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Terpene alcohol and phytosterol from the root of the *Commelina Diffusa* Burm. F

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Abstract
Commelina diffusa Burm. f. (*C. diffusa*) is the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. This study aims to isolate and characterize compounds from the methanol extract of the plant. Healthy roots of the plant were collected, chopped into small pieces, air-dried under a shade, segregated, pulverized, extracted with methanol using maceration technique and concentrated to obtain the plant extract. The results from the phytochemical screening of the plant extract revealed the presence of higher amount of steroids, terpenoids, alkaloids, phenolic compounds, tannins, saponins, amino acid and phytosteroids. While column chromatography was used for the isolation and purification of the pure compound. Silica gel chromatographic separation of the CH_3OH extract gave stigmasterol and 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol. A complete characterization of the isolated compound was done with the help of (UV-Vis, IR, 1D NMR) spectroscopic techniques.

Keywords: stigmasterol*; commelina diffusa*; phytochemical analysis; *commelinaceae*

Introduction

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 [1]. In 1997, it was reported that out of the 300,000 different plant species identified to be medicinal, only about 5,000 have been studied so far for their possible medicinal usefulness. This therefore, necessitate for continuous search for new antimicrobial agents especially from the root, stem and leaves of plants since the synthetic drugs have retarded in many respects. Research on plants with antimicrobial activities should indeed be a continuous one so as to reveal all hidden aspects of traditional medicine [2]. Medicinal plants have been used for centuries to treat numerous human diseases. Reports indicate that estimate of about 70-80% of world population; most of who are from developing countries still relies on medicinal plants for their primary health care systems [3-6]. Moreover, several drugs currently in use in modern medicine are originated from medicinal plants [7, 8]. *Commelina diffusa* belongs to family *commelinaceae*. It is also known as climbing dayflower or spreading dayflower. The weeds are commonly found in many crop fields during June to September 1998. In India, almost 95% of the prescriptions have been reported to be plant based in the traditional systems of unani, siddha, ayurveda and homeopathy [9, 10]. In different countries of Asia, Africa and America it is commonly used in urinary tract infections, to remove cough with sticky phlegm and in diarrhea, hemorrhoids, enteritis, eye irritation, conjunctivitis and other eye problems like ophthalmia [11, 12]. The leaves and stem of the plant are traditionally used for the treatment of abscess, boils, and malaria, for treatment of insect, snake and bug bites, edema, laryngitis, sorethroats, acute tonsillitis, otitis media and in nose bleeding [13]. This plant is also used for groin pain, wound dressing, influenza, dermatitis, dysmenorrhea, leprosy, kidney diseases and in treating many other diseases [14]. The wound healing property of the crude extract has been attributed to its antimicrobial and antioxidant properties [15]. Phytochemicals are present in plant have healing properties. *Commelina diffusa* contain minerals, vitamins, alkaloids, saponins, phenols, tannins, phytosterols, triterpens, terpenoids as secondary metabolites. Plants produce diverse array of low molecular weight products [16-18].

These phytochemicals are responsible for pharma industry and at higher concentration toxic to animals [19]. The present study aimed to extract, isolate and characterize the chemical constituents or bioactive components of the secondary metabolites from the root part of the *Commelina Diffusa*. The limitation of the current study is phytochemical investigation cannot be carried out on the root part of the *Commelina Diffusa* among the Ethiopians flora.

Materials and methods

Chemicals and apparatus

General laboratory grade solvents like methanol, dichloromethane, hexane and ethyl acetate were used for gradient extraction and column elution. The materials used for chromatographic analyses were silica gel (60-120 mesh size) and pre-coated TLC (silica gel, UV254). The UV-Vis spectrum was recorded on UNICAM UV-300 double beam spectrophotometer using $CHCl_3$ as internal standard. ^{1}H-NMR, ^{13}C-NMR and DEPT-135 were recorded using Bruker Advance 400 MHz spectrometer. $CDCl_3$ was used as a solvent in all NMR spectroscopic analyses. The Infrared (IR) spectra (KBr) data were obtained from Perkin-Elmer BX infrared spectrometer (400-4000 cm^{-1}). Melting point apparatus (Griffin) was used for melting point determination.

Plant material collection and authentication

The root of the *Commelina diffusa* (Figure 1) was collected fresh in April, 2020 from natural forest of Bonga town, Ethiopia. Taxonomical identification was done at the Biological Sciences Department by botanist Mr. Seyoum Robo at Bonga University, Bonga, Ethiopia. The collected plant material was chopped into small pieces and air-dried under a shade, segregated and pulverized by manual pounding using wooden mortar and pestle. The pulverized plant material was stored in a cool dry place to avoid moisture from the environment.

Fig 1: *Commelina diffusa* photo [Photo taken by Birhanu Bekele, April, 2020]

Extraction

Four hundred gram (400 g) of the pulverized stem bark *Commelina diffusa* was extracted by n-hexane (least polar), dichloromethane/methanol and methanol solvents using maceration technique with continuous shaking (at 25°C for 72 hours) using a shaker (GSL 400). The supernatant was filtered through Whatman No.1 filter paper. The extract was dried under reduced pressure using the rotatory evaporator at 40 ^{0}C.

Isolation

Thrteen grams (30 g) of the crude extract were subjected to the qualitative phytochemicals analysis using standard methods [20]. Phytochemical screening of the extracts indicated the presence of steroids, terpenoids, alkaloids, phenolic compounds, tannins, saponins, amino acid and phytosteroids. A small portion of the methanol crude extract was dissolved in n-hexane and the solution was spotted on precoated TLC plates. The TLC plates were developed by specific solvent systems and were viewed individually under UV light at 236 nm and also sprayed with the Vanillin-H_2SO_4 reagent. Through several pilot test experiments, it was found out that the compounds of this methanol extract were separated by the solvent system of n-hexane and ethyl acetate. Fractions 14-19 revealed single spot showing a white crystal solid with Rf of 0.45 on TLC in (90:10) and fractions 31-37 showing indicate single spot having as a yellowish liquid with Rf of 0.66 on TLC in (80:20) under UV light. The best solvent system for isolation of above compounds was n-hexane/ethyl acetate solvent system. After concentrating, the crystalline materials left were repeatedly washed with *n*-hexane to yield compound 1 (17mg) and compound 2 (13mg). The substances were further subjected to ^{1}H- NMR, ^{13}C-NMR, DEPT, UV-Vis and FTIR spectroscopy.

Preliminary phytochemical screening

Phytochemical screening tests were done to determine the class of secondary metabolites present in the crude extract following the standard protocols [21, 22].

Test for alkaloids

A small portion of the sample was stirred with few drops of dilute hydrochloric acid and was tested with Dragendroff's reagent for the presence of alkaloids; a white to buff precipitate was observed which proves the presence of alkaloids.

Tests for steroids and terpenoids (Salkowski test)

About 0.2 g of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated sulphuric acid (red color at lower layer indicates the presence of steroids and formation of yellow colored lower layer indicates the presence of triterpenoids).

Test for tannins

A small quantity of the sample was taken in water, and test for the presence of tannins was carried out with the dilute Ferric chloride solution (5%) resulted in a characteristic violet color.

Test for flavonoids (alkaline reagent test)

About 5 mL of dilute aqueous ammonia solution was added to a 0.2 g of the aqueous filtrate of the plant extract, followed by addition of concentrated H_2SO_4. The instant disappearance of yellow coloration indicated the presence of flavonoids in the crude extract.

Test for free anthraquinones

About 0.5g of the extract was boiled with 10% HCl for few minutes in a water bath and filtered. The filtrate was allowed to cool, and an equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% ammonia were added to the mixture and heated. The formation of rose-pink color was taken as an indication of the presence of anthraquinones.

Test for saponins (Froth Test)

Crude extract (0.1g) was dissolved in 20mL of water shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

Detection of glycosides

Glacial acetic acid (2mL) and 3 drops of 5% ferric chloride were added to 0.5 mL of an aqueous solution of plant extract. Then, 1 m of concentrated sulphuric acid was added. Formation of brown ring at the interface indicated presence of glycosides.

Results and Discussion

Phytochemical screening test results

The results from the phytochemical screening of the methanol extract revealed the presence of steroids, terpenoids, alkaloids, phenolic compounds, tannins, saponins, amino acid and phytosteroids.

Compound 1 was obtained as a white crystalline solid (17 mg) and Compound 2 was obtained as a yellowish liquid (13 mg) from the methanol extract with Rf value of 0.45 and o.66 respectively, in n-hexane/ethyl acetate (90:10) solvent system. Compound 1 was identified as stagmasterol (Figure 2) and compound 2 was identified as 3, 7, 11, 15-tetramethyl-2 hexadecen-1-ol (Figure 3)

with the following physical properties, chemical and spectral data.

Physical properties: Compound 1 was isolated as a white needle crystalline sold with a melting point of 166 ^{0}C and a molecular formula of $C_{29}H_{50}O$. Whereas Compound 2 was isolated as a yellowish liquid with a melting point of 203 ^{0}C and a molecular formula of $C_{20}H_{40}O$.

***Chemical test of the isolated compound*:** the isolated compound showed positive tests for Salkowski and Liebermann-Burchard test thus confirming the compound as phytosterol and Terpene alcohol.

Spectroscopic analysis: The UV spectrum (Appendix 1) indicated maximum absorbance (λ_{max}) (chloroform) at 290 and 261 nm which indicated the presence σ-π and π-π* transitions, respectively. IR spectrum compound 1 revealed (Appendix 2) at an intensely broad band at 3320 cm^{-1}, showed presence of OH stretching. The bands at 1631 cm^{-1} and 1052 cm^{-1} can be attributed to olefinic C= C stretch and carbon-oxygen (C-O) stretch, respectively. An intense peaks at 2850 cm^{-1}and 2930 cm^{-1}indicate methyelenes and methyls C-H stretching vibrations, respectively. IR spectrum compound 2 revealed (Appendix 3) the broad band at 3408 cm^{-1} is OH stretching in alcohol, 2924 cm^{-1} to 2853 cm^{-1} attributed to C-H stretching vibration in alkanes group. The ^{1}H-NMR spectrum (Appendix 4) compound 1: 1H NMR (400 MHz, $CDCl_3$): δ = 3.54 (m, 1H, 3-H), 5.39 (m, 1H, 6-H), 5.16 (dd, 1H, J = 15.3, 8.6 Hz, 22-H), 5.06 (dd, 1H, J = 15.3, 8.6 Hz, 23-H), 1.04 (s, 3H, 18-H), 0.75 (s, 3H, 19-H), 0.84 (d, 26-H) and 0.88 (d, 27-H), 0.94 (d, 3H, J = 6.5 Hz, 21-H), 0.82 (t, 3H, 29-H). The 1H-NMR spectrum (Appendix 5) compound 2: 1H NMR (400 MHz, $CDCl_3$), δ (ppm): 3.53 (t, J= 6.5), 1.98 (t, J= 6.5), 5.41 (dt, J= 3.6, 1.2), 1.95 (dt, 6.5), 1.28 (m), 1.55 (m), 1.20-1.30 (m), 1.55 (m), 0.87 (d, J= 6.0), 0.84 (d, J= 5.5) and 1.67 (s). The ^{13}C-NMR spectrum of compound 1 ($CDCl_3$, 150 MHz) (Appendix 6) showed twenty nine carbon signals assigned to six methyl, eleven methylene, nine methine groups and three quaternary carbon signals. Peaks at 30.4 (C-1), 31.8 (C-2), 71.7 (C-3), 41.9 (C-4), 140.9 (C-5), 121.9 (C-6), 40.3 (C-7), 34.1 (C-8), 54.6 (C-9), 35.3 (C-10), 20.2 (C-11), 37.6 (C-12), 41.6 (C-13), 58.8 (C-14), 25.3 (C-15), 27.7 (C-16), 56.7 (C-17), 21.1 (C-18), 23.5(C-19), 40.1 (C-20), 20.2 (C-21), 130.4 (C-22), 124.5 (C-23), 52.2 (C-24), 31.9 (C-25), 21.1 (C-26), 21.1 (C-27), 26.5 (C-28), 12.3 (C-29). The peak at δ71.7 (C-3) indicates sp3 oxygenated methine whereas peaks at δ140.9 (C-5) and δ121.79 (C-6) are attributed to olefinic carbons (C-5, 6). The ^{13}C-NMR spectrum of compound 2 ($CDCl_3$, 150 MHz) (Appendix 7) showed twenty carbon signals at 59.0 (C-1), 122.3 (C-2), 131.7 (C-3), 39.7 (C-4), 25.0 (C-5) 33.8 (C-6) 31.7 (C-7), 37.7 (C-8), 24.7 (C-9), 37.7(C-10), 33.2 (C-11), 37.7 (C-12), 24.4 (C-13), 39.9 (C-14), 28.2 (C-15), 23.2 (C-16), 23.2 (C-17), 21.2 (C-18), 21.0 (C-19),17.4 (C-20). Thus, based on the above spectral data and comparison with literature, the structure of compound 1 was similar to stigmasterol [23] and compound 2 was similar to 3, 7, 11, 15-tetramethyl-2 hexadecen-1-ol [24, 25] (Figure 2 and 3).

Table 1: ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR spectral data of isolated compound **1** compared with literature values or reference [23]

Position	^{33}C-NAM		uC-NMR (Reference)	'H-NNIR (Reference)
C-1	372		37.3	
C-2	282		282	
C-3	71.7	3.52 (broad m, 111)	71.8	3.55 (m, 1H)
C-4	422		42.3	
C-5	140.7		140.8	
C-6	121.7	5.34 (d, 113)	1213	5.37 (m, 111)
C-7	31.8		31.9	
C-8	332		33.0	
C-9	50.1		502	
C-10	36.4		36.5	
C-11	26.3		262	
C-12	39.7		39.8	
C-13	42.3		423	
C-14	56.7		56.8	
C-15	243		24.3	
C-16	29.1		292	
C-17	56.0		56.1	
C-18	122	0.67 (s, 311)	12.1	1.03 (s, 3H)
C-19	19.4	1.02 (s, 311)	19.4	0.72 (s, 311)
C-20	40.5		40.5	
C-21	21.3	0.92 (40,, 311)	21/	0.95(d, 3H)
C-22	138.4		138.3	5.18 41 111)
C-23	130.1		1293	5.04 (dsl, 1H)
C-24	512		51.3	
C-25	45.8		45.9	
C-26	19.5	0.83 (d, 311)	19.4	0.86 (d, 311)
C-27	19.8	0.82 (dA. 3H)	19.8	0.87 (d, 311)
C-28	24.6		24.4	
C-29	123	0.84 (04, 3H)	1/2	0.83 (t, 311) I

Table 2:.^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR spectral data of isolated compound **2** compared with literature values [24, 25]

Position	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR (Reference)	^{1}H-NMR (Reference)
C-1	59.0	4.2 (t, 2H), 2.0 (s, 1H)	59.8	4.1 (t, 2H), 1.6 (s, 1H)
C-2	122.3	5.3 (t, 1H)	123.4	5.4 (t, 1H)
C-3	131.7		130.9	
C-4	39.7	1.96 (t, 2H)	40.2	1.98 (t, 3H)
C-5	25.0	1.33 (m, 2H)	25.5	
C-6	33.8	1.25 (m, 2H)	33.2	
C-7	31.1	1.65 (m, 1H)	30.1	1.44 (m, 1H)
C-8	37.7	1.25 (m, 2H)	37.7	
C-9	24.7	1.29 (m, 2H)	24.8	
C-10	37.7	1.25 (m, 2H)	37.1	
C-11	33.2	1.65 (m, 1H)	33.1	1.35 (m, 1H)
C-12	37.7	1.25 (m, 2H)	37.7	
C-13	24.4	1.29 (m, 2H)	25.2	
C-14	39.9	1.25 (m, 2H)	39.7	
C-15	28.2	1.83 (m, 1H)	28.4	1.52 (m, 1H)
C-16	23.2	1.01 (d, 3H)	23.1	
C-17	23.2	1.01 (d, 3H)	23.0	
C-18	21.2	1.06 (d, 3H)	20.17	
C-19	21.0	1.06 (d, 3H)	20.14	
C-20	17.4	1.17 (s, 3H)	16.86	1.65 (s, 3H)

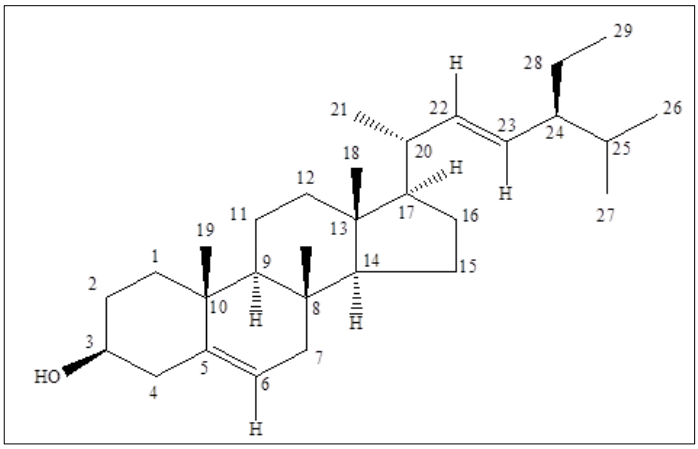

Fig 2: Compound **1** (Stigmasterol)

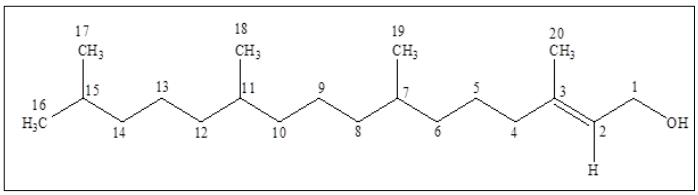

Fig 3: Compound **2** (3, 7, 11, 15-tetramethyl-3-hexadec-en-1-ol)

Conclusion

Currently most natural products have been the focus of scientific studies for ailments by different disease causing agent. Compound 1 and 2 were isolated from the methanol root extracts of the *Commelina diffusa* were terpene Alcohol and phytosterol named as stigmasterol and 3, 7, 11, 15-tetramethyl-3-hexadec-en-1-ol. The structure of the isolated compounds were identified on the basis of spectroscopic methods and by comparing its physical properties reported in the literature. To the best of our knowledge, this is the first report on the presence of such kind of compounds in the root of *Commelina diffusa.*

Recommendation

Stigmasterol (Compound-1) and 3, 7, 11, 15-tetramethyl-3-hexadec-en-1-ol (Compound-2) were isolated from the methanol root extracts of the *Commelina diffusa.* However, due to shortage of time and chemicals, we couldn't conduct the bioassay of the isolated compounds. Thus, we recommend further screening of the extract and compounds against selected strains of microorganism so as to validate the traditional use of the plant. As this work is the only study that attempted to phytochemically analyse the polar extract of the root the plant, further study is recommended on other parts of the plant such as aerial parts, leaf and stem bark. Finally, the crude TLC showed still coupled of unidentified compounds that we have missed during the chromatographic separation. However, we recommend a continuation of the work using high tech separation techniques such as RP-HPLC using reverse phase C8 and C18 column and test for their antimicrobial efficacy.

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