

Research Article

Assessment of Antibacterial Activity and Phytochemistry of Different Clones of Tea Grown and Processed at NTHRI

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A B S T R A C T

Background and Objective: Medicinal plants have been a major source of therapeutic agents to cure diseases. The present study was aimed to study phytochemistry and antimicrobial potential of tea clones i.e P3, P5, and P7 grown and processed in Black tea at NTHRI.

Materials and methods: Various analytical techniques were applied for phytochemical analysis to identify phytochemicals. The phytochemical analysis showed the presence of alkaloids, flavonoids, phlobatannins, glycosides, carotenoids, saponins, phenols, and terpenoids by changing the color of the medium when treated with respective reagents in aqueous extracts.

Results: Phlobatannins and steroids were found absent in all varieties in ethanolic extracts while seen positive in all varieties in aqueous extracts. The Minimum Inhibitory Concentration (MIC) for antimicrobial activity was determined by the agar well diffusion method against two bacterial strains *Staphylococcus aureus* and *Salmonella typhi*. P3 showed highest antimicrobial activity against both bacterial strains i.e 15.1±0.84 in 50 ppm and 17.3±0.62 in 100 ppm against *S. aureus*, while 7.9±0.32 in 50 ppm and 10.06±0.6 in 100 ppm against *S. typhi*. P7 also showed maximum activity against both strains i.e 13.3±1.53 in 50 ppm, 15.02±0.96 in 100 ppm against *S. aureus* and 6.16±0.76 in 50 ppm, 9.23±0.68 in 100 ppm against *S. typhi* and the low activity against both strains were observed in P5 i.e. 4.9±1.10 in 50 ppm, 5.6±0.36 in 100 ppm against *S. aureus* and 6.16±0.76 in 50 ppm, 9.23±0.68 in 100 ppm against *S. typhi*.

Conclusion: Our findings suggest that consumption of these varieties could be valuable to promote consumer health and also serve as a good source of energy and nutrients.

Keywords: Antimicrobial, Phytochemicals, Black Tea

Background

Herbal teas have been used up for centuries, not because of their taste, but the medical point of view as a disease-preventing agent. Herbal or traditional tea, derived from the plant *Camellia sinensis*, is frequently consumed beverage next to the water in the world (Kuroda and Hara, 1999). Herbal teas have been used for their medicinal properties for centuries. Studies showed a positive correlation between herbal tea consumption and the reduction of select chronic diseases. Herbal tea has antimicrobial, anticancer, hypotensive, and antimutagenic properties due to the presence of phytochemicals (De Vos and De Schrijver, 2003). Tea is composed of a large number of polyphenols (5–27%) consisting of catechins/ flavonols and gallic acid components along with free amino acids and alkaloids. In comparison with green tea amount of polyphenolic compounds are higher than those of black tea (Leung and Foster, 1996). Phytochemicals are a word originated from a Greek word phyto meaning plant chemical. It is considered as a purely active compound that occurred biologically and is present in plants, which is useful for the health benefits of man further than those credited to macronutrients and micronutrients (Halliwell, 1994). They prevent plants from disease and injury and subsidize the texture, color, and plant's aroma. Generally the plant chemicals that prevent plant cells from environmental threats such as a microbial attack, pollution, UV experience, stress and droughts are known as Phytochemicals (Hasler, 1999; Gibson, 1998). Recently, it is visibly identified that they have importance in the defense of human health when their nutritional consumption is important. Various plants have importance because of their antimicrobial characters, which are because of secondary metabolites manufactured by the plants. These behaviors are recognized by their vigorous substances like, phenolic substances which are a portion of the important oils, as well as in tanning. This has enforced scientist to exploration for new antimicrobial substances from numerous bases like the medicinal plants (Duncan et al., 1999).

The present investigation was carried to study the phytochemical screening and antimicrobial activity of tea clones grow in NTHRI.

Material and Methods

Collection

Tea samples of varieties P3, P5, and P7 were collected from National Tea and High Value Crops Research Institute, Shinkiyari, Pakistan. Fresh tea leaves were collected and processed in Black tea through different steps in the Turkish tea plant situated at NTHRI, Shinkiyari.

Antibacterial Activity

Antimicrobial activity was carried out against *S. aureus*

and *S. typhi*. All microorganisms were obtained from the Microbiology Laboratory, Hazara University Mansehra. The antimicrobial assay was carried out by using the agar well diffusion method (Zain et al., 2012). Nutrient agar media was prepared. The autoclave was carried out at 121°C for 15 minutes. After media plates preparation they were placed under UV light for more than 20 minutes for sterilization. The inoculum was spread over plates using a wire loop. A 6 mm standard cork barrier was used for good preparation. Sample extracts were introduced into wells by dropper. The filter paper discs of specific antibiotics were placed on nutrient agar surface and use as a standard. The inoculated plates were incubated at 37°C for 24 h and the inhibition zone was measured in mm by means of measuring scale after 24 h (Table 1, Figures 1&2).

Table 1. Zone of inhibition of tea varieties against Pathogenic Strains

Tea Varieties	Zone of Inhibition (mm)			
	S. aureus		S. typhi	
	50ppm	100ppm	50ppm	100ppm
P3	15.1±0.84	17.3±0.62	7.9±0.32	10.06 ±0.6
P5	4.9±1.10	5.6±0.36	5.9±0.4	8.2±0.5
P7	13.3±1.53	15.02 ±0.96	6.16 ±0.76	9.23 ±0.68

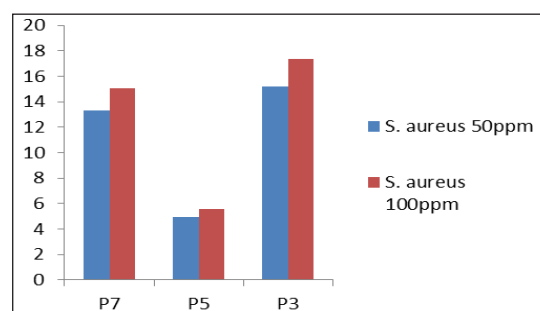


Figure 1. Zone of Inhibition against S. typhi

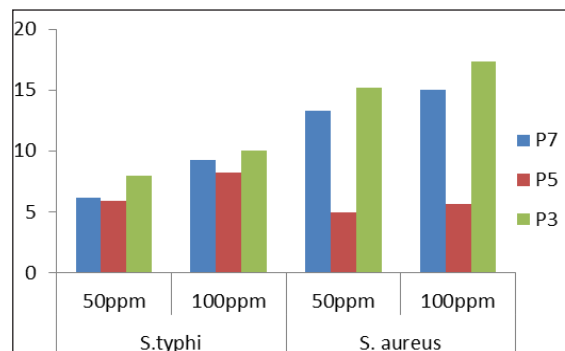


Figure 2. Zone of Inhibition against S. aureus

Phytochemical Screening

Phytochemicals viz. tannins, saponins, flavonoids, steroids, carotenoids, terpenoids, phenol, alkaloids, phlobatannins, and glycosides were identified qualitatively by applying the standard techniques prescribed by the scientists. Extracts were made in two solvents Distilled water and Ethanol.

Test of Tannins

KOH test was performed for the determination of tannins by Evans et al., 2002 method.

Test of Saponins

Froth test was done for detection of saponins by (Evan et al. 2012).

Test of Flavonoids

Method described by Trease and Evans. (1989).

Test of Steroids

H₂SO₄ test was performed described by Idu and Igeleke 2012, Trease and Evan 1983).

Test of Terpenoids

Method described by Harbone (1973).

Test of Carotenoids

Detection of carotenoids was performed by method described by Kudva et al. (1998).

Test of Phenol

Identification of phenol was performed by ferric test defined by Sofowora (1993).

Test of Alkaloids

Mayer's reagent test was done described by (Idu and Igeleke 2012).

Test of phlobatannins

Determination of phlobatannins was performed by test described by Sofowora (1993).

Test of Glycosides

Identification of glycosides was performed by method defined by Sofowora (1993).

Data Analysis

Qualitative and quantitative were used for data analysis. Standard deviations were applied for analysis

Results and Discussion

Antibacterial Activity

Antibacterial activity of three clones of tea i.e. P3, P5, P7 available at NTHRI, Mansehra was determined by using the agar diffusion method. Their antibacterial activity was determined against two bacterial strains *S. aureus* and *S. typhi*. 100 ppm and 50 ppm concentrations were made. P3 showed highest antimicrobial activity against both bacterial strains i.e 15.1±0.84 in 50 ppm and 17.3±0.62 in 100 ppm against *S. aureus*, while 7.9±0.32 in 50 ppm and 10.06±0.6 in 100 ppm against *S. typhi* (Figure 6). P7 also showed maximum activity against both strains i.e 13.3±1.53 in 50 ppm, 15.02±0.96 in 100 ppm against *S. aureus* and 6.16±0.76 in 50 ppm, 9.23±0.68 in 100 ppm against *S. typhi* (Figure 4) and the low activity against both strains were observed in P5 i.e. 4.9±1.10 in 50 ppm, 5.6±0.36 in 100 ppm against *S. aureus* and 6.16±0.76 in 50 ppm 9.23±0.68 in 100 ppm against *S. typhi* (Figure 5).

Phytochemistry of Processed Black Tea

Epidemiological studies suggest a reduction in the risk of chronic diseases, such as colon cancer, in populations with high consumption of plant foods containing phytochemicals (Liu, 2007). Various analytical techniques were applied for phytochemical analysis to identify tannins, saponins, flavonoids, steroids, carotenoids, terpenoids, phenol, alkaloids, phlobatannins, and glycosides. Extracts were prepared in two solvents i.e. distilled water and Ethanol. In aqueous extracts all phytochemicals were seen positive as shown in the Table 2.

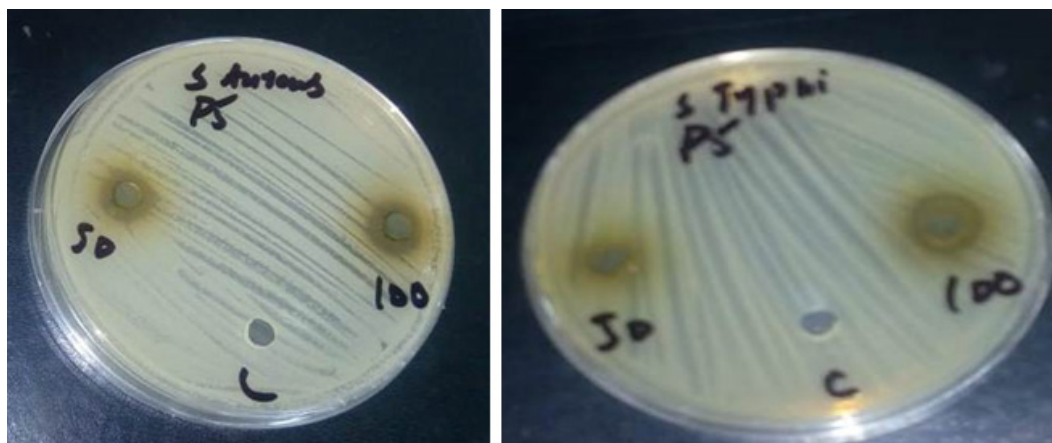


Figure 4. Zone of Inhibition of P7 Clone against *S. typhi* and *S. aureus*



Figure 5. Zone of Inhibition of P5 Clone against *S. typhi* and *S. aureus*

Table 2. Aqueous Extracts of tea varieties

Tests	P3(eth)	P5(eth)	P9(eth)
Tannins	++	++	++
Saponins	--	+	--
Flavonoids	++	++	++
Steroids	--	--	--
Carotenoids	+	+	+
Terpenoids	++	++	++
Phenol	++	++	++
Alkaloids	++	++	++
Phlobatannins	--	--	--
Glycosides	++	++	++

Table 3. Ethanolic Extracts of tea varieties

Tests	P3(dw)	P5(dw)	P9(dw)
Tannins	++	++	++
Saponins	++	+	++
Flavonoids	++	++	++
Steroids	+	++	++
Carotenoids	+	+	+
Terpenoids	++	++	++
Phenol	++	++	++
Alkaloids	++	++	++
Phlobatannins	+	+	+
Glycosides	+	+	+

++ = present, -- = absent

Ethanolic extracts of tea samples showed the presence of phytochemicals i.e. tannins, terpenoids, flavonoids, alkaloids, phenols, and glycosides (Table 3). Saponins were found positive in P5 variety while seen absent in P3 and P9. Tea saponin is a natural non-ionic surfactant. It not only possesses good emulsifying, separating, and dispersing

capability but is also good at foaming and forming foam stabilizer having good cleaning capacity (Wu and Raven, 1998). Phlobatannins and steroids were found absent in all varieties in ethanolic extracts while seen positive in all varieties in aqueous extracts.

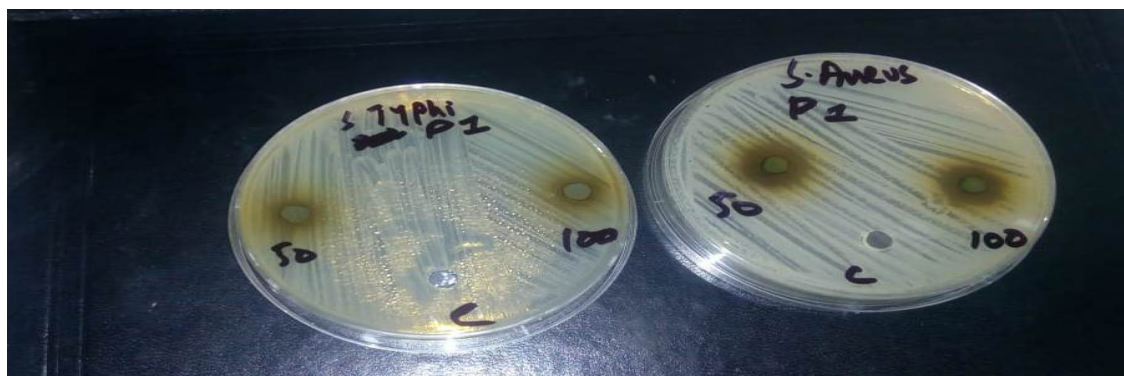


Figure 3. Zone of Inhibition of P7 Clone against *S. typhi* and *S. aureus*

Conclusion

The experimental data indicates the presence of essential phytochemicals like alkaloids, flavonoids, phlobatannins, glycosides, carotenoids, saponins, phenols, and terpenoids in processed black tea. It serves as a valuable starting material for drug and development. The presence of phytochemicals possesses an important source of the pharmacological effect that acts as new anti-infection, antioxidant, and anticancer agents. P3 showed the highest antimicrobial activity against both bacterial strains P7 also showed maximum activity against both strains and low activity against both strains were observed in P5. Hence processed black tea has antimicrobial potential. Our findings suggest that consumption of these varieties could be valuable to promote consumer health and also serve as a good source of energy and nutrients (Figure 3).

Abbreviations

MIC: Minimum inhibitory concentration

S. aureus: Staphylococcus Aureus

S. typhi: Salmonella typhi

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Availability of Data and Materials

The datasets supporting the results are included within the article

Authors' Contributions

All authors have contributed significantly to the conception and design of the study, the interpretation of data, and the drafting and revision of the manuscript. All authors read

and approved the final manuscript.

Ethics Approval and Consent to Participate

The manuscript does not contain studies involving human participants, human data, or human tissue. adore

Consent for Publication

The authors declare that the work has consent for publication

Competing Interests

The authors declare that they have no competing interests.

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