Research Article

Antibacterial, Antifungal and Anticorrosion Properties of Green Tea Polyphenols Extracted Using Different Solvents

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ABSTRACT

The purpose of this study was to examine the antibacterial, antifungal and anticorrosion properties of green tea polyphenols extracted using different solvents like water, 70% ethanol, 70% methanol and dimethyl form amide. The extracted polyphenols were separated using TLC analysis. The antibacterial and antifungal activities were done by well diffusion method and it was found that polyphenols extracted using methanol and ethanol showed increased inhibition activity. Anti-corrosion property was investigated using weight loss measurements and showed significant inhibition effect for DMF extract.

Key words: Antimicrobial activity, Anticorrosion property, Green Tea, Polyphenols, TLC analysis, Ethanol, Methanol.

INTRODUCTION

Green tea leaves contains a bio-active component called polyphenol with activity against wide spectrum of microorganisms. For the past two decades, several studies were conducted on the testing of antimicrobial properties of green tea polyphenols. A number of surveys have indicated that green tea consumption reduces the risk of cardiovascular disease,[1] stroke[2] and obesity.[3] In addition to the therapeutic and biological activities many studies found to show *in vitro* antimicrobial activity against many bacteria and virus, specifically *Helicobacter pylori* (responsible for gastritis and implicated as a risk factor for stomach cancer) and α-haemolytic streptococci (the main aetiological agents of dental caries),[4] rotavirus, enterovirus and influenza virus,[5] yeasts,[6] filamentous fungi,[7] chlamydia, mycoplasmas[8] and parasites.[9]

Hence the present work aims to extract high purity with good yield of polyphenols from green tea by using various solvents and the analysis of polyphenols fractions by TLC. The green tea polyphenols were tested for its antibacterial, antifungal by well diffusion method and anticorrosion properties by gravimetric analysis.

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MATERIALS AND METHODS

Plant Materials and Chemicals

Green tea (Camellia Sinensis) were purchased from local market and the solvents used were AR grade. Ethanol, methanol, chloroform, ethyl acetate and DMF were used of LR grade.

Extraction of Polyphenols from Green Tea

The extraction of polyphenols was done in three steps, viz., the sample for analysis were prepared following the conditions developed for optimized extraction of tea polyphenols in the laboratory. 25 g of coarse green tea was taken in round bottomed flask and 300 ml of water

was added to it. The flask was kept for refluxing for 2 hr at 80°C. After refluxing the contents were filtered and volume of the extract was measured. The extracted filtrate was partitioned with chloroform (1:1,vol %) ratio to remove caffeine and related impurities. The aqueous phase was collected and the organic layer (chloroform layer) were discarded. Then the aqueous layer is extracted with ethyl acetate (1:1 ratio) and then the ethyl acetate layer was collected. Then ethyl acetate layer was evaporated by using rotary vacuum evaporator, to remove ethyl acetate. Then the concentrated filtrate was obtained and it was freeze dried. The crude sample of polyphenol was obtained. Similar procedure was adopted for all the solvents.

Antibacterial and antifungal activities of green tea polyphenols

A fungus (Penicillium) and bacteria (*Staphylococcus aureus*) were inoculated into two different conical flasks Containing 50 ml of SDA and NA (medium) using a flamed loop. Drops of fungus/water culture was mixed with the warm, melted, autoclaved PDA and poured into separate plates under aseptic conditions. The plates were covered and allowed to cool. As soon as the agar was partly solidified, the plates were inverted and left few minutes. When cooled, 5 wells were made at the centre of the plate. The wells were made by using a 6 mm cork borer or puncher that was sterilized with alcohol and flame. Ethanol and methanol extracted samples were dissolved in a solvent at final concentration of 10mg/1mL was pipette into the different wells in a sterilized environment at different volumes (0.1-0.2-0.4-0.6 ml) in plates, using a micro plates. Control wells were inoculated by Chloromphenicol and Ketakonizol for bacteria and fungi, respectively. The plates were labelled, covered, inverted and kept for incubation for about 48h. Zone of inhibition was measured.

Anticorrosion property of green tea polyphenols

The anti-corrosion properties of green tea polyphenols extracted using different solvents were investigated using weight loss measurements at room temperature. The mild steel specimen of the dimensions 2.5 cm × 1 cm × 0.1 cm has been used and was polished using different grades of emery papers, washed with double distilled water, dried and finally degreased with the acetone. In this method previously weighed coupon was completely immersed in 100 ml 1 M hydrochloric acid with and without green tea extracts in an open beaker. After an hour, the corrosion product was removed by washing each coupon using double distilled water. The washed coupon was rinsed in acetone and dried in the air before reweighing. From the average weight loss (mean of three replicate analysis) results, the inhibition efficiency of the inhibitor, and the corrosion rate of mild steel were calculated.

RESULTS AND DISCUSSION

TLC analysis of polyphenols

TLC profile of the extracted polyphenols was carried out and it was observed that separation of catechins on silica using the mobile phase ethylacetate:n-butanol:formic acid:water(10:6:2:2 v/v/v/v) showed the presence of two major bands with ethanol and methanol. The results of TLC analysis of catechin compounds on silica with the above mobile phase are presented in Figure 1. The presented results of TLC analysis on silica gel was obtained after standardizing various mobile phases and the mobile phase comprising ethyl acetate:n-butanol:formic acid:water showed a higher sensitivity on development of the plate with vanillin:suplhuric acid reagent.

Two major spots were noted in all the samples; coloured green and orange with R_f value of 0.22 for the green spot and 0.90 for the orange spot for the water extract. The R_f values for ethanol and methanol extracts were also found to be 0.18 (green spot) and 0.91,0.85 (orange spot) respectively. More or less the R_f values for water, ethanol and methanol extracts were found to be same (Table 1), from this we can infer that the catechin fractions were separated on silica gel by the application of the mobile phase.

Antibacterial and antifungal activities of ethanol and methanol extracted green tea polyphenols

The methanol and ethanolic extract of polyphenols showed significant antibacterial activity and antifungal activity by agar gel diffusion method.[10] The methanolic

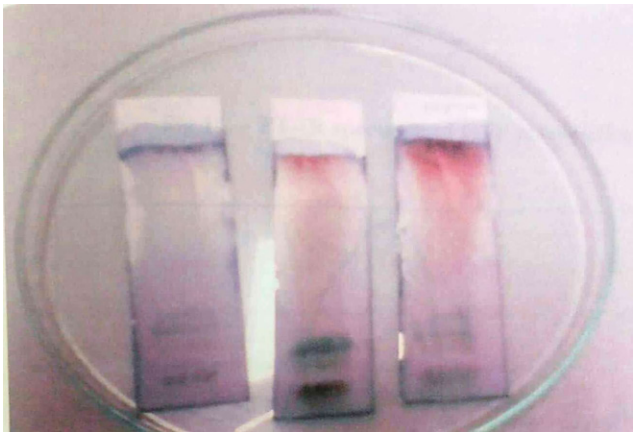

Figure 1: TLC Chromatogram of Catechin fractions of Green Tea Polyphenols water, ethanol and methanol.

extract showed marked antibacterial and antifungal activity against *Staphylococcus aureus* and Pencillium sps., respectively compared to other extracts. The antibacterial susceptibility of the methanolic and ethanolic extract of polyphenols seen in the Figure 2, 3 on *Staphylococcus aureus* indicate that even low concentrations of the extract was effective. Figure 4, 5 shows the antifungal susceptibility of the methanolic and ethanolic extract of the polyphenols on *Pencillium* sps., There was no antibacterial or antifungal activity for both the extracts when the concentration was 0.1 mg. The zones of inhibition of the extracts were distinct (Table 2) and comparable to the standard drug.

Table 1: R_f values of TLC Separated Polyphenols.

Type of extract	Colour of the spot	Rf value
Water	Green	0.22
	Orange	0.90
Ethanol	Green	0.18
	Orange	0.91
Methanol	Green	0.18
	Orange	0.85

The maximum inhibitory concentration (MIC) for antibacterial and antifungal activity of the methanol extract (0.4 ml) resulted in 26 mm and 15 mm respectively it was found to more or less similar to the inhibition levels of the standard chloromphenicol drug (43 mm). The result of the study showed that the solvent extracts of polyphenols produced prominent zones of inhibition against *Staphylococcus aureus* and *Penicillium*. This indicates the presence of potent antibacterial activity antifungal activity, and confirms reports on its use as anti- infective. Although both the methanol and ethanol extract of polyphenols produced inhibitory actions against the test organisms, methanol extracts showed more inhibitory effects than the ethanol extract. This tends to show that the active constituents in the leaves were better extracted with methanol than the other solvents.

Anticorrosion properties of green tea polyphenol extracts

The corrosion inhibition of green tea polyphenol extracted using water, 70% ethanol, 70% methanol and

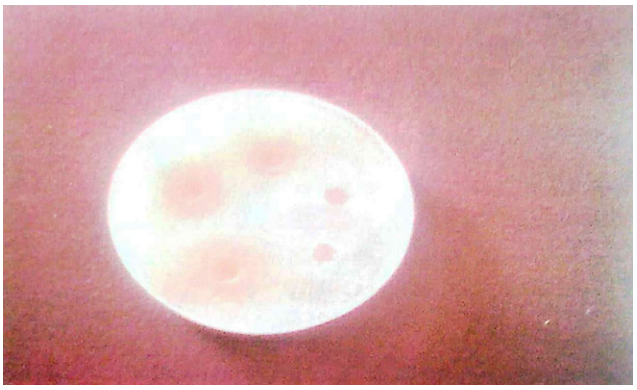

Figure 2: Antibacterial activity of Green Tea Polyphenols extracted with water.

Figure 3: Antibacterial activity of Green Tea Polyphenols extracted with Ethanol.

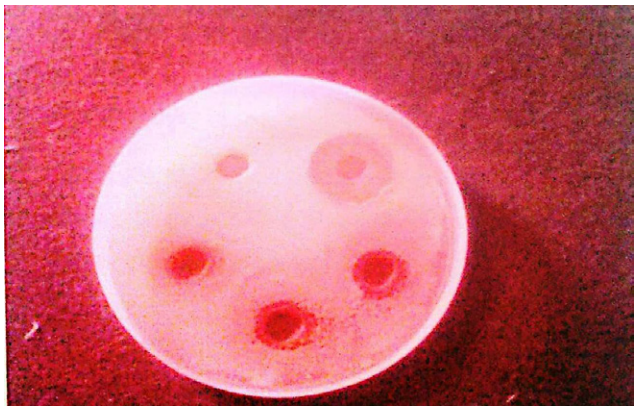

Figure 5: Antifungal activity of Green Tea Polyphenols with Ethanol.

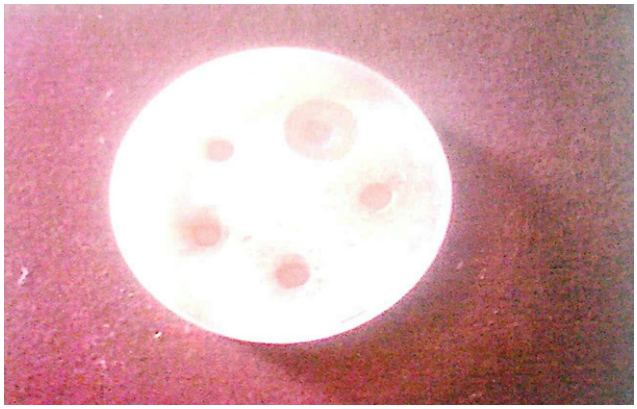

Figure 4: Antifungal activity of Green Tea Polyphenols extracted with Water.

Table 2: Antibacterial and antifungal activity of polyphenols.

Type of extract	Antibacterial activity		Antifungal activity	
	Concentration of the extract (ml)	Zone of inhibition (mm)	Concentration of the extract (ml)	Zone of inhibition (mm)
Ethanol	0.1	17.0	0.1	-
	0.2	18.0	0.2	17.0
	0.3	21.0	0.3	18.0
	0.4	32.0	0.4	20.0
Methanol	0.1	15.0	0.1	-
	0.2	24.0	0.2	18.0
	0.3	30.0	0.3	20.0
	0.4	26.0	0.4	15.0

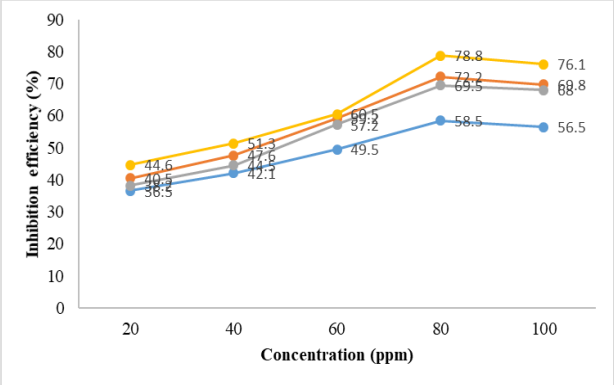

Figure 6: Plot of Inhibition efficiencies versus green tea polyphenols of different extracts on carbon steel in 1 M HCl.

dimethylformamide was investigated using weight loss measurements at room temperature on carbon steel in 1 M HCl. The inhibition efficiencies were calculated and its corresponding plot is given in the Figure 6. On analysing the graph it was found that inhibition efficiencies of green tea polyphenols extracted by all the solvents increases when its concentration increases. [11] But a significant corrosion protection of 78.8 % was found for DMF extract at 80 ppm. This may be attributed to the solvent effect which plays a greater role in the extraction of green tea polyphenols.

CONCLUSION

The antibacterial and antifungal activity carried out in this study revealed that even lower concentration (0.1 mg) of polyphenol extracted using ethanol and methanol were more effective against *Staphylococcus aureus* and *Pencillium* sps., It can be concluded that the green tea polyphenols not only possess antimicrobial property but also it have a strong efficiency towards corrosion on carbon steel in acid medium. Based on this study, it can be concluded that the extraction with ethanol and methanol are especially suitable for the antimicrobial and anticorrosion properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TLC: Thin layer chromatography; **DMF**: Dimethyl formamide; **AR:** Analar; **LR:** Laboratory; **SDA:** Sabouraud dextrose agar; **NA:** Nutrient agar; **Rf:** Retention factor; **HCl:** Hydrochloric acid.

REFERENCES

1. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. Lancet. 1993;342(8878):1007-11.
2. Keli SO, Hertog MG, Feskens EJ, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: The Zutphen study. Arch Intern Med. 1996 ; 156(6) :637-42.
3. Bell SJ, Goodrick GK. A functional food product for the management of weight. Crit Rev Food Sci Nutr. 2002; 42(2):163-78.
4. Taylor PW, Hamilton-Miller JMT, Stapleton PD. Antimicrobial properties of green tea catechins. Food Sci Technol Bull. 2005; 2: 71-81.
5. Song JM, Lee KH, Seong BL. Antiviral effect of catechins in green tea on influenza virus. Antiviral Res. 2005; 68(2) :66-74.
6. Hirasawa M, Takada K. Multiple effects of green tea catechin on the antifungal activity of antimycotics against *Candida albicans*. J Antimicrob Chemother. 2004; 53 (2):225-9.

7. Okubo S, Toda M, Hara Y, Shimamura T. Antifungal and fungicidal activities of tea extract and catechin against Trichophyton, Nihon Saikingaku Zasshi. Japanese Journal of Bacteriology. 1991; 46(2) :509-14.
8. Chosa H, Toda M, Okubo S, Hara Y, Shimamura T. Antimicrobial and microbicidal activities of tea and catechins against Mycoplasma, Kansenshogaku Zasshi. The Journal of the Japanese Association for Infectious Diseases.1992; 66(5) :606-11.
9. Paveto C, Güida MC, Esteva MI, Martino V, Coussio J, Flawiá MM, *et al*. Anti-*Trypanosoma cruzi* activity of green tea (*Camellia sinensis*) catechins. Antimicrob Agents Chemother. 2004; 48(1) :69-74.
10. Arun SD, Minal MK, Karibasappa GN, Prashanth VK, Girija AD, Harish CJ. Comparative assessment of antibacterial efficacy of aqueous extract of commercially available black, green, and lemon tea: an *in vitro* study. Int J Health Sci . 2017;11(4): 42-6.
11. Zhang B, Yao R, Li L, Wang Y, Luo R, Yang L, *et al*. Green Tea Polyphenol Induced Mg2+-rich Multilayer Conversion Coating: Toward Enhanced Corrosion Resistance and Promoted *in situ* Endothelialization of AZ31 for Potential Cardiovascular Applications. ACS Appl Mater Interfaces. 2019;11(44):41165-77.

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