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Extraction, isolation and characterization of the stem bark of *Combretum paniculatum* occurring in South West Ethiopia

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Abstract
Combretum paniculatum has been used widely in ethnomedicine in the treatment of leprosy, chronic diarrhea, dysentery, flatulence, vomiting, colic, enlarges spleen and liver in Ethiopia and tropical African country. However, despite its use in traditional medicine, to date, there appears to have been no chemical or biological studies published on the stem bark part of the plant species. Phytochemical screening tests of the methanol extract of the stem bark revealed the presence of steroids, alkaloids, terpenoids, phenols, flavonoids, saponins, tannins, glycosides and the absence of anthraquinones and coumarines. Silica gel chromatographic separation of the methanol extract gave Apigenin-7-O-β-D-glycoside (CPB) for the first time from the stem bark of *C. paniculatum*. A complete characterization of the isolated compound was done with the help of spectroscopic techniques.

Keywords: *Combretum paniculatum*, phytochemical screening, apigenin-7-O-β-D-glycoside

Introduction

Medicinal plants have been used since ancient times in virtually all cultures as a source of medicines and are of great importance to the health of individuals and communities [1]. Traditional medicine is used in all parts of the world and has a rapidly growing economic importance, mainly through the use of medicinal plants, especially in developing countries [2]. Some plants having the medicinal value in form of chemical substances that produce a definite physiological action on the human body are called phytochemicals. Since the ancient time these phyto chemicals are used to cure the disease in herbal and homeopathic medicines. These are non-nutritive substances, have protective or disease preventive property. There arises a need and therefore to screen medicinal plants for bioactive compounds as a basis for further biomedical studies. With advances in phytochemical techniques, several active principles of many medicinal plants have been isolated and introduced as valuable drug in modern systems of medicine. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds [3].

Combretum paniculatum is widely distributed in tropical Africa, from West Africa east to Ethiopia, south to Angola, Mozambique and South Africa. The use of plants for medicinal purposes is an important part of the culture and tradition in Africa. Thus, up to 80% of the population depends directly on the traditional medicine for the primary health care [4]. Medicinal plants represent a rich source of antimicrobial agents [5]. A high degree of antiviral activity against HIV-2 was achieved with the acetone extract of *Combretum paniculatum* [6]. The aqueous extract of inflorescences of the plant has anti-tumor activity against carcinoma of the lung. The antimicrobial, anti-inflammatory, antishistosomal, anti-HIV and central nervous system stimulation activities have been documented [7, 8].

Isolation of active compounds from a 70% acetone extract of *Combretum paniculatum* were cholest-5-en-3-ol, 2-phyten-1-ol, quercitrin-3-glucopyranoside, p-coumaric acid, 2, 3, 8-tri-O-methylellagicacid, β-sitosterol, gallocatechin, apigenin and apigenin-7-glucoside identified from the leaf of plants and two diglucosylated derivatives from cyanidin and pelargonidin (cyanidin 3, 5-O-β-D-diglu-copyranoside and pelargonidin 3, 5-O-β-D-diglucopyranoside) from flowers of *Combretum paniculatum* [9].

Majority of rural population in Ethiopia still uses traditional medicine for their healthcare need [10]. In Ethiopia, *Combretum paniculatum* grow in the warm, moist areas of Kaffa, Jimma, Wollega and Shewa Ethiopia, and flowers in January and February. The local name of this plant is “baggo” (Kafi Noono), “baggii” (Afaan Oromo) and “baye” (Amharic) (Fig. 1) [11]. The sap expressed from flowers is used to treat conjunctivitis and eye ailments. It is also externally applied to treat leprosy [12].

Fig 1: *Combretum paniculatum* (Baggo) [Photo taken by Birhanu Bekele, Dec, 2019]

Materials and Methods

Instrumentation

Column chromatographic separation was carried out on silica gel (230-400 mesh size, Merck). Thin layer chromatography was done on silica gel 60 F-254, 0.2 mm thick layer on aluminum sheets for detection of spots. The UV-Vis spectrum was recorded on UNICAM UV-300 double beam spectrophotometer using $CHCl_3$ as internal standard. The IR absorption spectrum was determined by Shimadzu 440 instrument using KBr disk in the range of 500-4000cm^{-1}. The 1H NMR, 13C NMR, DEPT-135, spectra were recorded using Bruker Avance 400MHz spectrometer using TMS as internal standard. Chemical shift values for all NMR data are reported in ppm relative to internal standard. All the chemicals used were analytical grade.

Plant material collection and authentication

The stem bark of *Combretum paniculatum* was collected in December, 2019 from the farm of Bonga University. The plant species was identified by botanist of the Biology department at this University.

Preparation of plant extract

Powdered stem bark of *Combretum paniculatum* (300g) were soaked with CH_3OH for 72 hours with occasional shaking. The extract was filtered and concentrated using rotary evaporator at 40 ^oC to give brown crude (45.6g, 9.12% yield).

Isolation and purification of compounds

Crude extract (25g) was subjected to silica gel column chromatographic separation (150g silica gel) and eluted with increasing gradient of methanol in dichloromethane. A total of 43 fractions (each 50mL) were collected. Out of the 43 fractions (dichloromethane/methanol) collected, fractions 22-27 revealed single spot showing yellow spot under UV light having the Rf value of 0.54 in (40:1) dichloromethane/methanol solvent system. After concentrating, the powder material left was repeatedly washed with n-hexane to yield compound CPB (15mg).

Results and Discussion

Mass and percentage yield of crude extracts

The most phytochemicals are extracted by polar solvents that are methanol, thus the chemical constituents of the stem bark of *Combretum paniculatum* majorly polar compounds. The result also indicated that methanol was the best solvent to extract this plant species. The percent yields of the crude extracts were calculated using the formula (Eq. 1 and Table 1).

$$\frac{\text{Mass of the extract}}{\text{Mass of the plant material used for extraction}} \times 100\% \quad \text{———Eq. 1}$$

Table 1: Percentage yield of crude extracts

Solvent used for extraction	Mass of crude extract (g)	% Yield
Methanol (100%)	45.6	9.12

Phytochemical screening test results

The results from the phytochemical screening of the methanol extract of the stem bark of *combretum paniculatum* revealed the presence of steroids, phytosterols, alkaloids, terpenoids, phenols, flavonoids, saponins, tannins, cardiac glycosides and the absence of anthraquinones and coumarines.

Structural elucidation of isolated compounds

Compound CPB was isolated as a yellow powder (15mg) with formula $C_{21}H_{20}O_{10}$ and molecular weight 432glmol from the methanol extract with Rf value of 0.54 in dichloromethane/methanol (40:1) solvent system. An FTIR spectrum (KBr) exhibited broad absorption at 3453 cm^{-1}can be attributed to the occurrence of hydroxyl moiety. The weak peak at 3119 cm^{-1} can be attributed to a stretching vibration of aromatic C-H, the bands at 2926 cm^{-1} is saturated hydrocarbon C-H stretching, the peak at 1663 cm^{-1} is γ-pyrone carbonyl stretch (C=O), the peaks at 1618, 1607, 1545cm^{-1} are aromatic C=C stretching, The bands at 1291, 1252 cm^{-1} are aromatic hydroxyl stretching, the peaks at 1487, 1446 cm^{-1} are aromatic C-H and peaks at 1110, 1076, 1035 cm^{-1} are glycoside C-O stretching.

The ^{1}H-NMR spectrum (DMSO-d_6, 400HZ) had further confirmed the presence of apigenin and glucose moieties in the compound CPB. The occurrence of an apigenin skeleton was viewed from a hydroxyl δ_H at 12.51 (s, 1H, OH-5), two doublets δ_H at 5.99 (d, 1H, J=2.0 Hz, H-6) and δ_H at 6.82 5.99 (d, 1H, J=2.0 Hz, H-8) on the A-ring; A_2B_2-type aromatic δ_H at 7.13 (d, 1H, J=8.7 Hz, H-2', H-6') and δ_H at 6.68 (d, 1H, J=8.7 Hz, H-3', H-5'), as well as a hydroxyl δ_H at 10.03 (s, 1H, OH-4') on the B-ring; together with an olefinic δ_H at 6.71 (s, 1H, H-3) on a flavone C-ring. This glycosidic δ_H at 5.88 (d, 1H, J=7.1 Hz, H-1") and δ_C at 101.8 (C-1") were evident in the ^{1}H and ^{13}C NMR spectra. The multiplet δ_H at 3.40-3.79 (5H, m, H-3",H-6") was assignable to the coupling between protons and methylene protons of the glucosyl ring. Proton δ_H at 3.91 (m, 1H,J=10.1Hz, H-2"), hydroxyl δ_H at 5.10 (s, 1H, OH-2"), 5.12 (s, OH-2"), δ_H at 5.05 (s, 1H, OH-3"), δ_H at 5.04 (s, 1H, OH-4"), and δ_H at 4.61 (s, 1H, OH-6") were assigned in the glycosidic ring.

Analyses of the ^{13}C-NMR spectrum ($CDCl_3$, 150MHz) (Table 2 and Fig. 2) revealed the existence of 21 carbons, including a hexose moiety at δ_C (101.8, 73.4, 75.4, 71.5, 77.7 and 62.3). The ^{13}C-NMR spectrum also exhibited the presence of δ_C (163.1, 98.8, 165.4 and 96.5) for the A-ring, δ_C (163.7, 104.5, 182.2 and 163.1) for the C-ring, and δ_C (123.0, 127.8, 115.8 and 158.7) for the B-ring of the flavone.

The ^{13}C-NMR spectrum ($CDCl_3$, 150MHz) led to the conclusion that compound CPB had 21 carbons with the following chemical shifts: One methylene group was C-6'' (62.3), twelve methine groups were C-2', 6' (127.8), C-3', 5' (115.8), C-3 (104.5), C-1" (101.8), C-6 (96.8), C-8 (96.5), C-5" (77.7), C-3" (75.4), C-2" (73.4), C-4" (71.5) and eight quaternary carbon atoms were C-4 (182.2), C-7 (165.4), C-2 (163.7), C-5 (163.1), C-9 (159.3), C-4' (158.7), C-1' (123.0), C-10 (103.5).

Thus, based on the above spectral data and comparison with reported data in the literature, the structure of compound CPB was elucidated as Apigenin-7-O-β-D-glycoside, in accordance with the reported data in the literature (Fig. 2, Table 2) [13, 14].

Table 2: ^{1}H-NMR (CDCl3, 400 MHz) and ^{13}-NMR (150MHz) spectral data of compound CPB

Position	^{1}H-NMR	^{13}C-NMR	Literature [13, 14] ^{1}H-NMR	^{13}C-NMR
1				
2		163.7		163.4
3	6.71 (s, 1H)	104.5	6.87 (1H, s)	103.5
4		182.2		182.5
5	12.51 (s, 1H, OH-5)	163.1		157.4
6	5.99 (s, 1H, J=2.0 Hz)	98.8	6.43 (1H, d, J=2.2 Hz)	100.3
7		165.4		164.7
8	5.99 (s, 1H, J=2.0 Hz)	96.5	6.82 (1H, d, J=2.2 Hz)	95.3
9		159.3		161.9
10		103.5		105.8
1'		123.0		121.4
2'	7.13 (d, 1H, J=8.7 Hz)	127.8	7.95 (1H, d, J=8.9 Hz)	129.1
3'	6.68 (d, 1H, J=8.7 Hz)	115.8	6.93 (1H, d, J=8.9 Hz)	116.5
4'	10.03 (s, 1H, OH-4')	158.7		161.6
5'	6.68 (d, 1H, J=8.7 Hz)	115.8	6.93 (1H, d, J=8.9 Hz)	116.5
6'	7.13 (d, 1H, J=8.7 Hz)	127.8	7.95 (1H, d, J=8.9 Hz)	129.1
1"	5.88 (d, 1H, J=7.1 Hz)	101.8	5.44 (1H, d, J=7.4 Hz)	99.9
2"	3.91 (d, 1H,J=10.1Hz)	73.4	3.71 (1H, d, J=10.3 Hz, H-2")	73.5
3"	3.40–3.79 (m, 5H, J=6.5 Hz glucose H 3", -4", -5", -6")	75.4	3.27–3.47 (5H, m, J=6.8 Hz, glucose H 3", -4", -5", -6")	77.6
4"	5.10 (s, 1H, OH-2"), 5.05 (s, 1H, OH-3"), 5.04 (s, 1H, OH-4"), 4.61 (s, 1H, OH-6")	71.5	5.12 (1H, s, OH-2"), 5.07 (1H, s, OH-3"), 5.05 (1H, s, OH-4"), 4.65 (1H, s, OH-6")	69.9
5"		77.7		76.9
6"		62.3		63.5

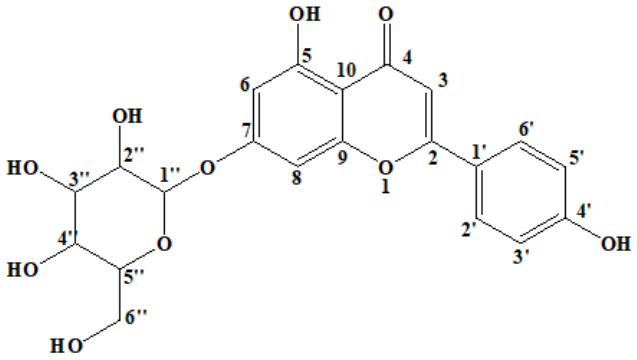

Fig 2: Structure of compound CPB (Apigenin-7-O-β-D-glycoside)

Conclusion

The qualitative preliminary phytochemical screening of the methanol stem bark extract of the *Combretum paniculatum* revealed the presence of steroids, alkaloids, terpenoids, phenols, flavonoids, saponins, tannins, glycosides and absence of anthraquinones and coumarines. Silica gel column chromatographic separations of the methanol extract of *Combretum paniculatum* gave Apigenin-7-O-β-D-glycoside. In agreement with the previous study, the wide traditional use of the plant may be attributed to its rich source of flavonoids constituents. The finding of these pharmacologically important secondary metabolites from stem bark extracts brings the attention of experts to look more on the medicinal importance of the plant. To the best of our knowledge, this is the first report on the presence of such kinds of compound in the stem bark of *Combretum paniculatum* among the Ethiopian flora.

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References

1. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J Biotechnol. 2005; 4:685-688.
2. Mallikharjuna P, Rajanna L, Seetharam Y, Sharanabas appa G. Phytochemical studies of Strychnospotatorum. Lf-A medicinal plant E-J Chem. 2007, 4(4): 510-518.
3. Li HB.; Jiang Y.; Chen F. Separation methods used for Scutellariabaicalensis active components. J Chromatogr B. 2004; 812(1-2):277-290.
4. WHO (World Health Organization). Malaria Control Today. Roll Back Malaria Department. World Health Organization. Geneva, 2005.
5. Khaing TA. Evaluation of the antifungal and antioxidant activities of the leaf extract of *Aloe vera*. World Acad Sci Eng Technol. 2011, 75.
6. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. J. Ethnobiol. Ethnomed, 2006.
7. Gansane A, Sanon, S, Ouattara LP, Traore A, Hutter S, Ollivier E *et al*. Antiplasmodial activity and toxicity of crude extracts from alternatives parts of plants widely used for the treatment of malaria in Burkina Faso: Contribution for their preservation. Parasitol. Res. 2010; 106:335-340.
8. Pietrovski E, Rosa K, Facundo V, Rios K, Marques M, Santos S *et al*. Antinociceptive properties of the ethanolic extract and of the triterpene 3β,6β,16β-trihidroxilup- 20(29)-ene obtained from flowers of Combretum leprosum in mice. Pharmacol. Biochem. Behav. 2006; 83:90-99.
9. Bajia SF. Characterization of antimicrobial compounds from Combretum paniculatum, plant with proven anti-HIV replication activity. Ph. D. Thesis, Departiment of paraclinical Sciences, University of Pretoria, South Africa, 2007.
10. Kassaye KD, Amberbir A, Getachew B, Mussema Y. A historical overview of traditional medicine practices and policy in Ethiopia. Ethiop. J Health Dev. 2006; 20:127-34.

11. Getahun A. Some common medicinal and poisonous plants used in Ethiopian folk medicine. Addis Ababa University, Addis Ababa, Ethiopia, 1976.
12. Schmelzer GH, Gurib-Fkim A. Combretum panIcul atum Vent. Prota, Medicinal plants, 2012.
13. Peng Hy, Zhang Xh, Xu Jz. Apigenin-7-O-β-D-glycoside isolation from the highly copper-tolerant plant Elsholtzia splendens. J Zhejiang Univ Sci B. 2016; 17:447-454.
14. Delazar A, Nazemiyeh H, Afshar FH, Barghi N, Esnaashari S, Asgharian P *et al*. Chemical compositions and biological activities of Scutellaria pinnatifida A. Hamilt aerial parts. Res. Pharma. Sci. 2017; 12(3):187-195.