

Antimicrobial activity of different tea extracts

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Abstract-Tea is a popular beverage known for its unique taste and vast health benefits. The main components in tea change greatly during different processing methods, which makes teas capable of having different biological activities.

Due to the presence of various bioactive compounds including catechin, polyphenols and saponins etc, tea exhibits antimicrobial activity against microorganisms. Catechins may be considered as the antimicrobial components of the tea extracts. Reports showed that the catechins damaged the cell membrane and increased cell membrane permeability, leading to changes in the relative electrical conductivity and the release of certain components into the cytoplasm.

This study was designed to investigate the antimicrobial activity of five tea extracts, including green, blue, black, hibiscus and chamomile tea extracts against standard strains of *Staph. aureus* (ATCC 6538) and

E. coli (ATCC 8739). Solvent extraction method was used to prepare the extracts and antimicrobial activity was determined by Agar well diffusion method.

Keywords: tea extracts; antibacterial activity; catechins; cell membrane; Solvent extraction method

Introduction

Tea is one of the most popular non-alcoholic beverages, consumed by over two-thirds of the world's population because of its refreshing, mild stimulant and medicinal properties. It is the second-most drunk and refreshing beverage after water since the time immemorial. Tea could be categorised as true tea and herbal tea based on the type of plant and part of plants used.

All “true” tea comes from the plant, called the *Camellia sinensis*. Any leaf, root, fruit or flower that comes from a different plant is considered an herbal tea. For example, chamomile flowers and peppermint leaves

are considered herbal teas because they do not come from the traditional tea plant. It is important to distinguish between real tea and herbal tea since the flavour, health benefits and nutritional characteristics vary from plant to plant

True tea or Tea

A beverage made from the leaves of *Camellia sinensis*, originated in China and has become increasingly popular worldwide in recent year⁽¹¹⁾. Indeed, tea, which is considered the most widely consumed beverage in the world after water, has been the subject of extensive research in recent years due to its chemical composition. Tea harbours more than 700 bioactive compounds viz, different classes of polyphenols, unique amino acid L-Theanine, alkaloids (Caffeine, Theobromine), and Volatile Flavor Compounds (VFC). In line with literature data, the most commonly consumed type of tea in Turkey is black tea, followed by green tea, yellow tea, and oolong tea, especially in the western regions (18)

Green tea

Green tea is rich in EGCG (Epigallocatechin gallate; one of nature's most potent antioxidants) and is gaining widespread popularity due to its natural ability to promote weight-loss and good health. As a result of minimal processing,

green tea retains its natural appearance and vibrant colour as well as high levels of the plant's healthy properties. Green tea varies dramatically in flavour from grassy and sweet, to floral and fresh, to nutty and roasted. Like fine wine, green tea's flavour depends on the plant varietal, season of harvest, soil, elevation, weather, cultivation and origin. Each region has its own distinct flavour and aroma.

Herbal Tea

Herbal teas are made from dried fruits, flowers, spices or herbs that comes from a plant other than the tea plant is considered as a herbal tea. For example, chamomile flowers, peppermint leaves, spice blends, and rooibos (which is often called red tea) are considered herbal teas because they do not come from the traditional tea plant. Most herbal teas are naturally 100% caffeine-free, making them an excellent choice for evening or for people with caffeine-sensitivities. Herbal teas like chamomile, hibiscus, blue tea offer several health-promoting properties and could help improve heart health, digestion, sleep quality and more.

Here are most popular herbal tea categories-

Chamomile tea- Chamomile is an aromatic perennial flower, producing feathery leaves and white, daisy-like flower heads with

yellow centers. Chamomile has a bright, golden-colored infusion and a fragrance reminiscent of honey, fruit blossoms and apples. It is often taken with honey and lemon.

THERAPEUTIC EFFECTS-Chamomile has been prized for thousands of years for its therapeutic effects. In fact, chamomile was dedicated to the ancient Egyptian gods for its ability to calm the mind and comfort the senses. Today, chamomile is used as a nurturing herbal tea ideal for countering PMS, easing stress, relieving headaches and enhancing a peaceful night's sleep. Chamomile is also known to have anti-inflammatory and anti-allergic properties as well as acting as a digestive stimulant, muscle relaxant and mild sedative. Chamomile may be a beneficial treatment for arthritis and is even used in lotions and cosmetics for its soothing, antiallergic properties on the skin

Hibiscus tea - Hibiscus tea has been extensively studied for its potential antihypertensive effects. Several randomized controlled trials have shown that regular consumption of hibiscus tea can lead to significant reductions in systolic and diastolic blood pressure (Herrera-Arellano et al., 2004; Mozaffari-Khosravi et al., 2009)^[1,2]. The hypotensive properties of hibiscus tea are believed to be attributed to its rich content of bioactive compounds,

including anthocyanins and polyphenols, which possess vasodilatory and diuretic effects (Hopkins et al., 2013)^[3]. Recent studies have highlighted the potent antioxidant activity of hibiscus tea, attributed to its phenolic compounds, such as flavonoids and anthocyanins (Ali et al., 2015)^[4]. Additionally, hibiscus tea has demonstrated anti-inflammatory properties by inhibiting inflammatory mediators and enzymes (Tseng et al., 2013)^[5]. Research has shown that regular consumption of hibiscus tea can lead to reductions in total cholesterol, LDL cholesterol and triglyceride levels, while increasing HDL cholesterol (Mozaffari-Khosravi et al., 2009; Serban et al., 2015)^[2,6]. These lipid-lowering effects are attributed to the presence of flavonoids and other bioactive compounds in hibiscus tea, which modulate lipid metabolism and inhibit cholesterol synthesis (Mozaffari-Khosravi et al., 2009)^[2]. Several animal and human studies have reported that hibiscus tea can reduce fasting blood glucose levels and improve insulin resistance (Herrera-Arellano et al., 2004; Mozaffari-Khosravi et al., 2009; Serban et al., 2015)^[7,2,6]. The hypoglycemic effects of hibiscus tea are attributed to its ability to enhance insulin secretion, inhibit carbohydrate absorption, and enhance glucose utilization (Hopkins et al., 2013)^[3]. Hibiscus Blood pressure regulation and antioxidant effects McKay et al., 2006^[8].

Blue tea- In Indian traditional medicine it is termed as Aparajit (Hindi), Kakkattan (Tamil) and Aparajita (Bengali)¹⁴. It is perennial twinning herbaceous plant and is widely distributed in tropical regions of India, Malaysia, Sri Lanka, Philippines islands and Burma^(12,13)

It's an herbal infusion native to South Asia made by steeping butterfly pea flowers (*Clitoria ternatea L*) in hot water. It contains wide range of phytoconstituents such as triterpenoids, flavanol glycosides, tannins, alkaloids, amino acids, proteins, ternatins (poly acylated anthocyanins) and carbohydrates etc. Its high anthocyanin (delphinidin) content gives the tea its characteristic bright blue colour and medicinal properties.

THERAPEUTIC EFFECTS: Blue tea is well known for its potential health advantages, it possess antioxidant, antimicrobial, antidiabetic, hepato-protective, nootropic, central cholinergic, antidepressant, antianxiety, analgesic, antipyretic, wound healing, improved heart and brain health, anti-diabetic, cancer fighting properties and many other medicinal properties. Additionally essential to preserving general health and wellbeing are antioxidants. Due to its propensity to increase collagen, blue tea is thought to have anti-inflammatory characteristics and

is linked to maintaining healthy hair and skin.

Material and Methods

Plant collection

Tea samples were collected from super market and authenticated by Pharmacognosy department HWC, Dehradun Unit.

Extract preparation

10g of each of the ground leaves were extracted by soaking for 2 days using 100ml of solvent in a 250ml sterile conical flask followed by intermittent shaking. Distilled water and methanol was used as solvent to prepare the extracts. Extracts were filtered using Whatman filter paper No 1. The filtrates were then concentrated by using water bath 60°-80°C and stored in screw-capped tubes in refrigerated prior to use.

Determination of anti-microbial activity

Well-Diffusion method

Pre-inoculated nutrient agar media was poured in Petri dishes, the plates were allowed to cooled and settled inside the laminar air flow. After the plates get solidified well was made using a well cutter. And 100µl sample was loaded in the well. The inoculated plates were incubated in incubator at 30-35°C for 12-24 hrs.

HPLC Analysis

Sample preparation (5mg/ml): Weight accurately about 0.5g of sample in a 100ml beaker. Add 50 ml of methanol and sonicate for 5-10 mins. Allow the residue to settle and decant the dissolved extract into 100ml volumetric flask. Repeat the same process with 15 ml methanol each, until methanol extract becomes colourless. Make the volume up to the mark with methanol. Filter through 0.45µ syringe filter.

Hplc conditions:

Column : C18 Phenomenex Luna (250 x 4.6mm 5µ)

Mobile Phase : Ortho Phosphoric acid 0.1% in water (solvent-A)

: Acetonitrile (solvent- B)

Flow rate : 1ml/minute

Wavelength : 210 nm

Injection volume : 20 µl

Temperature : 35°C

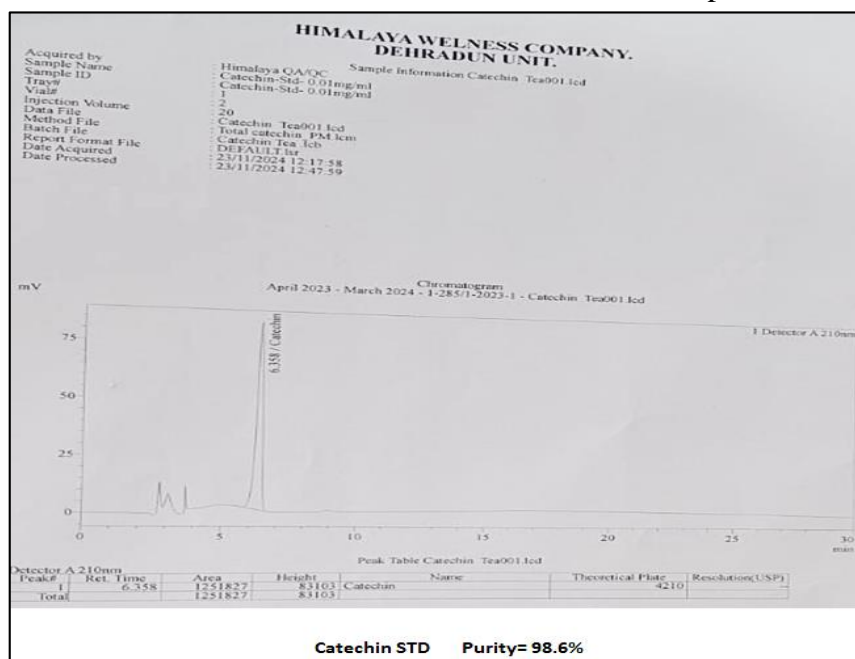
Standard catechins: weight 10mg of standard catechin into a volumetric flask add 7-8 ml methanol dissolve by sonication and make the volume up to the mark with methanol. Take 1 ml of above solution and dissolve in to 100 ml of methanol .

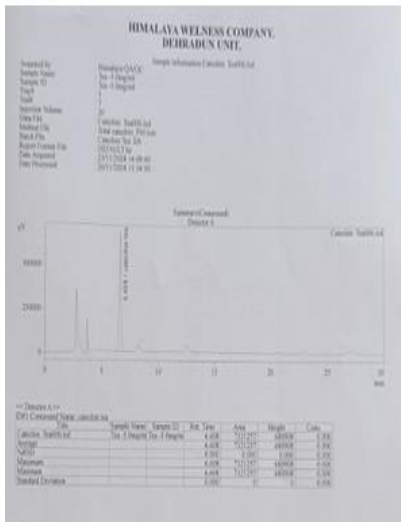
Calculation

$$\text{Catechin \%} = \frac{\text{Area of sample peak}}{\text{Area of standard peak}} \times \frac{\text{concentration of standard (mg/ml)}}{\text{concentration of sample (mg/ml)}} \times \% \text{ purity (std)}$$

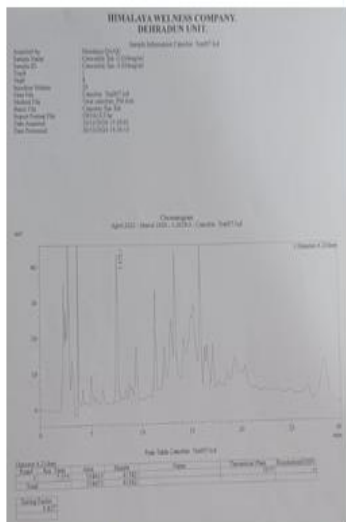
Results and Discussion

HPLC (High Performance Liquid Chromatography) is a powerful analytical technique used to separate, identify and quantify catechins in various Tea samples in different chromatogram. Table- 1 shows the catechin content in different tea samples. The table and chromatogram provided in the image summarize the catechin content in different tea samples. Antimicrobial activity of tea extracts was depicted in **Table-2**.

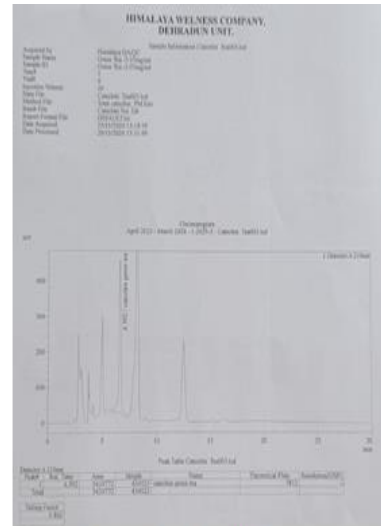




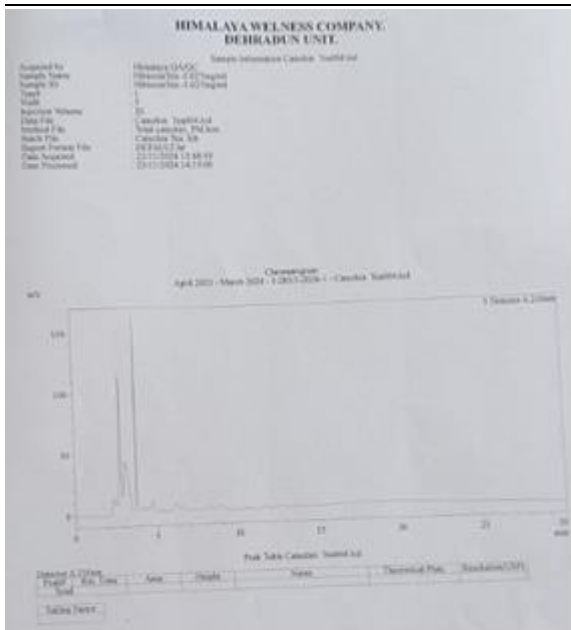
Tea (*Camellia sinensis*)



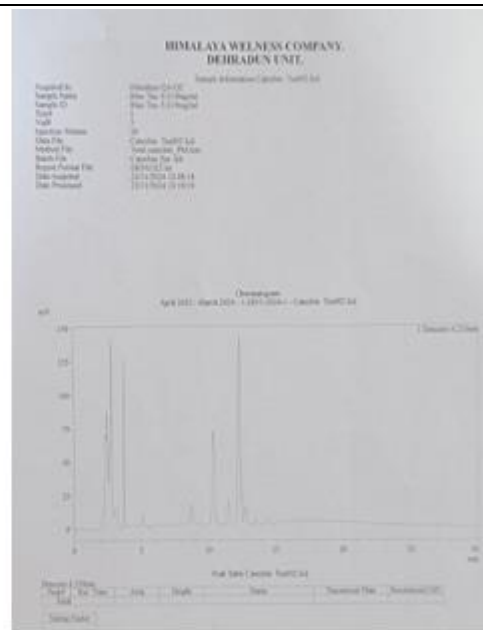
Camomile Tea (*Matricaria chamomilla*)



Green Tea (*Camellia sinensis*)



Hibiscus Tea (*Hibiscus rosa-sinensis*)



Blue Tea (*Clitoria ternatea*)

Table-1 Catechin content in Different Tea extract through HPLC

Sample	Catechin %
Tea	1.14
Green tea	0.84
Chamomile tea	0.087
Hibiscus tea	Absent
Blue tea	Absent

Catechin content is highest in Tea (*Camellia sinensis*), followed by green tea (*Camellia sinensis*). Chamomile tea (*Matricaria chamomilla*) has the lowest

catechin content among the listed samples, with hibiscus (*Hibiscus rosa-sinensis*) and blue tea (*Clitoria ternatea*) showing no detectable levels.

Table-2 Antimicrobial activity of Different Tea extract.

Sample	solvent	S.aureus	E.coli
TEA (<i>Camellia sinensis</i>)	Aqueous	20 mm	16 mm
	Methanol	24 mm	21 mm
GREEN TEA (<i>Camellia sinensis</i>)	Aqueous	23 mm	22 mm
	Methanol	27 mm	18 mm
BLUE TEA (<i>Clitoria ternatea</i>)	Aqueous	22 mm	NA
	Methanol	20 mm	NA
CHAMOMILE TEA (<i>Matricaria chamomilla</i>)	Aqueous	NA	NA
	Methanol	14 mm	13 mm
HIBISCUS TEA (<i>Hibiscus rosa-sinensis</i>)	Aqueous	25 mm	20 mm
	Methanol	28 mm	26 mm
Positive control		32 mm	30mm

The table-2 shows the antimicrobial activity of different tea extracts against two bacterial strains: *Staphylococcus aureus* and *Escherichia coli*. As its clear from the data that almost all the tea extracts found to be remarkably active against the tested pathogens except chamomile aqueous extract but its methanolic extract was active for both bacteria. Above all the tea extracts mentioned Hibiscus tea extract have highest antimicrobial activity followed by Green tea and Black tea.

Conclusion

Our results revealed that all the tea extracts were found active against *Staphylococcus aureus* and *E.coli*. Hibiscus tea had the highest activity for both bacteria in comparison to our true tea extracts i.e *Camellia sinensis* and further investigation is needed to explore the active phytochemical constituents responsible for antimicrobial activity of Hibiscus against *test pathogens*

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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