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# Comparative Analysis of Total Phenolic Content and Anti-Oxidant Activity of Vegetables

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***Abstract:***
*The antioxidant activity and total phenolic content of alcoholic extracts from 14 vegetables were evaluated by using a model system consisting of β-carotene, linoleic acid and Folin-Ciocalteau method. The total phenolics of the extracts was determined spectrophotometrically according to the Folin-Ciocalteau procedure and ranged from 28-74 mg /100 gm on a fresh weight basis. Broccoli, mint, holy basil, curry leaves, beetroot and black carrot have high antioxidant activity. The antioxidant activity expressed as percent inhibition of oxidation ranged from a high of 79% in broccoli extracts to a low of 57% in spring onion. Other vegetables found to have high antioxidant activity (>72%) were broccoli, mint, holy basil, curry leaves, beetroot and black carrot. Antioxidant activity correlated linearly significantly and positively with total phenolics. The results indicate that vegetables containing high phenolics may provide a source of dietary antioxidants. Using Thin Layer Chromatography (TLC), 14 vegetables were screened for the presence of total phenolics. Further, High Performance Liquid Chromatography (HPLC) was carried out for broccoli that showed the highest antioxidant activity but low phenolic content.*

***Key words:*** *Antioxidant, β-carotene, linoleic acid, Folin-Ciocalteau reagent*

## 1. Introduction

Today's society is characterized by having many unhealthy dietary habits. Not only snacking but also the inadequate intake of healthy foods triggers a major dietary imbalance, this being a major cause of chronic diseases such as obesity, diabetes mellitus, cardiovascular disease, hypertension, stroke, and several types of cancer. Therefore, it is vital to ascertain the composition and nutritional value of these products. To prevent the above-mentioned diseases, epidemiological studies recommend the consumption of whole fruits, vegetables, and legumes (Mertz et al. 2009; Espinosa-Alonso et al. 2008). Fruits and vegetables have conferred on them the status of functional foods (Hasler 1998). They seem to be capable of delivering health benefits besides fulfilling physiological needs. Routine or habitual consumption of fruits and vegetables confer significant benefits to human health (Steinmetz and Potter 1996). Epidemiological data as well as *in vitro* studies strongly suggest that foods containing phytochemicals with anti-oxidation potential have strong protective effects against major diseases risks including cancer and cardiovascular diseases (Steinberg 1991; Block et al. 1992; Ames et al. 1993; Hertog et al. 1993; Byers and Guerrero 1995; Knekt et al. 1997; Elliot 1999; Kaur and Kapoor 2001). The protective action of fruits and vegetables has been attributed to the presence of anti-oxidants, especially anti-oxidant vitamins, including ascorbic acid, α-tocopherol and β-carotene (Gey et al. 1991; Willet 1994; Kalt and Kushad 2000; Prior and Cao 2000). However, numerous studies have conclusively shown that the majority of the antioxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin rather than from Vitamin C, E and β-carotene (Wang et al. 1996; Kahkonen et al. 1999). Epidemiological studies have shown that consumption of food and beverages rich in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis by acting as antioxidants towards low-density lipo-protein (LDL) (Kinsella et al. 1993; Frankel et al. 1995; Landbo and Meyer 2001). Therefore, mostly, the current focus is on the anti-oxidant action of phenolics. Polyphenols, widely distributed in plants, contribute to fruit organoleptic and nutritive quality in terms of colour, taste, aroma, and flavour (Serrano et al. 2010) also being involved in astringent and bitter tastes. It is known that, amongst other factors, such as maturity stage or light exposure, phenolic composition varies with the cultivar. In addition, the phenolic profile has already been revealed to be a useful parameter for the discrimination of the different fruit parts. The species and levels of phenolic compounds vary dramatically among plants, and their different structures or levels are likely to have different functional properties (Magalhaes et al. 2009). Besides the general properties of the compounds, a number of polyphenolic compounds, especially catechins,

have been found to be potent antioxidants and to be effective in preventing cancer (Costa et al. 2009) while tannins have been reported to exert other physiological effects; e.g. they can reduce blood pressure, accelerate blood clotting, lower serum-lipid levels, modulate immunoresponses and cause liver necrosis (Muchuweti et al. 2006).The intake of these compounds is an important health-protecting factor. These bioactive compounds retard or inhibit lipid autoxidation by acting as radical scavengers and consequently, are essential antioxidants that protect against the propagation of the oxidative chain (Navarro et al. 2006). Evidence for their role in the prevention of degenerative diseases is emerging. Experimental studies on animal and human cell lines have demonstrated that polyphenols can play a role in preventing cancer and cardiovascular diseases, when taken daily in adequate amounts (Wijngaard et al. 2009).

The antioxidant activity of phenolics is mainly because of their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans et al. 1997). Antioxidants are naturally occurring or synthetic chemicals in foods that help to counter the detrimental effects of reactive oxygen species (ROS) and free radicals which causes degenerative human diseases such as cancer, heart diseases and cerebrovascular diseases. The recent growth in the knowledge of free radical and ROS in biology is producing a medical revolution that promises a new age of health. Reactive oxygen species have been implicated in the etiology of a host of a degenerative disease including cardiovascular disease, diabetes, cancer, Alzheimer disease and other neurodegenerative disorders, and in aging.

India is bestowed with diverse climatic conditions, conducive for the growth of different vegetables known for their nutritional values. So far, there has not been a study on anti-oxidant activity of vegetables grown and consumed here. The main objective of this study was to screen a large number of vegetables consumed in Indian diet with respect to their total phenolic content and antioxidant activity. The present study was planned to determine total phenolic content and antioxidant activity of given vegetables. Further, the polyphenols present in these vegetables were analyzed.

## 2. Materials and Methods

### *2.1. Sample Collection*

Fourteen vegetables were purchased fresh from different local markets of Jalandhar, Punjab (India). These vegetables consisted of mainly green leafy vegetables such as: mint, fenugreek, coriander, lettuce, curry leaves and holy basil ( tulsi), root vegetables such as: ginger, beetroot, red carrot, orange carrot, black carrot, spring onion ( bulb as well as stem) and other vegetables such as: bitter gourd (karela) and broccoli.

### *2.2. Sample Preparation*

The vegetables obtained from local market were washed with distilled water, cleaned and chopped into small pieces. The roots having dry and dead skin were processed after removal of their skin. Only edible portion of vegetables were weighed and homogenised with 80% ethanol.

### *2.3. Total Phenolic Content*

Total phenolics were determined using the Folin– Ciocalteau reagent (Kaur and Kapoor 2002). Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged at 10,000 rpm for 15 min and the supernatant was discarded. The residue was re-extracted twice with 80% ethanol and supernatants were pooled into evaporating dishes and evaporated to dryness at room temperature. Residue was dissolved in 5 ml of distilled water. 100 µL of this extract was diluted to 3 ml with water and 0.5 ml of Folin–Ciocalteau reagent was added to it. After 3 min, 2 ml of 20% of sodium carbonate was added and the contents were mixed thoroughly. The colour was developed and absorbance measured at 650 nm in spectrophotometer after 60 min using catechol as a standard. Total Phenolic Content was expressed as mg catechol/100 g of fresh weight material using the formula below.

$$\frac{\text{O.D (sample)} \times A_{650} \times 10 \times \text{volume made up} \times \text{dilution factor} \times 100}{\text{Weight of sample} \times 1000}$$

Where:

- O.D (sample) is Optical density of sample
- $A_{650}$ is Absorbance of standard at 650 nm

### *2.4. Antioxidant Activity*

For the determination of anti-oxidant activity, β-carotene bleaching method was used (Miller et al. 1993) with modifications (Wanasundara et al. 1994). Vegetable extracts were prepared by homogenizing 2 g of vegetable in the respective media (80% ethanol) and centrifuged at 10,000 rpm for 15 mins. Supernatants were stored in capped tubes until further use. β-Carotene (2 mg) was dissolved in 20 ml of chloroform. 4 ml aliquot of the above solution was added to a conical flask with 40 mg linoleic acid and 400 mg Tween-40. Chloroform was removed with a rotary evaporator at 50 °C. Oxygenated distilled water (100 ml) was added to the β-carotene emulsion, mixed well and aliquots (3 ml) of the oxygenated β-carotene emulsion and 0.2 ml of alcoholic extracts were placed in capped culture tubes and mixed well. The tubes were immediately placed in a waterbath and incubated at 50 °C for 10 mins.

Oxidation of the β-carotene emulsion was monitored spectrophotometerically by taking absorbance at 10-min interval at 470 nm for 100 min. A control consisted of 0.2 ml distilled water instead of vegetable extract. Antioxidant activity was expressed as per cent inhibition relative to control using the formula below:

$$1 - \frac{(\text{Abs sample 0min} - \text{Abs sample 100min})}{(\text{Abs control 0min} - {}^{*}\text{Abs control 100min})} \times 100$$

Where:

- Abs sample 0min is Absorbance of sample at 0 minute
- Abs sample 100min is Absorbance of sample at 100 minutes
- Abs control 0min is Absorbance of control at 0 minute
- Abs control 100min is Absorbance of control at 100 minutes

*2.5. Qualitative analysis of Polyphenols*

2.5.1. Thin Layer Chromatography (TLC)

A TLC apparatus was used to prepare thin layer (0.25 mm) of various adsorbents on 20x3cm glass plates. Glass jars (29x6cm) were used for the development of TLC plates. Standards of aromatic phenols used in the present study include: Catechol , Gallic acid , β-carotene and Quercitin . The test solutions (1 %) of all phenols were prepared in methanol. All phenols were detected by exposing TLC plates to iodine vapor in a closed chamber and phenols as dark brown/yellow spots were visualized. Stationary phase used was silica gel G and mobile phase was prepared at 30°C by emulsion of hexane (160 ml), water (8 ml) with butanol (25 ml). The TLC plates were prepared by mixing the sorbent with de-mineralized water in 1:3 ratio (by weight) with constant shaking to obtain a homogeneous slurry. The resultant slurry was applied to clean glass plates with the help of an applicator to give a 0.25mm thick layer. The plates were dried at room temperature and activated at 100±5°C by heating in an electrically controlled oven for 1 hour. The activated plates were stored in closed chamber at room temperature (30° C) until used. The activated plates were marked with horizontal line 2cm from the base. The test solutions (10µL) of phenols (1%) were spotted separately on the base line of the activated thin layer plates with the help of a micropipette. The spots were allowed to air dry and the plates were developed in chosen mobile phase by one dimensional ascending technique in glass jar. The solvent ascent was fixed to 12 cm from the point of application in all cases. After development, TLC plates were dried at room temperature. These plates were then exposed to iodine vapors for 10 min and then the spots were visualized, the phenols show yellowish brown spots. The Rf values were determined.

2.5.2. High Performance Liquid Chromatography

HPLC was carried out at NIPER (National Institute of Pharmaceutical Education), Mohali, India to analyse the phenolic content of sample having highest antioxidant activity. The test solutions (1 %) of all phenols were prepared in 80% methanol. Mobile phase was prepared at 30°C by emulsion of water: methanol: acetic acid (70:30:0.5). C18 column: 250mm x 4.6mm, 5µm, 100A was used. The chromatography of catechol and quercitin as standards and a sample of broccoli extract were performed. The flow rate was kept constant throughout analysis at 1ml/min. Injections were accomplished with 20µl fixed loop and the analysis was monitored with UV-Vis detector at 280nm.

*2.6. Statistical Analysis*

For determination of total phenolic content of three experimental values were taken. The results are mean ± standard deviation (S.D) of the values, Correlation and regression coefficient.

**3. Result and Discussion**

*3.1. Total Phenolic Content*

Considering a large variation in the total phenolics, the vegetables were divided into three groups namely, high (>55 mg catechol/100 g) (Table 1), medium (35-55 mg catechol/100 g) (Table 2) and low (<35 mg catechol/100 g) (Table 3). Total phenolic content of the vegetables varied from 74.24 mg catechol/100 g fresh weight in mint to 28.8 mg catechol/100 g fresh weight in spring onion bulb.

*3.2. Antioxidant Activity*

The antioxidant activities of ethanolic extracts of the 14 vegetables are given in Tables 1-3, wherein the vegetables have been placed in three different groups of high, medium and low antioxidant activities, respectively. The decrease in absorbance of β-carotene in the presence of different vegetable extracts was compared with the oxidation of β-carotene and linoleic acid (Table 1-3).

| Vegetables | Antioxidant activity (% inhibition of β- carotene bleaching) | Total phenolic content (mg of catechol/100gm of fresh weight) |
|---|---|---|
| Broccoli inflorescence (Brassica oleracea) | 79.1 | 34.86 ± 0.20 |
| Mint leaves (Mentha longifolia) | 79 | 74.24 ± 0.31 |
| Holy Basil leaves (Ocimum tenuiflorum) | 77.9 | 73.61 ± 0.19 |
| Curry Leaves (Murraya koenigii) | 75.6 | 69.03 ± 0.20 |
| Beetroot (Beta vulgaris) | 73.9 | 64.31 ± 0.19 |
| Black Carrot (Daucus carota) | 73.1 | 61.88 ± 0.49 |

*Table 1: Vegetables of high antioxidant activity (>70%) and high total phenolic content (>55 mg catechol/100 g) Data expressed as mean ± s.d. of three samples analysed separately*

| Vegetables | Antioxidant activity (% inhibition of beta- carotene bleaching) | Total phenolic content (mg of catechol/100gm of fresh weight) |
|---|---|---|
| Green Lettuce leaves (Lactuca sativa) | 72.3 | 51.46 ± 0.30 |
| Fenugreek leaves (Trigonella foenum-graceum) | 71.5 | 40.63 ± 0.29 |
| Ginger root (Zingiber officinale) | 70.9 | 39.86 ± 0.39 |
| Coriander leaves (Coriandrum sativum) | 70.3 | 35.58 ± 0.59 |

*Table 2: Vegetables of medium antioxidant activity (>60%) and total phenolic content (35 - 55 mg catechol/100 g) Data expressed as mean ± s.d. of three samples analysed separately*

| Vegetables | Antioxidant activity (% inhibition of β- carotene bleaching) | Totl phenolic content (mg of catechol/100gm of fresh weight) |
|---|---|---|
| Orange carrot (Daucus carota) | 69.2 | 32.43 ± 0.30 |
| Red carrot (Daucus carota) | 68.4 | 31.74 ± 0.10 |
| Bitter gourd (Momordica charantia) | 59.9 | 29.59 ± 0.39 |
| Spring onion leaves (Allium sps.) | 60.7 | 30.56 ± 0.19 |
| Spring onion bulb (Allium sps.) | 57.5 | 28.82 ± 0.30 |

*Table 3: Vegetables of low antioxidant activity (>55%) and total phenolic content (<35 mg catechol/100 g) Data expressed as mean ± s.d. of three samples analysed separately*

A large variation in the antioxidant activities, ranging from 79.1% in broccoli, to 57.5% in spring onion bulb was observed. A similar wide range in antioxidant activities has been reported earlier (Cao et al. 1996; Wang et al. 1996; Gazzani et al. 1998). Out of 14 vegetable extracts evaluated for anti-oxidant activity 6 vegetables were found in the group of high anti-oxidant activity (>70%) (Table 1). These included broccoli, mint, holy basil, curry leaves, beetroot and black carrot. Interestingly all vegetables, which were grouped in a high phenolic content group also had a high antioxidant activity and vegetables like bitter gourd, spring onion, orange carrot and red carrot, which had low phenolic contents, also had low anti-oxidant activities. On the other hand there were some vegetables like broccoli, which was characterized by moderate or low phenolic contents, but which had high antioxidant activities. Their high anti-

oxidant activities can be explained on the basis of high antioxidant activity of some individual phenolic units, which may act as efficient antioxidants rather than contributing to high total phenolics.
According to our results, a positive correlation of antioxidant activity with phenolic content was observed (Fig. 1).

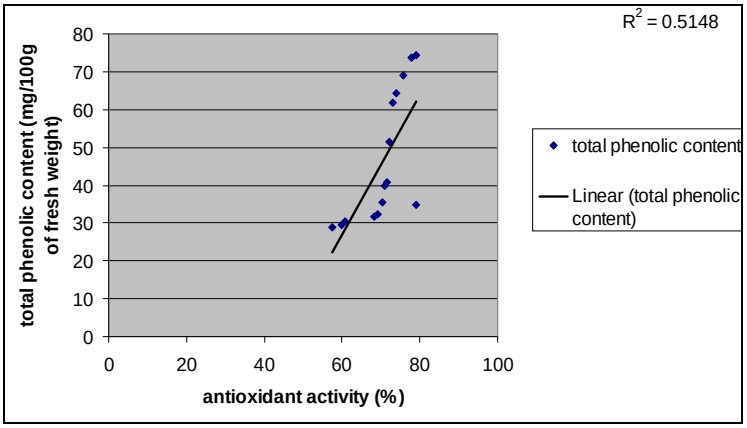



*Figure 1: Correlation of reducing power activity and total phenolic activity of different vegetables. ($p < 0.05$, $R^2 = 0.5148$)*

This correlation suggests that most of the vegetables which are having high antioxidant activity may also be having high phenolic contents. The vegetables may contain proteins and other anti-oxidants (ascorbate and carotenoids) but these do not contribute significantly to the anti-oxidant activity. Results with anti-oxidant activity correlated significantly and positively with total phenolics ($_r^2 = 0.5148$, $P < 0.05$). The results indicate that vegetables containing high phenolics may provide a source of dietary anti-oxidants.
The antioxidant activity of phenolic compounds is mainly due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers. Kaur and Kapoor, (2008) reported the total phenolic content of *Trigonella foenum graecum* to be 217.5 mg of catechol/100 g of fresh vegetable. Salvatore et al., (2008) in their study on antioxidant characterization of some Sicilian edible greens stated that the frequently consumed greens in the Mediterranean areas were very rich in antioxidants such as flavonoids and carotenoids. Therefore, it can be said that the total polyphenol content of vegetables varies widely depending on the variety of vegetable and a comparison is difficult, as different standard compounds have been used for their analysis. The presence of different antioxidant components in plant tissues especially fruits and vegetables make it relatively difficult to measure each antioxidant component separately. Therefore, several methods (Al-Saikhon et al.1995; Cao et al.1995; Furuta et al. 1997; Gazzani et al. 1998; Velioglu et al. 1998; Vinson et al. 1998; Kahkonen et al. 1999; Chu et al. 2000; Tang et al.β 2001) have been developed in recent years to calculate the total antioxidant activity of biological samples. In the present study, ethanol was used. Usage of ethanol was found to be more efficient than water in extracting the antioxidants present in the vegetables, especially the carotenoids. The bleaching of β-carotene is a free radical-mediated phenomenon, resulting from the hydroperoxides formed from linoleic acid. β-Carotene, in this model system, undergoes rapid discoloration in the absence of an antioxidant. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and characteristic orange colour, which is monitored spectrophotometrically. In the present study, it was observed that, the presence of different antioxidants of vegetables could hinder the extent of β-carotene bleaching by neutralising the linoleate free radical and other free radicals formed in the system.

*3.3. Qualitative Analysis of Polyphenols*
TLC is a relatively cheap but significant technique to screen plant extracts for the presence of different types of phenolic compounds. Four orange brown spots with Rf values 0.877, 0.873, 0.875, and 0.88, were detected that determined the presence of quercitin (Rf 0.875) in bitter gourd, broccoli, spring onion bulb and spring onion leaves respectively. Using iodine vapours, another two spots emerged in dormant samples with Rf values 0.958 and 0.955, determining the presence of β-carotene (Rf 0.954) in orange carrot and red carrot, respectively. Table 4 shows the presence of different polyphenols in the samples.

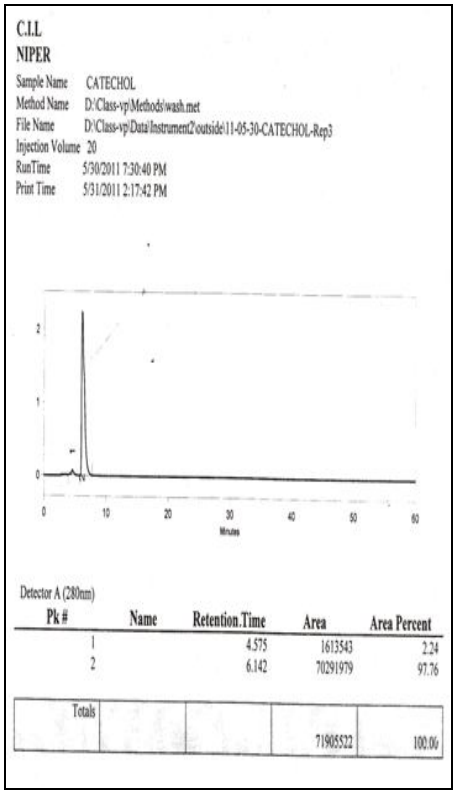
| | **β-carotene (Rf = 0.954)** | **Gallic acid (Rf = 0.347)** | **Catechol (Rf = 0.92)** | **Quercitin (Rf = 0.875)** |
|---|---|---|---|---|
| Black carrot | - | - | - | - |
| Red carrot | + | - | - | - |
| Orange carrot | + | - | - | - |
| Bitter gourd | - | - | - | + |
| Broccoli | - | - | - | + |
| Spring onion leaves | - | - | - | + |
| Spring onion bulb | - | - | - | + |

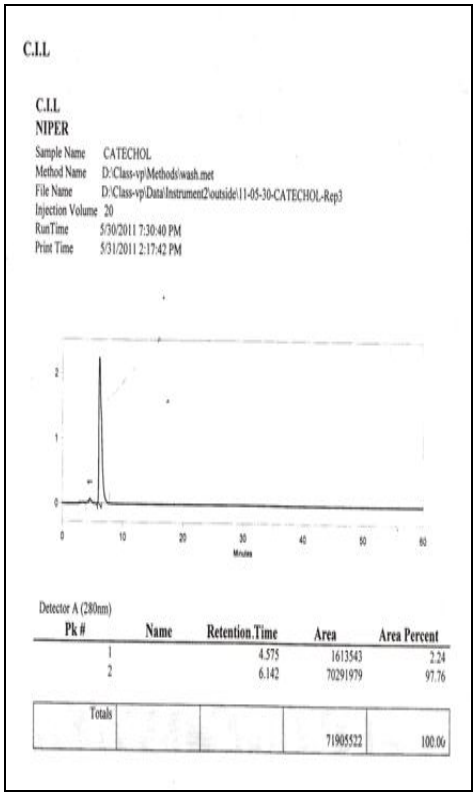
*Table 4: Rf values of different polyphenols.*

Reversed phase HPLC was performed for broccoli leaf extract. Preliminary separation and identification of individual phenolic compounds in broccoli leaf extract was conducted. Sample peaks were identified by comparing them with the known phenolic standards under same chromatographic conditions. According to the chromatograms (Fig. 2-4), it was observed that broccoli leaves having highest antioxidant activity out of 14 vegetables studied, had lower phenolic content. When compared with the standards i.e. catechol and quercitin, the area percent in sample peaks showed the presence of catechol and quercitin in broccoli leaves in small concentration.

The study clearly indicates that it is important to measure the antioxidant activity using various radicals and oxidation systems and to take both phenolic content and antioxidant activity into account while evaluating the antioxidant potential of plant extracts. However, the model system consisting of β-carotene and linoleic acid can be used to screen large number of sources for their antioxidant capacity. Further analysis by Thin Layer Chromatography and High Performance Liquid Chromatography confirms the presence of phenolic compounds in these vegetables. Furthermore, in order to realize the health benefits from potential plant sources, additional information on their dietary intake and enhancing bioavailability after various processing operations is required.

Research on polyphenol bioavailability must finally allow us to correlate polyphenol intake with one or several accurate measures of bioavailability (such as concentrations of key bioactive metabolites in plasma and tissues) and with potential health effects in epidemiologic studies. Knowledge of these correlations must be attained despite the difficulties linked to the high diversity of polyphenols, their different bioavailabilities, and the high interindividual variability observed in some metabolic processes, especially those in which the microflora is involved.



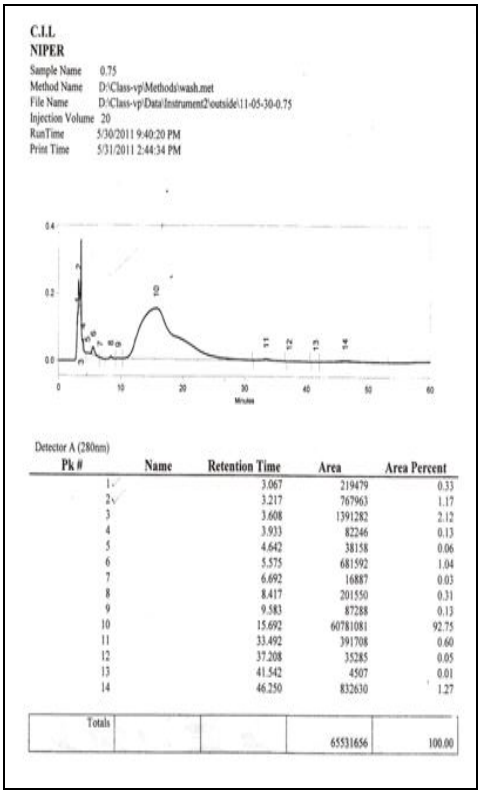




*Figure 2: HPLC graph of standard catechol*
*Figure 3: HPLC graph of standard Quercitin*
*Figure 4: HPLC of Broccoli (Brassica oleracea)*

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