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Antimicrobial Studies of the Aerial Part of Aeschynomene unifloraMey Phytochemical Screening

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Keywords: Aeschynomene uniflora; Aerial part; Antimicrobial activity; Phytochemical screening; Fabaceae

Abstract: Branches led to isolation of the flavonoid glycosides kaempferol 3,7-di- O-α-L-rhamnopyranoside, kaempferol 7-O-α-L-rhamnopyranoside, kaempferol 3-O-apiofuranosil- 7-O- rhamnopyranoside, quercitin 3-O-α-L-rhamnopyranoside, quercitin 3-O-arabinofuranoside, 8-β-D- glucopyranosyl 4',5,7 trihydroxyflavanone, the isoflavonoid 4',7-di- hydroxy-isoflavone, the dimer epicatechin-(2β, 4β)+- epicatechin, the polyol 3-O-methyl-chiro-inositol and two steroids in sitosterol and stigmasterol mixture [22]. In 2011, Chen et al. [23] reported novel compound monotetracontane from the dry leaves of Aeschynomene indica Linn.Materials and MethodsCollection of plant materials The aerial part of A. uniflora was collected during the rainy season in September, 2012 from Makurdi, Benue State, Nigeria. The plant was identified in the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria; and a Voucher specimen, number 2408, was deposited in the Herbarium. The plant materials were air-dried, pulverized and stored in air-tight containers until needed for further investigation.Extraction procedure.The air-dried pulverized plant material (200 g) was placed in a Soxhlet extractor, where it was exhaustively and successively extracted using petroleum ether (60-80°C), chloroform, ethyl acetate and methanol. The crude extracts of the plant were concentrated in vacuo at 40°C using a rotary evaporator. The crude extracts were subjected to phytochemical and antimicrobial studies.Phytochemical screening of the extracts was carried out using the standard procedures [7,24].The anti-microbial screeningThe anti-microbial activity of the petroleum ether, chloroform, ethyl acetate and the methanol extracts was determined using some pathogenic microbes, obtained from the Department of Medical Microbiology Ahmadu Bello University Teaching Hospital, Zaria. Each extracts (0.6 g) was weighed and dissolved in 10 ml dimethyl sulphoxide (DMSO) to obtain a concentration of 60 mg/ml. This initial concentration was used to determine the antimicrobial activity of the extracts. Mueller Hinton agar was the growth medium used for the microbes. The medium was prepared, sterilized at 121°C for 15 minutes and the sterilized medium was poured into sterile Petri dishes. The plates were allowed to cool and solidify. Agar diffusion method was used for screening of the extracts. The sterilized medium was seeded with 0.1 ml of the standard inoculum of the test microorganism; the inoculum was spread evenly over the surface of the medium with a sterile swab. Using a standard cork borer of 6 mm in diameter a well was cut at the center of each inoculated medium. Solution (0.1 ml) of each extracts of concentration of 60 mg/ml was then introduced into each well on the medium. The inoculated medium was then incubated at 37°C for 24 h after which each plate was observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the result recorded in millimeters [25].Minimum inhibition concentration of extractsThe minimum inhibition concentration (MIC) of the extracts was determined using broth dilution method. Mueller Hinton broth was prepared; 10 ml was dispensed into test tubes and was sterilized at 121°C for 15 minutes. The broth was allowed to cool. Mc- Farland turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared and 10 ml was dispensed into sterile test tubes. The test microbes were inoculated incubated at 37°C for 6 hours. Dilution of the test microbes was done in the normal saline until the turbidity matched that of the Mc - Farlands scale by visual comparison. At this point the concentration of

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the microbes was about1.5 × 108 cfu/ml. Two-fold serial dilution of the extracts in the sterile broth was made to obtain different concentration (60 mg/ml, 30 mg/ ml, 15 mg/ml, 7.5 mg/ml and 3.25 mg/ml). The initial concentration was obtained by dissolving 0.6 g of the extracts in 10 mL of the sterile broth. The broths were incubated at 37°C for 24 hours. The results were recorded after 24 hours. Thereafter 0.1 ml of the test microorganism in the normal saline was inoculated in to the different concentrations, incubations was made at 37°C for 24 h for bacteria and at 30°C for 48 h for fungi, after which each test tube of the broth was observed for turbidity (growth). The MIC was the value recorded from the test tube with the lowest concentration of the extract in the broth which showed no growth [25,26].Minimum bactericidal concentration/Minimum fungicidal concentrationThe minimum bactericidal concentration/ minimum fungicidal concentration (MBC/MFC) is the concentration that determines if the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared and sterilized at 121°C for 15 minutes, poured into Petri dishes and allowed to cool and solidify. The content of the MIC in the serial dilution was then sub-cultured onto the prepared medium and incubation was done at 37°C for 24 h. Thereafter each plate of the medium was observed for colony growth. The value obtained in the plate with the lowest concentration of the extract without colony growth was recorded as the MBC/MFC [7,25,26].Results Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [27]. The presence of steroids and triterpenes in all the four extracts were observed: cardiac glycosides were present in all the extracts except in petroleum ether extracts. Carbohydrates, cardiac glycoside, tannins, flavonoids, steroids and triterpenes were present in the ethyl acetate, chloroform, and methanol extracts. Saponins were present only in the methanol extract while alkaloids and anthraquinones were absent in all the four extracts (Table 1). The result of the antimicrobial screening is summarized in Tables 2 and 3. From the results of the antimicrobial screening (Table 2), the extracts showed activity against S. pyogenes, B. subtilis, C. albicans and S aureus. The petroleum ether extract was effective against S. pyogenes,B. subtilis, C. stellatoidea, K. pneumoniae, S aureus and C. albicans with a zone of inhibition of 16, 17, 17, 18 and 18 mm respectively. The MIC showed that the petroleum ether extract inhibited the growth of all the pathogenic microorganisms at a concentration of 30 mg/ml. The MBC/ MFC was found to be 60 mg/ ml for the petroleum ether extract against all the test microorganism (Table 3). The chloroform extract showed activity against S. pyogenes, C. albicans, C. stallatoidea, S. aureus and B. subtilis, with zones of inhibition of 22, 22, 23, 24 and 27 mm, respectively. At the MIC of 15 mg/ml the chloroform extract inhibited the growth of S. aureus, S. pyogenes, K. pneumoniae, C. albicans and C. stallatoidea, while at 7.5 mg/ml the growth of B. subtilis was inhibited. The MBC/MFC was found to be 30 mg/ml and at this concentration the extract exhibited activity against all the test microorganisms

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