

EFFECTS OF TANNINS AND POLYPHENOLS OF SOME MEDICINAL PLANTS ON BACTERIAL AGENTS OF URINARY TRACT INFECTIONS.

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ABSTRACT

Five medicinal plants, *Enantia chloranthia*, *Kigelia africana*, *Bridelia ferruginea*, *Trema nitens* and *Drypetes gossweileri* were screened for phytochemical components. The plants were found to contain tannins, phlobatannins, alkaloids, cardiac glycosides, anthranoids, anthraquinones, saponins and polyphenols. The crude aqueous and ethanolic extracts as well as tannins and polyphenols of the plants were tested on some bacterial agents of urinary tract infections (UTI). The results revealed that the extracts inhibited *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella sp.*, *Proteus sp.* and *Escherichia coli* to varying degrees. However, the ethanolic extracts showed greater degree of inhibitory activity on the organisms than the aqueous extracts. Similarly, the tannins and polyphenols inhibited *S. aureus*, *Klebsiella sp.*, *Proteus sp.* and *E. coli* but had no inhibitory effect on *Pseudomonas aeruginosa*. The minimal inhibitory concentration (MIC) values for the test bacteria varied but generally ranged between 3% and 7%. Mixing the ethanolic extracts of all five medicinal plants together resulted in lower MIC values for the organisms. The utilization of these plants for the manufacture of drugs for the treatment of UTI is highlighted.

Keywords: Tannins, polyphenols, bacteria, urinary tract infections

INTRODUCTION

Medicinal plants contain therapeutic substances in one or more of their organs (root, stem, bark, branches, or leaves and flowers) and these can also act as precursors for the synthesis of drugs (Sowofora, 1982). In recent times, many plants and plant products have been found to contain substances capable of inhibiting the growth of or killing pathogenic microorganisms. The antimicrobial substances include alkaloids, tannins, polyphenols, cardiac glycosides, saponins, anthranoids and anthraquinones (Ebana *et al.*, 1991; Irobi and Daramola, 1993; Ijah and Sar, 1998). Ebana *et al.* (1991) reported that alkaloids and cardiac glycosides from *Garcinia kola*, *Borreria ocyrnoides*, *Kola nitida* and *Citrus aurantifolia* inhibited bacteria causing urinary tract infections (UTI), a number of conditions affecting the urinary tract of man with significant counts of bacteria. A case of UTI is established when there are significant bacterial counts of up to 10^5 per millilitre of urine (Jan, 1980).

Urinary tract infections have over the years gained an increasing importance in human medicine because of the serious nature of complications and the increasing resistance of bacteria to antibiotics. This is the case in Nigeria

because of poor sanitary habits and poor health delivery systems (Leigh, 1984).

The most common microorganisms isolated from the urinary tract are *E. coli*, *Klebsiella spp.*, *Proteus spp.*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and other members of the Enterobacteriaceae (Princewil and Obiete, 1991; Ijah and Sar, 1996; Prescott *et al.*, 1999). These organisms, particularly *Klebsiella aerogenes* and *Proteus spp.* are resistant to antibiotics such as ampicillin and co-trimoxazole (septrin) commonly used in the treatment of UTI (Ijah and Sar, 1996). Thus many people rely on local herbs to cure UTI. Since medicinal plants are natural products, they are cheap to obtain (Sofowora, 1982; Gill, 1992).

The plants used for this study are *Enantia chloranthia*, *Kigelia africana*, *Bridelia ferruginea*, *Trema nitens* and *Drypetes gossweileri*. There is little or no information on the antibacterial activities or phytochemical components of the extracts, particularly tannins and polyphenols. Therefore, the effects of plant extracts particularly tannins and polyphenols, on bacterial pathogens of UTI were investigated. The phytochemical components of the plants were also determined.

MATERIALS AND METHODS

Source of bacteria

Five bacterial isolates from cases of urinary tract

infections were collected from the Microbiology Laboratory of the General Hospital, Minna, Nigeria. The pathogens were identified as *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella sp.* and *Proteus sp.* based

on colonial morphology and biochemical characteristics (Cowan, 1974). They were stored on Nutrient agar slants at 4°C prior to use.

Plant materials

The medicinal plants, *Enantia chlorantha*, *Kigelia africana*, *Trema nitens*, *Bridelia ferruginea*, and *Drypetes gossweileri* (Table 1) were collected in Minna and Ilorin after consultation with local herbalists. The plants were transported in polythene bags to the herbarium.

Extract preparation

The barks of the various plants were washed and dried in the sun for 3 days and in the hot air oven at 80°C for 24h. The dried plant materials were

crushed into fine powder using a Waring blender (Blender/Mill Mx-391 N). The powdered bark (100g) of each species was added to 250ml of distilled water and 25ml of ethanol (95%) (BDH Chemical, Poole, UK) in an Erlenmeyer flask and allowed to extract for 72h (Irobi and Daramola, 1993). The extracts obtained were passed through a Whatman/filter paper N0.1 (Whatman, UK) and concentrated in vacuo using a rotary evaporator (Buchii Laboratory Technique, Switzerland) at 40°C. The extracts were stored in the refrigerator (Thermocool Engineering Company Limited, Ikeja, Nigeria) for further use.

Phytochemical screening of medicinal plants

The phytochemical components of the medicinal plants were studied using the methods of Culei (1982) and Sofowora (1984). The plants were screened for the presence of alkaloids, anthroquinones, cardiac glycosides, phlobatannins, polyphenols, saponins and tannins.

Table 1. Taxonomy and local usage of plants whose extracts were studied

Plant Species	Plant code	Family name	Local name (Yoruba)	Part used	Usage (Treatment of:)
<i>Enantia chlorantha</i>	EC01	Annonaceae	Iyani	Bark	malarial fever, sleeping sickness
<i>Kigelia africana</i>	KA02	Bignoniaceae	Pandoro	Bark	typhoid fever and pile.
<i>Bridelia ferruginea</i>	BF03	Ulfobiaceae	Iraodan	Bark	diabetes and for cleaning dirt in the throat and tongue.
<i>Trema nitens</i>	TN04	Ulmaceae	Ayinyin	Bark	pile.
<i>Drypetes gossweileri</i>	BG05	Euphorbiaceae	Agawo	Bark	dizziness and diarrhoea

Isolation of tannins and polyphenols by thin layer chromatography (TLC)

Analytical thin layer chromatograph of the extracts was performed using coated aluminium chromatographic plate as absorbent while acetic acid-butanol, water were used in the ratio of 10:40:50 as the developing solvent system (Oloke and Kolawole, 1988). The samples were spotted on the coated aluminium chromatographic plates, which were developed inside a chromatographic tank with the appropriate solvent. The polyphenols and tannins were identified by comparing the spots of the samples with those of the controls (Resorcinol pyrogallol and tannic acid respectively). The spots were detected by exposing the chromatographic plates to ultraviolet light and marking the distance at which the samples moved.

Antibacterial susceptibility testing

The aqueous and alcoholic extracts as well as the tannins and polyphenols separated by TLC were tested for antimicrobial activity by the disc diffusion method of Baurer *et al.* (1966). Test organisms were subcultured in nutrient broth and incubated at 37°C for 6h to ensure that the organisms were in the logarithm phase of growth. They were then inoculated on nutrient agar. Sterile filter paper discs soaked in the plant extracts were placed on the surface of the inoculated medium and the plates were incubated at 37°C for 24h. After the incubation period, the diameter of zone of clearance was measured and recorded

Determination of minimum inhibitory concentration (MIC)

The MIC test was carried out using the method of Cruikshank *et al.* (1977). Different concentrations of the aqueous and ethanolic extracts were poured in separate petri plates in duplicates. The concentrations were 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%. Fifteen millilitres (15ml) of sterile nutrient agar was poured in each plate and swirled to ensure uniform mixture of the extracts. The medium was allowed to solidify. A serial dilution of overnight cultures of the test organisms was made and each dilution compared to a mixture of 5g BaCl₂ and 100ml distilled water kept in a separate test tube. The dilution, which had similar or close turbidity to that of the BaCl₂ solution was utilized to streak on the surface of nutrient agar plates containing appropriate concentrations of the extract. The inoculated plates were incubated at 37°C for 24h. At the end of the incubation period, the plates were observed for growth or growth inhibition. The lowest concentration that entirely inhibited the growth of the organism was recorded as the minimum inhibitory concentration of the extract.

RESULTS**Phytochemical screening of medicinal plants**

The results of phytochemical screening are shown in Table 2. The results showed that only one of the plants had anthraquinones. Other phytochemical components present were cardiac glycosides, phlobatannins, polyphenols, saponins

Table 2. Phytochemical components of ethanolic extracts of medicinal plants used

Phytochemical components	Medicinal plants				
	<i>Enantia chlorantha</i>	<i>Kigelia africana</i>	<i>Bridelia ferruginea</i>	<i>Trema nitens</i>	<i>Drypetes gossweileri</i>
Alkaloids	+	+	-	-	-
Anthranoids	-	-	-	-	-
Anthraquinones				-	
Cardiac glycosides	+	+	+	+	+
Phlobatannins	-	+	+	-	+
Polyphenols	+	+	+	+	+
Saponins	-	+	+	+	+
Tannins		+		+	+

+: Phytochemical component present.

-: Phytochemical component absent.

and tannins. Anthranoids were not detected in any of the five plants tested. Alkaloids were present in *Enantia chlorantha* and *Kigelia africana* (Table 2).

Inhibitory effects of ethanolic and aqueous extracts of medicinal plants on bacteria

Table 3 shows the results of the inhibitory effects of extracts of medicinal plants on *Klebsiella sp.*, *Proteus sp.*, *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The results revealed that all the extracts exhibited antibacterial effects. The ethanolic extracts however showed a greater degree of inhibitory activity on the test organisms than aqueous extracts. The aqueous extracts of two plants did not inhibit either *E. coli* or *Staphylococcus aureus*. *Klebsiella sp.*, *Pseudomonas aeruginosa* and *Proteus sp.* were not inhibited by crude extract of one of the plants. Similarly, *Proteus sp.* was not inhibited by ethanolic extracts of *Kigelia africana*, *Trema nitems* and *Drypetes gossweileri* (Table 3).

Effect of tannins and polyphenols on pathogenic bacteria

The results in Table 4 indicate the effect of tannins (4 plants had tannins) and polyphenols (all 5 plants had polyphenols) on pathogenic bacteria. Neither tannins nor polyphenols inhibited the growth of *P. aeruginosa*. All other four test bacteria were inhibited at varying degrees by tannins and polyphenols. However, tannins were more effective in inhibiting the growth of *E. coli* than the polyphenols.

DISCUSSION AND CONCLUSION

Extraction of bioactive agents from medicinal plants permits demonstration of their

physiological activity. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity (Ebana *et al.*, 1991; Manna and Abalaka, 2000). The five medicinal plants studied were found to contain one or more of the following phytochemical components: saponins, tannins, polyphenols, alkaloids, cardiac glycosides, anthraquinones and phlobatannins. The crude aqueous and ethanolic extracts of the plants inhibited the growth of test bacteria at varying degrees.

The inhibitory activities of the medicinal plants on the bacteria indicate that the plants possess active ingredients, which may be water or alcohol soluble. The results obtained, particularly regarding the effects of *Bridelia ferruginea* and *Trema nitems* on *Klebsiella* and *Proteus species* seem to agree with an earlier finding (Ijah and Sar, 1998) that the extracts of the roots of *Trema qwuineesis* and *Bridelia sp.* had inhibitory effects on *Klebsiella aerogenes* and *Proteus mirabilis* isolated from a case of UTI. However, only the aqueous extract of *Trema nitems* used in this study was able to inhibit the growth of *Proteus sp.* This means that the active substance is soluble in aqueous extract only.

The antimicrobial effects of the aqueous and ethanolic extracts of some medicinal plants used individually were negligible and may be due to the fact that the components that could cause antibacterial effects were present in trace amounts or that during extraction the components were diluted as to lessen their antimicrobial properties to a considerable extent. Another possible explanation is that, the part used for the extraction might not contain the active

Table 3. Effect of ethanolic and crude aqueous extracts of medicinal plants on test pathogenic bacteria

Test bacteria	Diameter of zone of inhibition (mm)									
	Ethanolic extracts					Crude aqueous extracts				
	EC01	KA02	BF03	TN04	DG05	EC01	KA02	BF03	TN04	DG05
<i>Klebsiella sp.</i>	26 ± 1.2*	26 ± 1.2	22 ± 0.5	27 ± 1.4	25 ± 1.2	-	11 ± 0.2	12 ± 0.6	14 ± 0.4	15 ± 1.6
<i>P. aeruginosa</i>	30 ± 1.5	32 ± 1.2	34 ± 0.8	30 ± 2.5	29 ± 1.6	15 ± 0.2	12 ± 0.4	-	11 ± 0.2	16 ± 0.8
<i>Proteus sp.</i>	19 ± 0.6	-	15 ± 0.6	-	-	12 ± 0.6	-	15 ± 0.8	15 ± 0.6	12 ± 0.6
<i>E. coli</i>	35 ± 3.2	30 ± 0.8	33 ± 2.1	35 ± 2.5	30 ± 2.0	-	-	13 ± 0.2	14 ± 1.0	15 ± 1.2
<i>S. aureus</i>	24 ± 2.2	25 ± 1.0	27 ± 1.6	-	26 ± 0.2	-	13 ± 0.4	-	15 ± 0.8	12 ± 0.4

EC01: *Enantia chlorantha*; KA02: *Kigelia africana*; BF03: *Bridelia ferruginea*; TN04: *Trema nitems*; DG05: *Drypetes gossweileri*.

*Standard deviations are based on two replications.

- Test bacteria not inhibited

Table 4 Effect of tannins and polyphenols on test bacteria

Test bacteria	Diameter of zone of inhibition (mm)									
	Ethanollic extracts					Crude aqueous extracts				
	KA02	BF03	TN04	DG05	EC01	KA02	BF03	TN04	DG03	
<i>Klebsiella</i> sp	22 ± 0.3	20 ± 0.2	23 ± 0.4	22 ± 0.4	27 ± 0.9	26 ± 1.2	20 ± 0.5	22 ± 1.0	28 ± 1.4	
<i>P. aeruginosa</i>										
<i>Proteus</i> sp	25 ± 0.5	22 ± 0.2	25 ± 0.5	27 ± 1.2	30 ± 1.0	28 ± 1.2	25 ± 0.7	23 ± 0.7	26 ± 1.2	
<i>E. coli</i>	23 ± 0.4	27 ± 0.7	29 ± 1.2	29 ± 1.4	22 ± 0.6	20 ± 0.7	24 ± 0.4	26 ± 0.5	21 ± 0.8	
<i>S. aureus</i>	-	26 ± 0.3	22 ± 0.6	24 ± 0.7	15 ± 0.5	22 ± 0.6	22 ± 0.2	23 ± 1.0	29 ± 0.6	

KA02, BF03, TN04, DG05 as in Table 3. Tannin was not detected in EC01

Standard deviations are based on two replications

- Test bacteria not inhibited.

components in sufficiently high amounts. A similar observation had been made by Asoradi (1989) who found that the unripe fruits of *Hunteria* plant possessed better antimicrobial properties than the leaves or roots of the same plant.

The minimum inhibitory concentration (MIC) is the lowest concentration of a drug that prevents growth of a particular microorganism (Prescott *et al.*, 1999). Thus, MIC test gives some idea of the effectiveness of a chemotherapeutic agent against a microorganism. The results of the MIC test indicated that different concentrations acted as the MIC values for the respective organisms. It is most probable that some of the organisms are more (sensitive) vulnerable to the action of the antimicrobial substance present in the extracts (which was manifested in their lower MIC values) than others (those having higher MIC values). The results indicate that the organisms were inhibited by a minimum concentration of between 3% and 7% extract. Higher MIC values were recorded for *Klebsiella* sp., *P. aeruginosa* and *E. coli* than for either *Proteus* sp. or *S. aureus*. The high MIC values for *Klebsiella* sp., *P. aeruginosa* and *E. coli* indicate that these organisms are more resistant to the extracts than *Proteus* sp. and *S. aureus*. This is probably due to differences in genetic constitution of the bacteria. However, this finding seems to show that *Klebsiella* sp., *P. aeruginosa* and *E. coli* may present difficulties in the therapy of urinary tract infections.

The high MIC values were reduced when the extracts were mixed. The decrease in MIC value observed could be attributed to additive effect between the phytochemical components of the extracts. This finding agrees with the report of Duguid *et al.* (1978) that combinations of drugs

were more effective in eliminating pathogens than a single drug. The finding in the present study may imply that extracts of the plants can be more effective when taken in a combined form (Gill, 1992; Bone, 1994).

Benjamin (1979) reported that chemical components of medicinal plants inhibit the test organisms in different ways. In the present study, tannins of *Kigelia africana* significantly inhibited *S. aureus*, *Klebsiella* sp. and *E. coli* but did not have any inhibitory effect on *P. aeruginosa*. This shows that tannins and polyphenols were not the active ingredients in these plants for *P. aeruginosa*. Its active principle may have been residing in those components which were not separated. This is confirmed by the results of the alcoholic extracts, which produced large zones of inhibition. The differences in the antibacterial activities to tannins and polyphenols could be due to the extent of solubility of these components in alcohol.

Nguji (1988) reported that tannins are important in herbal medicines. Marcerated bark or leaves or root of tanniferous plants is applied in arresting bleeding and as a healing dressing for wounds. The phenols in those plant tissues are usually oxidized to co-quinones which subsequently form effective cross-links with the serum proteins of the skin to arrest bleeding and effect healing. Some tissues of tanniferous plants are chewed as antiscrobutin while infusion of some bark and pods rich in tannin is used as tonic and sometimes provides remedy for dysentery, diarrhoea, cough, fever and venereal diseases (Dalziel, 1995). *E. coli* and *S. aureus* which tannins of the medicinal plants inhibit have been implicated in diarrhoea (Prescott *et al.*, 1999).

The occurrence of tannins in four (*Kigelia africana*, *Bridelia ferruginea*, *Trema nitemis* and *Drypetes gossweileri*) of the five plants used in this study also show that the plants may be useful in various industries considering the application of tannins in oil, food and pharmaceutical industries as well as in agriculture.

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