

Phytochemical Investigation and Characterization on the Stem Bark Extract of *Croton macrostachyus*

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Abstract

Croton macrostachyus which is called ‘rush foil’ or ‘broadleaved croton’ is a multipurpose, medium sized, drought-deciduous pioneer tree. It is a tall tree found in tropical regions of Africa. The genus *Croton* belongs to the family Euphorbiaceae, which commonly known as the ‘spurge’ family, and it is known as ‘Bisana’ (in Amharic). Traditionally, *C. macrostachyus* used for treatment of malaria, rabies, gonorrhoea, wound, diarrhoea, hepatitis, jaundice, scabies, toothache, abdominal pain, cancer, typhoid, pneumonia and gastrointestinal disorders and as ethno-veterinary medicine. The air dried and powdered plant material (400g) was first soaked with 500mL n-hexane for 48 hours and yielded 2g of n-hexane extract. Marc was soaked with 500mL of chloroform for 36 hours and afforded 3.5g of chloroform extract. Finally, Marc was soaked with 500mL of methanol and yielded 18g of methanol extract. The chloroform extract of the stem bark of *C. macrostachyus* afforded a compound coded as **EO**. Its structural determination was accomplished by means of spectroscopic techniques, namely IR, ¹H NMR, ¹³C NMR and DEPT-135. The compound, **EO** was isolated and characterized from the stem bark of *C. macrostachyus*. Generally, more advanced chromatographic techniques are required to isolate more compounds from stem bark of *C. macrostachyus*. Also MS and 2D NMR spectroscopic techniques are needed to fully characterize the isolated compound.

Keywords: *C. macrostachyus*; ethno-veterinary; characterization; chromatographic techniques; spectroscopic techniques.

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1. Introduction

Plants are invaluable and fundamental to almost all life on earth. They provide wide range of uses to human beings such as medicine, food, shelter, clothing, fuel wood for cooking, timber for construction, utensils, as well as fodder for cattle. They also recycle essential nutrients of ecosystems, establishing soils and maintaining soil fertility in addition to protecting areas of water catchments. Moreover, they keep ecological and climatic balance, facilitate, and control rainfall through the process of evaporative transpiration.^{1,2} *Croton macrostachyus* Hochst. ex Delile is a species of the genus *Croton* L., Euphorbiaceae family, commonly known as the spurge family. *Croton macrostachyus* is a medium sized, drought-deciduous pioneer tree which regenerates naturally in less productive sites including forest edges, mountain slopes, and waste grounds under a wide range of ecological conditions.³⁻⁵ *Croton macrostachyus* is regarded as a multipurpose tree by subsistence farmers in Ethiopia, Kenya, and Tanzania⁵⁻⁸, as it is often grown and managed in home gardens for provision of several ecosystem goods and services. In Ethiopia, for example, *C. macrostachyus* is a major tree intercropped in agroecosystems in order to increase soil productivity in midaltitude and semiarid areas.⁹

1.1 *Croton macrostachyus*

The genus *Croton* belongs to the family Euphorbiaceae (which commonly known as the ‘spurge’ family).¹⁰ It is known as ‘Bisana’ (in Amharic).¹¹ And consists of approximately 1300 species of trees, shrubs and herbs distributed in tropical and subtropical regions of the world.¹² *Croton macrostachyus* which is called ‘rush foil’ or ‘broadleaved croton’ is a multipurpose, medium sized, drought-deciduous pioneer tree. It is a tall tree found in tropical regions of Africa.¹³ Elsewhere in Africa, *C. macrostachyus* has been reported to occur in Angola, Burundi, Cameroon, Central Africa, Ghana, Guinea, Ivory Coast, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Sudan, Tanzania, Uganda, Zaire, and Zambia.¹⁴ It is native to Eritrea, Ethiopia, Kenya, Nigeria, Tanzania and Uganda.^{15, 16} In Ethiopia, *C. macrostachyus* occurs in regions between 1300 and 2500m with annual rainfall ranging between 750 and 2000mm. *Croton macrostachyus* is commonly found on forest edges along rivers, around lakes, woodlands, wooded grasslands and along roadsides.¹⁰ The tree is quite persistent, regenerating large numbers of coppices or shoots, even when it is repeatedly lopped or degraded. Provided that environmental and soil conditions are favorable, *C. macrostachyus* does establish well and can grow quite fast on reasonably good and well-drained soils, but prefers red or loam soils to vertisols. The latter soils are known

for their shrink-swell properties (during the dry and wet seasons, respectively), and for getting waterlogged during the rainy season.¹⁰ *Croton macrostachyus* (Euphorbiaceae) is a large tree with cylindrical trunk. The stem is more or less pyramidal in shape with widespread branches. The stem is gray clear, smooth and fissure with age. Leaves are almost as heart-shaped large that long, they have 10 to 15 cm of length; they are flexible, green or brunette according to the season and present some prominent ribs. Flowers are regrouped in inflorescence on stems of about 25 cm of long. They are visible but their life span is very short. They are colour creamy and slightly fragrant yellow. Fruits are regrouped along an axis.¹⁷

The name of Croton comes from a Greek word 'Kroton' which means ticks, because of the seeds' resemblance to ticks, the specific name "macrostachyus" is a contraction of two words, the Greek word "macro" meaning large and "stachyus" relating to the spike, hence, a species characterized by large spikes.¹⁸ *C. macrostachyus* is regarded as a multipurpose tree by subsistence farmers in Ethiopia, Kenya, and Tanzania and the species has potential in playing an important role in the primary healthcare. The bark, fruits, leaves, roots, and seeds of *C. macrostachyus* are reported to possess diverse medicinal properties and *C. macrostachyus* is used as herbal medicine for at least 61 human and 20 animal diseases and ailments. In the distribution area there is a high degree of medicinal use consensus for bleeding, blood clotting, cancer, constipation, diarrhea, epilepsy, malaria, pneumonia, purgative, ringworm, skin diseases or infections, stomach ache, typhoid, worm expulsion, and wounds.^{19,20}

1.2 Phytochemical constituent of *C. macrostachyus*

The genus Croton is rich in terpenoids (diterpenoids and triterpenoids), alkaloids, flavonoids, lignoids, proanthocyanidins and volatile oils containing monoterpenoids, sesquiterpenoids and some shikimate-derived compounds. Previous studies showed the existence of crotin (a chalcone), lupeol (a triperpene), crotepoxide (a cyclohexane diepoxide), proteins, fatty acids, saponins, resins and alkaloids.^{19, 21} The activity of *C. macrostachyus* stem bark extracts is comparable to studies where antiplasmodial activity has been related to a range of several classes of secondary plant metabolites including alkaloids and sesquiterpenes, triterpenes, flavonoids, inonoids, and quassinoids.²¹

1.3 Medicinal value of *C. macrostachyus*

Traditionally *C. macrostachyus* used for treatment of malaria, rabies, gonorrhoea, wound, diarrhea, hepatitis, jaundice, scabies, toothache, abdominal pain, cancer, typhoid, pneumonia and gastrointestinal disorders and as ethnoveterinary medicine.²²⁻²⁴ Pharmacological studies on *C. macrostachyus* indicate that it has a wide range of pharmacological activities such as anthelmintic, antibacterial, antimycobacterial, antidiarrheal, antifungal, anticonvulsant and sedative, antidiabetic, anti-inflammatory, antileishmanial, antioxidant, antiplasmodial, and larvicidal effects.¹⁹ The leaves and shoots of *C. macrostachyus* are used to treat fever and oedema and also mashed leaves used for treatment of hemorrhoids. Moreover, the maceration of *C. macrostachyus* stem bark is used as abortifacient and uterotonic to expel retained placenta.²⁵

2. Materials and Methods

2.1 Plant Material

The *C. macrostachyus* stem bark was collected from morsito Shero kebele Haqoro, Misha Woreda, Hadiya Administrative Zone, Southern Nations Nationalities and People Regional State (SNNPR). It was authenticated by a botanist and specimen was stored at the National Herbarium of Addis Ababa University (Voucher no.TA004), Addis Ababa, Ethiopia.

2.2 Instruments, Apparatus and Chemicals

¹H and ¹³C NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer (Germany) in CDCl₃ with TMS as internal standard. The ultra-violet and visible (UV-Vis) spectra were taken on GENESY'S 2PC UV-Vis scanning spectrometer (USA) (200-800nm). IR spectra were obtained on Perkin-Elmer BX infrared spectrometer (USA) (400-4000cm⁻¹) using KBr. Melting point was recorded using digital melting point apparatus;RV-10-basic Rotavapor (Germany) was used for separation of solvents. Analytical thin layer chromatograms were run on 0.2 mm thick layer of silica gel coated on aluminium foil. Column chromatography was performed using silica gel (70-230mesh). In this study, all the chemicals were provided by Hi-Media Co. including methanol, acetone, chloroform, n-hexane, and others.

Some of the apparatus were used: funnels, round bottom flasks, vials, glass wares, refrigerator, Whatman No.1 filter papers, grinder, drying oven, measuring cylinders, TLC Chamber, and others.

2.3 Extraction and Isolation

The air dried and powdered plant material (400g) was first soaked with 500ml n-hexane for 48 hours with frequently shaking and the extract was collected by filtering and concentrated under reduced pressure using the

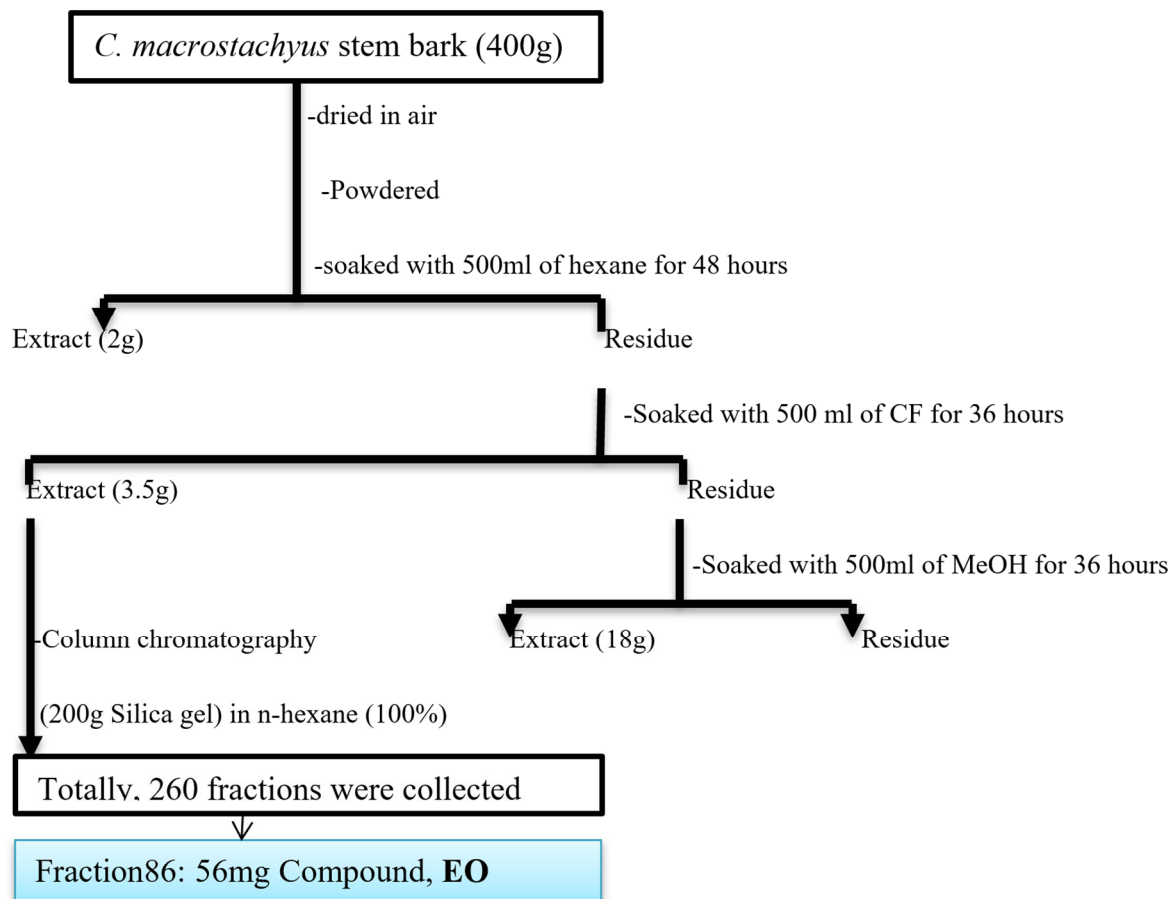
RV-10 basic Rotavapor. The solvent free residue was then soaked with 500ml of chloroform (CF) for 36 hours with frequently shaking and the extract was collected. This filtrate was evaporated under reduced pressure using the Rotavapor.



Fig. 1. *C. macrostachyus*: (Picture taken by Teshale A. during data collection)

Finally, the solvent free residue was soaked with 500ml of methanol, and then it was filtrated by using Whatman no.1 filter paper and concentrated under reduced pressure using the Rotavapor and its extract was afforded many spots on TLC. There was no visible spot for n-hexane extract, but chloroform extracts showed colored spots by using solvent system of n-hexane: chloroform (2:8) and crude extract was dissolved in itself with equivalent amount of silica gel, dried using Rotavapor and applied to a silica gel (200g) column chromatography which was packed with n-hexane (100%).

The scheme of extraction is shown below:



Scheme 1. Method used to extract and isolate of stem bark of *C. macrostachyus*

Table 1. Column chromatography of *C. macrostachyus* stem bark extract of chloroform by using solvent systems: n-hexane, chloroform and Methanol

S. no.	Solvent systems	Solvent ratio	Collected fractions
1	n-hexane	100%	1-4
2	n-hexane: chloroform	9:1	5-9
3	n-hexane: chloroform	8:2	10-16
4	n-hexane: chloroform	7:3	17-22
5	n-hexane: chloroform	6:4	23-31
6	n-hexane: chloroform	5:5	32-45
7	n-hexane: chloroform	4:6	46-58
8	n-hexane: chloroform	3:7	59-75
9	n-hexane: chloroform	2:8	76-96
10	n-hexane: chloroform	1:9	97-121
11	Chloroform	100%	122-143
12	chloroform: methanol	9:1	144-156
13	chloroform: methanol	8:2	157-165
14	chloroform: methanol	7:3	166-176
15	chloroform: methanol	6:4	177-186
16	chloroform: methanol	5:5	187-197
17	chloroform: methanol	3:7	198-220
18	chloroform: methanol	1:9	221-238
19	methanol	100%	239-260

Totally 260 fractions were collected. Out of 260 fractions which were collected using the solvent systems increased polarity, only those fractions from 76-96 showed the characteristic colored spots on TLC. The remaining fractions did not show the characteristic colored spots on TLC. Among fractions 76-96, fraction 86 showed single spot on TLC using the solvent system n-hexane: chloroform (2:8). Finally, the dried sample of this fraction was afforded 56mg of the compound, EO.

3. Results and Discussion

The air dried and powdered *C. macrostachyus* stem bark (400g) were extracted with solvents of n-hexane, chloroform, and methanol and their yields 2g, 3.5g, and 18g, respectively. These extracts when developed on TLC both the n-hexane and chloroform extracts have shown colored spots, but methanol extract was afforded many spots on TLC. The yellow organic extract of chloroform (3.5g) was subjected to column chromatography on silica gel and 260 fractions were collected.

Table 2. Results of phytochemical screening *C. macrostachyus* stem bark

Phytochemical constituents	Bark extracts	
	Chloroform	Methanol
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Terpenoids	+	+
Glycosides	+	+
Alkaloids	+	+
Steroids	+	+
Phenols	+	+

[NB : (+) indicate the presence of Phytochemical Constituents]

3.1 Characterization of Compound, EO

The compound, EO was obtained as a white solid that showed a characteristic color change to orange on TLC plate. It has retention factor, RF value 0.68 using hexane: chloroform (2:8) as solvent system. In the IR spectrum of the compound, EO the absorption band at 3300 cm⁻¹ showed the O-H stretching that indicated the presence of a hydroxyl group. The strong absorption band at 2900cm⁻¹ showed the presence of the C-H stretching of the olefinic group. The weak absorption band at 2886cm⁻¹ showed the presence of the C-H stretching for sp³ groups. The strong absorption band at 1610cm⁻¹, 1595cm⁻¹, and 1630cm⁻¹ showed the presence of the olefinic C=C stretching. The strong absorption band at 1720cm⁻¹ showed the presence of the ketone carbonyl group (-C=O) stretching. The absorption band at 1256cm⁻¹ showed the presence of the C-O bond stretching.

Table3: IR spectral peak values and functional groups obtained from the stem bark extract of *C. macrostachyus* (EO)

Extract prepared in	peak values in cm^{-1}	functional groups
Chloroform	3300	-OH (hydroxyl group)
	2900	Sp^2 C-H stretching
	2886	SP^3 C-H stretching
	1630, 1610 and 1695	Olefinic group of C=C stretching
	1720	Carbonyl group of -C=O stretching
	1472	Methylene group bending
	1363	Methyl group bending
	1256	CO bond stretching

The ^1H NMR spectrum showed, the coupled proton peaks, which is triplet peaks at δ 1.40; 1.17, integrating for two protons corresponded to the methylene proton which was attached to C-1. Coupled proton peaks, which is quartet peaks at δ 1.60; 1.43, integrating for two protons, which were corresponded to the methylene proton groups and assigned to C-2. The pentet peaks at δ 3.31, integrating for one proton, corresponded to the methine proton group that assigned at C-3. Coupled proton peaks, which is doublet peaks at δ 2.32; 1.95, which integrating for two protons corresponded to the methylene proton groups and attached to C-4. Triplet peaks at δ 5.34, integrating for one proton corresponded to the methine proton, which was assigned to C-6. Coupled proton peaks, which is triplet peaks at δ 2.15; 1.90, integrating for two protons corresponded to the methylene protons which were attached to C-7. The pentet peaks at δ 1.93, integrating for one proton, corresponded to the methine proton and assigned to C-8. The doublet peaks at δ 2.42, integrating for one proton, corresponded to the methine proton and assigned to C-9. The coupled proton peaks, which is doublet peaks at δ 2.41; 2.12, integrating for two protons corresponded to the methylene protons which were attached to C-12. The pentet peaks at δ 2.20 which integrating for one proton corresponded to the methine proton and attached to C-13. The quartet peaks at δ 2.30 which integrating for one proton corresponded to the methine proton and, which was attached to C-14. The triplet peaks at δ 5.54 which integrating for one proton corresponded to the methine proton and attached to C-15. The triplet peaks at δ 5.54 which integrating for one proton corresponded to the methine proton and attached to C-16. The quartet peaks at δ 2.83 which integrating for one proton corresponded to the methine proton and, which was attached to C-17. The triplet peaks at δ 5.37 which integrating for one proton corresponded to the methine proton and attached to C-18. The quartet peaks at δ 5.30 which integrating for one proton corresponded to the methine proton and, which was attached to C-19. The quartet peaks at δ 1.80 which integrating for two protons corresponded to the methylene protons and, which were attached to C-20. The quartet peaks at δ 1.26 which integrating for two protons corresponded to the methylene protons and, which were attached to C-21. The multiple peaks at δ 1.87 which integrating for one proton corresponded to the methine proton and, which was attached to C-22. Doublet peaks at δ 1.11, which integrating for six protons corresponded to the methyl protons and that were attached to C-23 and C-24. Singlet peak at δ 1.29, which integrating for three protons corresponded to the methyl protons and attached to C-25.

The ^{13}C NMR and DEPT-135 spectrum of compound, EO showed well resolved resonance of 25C atoms of which 3, 7, 12, and 3 of them were namely, methyl, methylene, methine, and quaternary carbon groups, respectively.

Table 4: ¹H NMR spectral data of compound (EO)

S. No.	Peaks (δ)	Peak multiplicities	Proton No.	Assigned carbon	Remark
1	1.40; 1.17	Triplet	Two	C-1	Methylene
2	1.60; 1.43	Quartet	Two	C-2	methylene
3	3.31	Pentet	One	C-3	Methine
4	2.32; 1.95	Doublet	Two	C-4	Methylene
5	5.34	Triplet	One	C-6	methine
6	2.15; 1.90	Triplet	Two	C-7	Methylene
7	1.93	Pentet	One	C-8	Methine
8	2.42	Doublet	One	C-9	Methine
9	2.41; 2.12	Doublet	two	C-12	methylene
10	2.20	Pentet	One	C-13	Methine
11	2.30	Quartet	One	C-14	Methine
12	5.54	Triplet	Two	C-15 and C-16	Methine
13	2.83	Quartet	One	C-17	Methine
14	5.37	Triplet	One	C-18	Methine
15	5.30	Quartet	One	C-19	Methine
16	1.80	Quartet	Two	C-20	Methylene
17	1.26	Quartet	Two	C-21	Methylene
18	1.87	Multiple	One	C-22	Methine
19	1.11	Doublet	Six	C-23 and C-24	Methyl
20	1.29	Singlet	Three	C-25	Methyl

The ¹³CNMR and DEPT-135 spectrum of compound (EO) showed well resolved resonance of 25 carbon atoms and from them were: three methyl groups, seven methylene groups, twelve methine groups and three quaternary carbons.

Table 5: ¹³C NMR and DEPT-135 (400 MHz, CDCl₃) spectral data of Compound (EO)

Carbon No.	¹³ C NMR (in ppm)	DEPT (in ppm)	Remark
1	30.60	30.62	CH ₂
2	32.71	32.70	CH ₂
3	70.00	70.00	CH
4	39.00	39.10	CH ₂
5	141.55	-	C(Quaternary carbon)
6	132.96	132.94	CH
7	28.87	28.85	CH ₂
8	31.91	31.90	CH
9	61.20	61.21	CH
10	39.05	-	C (Quaternary carbon)
11	210.00	-	C (Quaternary carbon)
12	48.21	48.22	CH ₂
13	43.00	43.10	CH
14	45.90	45.86	CH
15	133.90	133.83	CH
16	133.72	133.71	CH
17	50.40	50.40	CH
18	130.97	130.96	CH
19	130.87	130.86	CH
20	27.60	27.61	CH ₂
21	40.23	40.22	CH ₂
22	29.45	29.44	CH
23	24.67	24.67	CH ₃
24	24.67	24.67	CH ₃
25	23.56	23.55	CH ₃

Finally, from the all above data, namely IR spectral data, ¹³C NMR, DEPT, and ¹H NMR spectral data the suggested structure of compound, EO would be shown below:

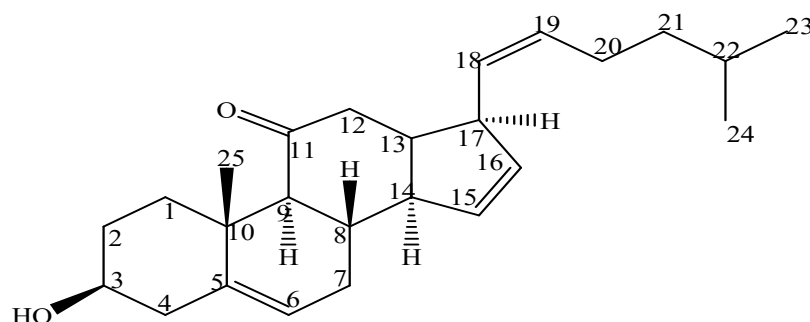


Fig. 2. The proposed structure of the compound, EO

4. Conclusions and Recommendations

C. macrostachyus used for treatment of malaria, rabies, gonorrhoea, wound, diarrhoea, hepatitis, jaundice, scabies, and as ethno-veterinary medicine. The *C. macrostachyus* stem bark extracts is comparable to studies where antiplasmodial activity has been related to a range of several classes of secondary plant metabolites including alkaloids and sesquiterpenes, triterpenes, flavonoids, inonoids, and quassinoids. In this study, *C. macrostachyus* stem bark were showed the presence of phytochemical constituents such as alkaloids, flavonoids, terpenoids, saponins, tannins, steroids, glycosides, and phenols of chloroform and methanol extracts. The *C. macrostachyus* stem bark was extracted with solvents of n-hexane, chloroform, and methanol and their yields 2g, 3.5g, and 18g, respectively. The organic extract of chloroform (3.5g) was subjected to column chromatography on silica gel and 260 of fractions were collected. From the IR spectrum of the compound, EO the absorption band at 3300 cm^{-1} showed the O-H stretching that indicated the presence of a hydroxyl group. The strong absorption band at 2900 cm^{-1} showed the presence of the C-H stretching of the olefinic group. The strong absorption band at 1610 cm^{-1} , 1595 cm^{-1} , and 1630 cm^{-1} showed the presence of the olefinic C=C stretching. The strong absorption band at 1720 cm^{-1} showed the presence of the ketone carbonyl group (-C=O) stretching. Lastly, from this study, the compound EO was elucidated and characterised by incorporating by spectroscopic techniques such as IR spectral data, ^{13}C NMR, DEPT, and ^1H NMR spectral data acquired.

Despite the traditional use of this plant for the treatments of various ailments, in many parts of the world there is limit report on phytochemical analysis on the *C. macrostachyus* stem bark extracts, context of our country. Thus, this study may oblige as point of departure for researchers who are inspired and interested to conduct such type of research in the future.

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Competing Interests

Authors have declared that no competing interests exist.

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