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Phytochemical investigation of the roots extracts of *Terminalia brownie* and isolation of dimethyl terephthalate

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Abstract
Terminalia brownie (*T. brownie*) is a plant traditionally used for treatment of human illnesses such as bronchitis, diarrhea, bacterial infections, fungal infections, viral infections, stomach ache, ulcers, sexually transmitted diseases, malaria, cough, hepatitis, jaundice and yellow fever. The objective of this study was to extract and isolate compounds from the root extract of *T. brownie,* and to characterize structures of isolated compounds.
The successive extraction of the roots was carried out using solvent systems n-hexane, dichloromethane: methanol (1:1) and methanol that afford 0.9 g, 42.0 g and 11.0 g of crude extracts, respectively. Phytochemical screening of the dichloromethane: methanol (1:1) root extract of the plant revealed the presence of terpenoids, saponins, steroids, flavonoids, tannins, phenols, and glycosides. Column Chromatographic separation on silica gel of dichloromethane: methanol (1:1) extract using chloroform: methanol solvent system with gradual increase in polarity led to isolation of compound 19 (dimethyl terephthalate) for the first time from the genus. The structure of this compound was elucidated using spectroscopic techniques (UV-Vis, IR, and NMR). The present study concluded that the secondary metabolites found in the dichloromethane: methanol (1:1) root extract of *T. brownii* are almost similar with that of the secondary metabolites reported in the previous studies of leaf and bark extract of the plant, and other species of genus *Terminaia*.

Keywords: Crude extract; *Terminalia brownii,* Dimethyl terephthalate; Phytochemical screening; Secondary metabolites

Introduction
Medicinal plants have been and being used in traditional medicine in most communities throughout the world for treatment of various human and animal illnesses [1]. According to the World Health Organization (WHO), about 80% of the world's population (especially those living in developing countries) depends on traditional medicine for their primary health care needs and traditional medical practice [2]. The medicinal uses or the pharmacological activities could be attributed to variety of secondary metabolites (biologically active compounds) present in those plants [3]. Medicinal plants also used as source of commercial drugs for several human diseases [4, 5]. Research reports indicated that approximately 25% of prescription drugs currently in use are originally derived from medicinal plants [6]. For instance, of new anticancer drugs marketed from 1981 to 2006, approximately 75% of them are derived from medicinal plants or are plant origins compounds [7]. Studies also indicate that only small proportion (10%) of available medicinal plants (250,000 species worldwide) have been studied so far, and there is a great potential to the discovery of commercially and/or therapeutically useful phytochemicals possessing a diverse range of activities against new diseases and exiting diseases that show resistance to the drugs currently in use [7, 8].
One of the plant species with big potential as source of modern drug is *Terminalia brownii* (*T. brownii*). It is one of the 30 *Terminalia* species [9], and is widely distributed in Eastern African countries such as Ethiopia, Tanzania, Sudan, Kenya, Eritrea, Democratic Republic of Congo and Djibouti [10] (Figure 1). The plant is known by different vernacular names in the countries where it is found in abundance [11], and it is well known for its use in traditional medicine [10]. Its different morphological parts are used by people of the region to treat a wide range of human illnesses. This includes its use for treatment of bacterial, fungal and viral infections, and also as a remedy for diarrhea and stomach ache, ulcers, sexually transmitted diseases, bleeding, malaria, cough, hepatitis, jaundice and yellow fever [10, 12-15]. It is also used for beautification or as local perfume for women [16].

Scientific studies showed that extracts from leaves, stem bark, wood and roots to possess biological activities such as antimicrobial (antibacterial and antifungal) [9, 11, 16-22], antimalarial (antiplasmodial) activity [23], used against allergic reactions, anti-inflammatory activity [24], antinociceptive activity [25] and radical scavenging activity [26]. The stem wood extracts also showed anticancer activity [11]. The observed (reported) biological activities can be used as justification for the use of the plant in traditional medicine such as for the treatment of conditions such as diarrhea, and gonorrhea, pain remedy (management), inflammation and treatment of oxidative stress [26].

Fig 1: Distribution of *T. brownii* in Africa (Source: *http://uses.plantnet-project.org/en/Terminalia_brownii_(PROTA); downloaded on 4 October 2017*).

There are several reports on isolation and evaluations of biological activities of isolated compounds from the bark, wood and root parts of the plant against pathogenic microorganisms [10, 25, 27]. For instance, isolation of five compounds namely β-sitosterol (1), stigmasterol (2), monogynol A (3), betulinic acid (4) and arjungenin (5) (Figure 2) has been reported from *n*-hexane, ethanoic acid and methanol extracts of the stem bark. Some of the isolated compounds showed comparable antifungal and antibacterial activities with that of the extracts [27]. Isolation 3β,24-O-ethylidenyl-2α,19α-dihydroxyolean-12-en-28-oic acid (6), tomentosic acid (7), sericoside (8) and arjunglucoside I (9), 3-O-β-D-glucopyranosyl-β-sitosterol (10), arjunic acid (11), sericic acid (12), 23-galloylarjunic acid (13), 3-*O*-methylellagic acid (14), 4-*O*-(3″,4″-di-*O*-galloyl-α-L-rhamnopyranosyl) ellagic acid (15), diellagic lactone (16), 3,3′-di-*O*-methylellagic acid (17) and 3,3′,4-tri-*O*-methylellagic acid (18) also reported from ethanolic extract of stem bark [10] (Figure 2). Moreover, the evaluation of isolated compounds revealed their antimicrobial and anti-plasmodial activities [10]. There are also other compounds isolated from the different parts of the plant [10, 28, 29]. Similar to that of other African countries, the morphological parts of *T. brownii* are widely used in Ethiopia for traditional medicine including its roots. The aim of the present study was to carry out phytochemical screening and isolation of compounds from root extract of the plant.

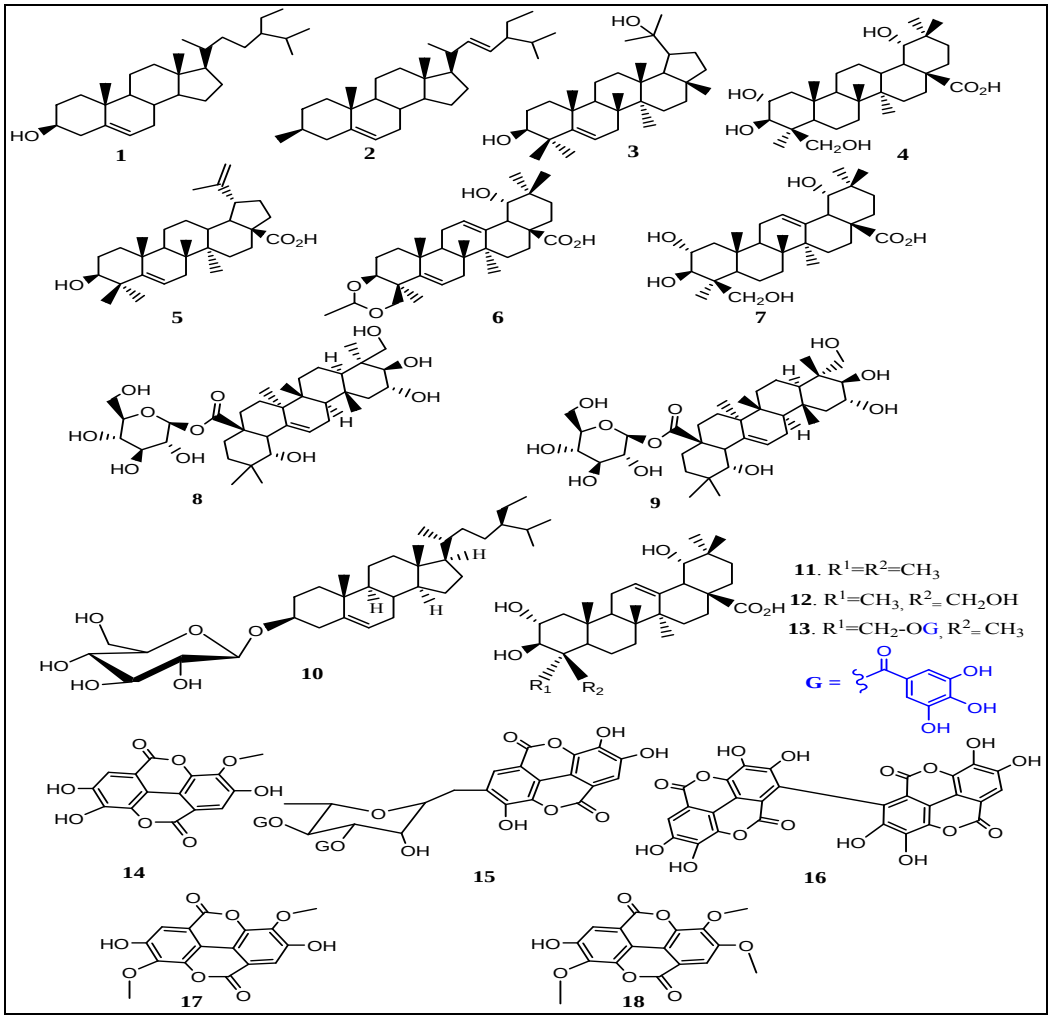

Fig 2: The chemical structures of some of the compounds isolated from *T. brownie*

3. Materials and Methods

3.1. General

Column chromatography (CC) was performed on Merk silica gel (60-120 mesh). Thin-layer chromatography (TLC) was carried out on aluminum sheets pre-coated with silica gel 60 F_{254} (20 x 20 cm, 0.2 mm thick; Merck) to monitor the purity of the compounds. The developed plates were visualized under UV light (254 and 366 nm). ^{1}H-NMR and ^{13}C-NMR spectra were recorded in on a Bruker Avance 400 MHz spectrometer with tetramethylsilane (TMS) as internal standard and $CDCl_3$ as solvent. IR spectrum was recorded as KBr pellets on Perk-Elmer BX Infrared Spectrometer in the range of 4000-400 cm^{-1}. All the spectral analyses were carried out at The Department of Chemistry, Addis Ababa University, Ethiopia.

3.2 Plant material collection and preparation

The roots of *T. brownie* were collected from *Selamber*, *Kucha* district, *Gamo Gofa* zone, South Nation and Nationality Peoples Region (SNNPR), Ethiopia. The area is 450 km South of Addis Ababa, capital of Ethiopia. It is also about 217 km far from Hawassa University. The fresh collected roots of *T. brownie* were dried under shade at room temperature for 20 days, and ground to powder form using Electric grinding machine.

3.3 Extraction

The powder of the roots (500 g) was divided in to two portions (250 g each) and soaked into 1.5 L n-hexane in two different 2000 ml Erlenmeyer flasks. After shaking well, the flasks containing the solution were put on orbital shaker and left for 24 hrs at a speed of 120 rpm. Then the solutions were filtered (after 24 hrs) using 15 cm size Whatman filter paper and suction filtration. The filtrate was dried using rotary evaporator at reduced pressure and at temperature of 40-45 ^{0}C. The marc of the n-hexane extraction was subjected to dichloromethane: methanol (1:1) extraction employing the above extraction procedures. Similarly, the residue of this extraction was subjected to further extraction in methanol, and employing the same procedure used above.

3.4 Phytochemical screening tests

Phytochemical screening tests were carried out on dichloromethane/methanol (1:1) extract to investigate the presence and absence of secondary metabolites such as terpenoids, saponins, steroids, flavonoids, tannins, phenols, glycosides, and alkaloids) present in those extract. Literature reported standard procedures [30-33] were used to carry out the screening tests.

3.5. Isolation and characterization of compounds

The mass of n-hexane extract was inadequate, and that methanol extract showed complex TLC profile of its components. So, these extracts were not used for column chromatographic separation. The dichloromethane: methanol (1:1) extract (28 g) was adsorbed onto 30 g of silica gel. It was applied to glass column (that was loaded with 160 g of silica gel) for chromatographic separation. The column was eluted with chloroform: methanol mixture staring from chloroform: methanol (100:0%) and then gradually increasing the polarity by adding methanol to chloroform until it reaches chloroform: methanol (0:100%). The column chromatographic separation results in isolation of a pure white crystalline compound. The compound was subjected for spectroscopic (IR, UV, IR and NMR) analyses.

4. Results and Discussion

4.1 Extraction Yields

The masses of extract yields were 0.9 g (0.18%), 42 g (8.4%), and 11.6 g (2.2%) for n-hexane, dichloromethane: methanol (1:1) and methanol extracts, respectively (Table 1). The data indicated that the medium polarity compounds. This is consistent with literature reports that indicate use of more polar solvents give high yield of extracts [34, 35]. The percent yields were calculated using the formula;

$$\% \text{ Yield} = \frac{\text{Mass of crude extract}}{\text{Mass of plant material used for extraction}} \text{ X } 100$$

Table 1: The percent yield of crude extracts

S. No	Solvent used for extraction	Mass of extract (g)	% yield of extracts
1	n-hexane	0.9	0.18
2	Dichloromethane: methanol (50:50%)	42.0	8.4
3	Methanol	11.0	2.2

4.2. Phytochemical screening test

Phytochemical screening tests were carried out on dichloromethane: methanol (1:1) root extracts of *T. brownii* using standard procedures to identify the class of secondary metabolites (terpenoids, saponins, steroids, flavonoids, tannins, phenols, glycosides and alkaloids) present in the extract. The result indicated the presence of terpenoids, saponins, steroids, flavonoids, tannins, phenols and glycosides, and absence of alkaloids (Table 2). The observations are consistent with litrature reports that showed the presence of tannins, saponins, steroids, flavonoids, polyphenols, phytosterols and terpinoids, and absence of alkaloids in extracts obtained from some parts of the plant [26, 36]. The presence of these secondary metabolites justifies various medicinal uses of the plant aforementioned (*See Introduction Section*).

Table 2: Phytochemical screening of the root extract of dichloromethane: methanol (1:1)

Phytochemical	Reagent used	Colr change	Result
Terpenoids	$CHCl_3$ and conc. H_2SO_4	Yellow to red	+
Saponins	Distilled water	Yellow to white froth	+
Steroids	Acetic anhydride ad H_2SO_4	Yellow to blue green	+
Flavonoids	NaOH solution	Yellow precipitate	+
Tannins	$FeCl_3$ solution	Yellow to brownish green	+
Phenols	$FeCl_3$ solution	Yellow to deep green	+
Glycosides	Acetic acid, $FeCl_3$ solution and conc. H_2SO_4	Appreance upper green layer and no color change in the lower layer	+
Alkaloids	Dragedoff's reagents	No color change	-

Present (+); absent (-)

4.3 Isolation and Characterization

As discussed in Section 3.4, compound F6 (compound 19) was isolated as pure compound from column chromatographic procedures, and was obtained as a white crystal solid with R_f value of 0.64 in chloroform: methanol (80: 20%) solvent system.

Its structure was elucidated based on its spectral data. Absorption at λ_{max} 240 nm in the UV spectrum (Appendix 1) of compound F6 (compound 19) indicates that the compound is an aromatic compound conjugated with carbonyl compound. A strong band at 1718 cm^{-1} on its IR spectrum (Appendix 2) indicated presence of carbonyl functional group. Similarly, bands at 3014, 2959 and 1503 cm^{-1} indicate C-H stretch of aromatic compound, C–H stretch of methyl group and C=C stretch of double bonds of alkene or aromatic compounds, respectively. A band at 3492 cm^{-1} could be attributed to C=O overtone.

The ^{1}H-NMR spectrum (Appendix 3) of the compound showed four protons of symmetric and para substituted aromatic (benzene) ring (at δ 8.1). Appearance strong singlet peak at δ 4.0 indicates presence of methoxy (OCH_3) group protons of ester functional group (Table 3). These data suggest that compound F6 (compound 19) could be an aromatic compound consists of a benzene ring carrying two ester functional groups attached at 1,4- positions of benzene. The ^{13}C-NMR spectrum (Appendix 4) also supports the above claim. The peaks at δ166.23 and 133.89 indicate quaternary carbons of carbonyl carbon of ester and aromatic ring, respectively. Moreover, the peaks at δ129.52 and 52.39 of ^{13}C-NMR spectra indicate substituted carbons of benzene and methoxy group attached to carbonyl group, respectively (Table 4). Based on the above spectral data and in comparison with literature reports [37, 38], compound **F6** (compound **19**) was proposed to be dimethyl terephthalate (DMT) (Figure 3). Though isolation the compound (DMT) is reported from other species such as ethanol leaf extracts Tree spinach (*Cnidoscolus aconitifolius*) [37] and the bark of *Goniothalamus tapis* Miq. and *G. uvaroides* [39], this is the first report for isolation DMT from *T. Brownii.*

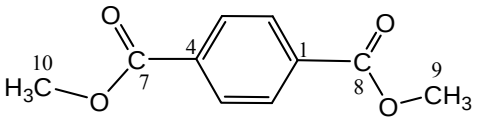

Fig 3: The proposed structure of compound 19 (DMT).

Table 3: ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$, 400 MHz) data of compound-19 and that of DMT reported in literature.

C/H atoms	^{1}H-NMR data of compound 19	^{13}C-NMR data of compound 19	Reported ^{1}H-NMR data of DMT [36, 37]	Reported ^{13}C-NMR data of DMT [36, 37]
Benzene ring				
1	-	133.89	-	133.94
2	8.1	129.52	8.10	129.57
3	8.1	129.52	8.10	129.57
4	-	133.89	-	133.94
5	8.1	129.52	8.10	129.57
6	8.1	129.52	8.10	129.57
Carbonyl group carbon	-	-	-	
7	-	166.23	-	166.30
8	-	166.23	-	166.30
Methoxy group carbon				
9	4.0	52.39	3.95	52.44
10	4.0	52.39	3.95	52.44

5. Conclusions

The present study concluded that phytochemical screening on *T. brownii*have revealed a variety of chemical constituents (terpenoids, saponins, steroids, flavonoids, tannins, phenols and glycosides) that justified the use of this plant in treatment of various diseases in traditional medicine. Chromatographic separation of the dichloromethane: methanol (1:1) yielded dimethyl terephthalate (compound 19). This is the first report on the presence of dimethyl terephthalate from *T. brownie* that is indigenous to Ethiopian flora. The isolation of this compound adds information to the list of compounds isolated from the plant, and also to justify some biological activities of extracts or use of this plant species in traditional medicine.

6. Acknowledgment

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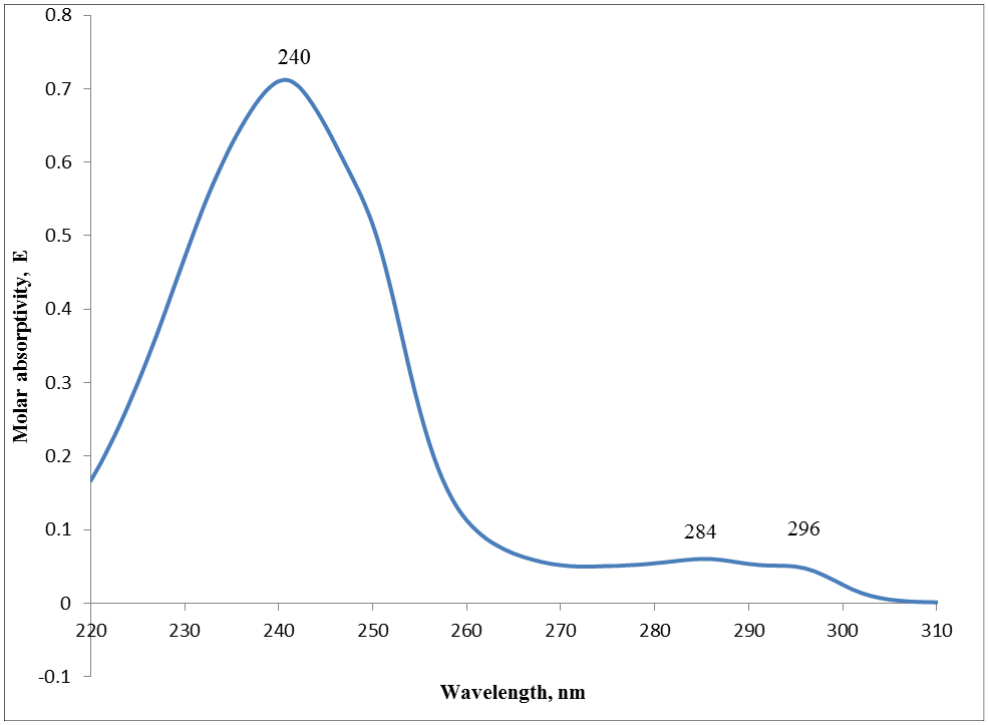
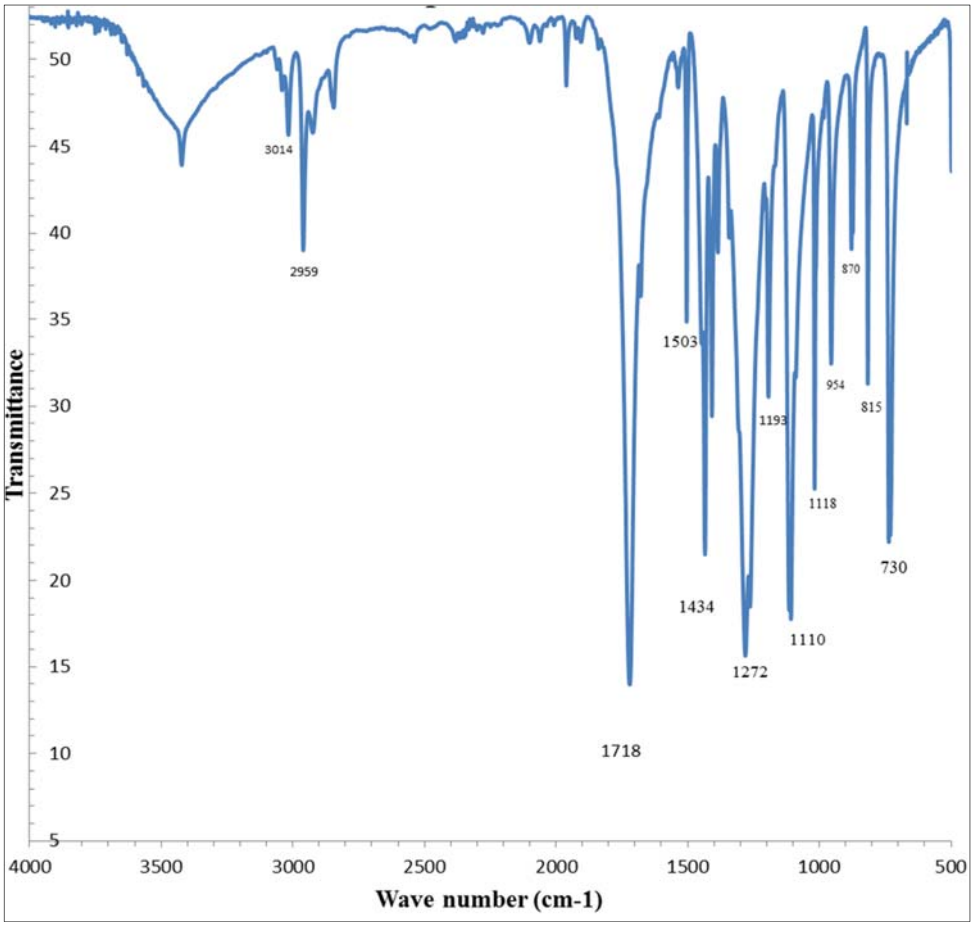
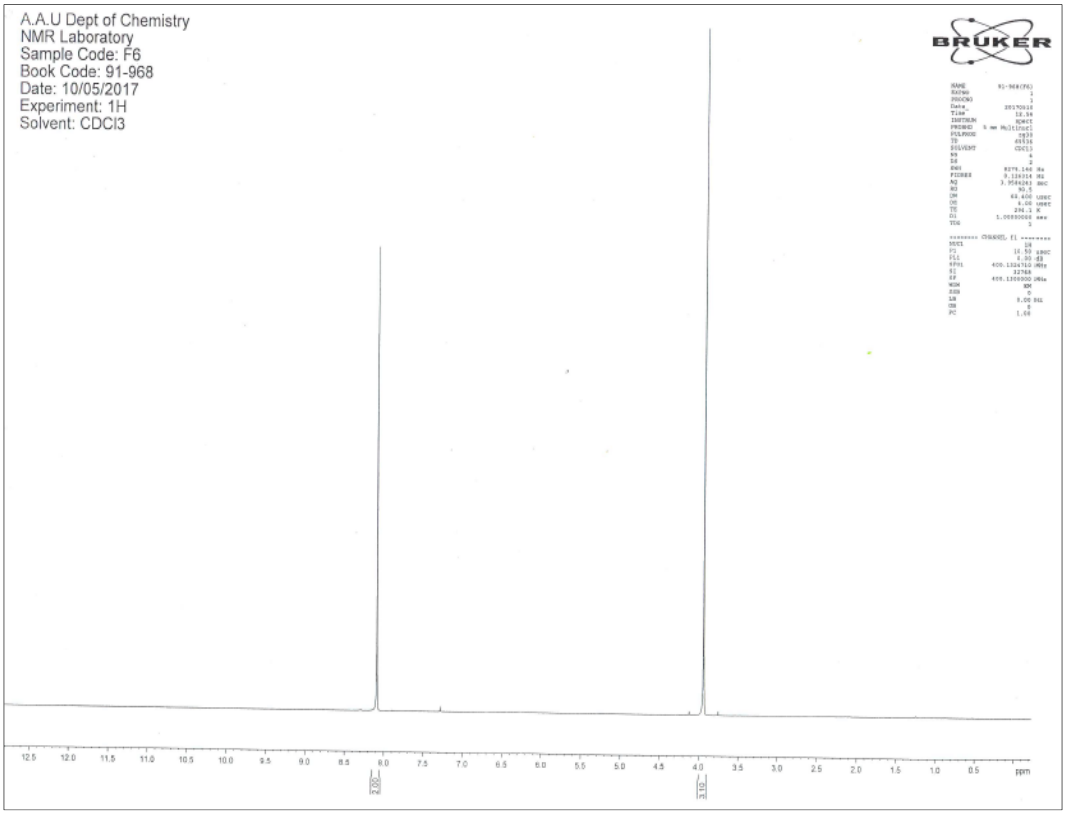


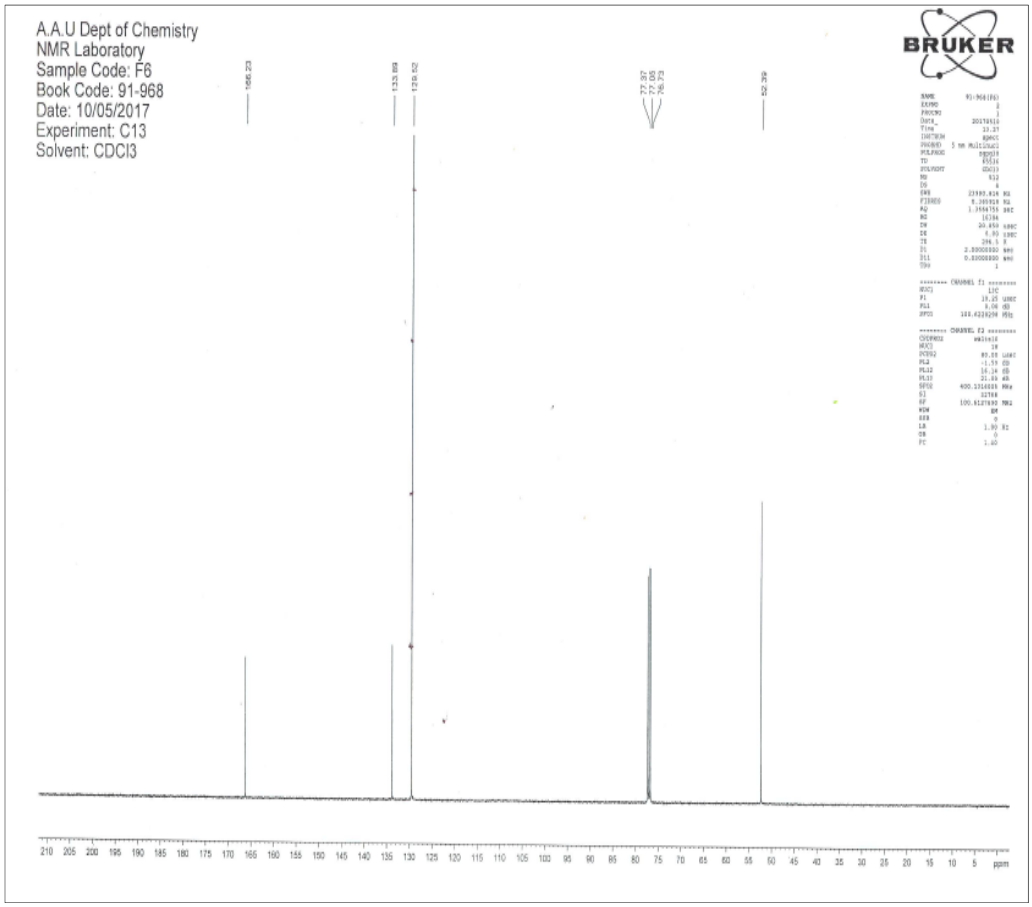
Appendix 1: The UV/Vis spectrum of compound **F6 (**Compound **19)**



Appendix 2: FT-IR spectrum of compound **F6 (**Compound **19)**



Appendix 3: ^{1}H-NMR spectrum of compound **F6 (**Compound **19)**



Appendix 4: ^{13}C-NMR spectrum of compound **F6** (Compound **19)**