Nigerian Journal of Pharmaceutical Research
Vol. 5 No. 1 pp 97-101 (September, 2006)

PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *Ficus iteophylla* LINN LEAVES (MORACEAE)

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Abstract

This study was performed to evaluate the antibacterial activity of the leaves of *Ficus iteophylla* against four pathogenic bacterial strain using agar diffusion method. The ethyl acetate, n-butanol and the aqueous portions of the crude ethanol extract were screened for anti-bacterial activities against *E. coli, Ps. aeruginosa, S. aureus,* and *B. Subtilis* using agar diffusion method. The Results showed that the aqueous portion has no activity against all the test organisms. Both the ethyl acetate and the n-butanol extracts showed activities with inhibition zone ranging from 11.3 mm to 12.9 mm for *E. coli,* 13.3 mm to 15.0 mm for *S. aureus* and 14.5 to 14.8 mm for *B. Subtilis,* but resistant to *Ps. aeruginosa.* The minimum inhibitory concentrations (MICs) were determined. The MIC of ethylacetate extract showed the best MIC of 2.5 mg/ml against *S.aureus,* followed by n-butanol extract 5 mg/ml against the same organism. The two fractions showed similar MICs against *B. subtilis* (5 mg/ml) and *E. coli* (10 mg/ml). The ethyl acetate extract is the most active extract. Fractionation of the n-butanol extract over silica Gel G column chromatography and sephadex LH-20 gave 2 Flavonoid glycosides identified as *Kaempferol-3-O-rutinoside* and *Quercitin-3-O-rutinoside.* The observed activity might justify the use of the plant for diarhoea, skin infection and sore throat.

Keywords: *Ficus iteophylla*; *antibacterial activity*; *flavonoid*; *kaempfero; quercitin-3-O-rutinosides*

INTRODUCTION

The genus *Ficus* L (Moraceae) consists of more than 850 species and is an important medicinal plant found in tropical and sub-tropical region, particularly Northern Nigeria (Pistili and Moelli, 2000; Dalziel, 1955). *Ficus iteophylla* belongs to the family Moraceae which has about 53 genera and about 800 species. Infusion from the leaves and bark are used in ethnomedicine for ailments which include: diarrhea, sore throat, skin infections, pile and diabetes (Irvine, 1961). Ever since the discovery of the first antibiotic, Penicillin, man's need for antimicrobial agents has not been satisfied. This has been attributed to the emergence of antibiotic resistance strains of microorganisms (Davies, 1994). Consequently in continuous search for novel compounds with biological activities including anti-microbial, considerable attention has focused on plant secondary metabolites as an alternative to and or combination with traditional antibiotics for treating bacterial infections (Collins, 1970). Previously we have isolated two furanocoumarins from the leaves of this plant (Ahmadu *et al.*, 2004). To our knowledge, there has been no documented evidence for the isolation of flavonoid glycoside from this plant. In this study, as part of our continuous search for bioactive compounds, we report here the isolation of two flavonoid glycosides and the antibacterial activity of the plant extracts.

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ISSN 0189-8434 © 2006 NAPA

MATERIALS AND METHODS

Plant Material

The leaves of the plant was collected in the month of June in Samaru, Zaria-Nigeria and authenticated at the herbarium section, Biological Sciences Department, Ahmadu Bello University, Zaria-Nigeria where a voucher specimen (Number 7167) is deposited.

*Extraction and Isolation***:** Air-dried powdered leaves of the plant (300 g) were exhaustively extracted with 95% ethanol at room temperature. The ethanol extract was concentrated at reduced pressure to give a dark green residue (25 g). 20 g of this was suspended in water and filter. The filtrate was successfully extracted with ethylacetate (1x1.5L) and n-butanol (2 L) gave a greenish mass of the former (1.7 g) and a brownish mass of the n-butanol (3.2 g). The n-butanol soluble fraction (1.5 g) was subjected to column chromatography over silica gel G (60-120 ìm) and eluted gradiently with chloroform and chloroform: Methanol mixtures. Elution with chloroform: Methanol (8:2) gave a mixture of flavonoids (65 mg) using the solvent system: Ethylacetate:Methanol:Water (100:16.5:13.5), and Ethyl acetate:Formic acid:Water (10:2:3). This was subjected to repeated purification over (Sephadex LH-20) eluted with methanol to give compound I (3.5 mg) and compound II (9 mg) identified as *Kaempferol-3-O-rutinoside* and *Quercetin 3-O-rutinoside* by spectroscopic method (1 and 2D ^{1}H-NMR and ^{13}C-NMR) spectroscopy.

Susceptibilty Testing

Test bacteria: *Staphylococcus aureus* (ATCC21001), *Escherichia coli* (ATCC11775) *Pseudomonas aeruginosa* (ATCC10145) and *Bacillus subtilis* (NCTC 8326B76) were all obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria-Nigeria.

The susceptibility test was done by the cup plate method (Collins, 1970). Prepared agar plates were flooded with diluted 24 hour broth culture of the test bacteria. The gram positive bacteria *Staph. aureus* (ATCC21001)and *Bacillus subtilis* (NCTC 8326876) were each diluted to 1:1000, while the gram negative bacteia: *Pseudomonas aeruginosa* (ATCC 10145) and *E.coli* (ATCC 1175) diluted to 1:5000). Excess of the cultures was sucked with syringe, there after holes were bored into inoculated nutrient agar with number 4 cork borer. The extracts were dissolved in 20% Dimethyl sulphoxide DMSO to give 20 mg/ml each were used to fill the holes in the nutrient agar plates and left four 1 hour to diffuse in to the agar medium. The nutrient agar plates were incubated at 37^{o}C for 24 hours. Ampicillin 10 ug/ml was used as standard. Zones of inhibition were measured to the nearest millimeter.

Determination of the Minimum inhibitory concentrations (MIC)

Two fold dilutions of the extract 40 mg/ml were made in sterile nutrient broth (2 ml in each tube). The diluted extracts were inoculated with 0.1 ml of each of the bacterial species and incubated at 37^{o}C for 24 hours. After incubation, sub cultures of the reaction mixtures were made on to nutrient agar plate and incubated at 37^{o}C for 24 hours to determine the presence or absence of growth. The least concentration, which showed absence of growth was considered as the minimum inhibitory concentration (MIC).

RESULTS

Table 1: NMR Spectra of compounds I and II

Position C/H	äH$_1$	äH$_2$	äC$_2$
2			158. 6
3			135.6
4			179.4
5			163.0
6	6.18, 1H, d(2.0Hz)	6.20, 1H, d(2.0Hz)	100.01
7			66.2
8	6.37, 1H, d(2.0Hz)	6.38, 1H, d(2.0Hz)	94.9
9			158.6
10			105.6
1'			123.15
2'	8.05, 2H, d, d(2, 8.5Hz)	7.66 1H (s)	117.7
3'	6.89, 2H, d, d(2, 8.5Hz)		145.8
4'			149.8
5'	6.89, 2H, d, d(2, 8.5Hz)	6.88, 1H, d(7.0Hz)	116.07
6'	8.05, 2H, d, d(2, 8.5Hz)	7.61, 1H, d, d(2, 7.0Hz)	123.56
Â-D-gluc			
1''	5.11d, 1H (J = 7.0Hz)	5.11 1H, d(7.0Hz)	102.4
2''			73.9
3''	Un-assigned glucose protons ä = 3.2 – 4.2		77.2
4''			71.42
5''			75.8
6''			68.57
á-rham			
1'''	4.51 1H (s)	4.52 1H (s)	101.4
2'''			71.42
3'''	Un-assigned rhamnose protons		72.26
4'''			72.13
5'''			69.73
6'''	1.13 3H d (6.0Hz)	1.13, 3H d(6.0Hz)	17.9

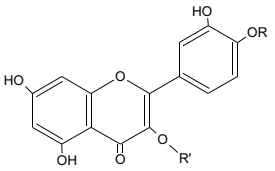
Antibacterial investigation

The activities of the extracts are presented in table 2. An extract was considered active when it produces zones of inhibition against the test organisms. From the table the aqueous extract has no activities against all the test organisms, while the n-butanol and ethyl acetate extracts at 20 mg/ml showed activities against *E. coli*, *B. subtilis* and *S. aureus* but not on *Ps. aeruginosa*. The activities of the n-butanol extracts ranged from 13.3 mm to 14.5 mm against *E. coli*, *S. aureus* and *B. subtilis*. The activities of the ethyl acetate extract ranged from 12.9 mm to 15.0 mm against the test pathogens. Only *S. aureus* was susceptible to ampicillin (10 ìg/ml). Table 2 also shows the MICs of the extract. The MIC of the n-butanol extract were 10, 5 mg/ml for *E. coli*, *S. aureus* respectively, while *B. subtilis* also gave 5 mg/ml as MIC. It can be seen that the n-butanol extract is more sensitive on *S.aureus* and *B.subtilis* than *E. coli*. Similarly the activity of the ethyl acetate is most on *S. aureus* followed by *B. subtilis* and then *E. coli*. Ampicillin which, was used as standard had activity only against *S. aureus* with zone of inhibition (17.8 mm).

Table 2: MIC of the extract

	Ethylacetate		N-Butanol		Aqueous layer		Ampicillin
S. aureus	15.0	250	13.3	500	-	-	17.8
B. subtilis	14.8	500	14.5	500	-	-	-
Ps. aeruginosa	-	-	-	-	-	-	-
E. coli	12.9	1000	11.3	1000	-	-	-

Zone of inhibition (mm), n = 3
Minimum inhibitory concentration (ìg/ml)
Ampicillin = 10 ìg/ml



Compound	R	R'
1	H	Glu-Rham
2	OH	Glu-Rham

DISCUSSION

Extensive chromatographic separation of the n-butanol extract over silica gel G and repeated purification over sephadex LH-20 led to the isolation of the flavonoid glycosides: compound I was identified as kaempferol-3-O-á-rhamnosyl (1 > 6)-â-D- glucopyranoside by comparism of the ^{1}H-NMR (1 and 2D) with that reported in literature (Ahmed and Nurdin, 1998; Claudia *et al.*, 2003]) while compound II was identified as Quercetin 3-O-á-rhamnosyl (1 > 6)-â-D-glucopyranoside by comparison of the ^{1}H-NMR (1D and 2D-^{1}H-H Cosy) and ^{13}C-NMR (Table 1) with that reported in literature (Mabry *et al.,*1965; Mabry and Markham, 1970), while flavonoids have been reported in other *Ficus species* (Pistili and Morelli, 2000), there is no documented evidence for the isolation of flavonoids from *Ficus iteophylla.*

Results of the antibacterial studies (Table 2) showed that the ethylacetate soluble extract showed the strongest antibacterial activity, followed by n-butanol extract while the aqueous extract did not shown any activity against the test pathogens. The presence of coumarins in the ethylacetate extract and flavonoids in the n-butanol extract might be responsible for the observed activity. The activity of both extract on *S. aureus* is comparable to that of ampicillin (17.8 mm). The present study has justified the ethnomedicinal uses of the plant in infections such as diarrhea, sore throat and skin infections, since all the susceptible bacterial species are implicated in similar health problems.

One of the undisputed functions of flavonoids and related polyphenols is their role in protecting plants against microbial invasion (Harbone, 2000). Flavonoids have been reported to possess antibacterial activity against gram positive and gram negative bacteria (Malterund, 1985; Liu *et al.*, 1999). Thus the presence of these compounds could be responsible for the observed activities. Work is underway to determine the activity of the isolated compounds.

Acknowledgements

The authors wish to thank Dr. Simon Gibbons of Centre for Phytotherapy Research, University of London for the NMR spectra of the isolated compounds.

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