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Full Length Research Paper

Protective role of gum arabic on modulation of indomethacin systemic toxicity

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This study was conducted to screen for the antimicrobial potency of the leaves of fermented and non-fermented *Camellia sinensis* on some pathogenic clinical isolates, notably: *coli*,*Pseudomonas aeruginosa*, *Klebsiella* spp., *Staphylococcus aureus, Streptococcus* spp. and *Candida albicans*; however, the major chemical compounds responsible for the activities was determined. The agar diffusion method was used to determine the inhibitory action of the leaves extracts on the tested pathogenic microorganisms. The Minimum Inhibitory Concentration (MIC) exhibited by the extracts against the isolates ranged between 3.125 and 6.25 mg/ml respectively. The zones of inhibition exerted by the extracts was significant ($P<0.05$) against the clinical isolates which ranged between 11.4 and 13.5 mm. The results revealed that the inhibitory activities displayed by these leaves extracts could be due to the presence of the phytochemical compounds found in the plant extracts.

Key words: Inhibition, phytochemical compounds, *Camellia senensis*, pathogenic, isolates.

INTRODUCTION

The development of resistance to multiple antibiotics among disease causing bacteria prompted many researchers to find new sources of non-antibiotic drugs, mainly plant extracts, in order to overcome the menace of antibiotic resistance (Davies, 1994; Marchase and Schito, 2001). Tea is the second commonly drunk liquid after water and the most common beverage drink (Okubo et al., 1989). Tea, the drink, is an infusion of various processed leaves and flowers of one of an evergreen shrub botanically called *Camelia sinensis*. It is cultivated in more than 30 countries worldwide and of the total amount of tea produced and consumed in the world, 78% is black, 20% is green and 2% is ooling (Graham, 1992; Khan et al., 2007). Tea is consumed everyday by billions of people worldwide demonstrating its safety (Kirk and Othmer, 1980). Black tea is consumed primarily in western countries and in South Asia such as India, Sri Lanka and Africa. Whereas green tea and ooling tea are consumed mainly in East Asian Countries like Japan, Taiwan and Africa (Khan et al., 2007). Chemical composition of *C. sinensis* is not completely known but it is understood to be quite complex. Black tea is more complex than green tea partly because of the oxidation process that occurs during fermentation (Hamilton-Miller, 1995). Tea contains many different compounds that grant it health-promoting properties including group of polyphenol compound called flavanoids. The flavanoids

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are made up of monolayers called catechin, natural plant antioxidant commonly found in tea (the flavins are the result of fermentation and they appear only in black tea) (Dufrence and Farnworth, 2011). Green tea comprised mainly of epigallocatechin, epicatechin gallate and epigallocatechin galate (Shimuzu et al., 2008). Many of green tea health promoting abilities are attributed to epigallocatechin gallate (Khan, 2007). Other compounds found in tea include caffeine, quinine and gallic acid (Dufrence and Farnworth, 2011).

C. sinensis has been used for different purposes which could be classified as: edible use, medicinal use and other uses which include dye, essential oil, food flavouring and perfumery (Samman et al., 2001). It has been reported to have physiological and pharmacological effect (Stagg and Millin, 1975; Min et al., 1991) such as strengthening of capillary, slowing down the catabolism of catecholamine, and exerting anti-inflammatory effects by enhancing the effect of vitamin C. It acts as an antioxidant and inhibits angiotensin converting enzymes and growth of implanted malignant cells (Hattori et al., 1990).

One of the earliest reports of *C. sinensis* regarding its antimicrobial activity is its recommendation by an Army surgeon in soldiers' water bottles as prophylactic against typhoid (McNaught, 1906). There are several reports on the antimicrobial effects of tea *in vitro* and *in vivo* mainly in intestinal pathogens (Das, 1962; Ryu, 1980; Scalbert, 1991). The need to search for other alternative sources of antimicrobial agents apart from the conventional antibiotic due to the ever increasing bacterial resistance necessitates this study.

METHODOLOGY

Plant material

In this study, the plant material used are leaves of *C. sinensis* commonly called Ahadar (green tea) in Arabic and Ahamar (black tea) in Arabic.

Sample collection

The dried leaves of black and green tea were purchased from the Maiduguri Monday market, Borno State, in a clean polyethene bag and transported to the herbarium of the Department of Biological Sciences, University of Maiduguri, for identification and authentication by a botanist.

Test organisms

Organisms used for the antimicrobial activity of black and green tea were obtained from different clinical pathogenic isolates notably: *Candida albicans*, *Escherichia coli, Staphylococcus aureus, Klebsiella* spp., *Pseudomonas aeruginosa* and *Streptococcus* spp. which were isolated from throat swab, *Pseudomonas aeruginosa* and *Klebsiella* spp. which were isolated from a wound swab, and *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* which were isolated from urine samples respectively.

The clinical samples were obtained from the Department of Medical Microbiology and Parasitology, University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria. Clinical isolates were sub cultured onto Nutrient and MacConkey agar medium respectively for purity and were maintained on nutrient agar slants at 4°C in the refrigerator until they were needed for use.

Sample preparation and extraction

About 300 g of dried leaves of the plant *C. sinensis* (black and green tea) each was added to 1500 ml of sterile distilled water in order to obtain the aqueous extracts (Spiro, 1995). To both black and green tea, the extraction was carried out at room temperature for 24 h. It was dried using the rotary evaporator at 50°C. For the ethanol extractions, 800 ml of ethanol was added to each 300 g of the dried leaves of *C. sinensis* (black and green tea) using Soxhlet method of extraction (Colle et al., 1989), and then dried in a rotary evaporator at 50°C. The stock solution of the extracts was then sterilized by filtration through Millipore membrane filter of 0.45 µm pore size. The sterile extract obtained was then stored in sterile capped flask and refrigerated at 4°C until use (Colle et al., 1989).

Sterility proofing of the extract

Both extracts of black and green tea of their respective ethanol and aqueous extracts were tested for sterility after Millipore filtration by introducing 2 ml of the sterile extract into 10 ml of sterile nutrient broth and were incubated at 37°C for 24 h. Sterile extract was confirmed by the absence of turbidity and clearness of broth after the incubation period.

Standardization of the bacterial cell suspension

To each test organism, five colonies were picked into sterile test tubes containing sterile nutrient broth and incubated at 37°C for 24 h. The turbidity produced by the organism was adjusted and used to match the turbidity standard prepared as described by Monica (1984).

Determination of minimum inhibitory concentration (MIC) of the extracts of black and green tea on the test organisms

Using the agar dilution method of Chung et al. (2004) and Chan et al. (2010), the minimum inhibitory concentrations

of the extracts was determined. The extracts were introduced into the growth medium at concentrations of 50, 25, 12.5, 6.25 and 3.125 mg/ml into wells bored using a cork borer of 8 mm in diameter. After 30 min of inoculation and introduction of extracts unto plates, it was allowed to diffuse and the plates were incubated at 37°C for 24 h. The minimum inhibitory concentrations were taken as the lowest concentrations of the plant extracts (Ghobasky, 1988).

Determination of zone of inhibition

About 15 ml of sterile nutrient agar was poured into each sterile Petri dish of equal size and Chocolate agar medium was used for *Streptococcus* spp. and these were allowed to solidify. The surface of this sterile nutrient and chocolate agar plates was streaked with the pure culture of the standardized bacterial cell suspension using a cork borer (8 mm in diameter) which was sterilized by flaming and was used to create a well at the centre of the plate. The well created was then filled with plant extracts, after which the plant was allowed to stand for 1 h for pre diffusion of the extract (Monica, 1984) and incubated at 37°C for 24 h. At the end of the incubation period, the diameter of the zone of inhibition was measured in millimeters using a meter rule (Mackeen et al., 1997).

Phytochemical analysis of the leaf extracts of black and green tea

The phytochemical screening of the leaves extracts of black and green comprising: flavonoids, alkaloids, saponins, phenol, glycosides, tannins, steroids, terpenoids and anthraquinones, was carried out using the method described by Sofowora (1993), Trease and Evans (1989, 2002) and Vishnoi (1979).

Test for flavonoids

Sodium hydroxide was used to test for flavonoids. 5 g sample of each extract was dissolved in water and filtered. To this sample, 2 ml of the dissolved extract was added to 10% aqueous sodium hydroxide which gives a yellow colouration. Change in colour from yellow to colourless on addition of dilute hydrochloric acid indicates the presence of flavonoids (Trease and Evans, 1989).

Test for alkaloids

About 2 ml of the extract was measured into a test tube to which a picric acid solution was added. The formation of orange colouration indicates the presence of alkaloids (Sofowora, 1993).

Test for saponins

About 1 g of each extract was boiled with 5 ml of distilled water, filtered and the filtrate was divided into 2 portions. To the first portion, about 3 ml of distilled water was added and shaken well for about 5 min; however, frothing which persist on warming indicates the presence of saponins (Sofowora, 1993).

Test for phenol

A total of 25 ml of extract was added to 2 ml ferric chloride solution, and a deep bluish green solution was formed which indicated the presence of phenol (Trease and Evans, 1989).

Test for glycosides

A total of 25 ml of 1 Molar Sulphuric acid was added to 5 ml of the extract in a test tube and boiled for 15 min, after which it was allowed to cool down and neutralized with 10% sodium hydroxide and then 5 ml of Fehling's solution A and B was added to it. A brick red precipitate of reducing sugars was formed thereby indicating the presence of glycosides (Vishnoi, 1979).

Test for tannins

About 3 g of the sample was boiled with 50 ml of distilled water for 30 min on a hot plate, after which the mixture was then filtered and the resulting filtrate was used. A portion of aqueous extract was diluted with distilled water in ratio 1:4 and few drops of ferric chloride were added. A blue or green colouration was formed indicating the presence of tannins (Trease and Evans, 2002).

Test for steroids

About 2 ml of acetic anhydride was added to 0.5 g of the extracts with 2 ml of Sulphuric acid. A colour change from violet to blue was formed indicating the presence of steroids (Sofowora, 1993).

Test for anthraquinones

The Borntrager's method was used for this test. 0.5 g of the sample was shaken in 10 ml of aqueous Sulphuric acid and then filtered. To the filtrate, 5 ml of benzene was added. The benzene layer was separated and half its own volume of 10% ammonia solution was added. The presence of pink, red or violet colouration in the ammoniac phase was taken as an indication of combined Anthraquinones (Trease and Evans, 2002).

Statistical analysis

The results obtained from these findings were subjected to ANOVA (Analysis of Variance) by Instant Graph Pad version 3.0 using randomized block design.

RESULTS AND DISCUSSION

The extracts of black and green tea possessed antimicrobial activities against the tested clinical pathogenic isolates and in some parts of the world, have been considered to have health promoting potentials (Johnson et al., 2012).

The results in this study showed that the ethanol black tea extract exhibited significant zone of ($P<0.05$) activity ranging from 11.4 to 13.5 mm and the aqueous extract ranging from 12.1 to 13.3 mm respectively. The ethanol black tea extract indicates significant ($P<0.05$) zone of inhibition with *Staphylococcus aureus* exerting the widest zone of inhibition of 13.5 mm, followed by *Klebsiella* spp. (12.9 mm), *Escherichia coli* (12.5 mm), *Pseudomonas aeruginosa* (12.2 mm), *Candida albicans* with 11.4 mm and the lowest potential activity was shown on *Streptococcus* spp. (10.5 mm) respectively (Table 1). The aqueous extract of black tea also exhibited significantly ($P<0.05$) wider zone of inhibition against all the clinical isolates compared to the ethanol black tea extracts on *Staphylococcus aureus* with 13.3 mm, followed by *Klebsiella* spp. (13.0 mm), *Escherichia coli* (12.6 mm), *Candida albicans* (12.1 mm) and *Pseudomonas aeruginosa* (12.1 mm) respectively (Table 2). The lowest inhibitory activity was observed on *Streptococcus* spp. with 12.0 mm zone of inhibition. This is in conformity with the fact that few studies have focused on the evaluation of antimicrobial activity of black tea, while several scientific studies have been conducted using green tea (Shimuzu et al., 2008).

Similarly, Attikh et al. (2013) reported in their study conducted on antibacterial and antifungal activities of black and green kombucha teas that the resulting kombucha antibacterial/antifungal activities against some pathogenic microorganisms, including human pathogenic bacteria and clinical *Candida* species, showed interesting antimicrobial potentials of both experimented kombucha teas against the tested microorganisms except *Candida krusei*. The green fermented tea exhibited the highest antimicrobial potential. Indeed, it showed large inhibition zones against *Staphylococcus epidermidis* (22 mm), *Listeria monocytogenes* (22 mm) and *Micrococcus luteus* (21.5 mm). Furthermore, interesting anti-*Candida* potential was revealed by the reaction of green tea kombucha against *Candida parapsilosis*. Moreover, the Minimum Inhibitory Concentration (MIC) of the aqueous and ethanol black tea extracts for the different clinical pathogenic isolates ranged between 3.125 and 6.25 mg/ml (Table 3).

The aqueous and ethanol extracts of green tea also showed appreciable significant ($P<0.05$) zone of inhibition ranging from 12.4 to 12.8 mm for ethanol extract and 11.8 to 12.5 mm for aqueous green tea extracts.

This study indicates that green tea extracts showed moderate zones of inhibitory effects compared to the black tea extracts. For the aqueous green tea extracts, *Candida albicans* showed the highest zone of inhibition of 12.5 mm, followed by *Klebsiella* spp. with 12.2 mm, *Pseudomonas aeruginosa* with 12.1 mm, *Escherichia coli* with 11.8 mm, *Staphylococcus aureus* with 11.7 mm and *Streptococcus* spp. 11.5 mm respectively. The ethanol green tea extracts also exhibited strong inhibitory activities against all the tested isolates when compared to the aqueous green tea extracts with *Klebsiella* spp. showing the highest zone of inhibition of 12.8 mm, followed by *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa,* all with the same zone of inhibition of 12.7 mm, and then *Candida albicans* (12.4 mm) and *Streptococcus* spp. with the lowest zone of inhibition respectively. This is in line with the study conducted by Peter et al. (2005) and Mckay and Blumberg (2002), who reported that green tea has been shown to have several antibacterial activities limiting bacterial growth and acting in synergy with β-lactam antibiotics. Sub inhibitory concentrations of green tea showed marked increase in the sensitivity of even the multiple resistant isolates to most of the antibiotics tested.

Olosunde et al. (2012) reported in their study on antibacterial activity of green tea (*Camellia sinensis*) extracts against various bacteria isolated from environmental sources that all the extracts were tested for antibacterial activity by disc diffusion method. Antibacterial assay was performed at $10<1$, $20<1$, and $30<1$ concentrations. Significant antibacterial activity was reported for all extracts with results. Aqueous extracts have shown little antibacterial activity against six bacteria isolated. Maximum antibacterial activity was found in methanolic extracts. In addition, the research by Johnson et al. (2012) indicates that green tea has numerous health benefits including the ability to kill oral bacteria and prevent cavities.

The Minimum Inhibitory Concentration (MIC) for the aqueous and ethanol green tea extracts against the different clinical pathogenic isolates also ranged between 3.125 and 6.25 mg/ml respectively (Table 4).

The phytochemical screening of black and green tea aqueous and ethanol extracts were also determined in this study (Tables 5 and 6). Phytochemical results of the tea extracts revealed the presence of flavonoids, alkaloids, saponins, tannins, steroids and terpenoids respectively. These phytochemical compounds exert antimicrobial activity through various mechanisms. These secondary metabolites are known to be biologically active and therefore play significant roles in bioactivity of medicinal plants because the medicinal values of medicinal plant lie in these phytochemical compounds which produced a definite and specific action on the human body. This finding is in agreement with the findings that the phytochemical screening of black tea and green tea has revealed the presence of saponins, polyphenol compounds (catechins) aflavins, arubigins,

Table 1. Antimicrobial activities of *black* and *green tea* ethanol extracts on selected clinical pathogenic isolates.

Test organisms	Zone of inhibition (mm)	
	Black tea (mm)	Green tea (mm)
Escherichia coli	12.5	12.7
Pseudomonas aeruginosa	12.2	12.7
Klebsiella spp.	12.9	12.8
Staphylococcus aureus	13.5	12.7
Streptococcus spp.	10.5	12.0
Candida albicans	11.4	12.4

Table 2. Antimicrobial activities of *black* and *green tea* aqueous extracts on selected clinical pathogenic isolates.

Test organisms	Zone of Inhibition (mm)	
	Black tea (mm)	Green tea (mm)
Escherichia coli	12.6	11.8
Pseudomonas aeruginosa	12.1	12.1
Klebsiella spp.	13.0	12.2
Staphylococcus aureus	13.3	11.7
Streptococcus spp.	12.0	11.5
Candida albicans	12.1	12.5

Table 3. Minimum inhibitory concentration (MIC) mg/ml of *black tea* ethanol and aqueous extracts on selected clinical pathogenic isolates.

Solvents (mg/ml)	Test organisms					
	E. coli	***P. aeruginosa***	***Klebsiella* spp.**	***S. aureus***	***Strept.spp.***	***C. albicans***
Ethanol extracts	3.125	3.125	3.125	3.125	3.125	6.25
Aqueous extracts	6.25	6.25	3. 125	3.125	3. 125	3.125

Table 4. Minimum inhibitory concentration (MIC) mg/ml of *green tea* ethanol and aqueous extracts on selected clinical pathogenic isolates.

Solvents (mg/ml)	Test organisms					
	E. coli	***P. aeruginosa***	***Klebsiella* spp.**	***S. aureus***	***Strept.* spp.**	***C. albicans***
Ethanol extracts	3.125	3.125	6.25	3.125	3.125	3.125
Aqueous extracts	3.125	6.25	3. 125	12.5	6.25	3.125

containing a trace of protein or not containing a trace of protein, amino acid, fibre and others to include: carbohydrates, pigments, minerals, phenolic compounds and lipids (Higdon and Frei, 2003).

Flavonoids constituent exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cystostatic antioxidant properties, and anti-cancer activities (Stagg and Millin, 1975; Hamilto-Miller, 1995). Furthermore, Nihal and Hassan (1999) reported earlier that antimicrobial activities are associated with the presence of tannins and flavonoids in black and green tea. Tannins have been found to form irreversible complex with proline-rich protein (Shimamura et al., 1990) resulting in the inhibition of cell protein synthesis.

Hara (2001) reported that tannins are known to react

Table 5. Phytochemical screening of *black tea* ethanol and aqueous extract.

Phytochemical screening test	Ethanol extracts	Aqueous extracts
Test	**Results**	**Results**
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Phenol	–	–
Cardiac Glycosides	–	–
Volatile Oil	–	–
Anthraquinone	–	–
Steroids	+	+
Terpenoids	+	+

Keys: + = Present.
- = Absent (not detected).

Table 6. Phytochemical screening of *green tea* ethanol and aqueous extract.

Phytochemical screening test	Ethanol extracts	Aqueous extracts
Test	**Results**	**Results**
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Phenol	–	–
Cardiac Glycosides	–	–
Volatile Oil	–	–
Anthraquinone	–	–
Steroids	+	+
Terpenoids	+	+

Keys: + = Present.
- = Absent (not detected).

with protein to provide the typical tannins effect which is important for the treatment of ulcer. Herbs that have tannins as their component are stringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery (Shetty and Shivananda, 1994). This observation therefore supports the use of black and green tea in herbal cure remedies (Diker et al., 1991).

Song et al. (2005) also confirmed the antiviral properties of steroids, whereas Mckay and Blumberg (2002) worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. However, phytosterols have cholesterol reducing properties and may play a role in cancer prevention.

Alkaloids which are one of the largest groups of phytochemical in plants have amazing effects on humans and have led to the development of powerful pain killer medicine (Katiyar et al., 1999). One of the most common biological properties of alkaloids is their toxicity against cells of foreign organism. Nonetheless, alkaloids have been reported extensively for their anticancer activities (Mackay and Blumberg, 2002).

Conclusions

Black and green tea ethanol and aqueous extracts possess antimicrobial activity against the selected clinical pathogenic isolates and it can be said that the leaves of fermented and non-fermented *C. sinensis* can be used for medicinal purpose. The demonstration of broad spectrum antimicrobial activities of black and green tea extracts could be due to the presence of the secondary

metabolites in the tea extracts and this might help in the discovery of new chemical classes of antimicrobial substance that could help serve as selective agents for infectious disease chemotherapy and control.

RECOMMENDATIONS

1. The daily habit of drinking tea does good to the body, as those drinking tea are at low risk of heart disease, stroke, cancer, hypertension, diabetes and obesity.

2. People should develop the habit of drinking tea because of the fact that it has many medicinal values in the treatment of various human pathogens.

3. Further research should be carried out on the mechanisms of action of other parts of the plant of *C. sinensis* (green and black tea) such as its root, stem, bark, etc.

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