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ARTICLES

Research Articles

Palliative treatment of cancer in resource poor settings: Traditional medicine Perspective
Florence Dushimemaria and Davis R. Mumbengegwi 73

Antibacterial attributes of extracts of Phyllantus amarus and Phyllantus niruri on Escherichia coli the causal organism of urinary tract infection 80
Idayat Titilayo Gbadamosi
Palliative treatment of cancer in resource poor settings: Traditional medicine perspective

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This study aimed to determine the suitability of ethnomedicinal plants as a suitable option for palliative care of cancer in Namibia. To achieve this, key informant interviews were conducted in central and northern parts of Namibia on the use of ethnomedicinal plant products for palliation of cancer. Information from surveys on the medicinal use of plants in Oshikoto and Zambezi regions of Namibia for ailments such as tumors were used to select plants for phytochemical analysis. Plants were collected and extracts were prepared for analysis for phytochemical detection using thin layer chromatography, anti-protease, antioxidant and phytochemical quantification of Colophospermum mopane and Shinziophyton rautanenii plants. Findings from key informant interviews revealed pain management for cancer patients was the primary form of disease management at health care facilities in contrast to an established holistic palliative care system. As a result, patients looked towards alternative treatment from ethnomedicinal plant sources in their bid to palliate cancer and seek hope. Phytochemical analysis of indigenous plants collected revealed the presence of class compounds such as flavonoids, alkaloids, triterpenes, coumarins and anthraquinones as well as biological activities such as anti-protease, antioxidant properties. In conclusion, phytochemical properties of the six plants were consistent with their ethnomedicinal use, making them a suitable option for treatment of cancer in resource poor settings such as Namibia. Further studies are required to evaluate safety and mode of action.

Key words: Palliative care, Namibia, medicinal plants, anti-cancer.

INTRODUCTION

Non-communicable diseases (NCD) such as cancer, diabetes, cardiovascular disease, as well as Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) and chronic respiratory disease were responsible for over 63% of the fifty seven million mortalities that occurred in 2008 (WHO, 2011). The World Health Organization (2011) report on NCD, shows that over 85% of premature deaths from NCD occurred in low-middle income countries. In Namibia, 19% of deaths were due to cardiovascular diseases while cancer, chronic respiratory disease and diabetes contributed 3% each respectively. The incidence of cancer...
in Namibia is on the rise. Kaposi sarcoma and prostate cancers are the leading cancers among the male population while breasts and cervical cancer are more common in females (Namibian Cancer Registry, 2011). Between 2006 and 2009, the frequencies were 22.1 and 19.2%, respectively, while statistics (Namibian Cancer Registry, 2009) showed a lower proportion of 19.2 and 12%, for Kaposi sarcoma and prostate cancer in males respectively. During the period of 2006 to 2009 (Namibian Cancer Registry, 2011), the most prevalent cancers in women were breast cancer (27.6%) and cervical cancer (17.1%). During the previous reporting period (Namibian Cancer Registry, 2009), a total of 4,949 cancer cases were recorded in comparison to 6,464 total cases between the period of 2006 to 2009 (Namibian Cancer Registry, 2011). These numbers reflect that cancer is a public health concern in Namibia, and hence emphasize the necessity of care and support for individuals with life-limiting conditions (Webster et al., 2007; Thomson, 2012).

Despite the evolution of treatment options of cancer such as chemotherapy, radiotherapy, surgery, hormonal treatment or a combination of these, effective and affordable cancer treatment is still illusive for many individuals. Factors such as disease progression, type of cancer, cost of treatment, lack of cancer management professionals and resources all hamper effective cancer treatment and management. In Namibia, a patient has to go through a long period of referrals before accessing proper treatment. First report is at a local clinic, from which patient is sent to a district hospital, followed by regional district, where one can consult with an oncologist; a patient may need to be referred to the central hospital in Windhoek before effective treatment and monitoring is initiated. By the time an opportunity for effective treatment is available, prognosis is often very poor and treatment options are expensive and beyond the reach of 56% of the Namibian population who live in rural resource poor settings.

Palliative care is a multi-dimensional, multi-disciplinary approach to treatment, which focuses on alleviating symptoms and distress arising from treatment serious disease and its treatments (Powell et al., 2010), for both the patient and their relatives (WHO, 2011). The African Palliative Care Association (APCA) of Namibia, in conjunction with partners such as the Cancer Association of Namibia, the Ministry of health and social services, United States Agency for International Development (USAID), Catholic AIDS Action (CAA) and Hospices of Hope, strive to increase awareness for the need for palliative care as well as access. Despite these efforts, access to effective palliative care is still illusive in Namibia (Palliative care training ends in capital, 2014). The APCA further advocates to avail morphine in order to effectively manage suffering of patients. Options for addressing chronic pain in palliative care involve use of opioids such as morphine (Webster et al., 2007). In resource poor settings, poverty makes it hard to access effective palliative treatment (Harding, 2008). In addition to this, legislative policies are not in place, especially when it comes to inclusion of alternative therapy options such as traditional medicines, further hampering effective palliative care (Webster et al., 2007; Powell et al., 2011; Logie and Harding, 2005).

Traditionally, plants have been a vital resource of medicines for health care provision (Thomson, 2010; Malwichi-Nyirenda and Malwichi, 2010), yet there is a renewed interest in research towards the discovery and development of plant-derived medicines for mainstream usage as, many medicines in clinical use owe their origin to plants (Thomson, 2010). In addition, Sharma et al. (2011) observed increased reliance on traditional medicine evident in developing countries as a primary healthcare provision. Medicinal plants owe their chemotherapeutic properties to the presence of bioactive constituents such as alkaloids, terpenes and particularly polyphenols, which are known for antioxidant plus anti-inflammatory qualities (Farombi and Owoeye, 2011). For instance, Bernard and Alayinka (2010) reported pharmacological effects that cucurbitacins, a triterpenoid steroid conjugate possess analgesic, anti-inflammatory, antioxidant and antiproliferative activities. The antioxidant properties of plant derived medicines are capable of palliative, curative and preventative pharmacological effects for chronic disease such as cancer (Thomson, 2010). Traditional medicine has two goals: to cure disease and thereby prolong life or provide comfort and relief, therefore making disease easier to live with (Mudigonda and Mudigonda, 2010; Powell et al., 2011).

In this study, the potential usage of Namibian indigenous plants for palliative care in a resource poor setting, with emphasis on cancer and other non-communicable diseases, is discussed. Furthermore, key informant interviews were conducted. Chemical profiles of plants used as baseline data for characterization and validation of plants’ usage in traditional settings.

MATERIALS AND METHODS

Key informant interviews

The Key informants (KI) interviews were conducted with practitioners in cancer treatment and care in the Khomas and Oshana regions of Namibia on the incidences of cancer in Namibia. Five key informants (referred to as KI1, KI2, KI3, KI4 and KI5) were interviewed on the use of alternative medicines in treatment of cancer within cancer patients in Namibia.

Plant species reviewed

Plants with an ethnomedicinal background were obtained using literature search (Watt and Breyer-Brandwijk, 1962; Palgrave, 1981; von Koenen, 2001; Cheikhyousssef et al., 2011a; Cheikhyousssef et al., 2011b), and based on responses obtained in a survey conducted in the Zambezi region of Namibia (Du Preez et al.,...
Further search revealed little or no evidence of proven medicinal evidence of plants. Plant material was sustainably harvested from the Zambezi region of Namibia, using guidelines from the National Botanical Research Institute (NBRI). Voucher specimens were prepared and sent for authentication at the NBRI as different ethnic names can refer to the same plant or different names to the same plant. Confirmation of scientific names can lead to proper identification of collected plants.

Preparation of bioactive extracts

Harvested plant material was cut into small pieces and dried at room temperature for two weeks before being ground to a fine powder. The ground plant material was then used to prepare organic plant extracts using methanol as solvent and sterilized distilled water to prepare aqueous extracts. After soaking ground plant for 7 days, mixture was filtered and solvent was evaporated. This was followed with dry freezing to totally remove solvent, resulting in dry crude extracts of various plant parts, such as leaf, root, twig and bark.

Chemical profile investigation

Plants were screened for known anti-cancer properties using chemical assays such as anti-protease activity, antioxidant activity and selected phytochemical compounds. Firstly, the antioxidant and anti-protease activity of the different plant extracts was investigated using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and radiograph film with gelatin respectively. Methanolic portions of different plant parts were screened for various selected phytochemical compounds, such as flavonoids, alkaloids, triterpenoids, coumarins and anthraquinones, according to Harborne (1998). Table 1 shows the various solvent systems and chromatographic reagent used to detect the listed phytochemicals using thin layer chromatography.

Phytochemical quantification

Plant material from Colophospernum mopane and Shinziophyton rautanenii were analyzed for the total content of saponins, alkaloids and phenols. Total saponin and alkaloid was quantified using methods adapted from Edeoga et al. (2005) to determine percentage yield. Total phenolic content was analyzed using a method contained in Jing et al. (2010) with slight modifications, and gallic acid was used to develop a calibration curve in order to expresses phenolic content as gallic acid equivalents (GAE) µg/ml. Antioxidant activity was investigated using a method adopted from Jing et al. (2010) and Re et al. (1999). A standard graph developed from ascorbic acid was used to estimate the antioxidant activity. Each phytochemical was quantified in triplicate from each sample.

RESULTS

Collected plants were identified (Table 2) using local names within the community and their identity was validated scientifically by the NBRI. The validation of plant names, by scientific means affords universal comparison of the same plant, even if it’s from different communities.

Chemical profiling of the plants revealed the presence of different phytochemicals. High anti-protease and antioxidant activity was detected in all plant parts, with the exemption of Acanthosicyos naudinianus shoots, which contained no detectable anti-protease activity. Different phytochemicals such as coumarin, anthraquinone, alkaloids, flavonoids and triterpenoids were detected and were observed to be distributed in different tissues of the plants (Table 3). This development implies that the local community members may sustainably utilize plant material with the assurance that either plant parts contain potentially therapeutic phytochemicals.

Furthermore, based on literature review (Watt and Breyer-Brandwijk, 1962; Palgrave, 1981; von Koenen, 2001; Cheihkyoussef et al., 2011; Cheihkyoussef et al., 2011), responses on the use of medicinal traditional plants and phytochemical profiles of the six collected plants (Table 2), two plants; S. rautanenii and C. mopane root ant bark extracts were analyzed for the total content of alkaloids, saponins, phenols and antioxidant activity (Figure 1). Both the roots of S. rautanenii (9.64±4.6% yield) and C. mopane (11.13±2.7% yield) displayed the highest alkaloid content while the bark extract of S. rautanenii (2.08±0.5% yield) had the lowest alkaloid content. Meanwhile, the C. mopane root (205.4±9.3 GAE µg/ml) had the highest phenol content and the C. mopane bark (12.6±1.7 GAE µg/ml) extract had the lowest phenolic content. Saponin quantification revealed that the bark of S. rautanenii (13.6±10.4 %) was the highest saponin content while the root extract of S. rautanenii (1.8±0.7%) was the lowest saponin yield. The S. rautanenii root (945.6±231.1 AAE µg/ml) and bark (226.7±17.6 AAE µg/ml) extracts had the highest and lowest antioxidant activity, respectively.

DISCUSSION

Namibia has a wealth of traditional practices, which includes substantial ethnomedicine knowledge. This is passed on from a traditional knowledge holder to younger members of the community. Sadly, this does not happen in all instances, which leads to loss of traditional heritage. Namibia needs to take advantage of its current wealth of information, to properly document and validate Namibia’s heritage. This will increase awareness of traditional based medicines in the country and potentially lead to inclusion of traditional plant based therapies in mainstream medicine.

In Namibia, some individuals suffer from cancer experience chronic pain, which is partly associated with the disease and also as a result of side effects from cancer treatments. Such pain makes living with cancer difficult and can be unbearable in advanced stages of cancer. In Namibia, since many patients present very late (K11) progressive cases, at which time pain levels need to be managed to bearable levels, the availability of affordable pain relieving medications is vital. In Namibia, morphine is the medication of choice for individuals experiencing excruciating pain, in cancer patients or...
Table 1. Solvent systems and chromogenic reagents for thin layer chromatography.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Solvent system</th>
<th>Chromogenic solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Ammonium hydroxide: Methanol, 3:200</td>
<td>Dragendorff reagent</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>Ethyl acetate: Methanol:Water, 100:17:13</td>
<td>10% KOH in methanol</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>Hexane:Ethyl acetate, 17:3</td>
<td>Liebermann burchard reagent</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Chloroform</td>
<td>10% KOH in methanol</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Butanol:Aceticacid:Water, 4:1:5</td>
<td>1% Aluminium chloride in methanol</td>
</tr>
</tbody>
</table>

Table 2. Medicinal plants collected for the study, their reputed use, local names and voucher specimen numbers deposited with NBRI.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Local name</th>
<th>Family</th>
<th>Traditional use</th>
<th>Voucher specimen #</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. rautanenii</td>
<td>Mankettii</td>
<td>Euphorbiaceae</td>
<td>Sores on body surface</td>
<td>FD03</td>
</tr>
<tr>
<td>C. mopane</td>
<td>Omusati</td>
<td>Fabaceae</td>
<td>Swollen testis</td>
<td>FD02</td>
</tr>
<tr>
<td>Capparis tomentosa</td>
<td>Mudyangwe</td>
<td>Capparaceae</td>
<td>Poison, wound healing and anti-inflammatory</td>
<td>FD08</td>
</tr>
<tr>
<td>Lessertia benguellensis</td>
<td>Ndendeoma</td>
<td>Fabaceae</td>
<td>Syphilis, bloody urine and tonsils</td>
<td>FD06</td>
</tr>
<tr>
<td>Commiphora africana</td>
<td>Muwowo</td>
<td>Burseraceae</td>
<td>Abdominal cramps, anticancer and snake bite remedy</td>
<td>FD05</td>
</tr>
<tr>
<td>Acanthosicyos naudinianus</td>
<td>Muputwi</td>
<td>Curcubitaceae</td>
<td>Poison, wound healing, edible and antiinflammatory</td>
<td>FD04</td>
</tr>
</tbody>
</table>

those suffering from other NCD, according to KI5. The APCA and Namibia’s Ministry of Health and Social Services are currently working on legislation which supports availability of morphine, even to the healthcare analyst I (MIA) levels in health care institutions (KI4).

However, the effectiveness of such a strategy relies hugely on the availability of such drugs. With lacking supporting legislation (Logie and Harding, 2005) coupled with lack of monetary funds in poor resource settings, palliative care is often unattainable. According to KI1, many cancer sufferers already seek assistance from traditional healers within the Namibian setting. KI2 and KI3 however presented a contradicting picture regarding the level to which individuals seek health care from elsewhere. According to KI1, mangosteen juice, Ativan (Lorazepam) and SOLAL are among natural products in use by cancer patients in Namibia. Mangosteen juice is derived from a fruit of *Garcinia mangostana*, a tropical tree, growing in Asian countries. Numerous studies have been conducted on the chemical properties of the “Queen of fruits”. These studies reveal compounds such as many variations of xanthones (Towatana et al., 2010). Its physiological properties include, high antioxidant activity, antitumor, anti-inflammatory, antiviral, antibacterial, anti-allergy properties but are not exclusive, (Pedraza-Chaverri et al., 2008). Use of combination treatments such as lorazepam, diphenhydramine plus metoclopramide and dexamethasone, to control vomiting and nausea as side effects arising from anticancer treatments are also common (Kris et al., 1987).

A source, (Olowokudejo et al., 2008) concedes that traditional medicine is a reliable source of effective, affordable and easily accessible therapeutic entities. However, it’s important to know that effective palliative care does not take the place of a prescribed treatment regimen. A look at these six Namibian plants (Table 2) revealed the presence of different bands of phytochemical compounds, using thin layer chromatography. Phytochemical compounds have been shown to confer anti-inflammatory (Bellik et al., 2013) and analgesic properties (Ojie et al., 2013), which are all beneficial to combat NCDs. Thin layer chromatography (TLC) results suggests that different kinds of triterpenoids and anthraquinones are present, as evidenced by the numerous bands observed on TLC plates (Table 3). It follows that, observed antioxidant activity may be due to the presence of phytochemicals (Table 3). In another study conducted in Botswana (Motlhanka, 2008), *C. mopane* methanolic extracts displayed radical scavenging activity. Antioxidant activity is commonly attributed to phenolic compounds, however, spearson correlation analysis revealed a correlation=0.58 (n=12, p=0.048) between alkaloid content and antioxidant activity. Plants from the euphorbiaceae family, such as *S.*
Table 3. Phytochemical profile of medicinal plants collected from the Zambezi region, Namibia.

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Anti-protease</th>
<th>anti-oxidant</th>
<th>Coumarin</th>
<th>Alkaloid</th>
<th>Flavonoid</th>
<th>Triterpene</th>
<th>Anthraquinone</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. rautanenii</em></td>
<td>+++++</td>
<td>+++</td>
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<tr>
<td><em>S. rautanenii</em></td>
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<td>-</td>
</tr>
<tr>
<td><em>C. mopane</em></td>
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<tr>
<td><em>C. mopane</em></td>
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<td>++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>A. naudinianus</em></td>
<td>-</td>
<td>+++</td>
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<tr>
<td><em>A. naudinianus</em></td>
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<td>-</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td><em>C. africana</em></td>
<td>+++++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>C. africana</em></td>
<td>+++++</td>
<td>+++</td>
<td>+</td>
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<tr>
<td><em>L. benguellensis</em></td>
<td>+++++</td>
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<td>+</td>
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<td>+++</td>
<td></td>
</tr>
<tr>
<td><em>C. tomentosa</em></td>
<td>+++++</td>
<td>+++</td>
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<td>-</td>
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<tr>
<td><em>C. tomentosa</em></td>
<td>+++++</td>
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</tbody>
</table>

Key: ++++ = high, + = low, - = no detection.

Figure 1. Quantification of phytochemicals in the root and bark of *C. mopane* and *S. rautanenii* depicted as depicted in A) Alkaloids, B) Antioxidant activity, C) Phenolic content and D) Saponin.
rautanenii have been found to confer antineoplastic activities (Soetan and Aiyelaagbe, 2009), antioxidant activity (Dasari et al., 2012) and this was attributed to the presence of phenolic acids. The phytochemical profile (Table 3) and quantification (Figure 1) suggests that the use of these plants in the traditional setting is rational and may be useful as an anticancer traditional alternative treatment (Wahab et al., 2010).

In the traditional setting, water is used as a solvent for preparation of medicinal treatments. However, organic solvents such as methanol, dichloromethane are used because they extract bioactive compounds more efficiently (Jo et al., 2011; Govindappa et al., 2011; Elkady, 2012). In addition to water, palm wine made from a tree, locally known as makalani palm (Hyphaene petersiana) is used to prepare medicinal portions, even though this method is rare. Therefore, with the wealth of opportunities presented by Namibia’s abundance of indigenous plants, there is a need for research into the safety and efficacy of plant derived extracts using in vitro assays to determine the anticancer effects of these plants. Further studies may also lead to standardization and dosage regimen of plant based supplements for use as alternative anticancer medicinal implements.

Conclusion

Cancer is an increasingly common cause of morbidity and mortality in Namibia. Providing healthcare to cancer patients in Namibia is a challenge not only because of the lack of treatment options, but also because of the lack of financial resources for treatment. This study shows the potential of medicinal plants to provide an option for palliative care of cancer patients. Further study of such medicinal plants may result in their becoming a mainstream as either a palliative treatment option or a potential cure for some early stage cancers in rural and even urban settings.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Antibacterial attributes of extracts of *Phyllanthus amarus* and *Phyllanthus niruri* on *Escherichia coli* the causal organism of urinary tract infection

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In view of the prevalence of urinary tract infection (UTI) worldwide, the increasing resistance of pathogenic bacteria to conventional antibiotics and their side effects, the aerial part and root of *Phyllanthus amarus* (Schum. and Thonn.) and *Phyllanthus niruri* (L.) were analysed for mineral and phytochemical constituents, and their ethanol extracts screened against five clinical isolates of *Escherichia coli* associated with UTI, to ascertain their effectiveness in UTI treatment, and provide basis for future clinical trials of the two plants. The isolates (10^1 × 10^6 cfu/ml) were tested against ethanol extracts (10 mg/ml) of plant parts using agar well diffusion method. Phytochemical and mineral analyses of the plant samples were done using standard protocols and all data were statistically analysed. The quantities of various phytochemical compounds and minerals were significantly (P<0.05) higher in *P. niruri* than *P. amarus*. At 10^6 cfu/ml inoculum concentration of all isolates, the inhibitory activities of extracts of *P. amarus* and *P. niruri* were the same, and significant (P<0.05) against isolates EC01, EC02, EC04 and EC05 compared to the control experiment. The inhibitory pattern varied against EC03, extract B (29.00 mm) was the most active, followed by extract C (24.00 mm), and extracts A and D gave the same diameter (19.00 mm) of inhibition. The two plants showed significant antibacterial activity against isolates and could be good alternatives to chemical antibiotics in the treatment of *E. coli* related UTI, however the mechanism of action of the plant extracts in treatment should be investigated.

**Key words:** Antibacterial activity, *E. coli*, *Phyllanthus amarus*, *Phyllanthus niruri*, urinary tract infection.

**INTRODUCTION**

A urinary tract infection (UTI) is a bacterial infection of the urinary tract consisting of the kidneys, ureters, bladder and the urethra. An infection of the lower urinary tract is a simple cystitis (bladder infection) of the upper tract pyelonephritis (a kidney infection). Common symptoms include burning with frequent urination (or an urge to urinate) in the absence of vaginal discharge and significant pain. These symptoms may vary from mild to severe and in healthy women lasting an average of six days (Nicolle, 2008; Lane and Takhar, 2011). People...
having pyelonephritis, may experience flank pain, fever, or nausea, and vomiting in addition to the classic symptoms of a lower urinary tract infection (Colgan and William, 2011). Rarely the urine may appear bloody or contain visible pus (Lane and Takhar, 2011; Salvatore et al., 2011). Urinary tract infections occur more commonly in women than men, with half of women having at least one infection at some point in their lives. Recurrences of infection are common and risk factors include female anatomy, sexual intercourse and family history (Salvatore et al., 2011).

The main causal agent of cystitis and pyelonephritis is *Escherichia coli*, which causes of 80 to 90% of UTI (Nicolle, 2008; Salvatore et al., 2011). The increasing prevalence of antimicrobial resistance and side effects of antibiotics are major health problem worldwide. Results of multidrug resistance in *E. coli* isolates from many parts of the world have shown that the choice of drugs for the treatment of UTI is quite narrow today. Many drugs which are considered effective against uropathogens are now rarely prescribed as empirical therapy in areas where resistance rate to these antibiotics is high (Rawat and Umesh, 2010; Shalini et al., 2011). The side effects of antibiotics such as fever, nausea, diarrhoea and neurotoxicity have been reported in literature (www.bestnaturalremedies.net; Grill and Maganti 2011).

*Phyllanthus amarus* originates from tropical America, and has spread as a weed throughout the tropics and subtropics. In Africa, the plant is useful in the treatment of gonorrhoea, diarrhoea, dysentery, stomach-ache and haemorrhoids. A suppository of the leaf paste is applied to the vagina to treat amenorrhoea and polyps. Leaf sap, mixed with palm oil or not, is applied as ear drops to treat otitis and applied to abscesses, sores and wounds (Burkill, 1994). *Phyllanthus niruri* is a widespread tropical plant. It is an important plant of Indian Ayurvedic system of medicine used for problems of the stomach, genitourinary system, liver, kidney and spleen. The plant has also been used in Brazil and Peru as an herbal remedy for kidney stones (Patel et al., 2011).

In view of the prevalence of UTI in the world, the increasing resistance of pathogenic bacteria to antibiotics and side effects of antibiotics due to prolonged use, this study screened *P. amarus* and *P. niruri* for phytochemical and mineral constituents. Also the ethanol extracts of aerial parts and roots of the two plants were tested against five clinical isolates of *E. coli* associated with UTI, to ascertain their efficacy in UTI treatment, and present them as alternatives to chemical antibiotics which could also be mammalian toxic and not easily biodegradable like the botanicals.

**MATERIALS AND METHODS**

**Identification and preparation of plant materials**

Whole plants of *P. amarus* and *P. niruri* were collected from the nursery of the Department of Botany, University of Ibadan, Nigeria. The plant samples were identified and deposited in the University of Ibadan Herbarium (UIH). They were then thoroughly washed, separated into aerial parts and roots.

**Phytochemical analysis of powdered plant samples**

Powdered samples were screened for the presence of active compounds such as alkaloids, saponins, tannins, phenols and glycosides, using standard techniques (AOAC, 2005).

**Mineral analysis of powdered plant samples**

The method of Walsh (1971) was used for digestion of the two plant samples. After digestion calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), sodium (Na) and potassium (K) were analysed using Atomic Absorption Spectrophotometer (FC 210/211 VGP Bausch & Lomb). Two methods of acid digestion, using Vanadomolybdate (Yellow method). Percent transmittance was determined at 400 nm using Spectronic 20 (Bausch and Lomb) Colorimeter (AOAC, 2005).

**Preparation of extracts**

The fresh aerial parts (500 g each) and roots (500 g each) of *P. amarus* and *P. niruri* were macerated and extracted in 1000 ml of 80% ethanol for a week using cold extraction method. The extract was concentrated at 40°C, and stored in the refrigerator (4°C) prior to use. The extracts were coded as follows: A= *Phyllanthus amarus* aerial parts; B= *P. amarus* roots; C= *P. niruri* aerial parts; D= *P. niruri* roots. 10 mg/ml of each extract was used for antibacterial screening against *E. coli* isolates.

**Source of *E. coli* isolates**

The test organisms were clinical urine isolates of *E. coli* associated with UTI in female patients, obtained through due process from the University College Hospital (UCH), Ibadan, Nigeria.

**Antibacterial assay**

The isolates were maintained in cultures on nutrient agar (Difco Laboratories, USA). They were grown in nutrient broth (Difco Laboratories, USA) for 18 h at 35°C. Six concentrations of each isolate were prepared from the broth in sterile distilled water to give a range of concentrations at 10⁻¹ to 10⁶ cfu/ml via serial dilution method prior to use. Exactly 1 ml of the inoculum was thoroughly mixed with 19 ml of sterile nutrient agar and poured into sterile Petri dish. The agar was left to solidify. Two wells of 6 mm in diameter were punctured in each agar plate and 60 μl of each extract was filled into the wells with the aid of a sterile micropipette. Sterile distilled water and ethanol were used instead of extract in the control experiment. Also, plates containing the test organisms in agar without extract were used as control. All experiments were done aseptically and each experiment was replicated three times. The plates were incubated at 37°C for 24 to 48 h. The zone of inhibition was measured and recorded in millimeters (mm).

**Statistical analysis**

Analysis of variance and comparison of means were carried out on all data using Statistical Analysis System (SAS). Differences between means were assessed for significance at P<0.05 by
### Table 1. Phytochemical components of *P. amarus* and *P. niruri*.

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Alkaloids (%)</th>
<th>Saponins (%)</th>
<th>Tannins (%)</th>
<th>Phenols (%)</th>
<th>Glycosides (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. amarus</em></td>
<td>0.096±0.002</td>
<td>0.190±0.002</td>
<td>0.022±0.002</td>
<td>0.067±0.002</td>
<td>0.077±0.002</td>
</tr>
<tr>
<td><em>P. niruri</em></td>
<td>0.122±0.002</td>
<td>0.214±0.001</td>
<td>0.040±0.001</td>
<td>0.079±0.002</td>
<td>0.090±0.002</td>
</tr>
</tbody>
</table>

Values within a column followed by the same superscript are not significantly different at P < 0.05.

Duncan’s multiple range test (DMRT).

### RESULTS

The two plants contained alkaloids, saponins, tannins, phenols and glycosides in varied quantity (Table 1). Saponin was the highest phytochemical in the two plants. The saponin content of *P. niruri* (0.214 %) was higher than *P. amarus* (0.190 %). Generally, the quantity of the various phytochemicals was significantly (P<0.05) higher in *P. niruri* than *P. amarus*. The mineral analysis revealed the presence of sodium (Na), potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg), zinc (Zn), copper (Cu) and iron (Fe) in both plants (Table 2). *P. niruri* contained 46.35% of Zn whereas *P. amarus* had 28.20%. Copper was higher in *P. niruri* (5.60%) than *P. amarus* (2.20%). Overall, *P. niruri* was significantly richer in all minerals than *P. amarus*.

The extracts of the two plants showed antibacterial activity against the test organisms (Table 3). On isolate EC01, extract C was the most active (21.00 mm), followed by extract D with 14.00 mm diameter of inhibition and the least (11.00 mm) activity was observed in extract B at 10⁻¹ cfu/ml. The highest (24.00 mm) inhibitory activity against isolate EC03 at 10⁻¹ cfu/ml was from extract B, followed by extracts A and C with 14.00 mm, extract D was inactive on isolate EC03 at 10⁻¹ cfu/ml. At high concentration (10⁻¹ cfu/ml) of inoculum of isolate EC04, extracts B and D were inactive, whereas extracts A and C gave the same diameter (19.00 mm) of inhibitory activity. Although, extract B and D were inactive against isolate EC04 at high inoculum concentrations (10⁻¹ and 10⁻³ cfu/ml), their activity increased along concentration gradient to 24.00 mm at 10⁻⁵ cfu/ml. All extracts (A, B, C and D) gave the same diameter (24.00 mm) of inhibition against EC04 at 10⁻⁵ cfu/ml. Isolate EC05 was susceptible to all extracts at 10⁻¹ cfu/ml with the same diameter (24.00 mm) and extracts C and D were the most active (24.00 to 29.00 mm) against EC05 at all inoculum concentrations (10⁻¹ – 10⁻⁵ cfu/ml). Overall, the extracts (C and D) of *P. niruri* were more active than extracts (A and B) of *P. amarus* on isolates EC01, EC02, and EC05.

The comparative inhibitory effect of extracts of the aerial part and root of *P. amarus* showed that the root was inactive on isolate EC04 at 10⁻² cfu/ml (Figure 1). The root extract of *P. niruri* was more active than the
aerial part extract on isolate EC04 at $10^{-4}$ cfu/ml (Figure 2). The collective antibacterial activity of all extracts of *P. amarus* and *P. niruri* against each isolate of *E. coli* at $10^{6}$ cfu/ml is presented in Figure 3. The inhibitory activity of all extracts was the same against isolates EC01, EC02, EC04 and EC05. The inhibitory pattern of the extracts varied against EC03, extract B (29.00 mm) was the most active against it, followed by extract C (24.00 mm) and extract A (19.00 mm) and D (19.00 mm) gave the same diameter of inhibition.

**DISCUSSION**

Although, *P. amarus* and *P. niruri* are often confused as the same plant species (Taylor, 2003), this study has shown clearly that the two plants are entirely different species. They differ in their phytochemical constituents. *P. niruri* contained significantly higher quantity of alkaloids, saponins, tannins, phenols and glycosides than *P. amarus*. Many valuable compounds isolated from the two plants have been reported to be responsible for their extensive pharmacological uses (Patel et al., 2011; Damle, 2008).

The mineral components of *P. niruri* were significantly higher than that of *P. amarus*. The occurrence of these minerals in both *Phyllanthus* species indicates that the plants have nutritional and therapeutic values. As an example Zn is an essential mineral required for normal growth and development, healthy skin, infection prevention and wound healing. A zinc deficiency might cause delayed growth and development in children and adolescents, hair loss, diarrhoea, delayed wound healing, loss of appetite and weight loss. Children in developing countries who are zinc deficient might be at increased risk of infections such as pneumonia (Kirby, 2011). Zn has application in wound healing and ulcers (Patel et al., 2011). Zinc could also play a role in pneumonia prevention, and is recommended by the World Health Organization (WHO) and United Nations Children’s Fund (UNICEF) as a treatment for acute diarrhoea (www.akiliinitiative.org). Copper is an essential trace element that is vital to the health of all living things. In humans, copper is essential to the proper functioning of organs and metabolic processes (Johnson, 2008). Iron is an essential mineral needed for the formation of hemoglobin; an iron deficiency can lead to anaemia, a condition characterized by fatigue, shortness of breath, dizziness, weight loss and headaches (Kirby, 2011).

There is dearth of information in the literature on the use of *Phyllanthus* species in UTI treatment. As shown by the present study, the significant antibacterial activity of ethanol extracts of the two plants against *E. coli* is an indication of their therapeutic potential in management of UTI. Results obtained in this work agree with the findings of previous authors on antimicrobial status of *P. amarus* (Alli et al., 2011; Elddeen et al., 2011; Njoroge et al., 2012). Although, there is scarcity of information on the antimicrobial activity of *P. niruri* in the literature, it has been reported to be effective against hepatitis B and other viral infections (Bhattacharjee and Sil, 2006; Bhattacharjee and Sil, 2007). The authors suggested that *P. niruri* species might inhibit proliferation of the virus by inhibiting replication of the genetic material of the virus (Thyagarajan et al., 1988). The lipid lowering activity of *P. niruri* has been reported (Chandra, 2000), as well as its antidiabetic, antimalarial, analgesic, and anti-spasmodic properties (Raphael and Sabu, 2000; Neraliya and Gaur, 2004; Santos, 1994). The therapeutic value of herbal remedies in UTI has been reported by previous authors. Ahmed et al. (2012) reported that the administration of aqueous extract of corn silk (*Zea mays*) significantly reduced the symptoms in patient with UTI in addition to reduction in the values of pus cells, red blood cells (RBCs), and crystals, without any reported side effect which indicated its efficacy and safety. Geetha et al. (2011) reported that *Vaccinium macrocarpon* (Cranberry), *Hydrastis canadensis* (Goldenseal), *Agathosma betulina* (Buchu), *Arctostaphylos uva-ursi* (Bearberry), *Echinacea purpurea* (Cone flower) and *Equisetum arvense* (Horsetail) have been clinically proven for urinary tract infection cure as well as bladder infection treatment.

Mustard oils prepared with *Moringa oleifera* (horseradish) and nasturtium (*Tropaelum*) and grapeseed (*Vitis vinifera*) extract are effective in the treatment of UTI (www.naturalnews.com). Goldenrod (Asteraceae) is widely used in Europe.

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>%Na</th>
<th>%K</th>
<th>%Ca</th>
<th>%P</th>
<th>%Mg</th>
<th>%Zn</th>
<th>%Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. amarus</em></td>
<td>0.03±0.002</td>
<td>0.19±0.001</td>
<td>0.03±0.001</td>
<td>0.19±0.001</td>
<td>0.26±0.002</td>
<td>28.20±0.141</td>
<td>2.20±0.141</td>
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<tr>
<td><em>P. niruri</em></td>
<td>0.06±0.001</td>
<td>0.12±0.001</td>
<td>0.07±0.002</td>
<td>0.21±0.002</td>
<td>0.34±0.002</td>
<td>46.35±0.212</td>
<td>5.60±0.141</td>
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Values within a column followed by the same superscript are not significantly different at P < 0.05.
Table 3. *In-vitro* antibacterial activity of ethanol extracts of *P. amarus* and *P. niruri* against *E. coli* isolates implicated in UTI.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>E. coli/isolate</th>
<th>Inoculum load (cfu/ml) / Zone of inhibition (mm)</th>
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<tr>
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<td>1 × 10⁻¹</td>
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<td>C</td>
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<tr>
<td>D</td>
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</table>

Values are mean ± SD of three replicates. Values within a column followed by the same superscript are not significantly different at *P* < 0.05. Diameter of cork borer = 6 mm. A = *Phyllantus amarus* aerial parts; B = *Phyllantus amarus* roots; C = *Phyllantus niruri* aerial parts; D = *Phyllantus niruri* roots.

**Figure 2.** Comparative antibacterial activity of aerial part and root of *P. niruri* against *E. coli* (1 × 10⁻⁴ cfu/ml). C = aerial parts; D = roots.
as an herb of choice for the treatment of urinary tract infections; it decreases inflammation and the painful spasms of bladder infections. Dandelion (Taraxacum sp) acts as a diuretic and flushes bacteria-causing microbes from the bladder. Dandelion also provides potassium, typically lost with diuretic use. Marshmallow (Althaea officinalis) root inhibits bacterial growth in urine by increasing its acidity (www.livestrong.com).

### Conclusion

The ethanol extracts of aerial parts and roots of *P. amarus* and *P. niruri* showed significant antibacterial activity against isolates of *E. coli* (the main causative organism of UTI). Comparatively, there was no significant difference in the antibacterial activities of the extracts of the two plants; they differ significantly in their chemical and mineral constituents. The antibacterial activities of the extracts of the two plants could be attributed to their phytochemical and mineral components. The inhibitory activities of *P. amarus* and *P. niruri* extracts were the same against four of the five *E. coli* isolates, this shows that either plant species could be used in the treatment of UTI. It could be suggested that a decoction or an infusion of either of the two herbs could help in the treatment of UTI. However, an investigation of the mechanism of action of the two plants could enhance the understanding of their role in UTI treatment. Furthermore, this article provides basis for future clinical trials of compounds and extracts of the two plants in UTI patients.

### Conflict of interest

The author declares no conflict of interest.

### REFERENCES


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