Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

https://doi.org/10.36673/AJPAMC.2023.v11.i02.A06

EVALUATION OF POLYPHENOL OXIDATION RATE AND METAL CHELATING/ SEQUESTERING POTENTIAL OF ASSORTED CAFFEINATED/NON-CAFFEINATED TEA

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ABSTRACT

Polyphenols and melanin both are important antioxidants with lot of biological activities. Since polyphenol undergoes oxidation, present study is focused on determination of amount of polyphenol present, rate of oxidation and subsequent generation of melanin in tea of various caffeinated and non-caffeinated natural products which comprises of seeds of fennel (*Foeniculum vulgare* Mill.), seeds of cumin (*Cuminum cyminum*), leaves of black tea (*Camellia sinensis* var.), leaves of green tea (*Camellia sinensis*), peels of orange (*Citrus reticulata*) and peels of banana (*Musa acuminata*). These estimations were done spectrophotometrically with 1:10 aqueous extract (w/v) of above samples against gallic acid and melanin as standards at 760-765nm, 475nm respectively. Rate of oxidation values ($P<0.05$) were noted from concentration of melanin formation at an interval of 5 mins. Sequestering potential/metal chelating activity against heavy metals (lead and cadmium) were studied from foam forming capacity of these substrates using laureth sulphate. Orange peel tea recorded highest content of polyphenol and least polyphenol oxidation. Green Tea was found to contain maximum melanin. Highest metal-chelating capacity with both lead and cadmium was found in banana peel tea. Cumin tea also recorded highest chelating potential with cadmium only.

KEYWORDS

Polyphenol, Melanin, Spectrophotometer, Sequestering potential and Rate of oxidation.

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INTRODUCTION

Polyphenols are natural products with "several hydroxyl groups on aromatic rings ", which are synthesized exclusively by plants. They are basically secondary metabolites found in nature, with chemical features possessing bioactivities to modulate oxidative as well as neuroprotective actions[1,2]. These group of compounds have powerful antioxidant properties along with capacity to form metal-chelate complex[3,4]. They are

popularly used as ethnomedicines due to various health benefits, such as reducing inflammation, improving heart health, and protecting against certain types of cancer[5,6].

Different parts of various plants consumed as food such as vegetables, fruits, spices and whole grains contain polyphenols. There are more than 8,000 types of polyphenols, which include: 1. flavonoids like quercetin and catechins in fruits 2. polyphenolic amides like capsaicin in chili peppers 3. phenolic acids like lignans and stilbenes in vegetables and whole grains 4. other like resveratrol in red wine and ellagic acid in berries. Polyphenol is also found commonly in tea. Some of the main polyphenols found in tea include catechins, epicatechines, flavonoids, theaflavins and tannins[7].

When polyphenols are exposed to oxygen, the PPO (Polyphenol Oxidase) enzyme present as a constituent darken the colour by converting the polyphenolic compounds to quinones[8]. Quinones subsequently react with other compound to from melanin. Melanin also contributes to the aroma and flavour of the tea.

Polyphenols are also found in several spices, leaves and fruits/ fruit peels. If polyphenol is readily oxidised in the presence of PPO, the benefits of the polyphenol are decreased as their metal-chelating activity reduces and they are not still in the phenolic form due to rapid formation of stable quinone[9]. More over these quinones react with other compound to from melanin, which has lots of biological activities such as antioxidant property, free radical-scavenging property, anti-HIV activity[10].

Hence estimation of melanin concentration needs to be monitored to determine the loss of polyphenols due to oxidation. Chelating activity of these polyphenolic substrates vary depending on their content of polyphenol. This can also be evaluated from foam index of these antioxidants against heavy metals which further helps us to predict the heavy metal scavenging potential.

So, the metal chelating capacity of some natural products have been evaluated and compared against EDTA from their foam forming ability against lead and cadmium which are most commonly occurring heavy metal pollutant[11]. The major sources of lead emissions today are ore and metal processing, automobiles[12]. Lead poisoning can cause a variety of symptoms such as insomnia, delirium, cognitive deficits, tremor, hallucinations, and convulsions[13]. Sources of cadmium emission are metal production and fossil fuel. Cadmium metal poisoning may lead to nausea and vomiting, diarrhoea, abdominal pain, cramping, tenesmus and also leads to the most severe form of chronic cadmium intoxication, Itai-Itai[14,15].

Samples investigated in this study are well known for their various antioxidants, polyphenols and hence biological importance. They are as follows**:**

SEEDS

Fennel (*Foeniculum vulgare* Mill.) is a biennial medicinal and aromatic plant containing different types of polyphenols such as epigallocatechin, gallate, flavonols, rutin, kaempferol-3-rutinoside, isorhamnetin, quercetin glucuronide etc[16] which are used for anti-cholinesterase activity[17], herbal remedy for poisoning and stomach condition and also helps in digestion.

Cumin (*Cuminum cyminum*) contains antioxidants such as phenolic acids, apigenin, luteolin, flavonoids, quercetin, tocopherol and coumarins[18]. The seeds contain phenolic compounds in suitable amounts that allow considerable radical scavenging and chelating activities[19]. The most important biological activities of cumin include antibacterial, anti-inflammatory, antidiabetic, immunomodulatory properties[20].

LEAVES

Black tea (*Camellia sinensis*) contain the polyphenolic compounds such as theaflavins, epigallocatechin gallate, theaflavins and thearubigins[21]. It also contains huge amount of caffeine. It has high inflammation power and provides protection against several chronic disorders, relieves stress and reduces risk of cancer.

Green tea (*Camellia sinensis*) contains polyphenolic compounds such as epicatechin, epigallocatechin, gallo catechin gallate, kaempferol, myricetin, flavanols, catechins, are flavonoid compounds with

a basic structure of α-phenyl-benzopyran, which is about 18% to 36% of the dry weight of tea leaves[22]. It helps with weight management, skin inflammation and type 2 diabetes[23,24].

FRUIT PEELS

Orange peels (*Citrus reticulata*) have theaflavins, thearubigens, rutin, epicatechin, quercetin, luteolin, naringin, hesperidin which are a large group of polymeric polyphenols in orange peel[25]. The biological activities of orange peel are aiding cancer, digestion, burning fat and reducing cholesterol, reducing stress, diabetes[26].

Banana peels (*Musa acuminata*) are rich in polyphenolic compounds like tannins, anthocyanins, gallic acid, epicatechin, catechin, quercetin, myricetin, kaempferol which have strong antimicrobial, antidiabetic and anti-inflammatory properties[27], regulate digestive system, reduce irritable bowel syndrome, have radical scavenging property[28].

Present study involves estimation of

Amount of polyphenol

Rate of oxidation of polyphenol and

Metal chelating activity

Which has been executed in various caffeinated/non caffeinated tea (aqueous extracts) of above mentioned assorted natural products.

MATERIAL AND METHODS

Collection of materials

Cumin seeds, fennel seeds, black tea, green tea were collected from more supermarket, Bally, Howrah, India. Orange and banana were procured from bally bazar, Howrah, India.

Chemical reagents and apparatus used

Lead acetate anhydrous, 99%, cadmium chloride 98% monohydrate, EDTA disodium salt dihydrate pure, 98%, sodium laureth sulfate, sodium carbonate was used as chemicals for this experiment. Gallic acid, folin ciocalteu reagent and sodium laureth sulfate were purchased from LOBA CHEMIE PVT. LTD.

SYSTRONICS Spectrophotometer 106 was used in this experiment.

EXPERIMENTAL PROCEDURE

Determination of polyphenol content in different caffeinated/non- Caffeinated tea

Equal amount of each sample (10gm) was taken in six different 250mL conical flasks by accurate weighing. Fennel and cumin seeds, orange and banana peels (cut into small pieces) were used as non-caffeinated herbal samples whereas black tea and green tea were used as caffeinated sample. To each sample was added 100mL distilled water to maintain 1:10 concentration ratio. These solutions were then heated for 2 minutes and filtered. Aqueous extracts were collected as filtrates which is referred to as tea.

To 5mL of the sample extract was added 2mL distilled water, 0.6mL sodium carbonate solution and 0.15mL folin ciocalteu reagent respectively. Transmittance of the solution was observed in spectrophotometer at 760-765 nm and compared against standard gallic acid curve (0-50mg)[29]. Thus, the polyphenol content was determined for all the sample-water extract[30,31].

Determination of concentration of melanin/polyphenol oxidation rate

Transmittance of the filtered aqueous extract of sample was observed using spectrophotometer at 475nm. And compared against a melanin standard curve from synthetic melanin. The solution was kept at room temperature for 5 minutes and was again measured. Thus, five readings of transmittance were measured at an interval of 5 minutes, keeping the water extract at room temperature. This protocol was followed for all samples extracts to record the transmittance.

Estimation of chelating/sequestering potential of tea /water extracts with heavy metals

Lead acetate and cadmium chloride solution was prepared separately in 10% concentration (w/v). EDTA solution of concentration 10% (w/v) was also prepared.

Different solutions were made in 5 sets.

Water extract of samples (10mL) + lead/cadmium solution (5mL) (6 sets+6 sets)

Water (10mL) + lead/cadmium solution (5mL) (1+1)

EDTA solution (10mL) + lead/cadmium solution (5mL) (1+1)
Water (5mL) + EDTA (10mL) solution
Only water (10mL) (as control)
In each of the above-mentioned solution was added 4 drops of laureth sulfate and were stirred at 830rpm using magnetic stirrer to form foam. Each solution was stirred for 10 minutes. Volume of foam formed in centimeter^3 was measured from the height and diameter of formed foam using a scale to determine the chelating / sequestering potential with corresponding metals.
Volume of the foam of each solution is calculated using the formula: ($V = \pi r^2 h$).
Where r = radius of beaker
h = height of foam

Statistical analysis

All the experiments have been done in triplicate. And data from two different experiments were subjected to analysis of variance (ANOVA) ($P< 0.05$).

RESULTS AND DISCUSSION

In the present study spectrophotometric method was applied to study amount of polyphenol in various tea at 760-765nm ($P<0.05$). From the experimental analyses, it is observed that the highest concentration of polyphenol is recorded in orange peel (227.9065mol/lit) and lowest in cumin seed (89.924mol/lit). Extent of increase in concentration of polyphenol along a concentration gradient is found to follow a pattern as: Cumin seed tea<fennel seed tea<banana peel tea<black tea<green tea<orange peel tea.
From the experimental analyses, it is observed that the concentration of melanin is increasing significantly ($P<0.05$) with increase in time. Highest melanin concentration is recorded in Green tea (339.136mol/lit) and lowest in orange peel (226.645mol/lit). Extent of increase in concentration of melanin along a concentration gradient is found to follow a pattern as: Orange peel <fennel<cumin<
banana<black tea< green tea. The rate of increase in concentration (%) is observed the highest in case of fennel seed (5.74 % after five minutes, 3.01% after ten minutes, 2.63% after fifteen minutes, 0.52% after twenty minutes). For banana peel no change in concentration is observed after 5 minutes, 2.78% after 10 minutes, no change after 15 minutes, 0.5% after 20 minutes. For green tea not much change is observed after 5 minutes, 0.73% change in concentration after 10 minutes, same concentration remained after 15 minutes, then it followed again 0.45% change in concentration after 20 minutes. Hence it is observed that percentage of melanin formation or disappearance rate of polyphenol gradually decreases with time. For black tea and cumin seed also trend of decrease in melanin formation or disappearance rate of polyphenol followed the same pattern.

METAL-CHELATE FORMING CAPACITY TEST

From the experimental analyses, it is inferred that banana tea has the highest chelating capacity/ sequestering potential with cadmium. In presence of lead, banana tea and fennel tea both showed similar sequestering potential and recorded a value which is higher than all other samples. Whereas cumin extract exhibited least sequestering potential /chelating capacity with both cadmium and lead.

Table No.1: Estimation of polyphenol content in different caffeinated / non- caffeinated tea

S.No	Sample	Fennel tea	Cumin tea	Black tea	Green tea	Orange tea	Banana tea
1	Concentration in Gallic acid equivalent	91.408± 0.067	89.924± 0.056	156.689± 0.064	177.461± 0.073	227.906± 0.082	112.179± 0.058

Table No.2: **Melanin concentration at 475nm/polyphenol content in different caffeinated / non-caffeinated tea**

S.No	Sample	Concentration (mol/lit) initial	Concentration (mol/lit) after 5 min	Concentration (mol/lit) after 10 mins	Concentration (mol/lit) after 15 min	Concentration (mol/lit) after 20 mins
1	Fennel tea	255.569±0.065	270.230±0.098	278.377±0.078	285.667±0.081	287.187±0.092
2	Cumin tea	272.878±0.059	275.593±0.076	278.377±0.067	281.234±0.077	282.692±0.064
3	Black tea	331.975±0.074	334.310±0.054	336.696±0.063	336.696±0.056	339.136±0.086
4	Green tea	339.136±0.073	339.136±0.068	341.632±0.057	341.632±0.063	343.187±0.056
5	Orange peel tea	226.645±0.069	227.523±0.085	228.410±0.088	229.303±0.085	230.204±0.064
6	Banana peel tea	288.725±0.083	288.725±0.065	296.771±0.099	296.771±0.093	298.455±0.068

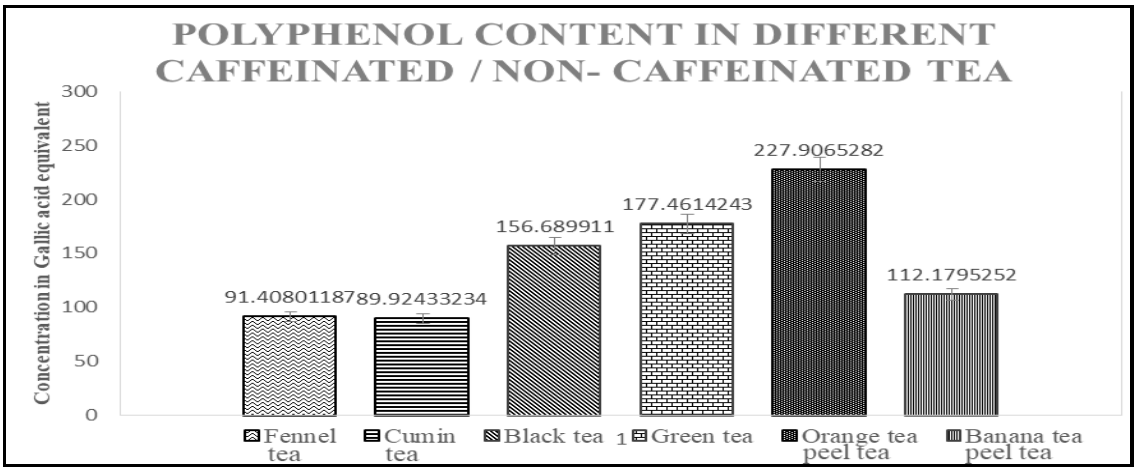

Figure No.1: Polyphenol content in different caffeinated/non- caffeinated tea

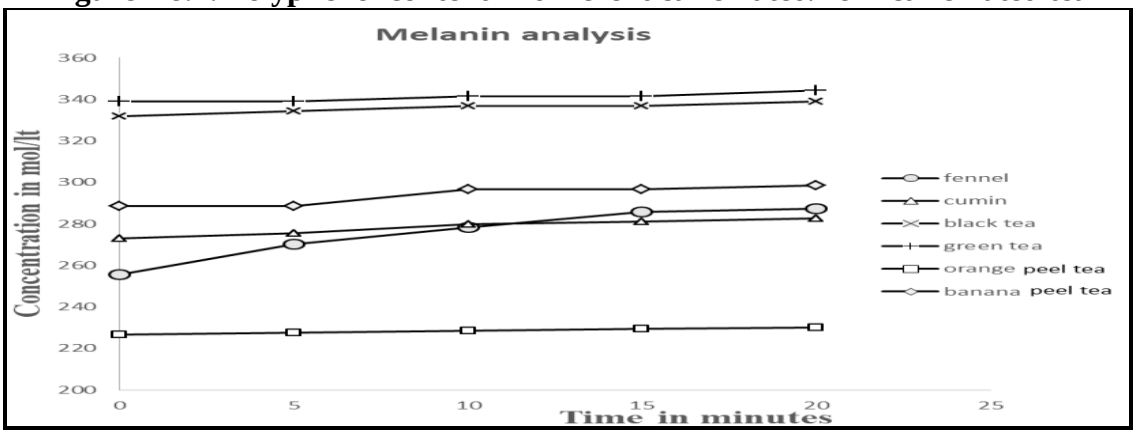

Figure No.2: Melanin concentration in various sample tea

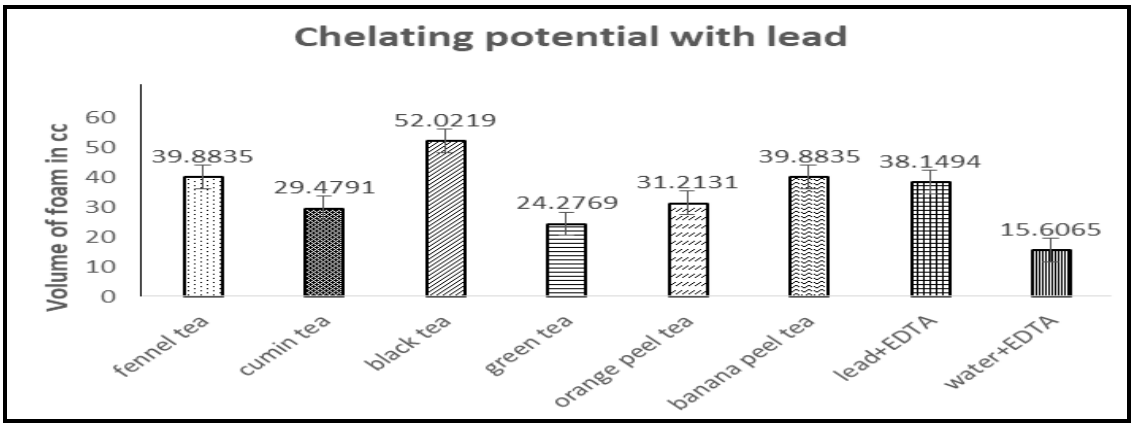

Figure No.3: Metal-chelating activity test on various sample aqueous extract with lead

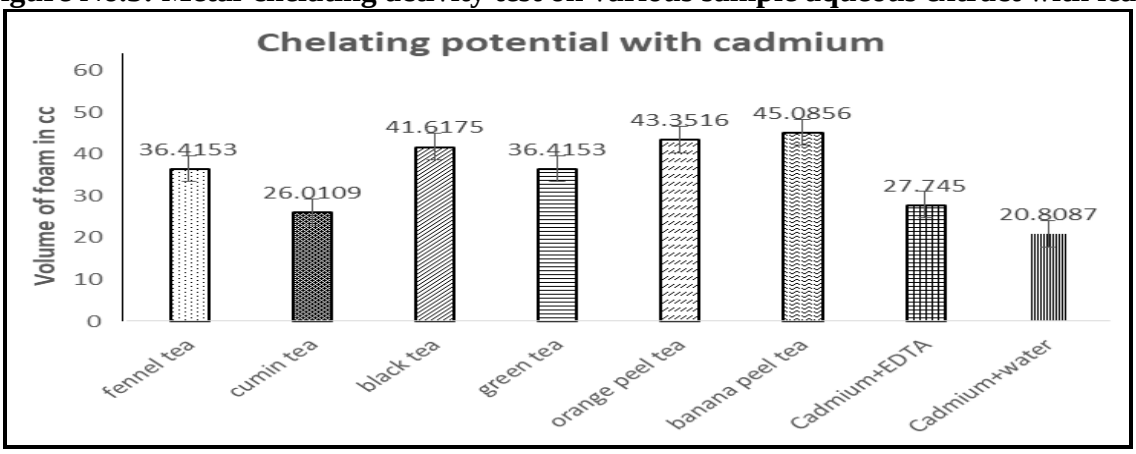

Figure No.4: Metal-chelating activity test on various sample aqueous extract with cadmium

CONCLUSION

The present study revealed estimation of amount of polyphenol is higher in orange peel aqueous extract in compared to other aqueous extract. Also, it is observed decrease in percentage of melanin formation or disappearance rate of polyphenol is found to be lower in orange peel aqueous extract compared to other extract. So, benefits of polyphenols are not as much decreased therefore, it can be concluded by consuming orange peel aqueous extract we will get such benefits of polyphenol more than the other samples. Melanin concentration is recorded to be higher in Green tea aqueous extract. Therefore Green tea extract will exhibit the benefits of melanin such as antioxidant, anti-inflammatory activity in our body. Metal-chelating activity is mostly found in banana peel extract and fennel seed extract with lead, for cadmium it is highest for banana peel. Therefore, consumption of these extracts will lower the risk of developing many diseases by chelating with these heavy toxic metals such as insomnia, delirium, cognitive deficits etc (associated with lead) and cramping, tenesmus, Itai-Itai (associated with cadmium). Further investigation is required to fully understand the chemical kinetics and confirm the order of the reaction.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Chemistry, Bijoy Krishna Girls' college, University of Calcutta, West Bengal, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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