

Full Length Research Paper

## 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 ( $10^{-1} \times 10^{-6}$  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

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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](http://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 prolong 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 scientific AAS). Phosphorus was determined 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 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  $10^{-6}$  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).

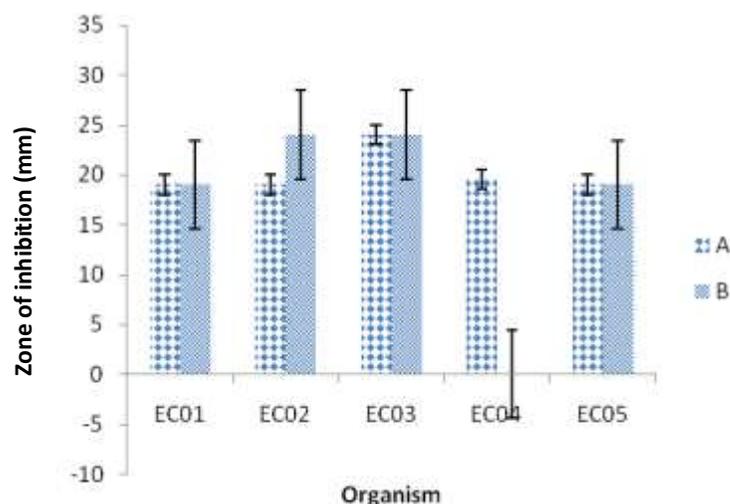
### Statistical analysis

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*.

Plant sample	Alkaloids (%)	Saponins (%)	Tannins (%)	Phenols (%)	Glycosides (%)
<i>P. amarus</i>	0.096 <sup>b</sup> ±0.002	0.190 <sup>b</sup> ±0.002	0.022 <sup>b</sup> ±0.002	0.067 <sup>b</sup> ±0.002	0.077 <sup>b</sup> ±0.002
<i>P. niruri</i>	0.122 <sup>a</sup> ±0.002	0.214 <sup>a</sup> ±0.001	0.040 <sup>a</sup> ±0.001	0.079 <sup>a</sup> ±0.002	0.090 <sup>a</sup> ±0.002

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



**Figure 1.** Comparative antibacterial activity of aerial part and root of *P. amarus* against *E. coli* ( $1 \times 10^2$  cfu/ml). A = aerial parts; B = roots.

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 (19.00 mm) and the least (11.00 mm) activity was observed in extract A at  $10^{-1}$  cfu/ml. On isolate EC02, extract C gave the highest (19.00 mm)

inhibition, 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 inhibition. 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

**Table 2.** Mineral constituents of *P. amarus* and *P. niruri*.

Plant sample	%Na	%K	%Ca	%P	%Mg	%Zn	%Cu	%Fe
<i>P. amarus</i>	0.03 <sup>b</sup> ±0.002	0.19 <sup>b</sup> ±.001	0.03 <sup>b</sup> ±0.001	0.19 <sup>b</sup> ±0.001	0.26 <sup>b</sup> ±0.002	28.20 <sup>b</sup> ±0.141	2.20 <sup>b</sup> ±0.141	12.65 <sup>b</sup> ±0.212
<i>P. niruri</i>	0.06 <sup>a</sup> ±0.001	0.12 <sup>a</sup> ±0.001	0.07 <sup>a</sup> ±0.002	0.21 <sup>a</sup> ±0.002	0.34 <sup>a</sup> ±0.002	46.35 <sup>a</sup> ±0.212	5.60 <sup>a</sup> ±0.141	37.40 <sup>a</sup> ±0.282

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

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](http://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; Eldeen et al., 2011; Njoroge et al., 2012). Although, there is scarcity of information on the antimicrobial activity

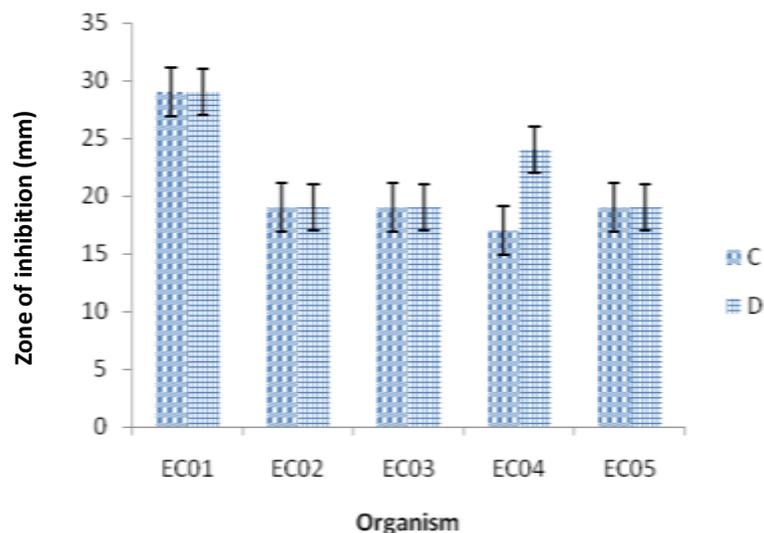
of *P. niruri* in the literature, it has been reported to be effective against hepatitis B and other viral infections (Bhattacharjee and Sil, 2006; Bhattacharjee and Sil, 2007). The authors suggested that *P. niruri* species might inhibit proliferation of the virus by inhibiting replication of the genetic 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), *Echinaceae purpurea* (Cone flower) and *Equisetum arvense* (Horsetail) have been clinically proven for urinary tract infection cure as well as bladder infection treatment.

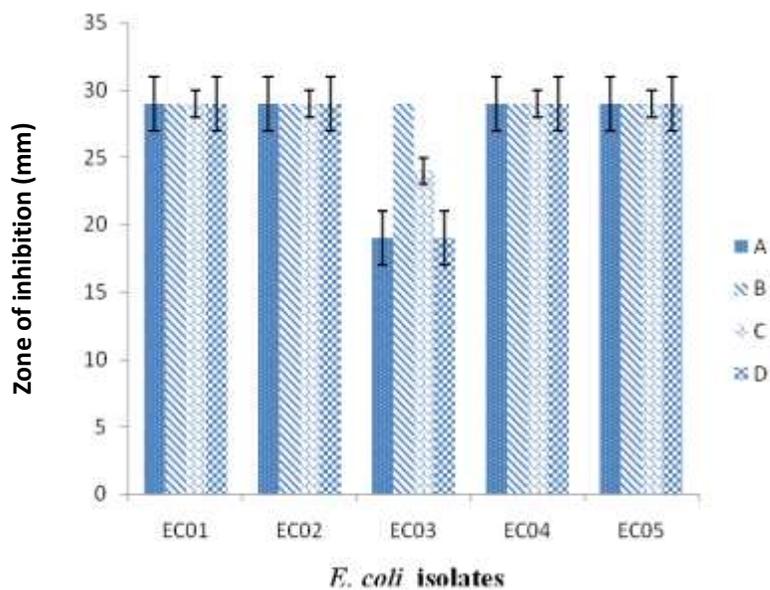
Mustard oils prepared with *Moringa oleifera* (horseradish) and nasturtium (*Tropaelum*), and grapeseed (*Vitis vinifera*) extract are effective in the treatment of UTI ([www.naturalnews.com](http://www.naturalnews.com)). Goldenrod (*Asteraceae*) is widely used in Europe

**Table 3.** *In-vitro* antibacterial activity of ethanol extracts of *P. amarus* and *P. niruri* against *E. coli* isolates implicated in UTI.

Plant extract	<i>E. coli</i> /isolate	Inoculum load (cfu/ml) / Zone of inhibition (mm)		
		$1 \times 10^{-1}$	$1 \times 10^{-3}$	$1 \times 10^{-5}$
A	EC01	11.00 <sup>e</sup> ±1.14	29.00 <sup>a</sup> ±1.14	29.00 <sup>a</sup> ±1.14
B		14.00 <sup>d</sup> ±1.14	29.00 <sup>a</sup> ±1.14	29.00 <sup>a</sup> ±1.14
C		21.00 <sup>b</sup> ±1.14	29.00 <sup>a</sup> ±1.14	29.00 <sup>a</sup> ±1.14
D		19.00 <sup>c</sup> ±1.14	29.00 <sup>a</sup> ±1.14	29.00 <sup>a</sup> ±1.14
A	EC02	14.00 <sup>d</sup> ±1.14	19.00 <sup>c</sup> ±1.14	19.00 <sup>c</sup> ±1.14
B		11.00 <sup>e</sup> ±1.41	24.00 <sup>b</sup> ±1.41	24.00 <sup>b</sup> ±1.41
C		19.00 <sup>c</sup> ±1.41	19.00 <sup>c</sup> ±1.41	24.00 <sup>b</sup> ±1.41
D		14.00 <sup>d</sup> ±1.41	19.00 <sup>c</sup> ±1.41	24.00 <sup>b</sup> ±1.41
A	EC03	14.00 <sup>d</sup> ±1.41	24.00 <sup>b</sup> ±1.41	19.00 <sup>c</sup> ±1.14
B		24.00 <sup>a</sup> ±1.41	24.00 <sup>b</sup> ±1.41	19.00 <sup>c</sup> ±1.14
C		14.00 <sup>d</sup> ±1.41	0.00 <sup>d</sup> ±0.00	29.00 <sup>a</sup> ±1.14
D		0.00 <sup>f</sup> ±0.00	24.00 <sup>b</sup> ±1.41	29.00 <sup>a</sup> ±1.14
A	EC04	19.00 <sup>c</sup> ±1.41	19.50 <sup>c</sup> ±0.70	24.00 <sup>b</sup> ±1.14
B		0.00 <sup>f</sup> ±0.00	0.00 <sup>d</sup> ±0.00	24.00 <sup>b</sup> ±1.14
C		19.00 <sup>c</sup> ±1.41	19.00 <sup>c</sup> ±1.41	24.00 <sup>b</sup> ±1.14
D		0.00 <sup>f</sup> ±0.00	19.00 <sup>c</sup> ±1.41	24.00 <sup>b</sup> ±1.14
A	EC05	24.00 <sup>a</sup> ±1.41	19.00 <sup>c</sup> ±1.41	29.00 <sup>a</sup> ±1.14
B		24.00 <sup>a</sup> ±1.41	19.00 <sup>c</sup> ±1.41	29.00 <sup>a</sup> ±1.14
C		24.00 <sup>a</sup> ±1.41	29.00 <sup>a</sup> ±1.41	29.00 <sup>a</sup> ±1.14
D		24.00 <sup>a</sup> ±1.41	29.00 <sup>a</sup> ±1.41	29.00 <sup>a</sup> ±1.14

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 = *Phyllanthus amarus* aerial parts; B = *Phyllanthus amarus* roots; C = *Phyllanthus niruri* aerial parts; D = *Phyllanthus niruri* roots.

**Figure 2.** Comparative antibacterial activity of aerial part and root of *P. niruri* against *E. coli* ( $1 \times 10^4$  cfu/ml). C = aerial parts; D = roots.



**Figure 3.** *In-vitro* antibacterial activity of ethanol extracts of *Phyllanthus amarus* and *Phyllanthus niruri* against *E. coli* ( $1 \times 10^6$  cfu/ml). A = *Phyllanthus amarus* aerial parts; B = *Phyllanthus amarus* roots; C = *Phyllanthus niruri* aerial parts; D = *Phyllanthus niruri* 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.

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