read at 695 nm for the above standard and samples. A graph of optical density versus concentration of Ascorbic acid (mM) was plotted and the unknown concentration of samples was determined from the above standard graph.

3.19.4 Cell Culture and Treatment
The human lung cancer cell line was obtained from the National Centre for Cell Science (NCCS), Pune, Maharashtra, India were free from any kind of bacterial and fungal contamination. It was grown in Eagles Minimum Essential Medium (EMEM) containing 10% Fetal Calf Serum (FBS) or Fetal Bovine Serum (FBS). All cells were maintained at 37°C, 5% carbon dioxide, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly and the culture medium was changed twice a week.

3.19.5 Determination of cell concentration and viability by Trypan Blue Exclusion Method
L cells were treated with different concentrations of spice extracts for 2 days. At the end of the treatment period, the cells were counted with the aid of a haemocytometer and cell viability was determined by trypan blue exclusion method. Trypan blue was prepared at a concentration of 0.4% in phosphate buffered saline. The percentage cytotoxicity is calculated using the following formula:

$$\% \text{ Cytotoxicity} = \frac{\text{Number of dead cells} - \text{Number of live cells}}{\text{Total cell count}} \times 100$$

3.19.6 MTT (3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) Assay
MTT salt or 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was reduced by mitochondrial dehydrogenases to water insoluble formazans. Viable cell number/well is directly proportional to formazans production. L cells were cultured in 96-well microtiter plates and were treated with various concentrations of spices extract and incubated for 48 hours. At the end of the treatment period, 20 µl of MTT was added and the plates were incubated for 3 h in dark followed by the addition of 200 µl of DMSO. The absorbance was read spectrophotometrically at 630 nm in an ELISA reader (Mosmann, 1983) [15]. The Percentage Inhibition is calculated using the following formula:

$$\% \text{ Inhibition} = \frac{1 - \text{Absorbance of the Control}}{\text{Absorbance of the Test}} \times 100$$

4. Results
4.1 Qualitative Analysis
The results concerning the qualitative analysis of the spice extract are presented in Table 1. The qualitative analysis carried out on the consortium of spices i.e., ginger, cinnamon and ajwain revealed the presence of the phytonutrients namely alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, steroids, terpenoids, tannins, coumarins, phlobatannins and anthraquinones. The identification of the above compounds supports the use of these spices in traditional medicine as these compounds have valuable antifungal, antibacterial and anti-inflammatory properties (Hassanshshian *et al.,* 2014; Gilani *et al.,* 2005) [7, 10].

Table 1: Qualitative Analysis

S. No	Phytoconstituents	Analysis
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Flavonoids	+
4.	Glycosides	+
5.	Saponins	+
6.	Steroids and Terpenoids	+
7.	Tannins	+
8.	Starch	+
9.	Phenolic Compounds	+
10.	Amino Acids	+
11.	Proteins	+
12.	Coumarins	+
13.	Phlobatannins	+
14.	Anthraquinones	+
15.	Quinones	+
16	Oxalate	-

Key: + = Present and - = Absent

4.2 Quantitative Analysis: Total Phenols, Tannins, and Antioxidants
The results of quantitative assay for total phenol and tannin content are shown in Figure 1.

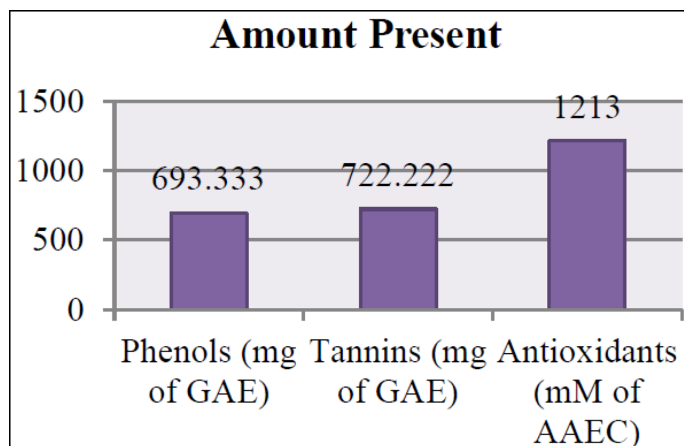

Fig 1: Total Phenols and Tannin Content

The total phenolic, tannin and antioxidants content of consortium of spices were 693.333 mg, 722.222 mg of gallic acid and 2.42% in terms of percentage of free radical scavenging capacity.

4.3 *In vitro* Cytotoxic Assay: Trypan Blue Test

Table 2: Results of Trypan Blue Test

Concentration (µg/ml)	% Cytotoxicity
Control	23.07
50	51.89
100	100
125	100
150	100
300	100

The percentage of cytotoxicity was tested on primary Peripheral Blood Mononuclear Cells (PBMC), for the toxic effect of the spice mix at varying concentrations between the chosen 50-300 µg/ml. Since Peripheral Blood Mononuclear Cells are mortals, prolonged exposure to extract may have resulted in cell death. In the untreated, the effect of the compounds showed 23.07% inhibition and as the concentration of the extract increased the percentage inhibition doubled from 50-100% at 100µg/ml concentration or above. 50µg/ml concentration showed 51% inhibition in cell viability. This could be partly due to the inherent senescence set in the primary cultures.

4.4 *In vitro* Cytotoxic Assay: MTT Assay

The Inhibition Percentage was tested by MTT assay. It was carried out on varying concentrations of the extract ranging from 50-300µg/ml. The concentrations of the extract ranges were 50µg/ml, 100µg/ml, 150µg/ml and 300 µg/ml exhibiting 58.72%, 58.28%, and 59.21% and 54.3% inhibition respectively.

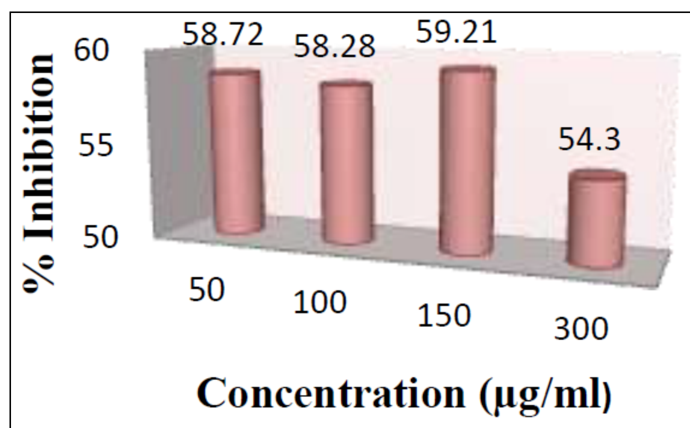

Fig 2: Results of MTT Assay

The concentration ranging from 25-50 µg/ml would be effective in inhibiting the proliferation of cancer cells. This is confirmed from Figure 2 wherein increasing the concentration of the spice extract has not markedly increased the percent inhibition. The trend of inhibition shows a plateaued effect despite increase it from 50 µg/ml to 150 µg/ml while it shows a steep drop in the percent inhibition level to 54.3%. Concentrations above 50 µg/ml are hazardous to normal healthy cells.

5. Discussions

5.1 Qualitative Analysis

Phenolic compound is a natural antioxidant originating from several fruits help in protection against chronic diseases (Proestos *et al.,* 2006) [17]. For example, alkaloids protect against chronic diseases (Mir *et al.,* 2013) [2]. Many of these compounds inhibit tumor growth, anti-parasitic effect, antibacterial, anti-neoplastic activity, anti-inflammatory, antiallergic and antiviral activity (Alan and Miller, 1996) [1].

Several studies on *Zingiber officinale, Cinnamomum verum* and *Trachyspermum ammi* revealed the abundant presence of phytochemicals such as alkaloids and saponins; tannins and glycosides in fair amounts; moderate amounts of terpenoids and phlobatannins and absence of steroids (Bhagya *et al.,* 2015) [4]. However, the current study showed the presence of all the phytochemicals listed in the table. Thus the present study showed the pharmacologically potential compounds like Tannins, Alkaloids, Anthocyanin, Carbohydrates, Glycosides, Flavonoids, Phenols, Steroids, Coumarins, Saponins, Terpenoids, phlobatannins and anthraquinones.

5.2 Quantitative Analysis
5.2.1 Total Phenols
The total phenolic content plays an important role in the composition of the samples. According to Denre (2014) [13], *Zingiber officinale* showed lower phenolic content of 15.52 mg/g as compared to the phenolic content of the consortium of spices of present study which had 693.333 mg GAE/g. The phenolic content of *Trachyspermum ammi* and *Zingiber officinale* as reported by Shahin and Ahmad (2014) [24] was found to be 13.56 and 10.3 in terms of mg of GAE/gram which was lower when compared with the results of the present study in the extract of consortium of spices. This higher level of phenols from the current study proves their effective role as anti-inflammatory, antioxidant and anti-analgesic effect in preventing chronic diseases including cancer.

5.2.2 Tannins
Tannins are natural polyphenol ubiquitously distributed in plants, such as vegetables, fruits and seeds (Chikezie *et al.,* 2008) [5]. It exerts anti-inflammatory, anti-irritant, anti-scretolytic, anti-parasitic, anti-microbial, anti-viral, antibacterial, antiparasitic activities, quickens the healing of wounds and is also beneficial when applied to the mucosal lining of the mouth (Stephane *et al.,* 2004) [26]. This justifies their usage in herbal medicine. According to Eleazu *et al.,* (2012) [6], the tannin content of ginger in terms of mg/100g was found to be 0.43 which is found to be extremely lower when compared to the current results in which 722.222 mg/100g of tannin was present in consortium of spices and according to Sakagami *et al.,* (2000) [21], tannins induced apoptotic cell death characterized by DNA fragmentation. Therefore, consumption of the spice extract prepared with the consortium of spices may be beneficial to cure cancer.

5.2.3 Antioxidants
Natural anti-oxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases and have been identified as potential modifiers of cancer (Prior and Cao, 2000) [20]. Antioxidant activity is due to the presence of phenolics, flavones, isoflavones, flavonoids, anthocyanin, coumarins, lignans, catechins and isocatechins. From the study conducted by Shahin and Ahmad (2014) [24], *Zingiber officinale* showed 49 mM Fe+2 / g which is lower compared to the antioxidant content of the extract of consortium of spices of the present study. It is therefore clear that since the antioxidant content of the consortium of spices of the present study is high, consumption of such a combination of spices may help in lowering cell damage and thus prevents the growth of cancer cells.

5.3 *In vitro* Cytotoxic Assay
5.3.1 Trypan Blue Test
The concentrations ranging from 25-50µg/ml could be effective in inhibiting the proliferation of cells which has to be validated by further experiments. A concentration above 50µg/ml has a possibility of affecting the primary cells. This effect can be prevented by the use of stabilizers and binders with a targeted release of the active principles present in spice mix. Examples of such binders include starch, matrices like poly (lactic-co-glycolic acid) (PLGA), nanoparticle conjugate, etc. The use of starch has both positive and negative effects on the targeted delivery of the active components. The positive effect is that it releases the active principles in a controlled manner while the negative effect was that it gets degraded by the intestinal flora and is unable to be utilized by all the subjects. Rhode *et al.,* (2007) [12] has concluded that continuous ginger exposure resulted in profound inhibition of cell proliferation and growth in ovarian cancer cells *in vitro* at doses of 50µg/ml which is comparable with the present study.

5.3.2 MTT Assay
The current study indicated that the proliferation of cancer cells will be inhibited effectively at concentrations ranging between 25-50µg/ml whereas the study by Abdullah *et al.,* (2010) [25] showed that ginger extract significantly inhibited the proliferation of both colon cancer cells in a dose dependent manner. Proliferation of HCT 116 cells decreased when treated with ginger extract resulting in a 50% reduction at 496 ± 34.2 µg/ml, while HT 29 cells showed significant decrease in proliferation with 50% reduction at 455 ± 18.6 µg/ml of ginger extract. It is therefore clear from the current study that the concentration ranging from 25-50 µg/ml would be effective in inhibiting the proliferation of cancer cells. Concentrations above 50 µg/ml may not be necessary.

6. Conclusion
Spices have a wide variety of phytochemicals that possess pharmacological activities. Individual spices have different chemical components that have beneficial effect on preventing diseases while the consumption of spices in combination shall have a synergistic effect on reducing the proliferation of cancer cells. In the long run, it is known to scavenge free radicals by producing antioxidants and preventing other diseases such as cardiovascular disease, diabetes, cataract, etc.

7. References

1. Alan L, Miller ND. Antioxidant Flavonoids: Structure, function and chemical usage. Alternative Medicine Review. 1996; 1(1):103-111.
2. Amin Mir M, Sawhney SS, Jassal MMS. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. Woodpecker Journal of Pharmacy and Pharmocology. 2013; 2(1):1-5.
3. Arshad Rahmani H, Fahad Al, Shalarmi M, Slalh Aly M. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. International Journal of Physiology Patho Physiology Pharmacology. 2014; 6(2):125-136.
4. Bhagya HP, Raveendra YC, Lalithya KA. Multibeneficial uses of spices: A brief review. Acta Scientifica Journal of Food and Nutrition. 2015; 1(1):3-7.
5. Chikezie PC, Agomuo EN, Amadi BA. Biochemistry, Practical/Research Method, a Fundamental Approach. 2nd (5), Mega soft publishers, New Delhi. 2008, 51-53.
6. Eleazu CO, Eleazu KC, Awa E, Chukwuma SC. Comparative study of the phytochemical composition of

the leaves of five Nigerian medicinal plants. Journal of Biotechnology and Pharmaceutical Research. 2012; 3(2):42-46.

7. Gilani AH, Jabeen Q, Ghayur MN, Janbaz KH, Akhtar MS. Studies on the antihypertensive, antispasmodic, bronchodilator and hepato protective activities of the *Carum copticum* seed extract. Journal of Ethno pharmacology. 2005; 98(5):127-135.
8. Harsharan Pal Singh, Sumeet Gullaiya, Ishpreet Kaur. Traditional Indian spices and their role as anti-cancer agents: A review and evidence based approach. International Journal of Pharmacy and Integrated Life Sciences. 2014; 2(2):1-10.
9. Haseena S, Manjunath Aithal, Kusal Das K, Shaik Hussain Saheb. Phytochemical analysis of *Nigella sativa* and its effect on reproductive system. Journal of Pharmaceutical Sciences and Research. 2015; 7(8):514-517.
10. Hassanshshian M, Bayat Z, Saeidi S, Shiri Y. Antimicrobial activity of *Trachyspermum ammi* essential oil against human bacterial. International Journal of Advanced Biological and Biomedical Research. 2014; 2(1):18-24.
11. Hossein Mostafavi, Sakha Pezhhanfar. Qualitative phytochemical analysis of ajwain (*Trachyspermum ammi*) from north-west Iran. International Research Journal of Pharmacy. 2015; 6(9):610-615.
12. Jennifer Rhode, Sarah Fogoros, Suzanna Zick, Heather Wahl, Kent Griffith A, Jennifer Huang *et al*. Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. BMC Complementary and Alternative Medicine. 2007; 7(44):2-9.
13. Manas Denre. The determination of vitamin-C, total phenol and antioxidant activity of some commonly cooking spices crops in West Bengal. International Journal of plant physiology and biochemistry. 2014; 6(6):66-70.
14. Mohan Reddy N, Rajashekhar Reddy N, Kaiser Jamil. Spicy anti-cancer spices: A review. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 7(11):1-6.
15. Mosmann TP. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983; 65(15):55-63.
16. Parvinnesh Kumar S, Paden Febriyanti M, Ferry Sofyan F, Dimas Luftimas E, Rizky Abdulah. Anticancer potential of *Syzygium aromaticum* L. in MCF-7 human breast cancer cell lines. Pharmacognosy research. 2014; 6(4):23-29.
17. Proestos C, Boziaris IS, Nychas GJE, Komaitis M. Aims and Scopes of food chemistry. Food Chemistry. 2006; 95(4):664-671.
18. Rajeev Nema, SaritaKhare, Dharmendra Singh, Parul Jain, Alka Pradhan, Abishek Gupta. Green source: A power of nature to cure cancer. International Journal of Phytotherapy. 2012; 2(2):111-116.
19. Robert Thomas, Elizabeth Butler, Fabio Macchi, Madeine Williams. Phytochemicals in cancer prevention and management. British Journal of Medical Practitioners; 8(2):1-8.
20. Ronald Prior L, Guohua Cao. Antioxidant phytochemicals in fruits and vegetables: diet and health implications. Hort Science. 2000; 35(4):588-592.
21. Sakagami H, Jiang Y, Kusama K, Atsumi T, Ueha T, Toguchi M *et al*. Cytotoxic activity of hydrolysable tannins against oral tumor cell lines-a possible mechanism. Phytomedicine. 2000; 7(1):39-47.
22. Samir Zaahkouk AM, Ezzat Aboul Ela I, Ramadan MA, Sayed Bakry, Ahmed Mhany BM. Anti-carcinogenic activity of methanolic extract of fennel seeds (*Foeniculum vulgare*) against breast, colon, and liver cancer cells. International Journal of Advanced Research. 2015; 3(5):1525-1537.
23. Sasikala Sasikumar, Kannan Eagappan. Nutri-Cognosy in cancer. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(3):23-29.
24. Shadma Shahin, Naheed Ahmad. Antioxidant Properties and Total phenolic content of herbs used in post-partum diet therapy in Patna (Bihar), India. IOSR Journal of Pharmacy and Biological Sciences. 2014; 9(2):17-20.
25. Shailah Abdullah, SitiAmalina Zainal Abidin, Noor Azian Murad, Suzana Makpol, Wan Zurinah Was Ngah, Yasmin Anum Mohd Yusof. Ginger extract (*Zingiber officinale)* triggers apoptosis and Go/G1 cells arrest in HCT 116 and HT 29 colon cancer cell lines. African Journal of Biochemistry Research. 2010; 4(4):134-142.
26. Stéphane Q, Tatiana V, Diana K, Michael J, Patrick P, Christian B. Main structural and stereo chemical aspects of the antiherpetic activity of nonahydroxyterphenoyl-containing C glycosidic ellagi tannins. Chemistry and biodiversity. 1(2):247-58.