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Screening of Flavonoids in Cauliflower Leaves (*Brassica Oleracea Var. Botrytis*) Using HPLC

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Abstract

Flavonoids have been characterized naturally from various plants. The majority of their functions result from their strong antioxidative properties. Stressing on the health complication in today's society, it is within reason to believe that flavonoids could play a significant role in combating the damage caused in cells and tissues due to oxidative stress. Brassica oleracea comprises several crop varieties of worldwide economic importance. Fresh leaves of cauliflower were purchased in bulk. The leaves were then washed, blanched, oven dried at 40^0c and finally ground into powder to screen for the presence of flavonoids using High Performance Liquid Chromatography. The results revealed the presence of fourteen flavonoids such as Gallic acid, Theanine, Theobromine and flavon 3ols compounds. Hence these locally available but highly neglected cauliflowers leaves possessing antimicrobial and antifungal properties due to flavonoids can be introduced in pharmacological, functional food and neutraceutical preparations thereby reducing the waste index of the vegetable.

Key words: Cauliflower Leaves, Flavonoids, Gallic Acid, Antimicrobial Antifungal

Introduction

Over 5000 naturally occurring flavonoids have been characterized from various plants. Flavonoids are small molecular secondary metabolites synthesized by plants with various biological activities. They are well known for their antioxidant activity. Due to their physical and biochemical properties, they are capable of participating in plants' interactions with other organisms and their reactions to environmental stresses[1]. In recent years, there is a raising interest in flavonoids, mostly because of their antioxidant, anti-inflammatory, antiallergenic, antimicrobal and anticancer activity[2]. The majority of their functions result from their strong antioxidative properties. Imbalance in the body mechanism to counterwork the production of free radicals has set in motion in initiating and development of many lifestyle diseases of modern time being inflammation, autoimmune diseases, cancer, coronary diseases and aging. Stressing on the health complication faced by

the present day society, it is within reason to believe that exogenous antioxidants like flavonoids could play a significant role in combating the damage caused in cells and tissues due to oxidative stress. Epidemiological studies have described the beneficial effects of dietary polyphenols (flavonoids) on reduction of chronic diseases, including cancer[3].

Brassica oleracea comprises several crop varieties of worldwide economic importance, such as kale, broccoli, brussels sprouts and cauliflower. Their high intraspecific variability extends to secondary metabolites produced by Brassica plants, among them glucosinolates and flavonoids[4,5].Flavonoids play an important role in ultraviolet (UV) protection since UV-B responsive flavonoids can reduce the risk of reactive oxygen species (ROS) generation and thereby prevent oxidative damage[6].Therefore, the impact of flavonoids on the human body after food consumption as well as their effect as pharmaceutical supplements was discussed in several reviews. Particularly relevant are their antioxidative activity and radical scavenging capacity[7].

Keeping in view the importance of relationship between flavonoids and brassica vegetables an attempt was made to screen for the presence of flavonoids in cauliflower leaves. Apart from consuming cauliflower as vegetable, their leaves that are edible for consumption are greatly ignored in our regular Indian diet. Hence identifying for the presence of these powerful antioxidant flavonoids in cauliflower leaves could positively influence an impact in pharmaceutical and health sectors thereby improving quality health of the individual and thereby reducing the waste index of the vegetable.

Materials and Methods

Fresh leaves of cauliflower were purchased in bulk from an organic farm in Ooty, Tamil Nadu. The stalks from the leaves were separated and the leaves were then washed in running water. They were then blanched for 10-15 sec and were spread in a filter paper for two hours at room temperature for the excess water to drain. Later the leaves were dried in hot air oven at 40^0c over night. Hot air oven drying method was applied because oven-dried (40^oC) cauliflower had the highest extraction yield while air-dried (ambient, approx 25^oC) had the lowest[8]. The dried leaves were then ground in a mixer and packed in an air tight container.

Sample Preparation

10gm powdered plant material was soaked in 80ml of ethanol overnight and then filtered through Whatmann filter paper No.41 along with 2gm Sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with ethanol. The filtrate is then concentrated by flushing nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non-polar phytocomponents. The extract was then injected into HPLC by autosampler for the screening of flavonoid s and the results were interpreted.

Results and Discussion

Table I and fig.1shows the screened list of fourteen different flavonoids present in cauliflower leaves powder using HPLC (High Performance Liquid chromatography).For more than 35 years HPLC has been used around for its largest separation technique optimum for chemical and biological compounds that are non volatile. HPLC is the method of choice for analysis of phenolic compounds, because of its extremely high versatility, precision and relatively low cost[9].Among the flavonoids detected Gallic acid was screened to be the highest with peak area (22.439%).

Medicinal plants are of great importance to health due to the presence of phytoconstituents. The most important of these constituents are alkaloids, glycosides, tannins, flavonoids, and phenolic compounds[10]. Phenolic acids are diverse group

that includes hydroxybenzoic and hydroxycinnamic acids. Various phenolic acids reported from plants are ferulic acid, ellagic acid, synergic acid, caffeic acid etc. They are also of interest in food, cosmetic and pharmaceutical industries as well as substitutes for synthetic antioxidants[11]. One such prominent phenolic acid is gallic acid which is found in a wide variety of vegetables, fruits, tea, coffee and wine[12]. Gallic acid has been reported to elicit various biological activities such as antibacterial, anti-fungal, antiviral, anti-inflammatory, antioxidant, anticancer, anti-diabetic etc[13]. On referring the biological activities of gallic acid. It was encouraging to note from the obtained results that cauliflower leaves also showed an appreciable amount of gallic acid. Hence cauliflower leaves can very well be introduced in pharmaceutical and food sectors due to the presence of a prominent phenolic acid namely gallic acid.

Flavan-3-ols from tea, cocoa, chocolate, fruits, vegetables and wine, are highly potent antioxidant compounds. They reduce incidence of stroke, heart failure and diabetes and cancer. Their anticancer effects are thoroughly investigated. Epigallocatechin 3-gallate and gallocatechan 3gallate induces reduction in experimental lung. Epigallocatechin 3-gallate is effective antiangiogenesis agent which inhibits tumor cell invasion and proliferation[14]. It, also, inhibits growth of the NBT-II bladder tumor cells and breast cancer cell lines. It is important to interpret that the cauliflower leaves also contains Flavan- 3- ols compounds like Epigallo Catechin, Catechin, EpiCatechin, Epi Gallocatechin Gallate (EGCG), Gallocatechin gallate (GCG), Epicatechin gallate (GCG), Catechin gallate (CG) that are present in tea leaves[15]. Owing to the above litreture reference cauliflower leaves could also be included in the list of highly potent antioxidant compounds.

Conclusion

There is a great interest in plant and plant derived phytochemical as food source because of its divergent nutritional function, antioxidant and other therapeutic properties[16]. Hence HPLC screening for the presence of flavonoids in cauliflower leaves confirms that gallic acid and tea phenolic compounds considered as a major antioxidant possessing antimicrobial and antifungal properties are strongly present in this cauliflower leaves powder. Hence these locally available but highly neglected cauliflower leaves can be introduced in pharmacological, functional food and neutraceutical preparations due to the presence of antioxidant flavonoids which in turn can help in reducing the waste index of the vegetable.

Table I

Ret. Time	**Area %**	**Name**	**Biological Activities**
0.464	22.439	Gallic acid	ACE-Inhibitor, Analgesic, AntiHIV, Antiadenovirus, Antiallergenic, Antianaphylactic, Antiangiogenic, Antiasthmatic ,Antibacterial ,Antibronchitic,Anticancer,Anticarcinomic, Antiescherichic, Antifibrinolytic,Antiflu, Antihepatotoxic, Antiherpetic, Antiinflammatory, Antileishmanic, Antimutagenic, Antinitrosaminic, Antioxidant**; 2/3 BHA,** Antioxidant**; 7 x quercetin,** Antioxidant**,** Antiperiodontitic,

Ret. Time	Area %	Name	Biological Activities
			Antiperoxidant,Antipolio, Antiproteolytic, Antiradicular; 7 x quercetin; JAF47:397,Antiradicular,Antiseptic,Antistaphylococcic, Antitumor, Antitumor-Promoter, Antiviral, Apoptotic, Astringent, Bacteristat, Bronchodilator, Cancer-Preventive, candidicide, Carcinogenic only to the affected cells, Choleretic, Cyclooxygenase, Cytotoxic , Floral-Inhibitor , Gram(+)icide , Gram(-)icide , Hemostat , Hepatoprotective , Immunomodulator, Immunostimulant, Immunosuppressant, Insulin-Sparing,Myorelaxant, Nephrotoxic, Pesticide, Styptic, Topoisomerase-I-Inhibitor, Xanthine-Oxidase-Inhibitor
0.661	14.727	Theanine	Antithromboxane, Hypocholesterolemic
1.221	15.219	Theobromine	Anorexic, Antiasthmatic,Anticellulitic, Arteriodilator,Bronchodilator,CNS-Stimulant, Cardiotonic, Diuretic, Emetic, Fetotoxic, Herbicide, Myocardiotonic, Myorelaxant, Myostimulant, Pesticide, Stimulant, Teratogenic, Vasodilator.
2.232	4.199	Theophylline	
3.275	13.594	Theacrine	
9.393	1.599	Gallo Catechin	Antiallergic
11.638	8.687	Caffeine	(+)-Inotropic, Adenosine-Antagonist, Analeptic, Analgesic-Synergist,Antiapneic, Antiapoptotic, Antiasthmatic, Anticarcinogenic, Anticariogenic, Antidermatitic, Antiemetic, Antifeedant, Antiflu, Antiherpetic, Antihypotensive, Antinarcotic, Antiobesity, Antioxidant, Antirhinitic, Antiserotonergic, Antiserotonergic, Antitumor, Antitumor (Lung), Antivaccinia, Antiviral, Apoptotic, Arrhythmigenic, CNS-Stimulant, Cancer-Preventive, Cardiotonic, Cardiotonic, Catabolic, Choleretic, Coronary-Dilator, Diuretic, Energizer,

Ret. Time	Area %	Name	Biological Activities
			Ergotamine-Enhancer; M29, FLavor; FEMA 125, Herbicide, Hypertensive, Hypoglycemic, Insecticide, Lypolytic, Myorelaxant, Neurotoxic, Pesticide, Phosphodiesterase-Inhibitor, Pyrogenic, Pyrogenic; 15 mg/kg ipr cat; EJP45:389, Pyrogenic; 60 mg/day oral hmn; ALG51:189;Respirastimulant, Spasmogenic, Stimulant, Tachycardic, Teratogenic, Topoisomerase-I-Inhibitor; 0.1 nM; JEM168:129, Topoisomerase-I-Inhibitor; 75 uM; TCM10:41, Topoisomerase-II-Inhibitor, Vasodilator, cAMP-Phosphodiesterase-Inhibitor; PAM, cGMP-Phosphodiesterase-Inhibitor; PAM.
12.528	3.681	Epigallo Catechin	Antimutagenic, Antioxidant,Antiradicular, Beta-Adrenergic Receptor Blocker, Lipoxygenase-Inhibitor, Xanthine-Oxidase-Inhibitor.
13.109	2.209	Catechin	Allelochemic, Antiaggregant, Antialcoholic, Antiarthritic, Antibacterial, Anticariogenic, Antiedemic, Antiendotoxic, Antifeedant, Antiflu, Antihepatitic, Antihepatotoxic, Antiherpetic, Antihistaminic, Antihyperlipidemic, Antiinflammatory, Antileukemic, Antilipoperoxidant, Antiosteotic, Antioxidant; 2.4 x Vit. E; BO2;Antioxidant; IC50=0.1 uM; JAF47:4821 PM56(6):695,Antiperiodontal, Antiplaque, Antiprostaglandin, Antiradicular, Antisclerodermic, Antiulcer; 1 g/5x/day/man/orl; PAM, Antiviral, Astringent, COMP-Inhibitor, COX-1-Inhibitor, Cancer-Preventive, Carcinogenic, Cyclooxygenase-Inhibitor,Fungicide, Hemostat, Hepatoprotective, Hypocholesterolemic, Immunostimulant, Lipoxygenase-Inhibitor, Pesticide, Propecic, Xanthine-Oxidase-Inhibitor.
14.302	1.171	EpiCatechin	Antiangiogenic, Antitumor, VEGF-Inhibitor

Ret. Time	Area %	Name	Biological Activities
14.742	1.776	Epi Gallocatechin Gallate (EGCG)	Antiallergic
15.953	0.556	Gallocatechin gallate (GCG)	Anticariogenic, Antihepatotoxic,Antimutagenic, Antioxidant; >10 x tocopherol,Antiperoxidant, COMP-Inhibitor, Cancer-Preventive, Glucosyl-Transferase-Inhibitor, Immunostimulant,Lipoxygenase-Inhibitor
16.681	9.356	Epicatechin gallate (GCG)	
17.901	0.786	Catechin gallate CG	

Source: Dr. Duke's data base

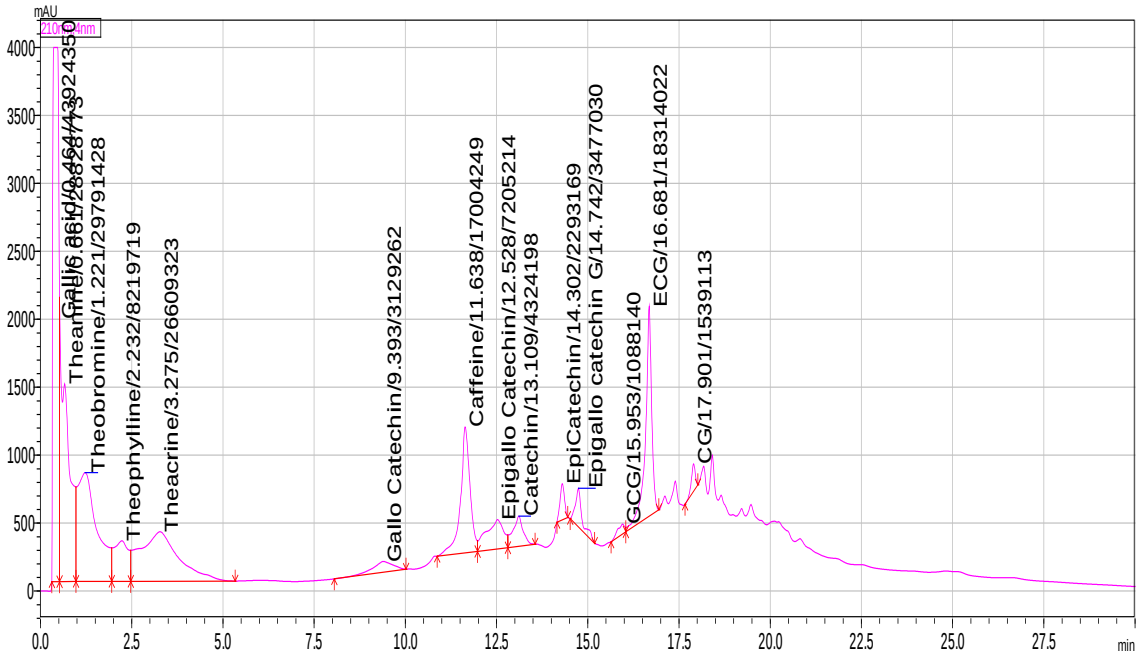

Fig 1: Graphical Representation of Flavonoids in Cauliflower Leaves

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