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PHYTOCHEMICAL ANALYSIS AND *IN-VITRO* ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF *CAMELLIA SINENSIS* (GREEN TEA) LEAF EXTRACT

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ABSTRACT: Plant-based or plant-derived drugs occupy 30% of the modern system of medicine. Several plants possess a variety of biologically active compounds. Among them *Camellia sinensis*, belonging to the family Theaceae. Green tea is one of the most ancient and popular therapeutic beverages consumed around the world. It can be prepared as a drink, which can have many systemic health effects or an “extract” can be made from the leaves to use as medicine. Green tea is reported to contain thousands of bioactive ingredients which are almost contributed by polyphenols, which play a key role in the prevention and treatment of many diseases. The aqueous and ethanolic extracts were used for their antioxidant potential was analyzed using DPPH assay and total phenolic content. The results of the present study revealed that the green extract possesses a good antioxidant property and in the antibacterial activity potential of following this extraction used well diffusion method against the microorganisms respectively *E. coli* and *Klebsilla sp.*

Keywords: *C. sinensis*, Phytochemical, DPPH, Aqueous and ethanolic leaf extract, Antioxidant activity, Antibacterial activity

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INTRODUCTION: Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea by Cabrera C *et al.*, 2006. ¹ The first green tea was exported from India to Japan during the 17th century. It is estimated that about 2.5 million tons of tea leaves are produced each year throughout the World, with 20% produced

As green tea, which is mainly consumed in Asia, some parts of North Africa, the United States, and Europe [Japanese Green Tea online.com]. The association between ² green tea and black tea are processed differently during manufacturing. To produce green tea, freshly harvested leaves are processed differently during manufacturing.

To produce green tea, freshly harvested leaves are immediately steamed to prevent fermentation, yielding a dry, stable product. This steaming process destroys the enzymes responsible for breaking down the color pigments in the leaves and allows the tea to maintain its green color during the subsequent rolling and drying processes. These processes preserve natural polyphenols concerning the health-promoting properties. As green tea is fermented to Oolong and then to black tea,

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polyphenol compounds (catechins) in green tea are dimerized to form a variety of theaflavins, such that these teas may have different biological activities.

MATERIALS AND METHODS:

Collection of *Camellia sinensis* Leaves: *Camellia sinensis* Commercial packet was brought in Supermarket. The leaves of *Camellia sinensis* was thoroughly washed and dried under oven (40 °C- 50 °C) for 5-7 days, pulverized by a mechanical grinder to the fine powder before analysis.

Preparation of Extracts:

Aqueous Extract: Aqueous extract was prepared mixing of 100 mg of dry powder of plant leaves with 100 ml of sterile distilled water and kept at room temperature for 24 h. The extract was filtered through a muslin cloth and finally filtered through Whatmann no.1 filter paper with the 150-micrometer diameter and stored in an airtight bottle at 4 °C until use.

Ethanol Extract: Ethanolic extract was prepared by mixing 100 mg of dry powder of plant leaves with 100 ml of 99% ethanol and kept at room temperature for 24 h. After 24 h, the extract was filtered through a muslin cloth and finally filtered through Whatmann no.1 filter paper and stored in an airtight bottle at 4 °C until use.

Antibacterial Activity: Sterilized nutrient agar plates were inoculated with a loop full of a freshly prepared suspension of each bacteria and aqueous and ethanolic extraction sample by the good diffusion method. The plates were then inverted and incubated at 37 °C for 18-24 h. Zone indicates the presence of antibacterial activity.

RESULTS:

Qualitative Analysis:

TABLE 1: PHYTOCHEMICAL SCREENING OF AQUEOUS AND ETHANOLIC LEAF EXTRACT OF *CAMELLIA SINENSIS*

S. no.	Test / Leaf extract	Aqueous	Ethanol
1	Test for Alkaloids		
	Mayer’s test	+	+
	Wagner’s test	+	+
2	Dragendorff’s test	+	+
	Test for flavonoids		
	Shinoda’s test	+	+
3	Alkaline reagent test	+	+
	Test for carbohydrates		
	Benedict’s test	-	-
4	Molisch’s test	-	-
	Test for glycosides		
	Borntrager’s test	-	-
5	Keller – Killani test	-	-
	Test for Proteins		
	Ninhydrin test	-	-
6	Biuret test	-	-
	Test for saponins		
	Froth test	-	-
7	Lead acetate test	-	-
	Test for Tannins		
	Ferric chloride test	+	+
8	Lead acetate test	+	+
	Test for Terpenoids		
	Salkowski test	-	-
9	Test for Anthraquinones		
	Ammonia test	-	-

TABLE 2: QUANTITATIVE ANALYSIS OF AQUEOUS AND ETHANOLIC LEAF EXTRACT OF *CAMELLIA SINENSIS*

S. no.	Substances	Aqueous extract	Ethanolic extract
		Leaf (mg %)	Leaf (mg %)
1	Total glucose	220	250
2	Total proteins	115	845

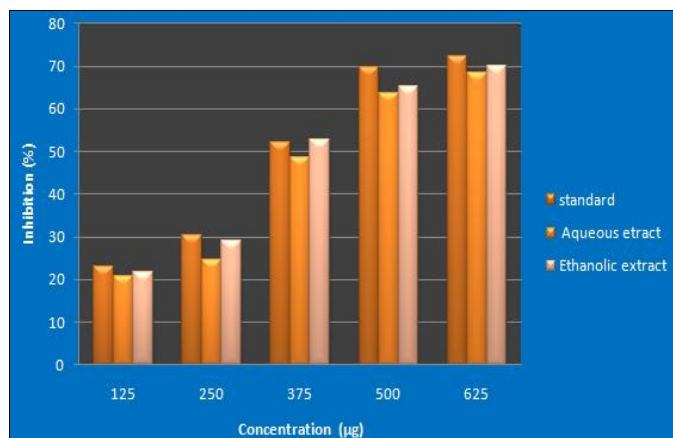


FIG. 1: DPPH RADICAL SCAVENGING ASSAY OF GREEN TEA (WITH MILK)

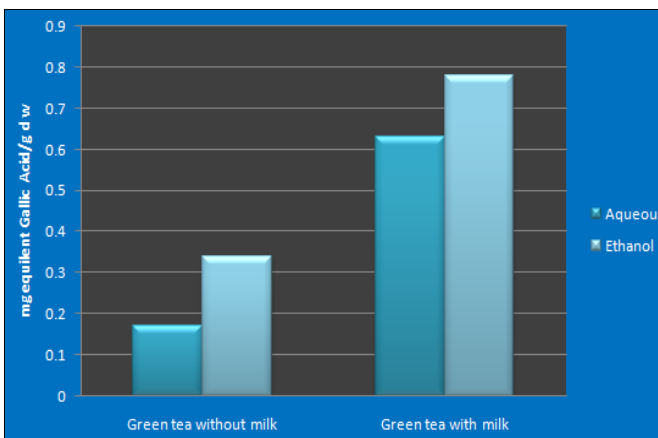


FIG. 2: TOTAL PHENOLIC CONTENT OF GREEN TEA WITHOUT AND WITH MILK



FIG. 3: ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACT OF GREEN TEA (*CAMELLIA SINENSIS*) USED IN *E. COLI*. ZONE INDICATES THE PRESENCE OF ANTIMICROBIAL ACTIVITY



FIG. 4: ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACT OF GREEN TEA (*CAMELLIA SINENSIS*) USED IN *PSEUDOMONAS*. ZONE INDICATES THE PRESENCE OF ANTIMICROBIAL ACTIVITY

C- Control; A- Aqueous extraction of green tea; E- Ethanolic extraction of green tea

TABLE 3: ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACTION OF *CAMELLIA SINENSIS*

Microorganisms name	Zone of inhibition in diameter		
	Control	Aqueous extraction	Ethanolic extraction
<i>Escherichia coli</i>	0 mm	30 mm	13 mm
<i>Klebsilla sps</i>	0 mm	24 mm	12 mm

DISCUSSION: Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infection, lung damage, inflammation, cardiovascular disorder, obesity, and weight loss, diabetes, cancer, atherosclerosis, neoplastic disease, Neurodegenerative disease such as aging, Parkinson's disease, Alzheimer's disease³. They are also involved in an autoimmune disorder like rheumatoid arthritis⁴. Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation⁵. Phenolic compounds and flavonoids are major constituents of most of the plants reported possessing antioxidant and free radical scavenging activity⁶.

Therefore, the importance of the search for natural antioxidants has increased in recent years, so many researchers focused the same⁷. DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. In this study, the antioxidant capacity of aqueous, ethanol extract of green tea leaves as

systematically evaluated. Ethanol extract of leaves of green tea possessed the highest DPPH scavenging activity in with milk (70.01% inhibition of the DPPH radical), followed by ethanol extract of leaves of green tea without milk (62% respectively), comparable to the commercial antioxidant ascorbic acid (72.12% inhibition of the DPPH radical). The antioxidant activity of the leaves of *Camellia sinensis* could be due to the presence of a wide variety of bioactive compounds, such as phenolics, flavonoids, carotenoids, and tannins in this plant.

Phenolic compounds are known as powerful chain-breaking antioxidants. Phenols are very important constituents because of their scavenging ability due to their hydroxyl group. The ethanolic extract of the whole plant of *Camellia sinensis* was found higher content of phenolic compounds with milk (0.78 mg of GAE/g extract), followed by the ethanolic extract without milk (0.34mg of GAE/g extract). *Camellia sinensis* leaf aqueous extraction and ethanolic extraction against the organisms showed 30mm, 24mm and 13mm, 12mm of the maximum zone of inhibition in well diffusion method respectively *E. coli* and *Klebsilla sps*.

CONCLUSION: *Camellia sinensis* commercial packet was brought in the Supermarket. The leaf of this plant was air-dried and ground into uniform powder using a milling machine. The present study was investigated to determine the phytochemicals and *in-vitro* antioxidant activity, which is present in leaves of *Camellia sinensis* with different extracts of ethanol and aqueous. Quantitative estimate the content like protein, carbohydrate, and qualitative analysis of alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannin, protein, and anthraquinone. *In-vitro* antioxidant activity was done by using a parameters DPPH radical scavenging activity and total phenolic content. *In-vitro* antibacterial activity was done by using *Escherichia coli* and *Klebsilla sp* in both aqueous and ethanolic extract of *C. sinensis*. These *in-vitro* assays indicate that this plant leaf extract is a significant source of natural antioxidant and antibacterial activity, which might help prevent the progress of various oxidative stresses.

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CONFLICT OF INTEREST: Nil

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