*European Journal of Medicinal Plants*
*2(3): 209-215, 2012*

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# Potential Antimicrobial Plant Extract Based Therapeutics from *Temnocalyx obovatus* Roots

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***Research Article***

***Received 10th March 2012***
***Accepted 4th April 2012***
***Online Ready 29th April 2012***

## ABSTRACT

**Aims:** The study was conducted to determine antimicrobial activity of *Temnocalyx obovatus* mature root extracts popularly used in folk medicine to treat diarrhoea in chickens, stomach disorders in turkeys, goats and cows, snakebites, asthma, ulcers and whooping cough in humans.
**Study Design:** Agar disk diffusion method and determining MIC.
**Place and Duration of Study:** Department of Chemistry (Natural product section) June 2011 and July 2011.
**Methodology:** Two methods were employed for the determination of antimicrobial activities; an agar paper disc diffusion method and determination of minimal inhibitory concentration (MIC). Methanol and ethanol extracts were assayed for antimicrobial activities. The following bacterial strains were employed in the screening studies: *Staphylococcus aureus, Escherichia coli, Clostridium perfringens* and fungi: *Aspergillus niger, Candida albicans* all from stock cultures of Bindura University department.
**Results:** Methanol extract exhibited the greatest extraction efficiency; 136,1g/Kg as compared to ethanol; 124,8 g/Kg. Both methanol and ethanol extracts showed significant antibacterial and antifungal activity. Sensitivity to the extracts was not similar for the chosen strains. The highest antibacterial activity of plant extract was 37,0 mm for methanol and 28,0 for ethanol extracts for diameter of zone inhibition found against *Clostridium perfringens* followed by 35,0 mm and 26,3 diameter of zone inhibition against *Escherichia coli* at a concentration of 100%. Antifungal activity was highest for *Aspergillus niger* at a concentration of 100%. The minimum inhibitory concentration (MIC) values against these bacteria ranged from 10 - 60 µg/ml. In comparison to reference standards Gentamicin, and miconazole, methanolic extracts showed significant antimicrobial activity, student t-test, p = 0,05.
**Conclusion:** The present results support the use of aqueous extracts of *Temnocalyx*

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*obovatus* roots in folk medicine for the treatment of conditions such as diarrhoea and stomach disorders. These results encourage follow-ups through bioassay-directed isolation of the phytocompounds responsible for the potency.

*Keywords: Antibacterial; antifungal; plant extracts; therapeutics; Temnocalyx obovatus.*

## 1. INTRODUCTION

The rise in multi-drug resistant strains of pathogens has become a worldwide public health concern (Cohen, 1992; Mora et al., 2005; Nascimento et al., 2000; Navon-Venezia et al., 2005). Globalization is making the problem worse since people, animals and food products can now be transported from one continent to another in a matter of hours. This has helped carry diseases into areas where they have never been before. Infections commonly associated with third world countries are on the rise in America and Europe WHO (2000). The progressively depletion of array of drugs available to treat infectious disease as a result of microbial resistance increases the need for alternative treatments Levy (1998). Fortunately, nature offers many effective therapies that have been used successfully for years in traditional medical practices and these await scientific validation and adoption (Lee et al., 2003; Tepe et al., 2004). The use of combinatorial chemistry and synthetic libraries to generate hits is fast becoming ineffective due to rapid development of resistant pathogens leaving natural source as the only answer to this dilemma. Advances in isolation, extraction, and verification of active compounds from various herbs allows for the production of safe, potent formulas that improve the body's own defenses and have direct antimicrobial action. *Temnocalyx obovatus* (family Rubiaceae) is a herb that grows widely in Zimbabwe mainly in clumps on open woodland or grassland. It is used to treat diarrhoea in chickens and stomach disorders in turkeys, goats and cows. It can also be used as an antidote for poisoning which includes snakebites Mahamadi et al. (2011). This makes the plant an interesting object of research. In this study, we evaluated the antibacterial and antifungal activity of extracts prepared from the mature roots of *Temnocalyx obovatus* against bacterial strains, *Staphylococcus aureus, Escherichia coli, Clostridium perfringens* and fungi strains, *Aspergillus niger, Candida albicans.* The choice was done in the knowledge that these are the major strains which are fast becoming resistant to current medicines Bax et al. (2000). These strains have become resistant to drugs such as erythromycin, ampicillin and tetracycline Box et al. (2000). The chosen bacterial strains cause a lot of infection in humans. The fungal species were chosen on the basis that they cause serious systemic and skin infections in humans, especially in people living with HIV/AIDS.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The plant material was collected in May and June 2011 in Chivhu, Zimbabwe in agreement with the United Nation Convention on Biodiversity of 2003. The plant was identified by botanists at the National Herbarium and Botanic Gardens in Harare. All plant materials were dried at room temperature, powdered and sifted in a sieve (0.750 µm). Sample specimens, 2011/5 were deposited in the Bindura University natural product section for future reference.

## 2.2 Extraction of Plant Material

The roots were dried at room temperature and powdered using sterilized mortar and pestle. Powdered root sample (100g) was extracted exhaustively with 50% methanol and ethanol (500 ml cold extraction) overnight. The extract was filtered using Whatman filter paper no. 1, and concentrated on a Büchi rotary evaporator at 40ºC. The extract was kept in a deep freeze waiting use.

## 2.3 Determination of Antimicrobial Activities

The following bacterial strains were employed in the screening studies: *Staphylococcus aureus* (ATCC29213), *Escherichia coli* (ATCC25922), *Clostridium perfringens* (ATCC 36200) and fungi: *Aspergillus niger* (135550/99), *Candida albicans* (ATCC90028) all from standard stock cultures of Bindura University department who in turn obtained them from Sigma and Roth (Strasbourg, France). The choice was done in the knowledge that these are the major strains which are fast becoming resistant to current medicines Bax et al. (2000). Two methods were employed for the determination of antimicrobial activities; an agar paper disc diffusion method and determination of Minimal inhibitory concentration (MIC).

## 2.4 Agar Paper Disc Diffusion Method

Antibacterial activity of the *Temnocalyx obovatus* extracts was determined by the agar disk diffusion method according to Rubio et al. (2003) with slight modifications. A suspension of each microorganism (1 ml) was carefully mixed in a tube with 18 ml of molten Agar and then pipetted into the appropriately labeled Petri dishes. Sterile filter-paper discs (Whatman no. 1, 6 mm diameter) were impregnated with the following concentrations: 10, 20, 50 and 100% (m/v) and placed on the inoculated plates. These plates were incubated at 37ºC for 24 h. The diameters of the inhibition zones were measured in millimeters and their means were calculated as reported by (Bagamboula et al., 2004; Rubio et al., 2003). Yeasts and hyphomycetes (1 x $10^6$ colony forming units per ml) were inoculated into sterile Mueller-Hinton-agar according to Al-Fatimi et al. (2007). The plates with yeast were incubated for 48h at 36ºC and the plates with hyphomycetes for 72 h at 30ºC. Inhibition zone diameters around each of the disc (diameter of inhibition zone plus diameter of the disc) were measured). An average zone of inhibition was calculated for the three replicates. Gentamicin and miconazole were used as positive control, (10µg/ml) dissolved in the same solvent like for extract. Ethanol and methanol (50%) were used as negative controls.

## 2.5 Minimal Inhibitory Concentration (MIC) Determination

Minimal inhibitory concentrations (MICs) were determined by the agar diffusion technique as described by Rajbhandari and Schöpke (1999) with slight modifications. The MIC (minimum inhibitory concentration) corresponds to the lowest concentration of the tested extracts, able to inhibit any visible microbial growth. Several concentrations of the extracts/control were prepared (5, 10, 20, 30, 50, 60, 70, 80, 100 (µg/ml). The different solutions were agitated vigorously and each concentration was used to impregnate paper disks. Then the disks were transferred into the Petri dishes containing the microorganism test mixtures. The plates were incubated for 24 h at 37ºC for bacteria, 48 h at 36ºC for yeast, and for 72 h at 30ºC for the fungal. After incubation, the results in each plate were recorded. MIC of the extract was taken as the lowest concentration that showed a visible no growth. Growth was observed in

plates where the concentration of the extract was below the inhibitory level as indicated by the broth medium becoming cloudy.

## 2.6 Statistical Analyses

Results are presented as mean value ± standard deviation (at least three replicate experiments). Statistical analysis between treatments were determined at the significance level of P = 0.05, student t test.

## 3. RESULTS AND DISCUSSION

## 3.1 Extraction Yield

In the present study methanol extract exhibited the greatest extraction efficiency; 136,1g/Kg as compared to ethanol; 124,8 g/Kg (Table 1).

**Table 1. Extraction yield of *Temnocalyx obovatus* roots**

| Sample | Solvent | Extract yield g/Kg DW | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D |
| Roots | Methanol | 138.5 | 135.5 | 134.2 | 136.1 ± 2.2 |
| | Ethanol | 128.9 | 124.3 | 121.1 | 124.8 ± 3.9 |

*DW = dry weight, S.D standard deviation*

## 3.2 Antimicrobial Activity

The results representing antibacterial and antifungal activity of *Temnocalyx obovatus* root extracts presented in (Tables 2 and 3) show that the extracts exhibited notable antimicrobial activity against all the species tested. Sensitivity to the extracts was not similar for the chosen strains. The highest antibacterial activity of plant extract was 37,0 mm for methanol and 28,0 for ethanol extracts for diameter of zone inhibition found against *Clostridium perfringens* followed by 35,0 mm and 26,3 diameter of zone inhibition against *Escherichia coli* at a concentration of 100%. Antifungal activity was highest for *Aspergillus niger* at a concentration of 100%.

**Table 2. Antimicrobial activity of methanolic extract of *Temnocalyx obovatus* root extracts**

| Test organisms | Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | 10% | 20% | 50% | 100% | Control (10µg/ml) |
| *Staphylococcus aureus* | 15,2 ± 0,0 | 16,2 ± 0,1 | 19,3± 0,8 | 34,1 ± 0,0* | 34,5 ± 0,0 |
| *Escherichia coli* | 15,0 ± 0,4 | 15,6 ± 0,0 | 18,8± 0,0 | 35,0 ± 0,1* | 35,1 ± 0,0 |
| *Clostridium perfringens* | 16,2 ± 0,1 | 16,3 ± 0,1 | 19,8± 0,1 | 37,0 ± 0,0 | 35,3 ± 0,1 |
| *Aspergillus niger* | 14,1 ± 0,7 | 15,0 ± 0,0 | 18,1± 0,1 | 23,0 ± 0,0* | 23,1 ± 0,0 |
| *Candida albicans* | 14,2 ± 0,0 | 15,4 ± 0,1 | 18,5± 0,1 | 34,2 ± 0,1* | 34,3 ± 0,1 |

** no significant difference as compared to control. P = 0,05 Student t-test.*

**Table 3. Antimicrobial activity of ethanolic extract of *Temnocalyx obovatus* root extracts**

| Test organisms | Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | 10% | 20% | 50% | 100% | Control (10µg/ml) |
| *Staphylococcus aureus* | 14,2 ± 0,0 | 16,7 ± 0,1 | 19,1 ± 0,0 | 26,2 ± 0,1 | 34,2 ± 0,0 |
| *Escherichia coli* | 14,0 ± 0,3 | 17,1 ± 0,2 | 19,2 ± 0,0 | 26,3 ± 0,3 | 34,4 ± 0,1 |
| *Clostridium perfringens* | 15,3 ± 0,2 | 18,0 ± 0,2 | 20,9 ± 0,1 | 28,0 ± 0,2 | 36,3 ± 0,2 |
| *Aspergillus niger* | 14,5 ± 0,0 | 16,3 ± 0,0 | 19,5 ± 0,0 | 23,1 ± 0,5 | 34,8 ± 0,0 |
| *Candida albicans* | 15,2 ± 0,0 | 16,4 ± 0,0 | 19,0 ± 0,1 | 15,9 ± 0,0 | 25,7 ± 0,7 |

## 3.3 Minimum Inhibitory Concentration (MIC) Measurement

The Minimum inhibitory concentration (MIC) values of the extract are shown in (Table 4). The extract exhibited different minimum inhibitory concentrations for both bacterial and fungal strains. The MIC values ranged from 10 - 60 µg/ml for both antibacterial and antifungal activities. The lowest MIC was recorded for *Escherichia coli*. Differences in MIC values of antibacterial activity may be attributed to differential susceptibility of bacterial cell walls, which is a result of slight differences inherent in the cell wall structure (Zhao et al. 2001). Gram-positive and gram-negative microorganisms differ in many features other than the structure of their cell walls, for example the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria form a barrier to hydrophobic compounds Mazutti et al. (2008). Many similar studies reported differences in antibacterial and antifungal activity of different medicinal plant extracts and the differences were rationalized as due to difference in morphological structure of the cell membranes (Mazutti et al., 2008; Rang et al., 2001; Zhao et al., 2001). In the present study methanol extracts showed better antimicrobial activity than ethanol extracts. The most susceptible organisms to the antimicrobial activity of *Temnocalyx obovatus* were *E. coli*, *Staphylococcus aureus* and *Clostridium perfringens.* Different plant extracts have been reported for their antifungal properties (Al-Fatimi et al., 2007; Bagamboula et al., 2004; Mazutti et al., 2008), which supports our present findings.

**Table 4. Minimum inhibitory concentration (MIC) of *Temnocalyx obovatus* root extracts**

| Test organisms | MIC values of methanol extracts (µg/ml) | MIC values of ethanol extracts (µg/ml) | Control (µg/ml) |
|---|---|---|---|
| *Staphylococcus aureus* | 20 | 30 | 10 |
| *Escherichia coli* | 10 | 20 | 30 |
| *Clostridium perfringens* | 20 | 30 | 30 |
| *Aspergillus niger* | 50 | 50 | 30 |
| *Candida albicans* | 60 | 60 | 50 |

## 4. CONCLUSION

The results of the antimicrobial activity tests indicate that *Temnocalyx obovatus* root extracts exhibited higher activity against the tested strains and confirms its uses in folk medicine. The present results suggest that *Temnocalyx obovatus* root extracts are beneficial to human

health. They have the potential to be used for medical purposes such as microbiostatics, antiseptics or as disinfectants.

## ACKNOWLEDGEMENT

The authors would like to thank Mr. Maphaka, Mr. Makahamadze for technical assistance and Harare botanical garden staff for their kind help.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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