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Influence of Geographical Location on the Antioxidant Activity of Green Tea

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ABSTRACT

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. This study was conducted in order to determine the impact of the geographical location on the antioxidant activity of five samples of green tea. The objective of this study was to extract the green tea, test the antioxidant activity of green tea extracts using, to screen the phytochemical elements using green tea extracts, as well as to study the chromatographical analysis based on the sampling location. Five green tea from different countries was obtained from Malaysia, China, Japan, London, and Australia. Then the green tea extracts was obtained by evaporation using rotary evaporator. The phytochemical elements were tested for saponin, flavonoid, alkaloid, tannin and phenol . The phytochemical screening had shown positive for all the elements tested. The antioxidant activity was done using 1, 1 –Diphenyl-2-picrylhydrazyl (DPPH) reagent which is a stable free radical. The antioxidant activity shown that the China green tea has the best scavenging activity of 97% while the scavenging activity of other green teas respectively are Malaysia green tea is 49% , Japan green tea has 77% , London green tea has 50% and Australia green tea has 70%. Finally, the thin- layer chromatography analysis revealed the compounds of flavonoid, catechin and phenol. This study found that there is a relationship between the geographical location and the antioxidant activity of green tea.

Key words: *Antioxidants, cell damage, geographical location, antioxidant activity, five green tea, DPPH, scavenging activity , thin-layer chromatography , flavonoid , catechin , phenol.*

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INTRODUCTION

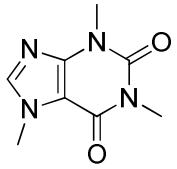
According to legend, as far back as 2700BC tea has been discovered and it is believed it was completely accidental. It all happen when Chinese Emperor Shen Nong was preparing boiling water, when a few leaves fell from the camellia bush into the water. The emperor then tasted the brew as he was attracted by the aroma given by the tea. He was also delighted by the fresh flavor that the tea exhibited. Next,the custom and love of tea soon spread to other cultures across the globe. By 300 AD, tea drinking had become a popular drink in China as it was popular for it's aroma, taste and freshness that it gave.

In the recent times, there was an increase in green tea drinking thanks to many studies that have proved this tea is one of the many healthy drinks available. Due to abundant amount of nutrients such as antioxidant, green tea works to promote glowing skin and allows the body to fight against cancer.

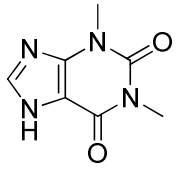
Fig. 1: World map showing the diversity of locations of the selected sample

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to anoxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions[3]. Antioxidants terminate these chain reaction by removing free radicals. Therefore, antioxidant acts as "free radicals scavengers" and repair the damages done by these free radicals. Oxidative damage plays a huge role in many of our modern-day diseases , such as muscle and tissue degeneration, heart disease, diabetes, cancer, as well as many other health problems.

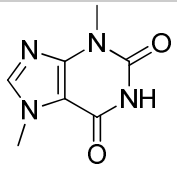
Other factors influencing catechin content and many other constituents isolated from the green tea leaves (See Fugure 1), are the geographical location and growing conditions soil, climate, agricultural practices, fertilizers), the type of green tea for example blended, decaffeinated, instant, and the preparation of the infusion for instance amount of the product used, brew time, temperature[12]. Unlike green tea and herbal infusions, the content of flavan-3-ols in white, Oolong and black tea was also significantly affected by water temperature[11].



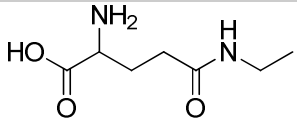
Caffeine



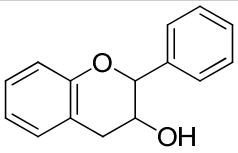
Theophylline



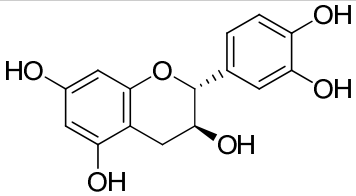
Theobromine



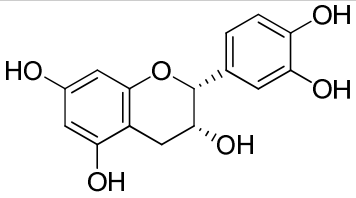
Theanine



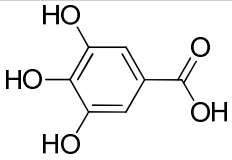
Flavan-3-ol



(+) Catechin (2R,3S)



(-) Epicatechin (EC)



Gallic acid

Fig. 2: Chemical Constituents Isolated From the Green Tea

MATERIALS AND METHODS

In this research the green tea was prepared and each tea was extracted to test antioxidant properties, phytochemical elements and analysis using thin-layer chromatography. The research was carried out in five phases.

Green tea sample preparation

The tea from Malaysia ,China and Japan were obtained at the local supermarket around Shah Alam. The tea from London was also obtained from the local supermarket Giant around Shah Alam. The remaining green tea from Australia were obtained from online purchase.

Fig. 1: The five types of dried green from different locationes

Phase 2 : Extraction of green tea

100g of each sample was weighed and to it 50ml of 70% methanol was added. Next ,the extract put into the ultrasonic cleaner. After cooling at room temperature, the extract was centrifuged at 2000rpm for 40min. The supernatant was collected in a centrifuge tube. Then, the supernatant is separated into a conical flask and the residue of green tea into another beaker. The supernatant is then put into the rotary evaporator to extract the crude green tea. The extraction step was repeated thrice. By using this apparatus a pure quality of semi – solid , tea extract was maintained and placed in small glass bottles that were weighed and then covered it with aluminium foil and refrigerated for further use later[5].

Measuring Antioxidant Activity of Green Tea

The DPPH. radical scavenging activity assay used by Chan *et al.* (2007) was adopted with slight modification. Different dilutions of the extract (0.001, 0.050 and 0.100 mg/mL) were prepared. DPPH solution was prepared by dissolving 6.0 mg of DPPH radical powder in 100 mL methanol. Then, 1 mL of extract from each dilution was added into the test tube containing 2 mL of DPPH solution. Control was prepared by adding 1 mL of methanol to 2 mL of DPPH solution , vitamin C were used as standards. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The scavenging activity of each extract on DPPH radical was calculated using the following equation:

Scavenging activity (%) = (1 -(Absorbance of sample)/(Absorbance of control) x 100)The results were then plotted in a graph to get the 〖MIC〗_50 (mg/mL) which is the concentration required inhibiting 50% of the DPPH[6].

Phytochemical Screening Of Green Tea

This test is done to detect the inorganic constituents of each extract. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, and phenols (Mbata. T.I,2008). Additional test of flavonoid was carried out . The procedure for all the constituents were carried out according to Godghate[7].

The tests are as follows :

a)- Test for alkaloid: A quantity (3ml) of concentrated extract was taken into a test and 1ml HCL was added,the mixture was heated gently for 20min cooled and filter, the filtrate was used for the following test.

Wagner test : the filtrate was treated with wagner's reagent, formation of brown reddish precipitate indicated presence of alkaloid

b)- Test for saponin: 5ml of extract were mixed with 20 ml of distilled water then agitated in graduated cylinder for 15min, formation of foam indicates Saponin.

c)- Test for tannin: 2ml of extract was added to 1% lead acetate ,a yellowish precipitate indicates the presence of tannin.

d)- Test for phenol: To the diluted extractions a few drops of lead acetate solution were added, blue/ green colour indicated a positive result.

e)- Test for flavonoid: 2g of green tea extracts was added to 10ml of methanol , and filtrated. Next, few drops of HCL is added to 2ml of filtrate and 0.5g Mg was added. Pink or red colour formation indicates positive result.

Measurement of thin-layer chromatography

The TLC sheet acts as a stationary phase support. The solvent used was chloroform-methanol water (65:35:10) as the mobile phase respectively which acted as mobile phase. 2µl of each sample control (solvent) were taken and spot in TLC sheet and kept in a beaker containing mobile phase. This set up was kept aside for 10-15 min. After that the TLC sheet was taken out and the bands were visualized. According to the number of component present bands were noted.

RESULTS

From table 1, it shows the extraction percentage yield of all the green teas which is calculated by using the formula. It shows the Malaysia green tea having a percentage yield of 19.35%, China green tea of 10.21%, Japan green tea of 10.95%, London green tea of 14.30% and lastly green tea of Australia green tea of 11.50%.

Table 1 : Percentage of extraction yield of green tea from various location

No	Sample	Amount of dried leaves (g)	Amount of extract yield (g)	Percentage of yield (%)
1.	Green tea From Malaysia (BOH)	100	19.35	19.35
2.	Green tea From China (TEN REN TEA)	100	10.21	10.21
3.	Green tea From Japan	100	10.95	10.95
4.	Green tea From London (AHMAD TEA)	100	14..30	14.30
5.	Green tea From AUSTRALIA (MADURA TEA)	100	11.50	11.50

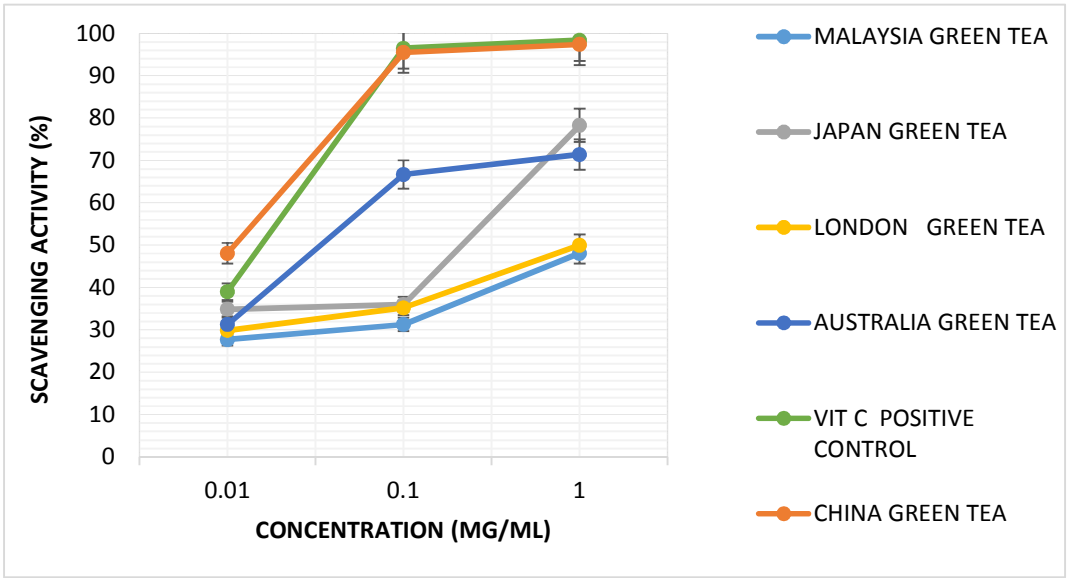


Fig. 1 : MIC_{50} antioxidant property graph versus scavenging activity (%) of the green tea

From figure 1, the graph and table 2 shows antioxidant activity against scavenging activity where China green tea has 97% and Japan green tea has 77% , London green tea has 50%, Australia green tea has 70% and lastly, Malaysia green tea has scavenging activity of 48%. Vitamin C which is used as a standard has scavenging activity of 98%.

Table 2 : MIC_{50} antioxidant property of the green tea

No	Green tea source	MIC_{50} (mg/ml)
1	Green tea from Malaysia (BOH)	NT / 48%
2	Green tea from China (TEN REN TEA)	0.46 / 97%
3	Green tea from Japan	NT / 77%
4	Green tea from London (AHMAD TEA)	NT/ 50%
5	Green tea from Australia (MADURA TEA)	0.75 / 70%
6	Vitamin C	0.40/ 98%

From table 3, the table shows the phytochemical elements tested on green tea extract. The elements tested were alkaloid, saponin, tannin, phenol and flavonoid. All were positive for the elements tested. Figure 3 shows all the positive results for the green tea extract.

Table 3: Phytochemical test results

No	Green tea location	Alkaloid	Tannin	Flavonoid	Saponin	Phenol
1	MALAYSIA GREEN TEA	+	+	+	+	+
2	JAPAN GREEN TEA	+	+	+	+	+
3	CHINA GREEN TEA	+	+	+	+	+
4	LONDON GREEN TEA	+	+	+	+	+
5	AUSTRALIA GREEN TEA	+	+	+	+	+

From table 4, the table shows Rf values of thin-layer chromatography of all the green tea extract tested. The values represents three bands of green tea components.

DISCUSSION

The green tea from China shows to have the best scavenging activity of 97% while the positive control shows 98% of scavenging activity. The factors such as climate are to be seen to affect the antioxidant activity. A short sun exposure time ,low temperature of the green tea leaves enables to yield a higher amount of catechins. A low amount of rainfall also contributes to a higher level of catechins which is an important contributions[8]. The Malaysia green tea shows scavenging activity lowest at concentration more than 1.0 mg/ml producing 48% scavenging activity. This shows that high antioxidant green teas mostly comes from subtropical climate with some seasonality (www.ratetea.com.my).

The types of phytochemical elements analyzed are Tannin, Saponin, Alkaloid, Flavonoid and Phenols. The same elements are tested in all the green tea from various location as the constituents in green tea are same. The major elements are tested. This phytochemical analysis functions to assess the chemical composition of crude extracts qualitatively by using specific reaction of precipitation and coloration reaction

to identify the major natural chemical groups such as starch, alkaloids, flavonoids, tannins, reducing sugars, and amino acids. General reactions in these analysis revealed the presence or absence of these compounds in crude extract tested.

Table 4 : Rf values of thin-layer chromatography

LOCATION	1ST SPOT	2ND SPOT	3RD SPOT
Green tea of malaysia	0.08	0.34	0.78
Green tea of china	0.10	0.49	0.92
Green tea of japan	0.15	0.38	0.82
Green tea of london	0.10	0.68	0.79
Green tea of australia	0.19	0.39	0.77
Ascorbic acid	0.06	0.55	0.97

This analysis showed three bands representing three constituents which are main in the green tea. As the green tea used was commercial green tea, the results matched with the supporting journal which showed results of fresh tea leaves having a seven bands , commercial green tea leaves extract shows three bands. The mobile phase used was chloroform: methanol: water in ratio of 65:35:10. Where the polarity of chloroform is the least polar followed by methanol which is medium polar and water is most polar. In comparision of the polarity , the flavonoid constituent has the least polarity which causes it to elucidates faster, the catechin has a medium polarity and the phenol has the most polarity elucidates slower.

CONCLUSION

Antioxidants plays an important role in oxidizing the oxidative stress in our body cells caused by free radicals. It is important to understand how geographical location factors may have effect on the antioxidant property. Based on the results obtained, the China green tea shows the best scavenging activity while Malaysia green tea shows the least scavenging activity. The lowest MIC_{50} was seen in the green tea from China which is seen to give 97% at 0.46mg/ml. Thus, the lowest minimum inhibitory concentration has the highest antioxidant activity. All the green teas were positive are shown to have abundant amount of phytochemical elements which consists of Alkaloid, Saponin, Tannin, Flavonoid and Phenol. The thin –layer analysis revealed three bands representing the major constituents in the commercial green tea extracts. It shows the flavonoids component which is less polar elucidating faster, catechin elucidating moderately and lastly the phenol component elucidating slowly but the furthers. As a conclusion it is found that there is relationship between geographical location and antioxidant activity of green tea.

REFERENCES

1. Aisyah, M.R. and Kamarul Rahim, K., Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves. *International Food Research Journal*, **17(4):** (2010).
2. Antioxidant and antibacterial activities of various seabuckthorn (Hippophae rhamnoides L.) seed extracts. *Food Chemistry*, **92(1):** 119-124.
3. Arash, K., & Fatiacad, F., Diabetic Nephropathy–using herbals in diabetic (2012).
4. Archana, S., & Abraham, J., Comparative analysis of antimicrobial activity of leaf Assays-onalternative therapy (2011).
5. Avneet Kaur, M. K., Estimation and Comparison of Total Phenolic and Total Antioxidants In Green Tea and Black Tea. Global *Journal of Bio-science and Biotechnology*, 116-120 (2015).

6. Azlim Almey, A. A., Ahmed Jalal Khan, C., Syed Zahir, I., Mustapha Suleiman, K., caffeine from a Japanese green tea (Sen Cha Uji Tsuyu) as a function of extracts from fresh green tea, commercial green tea and black tea on pathogens.fresh tea shoots and the theaflavin content of manufactured tea. *Journal of the Science of Food and Agriculture*, **24(7):** 813-818 (2010).
7. Godghate, A., Sawant, R., & Sutar, A., Phytochemical Analysis of Ethanolic Extract of Roots of Camellia sinensis Linn. *Rasayan J. Chem*, **5(4):** 456-459 (2012).
8. Hilton, P. J., & Palmer-Jones, R., Relationship between the flavanol composition of *Journal of Biotechnology*, ***7*(10):** (1973).
9. Mbata, T. I., Debiao, L. U., & Saikia, A., Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on Listeria monocytogenes. *African* onion (*Allium cepa*) in diabetics nephropathy. assays-on-alternative therapy,**7(10):** (2008).
10. Negi, P. S., Chauhan, A. S., Sadia, G. A., Rohinishree, Y. S., & Ramteke, R. S. Antioxidant and antibacterial activities of various seabuckthron seed extracts, *Journal of Food Chemistry*, **92(1):**119-124 (2005).
11. Price, W.E., & Spitzer, J.C., The kinetics of extraction of individual flavanols and temperature. *Food chemistry*, **50(1):** 19-23 (1994).
12. Wu, C. D., & Wei, G. X., Tea as a functional food for oral health. *Nutrition*, **18(5):** 443-444 (2002).
13. Heyam Ali, Rasha Saad, Babiker El-Haj: Prevention of Cap-Locking of Syrup Product by Treating the Manufacturing Process with Citric Acid Monohydrate. *International journal of pharmaceutical chemistry* **5(6):** 218 – 226 (2015).
14. Rasha Saad, Mohd Nazmi, Norfazrin Hanif, Fadli Asmani, Eddy Yusuf: Effect of different sampling locations on the antibacterial property of Centella asiatica leaves. *International journal of Pharmacy and Analytical Research*, **3(3):** 189 – 206 (2014).