Chemical Characterization and Antibacterial Activity of Black Tea Extract Components

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Summary:

Background: *Aqueous extracts of black tea exhibited antimicrobial activity against wide range of bacteria.*
Aim of study: *In this investigation the antimicrobially active components of this extract were identified and characterized. The minimal inhibitory concentration (MIC) for each one was determined.*
Subject & Methods: *the active components of the aqueous extract were identified and characterized. The minimal inhibitory concentration (MIC) for each was determined. Tannic acid, theophylline, caffeine and theobromine were isolated by thin layer chromatograhy (TLC) and purified by silica gel column. MIC was assessed by using agar dilution method.*
Results: *Broad spectrum activity of three components excluding theobromine against gram-negative and selective gram-positive organisms was observed. Tannic acid showed the greatest potency with MIC of 160 /ig/ml. whereas the MIC of theophylline and caffeine was 2.5 mg/ml and 10 mg/ml respectively.*

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Introduction

Thea or tea consists of the prepared leaves and leaf buds of the plant *Cornelia sinensis* (L.) O.Kuntze (Family Theaceae); a shrub or tree with alternate evergreen leaves. The generic name is from the Greek meaning goddness; *sinensis* refers to its Chinese origin. There are many types of tea available, the most common are the green tea which is grown and prepared in China and Japan, and the black tea which is grown in Ceylon and India

(Bently, 1957). In general, tea is used mainly as a bevarage containing (30-60) mg of caffein in a normal cup (Bentley,1965).

Antimicrobial activity of black tea extract, against wide range of both gram positive and negative bacteria was reported (Kitamura *etal,* 1990, Okubo *et al.,* 1991, Toda *et al.,* 1991 a,b, Nakahara *et* a/.,1993,Iwata *et al,* 1997). In previous studies in our laboratory, various concentrations of tea extracts were tested ranging from 5 to 500 mg/ml. MIC for each susceptible bacteria was determined (Ammash *et al.,* 1993).

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Tea constitutes of several pharmaceutically important compounds including caffeine (theine 1-4%) which induce stimulating action, adenine, theobromine, theophylline (1,3 - dimethylxanthine) which is a smooth muscle relaxant and xanthine. In addition to gallotannic acid (15%),yellow volatile oil (0.75%) and tannin which induce astringent properties.

In this investigation the antimicrobially active components of tea extracts were identified and characterized. The MIC for each one was determined.

Materials and Methods

Preparation of tea extract

Three types of commercial Ceylon tea leaves were mixed. A concentration of 50% was prepared by soaking 50 gm of dry tea in 100ml distilled water. The mixture was then boiled for two minutes. The extract was lyophilized for chemical analysis and antimicrobial activity.

Chemical analysis

Chromatographic analysis was performed as mentioned by (Kennethy, 1961, Hawarth,1963). Separation and isolation of the major active constituents of tea extract was done by ascending thin layer chromatography (TLC) using Kisel gel Gf (254) (type 6), Merek, West Germany.

Different developing solvents were tried but the most efficient system was chloroform, methanol, acetone, acetic acid (16: 12: 1: 2). Components were observed for their fluorescence or quenching by irradiation with U. V. light using an original hanan fluo test lamp, Heracus, West Germany or by

reactivity with iodine vapour.

Preparative thin layer chromatography was applied using silica gel Gf (234) 30 gm mixed with 130 ml distilled water to make a slurry which was spreed out in a layer of 0.5mm thick on a plate 20*20 cm. The same developing system was used as mentioned above in TLC. Separated bands were observed for their fluorescence or quenching as previously mentioned. The usual work-up was to scrap off the individual bands. The components were extracted by shaking the adsorbent for 30 minutes with mixture of hot water: ethanol(2:l). The mixture was then filtered and the filtrate was evaporated to dryness.

The components were purified by column chromatography using silica gel (60) Fluka and the column was eluted with the same solvent as mentioned in TLC.

Characterization of the main active constituents were performed as follows: Ultra-violet absorption spectra were recorded with a pye-Unicam SP 8-200 spectrophotometer within the range of (200-350 nm) in water. Two studies were done at two time intervals (June 1995 and December 1998) to test the stability of the separated components. Infra-Red (IR) spectroscopy was determined using pye-Unicam grating infra-Red spectrophotometer model PA - 4706 in nujol mulls. Melting points (Stuart apparatus) were determined in open - end capillaries. Elemental micro-analysis was performed by determining the CHN of the constituents.

Bacterial susceptibility

The four major tea extract components; tannic acid, theophylline, caffeine and theobromine, were studied to detect their activity against pathogenic bacteria. The susceptibility of *Salmonella typhi, Shigella dysentriae, Proteus mirabilis, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus* to each component of the extract was tested by using the agar diffusion method (Ericsson *et al.*, 1960)

Determination of MIC

Minimal inhibitory concentration (MIC) was determined by the two fold serial agar dilution method (Lannette, *et al.*, 1985). An overnight culture grown in brain heart infusion broth was diluted 100 times. About 10^4 colony forming unit (CFU) were inoculated onto a drug-containing Mueller-Hinton agar surface. Plates were incubated at 37°C and the MIC was evaluated after (18-24) hours of incubation. The MIC was determined as the lowest concentration that prevented growth of microorganisms.

Results and Discussion

The examination of the crude tea extract revealed the presence of four major components^. Their fluorescence or quenching under screened U.V. light and reaction with iodine vapour indicated that they were major in size and intensity. These components were lately identified as caffeine, theobromine, theophylline and tannic acid (tannin) with retarding factor (RF) values of 0.41, 0.59, 0.6 and 0.81 respectively, figure (1) They were identical to the authentic samples in their RF values and color reaction with iodine. Table (1) shows the physical properties of these components.

Table (2) and figure (2) show the U.V. spectra of each component of the tea extract at wavelength ranged between (274-272nm). Theophylline showed the highest absorbency (0.638) followed by caffeine (0.437), then theobromine

(0.429) and finally tannic acid (0.167). The same sequence was noticed at wavelength ranged between (203-206). The intensity of absorbency decreases according to the same manner. The results were 1.583, 1.164,1.044 and 0.565 respectivelly. The influence of time on the stability of the components indicated that their U. V. absorption spectroscopy was stable when tested at two time intervals.

Figure (3) shows the relationship between the wavenumber (CM^{-1}) and the percentage of transmittance of each component of the tea extract. The transmittance was monitored for wavenumbers ranging from (200-4000) CM^{-1} .

Table (3) shows the IR characteristic bands (CM^{-1}) of the four components. Weak (N-H) streching vibration resulted in the analysis of caffeine and theophylline at wavenumber (3550), while broad (N-H) or (OH) streching vibration resulted from theobromine between (3520-3380) and broad (OH) streching vibration of phenolic group indicating (H) bonding, between (3480-3120). All components (except tannic acid) showed(C=C-H) streching vibration at wavenumber ranging between (3080-3090), and (C=N) between (1680-1535). All four components showed (C=C) and (C=O) streching vibrations.

Table (4) shows the elemental micro-analysis of theobromine, caffeine and theophylline. No significant differences resulted between the calculated and the found values of (CHN). The molecular weight of each component was found to be 180.17,194.19 and 180.17 respectively.

The MIC of these four components against eight pathogenic bacterial species are shown in table (5). Theobromine was the only tested component which shows no activity. On the contrary, tannic acid was the strongest. Inhibition of *S. typhi, S.* dysenteriae, P. mirabilis, P. aemginosa and S. aureus was achieved by 160 //g/ml. Theophylline inhibited *S. aureus* with MIC of 2500 // g/ml. Caffeine was the least active among those three components. The MIC required

against *S. aureus* was 10.000 //g/ml, and that required for gram negative bacteria was 20.000 // g/ml.

Recent studies have reported antimicrobial activities of aqueous extracts of both black and green tea. This activity was attributed to epigallocatechin, epigallocatechin gallate and epicatechin gallate in green tea. In black tea extracts, theoflavin and its gallates were found to have such activity (Yam *et al.,* 1997). Polyphenol compounds, mainely catechin derivatives were also found to inhibit the cell growth of mutans streptococci (Kitamura *et al,* 1990), methicillin resistant *S. aureus* (Toda *et al,* 1991a) and *Vibrio cholera* 01 (Toda ef a/., 1991b).

Our results suggest that black tea or some of its components mainly tannic acid or theophylline can be used as prophylactic agents against wide range of bacterial infection. Further investigations are required to determine the mechanism of action of each active constituent of the tea which is responsible for the induction of growth inhibition of these bacteria.

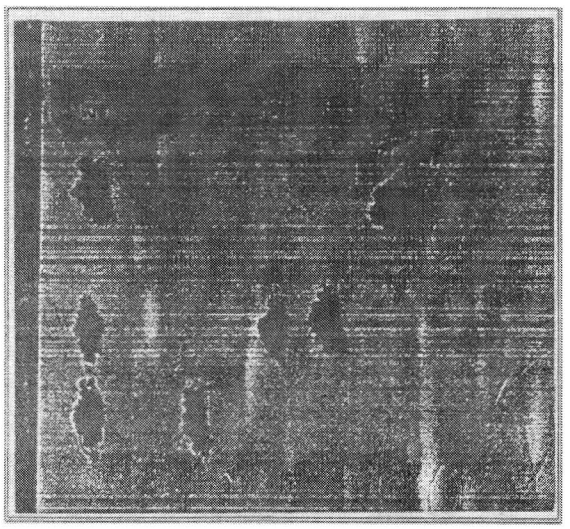

Figure (1): Thin layer chromatography of the major components of tea extract.

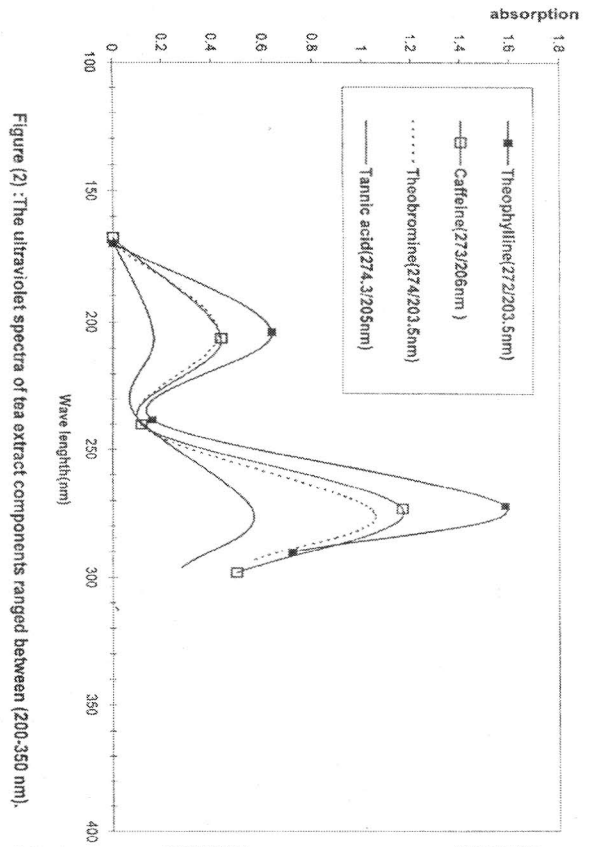

Figure (2) :The ultraviolet spectra of tea extract components ranged between (200-350 nm).

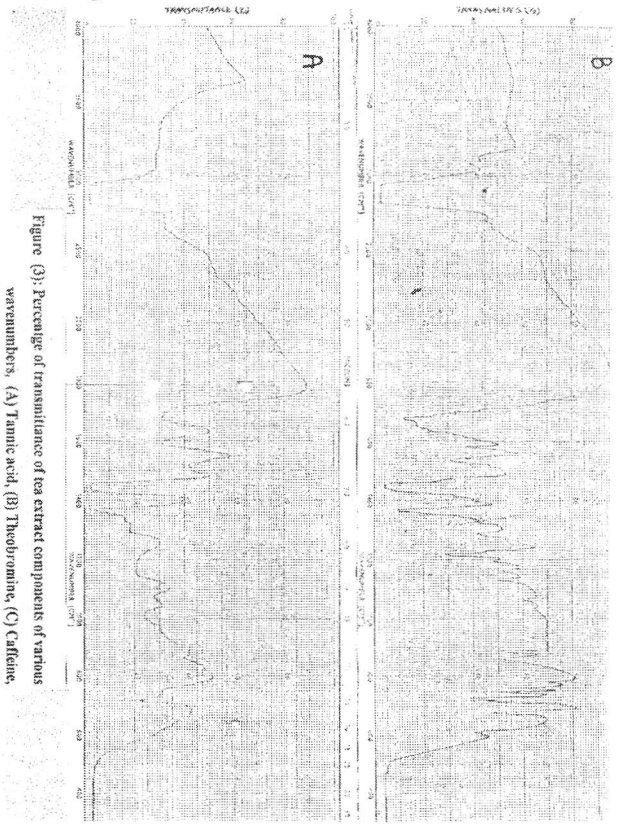

Figure (3): Percentage of transmittance of tea extract components of various wavenumbers. (A) Tannic acid, (B) Theobromine, (C) Caffeine.

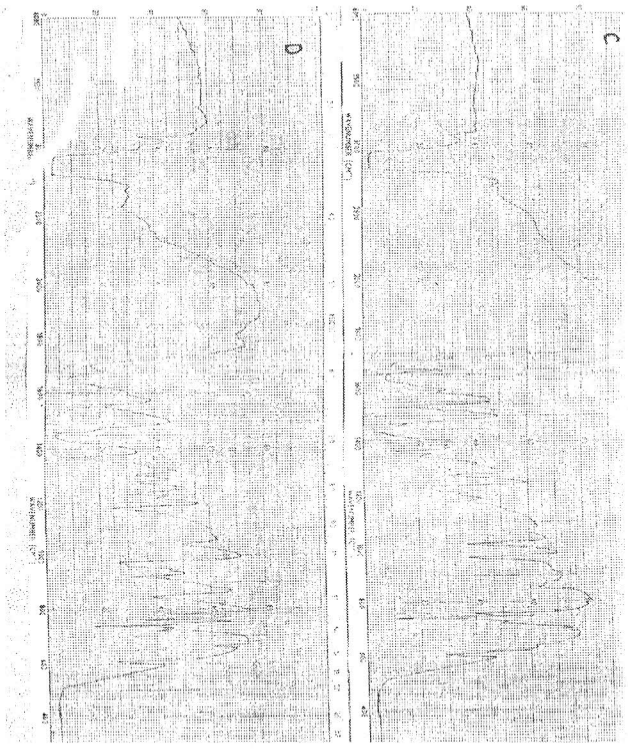

Table 1: The physical properties of the major active components of tea extract.

Components of tea extract[a]	Color	Appearance	Melting point
Tannic acid	Yellow to brown	Residue	Decomposed at (205-210) °C
Theobromine	White	Precipitate	(358-360) °C from water
Caffeine	White	Crystals	278 °C from water
Theophylline	White	Precipitate	280 °C from water

a. Ascending TLC was performed. The solvent system was chloroform, methanol, acetone and acetic acid (16: 12: 1: 2)

Table (2): Ultraviolet spectrophotometer of the isolated components of tea extract.

Tea extract components[a]	Wave length (nm)	Absorbance
Tannic acid	205	0.565
	274.3	0.167
Theobromine	203.5	1.044
	274	0.429
Caffeine	206	1.164
	273	0.437
Theophylline	203.5	1.583
	272	0.638

a. Seperation and isolation of tea extract components was performed by ascending TLC then they were purified by silica gel column.

Table (3): Infra-red spectrophotometry of the isolated components of tea extract.

Tea extract components[a]	Wavenumber (CM^{-1})	Characteristic band
Tannic acid	3550	Weak N-H stretching vibration
	3080	C = C-H
	1680	C = 0
	1635-1585	C = C and C = N stretching vibration
Theobromine	3520-3380	Broad N-H or OH stretching vibration
	3080	C = C-H
	1675	C = 0
	1680-1535	C = C and C = N stretching vibration
Caffeine	3550	Weak N-H stretching vibration
	3080	C = C-H
	1680	C = 0
	1635-1585	C = C and C = N stretching vibration
Theophylline	3550	Weak N-H stretching vibration
	3090	C = C-H
	1695	C = 0
	1650-1550	C = C and C = N stretching vibration

a. The samples were prepared as nujol pulls.

Table (4): Elemental micro- analysis of active components of tea extract.

Tea extract components	Chemical formula	CHN analysis		Molecular weight
		calculate	found	
Theobromine	$C_7H_8N_4O_2$	$C_{46.66}$ $H_{4.48}$ $N_{31.1}$	$C_{46.73}$ $H_{4.51}$ $N_{31.65}$	180.7
Caffeine	$C_8H_{10}N_4O_2$	$C_{49.48}$ $H_{5.19}$ $N_{28.85}$	$C_{49.31}$ $H_{5.1}$ $N_{28.7}$	194.19
Theophylline	$C_7H_8N_4O_2$	$C_{46.66}$ $H_{4.48}$ $N_{31.1}$	$C_{46.35}$ $H_{4.51}$ $N_{31.5}$	180.17

Table (5): Minimal inhibitory concentration of four components of tea extract.

Bacteria[a]	MIC (μg/ml) of the components[b]			
	Tannic acid	Theophylline	Caffeine	Theobromine
Enterobacter aerogenes	320	5.000	20.000	No inhibition
Escherichia coli	320	5.000	20.000	No inhibition
Klebsiella pneumonia	320	5.000	20.000	No inhibition
Proteus mirabilis	160	5.000	20.000	No inhibition
Pseudomonas aeruginosa	160	5.000	20.000	No inhibition
Salmonella typhi	160	5.000	20.000	No inhibition
Shigella dysentriae	160	5.000	20.000	No inhibition
Staphylococcus aureus	160	2.500	10.000	No inhibition

a. 10^4 CFU of overnight culture were grown in drug containing Mueller- Hinton agar for (18-24) hours at 37°C.

b. MIC was determined as the lowest concentration that prevented growth.

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