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In vitro Antibacterial Activity of Plants Used as Herbal Tea in Tanzania

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Authors' contributions

This work was carried out in collaboration between both authors. Authors OO and MC designed the study, performed the statistical analysis, wrote the protocol, managed analyses of the study, literature search and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was to evaluate antibacterial activity of *Rhus vulgaris*, *Sphaeranthus bullatus*, *Osyris lanceolata*, *Ocimum gratissimum, Cymbopogon citratus, Acacia nilotica* and *Tylosema fassoglensis* plants used as herbal teas in Tanzania and antibacterial synergic effect when combined with *Cymbopogon citratus*.
Study Design: *In vitro* antibacterial assay was employed to determine Minimum Inhibitory Concentration (MIC).
Methodology: Pulverized plant materials were sequentially extracted using dichloromethane, ethyl acetate and distilled water. Minimum Inhibitory concentration (MIC) was measured for antibacterial activity against gram negative bacteria using 96-well micro dilution method.
Results: The highest antibacterial activity with MIC value of 0.19 mg/mL was exhibited by *Camellia sinensis* against *Salmonella typhi*. About 3.7% of the extract exhibited antibacterial activity with MIC value of 0.3906 mg/mL whilst the remaining extract exhibited antibacterial activity with MIC values ranging from 0.781 to 25 mg/mL against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella kisarawe*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus mirabilis* and *Klebsiella oxytoca*. Thirty percent of the tested extracts exhibited antibacterial activity with MIC values below

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1 mg/mL indicating that they are potential antibacterial drug leads according to Rios and Recio (2005). The antibacterial activity of combined extracts of *C. citratus* and herbal teas revealed both antibacterial synergic and antagonistic effects.

Conclusion: The current study established that herbal teas can be consumed not only as refreshment but also as a remedy for Gram negative bacterial infections. It is therefore recommended that when herbal tea is consumed as a remedy, incorporation of *C. citratus* should only be necessary when it induces synergistic effects.

Keywords: Gram negative bacteria; extract; antibacterial; synergism; herbal tea.

1. INTRODUCTION

Herbal teas, infusions of a single plant or mixtures of plant parts including leaves, roots, seeds, bark or flowers used as tea for refreshment or for specific medicinal purposes [1]. These teas are not derived from the usual tea plant "*Camellia sinensis"* and in most cases they are caffeine free [2] which is contrast to *C. sinensis*, the most popular tea (both green and black) worldwide including Tanzania. Herbal teas have gained popularity recently due to their nutraceutical and health promoting properties [3]. They can be seen as general tonics, nutritional supplements and prevention of diseases, as well as treatment for various ailments, having been positioned anti-viral, antibacterial, antifungal, antioxidants or even digestive aids [4]. At the same time, the negative effects of high consumption of caffeine and high level of tannin and anti-nutritional factors in *C. sinensis* have been publicized [3]. Thus many people are shifting from black tea to herbal teas [5].

In Tanzania, popular herbal teas found in markets include chamomile tea, lemon grass tea, hibiscus tea and rosella tea. In addition to these, there are a number of herbal teas used by various ethnic groups which are used either as refreshment by people who wish to avoid black tea or as both refreshment and for medicinal purposes. These include *Rhus vulgaris* (Anacardiaceae), *Sphaeranthus bullatus*, (Asteraceae), *Osyris lanceolata* (Santalaceae)*, Ocimum gratissimum* (Lamiaceae)*, Cymbopogon citratus* (Gramineae)*, Acacia nilotica* (Mimosaceae) and *Tylosema fassoglensis* (Caesalpiniaceae).

Despite the long history of use of these plant species as traditional herbal tea in Tanzania, little or no attention has been paid to evaluate their health potential. The current study was thus designed to determine antibacterial effects of *R. vulgaris*, *S. bullatus*, *O. lanceolata*, *O. gratissimum, C. citratus, A. nilotica* and *T. fassoglensis*. Because *C. citratus* is used in combination with most part of plants used as herbal teas as a flavoring agent, the extracts from plants parts usually combined with *C. citratus* were combined with aerial parts of *C. citratus* to determine any synergetic antibacterial activity. Moreover, to provide a baseline for comparison, the antibacterial activity of black tea (*C. sinensis*) were also determined.

2. MATERIALS AND METHODS

2.1 Plant Materials

Plant materials were collected from Rorya, Same, Arumeru and Siha districts (Table 1) in Tanzania based on the available ethno herbal tea information. The collected plants were identified by Daniel Sitoni, a taxonomist in the herbarium department of the Tropical Pesticide Research Institute (TPRI), Arusha, Tanzania. Voucher specimens were deposited at Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha Tanzania.

2.2 Chemicals, Reagents and Bacterial Strains Used

Dichloromethane was purchased from LOBA® (Lobachemia laboratory reagents, India). Ethyl acetate was purchased from RFCL Limited, India. Dimethyl sulfoxide (DMSO) was purchased from AVANTOR® (Avantor performance material limited, India). Nutrient broth and nutrient agar were purchased from HIMEDIA® (Himedia Laboratories pvt Limited India). Iodonitrotetrazolium chloride (INT) and gentamycin, which were used as indicator and standards respectively, were purchased from SIGMA® (sigma –UK). Bacterial strains, namely *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC29953), *Salmonella kisarawe* (clinical isolate), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella typhi* (clinical isolate), *Proteus mirabilis* (clinical isolate) and *Klebsiella oxytoca* (clinical isolate), were obtained from the Department of Microbiology and Immunology at Muhimbili University of Health and Allied Sciences.

2.3 Preparation and Extraction of Plant Material

The leaves stem barks, roots and tuber of the plant species were air dried under shade and then pulverized into fine particles. For non polar and medium polar extraction, 250 g of pulverized material was sequentially macerated using dichloromethane, ethyl acetate for 48 h, filtered using Whitman paper number 1 and solvents removed under *vacuo* using a rotary evaporator. In the case of aqueous extraction the same pulverized material was soaked in boiled water (1500 mL) maintained at 60℃ in the incubator for 24 h. The extract were sieved and then centrifuged at 5000 rpm for 10 min. The supernatant was collected and then filtered using whatman paper 1. The process of centrifuge and filtration were repeated two times and final supernatant were collected and dried by freeze drier to eliminate water by sublimation. All extracts were stored in the deep freezer at -20℃ for further activity.

2.4 Test for Antimicrobial Activities

Minimum inhibitory concentrations (MICs) were created by serial micro dilution in duplicate using 96-well micro titer plates [6]. Initially, plates were loaded with 50 µL of either the nutrient broth in each well, followed by an addition of 50 µL of the extract (100 mg/mL) in the first wells of each row tested to make a total volume of 100 µL in the first wells. After thorough mixing, 50 µL was drawn from each of the first row wells and placed into the next row of wells. This process was repeated down the columns to the last wells, where 50 µL was discarded. Thereafter, 50 µl of the bacterial (approximately 0.5 McFarland standard turbidity) was then added to each well to make the final volume of 100 µL per well. The rows with gentamyicin were used as standard positive controls, with DMSO as negative control and a row with broth and bacteria used only to monitor bacterial growth. After serial micro dilution, plates were incubated for 24 h at 37℃. MICs were determined by adding 20 µl of 0.2% INT chloride dye in each well, followed by incubation at 37℃ for 1h. Bacterial growth was indicated by a pink color. The lowest concentration that showed no bacterial growth was considered as MIC.

2.5 Synergic Effects Antibacterial Activity

Dichloromethane, ethyl acetate and aqueous extracts of *O. gratissimum* leaves*; T. fassoglensis* tubers*, A. nilotica* barks and *O. lanceolata* root bark were selected and combined with *C. stratus* as usually combined within the ethnic groups to make herbal tea with flavoring agent. These extracts were mixed with corresponding extracts (dichloromethane, ethyl acetate and aqueous) of *C. stratus* in a ratio of 1:1 (50 mg/mL of other extract: 50 mg/mL of *C. stratus*). MIC values indicating the bacterial effects of these combinations were determined using the same procedure explained above.

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activity

Thirty nine extracts from seven plants used as herbal teas by different ethnic groups in Tanzania were evaluated for antibacterial activities and compared with antibacterial activity of extracts from *C.sinensi*s (black tea) and standard antibiotic gentamycin. Table 1 summarizes the ethnomedical use of the plants studied. All plant extracts tested demonstrated antibacterial potency against Gram negative bacteria with MIC range of 0.3906 - 25.5 mg/mL (Tables 2 and 3). The black tea which is widely consumed in the country exhibited the same antibacterial activity range as the herbal teas consumed in the ethnic groups with the exceptions of *C*. *sinensi*s dichloromethane extract which had MIC value of 0.1953 mg/mL against *S. typhi*. It is however evident that 3.7% of the tested extracts exhibited MIC value of 0.3906 mg/mL against the tested bacteria, 9.5% of the extracts was active with the MIC value of 0.781 mg/mL against the tested bacteria and 86. 8% of the extracts exhibited MIC values ranging from 1.5625 to 25 mg/mL. This paper elaborates the significance of extracts with MIC values less than 1 mg/mL as potential source of drug leads for the management of Gram negative bacteria.

The current study revealed an interesting bacterial susceptibility trends. The most susceptible bacteria were *S. typhi* (clinical isolate) which was inhibited by 9.52 and 19.05% of the tested extracts with MIC values of 0.3906 and 0.781 mg/mL respectively. The highest antibacterial activity demonstrated by *C. sinensis* dichloromethane extract (CSC) with MIC value 0.1956 mg/mL was against *S. typhi*. *Proteus mirabilis* was strongly inhibited by 4.76% of the tested extracts and moderately inhibited by 11.9% of the tested extracts with MIC values of 0.3906 and 0.781 mg/mL respectively. *K. pneumoniae* and *S. kisarawe* were the most

resistant as none of the extracts inhibited them with MIC values less than 0.781 mg/mL. *E.coli* and *P. aeruginosa* were only strongly inhibited by *T. fassoglensis* leaf dichloromethane extract and *R. vulgaris* leaf dichloromethane extract respectively with MIC value of 0.3906 mg/mL.

Findings emanated from this study revealed extracts with high selectivity against certain Gram negative bacteria while other extracts demonstrated broad herbal driven antibiotic. *Camellia sinensis* dichloromethane extract which was the most active extract selectively inhibited the growth of *S*. *typhi* with MIC value of 0.1953 mg/mL (Table 3). *T. fassoglensis* leaf dichloromethane extract, *C. sinensis* ethyl acetate extract and *Ocimum gratissimum* flowers aqueous extract had high selectivity against *S*. *typhi* with MIC values of 0.3906 mg/mL for the first two extracts and 0.781 mg/mL for the latter extract. *T. fassoglensis* leaf dichloromethane extract and *C. sinensis* ethyl acetate extracts had MIC range of 1.5625 – 12.5 mg/mL against the other tested bacteria whilst *O*. *gratissimum* flower aqueous extract, *R*. *vulgaris* leaf aqueous extract and *A*. *nilotica* roots dichloromethane extract had MIC range of 3.125 – 12.5 mg/mL, 1.5625 – 6.25 mg/mL and 6.25 – 12.5 mg/mL respectively.

A number of plant extracts demonstrated the ability to inhibit the growth of multiple Gram negative bacteria at MIC values below 1 mg/mL. They are therefore potential herbal driven antibiotics for the management of multiple bacterial infections. For instance, *O. gratissimum* root dichloromethane extract exhibited antibacterial activity against six bacteria namely *P*. *mirabilis, K*. *pneumoniae*, *E*. *coli* and *P*. *aeruginosa* with MIC values of 0.781 mg/mL and against *K*. *oxytoca* and *S*. *typhi* with MIC value of 0.3906 mg/mL. The second extract under this category was *T. fassoglensis* leaves aqueous extracts which inhibited five Gram negative bacteria namely *K*. *oxytoca*, *K*. *Pneumoniae*, *S*. *typhi* and *P*. *aeruginosa* with MIC value of 0.781 mg/mL and against *E. coli* with MIC value of 0.3906 mg/mL. *R. vulgaris* roots aqueous extract exhibited high potency against *P. mirabilis*, *S. typhi*, *E*. *coli* and *P*. *aeruginos*a with MIC value of 0.781 mg/mL. *R*. *vulgaris* leave dichloromethane extract and *R*. *vulgris* barks methanolic extract *T*. *fassoglensis* tubers aqueous extract, *S*. *ballatus* dichloromethane extract and *C*. *sinensis* aqueous extract inhibited three Gram negative bacteria each with MIC values below 1 mg/mL (Tables 2 and 3). It was however interesting to observe that six out of eight plants extracts demonstrated wide range of antibacterial activity was aqueous extracts. Plant extracts were tested for their significance in inhibiting bacteria tested. There was no significance difference between extracts in inhibiting bacterial growth ($P< 0.05$) except for extracts from *O. lanceolata* which showed significance difference ($P> 0.05$).

Table 1. Scientific name, location collected, parts extracted and medicinal use of herbal tea in the ethnic groups

Scientific name	Location collected	Part extracted	Ethno medicinal uses
Spheranthus bullatus	Arumeru district	Aerial parts	diarrhea, stomachache, malaria
Cymbopogon citratus	Siha districts	Aerial parts	Refreshment, cough, malaria
Rhus vulgaris	Rorya district	Roots	Wounds, dermal swelling
	Rorya district	Barks	Wounds, dermal swelling
	Rorya district	Leaves	Refreshment, dental problems
Tylosema fassoglensis	Rorya district	Tubers	Refreshment, HIV, digestive disorders in infants
	Rorya district	Leaves	Dermal swelling
Ocimum gratissimum	Siha district	Leaves	Refreshment, cough, diarrhea, wounds, amoebic infection, pneumonia
	Siha district	Flower	Cough, wounds, fever
	Siha district	Roots	Diarrhea, headache, fever
Acacia nilotica	Siha district	Barks	as a narcotic, increase libido, malaria, fever pneumonia
	Siha district	Roots	Pneumonia, wounds, stomach pain
Osyris lanceolata	Same district	Root barks	refreshment

3.1.1 Antibacterial synergic activity of combined herbal tea extracts with *Cymbopogon citratus* extracts

Cymbopogon citratus (lemmon grass) originated in India and adapted to other tropical countries including Tanzania contains lemony flavor which bring suitable flavor when mixed in a tea; both black and herbal tea. In this study, *C. citratus* extracts were combined with extracts of plant parts usually used in tea making and these are *O. gratissimum* leaf, *T. fassoglensis* tuber, *A. nilotica* stem bark and *O. lanceolata* root bark. The combined extracts were evaluated for antibacterial activity to establish the synergetic effects of the combined extracts (Table 4). Plants that are not usually combined with *C. citratus* in tea preparation by ethnic groups were not investigated for synergetic antibacterial effects and these are *R.vulgaris* and *S. bullatus*.

The findings revealed that some combined extracts had antibacterial synergetic effects, others antagonistic effects whilst some extracts maintained the antibacterial levels shown by the individual extracts. Combinationof *O. gratissimum* leaf and *C. citratus* dichloromethane extracts enhanced antibacterial activity of *O. gratissimum* leaves dichloromethane extracts from MIC value of 3.125 mg/mL to 0.781 mg/mL against *S. typhi*; and from 12.5 mg/mL to 1.5625 mg/mL against *K. oxytoca*. The synergistic antibacterial effect was also observed by *O. gratissimum* leaves / *C. citratus* ethyl acetate extracts and *O. gratissimum* leave /*C. citratus* aqueous extracts in which the former had effects on *P. aeruginosa* while the latter had effects on *K. oxytoca* and *P. aeruginosa* (Table 4). The aforementioned combined extracts lowered the antibacterial activity against a range of bacterial species as shown in Table 4. *T. fassoglensis* tubers aqueous extracts which had remarkable antibacterial activity against *K. oxytoca*, *S. typhi* and *P. aeruginosa* with MIC value of 0.781 mg/mL was lowered when combined with *C. citratus* aqueous extract to MIC value of 1.5625, 6.25 and 1.5625 mg/mL respectively. Surprisingly, the combinations of *O. lanceolata* and *C. citratus* extracts did not enhance or lower the antibacterial activity to MIC value lower than 1 mg/mL (Table 4). The same observation was observed with *A. nilotica/C. citratus* combined extracts.

4. DISCUSSION

Natural products derived from microorganisms, invertebrates and plants are extensively used and rapidly growing in health system in the world [7]. Among these, plants derived products have received more attention in promoting health and treatment of diseases [8]. Herbal tea is one of the plant products which have gained popularity and importance in human health system [3]. Besides of their use in refreshment, they are also used for protection and treatment of diseases worldwide [9]. The popular herbal teas such as peppermint, rosella, ginger, sage and cinnamon are involved in various human health management including, fever, cough and diarrhea [1]. In this study seven plants used as herbal teas by various ethnic groups were evaluated for antibacterial activities. The selection of these plants was based on the information obtained from the ethnic groups in Tanzania. Herbal teas which are usually used in combination with *C. citratus* to increase flavor were combined to study their synergic antibacterial effect. The result emanated from this study exhibited antibacterial activity from MIC value of 0.1953 – 25 mg/mL. Every bacterial species was susceptible to at least one plant extracts within MIC value ranging from 0.3906 – 0.781 mg/mL. According to [10] MIC values of crude extracts lower than 0.5 mg/mL are considered to show strong inhibition, 0.5 – 1.5 mg/mL moderate inhibition, and values from 1.6 mg/mL and above show weak inhibition. Hence, in the present study it was deduced that 4.8% and 24% of the tested extract exhibited strong and moderate activity respectively. Despite the fact the extracts with MIC value greater than 1 mg/mL have been reported to be of no interest in the drug discovery programmes, such extracts should also be reported as they can be incorporated with other extracts to improve it biological importance [11]. This paper elaborated the significance of extracts with MIC values less than 1 mg/mL as potential source of drug leads for the management of Gram negative bacteria.

The result obtained in this study, shown that *S. typhi* was the most susceptible to plant extracts compare to other bacteria species. *Salmonella typhi* has been reported to be resistant to many antibiotics due to its spore formation which increase its stability to external environments [12,13]. Thus extracts reported in this study to be potent against *S. typhi* are potential source of drug templates against this pathogen. On the other hand, antibacterial evaluation of plant extracts against *K. pneumoniae* was important because it has been reported by Sanchez et al. [14] to be resistant even to the third generation antibiotics like cephalosporins and carbapenem.

Table 2. Antibacterial activity of *Rhus vulgaris*, *Acacia nilotica* and *Tylosema fassoglensis*

Plant extracts	Minimum inhibitory concentration (mg/ml)						
	P. mirabilis	***K. oxytoca***	***K. pneumoniae***	***S. typhi***	***E. coli***	***P. aeruginosa***	***S. kisarawe***
RVBD	1.56	25	25	12.5	25	12.5	25
RVBE	3.13	1.56	12.5	12.5	25	3.13	12.5
RVBA	0.78	1.56	1.56	0.78	0.78	1.56	3.13
RVLD	0.39	3.13	1.56	1.56	12.5	0.39	1.56
RVLE	6.25	12.5	1.56	1.56	25	3.13	6.25
RVLA	1.56	3.13	3.13	1.56	0.78	1.56	6.25
RVRD	3.13	12.5	25	12.5	25	12.5	12.5
RVRE	3.13	6.25	25	12.5	25	12.5	6.25
RVRA	0.781	1.56	1.56	0.78	0.78	0.78	3.13
ANBD	1.56	12.5	12.5	6.25	25	3.13	25
ANBE	1.56	12.5	12.5	3.13	25	6.25	6.25
ANBA	3.13	6.25	12.5	1.56	1.56	6.25	12.5
ANRD	0.78	12.5	12.5	12.5	12.5	6.25	12.5
ANRE	3.13	6.25	12.5	12.5	25	6.25	12.5
ANRA	6.25	6.25	12.5	3.13	3.125	6.25	6.25
TFLD	1.56	1.56	1.56	0.39	12.5	6.25	1.56
TFLE	3.13	12.5	0.78	1.56	25	3.13	0.781
TFLA	0.78	1.56	0.78	0.78	0.3906	0.78	1.56
TFRD	6.25	12.5	12.5	6.25	12.5	3.13	6.25
TFRE	3.13	6.25	6.25	1.56	6.25	1.56	6.25
TFRA	6.25	0.78	1.56	0.78	1.56	0.78	3.13
GENT	0.25	0.25	0.06	0.004	0.003	0.016	0.004

Key: RVBD. R. vulgaris bark dichloromethane, RVBE. R. vulgaris bark ethyl acetate, RVBA: R. vulgaris bark aqueous, RVLC: R. vulgaris leaf dichloromethane, RVLE: R. vulgaris leaf ethyl acetate, RVLa: R. vulgaris leaf aqueous, RVRC: R. vulgaris root dichloromethane, RVRE: R. vulgaris roots ethyl acetate, RVRA: R. vulgaris roots aqueous, ANBD: A. nilotica bark dichloromethane, ANBE: A. nilotica bark ethyl acetate, ANBA: A. nilotica bark aqueous, ANRD: A. nilotica roots dichloromethane, ANRE: A. nilotica roots ethyl acetate, ANRA: A. nilotica roots aqueous, TFLD: T. fassoglensis leaf dichloromethane, TFLE: T. fassoglensis ethyl acetate ,TFLA: T. fassoglensis leaf aqueous, TFRD: T. fassoglensis root dichloromethane, TFRE: T. fassoglensis root ethyl acetate, TFRA: T. fassoglensis root aqueous, GENT: gentamycin

Table 3. Antibacterial activity of *Ocimum gratissimum*, *Sphaeranthus bullatus, Osyris lanceolata*, *Cymbopogon citratus* and *Camellia sinensis*

Plant extracts	Minimum inhibitory concentration (mg/ml)						
	P. mirabilis	***K. oxytoca***	***K. pneumoniae***	***S. typhi***	***E. coli***	***P. aeruginosa***	***S. kisarawe***
OGLD	3.13	12.5	3.13	3.13	12.5	3.13	6.25
OGLE	3.13	0.39	3.13	3.13	3.13	6.25	6.25
OGLA	6.25	6.25	12.5	1.56	6.25	6.25	12.5
OGFD	3.13	12.5	12.5	3.13	12.5	6.25	6.25
OGFE	3.13	3.13	6.25	3.13	6.25	3.13	6.25
OGFA	6.25	3.13	12.5	0.78	3.13	3.13	12.5
OGRD	3.13	>25	6.25	3.13	3.13	3.13	1.56
OGRE	1.56	6.25	6.25	3.13	6.25	6.25	6.25
OGRA	0.78	0.39	0.78	0.39	0.78	0.78	1.56
SBD	0.39	3.13	0.78	0.39	1.56	6.25	1.56
SBE	1.56	3.13	6.25	3.13	6.25	3.13	6.25
SBA	6.25	6.25	1.56	1.56	1.56	3.13	6.25
OLRD	12.5*	6.25	12.5	1.56	25	12.5	12.5
OLRE	12.5*	12.5	12.5	6.25	12.5	12.5	12.5
OLRA	12.5*	6.25	12.5	12.5	12.5	6.25	12.5
CCD	0.39	3.13	1.56	0.78	12.5	3.13	1.56
CCE	1.56	6.25	6.25	3.13	12.5	3.13	6.25
CCA	3.13	3.13	12.5	0.78	0.78	3.13	6.25
CSD	12.5	6.25	12.5	0.19	12.5	12.5	12.5
CSE	3.13	12.5	6.25	0.39	3.13	3.13	1.56
CSA	1.56	6.25	1.56	12.5	0.78	0.78	0.78
GENT	0.18	0.09	0.06	0.0039	0.006	0.003	0.0016

Key: OGLD: O. gratissimum leaf dichloromethane, OGLE: O. gratissimum leaf ethyle acetate, OGLA: O. gratissimum leaf aqueous, OGFD: O. gratissimum flower dichloromethane, OGFE: O.gratissimum flower ethyl acetate, OGFA: O. gratissimum flower aqueous, OGRD: O. gratissimum root dichloromethane, OGRE: O. gratissimum root ethyl acetate, OGRA: O. gratissimum roots aqueous, SBD: S. bullatus dichloromethane, SBE: S. bullatus ethyl acetate, SBA: S. bullatus aqueous, OLRD: Osyris lanceolata bark dichloromethane, OLRE: Osyris lanceolata bark ethyl acetate, OLRA: Osyris lanceolata bark aqueous, CCD: C. citratus dichloromethane, CCE: C. citratus ethyl acetate, CCa: C. citratus aqueous, CSD: C. sinensis dichloromethane, CSE: C. sinensis ethyl acetate, CSA: C. sinensis aqueous

: Significance difference at (P< 0.05)

Table 4. Antibacterial activity of *Ocimum gratissimum*, *Tylosema fassoglensis*, *Acacia nilotica* and *Osyris lanceolata* combined with *Cymbopogon citratus*

Comined plant extracts	Minimum inhibitory concentration (mg/ml)						
	P. mirabilis	***K. oxytoca***	***K. pneumoniae***	***S. typhi***	***E. coli***	***P. aeroginosa***	***S. kisarawe***
OGLD+ CCD	6.25	1.56	6.25	0.78	12.5	3.13	6.25
OGLE + CCE	6.25	12.5	3.13	3.13	6.25	3.13	12.5
OGLA + CCA	6.25	3.125	12.5	3.13	6.25	1.56	12.5
TFRD + CCD	12.5	6.25	3.13	3.13	12.5	12.5	12.5
TFRE + CCE	3.13	1.56	6.25	3.13	3.13	6.25	6.25
TFRA + CCA	3.13	1.56	12.5	6.25	3.13	1.56	6.25
OLRD+ CCD	12.5	6.25	12.5	6.25	12.5	12.5	12.5
OLRE + CCE	12.5	6.25	12.5	6.25	12.5	12.5	12.5
OLRA + CCA	12.5	12.5	12.5	12.5	12.5	12.5	12.5
ANBD + CCD	12.5	3.13	12.5	6.25	12.5	12.5	6.25
ANBE + CCE	12.5	6.25	6.25	3.13	6.25	6.25	6.25
ANBA + CCA	1.56	1.56	12.5	1.56	1.56	3.13	12.5
GENT	0.19	0.1	0.06	0.004	0.006	0.003	0.0016

Key: OGLD + CCD: O. gratisimum leaf dichloromethane + C. citratus dichloromethane, OGLE + CCE: O. gratissimum leaf ethyl acetate + C. citratus ethyl acetate, OGLA + CCA: O. gratissimum aqueous + C. citratus aqueous, TFRD + CCD: T. fassoglensis root dichloromethane + C. citratus dichloromethane, TFRE+ CCE: T. fassoglensis root ethyl acetate + C. citratus ethyl acetate: TFRA + CCA: T. fassoglensis root aqueous + C. citratus aqueous, OLRD + CCD: O. lanceolata root dichloromethane + C. citratus dichloromethane, OLRE + CCE: O. lanceolat root ethyl acetate + C. citratus ethyl acetate, OLRA + CCA: O. lanceolata root aqueous + C. citratus aqueous, ANBD + CCD: A. nilotica bark dichloromethane + C. citratus dichloromethane, ANBE+ CCE: A. nilotica bark ethyl acetate + C. citratus ethyl acetate: ANBA + CCA: A. nilotica bark aqueous + C. citratus aqueous, GENT: Gentamycin

It was interest of this study to establish the synergic antibacterial activity of *O. gratissimum* leaf, *O. lanceolata* root bark, *T. fassoglensis* tuber and *A. nilotica* bark combined with *C. citratus*. Apparently there is no report on the synergic antibacterial activity of these plant species when combined with *C. citratus*. It was observed that when some combinations of extract increased antibacterial activity, other combinations lowered the activity. For example *O. gratissimum* leaf and *C. citratus* extracts increased antibacterial activities against *S. typhi*, *K. oxytoca* and *P. aeuroginosa* whilst combination of *T. fassoglensis* tubers extracts lowered the antibacterial activities against the same bacterial species. The mechanisms underlying synergistic therapeutic actions of herbal medicines is that, different agents may regulate either the same or a different target in various pathways, and therefore cooperate in an agonistic synergistic way, regulating the enzymes and transporters involved in metabolism, improve oral drug bioavailability and overcome the drug resistance mechanisms, eliminates adverse effects or enhance pharmacological effectiveness [15]. Hence synergism action exhibited by the extracts in this study might be due to these factors.

5. CONCLUSION

The current study has established that herbal teas can be consumed not only as refreshment but also as a remedy for Gram negative bacterial infections. Addition of *C. citratus* to herbal tea either enhances or lowers the antibacterial activity of the herbal tea. It therefore recommended that when herbal tea is consumed as a remedy, incorporation of *C. citratus* should only be necessary when it induces synergistic effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ravikumar C. Review on herbal teas. J. Pharm. Sci. and Res. 2014; 6(5):236-238.
2. Hirata AH, Ayabe S. Anti-oxidative and antihydrogen peroxide activities of various herbal teas. Food Chemistry. 2007;103(2): 617–622.
3. Shaw YAB, Salmon CNA, Green CE, Hibbert SL, Smith AM, Williams LAD. Evaluation of the nutraceutical potential of *Rytidophyllum tomentosum* (L.) Mart. HPTLC fingerprinting, elemental composition, phenolic content and *in vitro* antioxidant activity. Pharmaceutical Crops. 2014;3(47):2210-12.
4. Marete EN, Jacquier JC, O'Riordan D. Feverfew as a source of bioactive for functional foods storage stability in model beverages. J. Funct. Foods. 2011;3:38-43.
5. CBI. Market Information Database. Coffee, tea and cocoa. CBI Market Survey: The EU Market for Herbal Infusion. 2009;18.
   Available:www.cbi.eu Or http://www.cadexco.bo/-
6. Ellof JN. A quick Micro plate Method to determine the minimum inhibitory sensitive and concentration of plant extracts for bacteria. Planta Medica. 1998;64:711-713.
7. Ji HF, Li XJ, Zhang HY. Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia?. EMBO Rep. 2009;10(3):194-200.
8. Nautiyal OH. Natural products from plant, microbial and marine species. Experiment. 2013;10(1):611-646.
9. Bansal S, Choudhary S, Sharma M, Kumar SS, Lohan S, Bhardwaj V, Syan N, Jyoti S. Tea: A native source of antimicrobial agents. Food Research International. 2013;53:568-584.
   DOI: 301 10.1016/j.foodres.2013.01.032
10. Aligiannis N, Kalpotzakis E, Mitaku S, Chinou IB. Composition and antimicrobial activity of the essential oils of two *Origanum* species. J. Agric. Food Chem. 2001;40:4168-4170.
11. Rios J, Recio M. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology. 2005;100(1):80-84.

12. Baltazar M, Ngandjio A, Holt KE, Leppilet E, Gandara MP, Collard JM, Bercion R, Nzounkeu A, Hello S, Dougan G, Fonkoua MC, Weill FX. Multidrug resistant *Salmonella enterica* serotype typhi. EID. 2015;21(4):1-5.
13. De Pasquale R, Germano MP, Keita A, Sanogo R, Lauk U. Anti ulcer activity of *pteleopsis suberosa*. J. Enthnopharmcol. 2001;47:55-558.
14. Sanchez GV, Master RN, Clark RB, Fyyaz M, Duvvuri P, Ekta G, Bordon J. *Klebsiella pneumonia* antimicrobial drug resistance. EID. 2013;19(1):1-4.
15. Ochwang'i DO, Kimwele CN, Oduma JA, Gathumbi PK, Mbaria JM, Kiama SG. Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. Ethnopharmacology. 2014; 15:1040–10 55.

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