Review article

ANTIFUNGAL ACTIVITIES OF Camellia sinensis CRUDE EXTRACT, MIXTURE WITH MILK, ON SELECTED PATHOGENIC AND MYCOTOXIC FUNGI

EROLLS CHERUIYOT SIGEI *, MARGRET MUTURI ¹, CHRISTINE BII

*Kenyatta University, School of Medicine, Department of Medical Laboratory Sciences, P. O. Box 43844, Nairobi, Kenya.
²Kenya Medical Research Institute, Centre for microbiology Research, Department of Infectious Diseases, Kenyatta National Hospital grounds, P. O. Box 54840-00200 Nairobi, Kenya.

ARTICLE INFO

Keywords:
Camellia sinensis
crude tea extracts
fungus species

ABSTRACT

Camellia sinensis extracts have been documented to have antibacterial activity but little knowledge on their antifungal activity. The aqueous extracts of Camellia sinensis (tea) both green and black, mixed with milk in equal ratio parts, referred as mixture for investigation for their antifungal activity and minimum inhibitory concentration (MICs) against seven fungal species. Green and black tea crude (100 mg mL−1) extracts were evaluated for antifungal activities. Quantitative bioassay was done using disc diffusion method and MIC done using broth dilution methods. The fungal isolates used for bioactivity testing were yeasts. Green tea crude extract mixture showed stronger inhibitory effect against the fungal strains tested than black tea crude extract mixture. There was a significant difference in inhibition zone of (T = 4.09, P < 0.05). Zone of inhibition exhibited by green tea crude extracts (33 ± 0.87 mm) were higher than black tea crude extracts (6.75 ± 0.66 mm). The pattern of activity by tea crude extracts mixture against ATCC standard fungal strains and clinical isolates was similar: Candida tropicalis, Candida lusitaniae, Candida parapsilosis ATCC 22019, Cryptococcus neoformans ATCC 66031 and Candida famata were inhibited by green tea crude extracts mixture (IZD ≥ 15 mm). Clinical isolates of Candida albicans (strain 5) showed susceptibility to Camellia sinensis green crude extracts mixture. The MIC of Camellia sinensis crude extracts mixture against fungal isolates tested ranged from 50 mg mL−1 to 1.6 mg mL−1, with green tea crude extract mixture showing highest MIC on clinical fungal isolates. The studies on Camellia sinensis have shown remarkable antifungal activity and highlighted its significance as potential health products.

Copyright 2011 CurrentSciDirect Publications. IJBMRI - All rights reserved.

Introduction

Human fungal infections pose serious medical issues. There is a general consensus among researchers, clinicians and pharmaceutical companies that new, potent, effective and safe antifungal drugs are needed. Tea is one of the most consumed drinks worldwide where green tea (Camellia sinensis) accounts for about 20% of the total tea consumption. In recent years, several studies have shown that green tea consumption can protect against diseases that are associated with free radical damage including atherosclerosis, coronary heart disease and cancer. Kenyan black tea has between 7-27% more polyphenols when compared with tea from China, Japan and Taiwan [26]. The Kenyan tea germ plasm has also been observed to be diverse in its polyphenol composition and contents and therefore provides raw material for production of different types of tea products including health drinks [15]. However, the state of research on tea regarding its pharmacological properties to fungi is limited and the majority of work has been conducted on green tea with very little on black and white tea against bacteria. New drug discoveries.

Beneficial effects of tea have been attributed to the strong antioxidant and free radical induced oxidative stress. In addition, many reports have presented data on the antimicrobial activity of different types of tea extracts on various pathogenic microorganisms [14]. Green tea elicits strong antibacterial activity including potential to inhibit gram positive cocci; gram negative bacilli. Studies have also shown that tea can inhibit and kill a wide range of pathogenic bacteria at or slightly below typical concentrations found in brewed tea [20]. Various studies have shown significant suppressive effects of green tea polyphenols against many microorganisms. Black tea, a major source of phenolic, including theaflavins and thearubigins, has also been shown to have antimicrobial properties both in vivo and in vitro [2]. Screening for antifungal properties of tea products is an important strategy for development of novel drugs or rational ways of managing fungal resistance to azoles group of compounds.

This study attempts to facilitate to unravel the potentiality of Camellia Sinesis plant product as novel modalities in the line of...
MATERIAL AND METHODS

Samples of Camellia sinensis

Processed Commercial Camellia sinensis (black and green tea) produced and packed by James Finlay (K) Ltd were purchased off shelf in retail outlet at the factory in Kericho County, Kenya.

Test Fungal Organisms

The standard test fungi of American Type Culture Collection (ATCC) was sourced from Kenya Medical Research Institute (KEMRI) and included: Cryptococcus neoformans ATCC 66031, Candida albicans ATCC 90028, Candida krusei ATCC 6258, Candida glabrata ATCC 24433, Candida tropicalis ATCC 750, and Candida parapsilosis ATCC 22019 as standard organisms. Clinical isolates included: Cryptococcus neoformans, Candida albicans, Candida famata, Candida lusitaniae, Trichophyton mentagrophytes, Microsporum gypseum. Mycotoxigenic fungi included: environmental pathogenic isolates includes: Fusarium moniliforme, Aspergillus flavus, Aspergillus niger and Penicillium chrysogenum. The selection of test strains was based on their significance as opportunistic pathogens and their resistance to conventional drugs.

Experimental design

Preparation of McFarland standard

McFarland standard is used as a reference to adjust the turbidity of fungal suspension so that fungal organisms will be within a given range. Exactly 0.5 McFarland equivalent turbidity standards was prepared by adding 0.6 ml of 1% barium chloride solution (BaCl2.2H2O) to 99.4 ml of 1% sulphuric acid (H2SO4) and mixed thoroughly. A small volume of the turbid solution was transferred to cap tube of the same type that was used to prepare the test and control inocula. It was then stored in the dark at room temperature (25°C). Exactly 0.5 McFarland gives an equivalent approximate density of fungi 1.5 x 10^8 Colony Forming Units per ml (CFU) mL^-1 [23].

Crude Extraction of Camellia sinensis (Teas)

The prepared soluble granules of both black and green tea samples sealed in silver lined sachets stored at room temperature were obtained. The mixture aqueous crude extract for each tea was prepared by mixing (50 ml) fresh milk with (50 ml) water in the ratio of 1:1, in a 250 ml conical flask. 10 grammes of tea sample was then weighed and added to the contained conical flask and boiled for 20 minutes. The aqueous mixture tea extract obtained was approximately more in the strength of normal “cup of tea”. The extracts were then filtered using sterile Whatman filter paper No.1 to exclude any suspending granules and filtrate of 100 mg/ml allowed to cool, then transferred to sterile screw cap bottles, labeled and stored under refrigerated condition (4°C) until use. Only fresh extracts was used in the experiment, as marked chemical changes occurred when tea was allowed to stand [28].

Preparation of tea extracts stock and working solutions

A two fold dilutions were obtained (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, 1.5625 mg/ml) concentrations. Antifungal activities of the above concentrations were determined.

Preparations of antifungal compounds stock and working solutions The antifungal compounds were removed from storage (-20°C) and allowed to come to room temperature. Each 250 µg of antifungal compound (Fluconazole) was weighed and dissolved in sterile distilled water to make a final 10 ml solution. The stock solutions ofazole group of compounds (Fluconazole) used was usually kept at -20°C until used. Doubling dilutions of stock solutions were made to obtain working solution.

Antimicrobial assay

The antimicrobial activities of the extracts were evaluated by the disc diffusion method [18]. The use of agar disc diffusion method to screen for antimicrobial activities of the crude tea extracts was done according to the National committee of clinical and laboratory standards now CLSI[19]. The fungal inoculums for susceptibility test were standardized using barium sulphate.
The MIC was recorded as the lowest extract concentration ≥ 15mm. However, black tea crude extract mixture on the other hand showed slightly moderate inhibition of 10mm for yeasts C. lusitaniae, C. famata and moulds Penicillium chrysogenum as well as Aspergillus niger showed no inhibition (6mm) in either of Camellia sinensis green and black crude extraction. Green tea crude extract mixture, showed greater antifungal activity for yeasts C. tropicalis ATCC 750, C. lusitaniae, C. parapsilosis ATCC 22019, C. famata and Cryptococcus neoformans ATCC 66031. None of the mould tested showed inhibitory above the cut-off point of IZD ≥ 15mm. However, black tea crude extract mixture on the other hand showed slightly moderate inhibition of 10mm for yeasts C. lusitaniae, C. famata and mould Fusarium moniliforme with IZD of 12mm as compared to break point of IZD ≥ 15mm.

The samples were analyzed using paired sample T-test to establish the differences in zones of inhibition caused by black tea crude extracts mixture from green tea crude extracts mixture. The results revealed that there was a significant difference in zones of inhibition (T = 4.09, P < 0.05). Zones of inhibition caused by green tea crude extracts mixture (8.33 ± 0.87 mm) were higher than inhibition by black tea crude extracts mixture (6.75 ± 0.66 mm).

From the table above, yeasts C. albicans ATCC 90028, C. glabrata ATCC 24433, C. krusei ATCC 6528 and moulds Penicillium chrysogenum as well as Aspergillus niger showed no inhibition (6mm) in either of Camellia sinensis green and black crude extraction. Green tea crude extract mixture, showed greater antifungal activity for yeasts C. tropicalis ATCC 750, C. lusitaniae, C. parapsilosis ATCC 22019, C. famata and Cryptococcus neoformans ATCC 66031. None of the mould tested showed inhibitory above the cut-off point of IZD ≥ 15mm. However, black tea crude extract mixture on the other hand showed slightly moderate inhibition of 10mm for yeasts C. lusitaniae, C. famata and mould Fusarium moniliforme with IZD of 12mm as compared to break point of IZD ≥ 15mm.

The samples were analyzed using paired sample T-test to establish the differences in zones of inhibition caused by black tea crude extracts mixture from green tea crude extracts mixture. The results revealed that there was a significant difference in zones of inhibition (T = 4.09, P < 0.05). Zones of inhibition caused by green tea crude extracts mixture (8.33 ± 0.87 mm) were higher than inhibition by black tea crude extracts mixture (6.75 ± 0.66 mm).

Figure 4.1: Mean zones of inhibition by green and black crude Tea Extracts mixture

Among the clinical isolates tested, Cryptococcus neoformans strain 5, Candida albicans strain 4 and strain 5, showed susceptibility to antifungal activity of green tea extracts mixture with inhibition zone diameter ≥ 10.0 mm each. This is moderately active as considered highest at 15.0 mm and least 6.0 mm. This conform to earlier studies that extracts of green tea have been reported to be more effective in inhibiting bacterial growth than black tea [24].

<table>
<thead>
<tr>
<th>FUNGI</th>
<th>Mixture extract</th>
<th>Black tea</th>
<th>Green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans ATCC 90028</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Candida parapsilosis ATCC 22019</td>
<td>6</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Candida glabrata ATCC 24433</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Candida famata</td>
<td>10</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Candida tropicalis ATCC 750</td>
<td>7</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Candida krusei ATCC 6258</td>
<td>6</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Cryptococcus neoformans ATCC 66031</td>
<td>6</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Microspora gyipseum (clinical isolate)</td>
<td>7</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger (clinical isolate)</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Fusarium moniliforme (clinical isolate)</td>
<td>12</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Penicillium chrysogenum (Clinical isolate)</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Trichophyton mentagrophytes (Clinical isolate)</td>
<td>6</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Zones of inhibitions (mm) by crude tea extracts mixture on the selected pathogenic and mycotoxigenic fungi

RESULTS AND DISCUSSION

4.1 In-vitro antifungal activities of Crude Extracts of green, black and mixture Camellia sinensis

The antifungal activities of green and black tea (Camellia sinensis) crude extracts having a concentration of 100 mg/ml of extracting solvent (sterile distilled water) and from the same tea mixture with milk with ratio of milk to extracting solvent is 1:1 are presented in the tables below. Their inhibitory effects against selected pathogenic and mycotoxic fungi were then compared.
4.2 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the crude tea extracts mixture both green and black was established. The MIC to the standard fungal test strains and clinical isolates was evaluated.

4.2.1 Minimum inhibitory concentration (MIC) to standard fungal test strains

Minimum inhibitory concentrations (MIC) of tea crude extracts mixture to the fungal strains were established. Tested at 15 mm diameter of inhibition zone diameter, the MIC of mixture tea crude extracts were recorded in mg/ml. Black tea crude extract was only tested against C. famata strain which was the only which showed inhibition activity (table 4.2 below).

Table 4.2: The Minimum Inhibition Concentration of tea crude extracts to the standard fungal test strains

<table>
<thead>
<tr>
<th>Fungal strain</th>
<th>Black tea</th>
<th>Green tea mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. lusitaniae</td>
<td>-</td>
<td>6.250</td>
</tr>
<tr>
<td>C. famata</td>
<td>50.00</td>
<td>1.600</td>
</tr>
<tr>
<td>C. parapsilosis ATCC 22019</td>
<td>-</td>
<td>50.00</td>
</tr>
<tr>
<td>C. tropicalis ATCC 750</td>
<td>-</td>
<td>8.250</td>
</tr>
<tr>
<td>C. neoformans ATCC 66031</td>
<td>-</td>
<td>1.600</td>
</tr>
<tr>
<td>M. gypseum</td>
<td>-</td>
<td>50.00</td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>-</td>
<td>6.250</td>
</tr>
</tbody>
</table>

NB: The black crude extracts mixture had no inhibitory activity

KEY: - No Inhibition activity

The MIC of the Camellia sinensis crude extracts mixture which had inhibition diameters of 15 mm and above (significance activity) was determined. Green tea crude extracts mixture had the least minimum inhibition concentration at 1.6 mg/ml against Cryptococcus neoformans ATCC 66031 and C. famata, and highest MIC against yeast C. parapsilosis ATCC 22019 and mould Microsporum gypseum. Green tea mixture minimum inhibition concentration of 1.6 mg/ml was adequate to inhibit growth of C. famata. However, at a concentration of 6.25 mg/ml of mixture green tea, 50% of the tested fungi were inhibited in growth; this gives the MIC50 of the test fungi when using green tea crude extract mixture.

4.2.1.1 Minimum Inhibition Concentration (MIC) at 50% of Green tea crude Extracts mixture

4.2.1.2 Minimum Inhibition Concentration (MIC90) of Green tea crude Extracts mixture

Green tea crude extract mixture minimum inhibition concentration of 50 mg/ml was adequate to inhibit growth of Microsporum gypseum. At this concentration of 50 mg/ml of mixture green tea, 90% of the tested fungi were inhibited, that is all fungi tested were inhibited.

4.3 Synergism/antagonism between crude extracts on Kenyan tea and conventional antifungal drugs on azoles resistant fungi

To establish synergism effect, fluconazole was mixed with tea crude extracts mixture (blended with milk) and zone of inhibition.
recorded. The findings shows that there was no significant difference in zones of inhibitions (F = 0.90, df = 3, P = 0.455). However, fluconazole alone (mean inhibition zone 20.00 ±1.29 mm) was greater than tea crude extract mixture inhibition zones

Table 4.3: Synergism/antagonism between Camellia sinensis Crude Extracts mixture and Fluconazole

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Mixture Extract +F (mm)</th>
<th>Fluconazole alone (mm) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans 4</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Candida albicans 15</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>C. tropicalis ATCC 750</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>Fusarium moniliforme</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Microsporum gypseum</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>17.96 ± 2.10</td>
<td>20.00 ±1.29</td>
</tr>
</tbody>
</table>

Using a combination of green tea crude extracts mixture (blended with milk) to Fluconazole mainly inhibited growth of C. neoformans 5, C. tropicalis ATCC 750 and C. albicans 15. The camellia sinensis crude extracts exhibited diminished activity when combined with Fluconazole (lesser inhibition zone diameters as compared to Fluconazole [IZD] as compared to activity by Fluconazole alone). This shows antagonism between the crude extracts mixture and conventional antifungal drug, Fluconazole.

4.4 Effect of Temperature and Addition of Milk to Crude Extracts

To test for effect of temperature on MIC of green and black tea crude extract mixture, a pair sample T-test was used to compare the MIC values. The result showed that there was no significant difference in MIC (t = 1.51, P = 0.182). Mean MIC of green tea crude extract mixture (mean 0.017 ± 0.008 mm) was higher than black tea (0.0143 ± 0.007 mm). Minimum fungicidal concentration (MFC) of green tea crude extract mixture was tested on C. tropicalis ATCC 750, C. neoformans ATCC 66031, C. lusitaniae, C. famata and C. parapsilosis ATCC 22019.

Table 4.4: Minimum fungicidal concentration of Green tea crude extract mixture

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Green tea Crude extract mixture (mg/ml)</th>
<th>extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. tropicalis ATCC 750</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>C. neoformans ATCC 66031</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>3.12</td>
<td></td>
</tr>
<tr>
<td>C. famata</td>
<td>50.00</td>
<td></td>
</tr>
<tr>
<td>C. parapsilosis ATCC 22019</td>
<td>8.25</td>
<td></td>
</tr>
</tbody>
</table>

Green tea crude extract mixture at 3.12 mg/ml was effective enough to kill C. lusitaniae while at 6.25 mg/ml C. tropicalis ATCC 750 and Cryptococcus neoformans ATCC 66031 was made static and could not grow. At a concentration of 8.25 mg/ml it was fungicial to C. parapsilosis ATCC22019. When the concentration reached 50 mg/ml all the tested fungi including C. famata were killed by the tea crude extract mixture.

DISCUSSION

According to world health report of infectious diseases[27], overcoming antimicrobial resistance is the major issue of W.H.O for the next millennium. Hence, the last decade witnessed an increase in the investigation of plants as a source of human disease management. In the present study, the crude extracts of Camellia sinensis (green and black) blended with milk, giving a mixture produced inhibitory activity against pathogenic and mycotoxicogenic fungi. The water crude extract produced yields enough for the experimental study and is the most commonly used and cost effective method of tea preparation. The choice for water extraction was due to the fact that water is very polar than organic solvents hence it is able to extract more polar compounds from a plant material. A previous study also found that water extracts was blended with milk as normal home-made tea giving strength of "normal cup of tea"[11]. The results obtained in this study indicate a considerable difference in antifungal activity of antymycotic activity of Camellia sinensis green and black crude extracts mixture. For all the yeasts tested, Candida tropicalis ATCC 750 was the most sensitive fungus to all the crude extracts (table 4.1). This conform to earlier studies that extracts of green tea have been reported to be more effective in inhibiting bacterial growth than black tea [24]. The results from the present study revealed that there was a significant difference in zones of inhibitions (T=4.09, P=0.05).

Zone of inhibition caused by green tea crude extracts (8.33 ± 0.87 mm) were higher than inhibition by black tea crude extracts (6.75 ± 0.66 mm) (figure 4.1). Candida albicans strain 4 and strain 5 among all the clinical isolates had greatest susceptibility to antifungal activity of green tea extract with IZD of 15mm (figure 4.2). C. neoformans strain 3, strain 5 and strain 12; showed susceptibility to antifungal activity of green tea extracts mixture with IZD ≥10.0 mm each. This is moderately active as considered highest at 15.0mm and least 6.0 mm. These findings are in line with other studies which indicate that the lowest activity was at 7.0 mm and the highest was at 18.0 mm in diameter[3]. The green tea crude extract mixture has shown higher antifungal activity than black tea. This difference in results is probably due to presence of different contents of active substances in the teas. Several studies have shown that the antimicrobial property is due to presence of polyphenols. Specific antioxidant polyphenols called catechins play an important role in green tea's inhibition of microbial growth. Several significant catechins include: EGC, EGC, ECG, EC and GCG. Antimicrobial activities of tea extracts are very selective. This difference in their activity depends upon the concentration and type of the extracts. These effects may also differ depending on (microbe) fungal species so that they may be either growth inhibitory or stimulatory [24].

Green tea and black tea crude extracts mixture tested in current study have also shown varying activities against fungal organisms. Other previous studies showed that the actions of catechins ECGG, EGC were fungicidal[9]. Studies of the antibacterial activity of catechins against phytopathogenic bacteria showed similar results to those against C. albicans. Catechins are known to have an affinity for proteins; this is clearly shown by a decrease in antibacterial activity of tea. This property is referred to as "astringency" contributes to the sensation known as "mouth feel" experienced when drinking tea. The mode of action involves inducing rapid leakage of small molecules entrapped in the intraliposomal space and aggregation of the liposomes. Thus, a number of membrane dependent cellular processes, such as cell signaling and cell cycle, arachidonic acid metabolism and cell
The resistant (least susceptible) fungal strains of clinical isolate Cryptococcus neoformans strain 3, strain 5, strain 12, strain 97, strain j076 and strain 065 (figure 4.2) was most probably because of the presence of mucopolysaccharide capsule. The polysaccharide capsular material in some of the pathogenic microorganisms is responsible for virulence and antimicrobial resistance [10]. The Candida species such as Candida albicans ATCC 90028, Candida glabrata ATCC 24433, Candida parapsilosis ATCC 22019 that showed less susceptibility to antifungal agents of Camellia sinensis crude extract mixture could be due to their outer membrane consisting of chitin binding proteins thus able to regulate the access of antifungal properties into the underlying structures. Candida species expresses multidrug efflux transporter (MET), which mediates the efflux of a broad range of compounds including antifungal agents [16]. But in this study, we found contradicting results among the Candida strains clinical isolates (figure 4.2). C. albicans strain 3, strain 6 and strain 20 showed least or no activity whereas strain 4, strain 5 and strain 15 had activity. The disparity in findings could be due to differences in strains of fungi used and susceptibility to antifungal drugs.

The preliminary screening assays for antifungal activity can largely be considered as qualitative assays and are used for identifying the presence or absence of bioactive constituents in the extracts. However, these methods of assays offer little information on these compounds. The minimum inhibition concentration (MIC) is a quantitative assay and provides more information on the potency of the compounds present in the extracts. Thus, the MIC values of the crude extracts of Camellia sinensis which had inhibition zone diameter of 15 mm and above was determined so as to demonstrate the potency of the extracts against the selected strains of fungi. The least the MIC the better the Camellia sinensis crude extract against the isolate in question. The green tea crude extract mixture had the least minimum inhibition of 1.6 mg mL-1 against yeast Candida famata, and Cryptococcus neoformans ATCC 66031 and the highest MIC against yeast C. parapsilosis ATCC 22019 of 50 mg mL-1 and mould M. gyipseum (table 4.2). When the green tea crude extract was mixed with milk at a ratio of 1:1, the MIC was established to be 1.6 mg mL-1 against C. famata and same concentration formed the MIC50 (figure 4.3); whereas, at a concentration of 50 mg mL-1, 90% of the fungal isolates tested were inhibited. At this concentration of mixture green tea crude extract, all the fungi tested were inhibited (figure 4.4).

Generally, the MIC of the Camelia sinensis crude extracts mixture were as high as 50 mg mL-1 as compared to the standard drugs which is 0.5 mg mL-1 for yeasts and 1.0 mg mL-1 for dermatophytes at 95% confidence interval (P=0.05 level of significance). Although this was significantly lower than that of Fluconazole (P<0.01), the extracts are promising since they are crude extracts compared to pure compound of Fluconazole. This is a clear indication that the active ingredient is present in low quantities which necessitate the use of large amounts of crude extracts to gain the desired therapeutic effects. The difference in bioactivities of green and black tea crude extracts mixture could be attributed to the facts that plants differ phytochemically and the blending with milk as well as boiling also affect/alter their composition (cold and hot water extraction). Absence of bioactivity does not warrant disapproval of ethno botanical utilization of the Camellia sinensis, simply because it may suggest that the extracts are reacting in an indirect way where active ingredient exists as a precursor requiring activation in vivo. The present study also showed antagonistic antifungal activity of the combination of tea crude extracts mixture and antimycotic, Fluconazole against tested fungal isolates. This is in contrary to earlier studies, since the arrival ofazole antifungal agents as first-line drugs; Fluconazole-resistant C. albicans has begun to appear.

Similar studies have been reported by [9], on the combined use of EGGG and Fluconazole was effective against Fluconazole resistant C. albicans. More detailed studies revealed that EGGG enhanced the antifungal activity of the drug Amphotericin B; and the combined use of EGGG and antifungal drug Fluconazole inhibited Fluconazole-resistant strains of this fungus. It is suggestive to have converted Fluconazole resistant phenotypes to sensitive ones. Earlier studies showed that EGGG converted a Methicillin-resistant phenotype to a Methicillin-sensitive one. EGGG synergizes the activity of β-lactam antibiotics against S.aureus by binding to the peptidoglycan component of the bacterial cell wall [30]. The wide ranging effects that catechins gallates have on modulation of drug resistance has recently been emphasized by the novel observation that sub-inhibitory concentrations of EGGG are able to reverse resistance by inhibition of efflux pump, in addition to further sensitizing susceptible isolates to antibiotic [21]. However, the present study findings established that using a mixture of tea crude extracts to Fluconazole mainly diminished inhibitory effect to fungal species. In terms of effects of inhibition as a result of difference in extraction temperatures, the present study revealed that higher temperatures reduces the polarity of water, thus increasing its extraction efficiency and capability to dissolve polar compounds [8].

Raising the temperature of water also reduces its surface tension and viscosity, which increases the diffusion rate and the rate of mass transfer during extraction. The Mean MIC of green tea crude extract mixture (mean 0.017 ± 0.008 mm) was higher than black tea (0.0143 ± 0.007 mm). When green tea crude extract was mixed with milk, the mixture crude extract at a concentration of 3.12 mgmL-1 was fungidal to C. lusitaniae but fungistatic to other fungal isolates tested. But at concentration of 6.25 mgmL-1 was fungidal to C. tropicalis ATCC 750 and C. neoformans ATCC 66031; while at 8.25 mgmL-1 was fungidal to C. parapsilosis ATCC 22019 but fungistatic to C. famata. The MFC of C. famata was 50 mg mL-1. These results are suggestive that addition of milk to blend the crude extracts altered the bioactive ingredients resulting to higher concentration for its MFC as compared to crude extracts alone. These results conform to earlier reported studies describing milk known to decrease antioxidant activity of Camellia sinensis [25]. The mechanistic aspect of fungicidal brought about by tea crude extracts is suggestive to be due to catechins and gallates. The bioactive ingredients in crude tea extracts binds to ergosterol, one of the cell membrane sterols, and damages the cell membrane directly, leading to fungicidal activity against fungi. Catechins regulate expression of the gene(s) coding for Cytochrome P450.

Detailed physiochemical studies suggest that fungicidal activities of galloylated tea catechins at the cell membrane level may be due to their specific perturbations of ordered structure of chitin binding proteins, a nitrogen containing polysaccharide constituting fungal cell wall. Differential effects of catechins on fungal cell walls compared to membrane of human cells may be due to differences in structures of the respective walls (membranes). The fungicidal action of EGGG may depend on hydrogen peroxide derived from the reaction EGGG with oxygen (Prooxidative activity) [1]. These observations suggest that
antifungal activity of antimycotic effect seem to arise from the interactions of catechins in crude extract with oxygen, genes, cell membranes and enzymes. This aspect merits further study. This predominately in vitro information has ramifications for Mycotic disease prevention in humans.

CONCLUSION AND RECOMMENDATIONS

1. The Camellia sinensis crude extract possess antifungal activity. In the present study, the crude extracts of Camellia sinensis (green and black) produced inhibitory actions against the fungal test strains.

2. The Minimum Fungicidal Concentration (MFC) of the Camellia sinensis crude extracts mixture with milk was slightly higher as compared to that of fluconazole drug. Therefore, addition of milk to the crude extracts altered the bioactive ingredients resulting to higher concentration for its MFC as compared to azole drug alone (it diminishes fungicidal activity).

3. The Plant based crude extracts represents unlimited sources of modern therapies therefore; a continued and regular exploration of Camellia sinensis for antifungal agent is required.

4. Tea is an infusion of the leaves of Camellia sinensis plant, and is one of the most widely consumed beverage in the world. For potential antifungal beneficial effects, the green tea should be consumed in preference to black tea. The green tea as beverage should also be consumed purely without blending with milk so as to achieve maximum health benefits.

5. The fractionation of crude extracts and purification of active compounds is needed to isolate these bioactive compounds to establish their mechanistic aspect of action against the fungal isolates and elucidate mechanism of synergism / antagonism.

6. Assayed antifungal were tested in vitro, but practically in human aspect both antifungal and polyphenolic compounds of Camellia sinensis undergo metabolic processes in the body; there is no information on the interaction of the related metabolites. This needs further studies.

Acknowledgments: The technical assistance of the staff of the Medical Mycology Department of the Kenya Medical Research Institute (KEMRI), Nairobi is appreciated.

Conflict of Interests

The authors did not declare any conflict of interest.

REFERENCES


10. Hooper, D.C. 2001. Emerging mechanisms of fluoroquinolone resistance. E m e r g i n g  I n f e c t i o u s  D i s e a s e s , 7 , 3 3 7 - 3 4 1 . http://dx.doi.org/10.1038/00378600a000000


16. Marchetti, G., Morello, E., Grazi, M.P. 2000. Potent synergism of the combination of fluconazole and cyclosporine in Candida albicans. A n t i m i c r o b a l  A g e n t s  a n d  C h e m o t h e r a p y , 4 4 , 2373–2381. http://dx.doi.org/10.1128/acs.44.9.2373-2381.2000


© Copyright 2019 BioMedSciDirect Publications IJBMR - ISSN: 0976-6685. All rights reserved.