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Chemical compositions and antimicrobial activities of *Athrixia phylicoides* DC. (bush tea), *Monsonia burkeana* (special tea) and synergistic effects of both combined herbal teas

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ABSTRACT

Objective: To determine the chemical compositions and evaluate the antimicrobial activity of bush tea (*Athrixia phylicoides* DC.), special tea (*Monsonia burkeana*) and synergy (combination of bush tea and special tea).
Methods: Total polyphenols were determined using the methods reported by Singleton and Rossi (1965) and modified by Waterman and Mole (1994). Tannins were determined using vanillin HCL methods described by Prince *et al.* (1978). Total antioxidants were determined using the methods described by Awika *et al.* (2004). The micro dilution technique using 96-well micro-plates, as described by Eloff (1998) was used to obtain the minimum inhibition concentration (MIC) and minimum microbicidal concentration (MMC) values of the ethanol extracts against the microorganisms under study. The microbes strain used was Gram negative bacteria such as *Escherichia coli, Klebsiella oxytoca, Proteus vulgaris, Serratia marcescens, Salmonella typhi, Klebsiella pneumonia*; Gram positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus* and a fungus *Candida albicans*.
Results: The results demonstrated that special tea contains significantly higher content of total polyphenols (8.34 mg/100 g) and total antioxidant (0.83 mg/100 g) as compared to bush tea [total polyphenols (6.41 mg/100g) and total antioxidant (0.63 mg/100g)] and combination of bush tea and special tea [total polyphenols (6.42 mg/100 g) and total antioxidant (0.64 mg/100 g)]. There was no significant difference in tannins between bush tea, special tea and synergy. The results of antimicrobial activity (MIC and MMC) demonstrated that the ethanol extracts of bush tea, special tea and synergy possessed antimicrobial activity against all microorganisms at different zones. The MIC of bush tea ranged from 1.56 to 12.50 mg/mL while the MMC ranged from 0.78 to 12.50 mg/mL. Special tea´s MIC ranged from 0.39 to 12.50 mg/mL while the MMC ranged from 0.01 to 12.50 mg/mL. The MIC of synergy ranged from 3.13 to 12.50 mg/mL while the MMC ranged from 3.13 to 12.50 mg/mL without positive synergistic effect recorded.
Conclusions: Both bush and special tea contain total polyphenols, total antioxidants and tannins with special tea containing a significantly higher total polyphenols and total antioxidant as compared to bush tea and synergy. Bush tea, special tea and synergy possess antimicrobial activity at various degrees.

1. Introduction

Plants have history in proving inspiration as source of novel drug compounds[1]. For centuries, medicinal plants have been the main source for drug development and for treatment of various human diseases[2,3]. Plant derived medicines have been reported to have large contribution towards human health and well being and play important role in traditional health care in most parts of the world[1,4]. Plants are used as alternative medicines against infectious diseases by those who cannot afford or don't have access to antibiotics[5]. Aromatic and medicinal plants are sources of

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diverse compounds that have strong antioxidant activities and display antimicrobial properties[6,7]. Medicinal plants are an important source of phytochemicals that offer traditional medicinal treatment of various ailments[8]. Medicinal plants have been in use in one form or another, under indigenous systems of medicine[9]. Herbal medicines that are extracted from different plant parts such as leaves, roots, bark seeds, and flowers contain a variety of naturally–occurring bio–chemicals, which contribute to the plant's medicinal benefits[10]. The crude plant extracts of herbal plant in the form of infusion, decoction, tincture or herbal extract are traditionally used for the treatment of diseases, including infectious diseases[3].

An herb is described as a plant or parts of a plant valued for its medicinal, aromatic, or savory qualities and are eaten, swallowed, drunk, inhaled, or applied topically to the skin[10]. The medicinal plants used as traditional medicine are rich in secondary metabolites that serve as the main indicators of the medicinal potential[11,12]. Plants secondary metabolites have therapeutic effect and pharmacological properties[2,13]. Herbal medicines are also known as herbal remedies, herbal medicinal products, phytopharmaceuticals, phytotherapeutic agents and phytomedicines[14]. *In vitro* studies showed that plants were rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids that possess antimicrobial properties[5]. In addition the presence of metabolites such as saponins, tannins, alkaloids, flavanoids, steroids and cardiac glycosides in the medicinal plants present pharmacological significance[15].

Bush tea is used as a herbal tea and traditional medicines[16,17], for treatment of various ailments such as boils, acne, infected wounds, cuts, headaches, colds, loss of voice and throat infection as a gargle[18–20], hypertension, heart disease and diabetes[17]. The Vhavenda people are reported to use extracts from soaked roots and leaves as antihelminthic[21]. It is also used for cleansing or purifying the blood[18,19]. Special tea is also used to cure various sexually transmitted diseases, for blood cleansing, to improve erectile dysfunction and to enhance libido in males[22,23].

The increasing number of diseases, challenges to access to modern healthcare and high cost associated with the use of modern healthcare providing a need to investigate the antimicrobial activities of indigenous plants. Data that describe the chemical compositions of bush tea and special tea have been well documented, however, the synergistic effect of bush tea and special tea as well as microbial activities is lacking. Therefore, the objectives of this study were to determine the chemical composition, antimicrobial activities of bush tea, special tea and synergistic effect of bush tea and special tea.

2. Materials and methods

2.1. Plant material

The plant materials were collected from Limpopo Province of South Africa. Bush tea was collected from Haenerstburg (23°56′ S; 29°54′ E; 890 m.a.s.l.) while special tea plant was collected from Lebowakgomo (−24.9′ S; 29.29′ E; 1 206 m.a.s.l) during May 2013.

2.2. Preparation of samples for phenolics, tannins and antioxidants

The 4 g of dried samples of bush tea leaves, special tea and bush and special tea (50:50, 4 g) were weighed and transferred to a warring commercial blender (Instrulab, Johannesburg, South Africa) containing 100 mL of methanol, and then blended at a high speed for 2 min (stopping occasionally to avoid accumulation of fumes). The mixture was removed and let to stand in the beaker to achieve separation. After 6–8 min, the supernatant was collected, centrifuged at 6000 r/min (SS34 rotor) for 10 min and stored. The residues were blended again with 50 mL methanol, supernatant collected as above, combined with the first one, filtered with MN–615 (240 mm) filter papers (Bethlehem, USA) and stored at −4 °C until analysis.

2.3. Determinations of total polyphenols, tannins and antioxidants

Total polyphenols were determined using the methods reported by Singleton VL *et al*.[24] and modified by Waterman PG *et al*[25]. Tannins were determined using vanillin HCL methods[26], and total antioxidants were determined using the methods described by Awika JM *et al*[27].

2.4. Preparation of extracts for antimicrobial activity

The plant materials were air dried at room temperature for two weeks and then ground to a fine powder. The 50 g powder of each plant was then separately soaked in 40 mL of ethanol and shaken at room temperature for 48 h. The extract was filtered and the solvent was evaporated on a rotary-evaporator under reduced pressure at 370 °C. The extract was stored in the cold room at 5 °C after which they were subjected to antimicrobial tests.

2.5. Determination of minimum inhibitory concentration (MIC) and minimum microbial concentration (MMC)

The *in vitro* antimicrobial activity of bush tea and special tea ethanol extracts was conducted against various microbes. The micro dilution technique using 96-well micro-plates, as described by Eloff JN was used to obtain the MIC and MMC values of the ethanol extracts against the microorganisms under study[28]. Extracts were serially diluted in the 96-well plate. The final concentration of extracts and positive control ranged from 0.196 mg/mL to 12.5 mg/mL. Microorganisms that were 48 h old were added into the 96-well plates and incubated for 24 h at 37 °C. The MIC was determined by adding 40 μL of (0.2 mg/mL) *p*-iodonitrotetrazolium violet (Sigma-Aldrich, South Africa) to micro-plate wells and incubated at 37 °C for 24 h. MIC is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms[13]. The MMC was determined by adding 50 μL of the suspensions from the wells, which did not show any growth after incubation during MIC assays, to 150 μL of fresh broth. These suspensions were re-incubated at 37 °C for another 24 h. The MMC was determined by adding 40 μL of (0.2 mg/mL) *p*-iodonitrotetrazolium violet (Sigma-Aldrich, South Africa) to micro-plate wells and incubated at 37 °C for 24 h. The MMC was determined as the lowest concentration of extract which inhibited 100% growth of microorganisms[29].

2.6. Microbial species

The microbes strains used were the Gram negative bacteria such as *Escherichia coli* (*E. coli*), *Klebsiella oxytoca* (*K. oxytoca*), *Proteus vulgaris* (*P. vulgaris*), *Serratia marcescens* (*S. marcescens*), *Salmonella typhi* (*S. typhi*), *Klebsiella pneumonia* (*K. pneumonia*); Gram positive bacteria such as *Bacillus cereus* (*B. cereus*), *Staphylococcus aureus* (*S. aureus*) and fungus such as *Candida albicans* (*C. albicans*). All organisms were grown in casein-peptone soy agar medium [Merck (Pty) Ltd.]. The bacterial concentration (4×10^7) was determined by using McFarland standard (1).

2.7. Statistical analysis

Data were subjected to analysis of variance using general linear model of statistical analyses system version 9.4.

3. Results

Table 1 indicates the chemical composition of bush tea, special tea and synergy. The results showed that special tea contains significantly higher content of total polyphenols (8.34 mg/100 g) compared to bush tea (6.41 mg/100 g) and synergy (6.42 mg/100 g). Total antioxidant content in special tea (0.83 μmol/g) was significantly higher than in bush tea (0.63 μmol/g) and synergy (0.64 μmol/g). The results also showed that there was no significant difference in tannin content of bush tea, special tea and synergy. However, tannins concentration on bush tea, special tea and synergy was less than total polyphenols and total antioxidant.

Table 1

Chemical composition of bush tea, special tea and the synergy.

Herbal tea types	Total polyphenols content (mg/100 g)	Total antioxidants (μmol/g)	Tannin contents (mg/100 g)
Bush tea	6.41^b	0.63^b	0.34^a
Special tea	8.34^a	0.83^a	0.34^a
Bush and special tea	6.42^b	0.64^b	0.34^a
Significant level	0.0001	0.0001	0.5038

Means with different letters along the same column are significantly different (P<0.01).

The results of MIC illustrated in Table 2 show the selective zones of bush tea (1.56 to 12.50 mg/mL), special tea (0.39 to 12.50 mg/mL) and synergy (3.13 to 12.50 mg/mL) against the microbes tested. Special tea was found to be significantly more effective than bush tea on *E. coli*, *K. oxytoca*, *P. vulgaris*, *B. cereus*, *S. marcescens*, *K. pneumonia* and *S. aureus*. There was no significant difference shown on the effectiveness of microbial inhibition by the two tea types on *S. typhi* and *C. albicans*. The results also showed that there was no positive synergistic effect found between bush tea and special tea on microbial inhibition (Table 2).

Table 2

MIC of bush tea, special tea and synergy against selected microorganisms.

Microbes	Minimum inhibition concentration (mg/mL)		
	Bush tea	Special tea	Synergy
E. coli	3.13^a	0.78^b	3.13^a
K. oxytoca	1.56^b	0.39^c	3.13^a
P. vulgaris	6.25^a	1.56^c	3.13^b
B. cereus	6.25^b	3.13^c	12.50^a
S. marcescens	12.50^a	6.25^b	12.50^a
S. typhi	12.50^a	12.50^a	12.50^a
S. aureus	12.50^a	6.25^b	12.50^a
K. pneumonia	12.50^a	3.13^b	12.50^a
C. albicans	12.50^a	12.50^a	12.50^a

Means with different letters along the same row are significantly different (P<0.01).

The results of MMC illustrated in Table 3 show that bush tea, special tea and synergy possess antimicrobial activity at selective zones against the microbes tested. The MMC values ranged from 0.78 to 12.50 mg/mL, 0.01 to 12.50 mg/mL and 3.13 to 12.50 mg/mL respectively for bush tea, special tea and synergy. Bush tea was found to be significantly effective than special tea on *S. typhi*, *S. marcescens* and *C. albicans* whilst special tea was more effective on *E. coli*, *K. oxytoca*, *P. vulgaris*, *B. cereus* and *S. aureus*. There was no significant difference shown on the effectiveness of microbicidal effect by the two tea types on *K. pneumonia*. There was no positive microbicidal synergistic effect found between bush tea and special tea.

Table 3

MMC of bush tea, special tea and synergy against selected microorganisms.

Microbes	Minimum microbicidal concentration (mg/mL)		
	Bush tea	Special tea	Synergy
E. coli	0.78[b]	0.01[c]	3.13[a]
K. oxytoca	0.78[b]	0.39[c]	3.13[a]
P. vulgaris	6.25[a]	1.56[c]	3.13[b]
B. cereus	12.50[a]	3.13[b]	12.50[a]
S. marcescens	1.56[a]	6.25[b]	12.50[a]
S. typhi	0.78[b]	12.50[a]	12.50[a]
S. aureus	12.50[a]	6.25[b]	12.50[a]
K. pneumonia	3.13[b]	3.13[b]	12.50[a]
C. albicans	3.13[b]	12.50[a]	12.50[a]

Means with different letters along the same row are significantly different ($P<0.01$).

4. Discussion

The results of this study presented concur with the study of Mamphiswana ND *et al*.[22] who reported that special tea leaves contained total polyphenols (1.23 mg catechin equivalents/100 g) and Maudu ME *et al*.[30] who reported that the wild bush tea contained total polyphenols (4.60 mg/100 g). Plant polyphenols are aromatic hydroxylated compounds that are most potent and therapeutically useful bioactive substances[31]. The difference in the concentrations of total polyphenols concurs with the results of Achakzai AK *et al*.[32] who reported that the secondary metabolites may vary between plant of the same species and of different species.

Antioxidants play an important role in neutralizing oxidative damage caused by free radicals in blood, cells, and tissue fluids[33]. Our results suggest that special tea contains a significantly higher content of antioxidant activities compared to bush tea and synergy. It has been reported that the primary source of total antioxidant activity on special tea was total polyphenols[22]. Therefore, the significantly higher antioxidant activity found in special tea could be related with the significantly higher content of polyphenols recorded.

The tannin contents of bush tea, special tea and synergy was found to be 0.34 mg/100 g. Tannins are found in all plants and are considered main indicators of medicinal potential of a plant[34,35]. They are plant polyphenols that either bind and precipitate or shrink proteins[34]. Low tannin content is considered advantageous for people with digestive problems because tannins bind iron and reduce the absorption of non-heme iron[36]. On the other hand, high tannin content contributes to bitter, astringent taste of tea[30]. The astringency from the tannins causes the dry and puckery feeling in the mouth after consumption of red wine, strong tea, or an unripened fruit[34].

Results of the present study show that bush tea, special tea and synergy possess antimicrobial activity on selected zones. This implies that the antimicrobial activities of the two types of herbal teas differ according to microbial species tested. Similar results were reported by Albayrak S *et al*.[37] on antimicrobial effectiveness of *Mentha piperita*, *Thymus vulgaris*, *Melissa officinalis*, *Ocimum basilicum*, *Rosmarinus officinalis* and *Salvia officinalis* herbal plants. Variation of antibacterial and antifungal activities has also been reported to occur even within plants of the same species[38]. Composition of active plant compounds has also been reported to play a role in the antimicrobial activity[3]. In addition, bacterial inhibition may also vary according to plant extract; the solvent is used for extraction and the organism tested[39]. Polyphenols content are related to the antibacterial activity of tea extracts[40]. Our results indicated that special tea had significantly high polyphenols content compared to bush tea. Therefore, the differences in antimicrobial activity between bush tea and special tea may be attributed to different concentration of polyphenols.

Synergistic effect occurs when the effect of two drugs together is greater than the effect of either alone while indifference occurs when the effect of two drugs together is less than the effect of either alone[41]. The results of our study revealed that the synergy of bush tea and special did not yield positive synergistic effect on microorganism tested. Similar results have been reported by Eja ME *et al*.[42] who reported that combination of herbal plant species (*Allium sativum* and *Gongronema latifolium*) may not always yield the desired effect on both Gram positive and Gram negative bacteria. The current results therefore suggest that the combination of bush tea and special tea possesses antimicrobial activity without positive synergistic effect. The results of synergy indicate an increased MIC and MMC zones against the microbes tested as compared to individual plant. Our results contrast those reported by Das S *et al*.[43] that combinations of the species in several cases demonstrated synergistic or additive effects on microorganisms. On the other hand, our results concur with previous studies which reported that the antimicrobial activity of synergy showed selective degree of activity[44,45]. Combinations of different plants were found to inhibit the bacterial growth with variation in their effectiveness[46].

In conclusions, bush tea contains total polyphenols (6.41 mg/100 g), total antioxidants (0.63 mg/100 g), tannins (0.34 mg/100 g); Special tea contains total polyphenols (8.34 mg/100 g), total antioxidants (0.83 mg/100 g), tannins (0.34 mg/100 g) and synergy of bush tea and special tea contains polyphenols (6.42 mg/100 g), total antioxidants (0.64 mg/100 g) and tannins (0.34 mg/100 g). The antimicrobial activity guided assay of the two plant extracts revealing significant antimicrobial activity against microbes tested. Results of synergy suggest that combination of bush tea and special tea also possesses antimicrobial activity without positive synergistic effect. However, it is worthy to determine the effects of agricultural practices on the antimicrobial activity of bush tea and special tea. The antimicrobial activity of the two plants evaluated suggests that they possess compounds with antibacterial properties that can be used as antimicrobial agents in the development of new drugs for treatment of infectious diseases. Pharmacological evaluation such as the cytotoxicity study of the bush tea, special tea and synergy of bush tea and special tea should also be evaluated.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Dey SK, Banerjee D, Chattapadhyay S, Karmakar KB. Antimicrobial activities of some medicinal plants of West Bengal. *Int J Pharma Bio Sci* 2010; **1**: 1–10.

[2] Alla AA, Ishak CY, Ayoub SM. Antimicrobial activity of four medicinal plants used by Sudanese traditional medicine. *J Forest Prod Ind* 2013; **2**: 29–33.

[3] Wendakoon C, Calderon P, Gagnon D. Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. *J Med Active Plants* 2012; **1**: 60–68.

[4] Gupta P, Sharma A, Verma AK. GC/MS profiling and antimicrobial effect of six Indian tropical fruit residues against clinically pathogenic bacterial strain. *Int J Adv Pharm Res* 2012; **3**: 1229–1235.

[5] Sapkota R, Dasgupta R, Rawat DS. Antibacterial effects of plants extracts on human microbial pathogens & microbial limit tests. *Int J Res Pharm Chem* 2012; **2**: 926–936.

[6] Shilpi A, Shivhare US, Basu S. Supercritical CO_2 extraction of compounds with antioxidant activity from fruits and vegetables waste. *Focusing Mod Food Ind* 2013; **2**: 43–62.

[7] Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak J Pharm Sci* 2009; **22**: 102–106.

[8] Maobe MA, Gatebe E, Gitu L, Rotich H. Preliminary phytochemical screening of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii Region, Southwest Kenya. *Eur J Appl Sci* 2013; **5**: 1–6.

[9] Mukherjee PK, Venkatesh M, Kumar V. An overview on the development in regulation and control of medicinal and aromatic plants in the indian system of medicine. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 2007; **6**: 129-136.

[10] Folashade KO, Omoregie EH, Ochogu AP. Standardization of herbal medicines. *Int J Biodivers Conserv* 2012; **4**: 101–112.

[11] Savithramma N, Rao ML, Suhrulatha D. Screening of medicinal plants for secondary metabolites. *Middle–East J Sci Res* 2011; **8**: 579–584.

[12] Mudau FN, Soundy P, du Toit ES, Olivier J. Variation in polyphenolic content of *Athrixia phylicoides* DC. (Bush tea) leaves with season and nitrogen application. *S Afr J Bot* 2006; **72**: 398–402.

[13] Sen A, Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. *Int J Curr Pharm Res* 2012; **4**: 67–73.

[14] Hussain K, Majeed MT, Ismail Z, Sadikun A, Ibrahim P. Complementary and alternative medicine: quality assessment strategies and safe usage. *South Med Rev* 2009; **2**: 19–23.

[15] Singh K, Tiwari V, Prajapat R. Study of antimicrobial activity of medicinal plants against various multiple drug resistance pathogens and their molecular characterization and it's bioinformatics analysis of antibiotic gene from genomic database

with degenerate primer prediction. *Int J Biol Technol* 2010; **1**: 15–19.

[16] Nchabeleng L, Mudau FN, Mariga IK. Effects of chemical composition of wild bush tea (*Athrixia phylicoides* DC.) growing at locations differing in altitude, climate and edaphic factors. *J Med Plants Res* 2012; **6**: 1662–1666.

[17] Rampedi I, Olivier J. The use and potential commercial development of *Athrixia phylicoides*. *Acta Academica* 2005; **3**: 165–183.

[18] Mabogo DE. The ethnobotany of Vhavenda [dissertation]. Pretoria: University of Pretoria; 1990.

[19] Roberts M. *Indigenous healing plants*. South Africa: Southern Book Publishers; 1990.

[20] Joubert E, Gelderblom WC, Louw A, de Beer D. South African herbal tea: *Aspalathus linearis*, *Cyclopia* spp. and *Athrixia phylocoides*–a review. *J Ethnopharmacol* 2008; **119**: 376–412.

[21] Mbambezeli G. *Athrixia phylicoides*. Kirstenbosch NBG: South African National Biodiversity Institute; 2005.

[22] Mamphiswana ND, Mashela PW, Mdee LK. Distribution of total phenolics and antioxidant activityin fruit, leaf, stem and root of *Monsonia burkeana*. *Afr J Agri Res* 2010; **5**: 2570–2575.

[23] Mamphiswana ND, Mashela PW, Mdee LK. Accumulative capabilities of essential nutrient elements in organs of *Monsonia burkeana*. *Afr J Biotechnol* 2011; **10**: 16849–16853.

[24] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am J Enol Vitic* 1965; **16**: 144–158.

[25] Waterman PG, Mole S. *Analysis of phenolic plant metabolites*. Oxford: Alden Press Limited; 1994.

[26] Prince ML, Van Scoyoc S, Butler LG. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J Agric Food Chem* 1978; **26**: 1214–1218.

[27] Awika JM, Rooney LW, Waniska RD. Anthocyanins from black sorghum and their antioxidant properties. *Food Chem* 2005; **90**: 293–301.

[28] Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med* 1998; **64**: 711–713.

[29] Cohen MA, Husband MD, Yoder SL, Gage JW, Roland GE. Bacterial eradication by clinafloxacin, CI–990, and ciprofloxacin employing MBC test, *in–vitro* time kill and *in–vivo* time–kill studies. *J Antimicrob Chemother* 1998; **41**: 605–614.

[30] Maudu ME, Mudau FN, Mariga IK. Quality profiles of cultivated and wild bush tea (*Athrixia phylicoides*) harvested at various phenological stages. *Int J Agric Biol* 2012; **14**: 552–556.

[31] Apak R, Güçlü K, Demirata B, Özyürek M, Çelik SE, Bektaşoğlu B, et al. Comparative evaluation of various total antioxidant apacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* 2007; **12**: 1496–1547.

[32] Achakzai AK, Achakzai P, Masood A, KayanI SA, Tareen RB. Response of plant parts and age on the distribution of secondary metabolites on plants found in Quetta. *Pak J Bot* 2009; **41**: 2129–2135.

[33] Saleh MA, Clark S, Woodard B, Deolu–Sobogun SA. Antioxidant and free radical scavenging activities of essential oils. *Ethn Dis* 2010; **20**: 78–82.

[34] Ashok PK, Upadhyaya K. Tannins are astringent. *J Pharmacogn Phytochem* 2012; **1**: 45–50.

[35] Hirasawa M, Takada K, Makimura M, Otake S. Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. *J Periodontal Res* 2002; **37**: 433–438.

[36] Bukochova MA, Skobeleva NI. The biochemistry and technology of tea manufacture. *Crit Rev Food Sci Nutr* 1980; **12**: 303–370.

[37] Albayrak S, Aksoy A, Albayrak S, Sagdic O. *In vitro* antioxidant and antimicrobial activity of some Lamiaceae species. *Iran J Sci Technol* 2013; **37**: 1–9.

[38] Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J Agric Sci* 2008; **4**: 839–843.

[39] Dogruoz N, Zeybek Z, Karagoz A. Antibacterial activity of some plant extracts. *IUFS J Biol* 2008; **67**: 17–21.

[40] Erol NT, Sari F, Polat G, Velioğlu YS. Antioxidant and antibacterial activities of various extracts and fractions of fresh tea leaves and green tea. *Tarim Bilimleri Dergisi* 2009; **15**: 371–378.

[41] Rakholiya K, Chanda S. *In vitro* interaction of certain antimicrobial agents in combination with plant extracts against some pathogenic bacterial strains. *Asian Pac J Trop Biomed* 2012; **2**: S876–S880.

[42] Eja ME, Arikpo GE, Enyi–Idoh KH, Ikpeme EM. An evaluation of the antimicrobial synergy of garlic (*Allium sativum*) and utazi (*Gongronema latifolium*) on *Escherichia coli* and *Staphylococcus aureus*. *Malays J Microbiol* 2011; **7**: 49–53.

[43] Das S, Anjeza C, Mandal S. Synergistic or additive antimicrobial activities of Indian spice and herbal extracts against pathogenic, probiotic and food–spoiler micro–organisms. *Int Food Res J* 2012; **19**: 1185–1191.

[44] Karmegam N, Karuppusamy S, Prakash M, Jayakumar M, Rajasekar K. Antimicrobial potency and synergistic effect of certain plant extracts against food–borne diarrheagenic bacteria. *Int J Biomed Pharm Sci* 2008; **2**: 88–93.

[45] Mabrouk MI. Synergistic and antibacterial activity of six medicinal plants used in folklore medicine in Egypt against *E. coli* O157: H7. *J Appl Sci Res* 2012; **8**: 1321–1327.

[46] Hariharan AG. Synergistic antibacterial activity of some medicinal plants against pathogenic bacterial and fungal strains. *Pharm Sci* 2012; **1**: 11–14.