Journal of Science and Technology 13 (03) December 2011
ISSN 1605 – 427X

www.sustech.edu

Studies on the Chemical Constituents of the Leaves of *Ficus bengalensis* and their Antimicrobial Activity

Hassan Abdalla Almahy[1*] and Nafisa Ibrahim Alhassan[2]

Department of Chemistry, Faculty of Education, Bahari University E. mail: hassan208@hotmail.com

ABSTRACT: Catechin and genistein flavonoids isolated from methanol extracts of the leaves of *Ficus bengalensis* which belongs to the family Moraceae are reported for the first time in Sudanese varieties. The isolation process involved extraction with various solvents and separation using column chromatography techniques. Their structural identifications were assigned on the basis of IR, UV, ^{1}H-NMR, ^{13}C-NMR, Distortionless enhanceement by Polarization Transfer, Correlated Spectroscopy, MS spectral data and on comparison with published data. However, bioactivity screening showed that both compounds possessed antibacterial activity on two species of bacteria *Bacillus cereus* NRRLUI-1447 (Gram-positive) and *Pseudomonas aeruginosa* UI-60690 (Gram-negative), but had no effect on four species of fungi (*Aspergillus ochraceus* NRRL 398, *Candida lipolytica* ATCC 2075, *Sacchromyces cereviseae* NRRL 2034 and *Sacchromyces lipolytica*).

KEYWORDS: *Ficus bengalensis, catechin, genistein, antimicrobial activity.*

INTRODUCTION

Ficus bengalensis belonging to the family Moraceae[(1)] is a small tree comm-only found throughout India, Malaya and east India. It has now been introduced through the tropics[(2)] and is known locally as Allabakh[(3,4)]. The palnt is employed frequently in the traditional medicine as eye-disease, constipation, toothache, inflammation, leucoderma, headache, fever, rheumatic affection-ns,.asthma cigarettes, antidote for snake bites and relieve stomachache[(5-8)]. We report in this paper the chemical constituents present in the leaves and determine the biological activities of the pure isolated compounds.

MATERIALS and METHODS

Instrumentation

Melting points were determined on a Kofler hot-stage microscope melting Point apparatus and were uncorrected. Infrared and ultraviolet spectra were obtained by Perkin-Elmer lambda model 1330 and 20 spectrometers, respectively. ^{1}H and ^{13}C-nuclear magnetic resonance were recorded by JEOL JNM-GSX 400 spectrometer. Mass spectra were obtained using GCMS-QP 5050 A Shimadzu mass spectrometer, column chromatography and analytical thin layer chromatography were carried out using Merck 77491 and Merck 7730, 60 F-254.

Plant material

The leaves of *Ficus bengalensis* were collected from Shambat-Bahri (Khartoum-North) in October 2007 and authenticated by Austaz Awad Hussein University of Juba, Department of Botany and a voucher specimen was

deposited at the Herbarium of the Department of Biology University of Juba. Air dried leaves of *Ficus bengalensis* were used in this work.

Extraction of the plant material

Air dried leaves of *Ficus bengalensis* (2.0 kg) were extracted twice by maceration at room temperature and soaking the plant material successively in petroleum ether (35-60 □C, 3.0 liters), chloroform (2.5 liters) and methanol (2.0 liters). The solvents were removed by using a rotatory evaporator to give various 8.8, 16.0 and 70.8 grams of viscous crude extracts, respectively. The methanol extract was redissolved in 95 % aqueous methanol (500 ml) and then extracted successively with n-hexane, chloroform, ethyl acetate and n-butanol. The n-butanol extract was chromatographed on column chromatography eluting with chloroform with increase amount of methanol to give fractions of 250 ml each. Fractions which gave similar spots on the thin layer chromatography with appropriate solvent system were combined.

Methods of isolation

The crude extracts (44.0 g) of the n-butanol of the methanol extracts of the leaves of *Ficus bengalensis* were subjected to column chromatography over Merck silica gel PF_{254} (70-230 Mesh ASTM) and eluting with petro-leum ether, petroleum ether/chloroform, chloroform and chloroform/methanol to give 24 fractions. Thin layer chroma-tography was performed on comm.-ercially TLC plastic sheets precoated with Merck silica gel 60 F-254 (0.2 mm thickness). It is routinely used to detect and separate the various components present in plant extracts and to monitor fractions collected during column chromate-graphy.

Isolation of catechin (I)

The fraction of the methanol extracts of the leaves of *Ficus bengalensis* were separated by using vacuum column chromatography on silica gel 60 PF-254. The column was eluted under vacuum with chloroform with increasing amount of methanol to give 32 fractions of 250 ml each. Fractions were concentrated and analysed by thin layer chromatography. Fractions 3-6 were combined and further purified by second column that gave solid which was recrystallised from ethanol to give catechin (**I**) as white powder (20.0 mg) with melting point 198-200 °C (200-202 °C)[9]. The R_F 0.74 (chloroform: methanol).

IR (KBr, cm-1): 3392, 3267, 2936, 1630, 1524, 1472, 1291, 1199, 1053, 959, 822, 675, 530. [α]28 +54.6 (C, 0.25, MeOH). UV λ_{max} nm, DMSO (log ε): 300.5 (0.61).

1H-NMR (400 MHz, DMSO) δ: 9.2 (1H, s, 5-OH), 9.0 (1H, 7-OH), 8.8 (2H, 3/-OH & 4/-OH), 6.7 (2H, dd, J=J = 8.5 HZ, H-2/ & H-6/), 6.5 (1H, d, J= 8.5 HZ, H-5/), 5.9 (1H, d, J=1.2 HZ, H-8), 5.7 (1H, d, J=1.2 HZ, H-6), 4.9 (1H, br, 3-OH), 4.5 (1H, d, J=7.3 HZ, H-2), 3.8 (1H, m, H-3), 2.7 (1H, m, H-4b), 2.3 (1H, m, H-4a).

13C-NMR (100 MHz, DMSO) ppm: 156.3 (C-7), 156.0 (C-5), 155.2 (C-9), 144.7 (C-3/ & C-4/), 130.5 (C-1/), 118.3 (C-6/), 115.0 (C-2/), 114.4 (C-5/), 99.0 (C-10), 95.0 (C-8), 93.7 (C-6), 80.9 (C-2), 66.2 (C-3), 27.7 (C-4). MS m/e (% intensity): 290 (M+, 2.4), 267 (0.1), 249 (1.2), 225 (0.8), 207 (2.5), 193 (1.6), 179 (4.0), 167 (4.8), 152 (8.6), 139 (16.5), 123 (12.5), 111 (19.4), 97 (31.9), 71 (48.9), 57 (96.7), 43 (100), 41 (50.6).

Isolation of genistein (II)

Fractions 9-12 of the above column gave similar R_F value on thin layer chrom-atography plate and were combined and rechromatographed with the same solvent to give 11 fractions. Fractions 3-5 gave solid which was recrystallised from methanol to

give genistein (**II**) as white powder (22.0 mg) with melting point 218-220 °C (217-218 °C)[10,15]. IR (KBr, cm^{-1}): 3413, 3133, 1652, 1570, 1504, 1476, 1274, 1044, 886, 790, 608, 516. $[\alpha]_{28}$ -25.5 (C, 0.85, MeOH). UV λ_{max} nm, MeOH (log ε): 301.0 (0.25). ^{1}H-NMR (400 MHz, DMSO) δ: 12.9 (1H, s, 5-OH), 8.1 (1H, s, H-2), 7.4 (2H, d, J=3.4 H_Z, H-2′ & H-6′), 6.9 (2H, d, J=3.4 H_Z, H-3′ & H-5′), 6.4 (1H, d, J= 1.7 H_Z, H-8), 6.3 (1H, d, J= 1.7 H_Z, H-6). ^{13}C-NMR (100 MHz, DMSO) ppm: 180.3 (C-4), 163.5 (C-7), 161.2 (C-4′), 157.6 (C-5), 156.2 (C-9), 153.8 (C-2), 130.2 (C-2′ & C-6′), 122.2 (C-1′), 121.6 (C-3), 115.2 (C-3′ & C-5′), 104.5 (C-10), 98.9 (C-6), 94.0 (C-8). MS m/e (% intensity): 270 (M^+, 33.3), 248 (4.4), 241 (2.2), 217 (3.3), 207 (3.3), 189 (3.3), 173 (4.4), 165 (4.4), 154 (8.9), 153 (61.1), 138 (4.4), 135 (13.3), 124 (61.1), 118 (100), 111 (20.0), 105 (13.3), 96 (30.0), 89 (56.7), 84 (41.1), 77 (26.7), 69 (61.1), 66 (77.8), 63 (35.6), 55 (25.6), 51 (30.0).

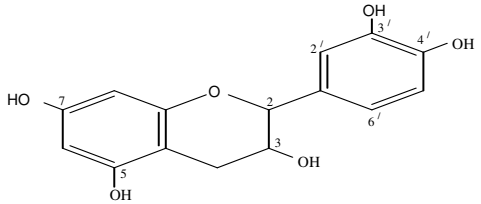
Evaluation of Antimicrobial Activity

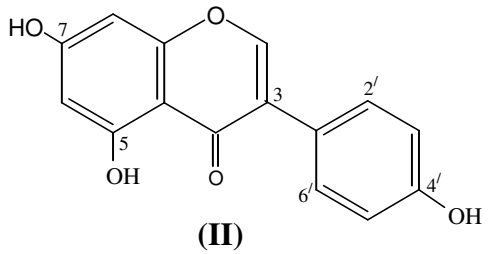
Microorganisms

The microorganisms used in this investigation were obtained from the culture collection of the Department of Biology-University of Juba. The stock cultures were grown on Potato Dextrose Agar (PDA) for 24 hours at 28 °C at which time the cells were harvested by centrifugation (4 °C, 2000 rpm, 3 min.). The cells were washed and suspended in sterile 0.9 % saline to give a final concentration of 10^5-10^6 CFU/ml using a haemocytometer [11]. The microbial strains used were *Bacillus cereus* NRRLUI-1447, *Pseudomonas aeruginosa* UI-60690 and four fungi (*Aspergillus ochraceus* NRRL 398, *Candida lipolytica* ATCC 2075, *Sacchromyces cereviseae* NRRL 2034 and *Sacchromyces lipolytica*). Antimic-robial activity of the crude extracts and isolated compounds were tested using the disc diffusion method[12]. The discs were prepared by impregnating them with an ethanolic solution of each sample (10 mg/ml). They were then evenly spaced out on the agar surface previously inoculated with the suspension of each microorganism to be tested. Standard discs of streptomycin sulphate (25 µg/discs) and nystatin (50 µg/discs) were used as positive controls. The plates were incubated at 37 °C for bacterial targets for 24 hours and the antimicrobial activity was recorded by measuring the width of the inhibition zones around each disc.

RESULTS and DISCUSSION

Extraction of the leaves of *Ficus bengalensis* by methanol followed by extensive chromatographic techniques resulted in the isolation of catechin (**I**) and genistein (**II**)





(II)

Catechin (**I**) was isolated as white powder with melting point 198-200 °C (200-202°C)[9,13] and R_F 0.74 (chloroform: methanol). The IR spectrum indicated the presence of hydroxyl group with the occurrence of strong and broad band at 3392 cm^{-1}. The presence of C-O-C stretching was suspected by the absorption at 1053 cm^{-1}, the absorption band at 3267, 2936 and 1524 cm^{-1} indicated the presence of aromatic CH, cyclic CH and double bond respectively. The UV spectrum indicated the presence of a flavonol skeleton by the occurrence of absorption maximum at λ_{max} 300.5 nm[14].

The ^{1}H-NMR spectrum of this compound integrated for the presence of 14 protons in which five of them occurred in the aromatic region. The doublet at δ 6.5 with coupling constant of 8.5 H_Z was assigned to proton at H-5′. This proton coupled with another proton at H-6′ which occurred as doublet of doublet at δ 6.7. This signal overlapped with another doublet assigned for proton at H-2′. Another *meta* coupling protons were observed as two seperate doublets at δ 5.9 and 5.7 with coupling constant *J*=1.2 H_Z assigned to H-8 and H-6, respectively. Proton at H-2 was observed to occur as doublet at δ 4.5 and it coupled with proton at H-3 which occurred as a multiplet at δ 3.8. The two methylene protons at H-4 occurred as two seperate multiplets at δ 2.7 and 2.3. The five hydroxyl groups occurred as broad signals at δ 9.2, 9.0, 8.8 (×2) and 4.9. The complete assignment of the proton signal was accomplished through ^{1}H-^{1}H correlated spectroscopy experiment.

The ^{13}C-NMR spectrum indicated the presence of fifteen carbons in which seven were quaternary carbons (156.3, 156.0, 155.2, 99.0, 130.5 and 144.7 (×2) ppm and assigned to C-7, C-5, C-9, C-10, C-1′, C-3′ and C-4′ respectively. The seven methine carbons occurred at 118.3, 115.0, 114.4, 95.0, 93.7, 80.9 and 66.2 ppm assigned for the C-6′, C-2′, C-5′, C-8, C-6, C-2 and C-3. The methylene carbon at C-4, occurred at 27.7 ppm and this assignement was based on Distortionless Enhanceement by Polarization Transfer experiments. The mass spectrum gave a molecular ion peak at m/z 290 which was consistent with the molecular formula $C_{15}H_{14}O_6$. The compound gave greenish colour on the TLC plate when sprayed with 1 % ferric chloride in methanol and yellow colour with 1 % aluminium chloride in ethanol. Based on these spectral data and chemical tests performed it was indicated that the compound was catechin.

Genistein (**II**) was isolated as white powder. The IR spectrum indicated the presence of hydroxyl group at 3413 cm^{-1} which occurred as a strong and broad band. The presence of carbonyl functionality and COC stretching were indicated by the absorptions at 1652 and 1044 cm^{-1}, respectively. The UV absorption maxima at 301 nm pointed to the presence of isoflavone nucleus [16].

The ^{1}H-NMR spectrum of genistein gave a low field one proton sharp singlet at δ 12.9 and this is considered as a characteristic of hydroxyl group chelated to the carbonyl functionality at C-4 position. The aromatic region integrated for the presence of seven aromatic protons with the occurrence of the two sets of doublets each integrated for two

protons. The doublet at δ 7.4 was assigned to the two equivalents protons at H-2′ and H-6′ and the other doublet at δ 6.9 to the two equivalent protons at H-3′ and H-5′. The low field aromatic singlet at δ 8.1 was assigned to H-2 due to deshielding effects of the oxygen atom in the fluran ring. The two meta-coupled protons at H-8 and H-6 occurred as doublets at δ 6.4 and 6.3 each with coupling constant of 1.7 H_Z, respectively.

The ^{13}C-NMR spectrum indicated the presence of fifteen carbons assigned by Distortionless Enhanceement by Polarization Transfer experiment which showed the presence of eight quaternary carbons at 121.6, 157.6, 163.5, 156.2, 104.5, 122.2, 161.2 and 180.3 ppm which were assigned to C-3, C-5, C-7, C-9, C-10, C-1′, C-4′ and C-4, respectively. The seven methine carbons occurred at 153.8, 98.9, 94.0, 115.2 (×2) and 130.2 (×2) ppm were assigned to C-2, C-6, C-8, C-3′ and C-5′, C-2′ and C-6′, respectively. Resonance for C-2′, C-6′ and C-3′, C-5′ appeared as two overlapped signals in the spectrum.

Mass spectrum gave a molecular ion peak at m/z 270 and is consistent with the molecular formula of $C_{15}H_{10}O_5$. Compound (**II**) gave positive test with 1 % ferric chloride in methanol and 1 % aluminium chloride in ethanol. Based on these spectral data, chemical tests and on comparison with previous studies[17,18] the isoflavone was found to be identical with genistein (i.e., 5, 7, 4′-trihydroxyisoflavone).

Preliminary screening of isolated compounds for antimicrobial activity was performed using the disc diffusion assay. The antimicrobial activity of the compounds isolated from the leaves of *Ficus bengalensis* revealed that catechin and genistein possess strong activity against Bacillus cereus (Gram-positive) and Pseudomonas aeruginosa (Gram-negative) but do not have any antifungal activity (Table 1, Figures 1and 2).

Table 1: Antimicrobial activity of catechin and genistein (100 µg/ml) isolated from the leaves of Ficus bengalensis

Microorganisms	catechin Inhibition zones (mm)	genistein inhibition zones (mm)	streptomycin (µg/discs)	nystatin (µg/discs)
Bacillus cereus	18.8	19.0	25	
Pseudomonas aeruginosa	19.0	18.5	25	
Aspergillus ochraceus	0	0		50
Candida lipolytica	0	0		50
Sacchromyces cereviseae	0	0		50
Sacchromyces lipolytica	0	0		50

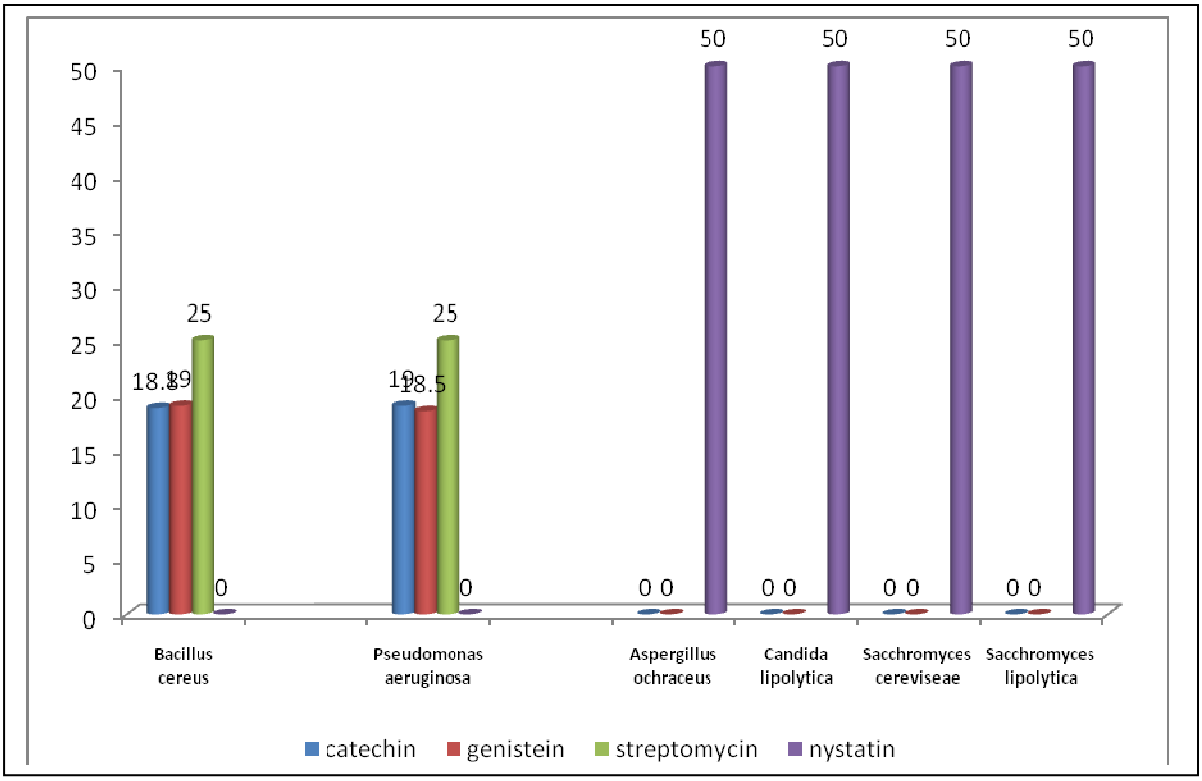


Figure. 1: Antimicrobial activity of catechin and genistein against two bacteria and four fungi compared with streptomycin and nystati*n*

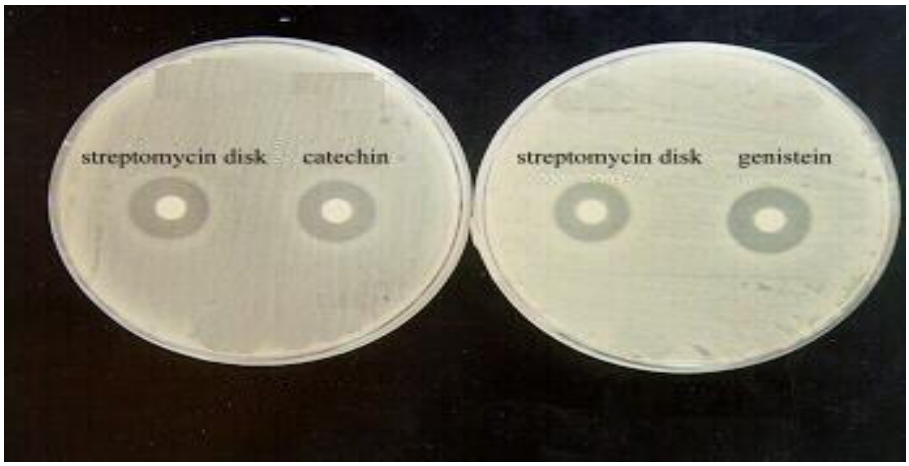


Figure.2: Inhibition zones of catechin and genistein against Bacillus cereus and Pseudomonas aeruginosa

REFERENCES

1. Hegnauer, R. (1983). *Chemical Plant Taxonomy*. p.389. Academic Press. London.
2. Corner, E. H. (1984). *Wayside Trees of Malaya*. Volume 2, p.752-753. United Selangor Press. Kuala Lumpur.
3. Nagiton, F .S. (1994). *Tree Flora of Malaya, A Manual for Foresters*. Volume 3, p.310 Berhad: Longman Malysia SDN. Kuala Lumpur.
4. Benoit, L. C., Georges, B. M. and Jean, M. N. (1997). *Carbohydrate Research*. **298**, 251-260.
5. Apichart, S. and Chatriwat, S. (1995). Ecdysteroids from *Vitex negundo*. *Phytochemistry* **32**, 303-306.
6. Kirtikar, K. R. and Basu, B. D. (1994). *Indian Medicinal Plants* 2nd edn..**3,** p.1943. Periodical Experts. Delhi.
7. Trease, G. E., and Evans, C. W. (1988). *Pharmacognosy Bailliere Tindall*. 12th edn. p.183 London.
8. Kubo, I. and Ayafor, T. (1990). Structure of a novel phytoecdysteroid from *Vitex fisherii*. *Journal of Chemical Ecology* **16,** 2581-2588.
9. Naherstedt, A., Prokch, P. and Conn, E. (1987). Flavones and Flavonol glycosides from *Chamaebatia* species. *Phytochemistry* **26,** 1546-1547.
10. Heibbron, D. S., Cadogan J. G. and Slater, C. A. (1974). *Dictionary of Organic Compounds* **4**, p.2054. Eyre and Spottiswood Publishers Ltd. London.
11. Bergeys, P. (1988). *Manual of Determinative Bacteriology* 7th edn. p.242 Williams and Wijkins Baltimore. New York.
12. Bauer, J., Kirby, G., Sherris, A. and Turck, M. (1986). Screening Classification and identification of microbial. *American Journal Clinical Pathology*. **45**, 493-496.
13. Munasingle, V., Pattendan, F., Rhodes, C. and Roberts, D. (1974). *Dictionary of Natural Products*. **4**, p.4889. Chapman and Hall. London.
14. Dayrit, F., Rosario, M. L. and Lolit, G. L. (1987). Phytochemical studies on the leaves of *Vitex negundo*, L. (Lagundi).*The Phillippine Journal of Science* **4**, 403-408.
15. Macwillian, C., Parsons, C., Ress, R. and Stater, A. (1982). *Dictionary of Organic Compounds*. **3**, p.1501. Eyre and Spottiswoode Publishers.Ltd. London.
16. Harbone, J. B. (1984). *Phytochemistry methods a Guide to Modern Techniques of Plant Analysis*. 2nd edn. p 225. Chapman and Hall. New York.
17. Pelter, A., Ward, R. and Bass, R. (1987). The ^{13}C-NMR spectra of isoflavones. *Journal Chemical Society Perkin Transiction* **1**, 666-668.
18. Andras, L. (1982). Synthesis of 3-O-methyl-L-xylose, a component of lipopolysaccharides of Gram-negative bacteria. *Carbohydrate Research* **107,** 296-299.