Garlic – A Natural Source of Cancer Preventive Compounds.

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Garlic – A Natural Source of Cancer Preventive Compounds

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Abstract

Several epidemiological observation and a number of laboratory studies have indicated anticarcinogenic potential of garlic, which has been traditionally used from time immemorial for varied human ailments in different parts of the globe. The anticarcinogenic properties of garlic have been attributed to a wide variety of chemical compounds identified to be present in garlic but most studies have focused on specific thioallyl constituents. Garlic components have been found to block covalent binding of carcinogens to DNA, enhance degradation of carcinogens, have antioxidative and free radical scavenging properties and to regulate cell proliferation, apoptosis and immune responses. In view of the variety of effects produced by garlic and its chemical constituents, renewed interest has been generated in investigating its medicinal properties, particularly with reference to cancer prevention and prophylaxis. There are a number of mechanisms at work which jointly are responsible for eliciting the anticarcinogenic effects noted in laboratory studies in a wide range of experimental systems. This has opened up a new avenue for researchers in the field of cancer chemoprevention and merits further scrutiny to establish the role of garlic in prevention of human cancers.

Key Words: Garlic - anticarcinogenesis - allylsulfides - alliin - allicin - ajoene - cancer chemoprevention

Introduction

Plants form an important source of novel chemical compounds with medicinal properties, many of which have been used for prevention and treatment of a variety of human ailments from time immemorial. Experimental and epidemiological studies over the past few decades have provided ample evidence in support of associations between plant food intake and reduced cancer risk. Many phytochemicals are proven to have anticancer activities and many are in use for cancer therapeutics. In view of the modulatory role of many minor dietary constituents and non-nutrient phytochemicals obtained from plants used as food, beverages and spices, on the detoxifying enzyme system, attention has focused on natural flavouring items in search of cancer chemopreventive compounds. Among them garlic has been of much interest, mostly due to the epidemiological reports which linked increased garlic consumption with reduced prevalence of many human diseases, particularly cancers of stomach, colon, breast and prostate and also because of the presence of a number of potential anticarcinogenic compounds viz. allylsulfides, quercen, kaempferol, and linolenic acid.

Garlic or Allium sativum Linn. (Alliaceae), originally indigenous to Asia, is now widely cultivated worldwide for the fleshy segments (clove) of its bulbs which are used as a condiment especially in Asian cuisine. Garlic and its various preparations may offer simple remedies for ailments from common cough and colds to whooping cough and other pulmonary diseases, skin troubles, gastrointestinal disorders, for averting premature ageing and for improving immunity. Scientific research has revealed that garlic or its constituents indeed have a broad range of biological activities including immune stimulation, anticarcinogenic and anti tumour activity besides being natural antibiotics with antiviral, antibacterial and antifungal actions. In recent times effects of garlic on cardiovascular diseases have been much publicized.

Chemical analyses have indicated that garlic bulbs are the source of many compounds with medicinal properties
Several experimental studies on animals and cultured cells have demonstrated the anticarcinogenic effect of garlic and its chemical compounds. Oral feeding of garlic extracts has been shown to reduce the incidence and growth of transplantable and spontaneous tumours in experimental animals and the active components were found to influence a number of physiological and immunological functions which account for their anticarcinogenic and antitumour effects.

When garlic oil was topically applied during the initiation phase of benzo(a)pyrene (BP) induced skin carcinogenesis in mice, a decline was noted in the incidence and multiplicity of tumours (Sadhana et al 1988). Oral administration of fresh water extract of garlic was shown to result in reduction of chemically induced cervical carcinomas in mice (Hussain 1990). Garlic treatment inhibited development of murine transitional cell carcinomas significantly (Riggs et al 1997) and an aqueous extract effectively suppressed dimethylbenz(a)anthracene (DMBA) induced oral carcinogenesis in hamsters by modulation of lipid peroxidation and glutathione (GSH), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) levels (Balasenthil et al 1999). Selenium enriched garlic was found to significantly suppress development of premalignant lesions and formation of adenocarcinomas in the mammary glands of carcinogen treated rats (Ip et al 2000). Our group has also observed that fresh garlic juice administered orally can prevent development of azoxymethane (AOM) induced aberrant crypt foci and adenocarcinoma in rat colon (Ghosh et al 2002, Sengupta et al 2002 a & b). Samaranayake and his group (2002) reported that garlic inhibited diethylnitrosamine (DEN) induced hepatocarcinogenesis in Wistar rats. Garlic juice given orally during exposure to DMBA could reduce clastogenicity in mice (Sengupta et al 2002 c) and also AOM induced micronuclei formation in the rat (Khanum et al 1998).

The anticarcinogenic action of garlic has been attributed to a number of factors. Dietary garlic can suppress production of DNA-adducts by DMBA and nitroso compounds (Liu et al 1992). Feeding of fresh garlic or garlic oil supplemented diet was found to modulate detoxification enzymes and reduce hepatic lipid peroxidation in rats treated with AOM (Khanum et al 1998). Our studies have also provided support for this observation. Liu et al (1992) further demonstrated that garlic powder increases glutathione content and GST activity in rat liver and mammary tissues. Oral administration of garlic was shown to significantly increase the level of many detoxification enzymes viz. GST, acid soluble sulfhydryl (-SH) cytochrome b5 and cytochrome P450 in murine liver (Singh and Singh 1997). Inhibition of cell proliferation and induction of apoptosis was also noted during colon carcinogenesis following treatment with fresh garlic juice (Sengupta et al 2002 a&b, Ghosh et al 2002).

Garlic is a rich source of a wide variety of organosulfur compounds which can undergo further chemical modifications when garlic is crushed, cut or minced. Allicin, a diallylsulfinothiolate which imparts much of garlic’s pungent characteristics (Stoll and Seebeck 1951), is considered to be the precursor compound from which other thioallyl compounds are derived (Block 1985). Experimental investigations have implicated specific thioallyl constituents and their derivatives regarding the anti-cancer actions of garlic (Dorant et al 1993, Milner 1996), although many other cancer chemopreventive compounds are also known to be present. The efficacy of various garlic derived compounds in inhibiting experimental carcinogenesis has been investigated by many. It was demonstrated that diallylsulfide (DAS), diallyltrisulfide (DAT), allylmethylidisulfide (AMD) and allylmethyltrisulfide (AMT) inhibit gastric malignancy induced by BP in mice (Sparnins et al 1986,1988). Wattenberg et al (1989) reported that DAS, disulfide derivatives (DADS), AMT and S-allylcysteine (SAC) could reduce nitroso compound induced forestomach tumours in mice and Wargovich et al (1988) had noted that these compounds inhibited squamous cell carcinoma in oesophageal tissue in rat. Fukushima et al (1997) analysed the potential of several organosulfur compounds present in garlic and onion and observed inhibitory effect of DAS on renal and colon carcinogenesis in rat induced by diethylnitrosamine (DEN). However they observed that DAS, DAT and AMT enhanced formation of altered hepatocellular foci and commented that some of the organosulfur compounds may have promotional potential at certain sites. In another study SAC failed to elicit any action during N-methyl nitrosourea induced mammary tumourgenesis in rat (Cohen et al 1999) although Amagase and Milner (1993) reported that garlic components could prevent DMBA induced mutagenesis and tumourgenesis in mammary gland. Several studies have indicated that allylsulfide derivatives can inhibit growth of transplantable tumours (Welch et al 1992, Takeyama et al 1993, Sundaram and Milner 1996) and can also ameliorate cardio and hepatotoxic effects of the potent anticancer drug Doxorubicin (Mostafa et al 2000). It was suggested that clinical efficacy of doxorubicin could also be improved by using the anticancer drug with DADS which inhibited liver microsomal lipid peroxidation (Dwivedi et al 1998). DADS was found to suppress growth of human colon tumour cell.  

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with varied pharmacological functions thus supporting many of the traditional medicinal uses of garlic (Ross, 1999). The major constituents of garlic are alliin, allicin, ajoene, diallylsulfide, diallyldisulfide, diallyltrisulfide, 2-vinyl-4H-1,2 dithin, linoleic acid, scordinins and selenium (Li, 2000). Recent studies, including experiments conducted in our own laboratory, have indicated a possible role of garlic and its components in modulation of carcinogenesis thereby drawing attention of researchers in cancer chemoprevention to this plant.

**Garlic and Anticarcinogenesis**

Garlic is a rich source of a wide variety of organosulfur compounds which can undergo further chemical modifications when garlic is crushed, cut or minced. Allicin, a diallylsulfinothiolate which imparts much of garlic’s pungent characteristics (Stoll and Seebeck 1951), is considered to be the precursor compound from which other thioallyl compounds are derived (Block 1985). Experimental investigations have implicated specific thioallyl constituents and their derivatives regarding the anti-cancer actions of garlic (Dorant et al 1993, Milner 1996), although many other cancer chemopreventive compounds are also known to be present. The efficacy of various garlic derived compounds in inhibiting experimental carcinogenesis has been investigated by many. It was demonstrated that diallylsulfide (DAS), diallyltrisulfide (DAT), allylmethylidisulfide (AMD) and allylmethyltrisulfide (AMT) inhibit gastric malignancy induced by BP in mice (Sparnins et al 1986,1988). Wattenberg et al (1989) reported that DAS, disulfide derivatives (DADS), AMT and S-allylcysteine (SAC) could reduce nitroso compound induced forestomach tumours in mice and Wargovich et al (1988) had noted that these compounds inhibited squamous cell carcinoma in oesophageal tissue in rat. Fukushima et al (1997) analysed the potential of several organosulfur compounds present in garlic and onion and observed inhibitory effect of DAS on renal and colon carcinogenesis in rat induced by diethylnitrosamine (DEN). However they observed that DAS, DAT and AMT enhanced formation of altered hepatocellular foci and commented that some of the organosulfur compounds may have promotional potential at certain sites. In another study SAC failed to elicit any action during N-methyl nitrosourea induced mammary tumourgenesis in rat (Cohen et al 1999) although Amagase and Milner (1993) reported that garlic components could prevent DMBA induced mutagenesis and tumourgenesis in mammary gland. Several studies have indicated that allylsulfide derivatives can inhibit growth of transplantable tumours (Welch et al 1992, Takeyama et al 1993, Sundaram and Milner 1996) and can also ameliorate cardio and hepatotoxic effects of the potent anticancer drug Doxorubicin (Mostafa et al 2000). It was suggested that clinical efficacy of doxorubicin could also be improved by using the anticancer drug with DADS which inhibited liver microsomal lipid peroxidation (Dwivedi et al 1998). DADS was found to suppress growth of human colon tumour cell.
xenografts in athymic nude mice without apparent ill consequences in the host and could reduce toxicity of the antitumour agent 5-fluorouracil (Sundaram and Milner 1996).

Mechanisms of Action of Garlic and Garlic Components

Researches in vivo and in vitro have brought to light several mechanism of action of garlic and its components which can be associated with elicitation of their anticarcinogenic activity noted in a wide range of experimental systems.

Garlic has been claimed to protect from diseases whose pathophysiology is associated with cellular damages due to free oxygen radicals. The organosulfur compounds of garlic have indeed been shown to be potent antioxidants and can also stimulate the detoxifying enzymes in liver. Natural compounds which elevate detoxification enzymes and/or reduce the carcinogen activating enzymes are good candidates for cancer chemoprevention. Shobana and Naidu (2000) reported that several spices including garlic possess antioxidant activity, thereby in addition to imparting flavour to food they provide health benefits by inhibiting lipid peroxidation. An investigation on the scavenging property of garlic preparations against oxygen radicals in human granulocytes, activated with phorbol myristyl acetate (PMA), suggested alliin metabolite allicin to be responsible for the oxygen radical scavenging property of garlic (Siegers et al 1999). Allicin was shown to scavenge hydroxyl radicals and prevent lipid peroxidation in liver homogenates (Prasad et al 1995). The garlic components DAS, DADS and DAT were shown to play a differential modulatory role on the GSH related antioxidant system in rat liver and red blood cells and increased hepatic GST activity. This was found to be accompanied by increased expression of GST ya, yb1 and yc proteins (Wu et al 2001). Protective effect of garlic extracts on hepatic lipid peroxidation during N-methyl-N-nitroso-N-nitrosoguanidine (MNNG) induced gastric carcinogenesis in Wistar rats was considered to be due to enhanced levels of glutathione and glutathione dependent enzymes noted in garlic treated group (Arivazhagan et al 2000). Aged garlic extract containing both water and lipid soluble organosulfur components including S-allylcysteine and S-allylmercaptocysteine is reported to have antioxidant action by enhancing cellular antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and GPx (Borek 2001). We also noted enhanced activity of GST and GPx and reduction in lipid peroxidation during AOM induced colon carcinogenesis in rat following treatment with fresh garlic juice (unpublished data). Garlic organosulfides DAS, DADS, DPS (dipropyl sulfide) and DPDS (dipropyl disulfide) induced the expression of NAD(P)H: quinone oxidoreductase (NQO) an enzyme implicated in the detoxification of activated quinone metabolite of BP (Singh et al 1998). Munday and Munday (1999) reported that DADS can increase tissue activities of phase II detoxification enzymes quinone reductase (QR) and glutathione transferase (GT) in forestomach, glandular stomach, duodenum, jejunum, ileum, caecum, colon, liver, kidney, spleen, heart, lungs and urinary bladder of rats and suggested that such enzyme induction contributed to the protective activity of garlic against GIT cancers. Studies on the effects of organosulfides from garlic against BP-induced forestomach neoplasia revealed a good correlation between their chemopreventive efficacy and their ability to induce mGSTP 1-1 expression in liver (Hu et al 1997). Helen et al (1999) reported that garlic oil supplementation increased antioxidant enzymes and concentrations of glutathione and protected from nicotine induced oxidative damage in rat tissues. Modulation of hepatic drug metabolizing enzymes was noted in rats treated with dimethyl sulfide (DMS), dimethyl disulfide (DMDS), methyl propyl disulfide (MPDS), DPS, DPDS and DADS. Siess et al (1997) suggested a protective effect of alkyl sulfides and diallyl sulfide on the first step of carcinogenesis via the modulation of enzymes involved in carcinogen metabolism. Khanum et al (1998) noted that ingestion of garlic caused a 40% increase in hepatic GPx activity but CAT activity was reduced and no change noted in GST activity by the same feeding regimen. They also recorded an increased gamma glutamyl transpeptidase activity in kidney of rats treated with AOM which could be reduced by 50% following treatment with fresh garlic juice or garlic oil.

The anticarcinogenic action of garlic can also be attributed to its role in preventing DNA-carcinogen adduct formation and activation of carcinogen (Liu et al 1992). It was revealed that water extract of raw garlic, DAS and SAC could reduce BP-DNA adduct formation in stimulated human peripheral blood lymphocytes in vitro (Hageman et al 1997). Garlic powder and allyl sulfur compounds were shown to modify selenite protection against DMBA induced mammary cancer by inhibition of DNA-adduct formation (Schaeffer et al 1997). Inhibition of DMBA-DNA adduct formation by selenium enriched garlic was also reported by Ip and Lisk (1997) and selenium enriched garlic was found to be very effective in prevention of premalignant lesion and formation of adenocarcinoma of rats (Ip et al 2000). DAS and DADS were reported to inhibit DNA adduct formation with N-acetyl-2-aminofluorene in human bladder tumour cells (Chung 1999).

It is interesting to note that production of heterocyclic aromatic amines (HAA-s) during high temperature cooking of meat and fish could be significantly reduced by marination with turmeric-garlic sauce before cooking (Nerurkar et al 1999). Production of N-nitrosomorpholine (NMOR), a known liver carcinogen, was reported to be reduced by water extracts of garlic and deodorized garlic powder (Dion et al 1997).

The anticarcinogenic action of garlic is most likely
mediated by several mechanism of actions of the different chemical components present in the bulb. Investigations have revealed that garlic and its chemical compounds were able to influence cell proliferation and programmed cell death. Experiments on human cancer cell lines have shown that alliin enriched garlic extract along with a garlic powder preparation inhibited or reduced cell proliferation of human hepatoma and colorectal carcinoma cell lines - Hep G2 and Caco 2 when used up to a concentration of 1000 g/ml although they were not effective individually (Siegers et al 1999). Antiproliferative effect of naturally occurring garlic derivatives and synthetic S-cysteinyl compounds resembling garlic constituent was noted on human prostate carcinoma cells – LNCaP and the effect was suggested to be by inhibition of polyamine synthesizing enzyme ornithine decarboxylase. Ajoene, a major compound of garlic was found to induce apoptosis in human leukaemic cells but interestingly not in normal peripheral mononuclear cells from healthy subjects. The effect was dose dependant (Dirsch et al 1998). A stable organosulfur compound of aged garlic extract, S-allylmercaptocysteine (SAMC) produced DNA fragmentation compatible with apoptosis in two erythroleukaemia cell lines HEL and OCIM-1 (Sigounas et al 1997). Exposure of cultured human neoplastic and non-neoplastic lung cells (A549 and MRC-S) for 24 hours to diallyl trisulfide (DATS) caused significant induction of apoptosis as indicated by increased DNA fragmentation (Sakamoto et al 1997). Garlic oil has been shown to induce differentiation and apoptosis of BGC-823, a human gastric cancer cell line and p21 and p53 genes played an important role in the process (Li et al 1998). Studies in our laboratory have shown that administration of fresh garlic juice during exposure to carcinogen AOM can inhibit proliferation as well as induce apoptosis at the target site - colon (Sengupta et al 2002). It was further noted that cyclooxygenase-2 (COX2) expression in rat colon that was enhanced following exposure to AOM could be reduced by garlic juice administered orally (Ghosh et al, unpublished data).

Reactive oxygen species are involved in signal transduction pathways leading to nuclear factor kappa B (NF kappa B) activation involved in regulation of gene transcription The garlic component S-allyl cysteine (SAC) has been shown to inhibit NF kappa B induced by TNF alpha and H2O2 in a dose dependant manner (Geng et al 1997).

Development of cancer is often attributed to a failure of immune surveillance and reduced competence of the immune effector cells like natural killer cells, lymphokine activated killer cells and tumour specific cytotoxic T lymphocytes and macrophages. Therefore agents that help in activating immune responses should also be useful in restraining abnormal cell proliferation and prevent development of tumour. Recent studies have focused on the immunomodulatory role of garlic and its components. It has been suggested that the effect of garlic in prevention of oral precancer in Wister rats induced by 4-nitroquinoiline 1-oxide (4NQO) was by activation of natural killer cells (NK) and T lymphocytes as well as level of IL 2 (Tang et al 1997). Oral administration of DAS, a flavouring component of garlic was found to protect from N-nitrosodimethylamine (NDMA) induced immunosuppression of humoral and cellular responses in BALB/C mice (Jeong and Lee,1998). It has been demonstrated that garlic extract and one of its components S-allyl cysteine (SAC) inhibited nitric oxide production through suppression of iNOS mRNA and protein expression in LPS and IF gamma stimulated murine macrophage cell line RAW264.7; garlic extract also inhibited NO production in activated peritoneal macrophages, rat hepatocytes and rat aortic smooth muscle cells (Kim et al 2001). Kyo et al (2001) noted anti-inflammatory action of aged garlic extract (AGE) as expressed by significant decrease in the antigen specific ear swelling induced by picryl chloride ointment in immunoglobulin IgE mediated allergic mouse model. They also reported increased natural killer and killer activities of spleen cells in Sarcoma 180 bearing mice receiving AGE, suggesting AGE could be a promising immune modifier for maintenance of homeostasis of immune functions. Garlic is reported to stimulate immunity including macrophage activity, natural killer cells, killer cells and LAK cells and to increase production of IL2, TNF and interferon gamma- the cytokine associated with beneficial Th 1 antitumour responses (Lann and Riggs 2000, 20001).

Epidemiological Studies

There are numerous studies to show an inverse relationship between garlic consumption and cancer risk although some population based studies failed to associate use of garlic supplements with increased or decreased prevalence of lung, breast, colon and rectal cancers (Dorant et al 1994; 1995;1996). Based on their studies in Shangdong Province of Northeastern China, a high incidence region for stomach cancer, You et al (1988; 1989) reported that garlic and other vegetables reduced the risk for stomach cancer. An inhibition of progression of precancer gastric lesion by garlic preparation was also noted with no difference of side effects between placebo and treatment groups (You et al, 2001). A multicentric study in Italy also revealed that risk of stomach cancer declined with increased intake of garlic (Buiatti et al 1989). Regular consumption of garlic was shown to be associated with decreased prevalence of adenomatous polyps in colon and rectum (Witte et al 1996), thereby implicating the role of garlic in prevention of colorectal cancer. Helicobacter pylori infection is now accepted as a high risk factor for gastric cancer and treatment of Helicobacter pylori infection is one approach for prevention of GIT cancers. You et al (1998) reported that garlic consumption had a protective effect and an inverse association with Helicobacter pylori infection and suggested that this flavouring plant may prevent development and progression of advanced gastric precancer lesions. O’Gara et al (2000) assessed the anti- Helicobacter pylori potential of a variety of garlic substances and noted widely differing

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anti-*Helicobacter pylori* effects and found allicin to be more potent than DAD. Sivam (2001) also demonstrated that *Helicobacter pylori*, including some antibiotic resistant forms are susceptible to garlic extract. From all these reports it may be suggested that garlic and its active compounds should be considered potential candidates for chemoprevention of GIT cancers and need to be explored further. However, Graham et al (1999) failed to observe any effect of garlic on *Helicobacter pylori* infection in otherwise healthy adults. Case referent studies of high epidemic and low incidence areas in Jiangson Province of China on the association of allium vegetable intake with GI tract cancers revealed an important and protective role of allium vegetables not only against stomach cancer but also oesophageal cancers (Gao et al 1999; Tajima 2001). In this connection it would be of interest to note the report by Nerurkar (1999) which shows reduced formation of heterocyclic aromatic amines (2-amino-1-methyl-6-phenylimidazo 4,5-f quinoxaline) produced during high temperature cooking of meat and fish, which is known to be associated with formation of potent mutagens implicated in the etiology of colorectal cancer, following marination with turmeric-garlic sauce before cooking. Reports are also available on the risk reducing influence of garlic on cancer at other sites. A case control study conducted in England (Key et al 1997) reported that garlic consumption could significantly reduce risk of prostate cancer. A French case-control study involving patients with primary breast cancer revealed that increased consumption of fibre and allium vegetables was associated with reduced risk (Challier et al 1998). A meta analysis of epidemiological literature on the association between garlic consumption and risk of head and neck, lung, breast, prostate, stomach and colon cancer suggested a possible protective effect of both raw and cooked garlic consumption against stomach and colorectal cancers only (Fleischauer et al 2000). A study to assess the anticarcinogenic potential of raw garlic (Hageman et al 1997) in humans using genetic biomarker revealed that carcinogen DNA adduct formation in ex-vivo Benzo-a-pyrene treated PBL obtained from human volunteers was significantly reduced in those receiving cucumber salad containing 3 gms of raw garlic. However in this study no evidence was found for antioxidative effect of garlic consumption. In another study, water extract of raw garlic and S-allylcysteine, an organosulphur compound of garlic, were shown to reduce BP-DNA adduct in stimulated PBL and this effect was attributed to increased GST activity as well as a more efficient repair of the adduct (Hageman et al 1997). The protective action of raw garlic and its components against carcinogen induced genetic damage may account for the risk reducing influence of garlic on human cancers. Habitual consumption of garlic was also reported to reduce gastric nitrite content and suppress urinary excretion of N-nitrosoproline (Mei et al 1982; 1989). These observations are possibly related to the protective effect of garlic on GIT cancers.

The information from human studies available to date, though limited, is enough to at least project the beneficial role of garlic against human cancer and attract attention for further studies to establish the role of garlic in prevention and prophylaxis of human cancers. The amount and duration of garlic intake (raw, cooked or processed) alone or in combination with other food components need to be considered at length and the major adverse effects if any need to be determined while trying to evaluate the cancer risk reducing capability of garlic and its active chemical compounds.

**Conclusions**

A survey of literature strongly indicates that garlic and its chemical compounds which elicit a wide range of biological activities associated with anticarcinogenesis and cancer prevention merit more focused attention. The Egyptians worshiped garlic and the Greek chewed it to get strength. In Asian countries it was recognized for its medicinal properties and also used for flavouring of food. Now scientists have revealed many health protective effects of garlic including prevention of cancer. It has become quite evident that the white bulb of garlic, though not a panacea for cancer, is packed with cancer chemopreventive substances and should prove to be not just a flavouring agent but also a natural cancer preventive formula. This beneficial plant part therefore is worthy of serious consideration for further investigation and clinical trials with respect to prevention and treatment of human cancer.

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