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Antibacterial Screening of Tea Leaves Extract on Selected Bacteria

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Abstract

Plants are naturally gifted with synthesis of bioactive compounds which shows medicinal activity. These bioactive compounds are also called phytochemicals and they demonstrate various activities such as; anti-oxidant activity, boosting the immune system, anti-inflammatory activity, anti-viral activity, anti-bacterial activity, and cellular repair activity. Tea has been used by human kind for long not only as a beverage but also for its perceived medicinal value. This study was undertaken to establish the antibacterial activity of tea methanolic extract. It demonstrated a broad spectrum antibacterial activity giving the highest inhibition zones in S. pneumonia (26 mm), E. faecalis (19.3 mm), E. coli (11.3 mm), S. typhi (10 mm) and S. aureus (8.7 mm). This activity can only be associated with phytochemicals such as catechins, gallates, flavonoids that are known to be present in tea. This study helps to confirm the therapeutic potential of tea as it has been stipulated through anecdotal evidence among the Kalenjin community of Kenya.

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1. Background Information

Medicinal plants are known to provide rich sources of raw materials for traditional medicine in the treatment of various ailments since time in memorial. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Herbalism has a long tradition of use outside the conventional medicine and it is becoming more of a mainstream as improvements in analysis and quality control along with advances in clinical research show its value in the treating and preventing disease (Carpinella and Rai, 2009).

The adverse impact of infectious diseases is most severe among the poor people who have few and limited resources have no access to integrated health care, prevention tools and medications and hence herbal plants becomes the cheap alternative to conventional medicine (Yemoa et al., 2010). Historically, plants have provided a good source of anti-infective agents; quinine, emetine and berberine remain highly effective agents in the fight against microbial infections. Phytomedicines derived from plants have shown great promise in the treatment of infectious diseases including HIV opportunistic infections (Turano et al 1989).

Plants remain a major source of most natural, semi-synthetic and synthetic drugs (Clark, 1996) and therefore the use of plant extracts or chemicals derived from them to treat diseases has stood the test of time (Chowdhury et al., 2002, Khan et al., 2006). Plant based antimicrobials represent a vast untapped source for medicines. Continued and further exploration of plant antimicrobials needs to be made a continuous process. Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of emerging and re-emerging infectious diseases while simultaneously mitigating many of the adverse effects that are often associated with synthetic antimicrobials. They are therefore effective, yet gentle (Ngoci et al 2011).

Natural plants contain phytochemicals which are considered to contribute to their bio-activity. There are over 10,000 phytochemicals and they have effects such as antioxidant activity, boosting the immune system, anti-inflammatory, anti-viral, anti-bacterial activity, cellular repair among others (Njeru et al. 2013; Ngoci et al., 2011). Camellia sinensis locally known as tumoek in Kalenjin community of Kenya is used to treat stomachaches, diarrhea, skin infections, cough and asthma. It pharmacologic roles have been associated with presence of polyphenols such as catechins, proanthocyanidins and gallates (Tayler et al., 2005). Therefore the aim of the study is to determine the antimicrobial activity and phytochemical determination of tea leaf extract.

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2. Materials and Methods

2.1 Collection and Identification of Plant sample

The plant leaves to be studied were collected from Kericho County of Kenya. The plant samples were taxonomically authenticated at Mount Kenya University, Department of Medical Laboratory Science.

2.2 Plant Leaf Preparation and Extraction

The plant leaves were air dried at room temperature in the dark to a constant weight before grinding to a powder with a mechanical grinder. The powder was then macerated in organic solvent (Methanol) and the extract was mixed with activated charcoal and filtered by Whatmann No. 1 filter paper and the filtrate concentrated and evaporated to dryness by a rotary evaporator.

2.3 Collection of Test Micro-organisms

A total of five standard microbial strains were used. These micro-organisms were obtained from Mount Kenya University laboratory - Microbiology Department. The bacteria included: three Gram positive bacterial species: Staphylococcus aureus, Enterococcus faecalis and Streptococcus pneumoniae and two Gram negative bacteria species: Escherichia coli, and Salmonella typhi.

Anti-Microbial Assays

This was done to evaluate in vitro activity of the anti-microbial agents. The results were reported qualitatively by taking the diameter of zones of inhition in disc diffusion method (Collins et al, 1995).

2.4.1 Culture Media

Nutrient agar was used for sub culturing of the test micro-organisms and incubated at 37 ^{0}C for between 18-24 hours. For drug sensitivity assay, Mueller Hinton agar was used.

2.4.2 Media Preparation

The Media was reconstituted following the Manufacturer's instructions, sterilized by autoclaving at 121 ^{0}C for 15 minutes and then dispensed aseptically into Petri dishes (9 cm diameter), a volume of between 20-25 milliliters molten agar, left to solidify and then stored in the refrigerator at 4 ^{0}C.

2.4.3 Preparation of Discs

Whatmann filter (No. 1) discs of 6 mm diameter were prepared by punching the paper and the blank disc were sterilized in hot air oven at 160 ^{0}C for 1 hour. The discs were then impregnated with 10 µl of the varying concentration of the extract solution made of DMSO diluents and then evaporated at 50 ^{0}C until it dried. The standard drug Chloramphenicol at 30 µg/disc was used as positive control and DMSO impregnated in a disc was used as a negative control.

2.4.4 Disc Diffusion Test

The anti-microbial activity was assayed by disc diffusion method according to CLSI (2007). The bacterial strains were activated by growing them in Nutrient agar at 37 ^{0}C for 18 – 24 hours and thereafter, a fresh inoculum was developed by suspending activated colonies in physiological saline solution (0.85% NaCl). The suspension was used to aseptically inoculate the surface of Mueller Hinton agar plates by swabbing and the impregnated discs were then planted at equidistant points on top of the inoculated agar medium using a sterile forceps. The controls; Chloramphenicol and DMSO discs were also used. The inoculated plates were then incubated at 4 ^{0}C for 2 hours to allow pre-diffusion of extracts into the media and thereafter transferred to the incubator at 37 ^{0}C for 18-24 hours. The results were read the following day for zone of inhibition. Anti microbial activity was evaluated by measuring the diameter of the zones of inhibition.

3. Results

The results of the sensitivity test showed that all the five micro-organisms exposed to the methanolic extract of tea leaves were sensitive to the plant extract. The micro-organisms studied had obvious differences in their susceptibility to the tea extract with Gram positive bacteria giving higher inhibition than Gram negative bacteria. S. pneumonia showed the highest zone of inhibition (26 mm), followed by E. faecalis (19 mm), E. coli (11 mm), S. typhi (10 mm) and S. aureus which gave the lowest inhibition of 8 mm as shown in table 1.

4. Discussion

The tea extract had broad spectrum activity. This is because the extract inhibited the growth of both Gram positive and Gram negative bacteria. Gram positive bacteria demonstrated higher susceptibility to the extract than Gram negative strains. This is in agreement with previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Parekh and Chanda, 2006; Mohamed et al., 2010). This high sensitivity to Gram-positive bacteria could be attributed to their outer peptidoglycan layer which is not an effective permeability barrier as the outer phospholipid membranes of Gram-negative bacteria (Trombetta et al., 2005; Tomczykowa et al., 2008; Kaur and Arora, 2009). The antimicrobial activity of tea extract especially against S. typhi and S. aureus has been established in other studies (Tayler et al., 2005). The pharmacological activity can only be attributed to the bioactive compounds found in tea. These include polyphenols such as catechin,

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flavanoids, gallates and proanthocyanidins. Catechins have been shown to act by damaging the membrane (Tayler et al., 2005), inhibiting bacterial enzyme glycosyltransferase as well as inactivating bacterial toxins (Njeru et al., 2013). Flavanoids works by complexing both intracellular and extracellular proteins as well as damaging bacterial membranes (Ngoci et al., 2013). The inhibition zones increased on increasing the concentration of the extract in the discs showing a concentration dependent activity. This is an indication that better activity can be achieved by increasing the concentration of extract or the bio-active compounds. The activity also varied with the kind of bacteria tested, indicating selectivity of the active compounds which is a significant principle of 'magic bullets' in drug discovery. Although the concentrations of the extract fractions were in the range of 100 times more than the concentration of standard antibiotic (chloramphenicol), they showed marked anti-bacterial activity as evidenced by their zones of inhibition. This could be due to the fact that the active components in the extract comprise only a fraction of the extract used. Therefore, the actual concentration of the individual active components in the extract could be much lower than the standard antibiotic used. It is important to note that, if the individual active components were isolated and purified, they would probably show higher antibacterial activity than those observed in this study.

5. Conclusions

The tea leaves extract has antimicrobial effect to some bacteria pathogens known to cause diseases to humans and other animals. This shows the potential of the plant as not only a refreshing beverage but also a therapeutic beverage. However more work is still required to isolate various active constituents and determine their molecular mechanisms of action in disease control.

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Njeru S. Ngoci
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Table 1. Anti-bacterial activity result for the methanol leaves extract.

Micro organism	Inhibition zones diameter in mm							
	Extract concentration (µg x 10^2)						STD_a	STD_b
	300	150	75	37.5	18.75	9.375	30µg	
Gram Negative								
E. coli	11.3±0.9	9.6±0.3	8.3±0.3	2.0±2.0	0.0	0.0	33.3±0.7	0.0
S. typhi	10.0±0	7.7±0.3	6.3±0.3	0.0	0.0	0.0	31.3±1.7	0.0
Gram Positive								
S. aureus	8.7±0.3	7.0±0.6	0.0	0.0	0.0	0.0	33.0±1.5	0.0
S. pneumoniae	26.0±0.6	25.0±0.6	22.7±0.7	20.3±1.2	16.0±2.0	12.3±1.2	36.7±0.7	0.0
E. faecalis	19.3±0.7	17.3±1.2	15.7±0.9	12.7±0.7	10.0±0	6.3±0.3	37.7±1.2	0.0

STDa – Represents positive control (Chloramphenical); STDb – Represents negative control (A disc loaded with 10 µl DMSO); Values of inhibition zones are in mm (mean±SEM, n=3)

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