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Original Article

Chemical Composition, Antioxidant and Anti-tumor Activities of the Aerial Parts of *Cometes abyssinica* R.Br. ex Wall

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ABSTRACT

An *in vitro* bioassay-guide revealed that the methanolic extract of the aerial parts of *Cometes abyssinica* had considerable inhibitory activity against colon (HCT-116) and hepatocellular (HepG2) carcinoma cell lines with IC_{50} 24.4 and 29.9 µg/mL, respectively, compared to the drug reference Cisplatin. On the other hand, all studied extracts exhibited weak antioxidant activities using the DPPH scavenging assay with IC_{50} values between 543-826.4 µg/mL. Phytochemical investigation of the methanol and ethyl acetate extracts of *C. abyssinica* led to the isolation of five flavonoid compounds: Formononetin 1, Daidzein 2, Eriodictyol 3, Taxifolin 4, and kaempferitrin 5. The chemical structure of the isolated compounds was identified based on their physicochemical and spectroscopic analyses.

Keywords: *Cometes abyssinica*, Aerial parts, Chemical constituents, Flavonoids, Antioxidant activity, Antitumor activity.

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INTRODUCTION

Naturally antioxidants are used widely as an ingredient in dietary supplement for promoting good health and preventing diseases like cancer, cardiovascular disease. In addition, they are also used as preservatives for foods.[1,2] Researchers in the middle of the last century after doing several studies found that life span of people increases by the normal consumption of antioxidants. In the 19th century, antioxidants are applied for many industrial processes like prevention of metal corrosion and rubber vulcanization.[3]

Antitumor agents used for the therapy of malignant and other diseases. They are designed to destroy quickly growing of the cancer cells. These agents have been revealed to be mutagenic, carcinogenic and/or teratogenic, either in therapy doses or bacterial assays.[4]

Always medicinal plants have played a vital role as sources for drug lead compounds. Over 1350 medicinal plants are used in European countries, about of them, 90% are from wild sources. According to the International Union for Conservation of Nature and the World Wildlife Fund, about 50,000–80,000 of flowering plants are used because of their medicinal values.[5]

Caryophyllaceae family present a huge group of plants spread around almost the whole world. Nowadays, this family contains more than one hundred genera with more 2000 species.[6]

Cometes abyssinica (*Caryophyllaceae* family) mostly distributed in open rocky slopes; near sea shores. It is distributed across Egypt, Palestine, Ethiopia, Sudan, Somalia, Saudi Arabia, Yemen, and Oman.[7] Its habitat is stony lands. Broad shrub to 50 cm high, sometimes flowering in first season as erect annual; all parts minutely erect puberulent.[8,9] According to literature, the MeOH extract of the leaf of *Cometes abyssinica* collected from Eritrea showed good antimicrobial activities against *S. aureus, E. coli* and *Candida albicans*.[8]

Till now, no studies published, providing full information about the phytochemical constituents of *Cometes abyssinica*. So, this research aimed to screen the aerial parts of *C. abyssinica* for its chemical constituents, antioxidant, and cytotoxic activities in a continuation of our studies on the medicinal phytotherapy.[10-14]

MATERIALS AND METHODS

All chemicals and reagent used were of high quality and analytical grade obtained from Sigma-Aldrich (USA). Melting points were determined on a A. KRÜSS Optronic (KSP1D). TLC was carried out on silica gel coated with flourescent indicator F_{254} aluminum plates (Merck). Compounds were detected by UV fluorescence before and after spraying with $AlCl_3$ and exposing

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to ammonia vapors. For column chromatography silica gel (Kieselgel 60, 230-400 mesh, Merck), Polyamide-6C (Fluca USA), and Sephadex LH-20 (Amersham Biosciences) were used as the stationary phases. UV-spectra (λ_{max}, nm) were recorded on a UV-spectrophotometer UV-1800 (Shimadzu, Japan). The Inferred spectra were measured using a Perkin-Elmer 1650 spectrometer (Waltham, MA, USA). NMR spectra were determined using a JEOL (JEOL, Tokyo, Japan) 300 MHz (^{1}H-NMR) and 75 MHz (^{13}C-NMR). Mass spectra were assessed by EI Ms-QP 1000 EX instrument (Shimadzu, Japan) at 70 eV.

Plant material

Aerial parts of *Cometes abyssinica* R.Br. ex wall (*C. abyssinica*) were collected from Gebel Elba region, southeast corner of Egypt in June 2019 and identified by Dr. Omran N. Ghaly, The Herbarium, Department of Plant Ecology and Range Management, Desert Research Center, Egypt. A voucher specimen (CAIH-1021-R) has been deposited in Desert Research Center Herbarium (CAIH).

Chemical studies

Air-dried finely powdered aerial parts of *C. abyssinica* (450 g) was extracted with MeOH (95%, 3 L) in a 5 L conical flask for 48 hr. The extraction was repeated three times under the same conditions. The aqueous methanol extracts were mixed, filtered, and evaporated under vacuum. The resulting methanol extract was suspended in H_2O and worked up successively with *n*-hexane, Chloroform ($CHCl_3$), Ethyl Acetate (EA), and Methanol (MeOH). The obtained extracts were evaporated under vacuum conditions.

The ethyl acetate extract (3.7 g) was subjected to column chromatography on silica gel and eluted with gradient: chloroform and methanol with increasing methanol concentration (0-100%) to afford 50 fractions. Compounds 1 and 2 (105 and 75 mg) were detected in $CHCl_3$-MeOH (7:3) fractions, compound 3 (95 mg) in $CHCl_3$-MeOH (6:4) fractions and compound 4 (100 mg) in $CHCl_3$-MeOH (5:5). MeOH extract (4.6 g) was subjected to polyamide column chromatography eluted with EtOH with increasing polarity by H_2O to afford 35 fractions. Compound 5 (95 mg) were detected in EtOH-H_2O (6:4) fractions. The qualitative composition of methanol and ethyl acetate fractions controlled by paper and thin-layer chromatography in a BAW solvent system (*n*-BuOH–CH_3COOH–H_2O) (4:1:5), CH_3COOH (15%), and $CHCl_3$-MeOH. The isolated compounds were purified by column chromatography using Sephadex LH-20 eluting with methanol.

For further analysis, the acid hydrolysis of some isolated compounds was conducted with 5% H_2SO_4 (2 mL) for 5 hr in the water bath. The solution was neutralized with Na_2CO_3 and extracted with ethyl acetate. Analysis of the substances was carried out by paper PC and TLC.

Compound 1

Amorphous powder, m.p. 254-257°C; UV (MeOH, λ_{max}, nm): 303 (sh), 251, 240, 205, 215; IR (KBr, ν_{max}, cm^{-1}): 2916 (OCH_3), 1599 (C=O), 1458, 1373 (C=C); ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 8.20 (1H, s, H-2), 7.98 (1H, d, J = 9.0 Hz, H-5), 7.51 (2H, d, J = 9.0 Hz, H-2′), 6.96 (2H, d, J = 9 Hz, H-3′), 6.90 (1H, dd, J = 9.0 Hz, J = 2.4 Hz, H-6), 6.85 (1H, d, J = 2.4 Hz, H-8), 3.77 (3H, s, 4′-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.35 (C-4), 162.85 (C-7), 159.10 (C-4′), 158.10 (C-9), 153.45 (C-2), 130.13 (C-2′ and C-6′), 127.55 (C-5), 124.6 (C-1′), 123.62 (C-3), 117.23 (C-10), 115.6 (C-6), 114.2 (C-3′ and C-5′), 102.3 (C-8), 56.10 (CH_3); MS (EI, *m/z*): 269 $[M + H]^+$, 268.1 $[M]^+$, 151 $[C_8H_7O_3]^+$, 137 $[C_7H_5O_3]^+$; Anal. calcd. for $C_{16}H_{12}O_4$: C, 71,64; H, 4.51. Found: C, 71.10; H, 4.15%.

Compound 2

Amorphous powder, m.p. 322-324°C; IR (KBr, ν_{max}, cm^{-1}): 3630 (OH), 1630 (C=O), 1595, 1518, 1455, 1188, (C=C); UV (MeOH, λ_{max}, nm): 212, 240 (sh), 250, 263 (sh), 306; + NaOH: 215, 260, 292 (sh), 332; + NaOAc: 256, 273 (sh), 312 (sh), 337; +H_3BO_3: 212, 240 (sh), 250, 263 (sh), 306; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25 (1H, s, H-2), 7.95 (1H, d, J = 9 Hz, H-5), 7.40 (2H, d, J = 8.0 Hz, H-2' and 6'), 6.97 (1H, dd, J = 9.0, 2.0 Hz, H-6), 6.90 (1H, d, J = 2 Hz, H-8), 6.83 (2H, d, J = 8 Hz, H-3' and 5'); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.6 (C-4), 162.2 (C-7), 157.4 (C-4'), 157.1 (C-9), 152.9(C-2), 130.3 (C-2'), 127.4 (C-5), 123.5 (C-1'), 122.7 (C-3), 116.3 (C-10), 114.9 (C-3'), 114.8 (C-6), 102.3 (C-8); MS (EI, *m/z*): 255,2 [M + H] $^+$, 254,06 $[M]^+$, 151 $[C_8H_7O_3]^+$, 137$[C_7H_5O_3]^+$; Anal. calcd. for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96. Found: C, 70.40; H, 3.60%.

Compound 3

White powder; m.p 259-261°C; IR (KBr, ν_{max}, cm^{-1}): 3353 (br OH), 1655 (C=O), 1636, 1604, 1596, 1450, 1311, 1274, 1259, 1159 (C=C); UV (MeOH, λ_{max}, nm): 214, 230 (sh), 287, 331.5 (sh); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.94 (1H, s, H-2'), 6.77-6.79 (2H, m, H-5' and H-6'), 5.89 (1H, d, *J*= 2.2 Hz, H-8), 5.85 (1H, d, J= 2.2 Hz, H-6), 5.19 (1H, dd, J=3.1, 12.7 Hz, H-2), 3.05 (1H, dd, J= 12.7, 17.2 Hz, H-3_{ax}), 2.70 (1H, dd, J= 3.0, 17.2 Hz, H-3eq);; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 197.9 (C-4), 168.6 (C-7), 164.8 (C-5), 162.3 (C-9), 148.1 (C-3'), 147.4 (C-4'), 131.6 (C-1'), 119.7 (C-6'), 116.1 (C-5'), 115.2 (C-2'), 103.9 (C-10), 97.1 (C-8), 96.5 (C-6), 80.7 (C-2), 44.0 (C-3); MS (EI, *m/z*): 289.2 $[M+H]^+$, 288.03 $[M]^+$, 179.2 [M- ring B]$^+$, 166.10, 153.13 [M - ring B - C_2H_2]$^+$, 136.05; Anal. calcd. for $C_{15}H_{12}O_6$: C, 62.50; H, 4.20. Found: C, 62.60; H, 4.00%.

Compound 4

Yellow powder, m.p. 219-221°C; IR (KBr, ν_{max}, cm^{-1}) 3366 (OH), 1640 (C=O), 16466, 1360, 1596, 1164, 1086(C=C); UV (MeOH, λ_{max}, nm): 290, 327; ^{1}H NMR ($CDCl_3$,300 MHz) δ 7.09 (1H, d, J= 1.7 Hz, H-2'), 6.85-6.9 (2H, m, H-5' and H-6'), 5.98 (1H, d, *J*= 1.7 Hz, H-8), 5.94 (1H, d, J= 1.7 Hz, H-6), 5.1 (1H, d, J=11.5 Hz, H-2), 4.65 (1H, d, J= 11.5 Hz, H-3); ^{13}C NMR ($CDCl_3$, 75 MHz)

δ 195.5 (C-4), 167.8 (C-7), 164.8 (C-5), 163.7 (C-9), 146.5 (C-4'), 145.6 (C-3'), 129.4 (C-1'), 120.6 (C-6'), 115.6 (C-2',5'), 101.4 (C-10), 96.0 (C-8), 97.1 (C-6), 83.9 (C-2), 73.0 (C-3); MS (EI, *m/z*) 305.2 $[M+H]^+$, 304.06 $[M]^+$, 286.6 $[M-H_2O]^+$, 250.2, 195.2 [M- ring B]$^+$, 153.2, 149.1; Anal. calcd. for $C_{15}H_{12}O_7$: C, 59.22; H, 3.98. Found: C, 59.40; H, 4.00%.

Compound 5

Pale yellow needles, m.p., 212-215°C; IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 1660, 1600; UV (MeOH, λ_{max}, nm): 263, 321 sh, 347; +NaOH: 247, 267, 300 (sh), 388; $AlCl_3$ 237, 273, 301, 348, 398; +HCl:237, 273 (sh), 300 (sh)h, 345, 397; +NaOAc: 263, 322 (sh), 360; +H_3BO: 263, 320 (sh), 347; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.79 (2H, d, *J* =8.7 Hz, H-2' and H-6'), 6.92 (2H, d, *J*=8.7 Hz, H-3' and H-5'), 6.77 (1H, d, *J*=2.0 Hz, H-8), 6.44 (1H, d, *J* =2.0 Hz, H-6), 5.55 (1H, d, *J* =0.9 Hz, H-1'''), 5.30 (1H, d, *J* =0.9 Hz, H-1''), 3.99 (1 H, s, H-2''), 3.85 (1H, s, H-2'''), 3.77 (1H, dd, *J*=2.9 and 9.0 Hz, H-3'') 3.64 (1H, dd, *J*=2.9 and 9.0 Hz, H-3'''), 3.18-3.33 (2H, m, H-4'' and H-5''), 3.09-3.15 (3H. m, H-3''', H-4''' and H-5'''), 1.13 (3H, d, *J* =6.0 Hz, Me-6'''), 0.80 (3H, d, *J* =5.3 Hz, Me-6''); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.6 (C-4), 166.1 (C-7), 162.1 (C-5), 161.6 (C-4'), 160.2 (C-9), 158.8 (C-2), 136.2 (C-3), 132.0 (C-2' and 6'), 122.6 (C-1'), 116.6 (C-3' and 5'), 105.8 (C-10), 100.5 (C-6), 101.9 (C-1''), 99.9 (C-1'''), 95.9 (C-8), 73.5 (C-4'''), 73.1 (C-4''), 72.1 (C-5''), 72.0 (C-3''), 72.0 (C-3'''), 71.6 (C-2'''), 71.8 (C-2''), 71.3 (C-5'''), 17.9 (C-6''), 17.5 (C-6'''); MS (EI, *m/z*): 577 $[M-H]^+$, 431 [M-rhamnosyl]+, 285 [M- rhamnosylrhamnose]+; Anal. calcd. for $C_{27}H_{30}O_{14}$: C, 56.06; H, 5.23. Found: C, 55.80; H, 5.10%.

Biological studies

Antitumor and antioxidant activity were evaluated at the Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt (RCMB-AzU).

Antitumor activity

The cytotoxic effect of chloroform, ethyl acetate and methanol extracts of *C. abyssinica* aerial parts were evaluated at RCMB-AzU according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay method.[15] The Mammalian cell lines: HepG-2 cells (human hepatocellular cancer cell line) and HCT-116 (human colon Carcinoma) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Briefly, the media was removed from the 96-well plate and replaced with 100 µL of fresh culture RPMI 1640 medium without phenol red then 10 µL of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96-well plates were then incubated at 37°C and 5% CO_2 for 4 hr. An 85 µL aliquot of the media was removed from the wells, and 50 µL of dimethyl sulphoxide was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and viability % was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between the surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with extracts. The IC_{50}, was estimated from graphic plots of the dose response curve for each concentration by GPP program software (USA).

DPPH Radical Scavenging Activity

The antioxidant effect of the studied extracts was studied at RCMB-AzU by the decoloration solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to Letelier *et al.*[16] This assay was realized essentially by the method described by Joyeux *et al.*,[17] and its modification by Viturro *et al.*,[18] Freshly prepared (0.004%w/v) MeOH solution of 2,2-diphenyl-1-picrylhydrazyl radical was prepared and stored at 10°C in the dark. A MeOH solution of the studied extracts was prepared. A 40 µL aliquot of the MeOH solution was added to 3ml of 2,2-diphenyl-1-picrylhydrazyl solution. Absorbance measurements were recorded directly by UV-visible spectrophotometer. The reduction in absorbance at 515 nm was concluded continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the 2,2-diphenyl-1-picrylhydrazyl radical without antioxidant (control) and the reference ascorbic acid were also measured. All the records were measured in thrice and averaged. The PI of the 2,2-diphenyl-1-picrylhydrazyl radical was calculated according to the formula:

$$PI = [\{(AC- AT)/ AC\} \times 100] \quad (1).$$

Where AC = Absorbance of the control at t = 0 min and AT = absorbance of the sample with DPPH at t = 16 min.

The IC_{50}, the concentration required to 50% 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity was estimated from graphic plots of the dose response curve by GPP program software (USA).[19]

RESULTS AND DISCUSSION

The lack of phytochemical studies overall the world on *C. abyssinica* is the main reason to focus some light on this plant species. The phytochemical studies of ethyl acetate and methanol extracts resulted in the isolation of five flavonoid known compounds. The ethyl acetate extract of *C. abyssinica* was subjected to successive chromatography on silica gel columns eluted with $CHCl_3$-MeOH then Sephadex eluted with MeOH for purification to obtain compounds 1-4. While the MeOH extract was subjected to repeated chromatography on polyamide column eluted with EtOH-H_2O with increasing polarity to afford compound 5. The structures of the obtained compounds were elucidated by interpretation of their spectral data, including ^1D

NMR (^{1}H and ^{13}C), UV, IR, and Mass spectra and by comparison with those reported data in the related literature. The chemical structure of the isolated compound illustrated in Figure 1.

Compounds 1 and 2 were isolated as a yellow amorphous powder. The EI-MS of these compounds showed the molecular ion peak $[M]^+$ at m/z 268.1 (1) and 254,1 (2), as well prominent fragments observed at m/z 137 $[C_7H_5O_3]^+$ and 151 $[C_8H_7O_3]^+$ in agreement with the isoflavone parent. ^{1}H NMR spectrum of compounds 1 and 2 showed eight aromatic protons in between δ 8.2-6.83 ppm as well three aliphatic protons (OCH_3) at δ 3.77 ppm from compound 1 only. At the same time, ^{13}C NMR spectrum identified out eight aromatic C-H carbon atoms of which C-2 at 153.4 (1) and 152.9 ppm (2) which characteristic of an isoflavonoid skeleton. Due to the influence of electronegative oxygen atoms in rings (A, B, and C) and the deshielding effect the chemical shift value of C-9, C-2, C-4 and C-4' are also attributed to the downfield ^{13}C NMR signals of these carbon atoms. Based on the above data and reported in literature,[20,21] compounds 1 and 2 were identified as Formononetin and Daidzein.

Compound 3 showed molecular ion peaks at m/z 289.2 $[M+H]^+$ and 288.03 $[M]^+$ with fragments observed at m/z 179.2 [M-ring B]$^+$, 153.13 [M - ring B - C_2H_2]$^+$. The IR spectrum showed absorption bands at 3353 (br OH), 1655 (C=O), and 1636–1159 cm^{-1}. The UV spectrum displayed absorptions at 331.5 and 287 nm. A comparison of the UV absorptions with the literature indicated the presence of a flavanone skeleton. The ^{1}H-NMR spectrum of compound 3 showed signals in the aromatic (δ 6.94-5.85), and in the aliphatic regions (δ 5.19-2.70). The two up-field ^{1}H-NMR signals appearing as doublets at δ 5.89 (H-8), and 5.85 (H-6) can be assigned to protons of an aromatic ring with a *meta* coupling between them. The up-field shifts of these signals revealed the presence of OH or ether groups at their *ortho* positions. The aromatic region of the ^{1}H-NMR spectrum also showed three downfield signals. This includes a broad singlet at δ 6.94 (br. s, H-2'), and two overlapping signals at δ 6.77-6.79 (H-5' and H-6'), these signals showed the presence of a tri-substituted aromatic ring. The aliphatic region showed the presence of three double doublets at δ 5.25, 3.05 and 2.70. The downfield shift of the signal at δ 5.25 indicated the presence of the electron withdrawing carbonyl group. UV, IR, MS, and NMR data consistent with literature values for Eriodictyol.[22-24] Compound 4 gave spectra data analogous to that of 3 and consistent with literature values for Taxifolin.[25,26]

Flavonoid 5 was obtained as pale-yellow needles. Its UV-spectrum (λ_{max} 263, 321sh, 347) suggested a flavonoid structure. In the ^{1}H NMR spectrum of 5, H-6 and H-8 protons appeared separately as doublets at δ 6.44 and 6.77 ppm. The B ring had four aromatic protons that split into two doublets (δ 7.79 [H-2' and H-6'] and 6.92 [H-3' and H-5']). The split of the signal of the anomeric proton H-1'' and H-1''' of the rhamnose moiety (d 5.30 and 5.55) showed the relayed proton signals at d 3.85 and 3.99 (H-2'' and H-2'''), and 0.80 and 1.13 (H-6'' and H-6'''). The methyl groups of sugar moieties showed doublets at δ 0.80 (3H) and 1.13 (3H). The ^{13}C NMR signal at δ 116.59 was assigned to an oxygen free aromatic carbon (C-3' and 5'). The ^{13}C NMR signals at δ 158.8 and 136.2 were assigned to C-2 and C-3, respectively, of the C-ring of a flavonol structure. 25 mg of a glycoside was refluxed with 5% sulphuric acid for 2 hr. Insoluble precipitate was filtered off, washed with water, and compared by coPC with standard of kaempferol. The sugars from the aqueous solution were identified by coPC and coTLC and visualized by spraying with aniline phthalate and heating at l05°C to give L-rhamnose. Complete acid hydrolysis of 5 led to kaempferol and L-rhamnose. The EI-MS showed the molecular ion at m/z 577 $[M-H]^+$ of 5 corresponded to $C_{27}H_{29}O_{14}$, as well as 431 [M-rhamnosyl]$^+$, 285 [M- rhamnosylrhamnose]$^+$. Therefore, flavonoid 5 was sugested as kaempferol 3,7-*O*-α-L-dirhamnoside (Kaempferitrin).

In vitro cytotoxic activity

The high toxicity of some cancer chemotherapy drugs, as well as their unfavorable side effects and drugs resistance, drives up the demand for natural compounds as new anticancer drugs. So, in this study, the cytotoxic effect of $CHCl_3$, EA and MeOH extracts of *C. abyssinica* against different human tumor cell lines of hepatocellular carcinoma cells (HepG2) and colon carcinoma (HCT-116) was determined by MTT viability assay and summarized in Table 1, the results revealed metabolic cytotoxicity of the tested extracts with IC_{50} ranged between 24.4 to 166 µg/mL. The methanolic extract showed the highest *in vitro* cytotoxic effect against HCT-116 and HepG2 cells with IC_{50} 24.4 and 29.9 µg/mL, respectively, compared to the drug reference Cisplatin, while the chloroform and ethyl acetate extracts were moderately affected against HepG2 cells (IC_{50} 71.9 and 74.5 µg/mL) and weekly affected against HCT-116 cells (IC_{50} 101 and 166 µg/mL). The remarked difference in cytotoxic activity of methanol extract

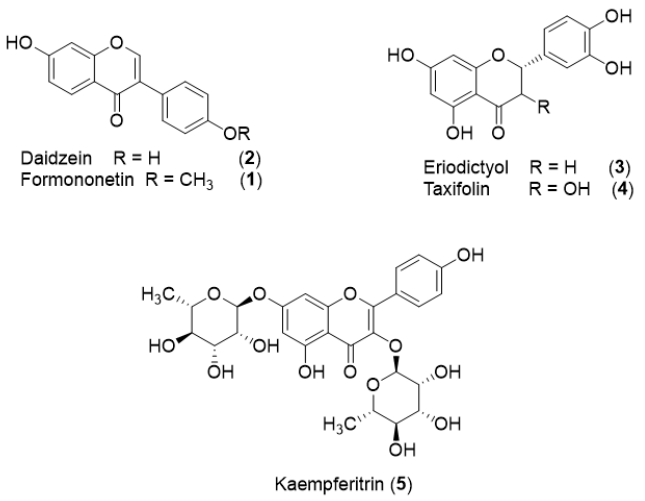

Figure 1: The chemical structure of the identified compounds from the aerial parts of *C. abyssinica.*

Table 1: Cytotoxic activity of chloroform, ethyl acetate and methanol extracts of *C. abyssinica*.

Sample conc. (µg/ml)	Viability % HepG-2				Viability % HCT-116			
	MeOH	EA	$CHCl_3$	Cisplatin	MeOH	EA	$CHCl_3$	Cisplatin
500	3.46	10.28	8.75	2.69	2.95	13.87	11.89	3.27
250	8.32	18.76	19.42	4.28	6.74	24.65	28.67	4.96
125	20.69	32.87	36.59	6.91	16.43	39.02	60.35	7.54
62.5	33.25	54.09	52.37	11.26	30.69	68.17	81.48	13.68
31.25	48.98	78.13	74.16	19.43	45.12	85.64	93.28	21.75
15.6	61.38	90.42	89.52	27.88	56.26	97.56	98.41	27.08
7.8	76.30	98.63	97.06	35.49	71.39	100	100	34.95
3.9	90.81	100	100	46.23	85.20	100	100	42.83
2	98.97	100	100	58.70	93.68	100	100	52.71
1	100	100	100	65.26	97.41	100	100	61.47
0	100	100	100	100	100	100	100	100
IC_{50} (µg/mL)	29.9	74.5	71.9	3.4	24.4	101	166	2.55

MeOH = Methanol, EA = Ethyl acetate, $CHCl_3$ = Chloroform.

Table 2: Antioxidant activity of chloroform, ethyl acetate and methanol extracts of *C. abyssinica*.

Sample conc. (µg/mL)	DPPH scavenging %			
	Chloroform	Ethyl acetate	Methanol	Ascorbic acid
1280	82.42	62.68	58.05	98.91
640	61.47	44.79	48.68	97.83
320	23.58	25.89	27.89	95.64
160	14.79	21.05	14.37	92.31
80	8.16	13.37	11.68	90.25
40	4.53	6.11	5.84	83.09
20	2.63	4.21	1.84	71.38
10	0.16	2.32	0.79	48.52
0	0	0	0	0
IC_{50} (µg/mL)	543	826.4	664.5	10.6

may be due the presence of flavonoids and phenolic compounds which are characterized by their powerful cytotoxic effect.

In vitro antioxidant Activity

Antioxidants are a class of substances that help trap and neutralize free radicals, thereby they can decrease the damage to the body caused by free radicals. Additionally, antioxidants play a protective role against certain diseases including inflammation and cancer caused by oxidative stress.[27-29] A variety of plant materials are known to be natural sources of antioxidants, such as herbs, spices, seeds, fruits, and vegetables. The antioxidant effect of *C. abyssinica* extract was assayed by the decoloration solution of DPPH radical method. The chloroform, ethyl acetate and methanol extracts of the aerial parts of *C. abyssinica* exhibited weak antioxidant activities using the DPPH free radical scavenging assay with IC_{50} values between 543-826.4 µg/mL as reported in Table 2.

CONCLUSION

The lack of phytochemical studies overall the world on *C. abyssinica* is the main reason to focus some light on this plant species. This study describes the isolation and identification of five compounds from the aerial parts of this plant including two isoflavonoid aglycones, Daidzein and Formononetin; two flavonoid aglycones, Eriodictyo and Taxifolin; one flavonoid-*O*-glycosides, Kaempferitrin. The methanolic extract showed potent cytotoxic effect against colon and hepatocellular carcinoma cell lines.

The significant *in vitro* antitumor effect of methanol extract against colon and liver cancer encourages more advanced phytochemical, pharmacological (*in vitro* and *in vivo*), and biosafety studied for the phenolic content of *C. abyssinica* as anticancer against another carcinoma cell lines. From *in vitro* cytotoxicity and biosafety studies, it can be concluded that *C. abyssinica* extracts are safe and can be considered for further development as a biomedicine.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest

ABBREVIATIONS

RCMB-AzU: Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl radical; **ATCC:** American Type Culture Collection; **HCT-116:** Human colon cancer cell line; **HepG-2:** Human hepatocellular cancer cell line; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **BAW: n-Butanol:** Acetic Acid; Water; **EA:** Ethyl acetate; **$CHCl_3$:** Chloroform; **MeOH:** Methanol; **CAIH:** Desert Research Center Herbarium; ***C. abyssinica*:** *Cometes abyssinica* R.Br. ex Wall; **CC:** Column Chromatography; **TLC:** Thin layer chromatography; **GPP:** GraphPad Prism; **PI:** The Percentage of inhibition.

SUMMARY

The aim of this study was to screen the aerial parts of *C. abyssinica* for its chemical composition, antioxidant, and antitumor activities. Phytochemical study of methanol and ethyl acetate extracts led to the isolation of five known flavonoid compounds named as Formononetin, Daidzein, Eriodictyol, Taxifolin, and kaempferitrin. The methanolic extract showed considerable inhibitory activity against colon (HCT-116) and hepatocellular (HepG2) carcinoma cell lines with IC_{50} 24.4-29.9 µg/mL compared to the drug reference Cisplatin. While all studied extracts exhibited weak antioxidant activities using the DPPH free radical scavenging assay with IC_{50} values between 543-826.4 µg/mL.

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