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COMPARATIVE STUDY BETWEEN GREEN AND BLACK TEA

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ABSTRACT

The objectives of this study are to investigate the qualitative and quantitive analysis of green and black tea that extracted by water, ethanol and petroleum ether. The results revealed that the presence of alkaloid, tannin, steroid, flavonoid and carbohydrate in green and black tea extracted by water ethanol and petroleum ether. In addition, extraction of water for green and black teat contains saponins while extraction of ethanol and petroleum ether for both teas not contains saponins. Extraction of ethanol and petroleum ether for green and black tea contains cardenolide and finally extraction of water, ethanol and petroleum ether for both teas not contains anthraquiaone and coumarin. The alkaloid content for extraction green tea by water is 5.4mg/g, by ethanol is 4.3mg/g and by petroleum ether is 4.6mg/g. While alkaloid content of extraction for black tea by water is 5.4mg/g, by ethanol is 4.3mg/g and by petroleum ether is 4.mg/g, respectively. The tannin content of extraction for green tea by water 1.2%, by ethanol is1.8% and petroleum ether is 1.5%. While tannin contents of extraction for black tea by water is 1.2%, by ethanol is 0.5% and petroleum is 1%.

KEYWORDS

Tea, Alkaloid, Tannin and Qualitative.

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INTRODUCTION

Green and Black Tea is made up from Camellia sinensis, which is characterized as a perennial evergreen shrub. Tea varieties reflected the growing region (Ceylon and Assam), districted (Darjeeling), form (pekoe and gunpowder) and processing method (green, black and oolong). India and Srilanka are major producers of green tea. The Turkish trader's reportedly introduced to Western culture in 6th Century (Berube *et al*, 2005)[1]. It has many uses for

its mild stimulant and medicinal properties for prevent cancer (Berube *et al*, 2005)[1], prostate cancer (Chiu *et al*, 2005)[2], asthma (Chow *et al*, 2005)[3], dental cavity, diabetes (Laurie and Miller, 2005)[4], heart attack (Fukino *et al*, 2005)[5], reduce high cholesterol (Riemersma *et al*, 2001)[6], improve fertility (Chow *et al*, 2005)[3], memory enhancement, mental performance and menopausal symptoms (Maron *et al*, 2005)[7]. The green tea is prepared by exposing the gathered leaves to air until superfluous moisture is eliminated, leaves roasted over a brick wood fire and continually stirred until leaves become moist and flaccid, after which leaves pass to rolling table and rolled into balls and subjected to pressure which twist them and gets rid of the moisture, then leaves shaking out on flat trays, again leaves roasted over a slow and steady charcoal fire and kept leaves in rapid motion for an hour A to hour and half, till leaves assume a dullish green colour after that the leaves winnowed, screened, and graded into different types. While black tea, gathered leaves are exposed to air for long period, gathered up, tossed until soft and flaccid, further exposure, roasted in an iron pan for about five minutes and after rolling and pressing the leaves are shaken out, exposed to the outer air for some hours, re-roasted for three or four minutes and recoiled, spread out in baskets and exposed to heat of charcoal fire for five or six minutes and then rolled for third time and again heat and finally dried in baskets over charcoal fire from which leaves become black in colour. Steroid compounds are containing the perhydrocyclopentano-phenanthrene skeleton and usually occur in glycosidal combination with sugar (AOAC, 1990)[8]. Alkaloids are group of natural products (atropine, quinine, morphine, etc). Which are widely used to treat Malaria and Cancer diseases (AOAC, 1990)[8]. Cardenolides are act on the heart direct or indirect mechanism to enhance the force and velocity of the contraction, therefore it is known as cardiotonic (AOAC, 1990)[8]. Carbohydrates are the most abundant organic constituents of plant, serve as the major source of chemical energy (sugar and starch), as well as important constituents of supporting tissue (cellulose in wood, cotton and flax) (AOAC, 1990)[8]. Anthraquinones are oxygenated derivative of pharmacological importance that is used as laxatives or cathartics, anti-inflammatory, antibacterial, antifungal and also as natural dyes (AOAC, 1990)[8]. The flavonoid, constitute one of most diverse and widespread group of natural products, found in fruit, vegetables, nuts, seeds, stem, flowers, as well as tea, and are one of the most important constituents of the human diet (AOAC, 1990)[8]. Coumarines are derivative of benz- pyrone, or lactones of o-hydroxcinnamic acid such as coumarin, umbelliferonee, aesculetin, daphnetin, fraxetin and scopolamine, they are found in both state in free or combination with glycosidic, not all members of the group are phenolic (AOAC, 1990)[8]. The tannin compounds are phenolic polymers precipitate protein from aqueous solution and it is reduce or inhibit enzyme activity (Goldstein and Swain, 1963)[9].

Objectives of this study are Phytochemicals screening of the green and black tea namely;

1. Qualitative analysis of Alkaloid, Anthraquiaones, Cardenolides, Tannin, Saponins, Coumarin, Flavonoid, Steroid and Carbohydrates in green and black tea extracted by water, ethanol and petroleum ether in green and black tea extracted by water, ethanol and petroleum ether.
2. Quantitive analysis of Alkaloid and Tannin content in black and green tea.

MATERIAL AND METHODS

Sample preparation

The dried green and black teas are collected from local market of Khartoum State, Sudan, the dried teas (green and black) are freed from foreign materials and ground into fine powder to pass 0.4mm mesh screen. The prepared samples are kept in tight containers and stored at room temperature until stared analysis. Preparation of e aqueous extraction of tea (green and black) by using water, ethanol and petroleum ether is carried out according to method described by (FAO, 1991)[10].

Qualitative analysis

Alkaloid, Saponins, Tannin, Steroid, Flavonoids, Comrine, Cardiac and Carbohydrates were identified according to method described by (FAO, 1991)[10].

Alkaloid

Two gram of well ground samples is extracted with 10ml of 1% HCL for 30 minutes in water bath. The suspension is filtrated through cotton whool into tube and the filtrate solution is divided into two portions A and B. Then saturated solution of sodium bicarbonate is added to the filtrate solution (B) until pH became 8 -9 and the solution is mixed with 3ml chloroform, then upper layer is removed by pipette and treated with acetic acid until pH became 5 and five drops of dragendroff's regent are added. If the precipitate is form. This indicated the presence of quaternary alkaloid. The lower layer (chloroform) is treated with 3ml of 1% HCL which separated the lower layer into two layers. Then upper layer is pipette and treated with dragendroff's regent, this indicated to presence of tertiary alkaloid.

Anthraquinones

0.5 gram of well ground sample is extracted in boiling water for two minutes with 5ml 0.5N KOH and 0.5ml H2O. Suspension is cooled and filtrate by using glass wool and six drops of acetic acid are added, then the solution is mixed with 5ml benzene, The upper layer is separated and pipette into test tube and then followed by adding of 2ml 0.0N KOH. If the solution gives red colour. This indicated the presence of Anthraquinones.

Cardenolides

3 gram of well ground sample is extracted 2ml of distil water in boiling water bath for 30 minutes, then allow the solution to cool and filtrate by using glass wool into test tube. 10 drops of lead acetate are added and then filtrate to tube (A) and (B). Four drops of Keddle's reagent are added. The solution (B) is mixed with 2 ml chloroform, and 4 drops of Keddle's reagent is added to lower layer. If the solution gives a violet colour. This indicated the presence of Cardenolides.

Tannin

3 gram of well ground sample is extracted with 1ml distil water in boiling water bath for 5 minutes, then filtrate the solution by using filter paper and allow a solution to cool . The filtration solution is divided into two portions A and B. 5ml of 2% NaCL was added to portion A. Suspended solution is filtrated by using filter paper, followed by adding 5ml 1% gelatin solution. Precipitate indicated the presence of Tannin. 1ml of 1% ferric chloride is added to portion B. The green blue colour indicated the presence of Tannin.

Saponins

3 gram of well ground sample is extracted with 5ml distil water in boiling water bath for 6 minutes, then filtrate the solution by using glass wool. 1ml of sample extracted is shaken for 5 minutes and finally the persistent and voluminous froth is formed.

Coumarin

3 gram of well ground sample is extracted with 5ml distil water in boiling water bath for 6 minutes, then filtrate the solution by using filter paper and allow a solution to cool. Then the sample extracted shows a blue fluorescence, but it changed into greenish - blue on addition of few drops of ammonia. This indicated to presence of coumarin.

Flavonoid

3 gram of well ground sample is extracted 2ml of distil water in boiling water bath for 30 minutes, then allow the solution to cool and filtrate by using glass wool into test tube. 10 drops of lead acetate are added to filtration. The yellowish precipitate is formed. This indicated to presence of Flavonoid.

Steroid

3 gram of well ground sample is extracted with 5ml ethanol with 1% acetic acid in boiling water bath for 5 minutes, then filtrate the solution by using filter paper and allow a solution to cool. The filtration solution is divided into two portions A and B. Few drops of SbCL3 are added to portion A and few drops of Marquis's reagent are added to portion B. Red colour develop in portion A and violet to red colour develop in portion B. This indicated to presence of Steroid.

Carbohydrates

3 gram of well ground sample is extracted with 5ml distil water in boiling water bath for 6 minutes, then filtration the solution by using glass wool. Few drops of Molish, s reagent are added to filtration solution. Then violet colour appeared, this indicated to presence of carbohydrates.

Quantitive analysis

Alkaloid content

It is determined according to method described by (AOAC, 1990)[8].

Evaporated combined extract on steam bath with air current to about 10ml add measured excess 0.2N H2SO4 and continue evaporation to remove the solvent, cool and add methyl. Then the excess acid (H2SO4) was titrated with 0.02 N NaOH.

$$V1 \times M1 = V2 \times M2$$

$$Mg/g = \frac{V2 \times D.F. \times M.WT}{103 \times S}$$

Where: V1 = Volume of 0.20.2N H2SO4, M1 = Concentration of H2SO4 (0.2N), V2 = Volume of NaOH, M2 = Concentration of NaOH (0.02 N), D.F. = Dilution factor, M.WT = Molecular weight of NaOH, S= Weight of sample and 103 = conversion factor from volume to weight.

Tannin content

It is determined according to method described by (Price and Butler, 1987)[11].

0.2 gram of extracted sample was placed in 50ml of conical flask and 10ml of 1% HCL was added. The contents of flask were shaken for 20 minutes, and then contents of the flask were centrifuged at 3000rpm for 5 minutes. Then contents of flask were incubated at 30^{o}C for 20 minutes. The spectrophotometer was read at 500nm and then scale read zero with the blank solution. Finally, readings were taken three times and averaged.

$$C.E.\ (\%) = \frac{C \times 100}{10 \times S}$$

Where: C.E. = Catechin equivalent, C = Concentration corresponding to optical density, 10 = Volume of extract (ml) and S = Weight of sample.

RESULTS AND DISCUSSION

Qualitative analysis

The qualitative test of green and black tea that extracted by water, ethanol and petroleum ether for the alkaloid, anthraquiaone, saponins, tannin, Cardenolides, steroid, flavonoids, coumrine, and carbohydrates were shown in Table No.1.

The findings revealed that the alkaloid, tannin, steroid, flavanoid in three extractions (water, ethanol and petroleum ether) for green and black tea are available. The cardenolide in two extractions (ethanol and petroleum ether) for green and black tea are presence, but the cardenolide in water extraction is not found in green and black tea. These results indicated that cardenolide is not dissolved in water, but dissolved in ethanol and petroleum ether. In addition to that the anthraquiaone and coumarin in three extractions (water, ethanol and petroleum ether) for green and black tea is not available. The saponins in green and black tea extracted by water are present, but saponins in green and black tea extracted by ethanol and petroleum ether are not present. These findings revealed that green and black tea do not contain anthraquiaone and coumarin. These results are supported by those results given by (Balbaa *et al*, 1981)[12].

Quantitive analysis

Table No.2 indicated that the alkaloid content in green tea in water, ethanol and petroleum extracted is 5.4, 4.3 and 4.6%, respectively. While the alkaloid content in black tea in water, ethanol and petroleum extracted is 6.4, 4.5 and 5.2%, respectively. The results revealed that alkaloid in both green and black tea in water extracted is higher than in ethanol and petroleum ether extracted, but there is no significant difference in three solvents extracted for green tea at ($P \leq 0.05$). This indicated that extraction of alkaloid is not effected by types of solvents. In addition to that the results indicated that tannin content in green and black tea for water extracted is similar, tannin content in green tea for ethanol extracted is higher than in black tea. The results revealed there significant difference at ($P \leq 0.05$). The variation in alkaloid and tannin content is attributed to type of solvent, type of tea and process of tea.

Table No.1: Chemical constituent of green and black tea

S.No	Sample	Green tea			Black tea		
	Solvent \ compounds	Water	Ethanol	Petroleum ether	Water	Ethanol	Petroleum ether
1	Alkaloids	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve
2	Anthraquinones	(-) ve	(-) ve	(-) ve	(-) ve	(-) ve	(-) ve
3	Cardenolides	(-) ve	(-) ve	(+) ve	(+) ve	(+) ve	(+) ve
4	Tannin	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve
5	Saponins	(+) ve	(+) ve	(-) ve	(-) ve	(-) ve	(-) ve
6	Coumarin	(-) ve	(-) ve	(-) ve	(-) ve	(-) ve	(-) ve
7	Flavonoids	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve
8	Steroid	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve
9	Carbohydrates	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve	(+) ve

*Chemical constituents that available (+ ve), while it is not available (- ve) in green and black

Table No.2: Alkaloid and tannin content in extracted tea solvent (water, ethanol and petroleum ether of green and black type

S.No	Sample	Green tea			Black tea		
	Solvent \ compounds	Water	Ethanol	Petroleum ether	Water	Ethanol	Petroleum ether
1	Alkaloid mg/g	5.4±1.0a	6.4 ±0.5a	4.3 ±0.3a	4.5 ±1.2a	4.6 ±1a	5.2 ±1.4a
2	Tannin %	1.2 ±0.5a	1.2 ±1a	1.8 ±2a	0.5 ±0.01b	1.5 ±1a	1.0 ±0.8a

*Each value is average of three replicates expressed on dry weight basis, row with the same litter are not significant while rows with different litters are significant at $P \leq 0.05$

CONCLUSION

The results concluded that the green and black tea contain alkaloid, tannin, steroid, flavonoid, Cardenolides, saponins and carbohydrate. In addition amount of alkaloid and tannin are varied according to type of solvent used and processing of tea.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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