

Evaluation of Antioxidants and Anti-Cancer properties of selected Medicinal Plants

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Abstract

Cancer is a disease which severely effects the human population. Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body .There is a constant demand for new therapies to treat and prevent this life-threatening disease. Scientific and research interest is drawing its attention towards naturally-derived compounds as they are considered to have less toxic side effects compared to current treatments such as chemotherapy. The medical plants such as cinnamon , green tea and turmeric produce naturally effect occurring secondary metabolites which are being investigated for their anticancer and antioxidant activities leading to the development of new clinical drugs. With the success of these compounds that have been developed into staple drugs for cancer treatment new technologies are emerging to develop the area further . The aim to enhance anticancer and antioxidant activities of plant-derived drugs by controlling the release of the compound and investigating new methods for administration . Although many biological activities including antioxidant and anticancer properties have been reported . the antioxidant and anticancer effects were evaluated by using The MTT activity , DPPH radical scavenging activity, ABTS radical scavenging activity and Hydroxyl radical scavenging assay . This review discusses the demand for naturally-derived compounds from medicinal plants and their properties which make them targets for

potential anticancer and antioxidant treatments.

Keywords: Cancer, Ayurveda, Inflammation,

Introduction

Cancer is a disease characterized by unregulated proliferation of cells. Natural products have been regarded as an important source that could contribute to potential chemotherapeutic agents. In particular, plant derived compounds have gained importance in anticancer therapy and some of the new chemotherapeutic agents currently available for use includes paclitaxel, vincristine, podophyllotoxin, and camptothecin (Mitra et al., 2012). Spices and herbs are well known for their antimicrobial and antioxidant properties, besides having the ability to produce multidimensional flavours in food (Bonilla et al., 2012; Hoque et al., 2011; Tongnuanchan et al., 2012). Weerakkody et al., (2010) concluded that there was a highly positive linear relationship between the antioxidant and antibacterial activities and the total phenolic content in some spices and herbs. According to Moure et al., (2001), of the different parts of the plant, the leaves deserve especial attention, as well as the roots and seeds, due to their antioxidant activity, detected and reported in several studies (Bonilla and Sorbal, 2017).

Curcuma longa, a perennial herb and member of the Zingiberaceae (ginger) family, grows to a height of three to five

feet and is cultivated extensively in Asia, India, China, and other countries with a tropical climate (Dobelis, 1986). Curcumin, a polyphenol with a diarylheptanoid structure that contains two α , β -unsaturated ketones, is considered to be the major active constituent of turmeric. The chemical properties and the historical background of Curcumin have been reviewed elsewhere (Sharma et al., 2005; Agarwal et al., 2003). This nontoxic natural compound has been reported to possess several biological activities that are therapeutically beneficial to cancer treatment. Turmeric is used extensively in foods for its flavor and color, as well as having a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory, and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic (Ravindranatha and Chandrashekhara, 1980; Wahlstrom and Blennow, 1978). Turmeric can also be applied topically in poultices to relieve pain and inflammation. The active constituents of turmeric are the flavonoid Curcumin. Current research has focused on Curcumin antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, and antimicrobial properties, in addition to its use in cardiovascular disease and gastrointestinal disorders (Dikshith et al., 1995; Toda et al., 1985).

The traditional knowledge on the use of cinnamon for a variety of diseases has been scientifically proven by many researchers. *Cinnamomum zeylanicum* has been reported to have many biological activities such as being an antioxidant (Prasad et al., 2009), antimicrobial and anticancer (Unlu et al., 2010), anti-inflammatory (Chao et al., 2008), antidiabetic (Adisakwattana et al., 2011), anti-mutagenic (Jayaprakasha et al., 2007) and anti-tyrosinase (Marongiu et al., 2007). However, none of these activities are

reported from Sri Lanka and there is no evidence for whether those are from Ceylon cinnamon, including for antioxidant activity. Limited research has also been conducted worldwide on *Cinnamomum* leaf as a natural antioxidant.

In ancient times, cinnamon (from the Greek *kinamon*), also known as a sweet wood, was greatly treasured by Egyptians, Israelites and Phoenicians. It was commonly used as a body perfume and medicine, and proclaimed as one of the main ingredients in the love potions prepared by the Greek and Romans as well. Several varieties of cinnamon can be distinguished, but only two types are generally used: Cassia (Chinese cinnamon) and Canela (Ceylon cinnamon) (Raghavan, 2007). *Cinnamomum cassia* (Lauraceae) belongs to an evergreen tree originating in southern China, and widely cultivated in the countries of southern and eastern Asia (India, Malaysia, Thailand, Vietnam, Indonesia, and Laos) (Yan et al., 2015). Cassia is characterized by a sweet spicy aroma, more pungent bitter taste and thicker bark than Ceylon cinnamon (Li et al., 2013). The dried barks of Cassia are not only known as a spice and flavoring agent, but also are regarded as a traditional medicine in the world (He et al., 2016). It is commonly used for the treatment of amenorrhea, rheumatoid arthritis, cardiac palpitation, diarrhea, and gastrointestinal neurosis (He et al., 2016; Lin et al., 2003). Pharmacological studies have demonstrated that Cassia cinnamon possesses antidiabetic (Kumar et al., 2014; Andrew 2013), antioxidant, antibacterial (Haung et al., 2014), antifungal (Giordani et al., 2006), antiulcer (de Assis Oliveira, 2014), anti-hyperlipidemic (Mhammad et al., 2015), and analgesic activity (Yan et al., 2015; Wu and Jia, 2012). Recently, an increasing number of investigations on antioxidant activity of spices have been reported (Brodowska et al., 2014, 2015; Stoilova et al., 2007; Spiridon et al., 2011).

Cinnamon has been extensively researched since it has many benefits for human life. The plants spread across Southeast Asia, China and Australia with different types and varieties such as true cinnamon and *Cinnamomum zeylanicum* from Srilanka; *Cassia cinnamomum* from China and Vietnam; *Cinnamomum tamala* from India and Myanmar (Burma); and *Cinnamomum burmannii* from Indonesia. Indonesian cinnamon is especially found in the area of Sumatra and Java islands (Ravindran et al., 2004). Cinnamon bark is one of the most popular herbs utilized as a spice in cooking. In addition, its processed products in the form of essential oils and oleoresins have been widely used in pharmaceutical, cosmetic, food, beverage, and cigarette industries; also in traditional and modern medicine (Heyne, 1987; Sangal, 2011). Several compounds of cinnamon, include essential oil, eugenol, safrole, cinnamaldehyde, tannin, and calcium oxalate. Cinnamaldehyde is potential antioxidant compounds with the ability to scavenge free radicals (Thomas and Duethi, 2001).

Tea is produced from the leaves and buds of the *Camellia sinensis* (Theaceae) plant, and is one of the world's most popular beverages. Tea can be broadly classified according to the production method as green tea, oolong tea, black tea, or pu-erh tea. Green tea is mainly consumed in Japan and China, whereas black tea is primarily consumed in Western countries, India, and other parts of the world (Hayakawa et al., 2016)

Green tea (*Camellia sinensis*, Theaceae) is the most widely consumed beverage, following water (Yang et al., 2009), and may have cancer preventive effects in vivo. Polyphenols in green tea are thought to be responsible for the cancer preventive effects observed in laboratory and epidemiological

studies. Green tea and green tea polyphenols have been shown to have anti-cancer activity in a number of laboratory studies, which could be mediated through antioxidant or pro-oxidant mechanisms. Green tea polyphenols such as EGCG inhibit cell viability and induce apoptosis in a number of cancer cell lines such as osteogenic sarcoma (Ji et al., 2006), lymphoblastoid cells (Noda et al., 2007), leukemia cells (Nakazato et al., 2005), melanoma cells (Nihal et al., 2005), T lymphocytes (Li et al., 2000), and larynx carcinoma (Lee et al., 2010). EGCG can inhibit breast cancer cell viability through induction of apoptosis, yet not in normal breast cells (Vergote et al., 2000). Apoptosis by green tea polyphenols may occur independent of caspase-3 induction, through activation of p53 (Lee et al., 2010). Evidence for cell cycle modulation also exists. EGCG in green tea causes a reduction in cell viability through G1 growth arrest in human breast cancer cells (Kavanagh et al., 2001; Lin et al., 1999), which likely occurs through suppression of cyclin D1 (Nihal et al., 2005). Green tea polyphenols can even cause differentiation of cancer cells into slower proliferating cells (Zhou et al., 2004). Since, the literature shows that turmeric, cinnamon and green tea are good source of antioxidants, there by inducing anti-cancer activities, the objectives of the present study includes:

1. Testing the anti-cancer properties of turmeric, cinnamon and green tea on breast cancer (MCF-7) cell lines.
2. Testing the anti oxidant properties of turmeric, cinnamon and green tea.
3. Characterization of turmeric, cinnamon and green tea extracts by HPLC method.

MATERIALS AND METHODS

Plant selection and extraction of phytochemicals

Based on the review of literature, turmeric, cinnamon and green tea leaves were

procured from local medicinal plant depot. The samplers were completely shade dried and 10g dry weight of samples were powdered and used for extraction. Extraction was carried out by conventional decoction method using analytical grade methanol for 4hrs. the samples were then filtered and evaporated to obtain the crude extracts of the samples.

Cytotoxicity Studies By MTT Assay:

For cytotoxicity studies, MCF-7 cell line was procured from ATCC, stock cells was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further, 50,000 cells /well was seeded in a 96 well plate and incubated for 24 hrs at 37°C, 5 % CO₂ incubator. 32mg/ml stocks were prepared using DMSO. Serial two fold dilutions were prepared from 3.2mg/ml to 10mg/ml using DMEM plain media for treatment. 320µg/ml, 160µg/ml, 80µg/ml, 40µg/ml, 20µg/ml, 10µg/ml were used as test concentrations. The monolayer cell culture was trypsinized and the cell count was adjusted to 5.0 x 10⁵ cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 24hrs in 5% CO₂ atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in

PBS) was added to each well. The plates were incubated for 4 h at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC₅₀) values is generated from the dose-response curves for each cell line. % Inhibition calculated by using following formula:

ABTS Radical Scavenging Assay

The assay is performed as per Auddy (2003). Different concentrations of the test sample and the reference standard Quercetin (highest volume taken was 25l) are added to 225l of ABTS working solution to give a final volume of 1ml, made up by adding PBS. The absorbance is recorded immediately at 595nm. The percent inhibition is calculated at different concentrations and the IC₅₀ values are calculated using Graph pad prism software. Percentage growth inhibition calculated by using following formula:

DPPH Radical Scavenging Assay:

DPPH assay was carried out as per the method of Rajakumaret al., 1994. In brief, 80l of DPPH solution; various concentration of test solution and quantity sufficient to 240 l with HPLC grade methanol. The different concentrations tested for reference standard are 0.3125, 0.625, 1.25, 2.5, 5, 10 µg/ml. The different concentrations tested for test samples are 15.625, 31.25, 62.5, 125, 250 and µg/ml. The reaction mixture is mixed and incubated at 25°C for 15 minutes. The absorbance is measured at 510nm using semi-autoanalyzer. A control reaction is carried out without the test

sample. Percentage growth inhibition calculated by using following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test sample)}}{\text{Absorbance (control)}} \times 100$$

Determination of Catalase Activity

Catalase assay was carried using Abnova Catalase assay kit (Cat No: KA0884). 5 µl of 0.88M H₂O₂ was diluted into 215 µl dH₂O to generate 20 mM H₂O₂, then take 50 µl of the 20 mM H₂O₂ and diluted into 0.95 ml dH₂O to generate 1 mM H₂O₂. Add 0, 2, 4, 8, 16 µl of 1 mM H₂O₂ solution into 96-well plate to generate 0, 2, 4, 6, 8, 16 nmol/well H₂O₂ standard. The final volume adjusted to 90 µl with Assay Buffer. 10 µl Stop Solution was added into each well.

78 µl samples and 12 µl fresh 1 mM H₂O₂ were added to each well and OD recorded immediately at 570nm absorbance (A-before). Incubated at 25°C for 30 min and OD taken at 570nm (A-after).

Hydroxyl radical scavenging assay

The deoxyribose assay is performed as described by Halliwell et al., (1987). The reaction volume of 1.0 mL was contained 5.6 mM deoxyribose, 2.8 mM H₂O₂, 40 M FeCl₃, 100 M EDTA, and varying concentrations of the sample in 2.5 mM phosphate buffer, pH 7.4. Initiation of the reaction was by the addition of 0.1 mM Ascorbic acid. The mixture was incubated for 90 minutes at 37°C. After incubation, 1 mL of TBA (0.7% in 0.05 N KOH) and 1 mL of 2.5% TCA was added. The mixture was heated at 100°C for 8 minutes, cooled and the pink color formed is measured at 532 nm. Controls were devoid of test samples. Catechin was used as the reference standard. The percentage inhibition of hydroxyl radicals is calculated as follows:

HPLC

Test samples (10mg/mL) were prepared from stock with HPLC grade Methanol, Chloroform and Hexane (based on solubility, table no:) and used for HPLC analysis. Shimadzu LC- Prominence 20AT with C18 column 250 mm x 4.6 mm, 5µ particle used for analysis. 10µl injected per volume at the speed of 1mL/min. HPLC grade Aceto nitrile (50%) used as mobile phase A and HPLC grade Water (50%) as mobile phase B.

RESULT

Cytotoxicity assay:

Table No. 1: MTT assay of test samples against MCF-7 cell line

Compound name	Conc. µg/ml	OD at 590nm	% Inhibition	IC ₅₀ µg/ml
Control	0	0.773	0.00	
Cinnamon	10	0.726	6.08	211
	20	0.682	11.77	
	40	0.616	20.31	
	80	0.513	33.64	
	160	0.412	46.70	
	320	0.242	68.69	
Turmeric	10	0.696	9.96	112.2
	20	0.608	21.35	
	40	0.543	29.75	
	80	0.404	47.74	
	160	0.278	64.04	
	320	0.139	82.02	
Green Tea	10	0.655	15.27	109.5
	20	0.598	22.64	
	40	0.522	32.47	
	80	0.423	45.28	
	160	0.345	55.37	
	320	0.215	72.19	

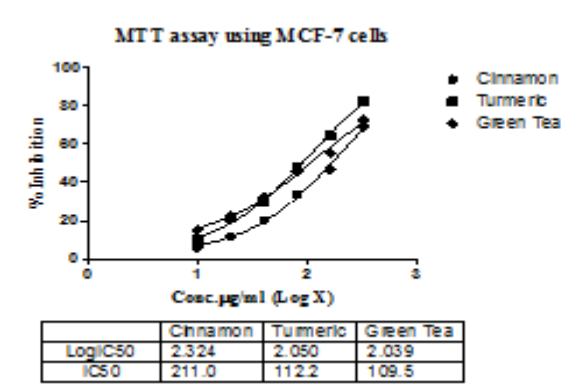


Fig.1 : Graph showing IC₅₀ of test samples
Table No. 2: MTT assay of positive control against MCF-7 cell line

MCF-7	Standard			
Compound name	Conc. µM	OD at 590nm	% Inhibition	IC ₅₀ µM
Control	0	0.768	0.00	
Doxorubicin	3.12	0.524	31.76	24.6
	6.25	0.518	32.55	
	12.5	0.421	45.18	
	25	0.265	65.44	
	50	0.251	67.32	
	100	0.124	83.85	

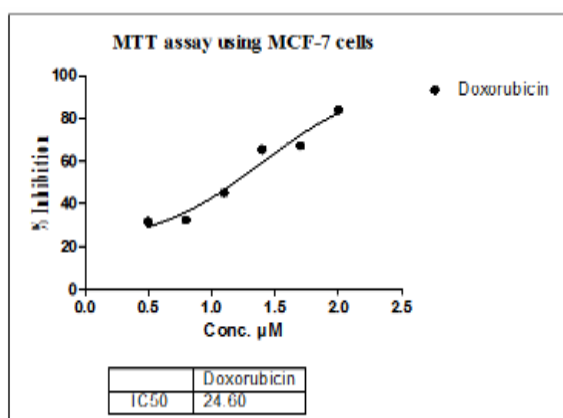
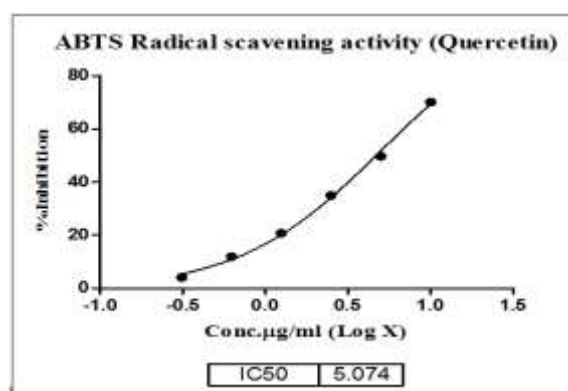


Fig. 2: Graph showing IC₅₀ of Doxorubicin

Sample Cinnamon and Turmeric showed IC₅₀ value of 210.4µg/ml and 111.7µg/ml inhibition in MCF-7 cells when compared to standard doxorubicin which is having IC₅₀ value of 24.6µM.

Sample	Conc (µg/ml)	OD @ 595nm	% Inhibition	IC ₅₀ (µg/ml)
C	0	0.823	0	
Quercetin	0.3125	0.789	4.12	5.074
	0.625	0.725	11.91	
	0.125	0.652	20.77	
	2.5	0.535	34.96	
	5	0.414	49.72	
	10	0.246	70.12	
Turmeric	0	0.774	0	94.23
	6.25	0.656	15.25	
	12.5	0.589	23.92	
	25	0.554	28.41	
	50	0.510	34.07	
	100	0.326	57.91	
Cinnamon	0	0.774	0	39.03
	6.25	0.703	9.17	
	12.5	0.652	15.75	
	25	0.494	36.14	
	50	0.348	55.05	
	100	0.254	67.15	
Green tea	0	0.774	0	31.74
	6.25	0.680	12.17	
	12.5	0.590	23.75	
	25	0.425	45.14	
	50	0.278	64.05	
	100	0.169	78.15	



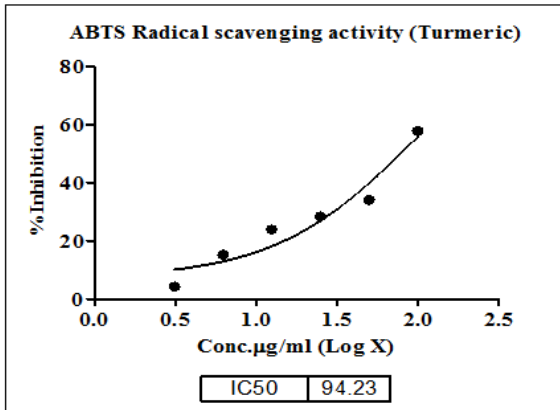


Fig. 4: IC50 of Turmeric

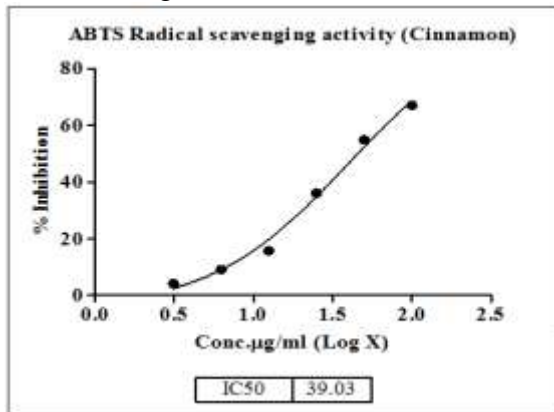


Fig. 5 : IC50 of Cinnamon

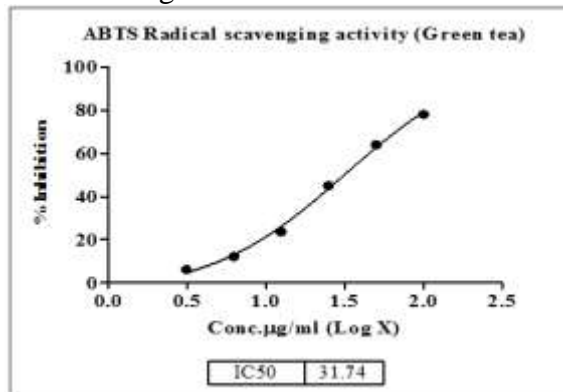


Fig. 6: IC50 of Green Tea

Among the samples tested above sample Green tea has showed maximum activity having an IC50 value of 31.74µg/ml while the sample Cinnamon has showed minimum activity having an IC50 value of 39.03µg/ml. Quercetin used as the standard has showed IC50 value of 5.074 µg/ml.

DPPH Radical Scavenging Assay:

Table 4: IC50 of Standard and test samples

Sample	Conc (µg/ml)	OD @ 510nm	% Inhibition	IC50 (µg/ml)
C	0	0.767	0	1.413
Quercetin	0.3125	0.706	8	
	0.625	0.627	18.3	
	0.125	0.504	34.3	
	2.5	0.341	55.5	
	5	0.242	68.4	
10	0.184	76		
Turmeric	0	0.711	0.00	38.64
	3.125	0.646	9.12	
	6.25	0.595	16.26	
	12.5	0.535	24.68	
	25	0.432	39.25	
	50	0.296	58.35	
	100	0.205	71.21	
Cinnamon	3.125	0.625	12.12	20.81
	6.25	0.560	21.26	
	12.5	0.450	36.68	
	25	0.339	52.25	
	50	0.275	61.35	
	100	0.148	79.21	
Green tea	3.125	0.631	11.22	34.07
	6.25	0.568	20.17	
	12.5	0.507	28.75	
	25	0.404	43.14	
	50	0.291	59.05	
	100	0.191	73.15	

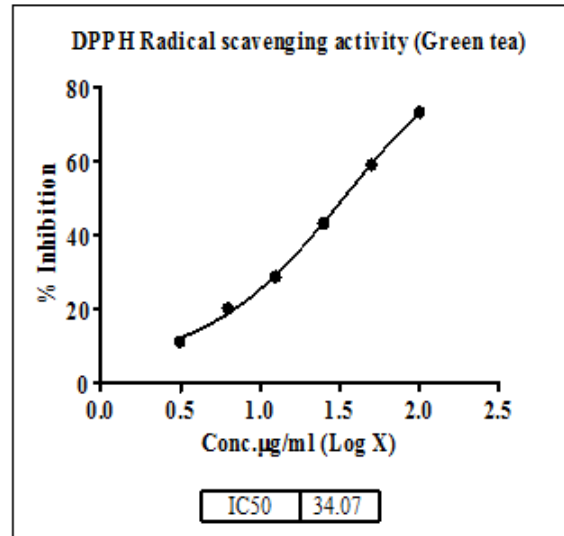
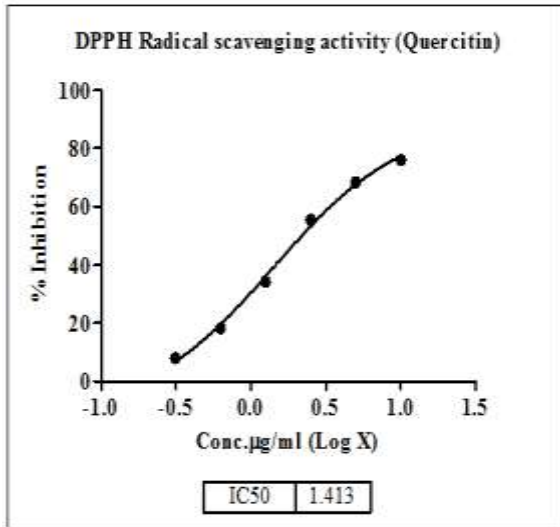
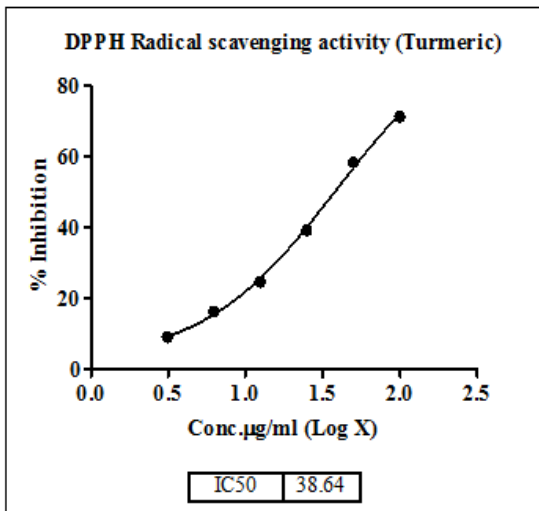


Fig. 10: IC50 of Green Tea



Among the samples tested above, Sample Cinnamon has showed maximum activity having an IC50 value of 20.81 µg/ml while the sample Turmeric has showed minimum activity having an IC50 value of 38.64 µg/ml. Standard Quercetin has showed IC50 value of 1.413 µg/ml.

Determination of Catalase Activity
Table 5: Standard (H2O2) absorbance

Hydrogen peroxide (nM)	Abs (570)
0.000	0.000
2.000	0.019
4.000	0.031
8.000	0.079
16.000	0.130

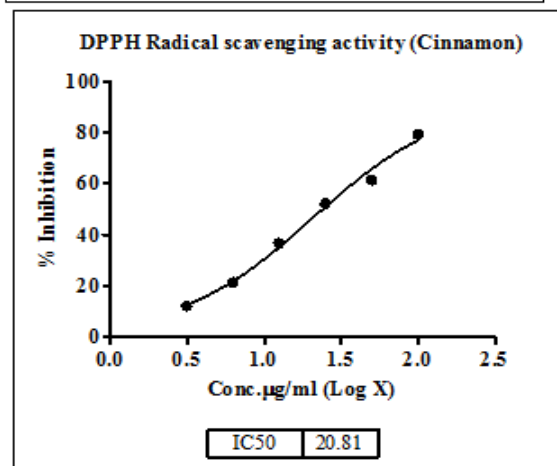


Fig. 9: IC50 of Cinnamon

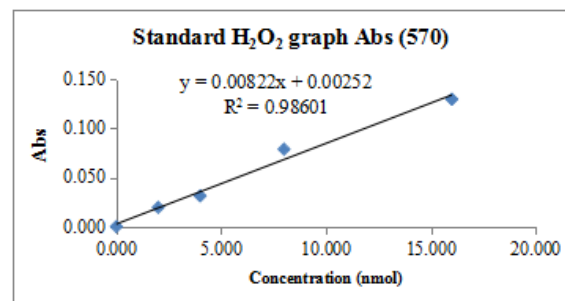


Fig. 11: Absorbance of the H2O2 vs. their concentration

Table 6: Catalase activity (mU/ml) in samples

Sample	Conc (µg/ml)	Abs _{before}	Abs _{after}	ΔA	Conc. H ₂ O ₂ nmol	Catalase activity mU/ML
Control	0	0.0023	0.0029			
Green Tea	10	0.0283	0.0356	0.0320	3.74	1.87
	20	0.0305	0.0383	0.0344	4.05	2.03
	40	0.0498	0.0485	0.0492	5.89	2.95
	80	0.0538	0.0509	0.0524	6.29	3.15
	160	0.0658	0.0682	0.0670	8.13	4.06
	320	0.0793	0.0732	0.0763	9.28	4.64
Turmeric	10	0.0136	0.0392	0.0264	3.05	1.53
	20	0.0304	0.0437	0.0371	4.38	2.19
	40	0.0395	0.0499	0.0447	5.34	2.67
	80	0.0436	0.0537	0.0487	5.83	2.92
	160	0.0492	0.0557	0.0525	6.31	3.15
	320	0.0542	0.0618	0.0580	7.00	3.50
Cinnamon	10	0.0221	0.0387	0.0304	3.55	1.78
	20	0.0406	0.0489	0.0448	5.34	2.67
	40	0.0532	0.0598	0.0565	6.81	3.41
	80	0.0699	0.0756	0.0728	8.84	4.42
	160	0.0764	0.0814	0.0789	9.61	4.81
	320	0.0801	0.0855	0.0828	10.10	5.05

Catalase activity mU/ML = Conc. H₂O₂ nmol/(min*0.1)

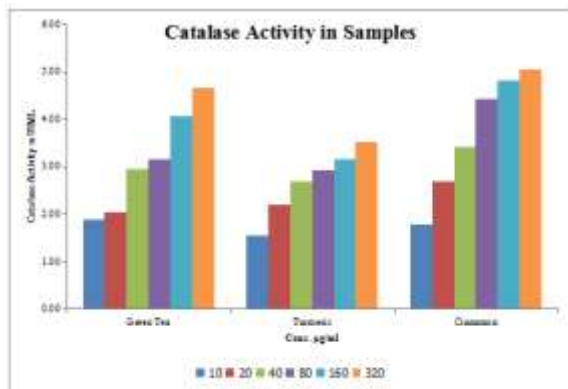
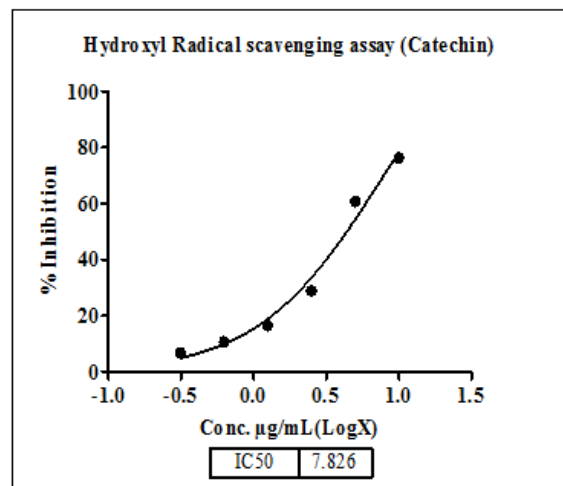


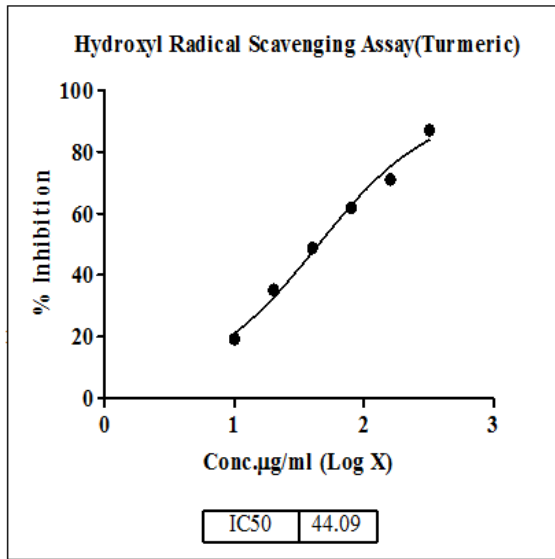
Fig. 12: Graphical representation- Conc. µg/ml plotted against Catalase activity.

Samples Green tea, Turmeric and Cinnamon tested for catalase activity has showed 4.64, 3.50 and 5.05 mU/ML of catalase activity at the maximum concentration tested (320 µg/ml).

Table 7: Hydroxyl radical scavenging assay

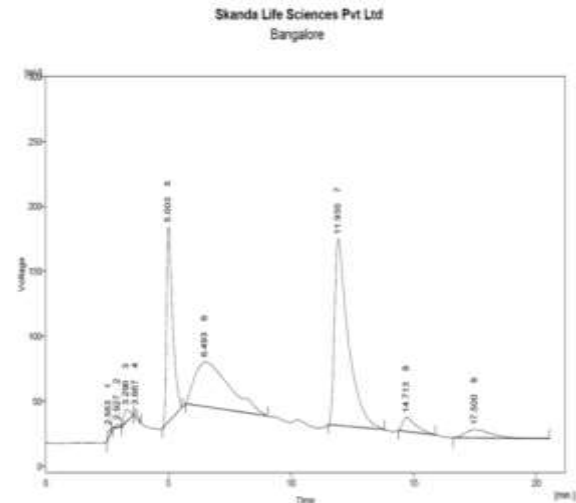
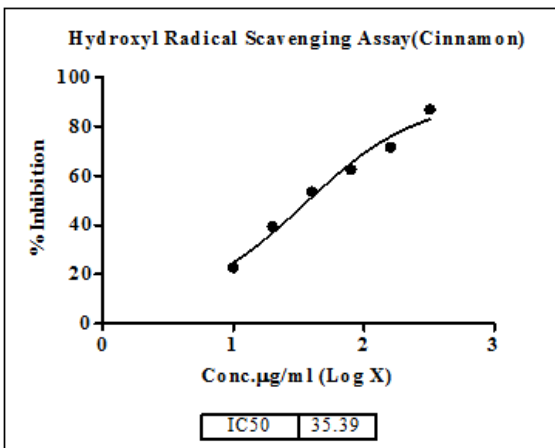
Plants Name	Concentration (µg/ml)	Absorbance 532nm	% Inhibition	IC ₅₀
Control	0	0.54	0.00	7.826 µg/ml
Standard (Catechin)	0.3125	0.51	6.74	
	0.625	0.49	10.61	
	1.25	0.45	16.43	
	2.5	0.39	28.89	
	5	0.21	60.72	
	10	0.13	76.27	
Control	0	0.59	0	44.09 µg/ml
Turmeric	10	0.48	19.22	
	20	0.38	35.17	
	40	0.30	48.75	
	80	0.22	61.896	
	160	0.17	71.05	
	320	0.08	87.15	
Cinnamon	10	0.46	22.68	35.39 µg/ml
	20	0.36	39.47	
	40	0.27	53.565	
	80	0.22	62.41	
	160	0.17	71.42	
	320	0.08	86.81	
Green tea	10	0.43	26.72	56.7 µg/ml
	20	0.38	35.38	
	40	0.31	47.73	
	80	0.23	60.82	
	160	0.15	73.74	
	320	0.11	81.92	





having an IC₅₀ value of 35.39 µg/ml while the sample Turmeric has showed minimum activity having an IC₅₀ value of 56.70 µg/ml. Standard Catechin has showed IC₅₀ value of 7.826 µg/ml.

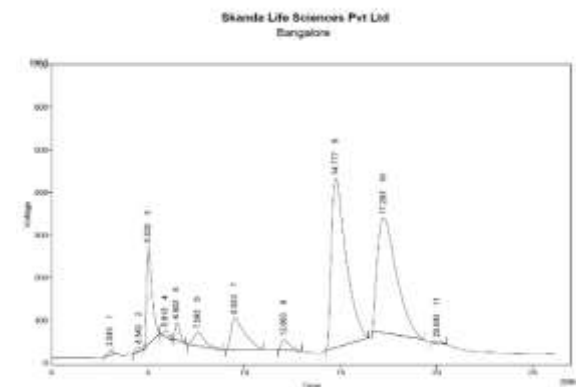
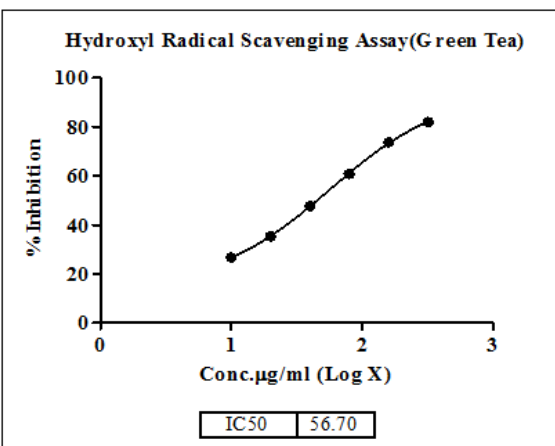
Fig.17: HPLC data of Turmeric



Result Table (Linear - C:\SPINCHROM\WORK\DATA\2019\APRIL\HASAN\CINNAMON)

Reten. Time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	Wt% (µg)
1	2563	35470	0.003	0.3	1.7
2	2927	29045	0.2	0.7	0.16
3	3290	148897	0.371	1.2	2.5
4	3667	49681	0.153	0.4	1.7
5	5003	2577168	149.960	20.2	40.6
6	6493	3434421	34.412	25.9	9.3
7	11930	5601342	143.539	43.9	38.9
8	14713	421294	10.934	3.3	3.0
9	17500	462360	6.323	3.6	1.7
Total	12769677	369160	100.0	100.0	

Fig.18: HPLC data of Cinnamon



Among the samples tested above, Sample Cinnamon has showed maximum activity

Result Table (Uncal - C:\SPINCHROM\WORK\DATA\2019\APRIL\HASANTURMERIC)

	Reten Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.043	151.200	11.833	0.3	1.1	0.18
2	4.643	188.076	9.960	0.4	0.9	0.31
3	5.030	4449.724	230.610	8.6	20.7	0.30
4	5.913	308.804	15.404	0.6	1.4	0.34
5	6.503	678.899	41.144	1.3	3.7	0.25
6	7.593	1308.179	32.102	2.5	2.9	0.57
7	9.523	3644.904	75.576	7.1	6.8	0.74
8	12.083	731.800	23.836	1.4	2.1	0.45
9	14.777	21121.185	396.441	40.9	35.6	0.83
10	17.263	18827.005	270.216	36.5	24.3	1.08
11	20.043	179.844	5.804	0.3	0.5	0.50
	Total	51589.714	1112.906	100.0	100.0	

Fig.19: HPLC data of Green tea

Table 8 : HPLC summary

sample	RT	Area%	peak
GA	5.186	96	2
Turmeric	14.77	40	11
	17.26	36	
Cinnamon	11.93	43.9	9
	6.49	26.9	
Tea	5.193	69.1	5

Table 9: Comparative result of Turmeric Cinnamon to Gallic acid

Sample	Stock	R.T	Area (mv*s)	GA ug/ml of extract	Dilution	GA mg/g	Chromatogram Reference
GA	100ug/ml	5.186	1786.55	-	1		1
Turmeric	10mg/ml	5.05	4449.60	249.06	1	249.061	2
cinnamon	10mg/ml	5	2577.16	144.25	1	144.253	3
Tea	10mg/ml	5.193	900.12	20.23	20	404.586	4

DISCUSSION

Acharya et al., (2015) showed that, Curcumin and Berberine are of extreme antitumor agents. Ji et al., 2012 showed that curcumin is a potential anti- cancer activity. Rachana and Venugopalan (2014) showed that ethyl acetate and dichloromethane extracts have the maximum antioxidant activity. Among the extracts screened for antimicrobial activity, hexane, dichloromethane and ethyl acetate extracts showed maximum activity against all tested organisms.

The cytotoxicity of the developed conjugate against some cancerous cell lines

including HeLa, Saos-2 and MCF-7 cells as well as normal fibroblast cells was studied (Razaei et al., 2018). Promising results were achieved where the conjugate could effectively kill the growing cancerous cells, while its impact on healthy cells was much less.

In the present study, sample Cinnamon, Turmeric and Green Tea tested on breast cancer cell lines (MCF-7). Samples showed IC50 value of 211µg/ml, 112.2µg/ml and 109.5µg/ml inhibition in MCF-7 cells when compared to standard doxorubicin which is having IC50 value of 24.6µM showing that all the cells are potentially inducing breast cancer.

Reactive oxygen species (ROS) and free transition metal ions cause extensive oxidative damage to cellular biomolecules such as DNA, proteins and lipids. Oxidation of these biomolecules often leads to oxidative stress, which can cause cell damage and contribute to aging and the development of many chronic and degenerative diseases such as cancers, coronary heart diseases, Alzheimer's disease and Parkinson's disease. Antioxidants are substances that counteract free radicals and prevent the damage caused by them. In recent years, antioxidants have gained a lot of importance due to their potential as prophylactic and therapeutic agents in many diseases (Abeysekera et al., 2013). Abeysekera et al., (2013) showed that the leaf and bark extracts of Ceylon cinnamon possess marked antioxidant properties, the leaf showing the highest activity.

Bark of Ceylon cinnamon in the day-to-day life for prevention of oxidative stress-associated chronic diseases and for development of functional foods, nutraceuticals and drug discovery.

Tanvir et al., 2017 showed that the turmeric varieties from Bangladesh are promising sources of natural antioxidants, as indicated by their high contents of polyphenols, flavonoids, tannins, and ascorbic acid and by their considerable DPPH free radical-scavenging activities and FRAP values. The extraction yields investigated in both aqueous and ethanol extracts of the turmeric varieties suggest that higher antioxidant compounds could be obtained with ethanol.

Cinnamon extracts possess antioxidant activity. By these findings and purification of the active substance(s) present in the extracts of Cinnamon, it will be possible to discover new natural drugs serving as antioxidant agents for application in the nutritional or pharmaceutical fields, in the prevention of free radical-mediated diseases. Cinnamon barks could act as a better antioxidant agent than other parts of Cinnamon based on their radical scavenging abilities Yang et al., 2012).

The results obtained by the use of two different methods (DPPH and ABTS) proved that cinnamon bark, its methanolic extracts and essential oils can be considered as a good source of natural antioxidants. High correlation between antioxidant activity and cinnamon oil and extract may be attributed either to high concentration of the bioactive constituents or to synergy among different oil and extract constituents. Both extract and essential oil of cinnamon bark could be applied in cosmetic and pharmaceutical branches of industry (Brodowska et al., 2016).

Wijayanti et al. (2011) reported that ethanolic extract of cinnamon bark collected from different areas possess antioxidant activity with various IC₅₀ values in a range of 75.48 µg/mL and 136.88 µg/mL. In the study on beneficial effects of cinnamon to prevent diabetes and Alzheimer's diseases. The phytochemical analysis results indicated that polyphenols

(including flavonoids, tannin) and phenolic volatile oil compounds as the major antioxidant compounds. This result suggested that the simple traditional method of preparation of infusion commonly applied by people, yielded the highest antioxidant activity (Ervina et al., 2017). Recent studies have suggested that cinnamon extract exerts chemopreventive properties such as anti-proliferative and anti-oxidative effects (Schoen et al., 2005; Dhuley, 1999).

Kim et al., 2010 showed that 2'-hydroxycinnamaldehyde (HCA) shows antitumor activity in SCC-15 and HEP-2 oral carcinoma cells and inhibits cell proliferation by inducing cell cycle arrest and apoptosis.

Antioxidants are a group of substances which, when present at low concentrations, in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves. Plants possess antioxidant principles. Various classes of phytochemicals have been shown to have antioxidant properties which are due to the presence of substituted groups such as carbonyl, phenolic, phytol side chain, electron withdrawing group, electron donating group etc.

The DPPH scavenging assay is a simple chemical experiment for the primary evaluation of any compound for its simplicity and low cost for free radical scavenging activity. In the present study, among the samples tested above, Sample Cinnamon has shown maximum activity having an IC₅₀ value of 20.81 µg/ml while the sample Turmeric has shown minimum activity having an IC₅₀ value of 38.64 µg/ml. Standard Quercetin has shown an IC₅₀ value of 1.413 µg/ml.

ABTS assay is based on the scavenging of light by ABTS radicals. An antioxidant

with an ability to donate a hydrogen atom will quench the stable free radical, a process which is associated with a change in absorption which can be followed spectroscopically. The relatively stable ABTS radical has a green colour and is quantified spectrophotometrically at 734nm.

In the present study, Among the samples tested above sample Green tea has showed maximum activity having an IC₅₀ value of 31.74µg/ml while the sample Cinnamon has showed minimum activity having an IC₅₀ value of 39.03µg/ml. Quercetin used as the standard has showed IC₅₀ value of 5.074 µg/ml in case of ABTS assay.

In th present study, samples Green tea, Turmeric and Cinnamon tested for catalase activity has showed 4.64, 3.50 and 5.05 mU/ml of catalase activity at the maximum concentration tested (320 µg/ml) in Catalase activity assay.

Hydroxyl radical scavenging activity can be measured using the deoxyribose assay, a Fenton type reaction system which is considered one of the best known models for determining the hydroxyl radical scavenging activity.

In th present study, aAmong the samples tested above, Sample Cinnamon has showed maximum activity having an IC₅₀ value of 35.39µg/ml while the sample Turmeric has showed minimum activity having an IC₅₀ value of 56.70µg/ml. Standard Catechin has showed IC₅₀ value of 7.826µg/ml in hydroxyl scavenging assay.

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