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Original Article

PHYTOCHEMICAL SCREENING AND IN VITRO ANTIFUNGAL ACTIVITY OF CAMELLIA SINENSIS

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ABSTRACT

Objectives: The aim of the present work was to evaluate the phytochemical composition of *Camellia sinensis* and to assess the antifungal activities of *Camellia sinensis* using in vitro antifungal screening techniques. The ability of tea plant extract to inhibit the growth of fungal strains is an indication of its antifungal property that might be used in the management of fungal infections in future.

Methods: Extracts of leaves from the tea plant *Camellia sinensis* contain polyphenolic components with activity against a wide spectrum of microbes. Studies conducted over the last 20 years have shown that the green tea polyphenoliccatechins, in particular (−)-epigallocatechingallate (EGCg) and (−)-epicatechingallate (ECg), can inhibit the growth of a wide range of Gram-positive and Gram-negative fungal species with moderate potency. The leaves were collected from the market and identified by the Pharmacognosy department of our own college. Phytochemical analysis revealed the presence flavonoids.The study was carried out on various species of fungi including *Candida albicans*(MTCC No.183), *Candida tropicalis* (MTCC No.184),and *Saccharomyces cerevisiae* (MTCC No.170)using cup plate method. The results obtained were compared against standard antibiotic streptomycin.

Results: The aqueous extract is effective against *saccharomyces cerevisiae* and very less against *Candida albicans*and no effect was found in *Candida tropicalis.* The alcoholic extract is found effective against *Candida albicans and Saccharomyces cerevisiae* and very less effect was seen against *Candida tropicalis.*

Conclusion: The study illustrates that *Camellia sinensis* has shown a significant antifungal activity against various species of fungi like *saccharomyces cerevisiae, Candida albicans* and very less effect was seen against *Candida tropicalis;* both in aqueous as well as methanolic extract. Along with that it is a good source of Flavonoid.

Keywords: Green tea, *Camellia sinensis* Antifungal, Phytochemical screening, Zone of inhibition.

INTRODUCTION

In the world, phytomedicines have been used in past to treat various ailments long before the introduction of modern medicine. Herbal medicines are still widely used in many parts of the world especially in areas where people do not have contact to modern medicines [1, 2]. In most Asian countries where herbal medicines are still heavily relied upon because of high cost of chemotherapeutic drugs, there is a need for scientific research to estimate the biological activities of medicinal plants. The results obtained from such research may lead to the development and validation of traditionally used medicinally important plants and enable full usage of the properties of these plants [3].Green tea is selected for the study because;tea consumption has its legendary origins in China of more than 4,000 years ago. Green tea has been used as both a beverage and a medicine in most of Asia, to help everything from controlling bleeding and helping heal wounds to regulating body temperature, blood sugar and promoting digestion[4]. The most abundant components in green tea are polyphenols, in particular flavonoids such as the catechins, catechingallates and proanthocyanidins [5]. Tea polyphenols are well-known for their antioxidant properties. Green tea has greater antioxidant potential than oolong and black teas [6-10]. Studies have shown that the strong antioxidant properties of green tea are attributed to catechins of EGCG and EGC[11–14] The three adjacent hydroxyl groups on the B-ring of EGCG, GCG, EGC, and GC are more effective in scavenging free radicals than the two adjacent OH groups of ECG, CG, and EC[15]. Black tea is also known to have potent antioxidant properties which are manifested by its ability to scavenge free radicals, inhibit lipid peroxidation, and chelate metal ions[16, 17]. Although green tea has higher total phenolic content (TPC), free radical scavenging activity, and ferric reducing power, its ferrous ion-chelating ability is poorer than black tea[9, 10]. Tea polyphenols are also known for their antifungal activity. In general, antifungal activity decreases when the extent of tea fermentation is increased, implying stronger activity in green tea than black tea[18, 19].

MATERIALS AND METHOD

Collection of plant materials

The experiment was conducted in the year 2013 in the college. Leaves of *Camellia Sinensis* were collected from the Herbal Garden. The species was identified and authenticated at the Herbal department of Pioneer Pharmacy Degree College, Vadodara, Gujarat. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and dried for 3 days.

Source of Microorganisms

The organisms studied *Candida albicans*(MTCC No.183), *Candida tropicalis* (MTCC No.184), and *saccharomyces cerevisiae* (MTCC No.170). The organisms were obtained from the MTCC, Chandigarh. The Sub culturing was done after interval of 15 days.

Preparation of methanolic and aqueous leaf extract

Fresh leaves (500gm) of *Camellia sinensis* were shade dried at room temperature (32 – 35 °C) to constant weight over a period of 3 days. 45 g of the powdered leaves were separately extracted in 500 ml conical flasks with 90% methanol (methanolic extraction) and slightly warm water (aqueous extraction) for overnight. The extracts were separately filtered using sterile Whatman no.1 filter paper. These extracts were used in further process.

Storage conditions

The extract was stored in a cool condition protected from direct sunlight.

Phytochemical analysis

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins was performed by the extracts.

Alkaloids [31]

Wagner's test: 1 ml of extract, add 2 ml of Wagner's reagent (iodine in potassium iodide).

Dragandroff's test: 1 ml of extract, add 1 ml of Dragandroff's reagent (potassium bismuth iodide solution).

Hager test:-1 ml of extract, add 3 ml of Hager's reagent (saturated aqueous solution of picric acid).

Mayer's test:-1 ml of extract, add 1 ml of Mayer's reagent (potassium mercuric iodide solution).

Flavonoids: 3ml of each extract was added to 10ml of distilled water the solution was shaken. 1ml of 10% NaOH solution was added to the mixture. [32]

Saponins: 3ml of each extract and dilute with 2ml of distilled water was added in a test tube. The mixture was shaken vigorously.[33]

Salkowski Test: 5 drops of concentrated H_2SO_4 were added to 1ml of each extract in a separate test tube were boiled gently for 2min and allowed to cool. 3 drop of ferric chloride solution were added to each extract.

Glycosides: 25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added.[32]

Reducing Sugars: To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath.[32]

Terpenoids: Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly. [34]

Determination of Antifungal Activity

The antifungal activity of the leaf extracts was determined using agar well diffusion method by following the known procedure. Briefly, Nutrient agar was inoculated with the given microorganisms by spreading the fungal inoculums on the media. Wells were made in the agar using the stainless steel borer of 8mm and filled with 300µl of plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 28°C for 72 hours and the antimicrobial activity was assessed by measuring the diameter of the zone of inhibition. Same method was applied for the standard antibiotic Streptomycin.

RESULT AND DISCUSION

Qualitative phytochemical analysis

The present study reveals that plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthraquinones in different solvent extracts as shown in Table 1.

Antifungal Activity

Antifungal activity of *Camellia sinensis* was seen against organisms namely *Escherichia coli* (MTCC No.40), *Staphylococcus aureus* (MTCC No.87), *Proteus vulgaris* (MTCC No.742), *Pseudomonas aeruginosa* (MTCC No.424), *Bacillus subtilis* (MTCC No.441), *Staphylococcus epidermidis* (MTCC No.9041), and *Micrococcus luteus* (MTCC No.106).

The Aqueous extract exhibits inhibition zone on *Proteus vulgaris* (4mm).Same procedure was also applied for the standard drug streptomycin and the results obtained are also presented in Fig.1.While the methanolic extract exhibit effective antifungal activity against the organisms like *Staphylococcus aureus*(2mm), *Bacillus subtilis*(3mm), *Micrococcus luteus*(2mm), *Staphylococcus epidermidis*(4mm) as shown in Fig.2.

CONCLUSION

Extracts of leaves from the tea plant *Camellia sinensis* contain polyphenolic components with activity against a wide spectrum of microbes. Studies conducted over the last 20 years have shown that the green tea polyphenoliccatechins, in particular (−)-epigallocatechingallate (EGCg) and (−)-epicatechingallate (ECg), can inhibit the growth of a wide range of Gram-positive and Gram-negative fungal species with moderate potency. The study was carried out on various species of fungal *Candida albicans*(MTCC No.183), *Candida tropicalis* (MTCC No.184), and *Saccharomyces cerevisiae* (MTCC No.170) using cup plate method.

The results obtained were compared against standard antibiotic streptomycin.The results obtained were compared against standard antibiotic streptomycin. The zone of inhibition is seen in *Saccharomyces cerevisiae* and very less inhibition is seen against *Candida albicans*and no effect is foundin *Candidatropicalis* inaqueous extract. The alcoholic extract is found effective against *Candida albicans*and*Saccharomyces cerevisiae* and very less effect was seen against *Candida tropicalis* by getting the above results, author suggest further detail phytochemical investigation and work.

Table 1: Qualitative Phytochemical analysis of *Camellia sinensis*

S. No.	Phytoconstituent	Aqueous extract	Methanolic extract
1.	**Alkaloids**		
	Dragondroff's test	-	-
	Wagner's test	-	-
	Mayer's test	-	-
	Hager's test	-	-
2.	**Carbohydrates**		
	Molisch Test	-	-
	Benedicts Test	-	-
3.	**Glycosides**		
	Legal test	-	-
	Baljet test	-	-
4.	**Steroids**		
	Lieberman Burchard Test	-	-
5.	**Proteins & Amino acids**		
	Biuret test	-	-
	Xanthoproteic test	-	-
	Lead Acetate test	-	-
8.	**Saponins**		
	Foam test	-	-
9.	**Flavonoids**		
	Shinoda test	+	+

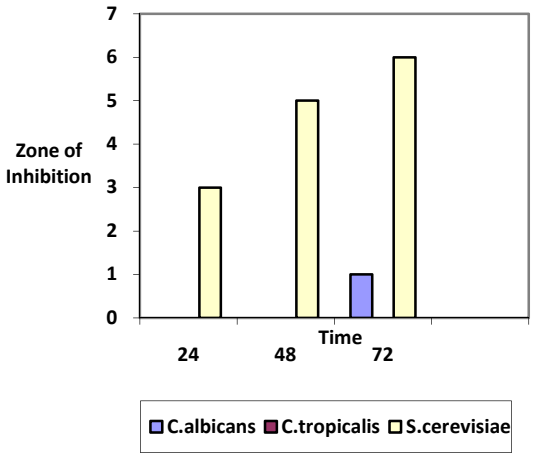

Fig.1: Zone of Inhibition seen on various fungal species in aqueous extract of *Camellia sinensis*

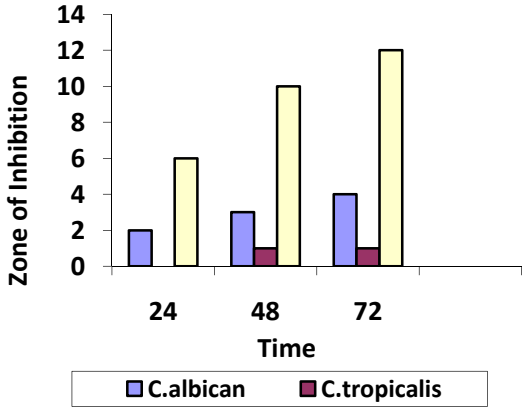

Fig. 2: Zone of Inhibition seen on various fungal species in methanolic extract of *Camellia sinensis*

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCE

1. Hoareau L & Da Silva E J. Medicinal plants. A Re-emerging Health Aid. Electronic J Biotech.1999;2(2).
2. Ajibad L.T, Fatoba P.O,Raheem U.A & Odunuga, B.AEthnomedicine and primary healthcare in Ilorin. Nigeria. Ind. J. Trad. Knowl. 2005; 4(2): 150-158.
3. Adde-Mensah. I. Towards a rational scientific basis for herbal medicine. a phytochemist's two decades contribution. Ghana University Press . Accra.1992.
4. Anderson JC, Headley C, Stapleton PD, Taylor PW. Synthesis and antifungal activity of a hydrolytically stable (−)-epicatechingallate analogue for the modulation of β-lactam resistance in *Staphylococcus aureus*. Bioorganic and Medicinal Chemistry Letters. 2005b;15:2633–2635.
5. Brantner A, Grein E. Antifungal activity of plant extracts used externally in traditional medicine. Journal of Ethnopharmacology. 1994;44:35–40.
6. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem. 1995;43:27–32.
7. von Gadow A, Joubert E, Hansmann CF. Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong, and black tea. Food Chem.1997;60:73–7.
8. Yokozawa T, Dong E, Nakagawa T, Kashiwagi H, Nakagawa H, Takeuchi S, et al. *In vitro* and *in vivo* studies on the radical scavenging activity of tea. J Agric Food Chem. 1998;46:2143–50.
9. Chan EW, Lim YY, Chew YL. Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. Food Chem. 2007;102:1214–22.
10. Chan EW, Lim YY, Chong KL, Tan JBL, Wong SK. Antioxidant properties of tropical and temperate herbal teas. J Food Compos Anal. 2010;23:185–9
11. Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Radical Biol Med. 1996;21:895–902
12. Kondo K, Kurihara M, Miyata N, Suzuki T, Toyoda M. Scavenging mechanisms of (−)-epigallocatechin gallate and (−)-epicatechin gallate on peroxyl radicals and formation of superoxide during the inhibitory action. Free Radical Biol Med. 1999;27:855–63.
13. Farhoosh R, Golmovahhed GA, Khodaparast MH. Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.) Food Chem. 2007;100:231–6.
14. Horžić D, Komes D, Belscak A, Ganic KK, Ivekovic D, Karlovic D. The composition of polyphenols and methylxanthines in teas and herbal infusions. Food Chem. 2009;115:441–8.
15. Sharma A, Wang R, Zhou W. Functional foods from green tea. In: Shahidi F, editor. Functional foods of the east. United States: CRC Press; 2011. pp. 173–95.
16. Łuczaj W, Skrzydlewska E. Antioxidative properties of black tea. Prev Med. 2005;40:910–8.
17. Wiseman SA, Balentine DA, Frei B. Antioxidants in tea. Crit Rev Food Sci Nutr. 1997;37:705–18. [PubMed]
18. Kokate CK. 1994. Practical Pharmacognosy, 4thedition, VallabhPrakashan, New Delhi, pp. 4, 29.
19. Jones C, Woods K, Whittle G, Worthington H, Taylor G. Sugar, drinks, deprivation and dental caries in 14-year old children in the north west of England. Community Dental Health.1999;16:68–71
20. Ansari SH. 2006. Essentials of Pharnacognosy, 1stedition, Birla publications, New Delhi, pp. 357-359, 588-590.
21. Mukherjee PK. 2002. Quality Control of Herbal Drugs, Business Horizons Pharmaceutical Publishers, New Delhi, pp. 356-358.