

## **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:** *Athrixia phylicoides* and *Monsonia burkeana* are indigenous plants of South Africa predominately used as traditional herbal teas. The objective of current study was to evaluate and compare the antimicrobial activity of *A. phylicoides* and *M. burkeana* on various selected human pathogenic bacteria such as *Escherichia coli*; *Klebsiella oxytoca*; *Proteus vulgaris*; *Serratia marcescens*; *Salmonella typhi*; *Staphylococcus aureus*; *Klebsiella pneumoniae* and fungi such as *Candida albicans*. The minimum inhibition concentration (MIC) and minimum microbial concentration (MMC) of ethanol extract were determined against human pathogens using micro-plate's dilution method. The results have demonstrated that the ethanol extracts of selected plants possessed antimicrobial activity of selected pathogens at certain different level of concentrations. In addition the results demonstrated that the MIC and MMC was also different according to the pathogens, plants species and concentration of plant extract. The future prospects will be to determine the effects of agricultural practices on the antimicrobial activity of *A. phylicoides* and *M. burkeana*.

### **1. Introduction**

Plants have history in proving inspiration as source of novel drug compound (Dey et al., 2010). For centuries, medicinal plants have been main source for drug development (Alla et al., 2013) and for treatment of various human diseases (Wendakon et al., 2012). Plant derived medicines have been reported to have large contribution towards human health and well being (Dey

et al., 2010). According to (R'ios and Recio, 2005) it was accepted that certain plants had healing potential and contained antimicrobial principles before mankind discovered the existence of microbes.

Plants with therapeutic properties are known as medicinal plant (Akharaiyi et al., 2012) or herbs (Walter et al., 2011). Herbal medicines are also known as herbal remedies, herbal medicinal products, phytopharmaceuticals, phytotherapeutic agents and phytomedicines (Hussain et al., 2009). Medicinal plants have been in use in one form or another, under indigenous systems of medicine (Mukherjee et al., 2007). 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 (Wendakoon et al., 2012). Plants are used as alternative medicines against infectious diseases by those who cannot afford or don't have access to antibiotics (Sapkota et al., 2012). Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, which display antioxidant and antimicrobial properties (Sengul et al., 2009). Medicinal plants are important source of phytochemicals that offer traditional medicinal treatment of various ailments (Maobe et al., 2013). It has been scientifically proven that plants secondary metabolites have therapeutic effect (Alla, 2013) or pharmacological properties (Sen and Batra, 2012). In-vitro studies have proved that plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which possess antimicrobial properties (Sapkota et al., 2012). In addition the presence of metabolites such as saponins, tannins, alkaloids, flavanoids, steroids and cardiac glycosides in the plant are important for various pharmacological uses (Singh et al., 2010). The different plant parts used to extract for raw drugs include root, stem, flower, fruit and twigs exudates (Al-daihan and Bhat, 2012).

Many countries have been reported to predominantly use plants as their sources of medicines for treatment of various infectious diseases (Akharaiyi et al., 2012; Dogruoz et al., 2008). According to World Health organization (1998) in most developing countries about 80% of population rely on traditional medicine. Many plants are used for folk medicinal purposes (Islam

et al., 2008). It has been reported that more than 60% of the medicines currently available on the market and most of those in the late stages of clinical trials are derived from natural products (Mukherjee et al., 2007). With increasing incidence of multiple resistances in human pathogenic microorganisms there have been continuous efforts to find plants with compounds that have potential to act against multi-resistant bacteria (Aliyu et al., 2009). As a result it has become important to study the efficacy of medicinal plants extracts as potential antibacterial agents (Murali et al., 2012). A scientific and systematic investigation with regard to the various biological activities of *A. phyllicoides* and *M. burkeana* is lacking. The rationale of this study was to scientifically evaluate the traditional use of the two selected plants species predominately used as herbal teas in South Africa, by determining their antimicrobial activity against selected human pathogens.

## **2. Materials and methods**

### **2.1. Plant material**

Twigs from two selected plant species (*A. phyllicoides* and *M. burkeana*) traditionally used as herbal teas were collected during May 2013.

### **2.2. Preparation of extracts**

The plant materials were air dried at room temperature and ground in juke and kunkel grinder to a fine powder. The 50 g of powder was then soaked in 40 ml of ethanol and was shaken with a shaker 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 (refrigerator at 5°C after which they were subjected to antimicrobial tests.

### **2.3. Determination of minimum inhibitory concentration (MIC) and Minimum microbial concentration (MMC)**

The *In vitro* antimicrobial activity of *A. phyllicoides* and *M. burkeana* ethanol extracts was conducted against various human pathogens. The micro dilution

technique using 96-well micro-plates, as described by Eloff (1998) was used to obtain the MIC and MMC values of the ethanol extracts against the microorganisms under study. Selected extracts were serially diluted in the 96-well plate. The final concentration of extracts and positive control (CHX) ranged from 12.5 mg/ml to 0.196 mg/ml. Microorganisms (0.217 CFU/ml), that was 48 h old was added in to the 96-well plates and incubated for 24 h at 37°C. Microbial growth inhibition was determined by adding 40 µl of (0.2 mg/ml) *p*-iodonitrotetrazolium violet (INT) (Sigma–Aldrich, South Africa) to micro-plate wells and incubated at 37 °C for 24 h. Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms (Sen and Batra, 2012). 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 (INT) (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 (Cohen et al., 1998).

#### **2.4. Microbial species**

The microorganism used in the study were *Escherichia coli*; *Klebsiella oxytoca*; *Protous vulgaris*; *Serratia marcescens*; *Salmonella typhi*; *Staphylococcus aureus*; *Klebsiella pneumonia* and the fungus *Candida albicans*. All organisms were grown in casein-peptone Soy Agar medium (CASO) (Merk) SA (Pty) Ltd. The bacterial concentration was 0.217 measured using McFarland.

### **3. Results**

Table 1 presents the results of both MIC and MMC of *A. phylloides* and *M. burkeana* ethanol extracts. The results showed that both plants extract have antimicrobial activity at certain level of concentration. The MIC and MMC for both plant species ranges from 0.091 to >12.5. The MIC and MMC was

different depending on the microbes, plant species extract used and extract concentration.

The lowest concentration of *A. phyllicoides* achieved to inhibit the growth of pathogens was 1.563 mg/ml against *K. oxytoca* while the lowest concentration achieved for *M. burkeana* was 0.781 against *E. coli*. The most resistant pathogens against *A. phyllicoides* were *S. aureus* and *S. marcescens* while the most resistant pathogens for *M. burkeana* extract were *S. aureus*. The highest concentration (12.5 mg/ml) used was not efficient for inhibiting these pathogens growth. On the other hand the lowest concentration of *A. phyllicoides* achieved as microcidal was 0.781mg/ml against *E. coli*, *K oxytoca* and *S. typhi* while the lowest concentration of *M. burkeana* achieved as microcidal was 0.096 mg/ml against *E. coli*. The most resistance pathogens against *A. phyllicoides* extract was *S. aureus* while the most resistance pathogen against *M. burkeana* was *S. typhi*. The highest concentration used (12.5 mg/ml) was not efficient microcidal against these pathogens.

Table 1: Antimicrobial activity of plants extracts tested against human pathogens with zones in mg/ml

Pathogens	<i>A. phyllicoides</i>		<i>M. burkeana</i>	
	MIC	MMC	MIC	MMC
<i>E. coli</i>	3.125	0.781	0.781	0.096
<i>K.oxytoca</i>	1.563	0.781	3.125	0.391
<i>P. vulgaris</i>	6.25	6.25	1.563	1.563
<i>B. cereus</i>	6.25	12.5	12.5	3.125
<i>S. marcescens</i>	>12.5	1.563	6.25	6.25
<i>S. typhi</i>	12.5	0.781	>12.5	12.5
<i>S. aureus</i>	>12.5	>12.5	6.25	6.25
<i>K. pneumonia</i>	12.5	3.125	6.25	3.125
<i>C. albicans</i>	12.5	3.125	12.5	12.5

#### 4. Discussion

The antimicrobial activity of two selected plant species was conducted against selected human pathogens. The (MIC) and (MMC) of both *A. phyllicoides* and *M. burkeana* was determined. The results suggest that both plant species have positive antimicrobial activity against most human pathogen tested. However the antimicrobial activity of the two plant species was different of which various factors have been reported to effect antibacterial activity of plant extracts. According to Dogrouz et al., (2008) the bacterial inhibition can vary with the plant extract, the solvent used for extraction, and the organism tested. Variation in composition of active compounds and the concentration level of ethanol to achieve maximum recovery of bioactive components may also play role (Wanderkoon et al., 2012). The results of the study conducted by Wonderkoon et al., (2012) shows that the antimicrobial activity of one plant species may not be the same on different microbes. In the other hand one micro organism species may not respond to different plants in the same way. The results demonstrated that the MIC and MMC zones were decreased were the concentration of plant extract was high. This results support the findings by Shahid (et al 2013) that the inhibition zones were decreased by increasing concentration of the extract.

Plants contain natural bioactive compounds such as secondary metabolites and antioxidants (Ghasemzadeh and Ghasemzadeh, 2011). The medicinal plants used as traditional medicine are rich in secondary metabolites (Savithamma et al., 2011). According to Mudau et al., (2006) the active chemical compounds present in herbal tea serve as the main indicators of the medicinal potential due to their antioxidant activities. The antibacterial activity of tea extracts is related to the polyphenol content (Erol et al., 2009). The MIC and MMC of both *A. phyllicoides* and *M. burkeana* extracts could be attributed to the chemical properties of the two plant species. Mamphiswana et al., (2010) reported that *M. burkeana* contain total phenolics while Mudau et al., (2007) reported that *A. phyllicoides* contain high concentration of polyphenols (compare phenolics to phenolics, and polyphenols to polyphenols). The results may be linked to the observations by Archana and Abraham (2011) that green tea catechin compounds and polyphenols possess antibacterial action. This might explain the antibacterial activity of both *A. phyllicoides* and

*M. burkeana*. In addition the results also justify the traditional use of the two plants as herbal tea.

According to Savithramma (et al., 2011) plants may have different level of natural compounds. Therefore it is anticipated that different plants may have different antimicrobial activity or efficacy. The antibacterial activity guided assay of the two plant extracts have revealed highly significant antibacterial activity against most human pathogens tested. These results further support the medicinal usage of the two plant species studied. However, pharmacological evaluation such as the cytotoxicity study of the extracts should be studied.

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