JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Comparative Studies Of Phytochemical Screening And Antioxidant Analysis Of Green Tea(leaves) And Black Tea(dust) - (Camellia sinensis)

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ABSTRACT:

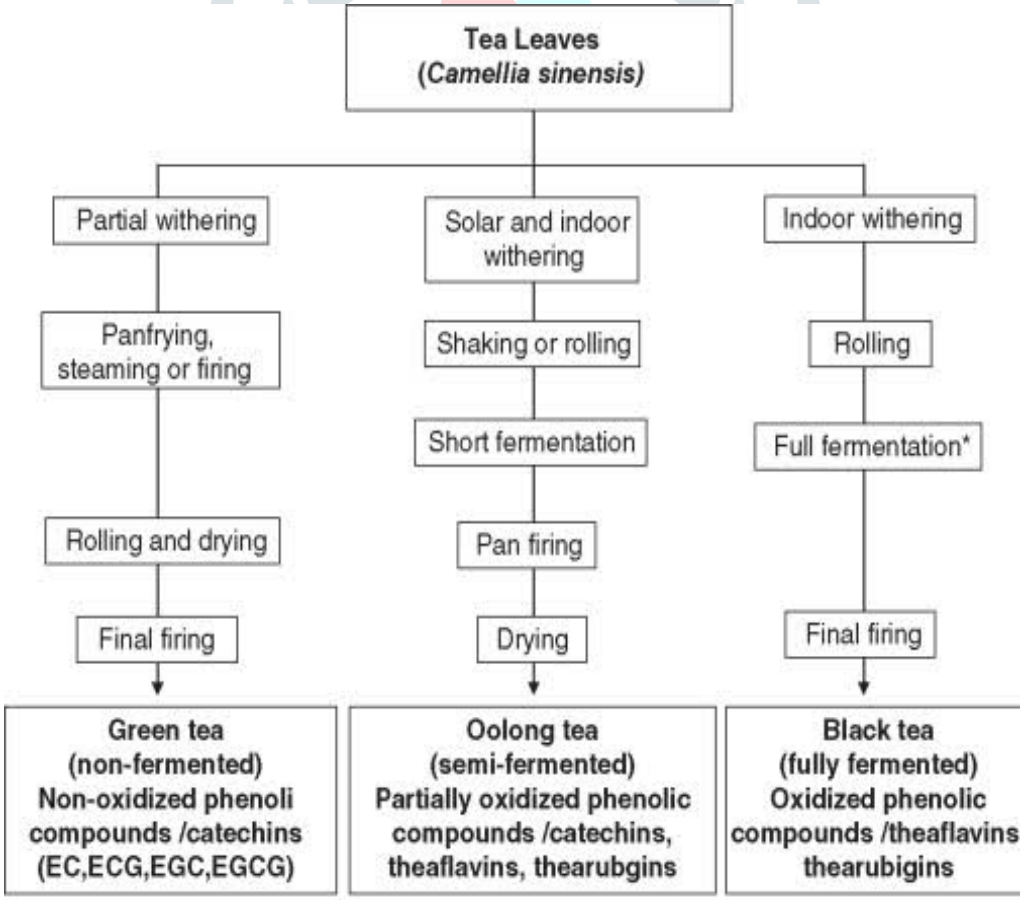
In recent years, researchers have looked at the discovery of new alternative sources of Phytochemicals and antioxidant agents, especially from plant sources. *Camellia sinensis* has served humanity as the source of medicinal agents from the very beginning. Phytochemical agents present in the leaves to mark the compounds responsible for the antioxidant activity. Phytochemical screening of plant leaves reveals the presence of saponins, alkaloids, flavonoids, steroids, phenols, tannins and glycosides.Leaf extracts of *Camelia sinensis* were prepared and their phytochemical and antioxidant activity was evaluated against the DPPH radical scavenging and Nitric oxide (NO) radical scavenging method .

Keywords: *Camellia sinensis*, saponins, alkaloids, flavonoids, steroids, phenols, tannins , glycosides, DPPH, Nitric oxide

INTRODUCTION:

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These are non-nutritive chemicals that have protective or disease preventive property. The most important of these phytochemicals are alkaloids, flavonoids, tannins and phenolic compounds (Hill, 1952). Many of these indigenous plants are used as spices and food plants. Current research has shown that polyphenols contribute to the prevention of cardiovascular diseases, cancers, osteoporosis and antioxidant character with potential health benefits (Arts & Hollman, 2005; Lambert et al., 2005; Joseph et al., 2005). They are known to have beneficial effects on cardio vascular system. (Keen et al., 2005; Sies et al., 2005;

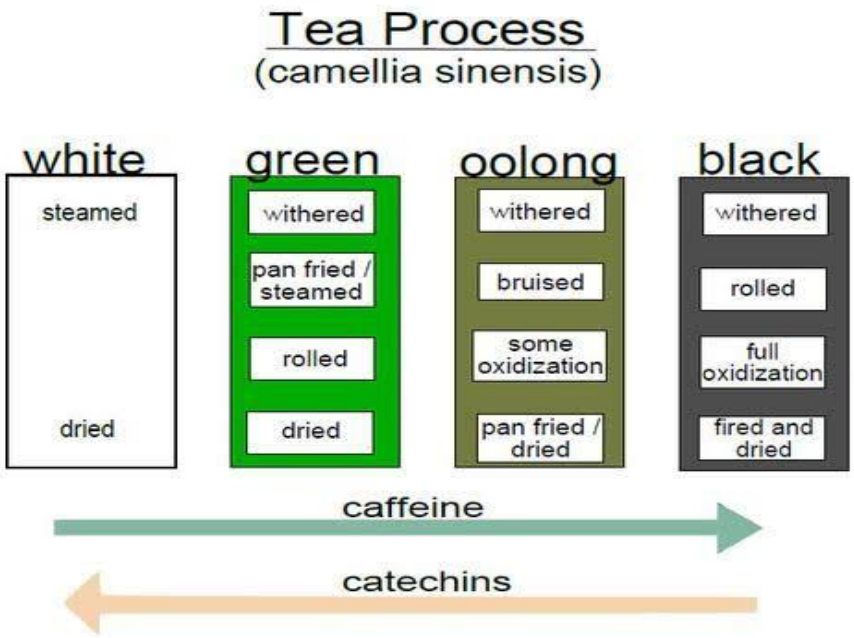
Vita, 2005) and have a role in the prevention of neurodegenerative diseases and diabetes mellitus (Scalbert et al., 2005). Tea is an infusion of the leaves of the *Camellia sinensis* plant and was first discovered in china where it has been consumed for its medicinal properties since 3000 BC (Cheng & Chen, 1994; Balentine, 1997). It is one of the most widely consumed beverages after water. Green tea is made from more mature tea leaves by withering followed by steaming or firing which inactivates the enzyme to prevent the enzymatic oxidation of catechins. Though mode of consumption of tea differs worldwide, one serving (1cup) of tea beverage is prepared from 2g of tea. Green tea leaves (GL) consist of polyphenols, which can make up to 30% of the fresh leaves by dry weight, but only 10% by dry weight of Black tea. There are three basic polyphenol groups in tea leaves: Catechin, Theaflavins and Thearubigins (Yanishlieva-Maslarowa & Heinonen, 2001). Tea catechins can act as antioxidants by donation of a hydrogen atom, as an acceptor of free radicals, interrupting chain oxidation reactions, or by chelating metals (Gramza et al., 2004). It was found that the antioxidant activities of these compounds were higher than those of glutathione, ascorbic acid, and α-tocopherol. Recent research papers have reported effects on coronary heart disease in experimental animals by tea or tea catechins. (Tijburg et al., 1997). Tea catechins were responsible for the inhibition of carcinogenesis at all 3 levels (Sakanaka, 1991; Blot et al., 1996; Dreostic et al., 1997; Jankun et al., 1997; Yang,
1997).



Tea is the most popularly consumed beverage worldwide after water with a per capita consumption of 120 mL/day. It is the cheapest beverage, that human consumes about two-thirds of the world's population. Tea types, Based on

processing (drying & fermentation) or harvesting leaf, tea classified into three major types. They are Black (fermented), Green (non-fermented) and Oolong (semi-fermented) and all the types are made from the processed leaf of *Camellia sinensis*. Approximately 76% to 78% of the tea produced and consumed worldwide is Black, 20% and 22% is Green and less than 2% is Oolong.

TYPES OF TEA BASED ON PROCESSING TECHNIQUES:

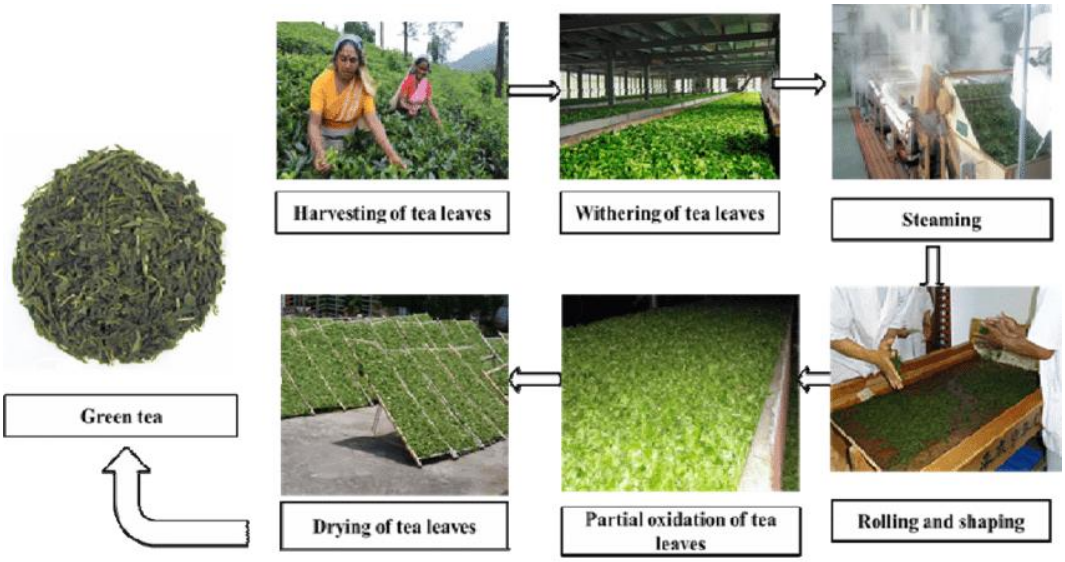


Tea processing involves five basic steps : some teas don't utilize all of these steps, while other teas repeat them several times. Basic processing includes plucking, withering (allowing the leaves to wilt and soften), rolling (to shape the leave and wring out the juices), oxidizing, and firing (i.e: Drying).

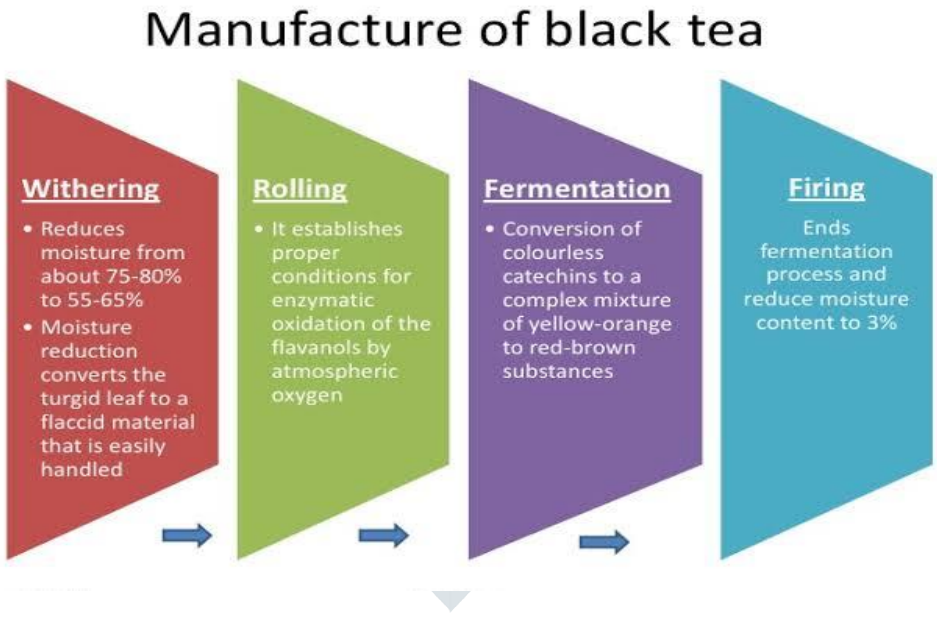
TYPES OF TEA:

GREEN TEA:

Green tea is made by withering tea leaves – and then steaming or pan firing, rolling and drying them without fermentation. Pan firing is required to prevent the tea leaves from fermenting by the natural enzyme activities. It undergoes minimal processing, and contains 80-90% catechins and flavanols (10% of total flavanoids). The infused leaf is green, and the liquor is mild, pale green or lemon-yellow (Mukhtar and Ahmad 2000). The bypass of oxidation allows green tea to retain most of its natural dark green color, tannins, vitamin-C, chlorophyll and minerals. The taste of green tea is therefore more astringent and subtler than oolong or black tea.

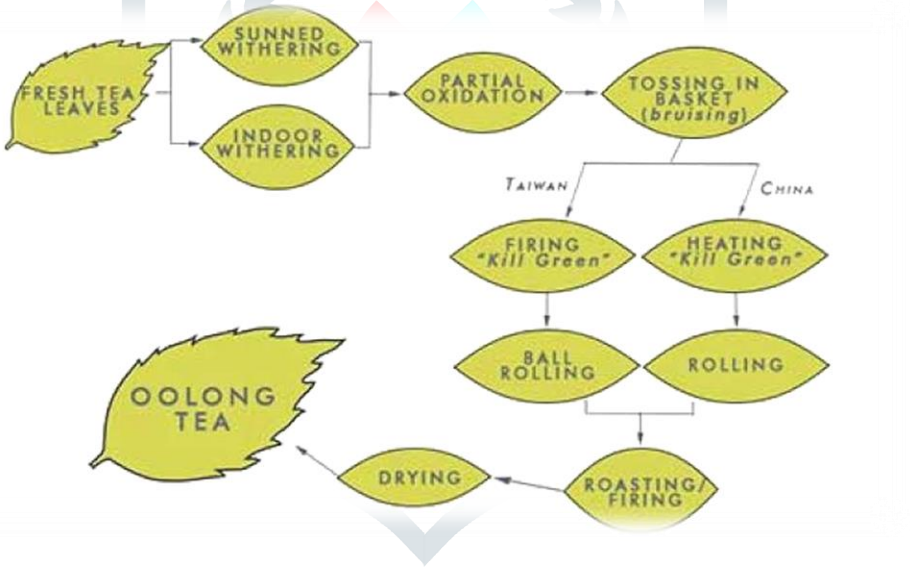


BLACK TEA:



Production of black tea involves additional processing (i.e: aeration and withering). Aeration is the process of exposing the tea leaves to air, causing them to oxidize. Black teas are fully oxidized teas. This oxidation process turns leaves into a deep brown color and during this process, the flavor is intensified (Mukhtar and Ahmad 2000). The leaves are then left as such are heated, dried and crushed. As a result, it has different levels of catechin (20% - 30%) and flavanoid content (theaflavins and thearubigins represent 10% and 50 – 60% of total flavanoids respectively). They are the most popular type of tea in the western world. Black teas range from 40 – 60 milligrams of caffeine per 8 oz cup.

OOLONG TEA:



Oolong teas are semi-oxidized (partial oxidation of the leaf), which places them mid-way between gren and black teas (Mukhtar an Ahmad 2000). The leaves are withered and then rolled, often by hand. The leaves are allowed to partially oxidized and then are fired in pan or basket to arrest the oxidation process. Oxidation may range from 12 – 85%. Sometimes charcoal smoke is used to impart a flavor to the tea. This gives them the body and complexity of a black tea, with the brightness and freshness of a green tea. The caffeine content and antioxidant level is also mid-way between that of green and black teas, making them most healthy and palatable.

TEA QUALITY:

Tea quality not only varies from one garden to another, but also between the same type of tea manufactured at different times within a particular garden.

Aside from processing, the quality of tea can be affected by genetic, environmental and cultural factors, i.e:

- Genetic properties of the tea plant and tea bush
- Soil and climate conditions, including temperature, humidity, sunshine duration, rainfall, etc.
- Field operation such as pruning, fertilizing, shading , plucking round and plucking standards.

TEA PRODUCING REGIONS OF INDIA:

Following the success of tea cultivation experiments in Darjeeling and Assam in the 1800s, endeavours in other parts of India with similar natural conditions were undertaken. These efforts led to a thriving tea industry in at least ten distinct tea producing regions of North-East and South India : Darjeeling, Assam, Dooars and Terai, Kangra, Nilgiri, Annamalais, Wayanaad, Karnataka, Munnar, Travancore.

HEALTH BENEFITS OF TEA:

Tea is a rich source of polyphenolics, particularly flavanoids. The major flavonoids present in green tea include catechins (flavan2-ols) such as epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). In black tea the polymerized catechins such as theaflavins and thearubigens predominate. Arts and his coworkers (2000) reported that the flavonoid concentration of any particular tea beverage depends upon the type of tea (e.g: blended, decaffeinated instant) and preparation (e.g: amount used, brew time, temperature). The highest concentration of flavonoids are found in brewed hot tea (541 – 692ug/mL), less in instant preparations (90 – 100 ug/mL) and lower amounts in iced and ready-to-drink tea (Arts et al. 2000; Hakim et al. 2000). Still, there is controversy on the antioxidant role of tea on the preparation of tea with milk. Few studies reported that the addition of milk or water (e.g: to iced tea) can reduce the flavonoid concentration per serving. In

contrast, recent research studies demonstrate that the addition of milk to tea does not interfere with catechin absorption (Leenen et at. 2000; Hollman et al. 2000). Milk may affect the antioxidant potential of tea, depending upon its fat content, the volume added and the method used to assess this parameter (Evans 2000a,b; Serafini et al. 1996; Tewari et al. 2000).

Repeated consumption of tea and encapsulated tea extracts for one to four weeks has been demonstrated to decrease biomarkers of oxidative status. Health benefits of tea for human was well reviewed in a research article (Diane and Jeffrey 2002) where the authors reported tea and its polyphenols have beneficial role such as cardiovascular diseases (coronary heart disease, hypertension, atherosclerosis, endothelial cell function), cancer (breast cancer, esophageal cancer, lung, stomach, colorectal, bladder and kidney, prostate, skin etc.,), diabetes mellitus, oral health, bone health, thermogenesis, cognitive function, kidney stones.

During the first millennium tea was primarily considered to be medicine rather than an ordinary drink. Over the last few years a large number of epidemiological studies have conclusively proven that tea contains polyphenols and other components that may reduce the risk of developing chronic diseases such as cancer, cardiovascular diseases, arthritis and diabetes (Khan and Mukhtar 2013). Green tea is best studied for its health benefits, including cancer chemopreventive and chemotherapeutic effects (Khan et al. 2008; Khan and Mukhtar 2008), but emerging data is showing that black tea may possess similar health promoting attributes. In agreement with this, Stangel and his colleagues (2006) reported that the total polyphenol content of green and black teas is similar, but with different types of flavonoids present due to the degree of oxidation during processing.

These antioxidant properties are manifested by its ability to scavenge free radicals, inhibit lipid peroxidation, and chelate metal ions (Luczaj and Skrzydlewska 2005; Wiseman et al. 1997; Chan et al. 2007; 2010). Hence, we aimed to determine the free radical (DPPH, nitric oxide, superoxide, hydroxyl radicals) scavenging property of both black tea and green tea extracts. Although much work has been done on the antioxidants/flavanoids identification/qualification and antimicrobial effect of the extracts of black tea and green tea, still it remains unclear whether black tea and green tea exhibit similar or different phytochemical and antixodant property.

ANTIOXIDANTS:

Antioxidants are chemical substances used for treating various human diseases related to heart, lungs, kidney, muscle, brain and helps to control aging process. Antioxidants effectively function in human body by inhibiting or delaying the formation of free radicals and lipid peroxidation that are mainly responsible for many human diseases and aging process. Plant based natural compounds have been accounted for a wide range of biological properties such as antioxidant, anti-inflammatory and antimicrobial activities. The presence of different phytochemicals such as ascorbic acid, tocopherols, carotenoids, and polyphenolic compounds and their combined activities result in the total antioxidant activity of a plant. However, polyphenolic compounds from plants appear to have the greatest antioxidant potential and could be the most beneficial antioxidants . Many of these common antioxidant compounds

are found in fruits and vegetables. Plants are known to possess polyphenolic compounds such as flavonoids, and other phytochemicals such as carotenoids . Karimi et al. proposed that plant fruits contain a variety of (poly) phenolics and (poly) phenolic derivative compounds and many of these compounds could be potential antioxidant sources. Tea (*Camellia sinensis*) plants belonging to Theacea family are known to contain higher antioxidant compounds. Tea is been one of the widely consumed beverages in the world. Tea is a perennial evergreen plant that requires humid and warm environmental conditions. Native to Southeast Asia, tea has been planted widely in tropical and subtropical areas. Near the equator, it ranges up to nearly 2000 m elevation. It thrives well on well-drained acidic soils (pH 4.5–6.0) and requires temperatures ranging from 13°C to 30°C with an annual rainfall of about 120 cm or more. The quality and uniqueness of each tea brand depends on many parameters including the growing seasons, geographic regions, processing and fermentation methods. Iran is one of the 13 major tea producers in the world. Two evergreen regions in the Northern region of Iran are Gulian and Mazandaran (36°/31 to 37°/25 N, and 49°/15 to 51°/15 E) and these are the important regions contributing to tea production. This land stretches to about 34,000 ha and is used for the production of tea . There are many types of tea, all classified based on how they are processed. Green tea and black tea are two of the major commercial types of tea. Among the daily food and beverage products, tea is very rich in flavonoid compounds mainly catechins which mainly accumulate in growing tea leaves. Catechins are naturally occurring polyphenols found in tea, red wine, chocolates and many fruits. They belong to the flavonoid group and are considered as flavan-3-ols. Common catechins found in tea are (−)epigallocatechin (EGC), (−) epigallocatechin-3-gallate (EGCG), (−)epicatechin-3-gallate (ECG) and (−)epicatechin (EC).

Tea is an important beverage in Iran and to date the demand for tea is increasing. However, most tea plantations are old resulting in low productivity. It is therefore, necessary to replace the existing plantation with elite clones with the best quality. Presently, there are more than 20 registered high yielding clones in Iran such as 102, 449, 219 and clone Iran 100. Selection of elite planting material for commercial plantation from the above mentioned clones can be better achieved based on chemical profiling and studying their biological activities. Therefore, the present study was aimed at characterizing the phytochemical constituents and antioxidant activity in different tea clones of Iran to compare their quality attributes.

MATERIAL AND METHODS :

RAW MATERIALS AND SAMPLE PREPARATION:

The quality of an extraction is influenced by many factors, such as the solvent used for the connection between parts of the plant, extraction, extraction process and plant and solvent used to be used as an early use of the material. From the lab scale to the pilot scale, all the parameters are optimized and controlled during the extraction. Soluble plants separate metabolites through extraction techniques Selection of solvents (Wichowski et al., 1908).

Fig. 1: Tea Leaf Powder

SAMPLE EXTRACTION:

The healthy and disease free mature leaves of *Camellia sinensis* L plant material was collected from the botanical garden. Collected plant material was washed thoroughly in running tap water, shade dried in open air separately. Powder of the leaf is obtained by grinding them mechanically. Blended samples of green tea and black tea were extracted with 50 ml of 50% ethanol, water and chloroform for 1 hour on an orbital shaker. The mixture was centrifuged at 8500 rpm for 10 min. The pellets were re-extracted at identical conditions. After a day the plant extracts were subjected to filtration, filtered with No 42 whatman filter paper separately. Concentrated extracts was preserved in sterilized air tight labeled bottles and preserved in refrigerator at 4˚C until required for further use. The extract was filtered under reduced pressure using rotary flash evaporator and subjected for further preliminary phytochemical and antioxidant Tests.

PRELIMINARY PHYTOCHEMICAL SCREENING:

1.TEST FOR ALKALOIDS:

MEYER'S TEST:

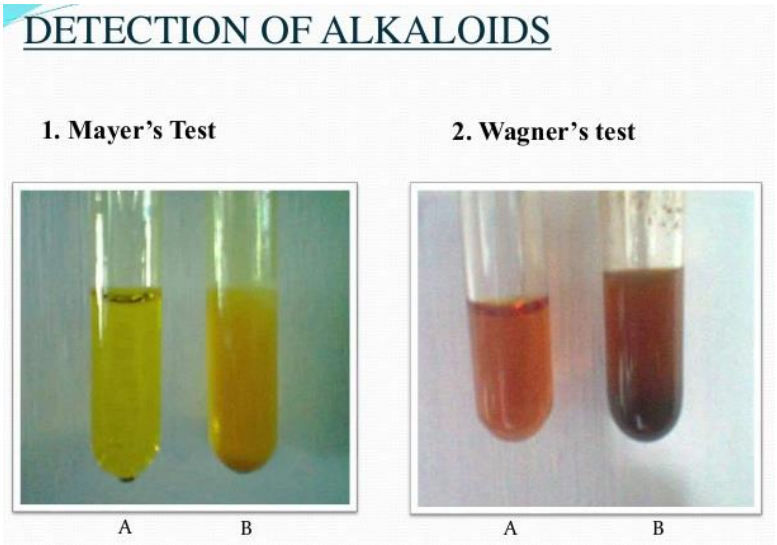
To the 5ml of extract 5ml of 2N HCL is added and boiled and then the mixture is filtered.

To the filtrate a few drops of Mayer‟s reagent is added.

A cream colour precipitate was produced immediately indicating the presence of alkaloids.

WAGNER'S TEST:

About 10 mg of tea leaf and tea dust extract was taken and few drops of Wagner's reagent was added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

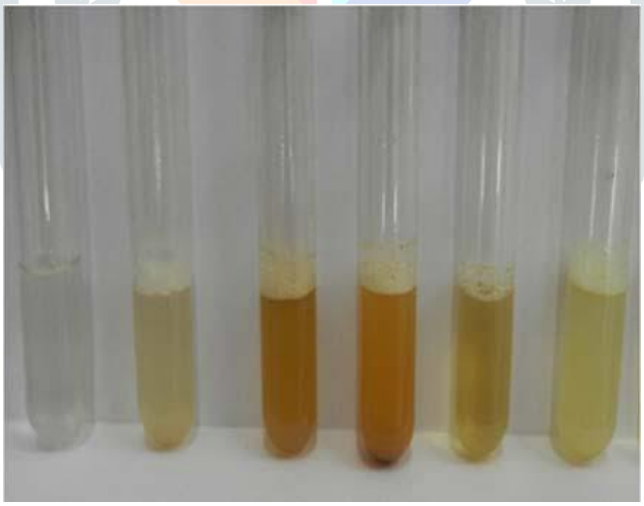


2.TEST FOR SAPONINS:

FROTHING TEST:

Saponins are tested by boiling 5ml of extract in 10ml of distilled water in a test tube and are shaken vigorously for about 30 seconds.The test tube is allowed to settle for half an hour.

Formation of froth indicates the presence of saponins.



3.TEST FOR TANNINS:

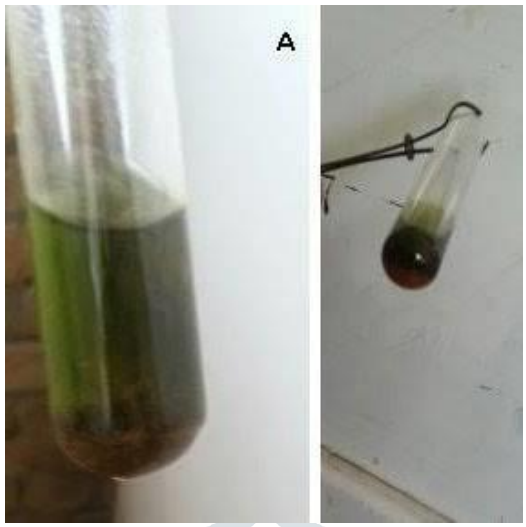
FERRIC CHLORIDE TEST:

2 ml of the leaf extract and dust extract were added to a few drops of 10% Ferric chloride solution (light yellow).The occurrence of blackish blue colour showed the presence of Gallic tannins and a green-blackish colour indicated presence of catechol tannins.

LEAD ACETATE TEST:

Tannins are tested by adding a few drops of 1% lead acetate to 5 ml of plant extract.

Appearance of yellow precipitate indicates the presence of tannins.

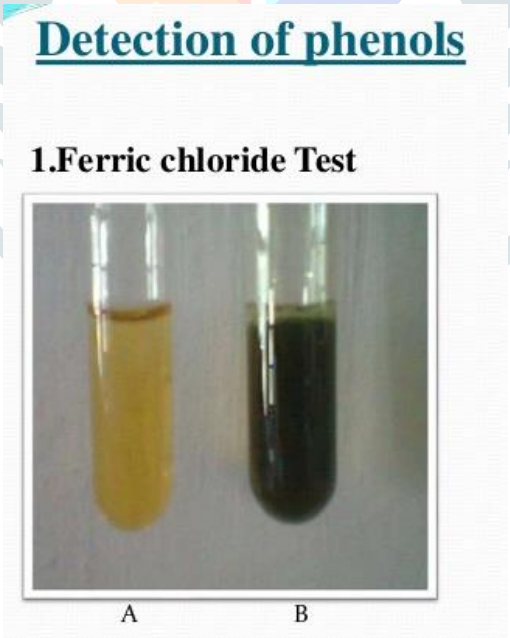


4.TEST FOR PHENOLS:

FERRIC CHLORIDE TEST:

Phenols are tested by adding 2ml of ferric chloride solution to 2ml of plant extract.

Appearance of bluish green colour solution indicates the presence of phenols.



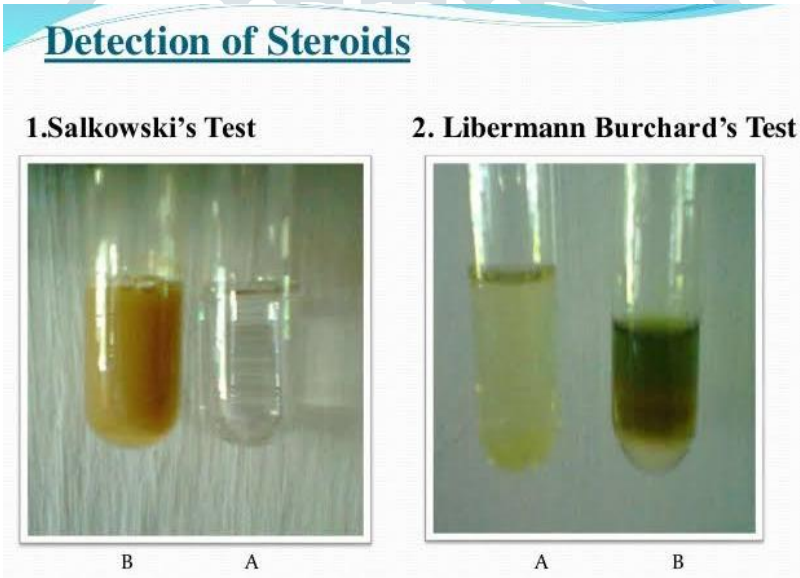
5.TEST FOR STEROIDS:

SALKOWSKI'S TEST:

The amount of 0.5g of the extract was dissolved in 10 ml of anhydrous chloroform and filtered. The solution was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish brown colour indicative a steroid ring.

LIEBERMANN – BURCHARD'S TEST:

The amount of 0.5g of the extract was dissolved in 10 ml of anhydrous chloroform and filtered. The solution was mixed with 1 ml of acetic anhydride followed by the addition of 1 ml of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration as indicative of steroids.



6.TEST FOR CARDIAC GLYCOSIDES:

KELLER-KILIANI TEST:

To 5ml of extract and add a 4 ml of glacial acetic acid, few drops of ferric chloride and then finally concentrated sulphuric acid were added from the walls of the test tube.

Appearance of the reddish brown at the junction of two layers and the bluish green colour in the upper layer indicates the presence of cardiac glycosides.

7.TEST FOR ANTHRAQUINONES:

BORNTRAGER'S REACTION FOR FREE ANTHRAQUINONES:

5ml extract was boiled with 10ml of sulphuric acid and filtered while hot.The filtrate was shaken with 5ml of chloroform the chloroform layer was pipette out into another test tube then 1ml of dilute ammonia is added.This was shaken and the upper layer was observed for bright pink colouration as indicative of the presence of Anthraquinones.

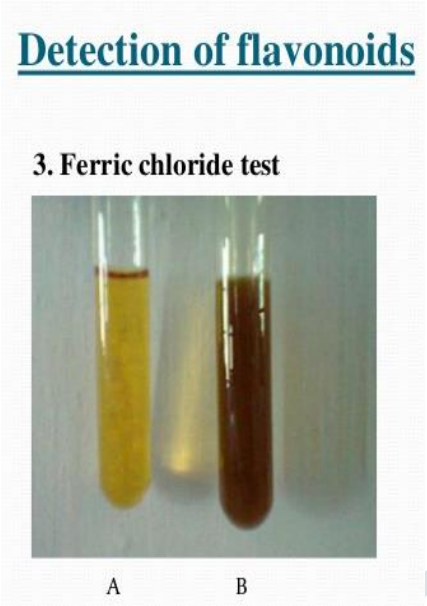
8.TEST FOR FLAVONOIDS:

ALKALINE TEST:

2 ml of 2% sodium hydroxide mixture was mixed with tea leaf and tea dust extract; concentrated yellow colour was produced, which became colourless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

LEAD ACETATE TEST:

10 mg of tea leaf and tea dust extract was taken and few drops of 10% lead acetate solution was added.Appearance of yellow colour precipitate indicates the presence of flavonoids.



9.TEST FOR TERPENOIDS:

1ml of the extract was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid.

Formation of reddish colour indicates the presence of terpenoids.

10.TEST FOR CARBOHYDRATES:

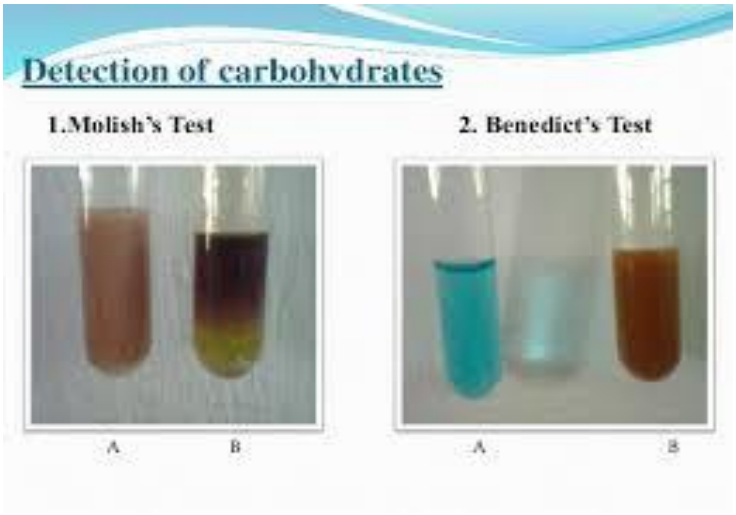
MOLISCH'S TEST:

A Few drops of molischs solution was added to 2 ml of extract, thereafter a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking.

The interface was observed for upper phase brown colour ring was indicative for carbohydrate.

BENEDICT'S TEST:

3 ml of extract and add a few drops of boiled benedicts reagent and then heated.The interface was observed for blue colour was indicative for carbohydrate.



11.TEST FOR PROTEINS:

NINHYDRIN TEST:

About 5 mL of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent were added and heated.The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

12.TEST FOR AMINO ACIDS:

MILLION TEST:

2ml of the extract, added the million's reagent (mercuric sulphate and concentrated sulphuric acid) was added and heated for 10 minutes.

After cooling few drops of 1% sodium nitrate was added.

It gives the color of red brick indicates the presence of amino acids.

BIOACTIVE COMPOUNDS:

1. DETERMINATION OF FLAVONOID CONTENT (TFC)

Flavonoid content of isolated crude (tea leaves and coffee seeds) was determined with this method (Gia et al., 1999). Take a clean test tube and add 0.5 ml of the specimen (extract) to 1.25 ml of distilled water. Then 0.075 ml of 5% sodium nitrite solution was added and allowed to stand for 5 minutes. 0.15 ml of 10% aluminum chloride was added, after 6 minutes, 0.5 ml of 1.0

ml sodium hydroxide was added and the mixture was diluted with 0.275 ml of distilled water. The absorption of the mixture was immediately measured at 510 nm. Flavonoid content was expressed in the form of sample mg of equivalent catechins/g and the same process was done with extract of tea leaves and coffee seeds.

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

Total phenolic contents of each extract were determined using a Folin-Ciocalteu colorimetric method (Singleton et al., 1999). 1 mL properly diluted of each extract solution were mixed with 0.5 mL of Folin-Ciocalteu reagent. The reagent was pre diluted, 10 times, with distilled water. After standing for 8 min at room temperature, 2 mL of (7.5% w/v) sodium carbonate solution were added. The solutions were mixed and allowed to stand for 30 min at room temperature. Then, the absorbance was measured with a spectrophotometer UV–visible Genesys 10 BIO at 765 nm. A calibration curve was prepared, using a standard solution of gallic acid. Results are expressed as mg of gallic acid equivalents (GAE)/100 g dry weight (dw) extract. Data is reported by means of at least two replications.

ANTIOXIDANT ACTIVITY:

Method used for antioxidant activity was radical scavenging activity methods are:

A) DPPH (2, 2 -DIPHENYL-1-PYCRYL-HYDRAZYL) FREE RADICAL SCAVENGING ACTIVITY:

The free radical- scavenging capacity of the extract was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl). One milliliter of methanol DPPH solution (0.12mm) was mixed with 0.002% of black tea extract and green tea extract. After 10 minutes of incubation, the absorbance was read at 515 nm using a spectrophotometer (Perkin-Elmer Lambda 20 UV visible spectrophotometer). Vitamic C was used as a standard. The inhibition curve was plotted and comparison was made for the green tea extract and black tea extract (Viturro et al. 1999).

B) NITRIC OXIDE RADICAL SCAVENGING ACTIVITY:

The NO radical scavenging activity was estimated using Griess Illosvoy reaction (Garrat, 1964) with some modifications using naphthyl ethylene diamine dihydrochloride(01% w/v) and sulfanilic acid reagent (0.33% in 20% glacial acetic acid). The absorbance of the pink chromophore formed was measured at 540 nm against the corresponding blank solution. Rutin was used as a standard.

C) TOTAL ANTIOXIDANT CAPACITY ASSAY:

The assessment of the total antioxidant capacity was determined by the Phosphomolibdenum Method (Preto et al., 1999). 0.3 ml of extracts and sub-parts were mixed in ethanol; ascorbic acid was used separately with standard (5 to 200 g / mL) and 3 ml reagent mixture with white (ethanol) and 95 degrees Celsius But incubation was done. 90 min After cooling the room temperature, the absorption of each sample was measured against the vacuum at 695 nm. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalent
to ascorbic acid or galic acid .

RESULTS:

DETERMINATION OF TOTAL ANTOXIDANT ACTIVITY BY DPPH RADICAL-SCAVENGING ACTIVITY:

DPPH radical scavenging activity was represented in Figure 1. It depicts that black tea (35%) exhibits a significant DPPH radical scavenging activity than green tea extracts (22%). Vitamin C as the positive control which depicts the 45% DPPH radical inhibition.

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY BY NITRIC OXIDE (NO) RADICAL SCAVENGING ACTIVITY:

Nitric oxide radical scavenging activity was represented in Figure 2. It presents that block tea (15%) exhibits a significant nitric oxide radical scavenging activity than green tea extracts (13%). Rutin was used as the positive control which displays the 30% nitric oxide radical inhibition.

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY ASSAY:

Total antioxidant capacity assay was represented in Figure 3. It presents that black tea (30%) exhibits a significant total antioxidant capacity assay than green tea extracts (31%) . vitamin E was used as the positive control which displays the 47% total antioxidant capacity.

Figure 1: DPPH RADICAL-SCAVENGING ACTIVITY OF METHANOL EXTRACTS OF BLACK TEA AND GREEN TEA. (values are mean + or – SD)

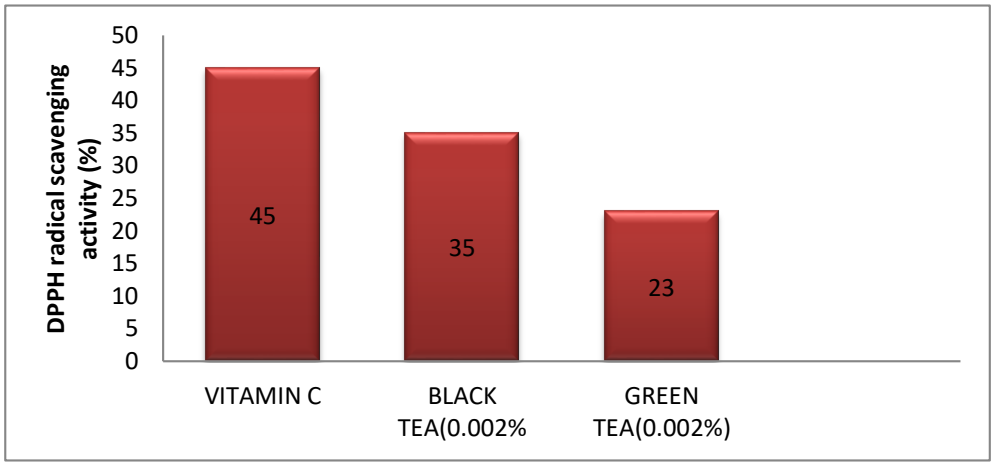


Figure 2: NITRIC OXIDE (NO) RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACTS OF BLACK TEA AND GREEN TEA. (values are mean + or – SD)

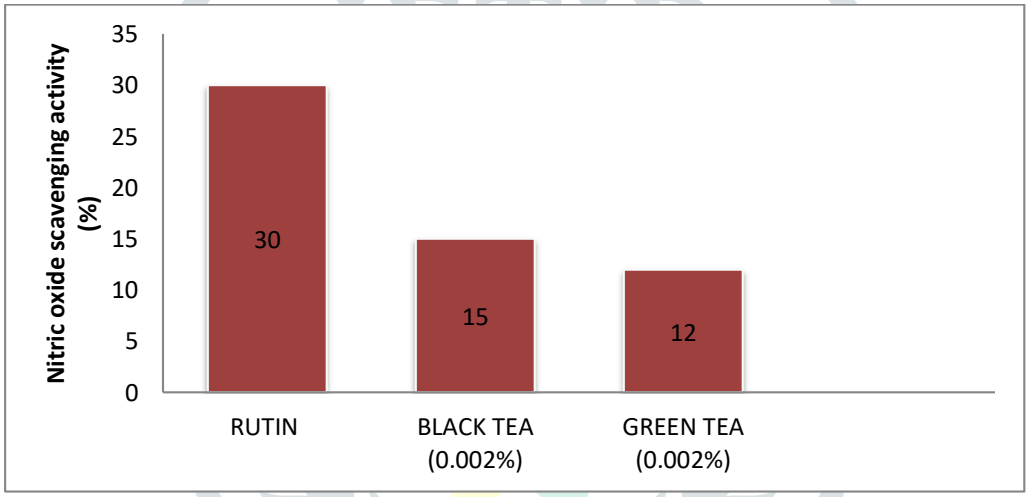
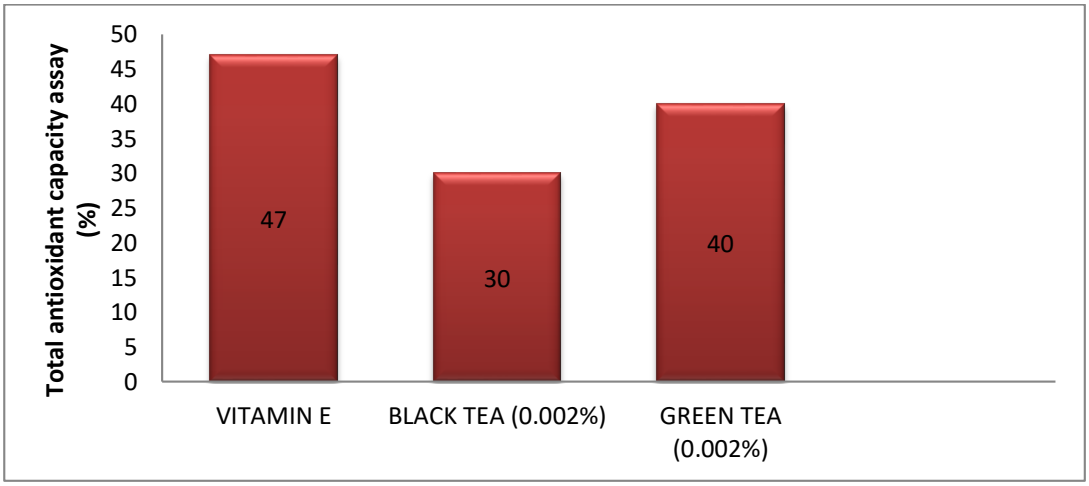


Figure 3: TOTAL ANTIOXIDANT CAPACITY ASSAY OF METHANOL EXTRACTS OF BLACK TEA AND GREE TEA. (Values are mean + or – SD)



PHYTOCHEMICAL INVESTIGATION:

1. EXTRACTION YIELD:-

Extraction Yield for Leaf and dust was found to be higher for methanolic extract. The total % of yield for Soxhlet (solvent-300ml) was (Soxhlet- 10 %) respectively.

Table.1: The total % of yield was as follow

EXTRACTION	% OF EXTRACT
METHANOLIC EXTRACT	60%

The result of phytochemical screening reveals alkaloids, steroids and saponins were found to be present in aqueous extracts of Leaf & Seed Powder of Tea leaves and Tea dust (Table 2)

Table.2: Phytochemical screening of methanolic extract of tea leaves

S.NO	PHYTOCHEMICAL TEST	TEA LEAVES AND DUST EXTRACT
1.	ALKALOIDS	POSITIVE
2.	SAPONINS	POSITIVE
3.	TANNINS	POSITIVE
4.	PHENOLS	POSITIVE
5.	STEROIDS	POSITIVE
6.	CARDIAC GLYCOSIDES	POSITIVE
7.	ANTHRAQUINONE	NEGATIVE
8.	FLAVONOIDS	POSITIVE
9.	TERPENOIDS	NEGATIVE
10.	CARBOHYDRATES	POSITIVE
11.	PROTEIN	NEGATIVE
12.	AMINO ACIDS	NEGATIVE

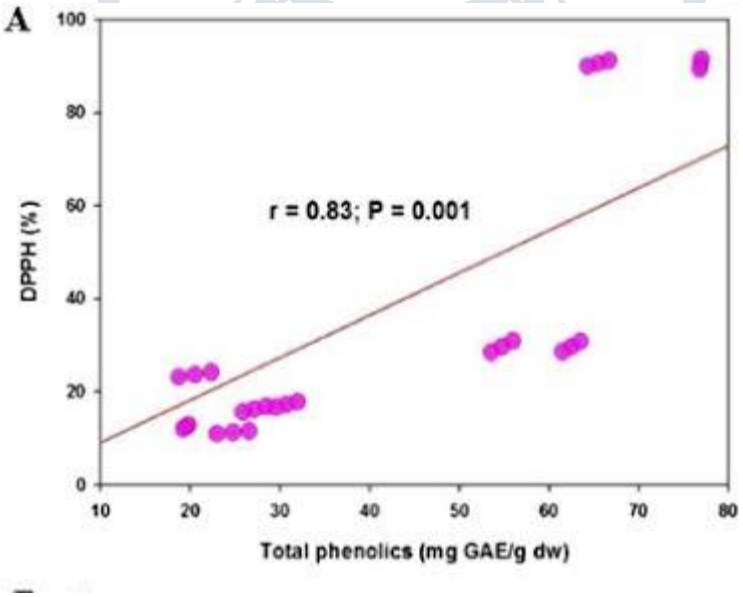
Total phenolic (TPC) assay:

Table.3: Total Phenol and Flavonoid Levels of Selected Tea Varieties

QUANTITATIVE ANALYSIS	TEA
TOTAL PHENOLS	0.03 ± 0.10

The results of the Folin-Ciocalteu total phenol assay with aqua's extract were reported in Table.3. Sample of Tea leaf was show phenol compound.

The Total Phenolic content of Tea leaves contained the considerable amount show in graph:



TOTAL FLAVONOID (TFC) ASSAY:

Table.4: Total Flavonoides in Levels of Selected Tea leaf extract

QUANTITATIVE ANALYSIS	TEA
TOTAL FLAVONOIDS	0.02 ± 0.09

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The values of flavonoid content was observed in tea leaves 0.02±0.09mg/gm.

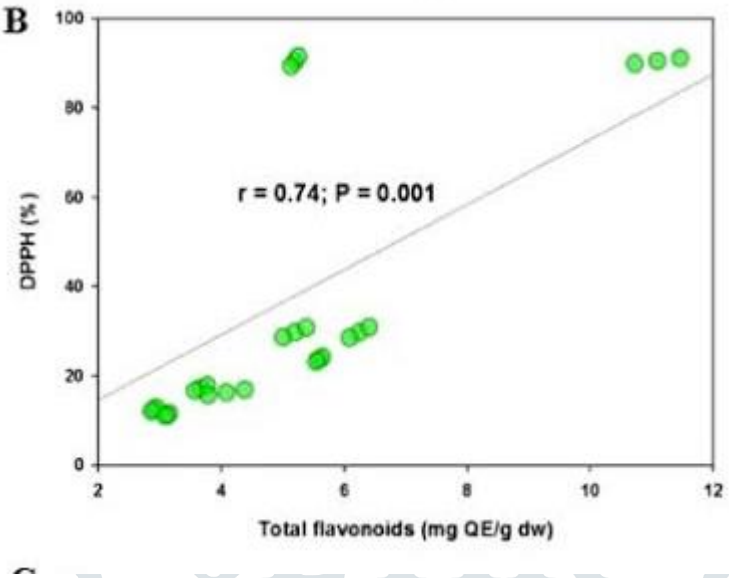


Table. 7: Total Antioxidant scavenging activity

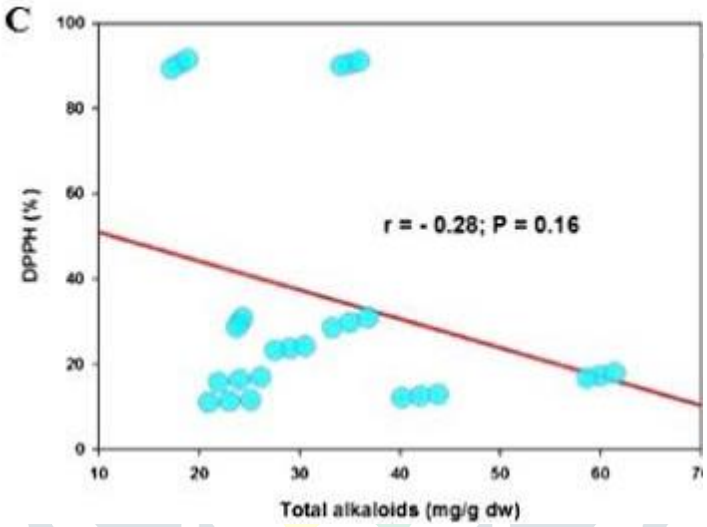
S.NO	CONCENTRATION (µg)	STANDARD	VALUE (LEAF)	VALUE (POWDER)
1.	20	0.652	0.678	0.489
2.	40	0.692	0.563	0.234
3.	60	0.723	1.202	0.214
4.	80	0.793	0.215	0.745
5.	100	0.813	0.577	0.833

Total antioxidant capacity equivalent of ascorbic acid was 0.996 mg/g of extract. Concentration ranging from 20-100 µg /ml of the aqueous extract of Tea Leaves was tested for their antioxidant activity in different in vitro assay. It was observed that the free radical scavenged by the extract was show in table-7.

Table. 8: DPPH scavenging activity

S. No.	Concentration	Standard Ascorbic acid	Tea
1.	20 µg	0.994±0.40	0.224±0.69
2.	40 µg	1.251±0.61	1.266±0.19
3.	60 µg	1.622±0.24	0.301±0.35
4.	80 µg	1.174±0.50	0.065±0.22
5.	100µg	1.977±0.52	1.789±0.61

DPPH scavenging activity equivalent of ascorbic acid was 0.994 mg/g of extract. Concentration ranging from 20-100 µg /ml of the methanolic extract of Tea leaf and Coffee seed was tested for their antioxidant activity in different in vitro assay. It was observed that the free radical scavenged by the extract was show in table-7 and in graph.



DISCUSSION:

Aqueous extracts of *Camellia sinensis* (Tea), in particular green and black tea, have been studied due to their potent antioxidant and phytochemical effects. Though they are varied in production process, they are found to have a potential antioxidant activity due to the enriched bioactive components. In overall radical scavenging activities, black tea takes a higher rank than green tea. Yashin et al. (2011) reported that this antioxidant activity of black tea is effected mainly by flavanoids (theaflavins and theaarubigings), tannins whereas in green tea by catechins(90%).

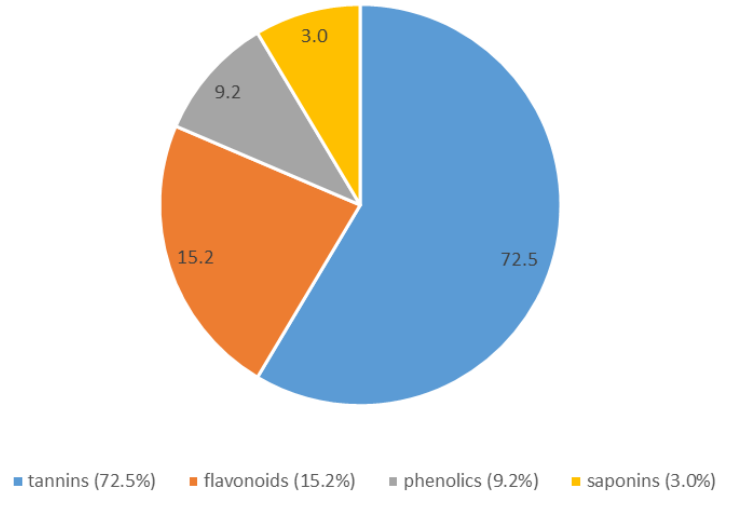
Considering the increasing interest in the commercialization and consumption of green tea, present study has been designed to evaluate whether black tea comparatively higher/equal/lesser antioxidant efficacy of that of green tea. The strong antioxidant properties of black tea may be attributed to its chemical components of flavanoid(thearubigins), terpenoid, saponnins, phenolic acid, and catechins. Flavanoids(theaflavins) are the major part of black tea; impart color, brightness and astringency. They also render potent antioxidant properties (Luczaj

and Skrzydlewska 2005; Ngure et al. 2009). Studies also reported that tannins has been shown to have higher antioxidant activity than EGCG (strongest antioxidant among the green tea catechins) (Engelhardt et al. 2003; Leung et al. 2001). These antioxidant properties of tannins and theaflavins have been attributed to their gallic acide moiety (Shiraki et al. 1994 Miller et al. 19960). Since, tannins and theaflavins are dimmers of catechins, they have more OH groups than catechins and thus contribute more antioxidant efficacy (Luczaj and Skrzydlewska 2005).

Another major constituent present in black tea are flavanoids(thearubigins). Like, theaflavins, thearubigins also posses hydroxyl and galloyl groups which may be involved in the enhancement of the scavenging activity of black tea. The DPPH radical scavenging activity of black tea extracts may be attributed to the rich theaflavins and thearubigins. These flavanoids donates hydrogen atom and exhibits good scavenging activity against DPPH. It was observed that extracts of black tea also exhibit significant Nitricx oxide radical scavenging activity.

The nitric oxide radical scavenging activity on tea extract incubation which reveals that there was a high significant inhibition of nitric oxide by both tea extracts. Consistent with this Yang et al. (2011) reported a high significant inhibition of nitric oxide with tea infusion incubation. In contrast, nitric oxide radical scavenging activity of both tea extracts is significantly greater over the green tea extracts.

Though green tea extracts was found to have free radical scavenging activity, it was less compared to black tea extracts at the concentration of 20 µg /mL of both tea extracts. In addition, it is known that consumption of black tea is more than the green tea and also easily affordable for comman man.



SUMMARY AND CONCLUSION:

This work reveals the preliminary phytochemical screening and radical scavenging activity of tea is mainly due to their inbuilt polyphenols and their properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. The rich fractions of thiaflavins and thearubigns in black tea; and catechins in green tea enhanced the antioxidant properties of tea extracts. Present study also suggests that black tea exhibits significant radical scavenging activity than green tea portrayed from the DPPH and Nitric oxide radical scavenging activity. It may be attributed to the presence of higher level of gallic acid in black tea, an essential polyphenol and phytochemicals having significant antioxidant activity than catechins present in green tea.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