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Research article

Flavonoids with antibacterial and antioxidant potentials from the stem bark of *Uapaca heudelotti*

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ABSTRACT

Two flavonol glycosides; U1: naringenin-7-*O*-glucoside and U2: kaempferol-3-*O*-glucoside were isolated for the first time, from ethyl acetate fraction of the stem bark of a traditional medicinal plant called *Uapaca heudelotti*. IR and NMR spectroscopy were used to elucidate the structures of the isolated compound. The two compounds were active against the 7 tested microorganisms; *Escherichia coli, Bacillus subtilis, Salmonella typhi, Streptococcus pyogenes, Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus mirabilis*. The zones of inhibition of the compounds ranged from 16 to 23 mm. The MIC value was as low as 6.25 μg/mL against *Salmonella typhi, Streptococcus pyogenes,* and *Bacillus subtilis*. The radical scavenging activity of compound U1 and U2 was 80 and 85 % at 240 μg/mL, while that of the standard drug was 98% at 240 μg/mL. The results show an existent possibility of using the plant for the treatment of microbial diseases.

1. Introduction

There is little or no literature report on the phytochemistry and pharmacology of *Uapaca heudelotti* (Euphorbiaceae). No attempt has previously been made to isolate and characterize some bio-active compounds from this plant until now. It is against this background that *Uapaca heudelotti,* used extensively in ethnomedicine for the treatment of oxidative stress and infectious diseases in some parts of Africa and Nigeria in particular, was chosen for this study. This research is aimed at isolating and characterizing bioactive compounds from the stem bark of *Uapaca heudelotti*. The root preparation of *Uapaca heudelotii* is taken as expectorant, and to treat fever and headache. The decoction of the bark is used to treat dysentery, food poisoning, female sterility, ovarian disorder, toothache, rheumatism, oedema and hemorrhoids. The decoction is also applied as enema to treat constipation, and rubbed against swellings (Burkill, 1985). The bark-extract of *Uapaca heudelotii* is used on skin-infections and as an emetic (Cooper and Record, 1931).

Plants are sources of medicinal ingredients that have been used by humans as natural medications (Mita et al., 2009). Phenolic compounds in plant protect plants from antimicrobial effects (Gulcin et al., 2010). Many studies have been tailored towards discovering new drugs from traditionally used medicinal plants (Govindarajan et al., 2016). Interest in studying phytochemistry and pharmacology of chemical compounds isolated from different plant species have increased significantly over the years (Karapandzova et al., 2011). Flavonoids are produced from the phenylpropanoid and malony-CoA pathway, and are phytochemicals which defend pathogenic infection (Jasiński et al., 2009). Scientists have confirmed that flavonoids could inhibit diseases, such as dysentery, diarrhoea, hypertension, liver, urinary and cerebral disorders (Azuma et al., 2012; Jennings et al., 2012; Chang et al., 2013).

Plants contain natural antioxidants but, there is limited scientific information on the practical usefulness of these plants. Phytochemicals are commonly found in various fruits, vegetables and herbs and help to fight against oxidative stress (Antolovich et al., 2000; Sarikurkcu et al., 2009). Herbal preparations, commonly used at home, have antioxidative properties due to the presence of phenolic compounds (Fecka et al., 2007).

2. Methodology

2.1. Plant material

Uapaca heudelotti stem bark was collected from Benue State, Nigeria. It was dried at 27 °C and pounded to uniform powder. The dried powdered plant materials was extracted with methanol at room temperature for 48 h and concentrated using a rotary evaporator. A portion of this extract was dissolved in water to yield a water-soluble fraction and water-insoluble fraction. The two fractions were subsequently partitioned successively and exhaustively using hexane and ethyl acetate.

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2.2. Separation techniques

2.2.1. Thin layer chromatography

Thin layer chromatography was performed using precoated aluminium TLC plates. They were developed by using the ascending techniques. Spots were applied on the TLC plates and developed at room temperature using a chromatographic tank.

2.2.2. Column chromatography

Column chromatography was carried out using the gradient elution technique. The column was 75 cm in length and 3.5 cm in diameter. The stationary phase used was silica gel of 60 -120 mesh size. The wet slurry method was employed during the column packing. 7.5 g of ethyl acetate extract was dissolved in ethyl acetate and a small quantity of silica gel was added to the dissolved extract. It was dried and loaded on top of the previously packed column (Cannell, 2000).

1.5 L of various solvents comprising absolute hexane, hexane and ethyl acetate mixtures (80:20 and 60:40 respectively), absolute ethyl acetate and absolute methanol was used as solvent systems. A total of 53 collections was obtained and pooled together into 4 sub-fractions, 'a', 'b', 'c' and 'd' (due to similarities of their TLC chromatograms).

2.2.3. Gel filtration chromatography

10 g of Sephadex LH-20 was soaked in methanol and allowed to swell for 24 h prior to use. It was then introduced into a glass column and allowed to settle tightly. Sub-fractions 'c' (3.0 g) was dissolved in a minimum amount of methanol which was used as the eluting solvent and applied on top of the column. Several gel filtration chromatography of sub fraction 'c' gave another 21 collections. Collections 3 to 8 consisted of just one spot. They were pooled together and labelled as U1. Collection 9 to 14 had a different single spot with a different R_f value. Collection 9 to 14 were pooled together and labelled as U2.

2.3. Determination of antioxidant activity

The antioxidant activity of the isolated compounds was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The antioxidant activity was carried out by a method described by Mensor et al. (2001). 15, 30, 60, 120 and 240 μg/mL of the isolated compounds was prepared and ascorbic acid was used as the antioxidant standard. Same concentrations of each solution of the compound was placed in a test tube, and 1 mL of 0.3 mM methanolic solution of DPPH was added. The experiment was done in triplicates for each of the concentrations. The percentage antioxidant activity was calculated using the following formula:

$$\%\ \text{antioxidant activity} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2.4. Susceptibility test

The antibacterial activity was evaluated using clinical strains of *Klebsiella pneumonia Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Streptococcus pyogenes, Salmonella typhi* and *Proteus mirabilis.* Culturing and identification of bacterial species was carried out using standard method (Colle et al., 1996).

The antimicrobial screening was performed using the agar well diffusion technique as described by NCCLS, 2002. The culture media was sterilized for 15 min at 121 °C. 0.1 mL standard inoculum of the test microorganism was introduced into the sterilized media. The inoculum was spread all through the media. 6 mm in diameter well was cut at the centre of each seeded medium, and 0.1 mL solution of the compound was then introduced into each well on the medium. The inoculated plates were incubated at 37 °C for 24 h, after which the plates were observed for the zone of inhibition of growth of microorganisms. The zone was measured with a transparent ruler in millimetres.

2.5. Minimum inhibitory concentration (MIC) of the compounds

Minimum inhibitory concentration of the compounds was carried out using standard method by Vollekova et al. (2001). 10 mL nutrient broth was transferred into test tubes, they were sterilized for 10 min at 121 °C and cooled. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. 10 mL normal saline was dispensed into

Table 1. NMR spectra data of U1 (MeOD, 600 Hz) and comparison with literature data.

Position (U1)	^{13}C NMR Experimental	^{13}CNMR (Andersen and Markham, 2006)	^{1}H NMR Experimental	^{1}H NMR (Andersen and Markham, 2006)
2	79.07	80.62	5.385 J = 10.0	5.46 dd, J = 13.0
3	42.58	44.13	2.761 (3.1648) d, J = 17.6	2.83 (3.23) J = 17.0,12.5
4	196.49	198.52		
5	157.56	159.07		
6	94.88	96.92	5.93(1H, d, J = 7.14 Hz)	
7	167.03	167.00		
8	95.74	97.99	5.93(1H, d, J = 7.14 Hz)	
9	163.49	164.0		
10	102.01	104.4		
1′	127.64	130.85		
2′	129.74	129.09	7.35 J = 8.16	7.4,d 8.6
3′	115.03	116.33	6.865 (2H, d, J = 8.18 Hz)	
4′	164.01	164.59		
5′	115.03	116.33	6.865 (2H, d, J = 8.18 Hz)	6.90 d 8.6
6′	129.74	129.09	7.35 J = 8.16	7.4, d 8.6
1″	102.01	102.3	4.54(d)	4.47 (d)
2″	72.47	74.50	3.20 (m)	3.23 (m)
3″	79.06	77.80	3.26 (m)	3.26 (m)
4″	72.47	71.10	3.14 (m)	3.12 (m)
5″	79.06	78.30	3.34 (m)	3.37 (m)
6″	63.04	62.40	3.67 (m)	3.67(m)

Table 2. NMR spectra data of U2 (MeOD, 600 Hz) and comparison with literature data.

(U2)	^{13}CNMR	^{13}C NMR (Furusawa et al., 2005)	^{1}H NMR	^{1}H NMR (Furusawa et al., 2005)
2	159.16	157.5		
3	135.73	134.4		
4	176.02	178.57		
5	161.17	161.9		
6	97.93	100.8	6.22 (d)	6.44(d)
7	164.2	158.57		
8	93.11	95.2	6.43 (d)	6.76 (d)
9	156.9	156.1		
10	103.19	107.2		
1′	122.37	121.3		
2′	131.47	131.4	8.12, d, $J = 8.34$ Hz	8.04, d, $J = 8.8$ Hz
3′	115.93	116.5	6.945, d, $J = 8.24$ Hz	6.88, d, $J = 8.8$ Hz
4′	161.17	161.4		
5′	115.93	116.5	6.949, d, $J = 8.24$ Hz	6.89, d, $J = 8.8$ Hz
6′	131.47	131.4	8.12, d, $J = 8.34$ Hz	8.04, d, $J = 8.8$ Hz
1″	103.19	106.7	5.20 (d)	5.20 (d)
2″	72.5	74.8	3.19 (m)	3.19 (m)
3″	76.7	77.4	3.02 (m)	3.22(m)
4″	72.47	70.8	3.13 (m)	3.03(m)
5″	79.3	77.2	3.01 (m)	3.07(m)
6″	63.06	61.7	3.50 (m)	3.54 (m)

sterile test-tube and the test microbes inoculated and incubated for 6 h at 37 °C. Dilution of the test microbes in the normal saline was made until the turbidity matched that of the McFarland scale by visual comparison. The concentration of the test organism was about 1.5×10^8 cfu/mL. Serial dilution of the compounds in the sterile broth was made to obtain concentrations of 20 μg/mL, 10 μg/mL, 5 μg/mL, 2.5 μg/mL and 1.25 μg/mL. Stock concentration was obtained by dissolving 20 μg of the compound in 1 mL of the sterile broth. Having obtained the different concentration of the compound in the serial broth, 0.1 mL of the test microbes in the normal saline was then inoculated into the different concentrations of the compounds. Incubation was for 24 h at 37 °C, and each test-tube was observed for growth of microbes. The least concentration of the compounds in the broth which shows no turbidity (microbial growth) was taken as the minimum inhibitory concentration. The experiment was conducted in triplicates for all the concentrations.

2.6. Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was performed to determine the concentration the test microbes were killed. Mueller-Hinton media was prepared, sterilized and allowed to cool. The plates were allowed to cool and solidify. The content of the test tube with MIC were then sub-cultured onto the prepared media. Incubation was made for 24 h at 37 °C and the plate were observe for microbial growth. The MBC were the plates with least concentration of the compound without microbial growth (NCCLS, 2002).

2.7. Spectral analysis

FTIR analysis was performed on a Fourier Transform Infra-red Spectrophotometer at the Multiuser Laboratory, Department of Chemistry, ABU, Zaria. ^{1}H and ^{13}CNMR spectroscopy was carried out at the School of Chemistry and Physics, University of *Kwazulu-Natal,* Durban South Africa on a Bruker ADVANCE spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C). Chemical shifts (ppm) relative to TMS standard and coupling constant are recorded in Hertz. Samples were dissolved in deuterated methanol.

3. Results

The aromatic protons in the ^{1}HNMR spectrum of U1 were assigned as follows: 5.385 ppm (1H, d, $J = 10.0$ Hz, H-2), 2.761 ppm (1H, d, $J = 17.0$ Hz, H-3), 5.93 ppm (1H, d, $J = 7.14$ Hz, H-8) and ppm (1H, d, $J = 7.14$ Hz, H-6) on ring and 6.865 ppm (2H, d, $J = 8.18$ Hz, H-3′,5′). The resonance at 4.5 ppm is characteristic of an – anomeric proton (Table 1). The ^{13}CNMR spectrum of compound U1 revealed the presence of carbonyl carbon at 196.49 ppm, in addition to 12 aromatic carbon atoms at 94.5–165.6 ppm. In the ^{13}CNMR spectrum of U1, the presence of a signal 157 ppm was observed which is characteristic of glycosylation at C-7. Anomeric carbon signal showed at 100.7 ppm. The remaining sugar carbons appeared at 68–77 ppm (Table 1). ^{1}HNMR spectrum of U2 showed that the ring B protons showed a doublet (2H, d, $J = 8.34$ Hz) at δ 8.12 and 6.945, which is characteristic of the presence of a para-

Table 3. Zone of inhibition of the isolated compounds (mm).

Micro organisms	U1	U2	Ciprofloxacin
Bacillus subtilis	23 ± 1	22 ± 1	37 ± 1
Proteus mirabilis	21 ± 1	15 ± 1	17 ± 1
Staphylococcus aureus	22 ± 1	21 ± 1	28 ± 1
Klebsiella pneumonia	16 ± 1	20 ± 1	25 ± 1
Streptococcus pyogenes	17 ± 1	21 ± 1	30 ± 1
Salmonella typhi	23 ± 1	20 ± 1	35 ± 1
Escherichia coli	17 ± 1	15 ± 1	32 ± 1

Table 4. Minimum inhibitory concentration of the isolated compounds (μg/mL).

Micro organisms	U1	U2	Ciprofloxacin
Bacillus subtilis	6.25 ± 0.03	6.25 ± 0.03	3.12 ± 0.03
Proteus mirabilis	6.25 ± 0.03	12.5 ± 0.03	6.25 ± 0.03
Staphylococcus aureus	6.25 ± 0.03	12.5 ± 0.2	6.25 ± 0.03
Klebsiella pneumonia	12.5 ± 0.2	12.5 ± 0.2	6.25 ± 0.03
Streptococcus pyogenes	6.25 ± 0.03	6.25 ± 0.03	6.25 ± 0.03
Salmonella typhi	6.25 ± 0.03	6.25 ± 0.03	12.5 ± 0.2
Escherichia coli	3.12 ± 0.02	3.12 ± 0.02	6.12 ± 0.03

Table 5. Minimum bactericidal concentration the isolated compounds (μg/mL).

Micro organism	U1	U2	Ciprofloxacin
Bacillus subtilis	12.5 ± 0.2	12.5 ± 0.2	12.5 ± 0.2
Proteus mirabilis	6.25 ± 0.03	12.5 ± 0.2	6.25 ± 0.03
Staphylococcus aureus	6.25 ± 0.03	12.5 ± 0.2	12.5 ± 0.2
Klebsiella pneumonia	6.25 ± 0.03	12.5 ± 0.2	6.25 ± 0.03
Streptococcus pyogenes	12.5 ± 0.2	6.25 0.03	6.25 ± 0.03
Salmonella typhi	6.25 ± 0.03	12.5 ± 0.2	12.5 ± 0.2
Escherichia coli	6.25 ± 0.03	6.25 0.03	6.25 0.03

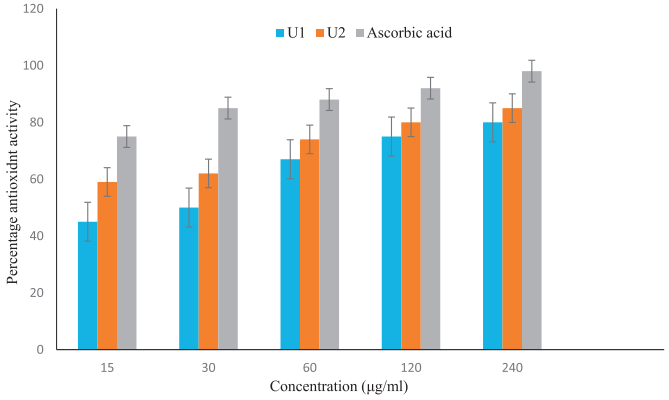
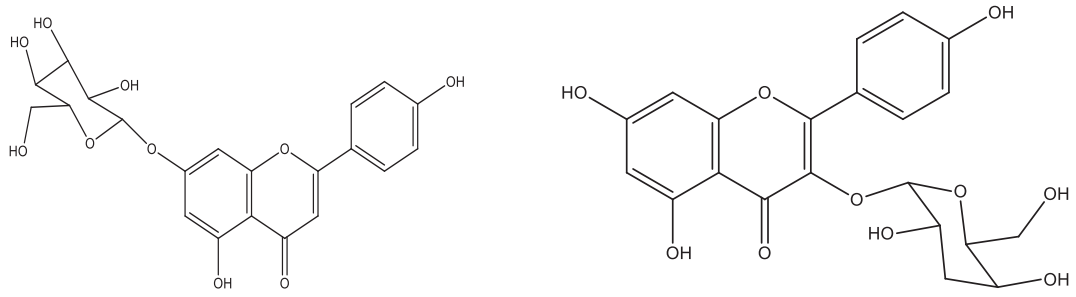


Figure 1. Antioxidant activity of U1 and U2.

substituted benzene ring. Ring A Protons at δ 6.22 (1H, d, $J = 1.90$ Hz) and 6.43 (1H, d, $J = 1.90$ Hz), respectively revealed meta-aromatic protons at H-8 and H-6 (Table 2). The ^{13}CNMR spectrum of U2 revealed 21 carbon signals - one anomeric carbon, one carbonyl, one oxygenated sp^3 methylene carbon, four oxygenated sp^3 methines, six sp^2 methines and eight sp^2 quartenary carbon (Table 2). The diameter of zone of inhibition of U1 was 23, 21, 22, 16, 17, 23 and 17 mm against *Bacillus subtilis*, *Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pyogenes, Salmonella typhi* and *Escherichia coli* respectively (Table 3). While 22, 15, 21, 20, 21, 20 and 15 mm was recorded for compound U2 against the same bacteria respectively. The diameter of zone of inhibition of ciprofloxacin against these bacteria was 37, 17, 28, 25, 30, 35 and 32 mm respectively (Table 3). Compound U1 had MIC of 3.12 μg/mL against *Escherichia coli,* 6.25 μg/mL against *Bacillus subtilis*, *Proteus mirabilis, Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi*, and 12.5 μg/mL against *Klebsiella pneumonia* (Table 4). Compound U2 had a MIC of 3.125 μg/mL against *Escherichia coli,* 6.25 μg/mL against *Bacillus subtilis*, *Streptococcus pyogenes, Salmonella typhi,* and 12.5 μg/mL against *Klebsiella pneumonia, Proteus mirabilis* and *Staphylococcus aureus* (Table 4). U1 had MBC of 6.25 μg/mL against *Salmonella typhi*, *Proteus mirabilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia,* and 12.5 μg/mL against *Bacillus subtilis* and *Streptococcus pyogenes* (Table 5). U2 had MBC of 6.25 μg/mL against *Streptococcus pyogenes* and *Escherichia coli,* and 12.5 mg/mL against *Salmonella typhi, Proteus mirabilis, Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumonia* (Table 5). The radical scavenging activity of compound U1 was 45, 50, 67, 75 and 80% at 15, 30, 60, 120 and 240 μg/mL respectively (Figure 1). The antioxidant activity of compound U2 was 59, 62, 74, 80 and 85% at 15, 30, 60,120 and 240 μg/mL respectively (Figure 1).



(U1) (U2)

Figure 2. (U1) naringenin -7-*O*-glucoside and (U2) kaempferol-3-*O*-glucoside.

4. Discussion

Compound U1 was isolated as a greenish yellow amorphous substance with a sharp melting point of 124 °C also indicating its purity. Compound U1 tested positive to Shinoda test, indicating that it is a flavonoid (Sofowora, 1993).

The band at 3343 cm^{-1} in the IR spectrum of U1 revealed O–H stretching on a benzene ring, also the absorption peak at 1684 cm^{-1} is evident of carbonyl functional group (Wade, 2006).

Based on spectra data and comparison with available literature values (Table 1) (Andersen and Markham, 2006), it was observed that U1 had a flavonoid skeleton consisting of naringenin aglycone moiety attached to β-D-glucoside. Consequently, the structure of U1 was identified as naringenin-7-*O*-glucoside (Figure 2) isolated for the first time from *U. heudelotti*.

Compound U2 is yellow and amorphous with a sharp melting point of 147 °C also indicating its purity. Compound U2 also tested positive to Shinoda test, indicating that U2 is a flavonoid (Sofowora, 1993).

Hydroxyl group (3354 cm^{-1}), carbonyl group (1684 cm^{-1}), conjugated olefinic bond (1607 cm^{-1}) and ether groups (1078 cm^{-1}) were observed in the IR spectrum of U2.

The ^{1}H-NMR of U2 showed ring B protons as a doublet (2H, d, J = 8.34 Hz) at δ 8.12 and 6.945, signifying a para-substituted aromatic ring. Ring A protons at δ 6.22 (1H, d, J = 1.90 Hz) and 6.43 (1H, d, J = 1.90 Hz), respectively suggests meta-aromatic proton H-8 and H-6. From these observations and comparisons with literature data, U2 is a kaempferol aglycone (Figure 2). The ^{1}HNMR spectra of U2 also showed the presence of an anomeric proton as a doublet at δ 5.20 suggesting a sugar residue (Furusawa et al., 2005).

From the ^{13}CNMR of U2 eight olefinic bond equivalents was revealed out of the total twelve olefinic bond equivalents. The remaining four olefinic bond equivalents were characteristic of the flavone skeleton with a β-glucose residue (Nakano et al., 1983). Based on the above NMR data and the comparison with literature data, compound U2 was discovered to have flavonoid structure (Toker et al., 2004: Furusawa et al., 2005) and consists of kaempferol aglycone attached to D-glucoside as a sugar residue. Consequently, U2 was identified as kaempferol-3-O-glucoside (Figure 2) consistent with reported literature values (Kuruuzum-uz et al., 2013).

The highest sensitivity was observed against *Salmonella typhi* and *Bacillus subtilis* at a zone of inhibition of 23 mm and MIC of 6.25 μg/mL (Tables 3 and 4) for compound U1, which was elucidated as a flavonoid. This antibacterial activity could be due to the fact that flavonoids have been shown to act by complexing proteins and breaking microbial membranes (Samy and Gopalakrishnakone, 2008). Antimicrobial activity for compound U2 ranged from 15 to 22 mm. This activity may be due to the fact that phytochemicals such as flavonoids act by complexing bacterial proteins, interfering with bacterial adhesion, inactivating enzymes and disrupting bacterial cell membrane (Cowan, 1999; Okuda, 2005; Victor et al., 2005; Biradar et al., 2007). These chemicals penetrate cell membranes up to the cytoplasm and interact with intracellular targets critical for antibacterial activity (Trombetta et al., 2005). They are also used to manage acute bronchial disease, epilepsy, cold, cough, and influenza (Victor et al., 2005).

The radical scavenging activity of compound U1 was 80 % 240 μg/mL (Figure 1). Antioxidant effect of chemical compounds is mainly due to the presence of hydroxyl functional groups (Mallick et al., 2016). The high polyphenolic content in compound U1 could be responsible for the high antioxidant activity observed (Mallick et al., 2016). Polyhydroxy compounds are good antoxidants, due to their hydroxyl groups. These polyhydroxy groups present in compound U2 may be responsible for the antioxidant activity of compound U2 (a flavonoid) which was 85 % at 240 μg/mL (Figure 2).

In conclusion, the antimicrobial and antioxidant activities of the plant may be due to the presence of the isolated compounds.

Declarations

Author contribution statement

Jonathan Achika: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Rachael Ayo: Conceived and designed the experiments.

Adebayo Oyewale: Contributed reagents, materials, analysis tools or data.

James Habila: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Andersen, O.M., Markham, K.R., 2006. FLAVONOIDS: Chemistry, Biochemistry and Applications. CRC PressTaylor & Francis Group 6000 Broken Sound Parkway NW, Suite 300 Boca Raton, FL 33487-2742.

Antolovich, M., Prenzler, P., Robards, K., Ryan, D., 2000. Sample preparation in the determination of phenolic compounds in fruits. Analyst 125 (5), 989–1009.

Azuma, A., Yakushiji, H., Koshita, Y., Kobayashi, S., 2012. Flavonoid biosynthesis-related genes in grape skin are diferentially regulated by temperature and light conditions. Planta 236, 1067–1080.

Biradar, Y.S., Jagatap, S., Khandelwal, K.R., Singhania, S.S., 2008. Exploring of antimicrobial activity of Triphala Mashi—an ayurvedic formulation. Evid. Base Compl. Alternative Med. 5 (1), 107–113.

Burkill, H.M., 1985. The Flora of West Tropical Africa. Royal Botanic Gardens, Kew.

Cannell, R.J.P., 2000. How to approach the isolation of natural Product. In: Cannell, R.J.P. (Ed.), Natural Product Isolation. Human Press Totowa, New Jersey (USA), pp. 1–52.

Cooper, G.P., Record, S.J., 1931. The Evergreen Forests of Liberia: A Report on Investigations Made in the West African Republic of Liberia by the. Yale University School of Forestry in Cooperation with the Firestone Plantations Company.

Chang, J.J., Hsu, M.J., Huang, H.P., Chung, D.J., Chang, Y.C., Wang, C.J., 2013. Mulberry anthocyanins inhibit oleic acid induced lipid accumulation by reduction of lipogenesis and promotion of hepatic lipid clearance. J. Agric. Food Chem. 61 (25), 6069–6076.

Colle, J., Fraser, A., Marmion, B., Simmans, A., Mackie, McCartney, 1996. Practical Medical Microbiology, fourteenth ed. Churchill Living ston., New York, USA, p. 978.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12 (4), 564–582.

Fecka, I., Raj, D., Krauze-Baranowska, M., 2007. Quantitative determination of four water-soluble compounds in herbal drugs from Lamiaceae using different chromatographic techniques. Chromatographia 66 (no. 1-2), 87–93.

Furusawa, M., Tanaka, T., Ito, T., Nishikawa, A., Yamazaki, N., Nakaya, K.I., et al., 2005. Antioxidant activity of hydroxyflavonoids. J. Health Sci. 51 (3), 376–378.

Govindarajan, M., Rajeswary, M., Benelli, G., 2016. Chemical composition, toxicity and non-target effects of Pinus kesiya essential oil: an eco-friendly and novel larvicide against malaria, dengue and lymphatic filariasis mosquito vectors. Ecotoxicol. Environ. Saf. 129, 85–90.

Gulcin, I., Bursal, E., Şehitoğlu, M.H., Bilsel, M., Gören, A.C., 2010. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food Chem. Toxicol. 48 (8-9), 2227–2238.

Jasiński, M., Kachlicki, P., Rodziewicz, P., Figlerowicz, M., Stobiecki, M., 2009. Changes in the profile of flavonoid accumulation in Medicago truncatula leaves during infection with fungal pathogen Phoma medicaginis. Plant Physiol. Biochem. 47 (9), 847–853.

Jennings, A., Welch, A.A., Fairweather-Tait, S.J., Kay, C., Minihane, A.M., Chowienczyk, P., et al., 2012. Higher anthocyanin intake is associated with lower arterial stiffness and central blood pressure in women. Am. J. Clin. Nutr. 96 (4), 781–788.

Karapandzova, M., Stefkov, G., Trajkovska-Dokic, E., Kaftandzieva, A., Kulevanova, S., 2011. Antimicrobial activity of needle essential oil of Pinus peuce Griseb.(Pinaceae) from Macedonian flora. Maced. Pharmaceut. Bull. 57 (1), 2.

Kuruüzüm-Uz, A., Güvenalp, Z., Kazaz, C., Demirezer, L.Ö., 2013. Phenolic compounds from the roots of Anchusa azurea var. azurea. Turk. J. Pharm. Sci. 10 (2), 177–184.

Mallick, M., Bose, A., Mukhi, S., 2016. Comparative evaluation of the antioxidant activity of some commonly used spices. Int. J. PharmTech Res. 9 (1), 1–8.
Mensor, L.I., Menezes, F.S., Leitao, G.G., Reis, A.S., Dos Santos, T., Coube, C.S., Leitao, S.G., 2001. Screening of Brazilian plants extracts for antioxidants activity by the use of DPPH free radical method. Phytother. Res. 15, 127–130.
Mita, T., Tanabe, K., Kita, K., 2009. Spread and evolution of Plasmodium falciparum drug resistance. Parasitol. Int. 58 (3), 201–209.
Nakano, K., Takatani, M., Tomimatsu, T., 1983. Four kaemferol glycosides from leaves of Cinnamomum sieboldii. Phytochemistry 22 (12), 2831–2833.
NCCLS, 2002. Performance Standard for Antimicrobial Susceptibility Testing, 12th Informational Supplements. NCCLS Document M100-512. National Commettee For Chemical Laboratory Standards, Wayne, P.A.
Okuda, T., 2005. Systematics and health effects of chemically distinct tannins in medicinal plants. Phytochemistry 66 (17), 2012–2031.
Samy, R.P., Gopalakrishnakone, P., 2008. Review: therapeutic potential of plants asantimicrobials for drug discovery. J. Evidence-Based Compl. Alternative Med. 10, 1093.
Sarikurkcu, C., Arisoy, K., Tepe, B., Cakir, A., Abali, G., Mete, E., 2009. Studies on the antioxidant activity of essential oil and different solvent extracts of Vitexagnuscastus L. fruits from Turkey. Food Chem. Toxicol. 47 (10), 2479–2483.
Sofowora, L.A., 1993. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan Nigeri, p. 130.
Toker, G., Memisoglu, M., Yesilada, E., Asian, M., 2004. Mainflavonoids of *Tilia argentea* DESF ex DC leaves. Turk. J. Chem. 28, 745–749.
Trombetta, D., Castelli, F., Sarpietro, M., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G., Bisignano, G., 2005. Mechanisms of anti-bacterial action of three monoterpenes. J. Antimicrob. Agents Chemother. 49, 2474–2478.
Victor, J., Siebert, S., Hoare, D., Wyk, B.V., 2005. Sekhukhuneland Grasslands: A Treasure House of Biodiversity. FAO, Rome.
Vollekova, A., Košťálová, D., Sochorova, R., 2001. Isoquinoline alkaloids from Mahonia aquifolium stem bark are active against *Malassezia* spp. Folia Microbiol. 46 (2), 107–111.
Wade, L.G., 2006. Organic chemistry. In: International Editions, pp. 396–400.