Research Article

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Phytochemical Investigation on The Root Extract of Teclea Nobilis

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Abstract

*Teclea nobilis is one of an endemic species to Ethiopia. It is used as a traditional medicine to treat analgesics and other disorders. Although many species of the genus Teclea have been investigated for their chemical constituents, there is no much prior report on the investigation of chemical constituents of root of Teclea nobilis. Phytochemical screening of the C_6H_{14}, CH_2Cl_2,CH_2Cl_2/CH_3OH (1:1) and CH_3OH root extracts of the plant revealed the presence of alkaloids, flavonoids, terpenes, phenols, tannins and glycosides.Chromatographic separation using column chromatography of CH_2Cl_2/CH_3OH (1:1) result in isolation of three compounds namely compound **TN1**(0.06%), **TN2**(0.04%)and **TN3**(0.05%).Structure are proposed for Compound **TN1** and compound **TN2** .Compound **TN3** was not fully characterized due to lack of adequate spectral data.*

Keywords: Rutaceae, Tecleanobilis, Alkaloids, Phytochemicals

Introduction

Background Information

For thousands of years natural products have played a very important role in health care and prevention of diseases. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases [1]. Chemicals of plant origin, commonly referred to as natural products, are mainly organic compounds that are unique to one organism or common to a small number of closely related organisms. In most instances, their functions in the organism producing them are not yet known. But these naturally occurring compounds have significant effects on the physiological functioning of other organisms including human being [2].

Medicinal plants have always been of great importance to mankind in preventing and curing of various diseases [3]. Globally, nearly three quarters of drugs are derived from plants [4]. In Sub-Saharan Africa, over 80% of the population depends largely on plant based medicine in meeting their basic health care needs. The heavy reliance on herbal remedies is increasing from time to time due to resistance of microbial pathogens to the existing convengtional drugs [5]. In the developing countries, these alternative drugs are neither readily available nor affordable and thusmedicinal plants have been used as replacements [6]. These plants are, thus considered as rich resources of ingredients which can be used in drug development and synthesis. Moreover,they can also be used as rawmaterials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti-malarial medications, contain ingredients from plants [7].

Medicinal plants have been utilized as medicines for thousands of years. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations. The specific plants to be used and the methods of application for ailments of a diseaseare passed down orally from generation to generation. Through time, with advent of scientific instruments the use of plants as medicines has involved the isolation of active compounds that have been used as drugs in modern medicine [8]. Today, there are several modern drugs in market are obtained from medicinal plants. Cocaine (1), codeine (2), dig toxin (3), penicillin (4),morphine(5)and quinine(6) (Figure 1) are some examples of modern drugs that have been obtained from medicinal plants.Researches carried out in on medicinal plants by academic institute and pharmaceutical drug discovery programs focused on the determination of the phytochemical constituents of plant extracts. This is very essential in order to ensure the reliability and repeatability of pharmacological and clinical researches in order to understand their bioactivities and possible side effects and also to enhance product quality control [9].

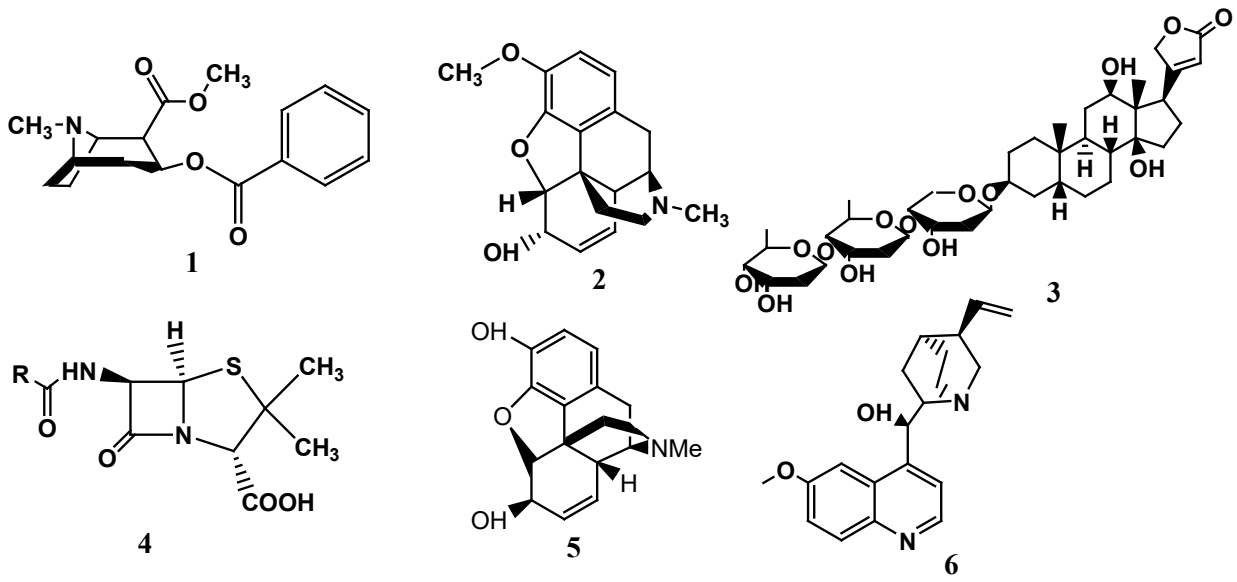

Figure 1: The chemical structures of some modern drugs (1-6) isolated from medicinal plants

Ethiopia is one of the biodiversity rich countries of the world. Its climatic and topographic conditions are believed to contribute for the existing rich biodiversity. Reports show that the country has about 6,000 species of higher plants with approximately 10% endemism [10].Similar to other countries most of the plant species in Ethiopia have medicinal values, and greater concentration of these plants is found in the southwestern parts of the country following the concentration of biological and cultural diversity. Many reports also showed that small proportion are formed in central, north and northwestern part of Ethiopia [11]. Even though, the country is considered as the home of most diverse plant species that could serve as sources of many traditional medicinal plants, scientific screening and the development of their therapeutic products is still limited. No single modern drug is available in market from Ethiopian medicinal plants. Thus, it is important to investigate medicinal plants of Ethiopia to get drugs or identify compounds responsible for their medicinal usesand also to validate traditional medicinal uses of such plants [12].

1.2 The Genus Teclea

Teclea is a genus in the subfamily Toddaliea of the family Rutaceae. There are about 30 species in Africa.The genus Tecleahas been reported to contain diverse classes of secondary metabolites such as quinolines alkaloids, acridone alkaloids, triterpenes, flavonoid glycosides.a number of Teclea species are widely used by various communities in treating a range of ailments. In Kenya, herbalists in the Akamba community useT. trichocarpa roots in the treatment of malariawhile in Ethiopia, the bark and leaves of T. nobilisare used as analgesics.Teclea simplicifolia has several uses such as treatment of malaria by the Maasai community in Kenya, while the wood of the plant is used in making roof beams, walking sticks and bows in addition to this there are many biological activities of genus teclea for instance The essential oils of the leaves of Teclea nobilis showed significant analgesic and antipyretic activity in mice [13, 14].

Teclea nobilisalso known asveprisnobilis is one of the medicinal plants available in Ethiopia. It is a member of genus Teclea, and widely distributed in tropical Eastern Africa, namely Ethiopia, Sudan, Somalia, Kenya, Uganda, Tanzania and also in Arabia. This plant is used in folk medicine of many African societies. For instance, in South Africa the bark of Teclea nobilis is reported to be a remedy for gonorrhoea while in Tanzanian folk medicine the leaves are used as cure for fever [15]. Similarly, in Ethiopian traditional medicine it is used to treat many ailments For example, the bark, leaves and root of *Teclea nobilis (locally known as 'Hadessa' in sidama zone,Atesa/sni in Amharic, tsihila in Tegray)* areused as analgesics, and also used to stomach ache and cough. The aerial parts of Teclea nobilis are shown below (Figure 2)

Figure 2: Aerial parts of Tecleanobilis (Had'essa) [photo taken by Tewodros.M, from Yergalemtown, SNNPR, Eth, Sept, 2016

Literature Review

2.1 Ethnomedicinal Uses Of The Genus Teclea

The genus Teclea, is widely distributed in wet highland forests, particularly in the Lake Victoria basin. It prefers sites in highland forests between the altitudes of 1700 and 2700 meters. Both the leaves and roots are used in local medicine. The roots are used to treat colds and chest problems. The wood is moderately hard, tough, and pale and is used for walking sticks, tool handles, bowls, clubs, spear shafts, poles, and hoe pins [16].

Ethnomedical survey reveals widespread and diverse medical usage of different species of the genus Teclea. Some of the medicinal uses of species in this genus are antibacterial, anti-malarial, antipyretic, anti-inflammatory and analgesicactivity].Teclea nobilis is a plant used in folk medicine as an analgesic and antipyretic agent. In Ethiopian folk medicine the leaves are used to control pain [17]. T. trichocarpa is used by traditional healers belonging to the Akamba tribe o f East Africa for malaria treatment, as an antihelmintic and the vapour is inhaled as a cure for fever The various parts o f the plant including leaves and stem bark are said to be a remedy for gonorrhea and pain.Teclea ouabanguiensis is used as a remedy for coughs and asthma in Cameroon [18].

2.2. Phytochemistry of the genus Teclea

Phytochemical investigations on genus teclea have revealed the presence of quinoline and furoquinoline alkaloids. while limonoids, tetranortriterpenes, triterpenes, alkaloids, and flavonoid glucosides were isolated from Teclea ouabanguiensis, Teclea grandifolia, Teclea verdoorniana, and Teclea sudanica,respectively [19]. Some chlorinated compounds have been isolated from this genus. Chlorodesnkolbisine is one of isolated compound from the aerial parts of Teclea nobilis. These chlorinated compounds have not been reported from any other source. Teclea nobilisis a big tree not a herb. The genus Teclea is well known for alkaloids derived from anthranilic acid, limonoids, coumarins and triterpene derivative [20].

As discussed in previous pages plants from this genus have several medicinal uses. They are used for treatment of a wide variety of medicinal disorders and conditions. Here, brief summaries of investigations on medicinal uses, phytochemical studies, biological activity tests/studies, and compounds isolated from some species in genus Tecleaare given. The species discussed in this section are Teclea trichocarapa, Teclea amanuensis, Teclea natalensis, Teclea verdooniana, Teclea simplicifolia, Teclea gerrardii and Teclea nobilis.

2.2.1.Teclea trichocarapa

Teclea trichocarpa is a small evergreen tree which in Kenya grows mainly in forest areas of the Coast and Central Provinces.It is used by traditional healers belonging to the Akamba tribe of East Africa for malaria treatment, as anthelmintic and inhale the vapour as a cure for fever .Itis widely used in Kenya for the treatment of malaria, fever and helminthes. The last three are acridone alkaloids while the first one is a quinoline alkaloid all of which exhibit bioactivity against bacteria and the malaria causing plasmodia .The leaves of T. trichocarpa have been found to contain antiplasmodial acridone alkaloids. Four alkaloids, namely, skimmianine (7) melicopicine (8), arborinine (9), normelicopicine (10) tecleanthine (11), and dictamnine (12), and threeacridones and 6-methoxytecleanthine (13) (Figure 3) have been reported from the leaves of T. trichocarpa. Ithas yielded a number of bioactive phytochemicals which include skimmianine (7), melicopicine (8), arborinine (9),tecleanthine (11) and many others [20].

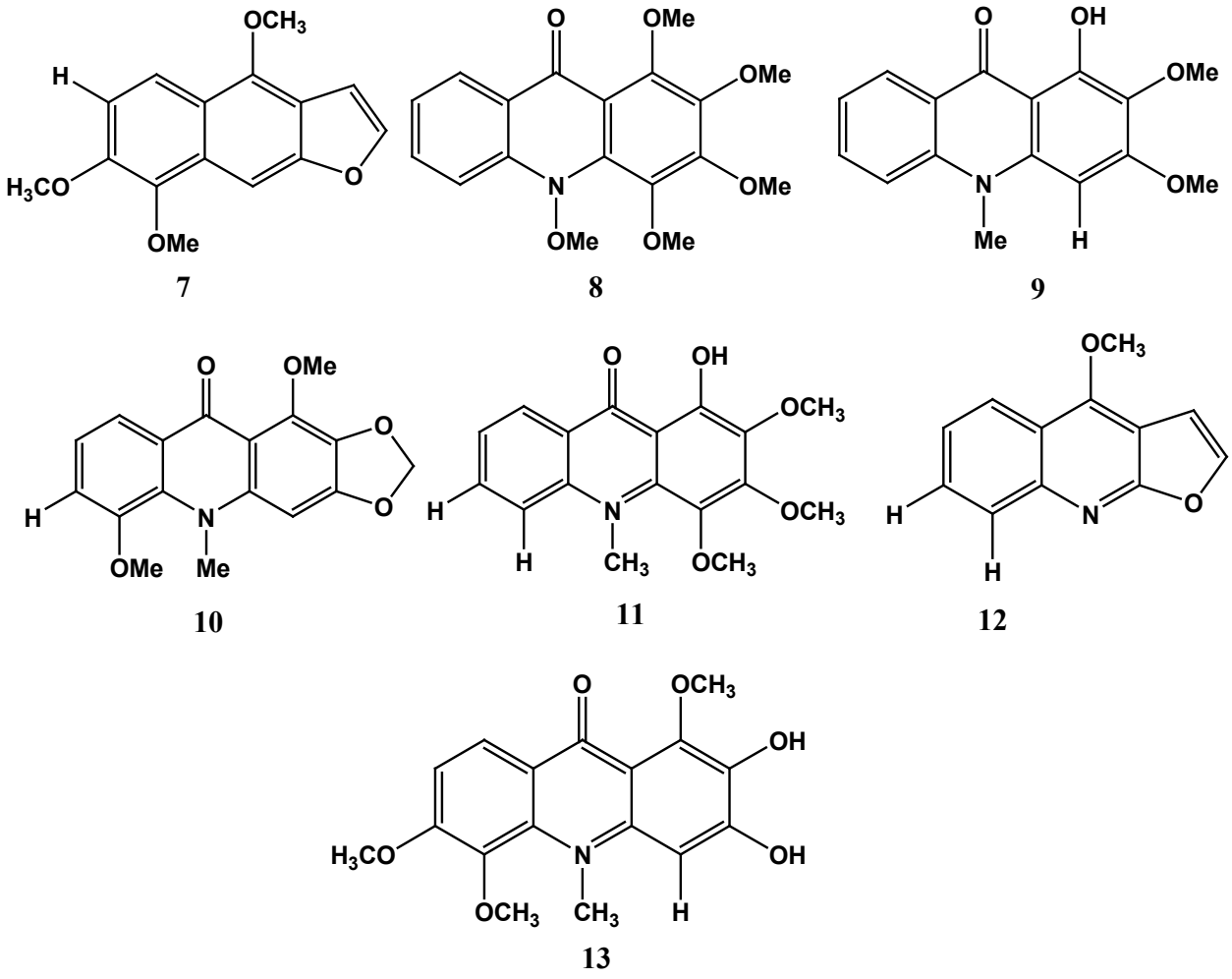

Figure 3: The chemical structures of compounds (7-13) isolated from the leaves of Teclea trichocarpa

2.2.2.Teclea amanuensis

T. amaniensis is a small tree that isendemic to the, Tanga region in Tanzania. Little has been done to investigate its phytochemical constituents.The report by Magadula and coworker's revealed the presence of furanoquinoline and acridone alkaloids which is a typical chemo taxonomical characteristic of the genus Teclea,Although there is only one report on its chemical constituents, nothing is known about pharmacological properties of its extracts and compounds. In the plantT. Amaniensisthere is a an interest to search for potential anti-mycobacterium natural products, also the isolation and in vitro antimycobacterial activity of veprisine(14), quinoline (15)(figure 4)alkaloid isolated from the root wood of T. amanuensis [21].

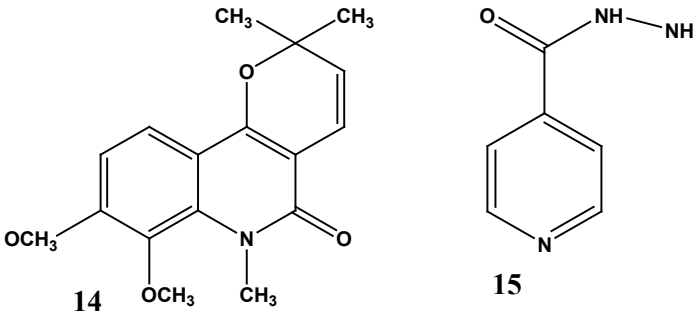

Figure 4: The chemical Structures of compounds veprisine(14)and quinoline(15) isolated from the root wood of *Teclea amanuensis*

2.2.3.Teclea natalensis

Teclea natalensis(T. natalensis) is a shrub or small (2-8 m) tree widespread in southern and eastern Africa,where it occurs on rock outcrops and forest margins .Previous investigations of the root bark of T. natalensisrevealed the presence ofacridone alkaloid such astecleanthine(11),arborinine(9), evoxanthine(25), melicopcine(7) and tecleanine(33), were reported the isolation ofalkaloids such asflindersiamine(16) and dictamnine(17)Figure 5 [22].

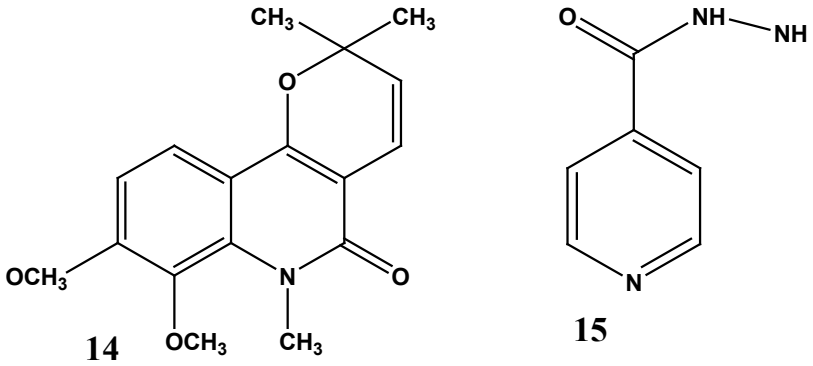

Figure 4: The chemical Structures of compounds veprisine(14)and quinoline(15) isolated from the root wood of *Teclea amanuensis*

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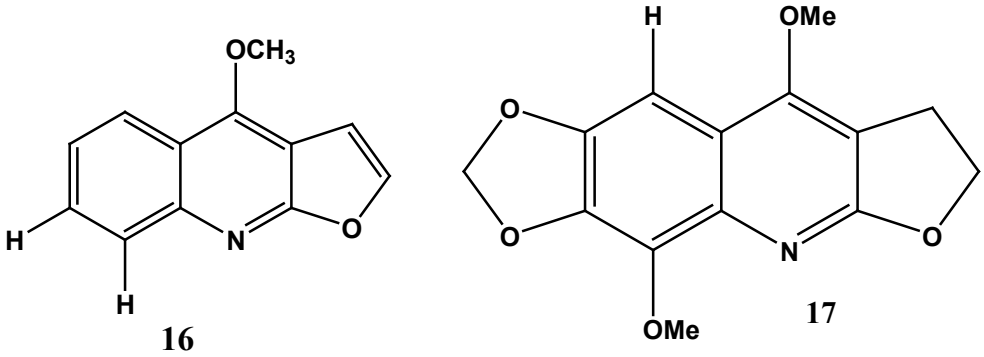


Figure 5: The chemical structure of compounds (16 and17) isolated from Teclea natalensis

2.2.4.Teclea verdooniana

Teclea verdooniana a plant that is predominant in dry rocky areas of the tropics, especially in North and West Africa areas.the plant contain a number of terpenoids and quinoline alkaloids just like other members of thes genus. Some of these phytochemicals include flindersiamine(18), kokusaginine(19), tecleaverdoornine(20) and the terpenoid lupeol(21) (Figure 6) [23].

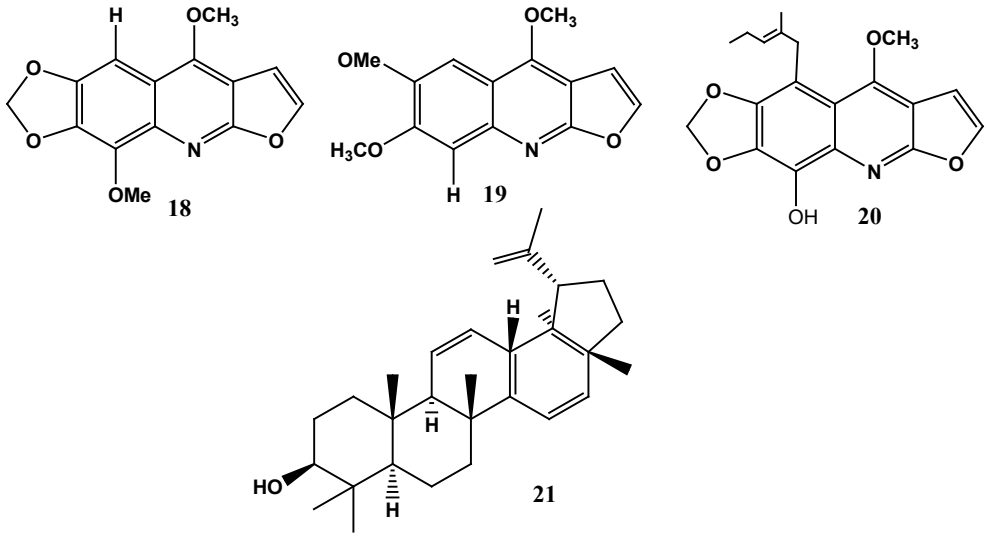


Figure 6: The chemical structures of compounds (18-21) isolated from Teclea verdooniana

2.2.5. Teclea simplicifolia

Teclea simplicifolia is a shrub or medium-sized tree of 2-9 m. It is an evergreen plant with a smooth bark, yellow-green flowers and orange or red fruits. This plant is also widely distributed in the tropical Eastern Africa regions such as Kenya, Uganda,Ethiopia and Tanzania.It has several medicinal uses such as treatment of malaria(by the Maasai community in Kenya), while the crude extracts of the stem bark and leaves of T. simplicifolia demonstrated significant analgesic activity.Furoquinoline alkaloids such as maculine (22) flindersiamine (18), kokusaginine(23), maculosidine(24), 4,5,6,7-tetramethoxyfuro[2,3-b]quinolines (25) nobiline (26) and triterpene lupeol (27) were reported from stem bark of this plant specie (Figure 7) [24].

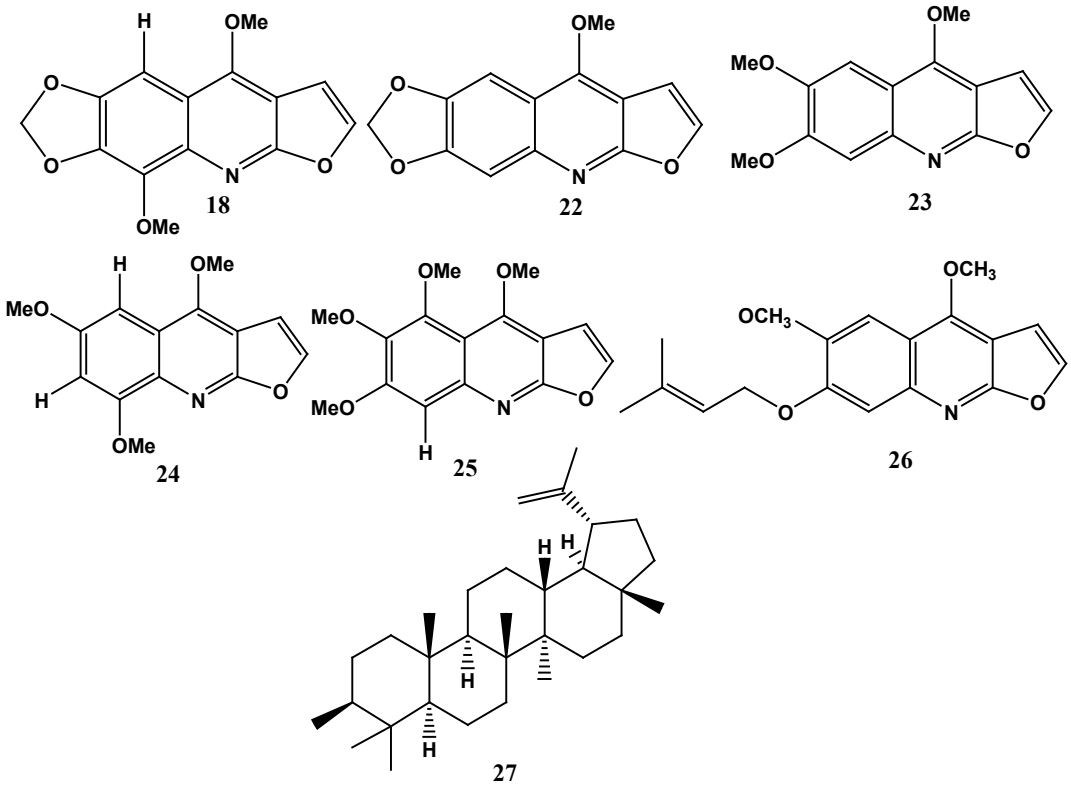


Figure 7: The chemical structures of compounds 18,22-27 isolated from stem bark ofTeclea simplicifolia

2.2.6.Teclea gerrardii

Teclea gerrardii grows in riverine thicket and dry forest along the eastern seaboard of Southern Africa (South Africa, Swaziland and Southern Mozambique) known to have many medicinal uses.For instance,bark decoctions of the plant are employed traditionally by the Zulu people (South Africa) for chest complaintsFuroqunoline alkaloids such as evoxine (28) and 7-(g,g-dimethylallyloxy)-g-fagarine(29),acridone alkaloids,Teclea gerrardin A (30), Teclea gerrardin B (31), arborinine (9), evoxanthine (25), 1,3-dimethoxy-N-methylacridone(32) and tecleanine (33)were reported from the stem bark of Teclea gerrardii(Figure 8)[25].

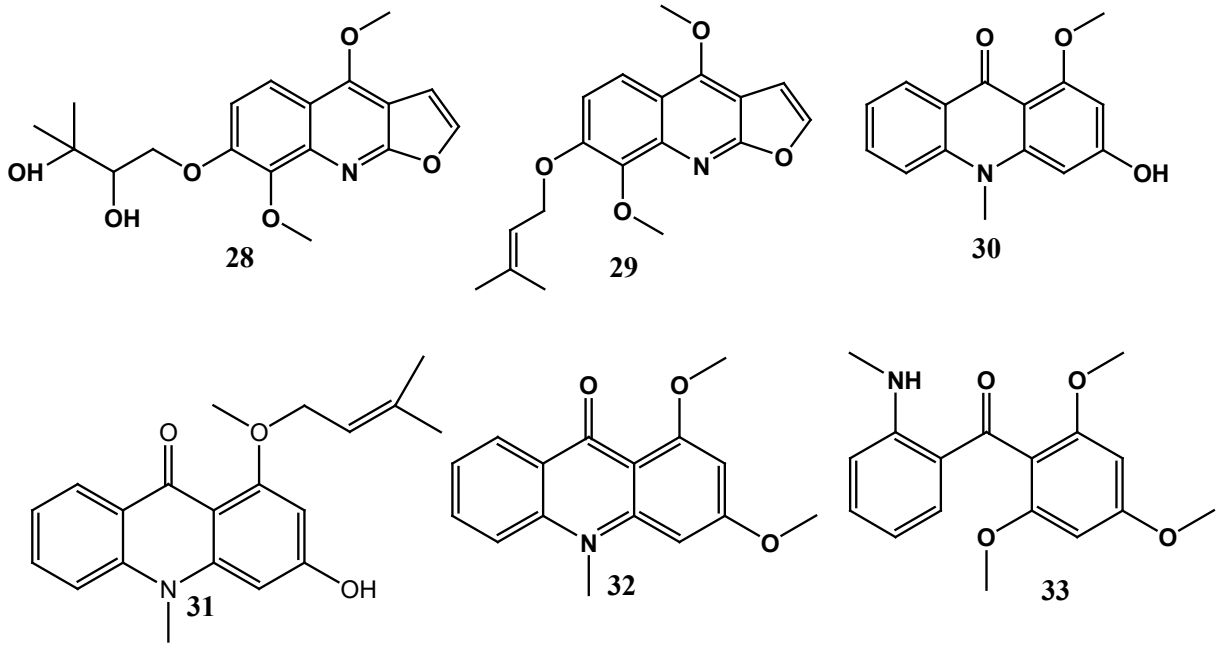


Figure 8: The chemical structures of compounds 9,25,(28-33) isolated from the stem bark ofTeclea gerrardii

2.2.7.Teclea nobilis

Teclea nobil is is a rutaceous plant that widely distributed in tropical Africa it is known in many African societies as a medicinal plant. For instance South Africa the bark is used for treatment of gonorrhoea, in Tanzania folk medicine, the leaves are used as cure for fever. Similarly, in Ethiopian traditional medicine, the bark and leaves are used as analgesics. There is no prior report on the chemical constituents of T. nobilis. The leaves and fruits of T.nobilis plant, since preliminary chemical screening showed both to be rich in alkaloids.Nobiline(34)and ribalinine(35)(figure 9) were isolated from both the leaves and fruits of T.nobilis.The leaves and fruits of T. nobilis also yieldes edulinine (36).The occurrence of the furoquinoline alkaloids skimmianine (7),montrifoline (37) flindersiamine (18) and maculine (38)(Figure 9) was reportedin the leaves of T. nobilis further rich this plant in quinoline alkaloids (Figure 9) [26].As the literature review indicated, there are no phytochemical studiesand attempts to isolate compounds from the root extracts of Teclea nobilis. The aim of this study is to conduct phytochemical screening tests and isolation of chemical constituents from root part of Teclea nobilis.

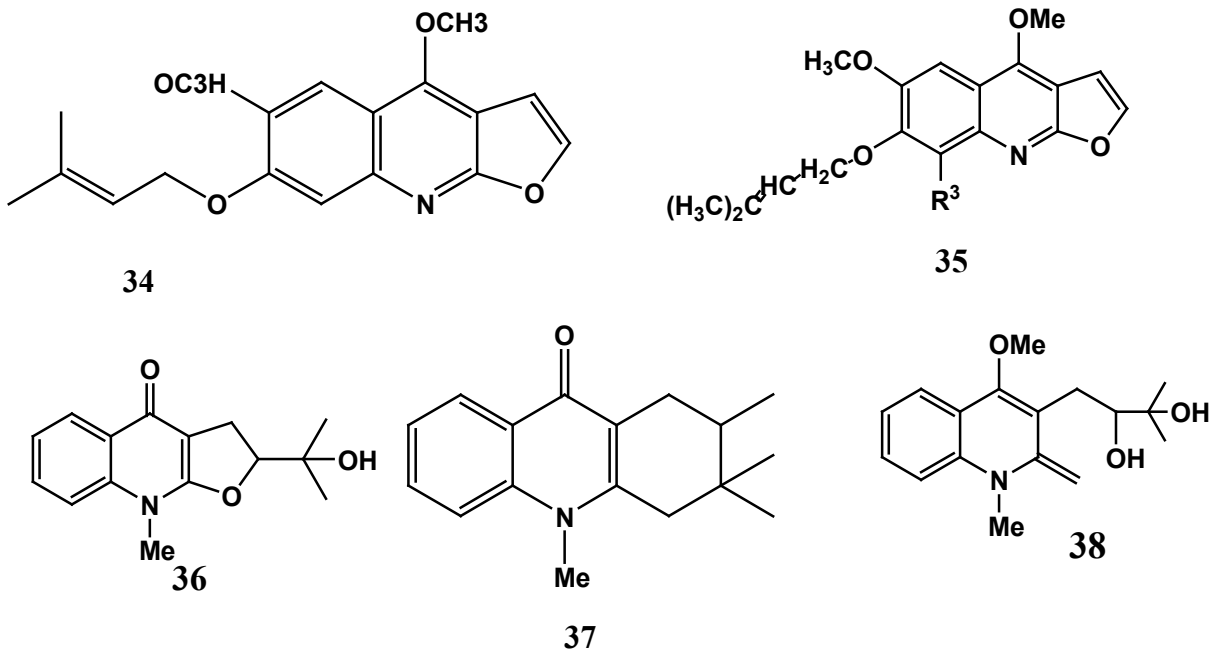


Figure 9: The chemical structures of some compounds (34-38) isolated from the leaves of Teclea Nobilis

2.3. Biological activity of the genus Teclea

The crude extracts and Some compounds isolated from Teclea nobilisalso showed anti inflammatory activity on rats without causing apparent deleterious effects Teclea trichocarpa was reported to havesignificant antiplasmodial, antifungal, antibacterial activities. Insect anti feedant activity against the African army worm has also been reported for this plant Isolation and in vitro antiplasmodial, anti-bacterial and anti-fungal activities of maculine and kolbisine of Tecleaafzelii have been documented[14].Antipyretic and analgesic activities of the ethanol extract of Teclea nobilis have been reported. Further pharmacological studies indicated that quinoline alkaloids are responsible for the observed analgesic and antipyretic activities of this plant[17].Antifungal, antibacterial and in vitro anti-plasmodial activities of Teclea trichocarpahave also been reportedOther biological studies of this plant revealed potent insect anti-feedant activity against the African armyworm. [27].

Expiremental

3.1 Chemicals, Materials and Methods

3.1.1 Chemicals

n- hexane, dichloromethane, methanol, ethyl acetate, chloroform, ethanol, and acetone,CDCl3.1% HCl,Wagner reagent,sodium hydroxide,ferric chloride,glacial acetic acid, concentrated sulpheric acid,0.1 % ferric chloride,acetic anhydride,sodium bicarbonate and nitric acid were used for phytochemical Screnning tests. pre-coated TLC (silica gel, Uv 254).all chemical used wereanalytical greads purchased from Rancheme chemicals Co. Ltd. Agents in Addis Ababa, Ethiopia.

3.1.2 Materials

Rotary evaporator (Heidolph, UK) for concentration of crude extracts, Grant (GLS 400) thermostatic bath shaker (for maceration of plant materials) was used. Oven (model N50L, GENLAB, WIDNES, ENGLAND), Analytical Balance ADAM (AFP-110L), Iodine chamber, UV chamber (Uvitec), 1H-NMR, 13C-NMR and DEPT-135 were recorded using Bruker Advance 400 MHz spectrometer for characterization of isolated pure compounds. For Infrared (IR) spectra were obtained from Perkin Elmer BX infrared spectrometer (400 - 4000cm-1). All spectroscopic analyses were carried out at Addis Ababa University, Department of Chemistry

3.2. Methods

3.2.1. Collection Of Plant Materials

The roots of Teclea nobilis were collected in September, 2016 from Yirgalem ,Sidama zone, SNNPR, Ethiopia, 335 km from Addis Ababa, (capital of Ethiopia) the area is also 47 km from Hawassa University. The plant was authenticated by botanist Mr. Reta Regasa Department of Biology at Hawassa College of Teachers Education and deposited at Hawassa University chemistry laboratory, Hawassa, Ethiopia.

3.2.2 Preparation Of Plant Specimen

The collected plant morphological parts of root was chopped into small pieces and air-dried under shade for 3 monthes and milled to suitable size with a grinding machine at Department of chemistry,College of Natural Science Hawassa University chemistry laboratories. 750 gm of plant materialwas prepared, and the prepared sample was stored under refrigerator below 4oC until is used for extraction.

3.3. Extraction

500 gm of powdered form the roots of Teclea nobilis was sequentially

extracted with n-hexane, dichloromethane, dichloromethane/ methanol (1:1) and methanol and using maceration techniques for 48 hrs with continuous shaking. The extracted matter was filtered using Whatmann No.1 filter paper, and the residual solvent in each gradient extract was removed using Rotary evaporator under reduced pressure. The mass of the crude extracts of each solvent was determined using analytical balance and stored in hood for further analysis. General schematic flow chart indicates general extraction techniques (Figure 10).

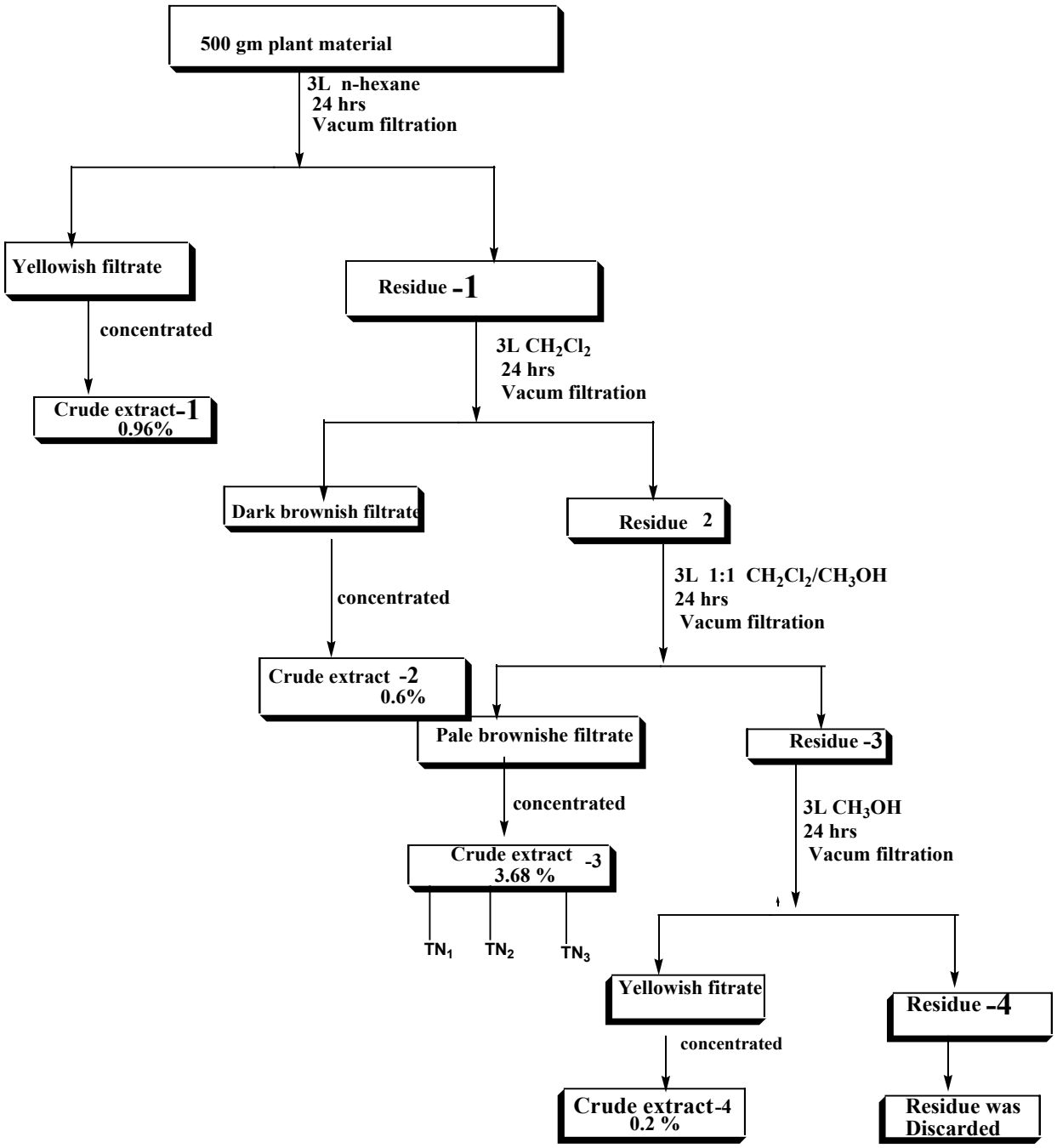


Figure 10: General Procedure Of The Extraction Of Root Of Teclea Nobilis

3.4. Phytochemical Screening

Photochemical examinations were carried out for all the extracts as per the standard methods reported in the literature [28-29].

3.4.1. Detection of Alkaloids

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

3.4.2. Detection of Flavanoids

Alkaline Reagent Test:Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

3.4.3. Detection Of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chlorides solution. Formation of green or bluish black color indicates the presence of phenols.

3.4.4. Detection of Glycosides

Keller-Killani test: Extracts were treated with, glacial acetic acid, traces of ferric chloride and conc. Sulphuric acid was added to extract formation of reddish brown color at the junction of two layers and the upper layer turn bluish green indicates the presence of glycosides.

3.4.5. Detection of Terpenoids

Salkowski test:Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated H_2SO_4, shaken and allowed to stand. Appearance of a reddish brown coloration indicates the presence terpenoides.

3.4.6. Detection of Test for Tannins

Extracts were boiled in a boiling tube by using distilled water and then filtered, add drops of 0.1% ferric chloride (FeCl3) solution mix well and allowed to stand some time. Appearance of a brownish green or a blue-black coloration for confirmation of tannins.

3.4.7. Detection of Saponins

Froth test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

3.4.8. Detection of Steroids

Liebermann Burchard reaction: 200 mg plant extracts treated in 10 ml chloroform then filtered. In 2 ml filtrate, 2 ml acetic anhydride and 2 ml H_2SO_4 was added. Appearance of a blue-green ring indicates the presence of steroids.

Result And Discussions

Based on the objective of the study, all the crude extracts of Teclea nobilis were subjected to preliminary identification of phytoconstituents. The structural elucidation of the isolated compounds was performed based on the spectroscopic data (UV-vis, FTIR and NMR) data.

4.1 Mass Of Crude Extracts

Dry powdered root of Teclea nobilis weighing 500 gm were soaked in the foursolvents, in order of increasing polarity starting from hexane, dichloromethane, dichloromethane: methanol 1:1 and finally methanol. The amount of extract obtained was recorded and tabulated as in Table 1 below.

Table 1: Wight of crude extracts

Solvent	Mass of Crude Extract	Yield (%)
n-hexane	4.8g	0.96%
Dichloromethane	3.8g	0.6%
Dichloromethane: Methanol 1:1	18.4g	3.68%
Methanol	6g	1.2%

The dichloromethane: methanol(1:1) extracts had the highest percentage yield(3.68%) than the rest while the dichloromethane had the least (0.6%).

4.2. Phyochemical Screening Tests

Phytochemical screening tests was carried out on all the crude extracts of (n-hexane, dichloromethane, dichloromethane: methanol (1:1) and 100% methanol) following standard procedures,The focus was made on testing presence or absence of secondary metabolites such as flavonoids, phenols, glycosides, terpenoids, tannins, saponnins and steroids.The result of the n-hexane, dichloromethane, dichloromethane: methanol 1:1 ratio and methanol extracts withtheir phytochemical analysis of the root of Teclea nobiliswere presented below (Table 2).

Table 2: Phytochemical screening on the root of Teclea nobilis

No	Phyto constituents	Tests	n-hexane		Dichloromethane		Dichloromethane: Methanol 1:1 ratio		Methanol	
			Result	Color observed	Result	Color observed	Result	Color observed	Result	Color observed
1	Alkaloids	Dragendroff's test	+ ve	Deep brown	+ ve	Reddish brown	+ ve	Brown reddish	+ ve	Deep Brownishe
2	Flavanoids	Alkaline test	+ ve	Intense yellowishe	+ ve	Brownishe yellow	+ ve	Dark brownishe	+ ve	Intense brownishe
3	Terpinoids	Salkowski test	+ ve	Dark brownishe	+ ve	Dark brownishe	+ ve	Deep brownishe	+ ve	Dark brown
4	Phenols	$Fecl_3$ test	+ve	Light yellow	+ve	Intense yellowishe	+ ve	Dark reddish	+ ve	Dark green
5	Glycosides	Keller-Killani test	+ve	Reddish brown	+ve	Reddish brown	+ ve	Reddish brown	+ ve	Reddish brown
6	Tannins	Fecl3 test	+ve	yellowish	+ve	Brownish green	+ ve	Brownishe green	+ ve	Brownishe green
7	Saponnins	Froth forming test	-ve	No foam	-ve	Little foam	- ve	Little foam	- ve	Little foam
8	Steriodes	Liebermann Buchard rxn	-ve	Dark brown	-ve	Dark brown	- ve	Dark brown	- ve	Dark brown

Preliminary screening tests of the crude extract of (n-hexane, dichloromethane, dichloromethane :methanol(1:1) and 100% methanol) revealed the presence of Alkaloids, Flavanoids, Glycosides, phenols, Terpenoids, Tannins and the remaining bioactive components such as saponnins and steroids are absent in the plant material.

4.3. Isolation of compounds from crude dichloromethane/ methanol (1:1) ratio extract of root of Teclea nobilis.

An 18g of dried dichloromethane/methanol (1:1) ratio crude extract was adsorbed on20 g of silica gel and loaded on column packed with 160 g silica gel using n-hexane to achieve least polarity medium of the beginning of elations.The extract was then eluted with n-hexane: ethyl acetate and reverse, finally methanol in ethyl acetate solvent system. The elution process was started by pure n-hexane (100%) and followed by Ethyl acetate/ n-hexane mixture in different ratios.This successive elution was repeated for many times and a total of 136 fractions were collected as shown in Table 3.

Table 3: Column chromatography fractionation of the crude using n-hexane/ethyl acetate solvent system

	Solvent system	Solvent Ratio	Volume
1	Ethyl acetate in n-hexane	20:80	100
2	Ethyl acetate in n-hexane	30:70	100
3	Ethyl acetate in n-hexane	40:60	100
4	Ethyl acetate in n-hexane	50:50	100
5	Ethyl acetate in n-hexane	60:40	100
6	Ethyl acetate in n-hexane	70:30	100
7	Ethyl acetate in n-hexane	80:20	100
8	Methanol in ethyl acetate	5:95	100
9	Methanol in ethyl acetate	10:90	100
10	Methanol in ethyl acetate	20:80	100
11	Methanol in ethyl acetate	30:70	100
12	Methanol in ethyl acetate	40:60	100
13	Methanol in ethyl acetate	50:50	100
14	Methanol	100%	100

Most fractions were combined and concentrated using rotary evaporator.

- Fractions 1-8 were light white colored and precipitate obtained from fraction 4 showed major spot with few impurities. Thus, this precipitate was purified by washing continuously with n-hexanebeing monitored by TLC and visualized under UV-lamp at 365nm and254nm.A 12 mg of purified yellowish solids was obtained as a single spot at Rf value 0.62 in n-hexane/ ethyl acetate(80:20)solvent system and coded as TN-1.
- Fractions 9-22 were discarded due to absence of any spot to be observed on the TLC under different solvent systems.
- Fraction 23 was crystallized as white crystal and washed with n-hexane for several times to remove impurities. A8 mg of purified white crystal was obtained as a single spot at Rf value of 0.46 in n-hexane/ethyl acetate(70:30) solvent system and coded as TN-2.

Fractions 24-45 revealed as two major spots with few impurities on TLC in (70:30) n-hexane/ethyl acetate solvent system. Based on the Rf values of the spots, fractions from 24-45 were combined and subjected to further fractionation by repeated column chromatography in the solvent mixture of ethyl acetate/n-hexane (70:30)proportion.

Based up on their TLC results of further fractionation the first six fractions (1-6) showed the same and one major spot at (Rf=0.72) in (70:30) ethyl acetate/n-hexane solvent system. yellowish powder was obtained after washing continuously with n-hexane by monitored with TLC and coded as compound TN-3.

4.4. Structural Elucidation Of Isolated Compounds

All isolated compounds are fromdichloromethane/methanol (1:1) ratio extract of root of Teclea nobilis.The1H -NMR, 13C-NMR, DEPT-135, UV andIR spectra was recorded for both compound-TN1 and compound TN2.1H –NMR ,UV and IR spectra was recorded for TN3. Therefore, this thesis constitutes full characterization of compounds-TN1 and TN2. partial forcharacterization ofcompound TN3.

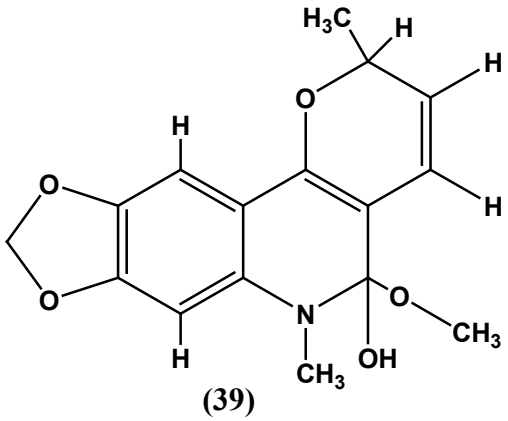
4.4.1. Characterization of Compound -TN1

Compound TN1was isolated as white crystal from dichloromethane: methanol (1:1 ratio) extract. Structural elucidation of this compound was done based on the spectroscopic data obtained from UV-vis, IR, 1H NMR, and 13C NMR and DEPT spectrum.

The UV spectrum (Appendix1) shows strong absorption pick between 230nm-260nm shows the presence of $\pi \rightarrow \pi^*$ transition of C=C double bond and $n \rightarrow \sigma^*$ transition due to the presence of -CH-OH, this confirm the presence of conjugation in the structure. The IR spectrum (appendix2) shows the presence of OH functionality with strong absorption at about 3490 cm^{-1}, aliphatic -CH strong stretching at about 2900 cm-1, aromatic =C-H stretching at about 2450 cm-1and C-O stretching at about 1100 cm^{1}.

13 C NMR spectrum (Appendix4)of compound TN1, (39) showsfifteen carbon peaksand according to its phytochemical screening test it is expected to incorporate Nitrogen to be an alkaloid class of organic compound..The 13C NMR/ DEPT-135 spectra displayed six quaternary carbons at δC 99.6,104.7,114.2,146.5,151.5 and 153.9, two olifinic carbons at δC 124.3 and 136.5, one methyl group attached to Nitrogen group at δC 26.5, one single methoxy at δC 49.1, one methyleneat 101.9 and five methine carbons at δC 70.5,99.8,112.5,128.1 and 128.5.

1H NMR spectrum showing singlet signal at δH 5.9, ortho-para coupling at δH 6.5 to 5.94, one single (CH3 proton) attached to nitrogen group at δH 2.85, one single proton methoxy at δH 3.24, one proton ortho-para coupling doublet of doublet at δH 6.47, 5.84 and 1.47, one olifinic proton triplet at 5.84, five CH protons at δH 1.47,5.84,6.4,5.94 and 6.52, three CH3 at δH 2.85,3.24 and 1.47. Based on the above spectroscopic data(UV-vis, IR, 1H NMR, 13C NMR and DEPT-135) spectrum the structure of compound TN1, (39) is most probably as shown below.



(39)

Figure 11: Proposed Structure Of Compound-Tn1

4.4.2. Characterization of Compound -TN2

Compound TN2 was isolated as white crystals. The UV spectrum (appendix-6) shows strong absorption pick below 250nm shows the presence of $\pi \rightarrow \pi^*$transition of C=C double bond, $n \rightarrow \sigma^*$transition due to CH-OH and $\sigma \rightarrow \sigma^*$ transition due to the presence of –C-C, C-H single bonds. This might confirm the absence of conjugation in the structure. The IR spectrum (appendix-7)shows the presence of OH functionality with strong absorption at about 3500 cm^{-1}, aliphatic –C-H stretching at about 2900 cm-1and -C-O stretching at about 1350 cm^{1}.

On examination of the 13C NMR (appendix -8) spectrum there were thirty carbon signals which is a characteristic feature of triterpene. The 13C NMR/DEPT(appendix -9) spectra displayed the presence of seven methyl carbons which resonated at δC 14.55, 15.37, 15.97, 16.12, 18.00, 19.3 0and 27.99 which was confirmed by the 1H NMR spectrum which contained seven singlet signals at δH 0.77, 0.80, 0.84, 0.96, 0.98, 1.04 and 1.27.

The13C/ DEPT spectrum also showed ten methylene carbon atoms

with the signals appearing at δC 18.32, 20.93, 25.14, 27.41, 29.70, 29.85, 34.28, 35.58, 38.70 and 40.00. Two of the methylene protons signals in the 1H NMR spectrum appeared at δ 4.58 for H-29a and 4.70 for H-29b and δ 3.14 for H-3. Presence of five methine carbons (resonating at δC 38.05, 47.99, 48.30, 50.44and55.29), olefiniccarbonsδC 151.00 and 109.33), quaternary carbon peaks (at δC37.17, 38.86, 40.83, 42.83and43.01) and an oxymethine signal δC 79.02 for the C-3 were also apparent from 13C/DEPT spectrum. Using the data obtained and comparing with literature compound TN2 (40)was identified as lupeol as shown below Table 4.

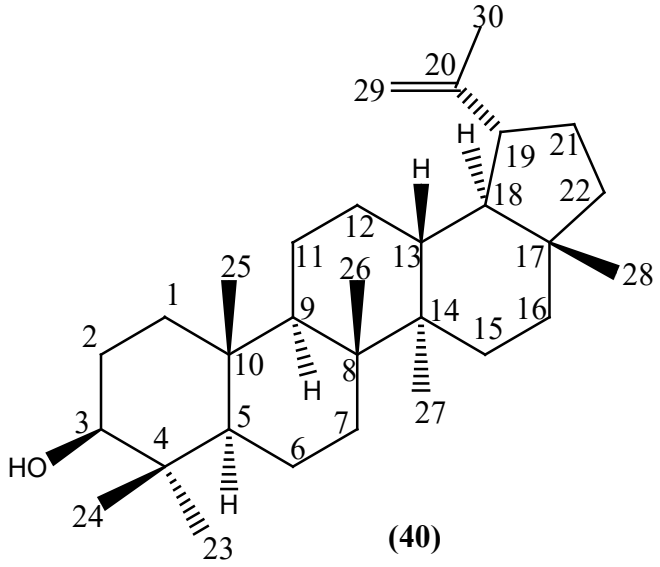


Figure 12: Proposed structure of compound-TN2

Table 4: The 13 C-NMR and 1H NMR data of compound TN2 and reported NMR data of lupeol

Position	Isolated (40)	Litrature report
	^{13} C NMR	^{13} C NMR
1	33.4	38.94
2	27.4	27.65
3	80.6	79.24
4	41.0	39.09
5	44.8	55.53
6	17.5	18.55
7	35.3	34.52
8	36.1	41.07
9	50.44	50.68
10	37.17	37.40
11	27.41	21.16
12	29.85	25.38
13	38.05	38.29
14	43.01	43.06
15	20.93	27.68
16	38.70	35.81
17	42.83	43.23
18	55.29	48.54
19	29.70	48.21

20	151.00	151.20
21	47.99	30.08
22	40.00	40.23
23	15.97	28.21
24	16.12	15.29
25	27.99	16.34
26	14.55	16.20
27	15.37	14.78
28	18.00	18.23
29	109.33	109.54
30	19.30	19.53

The proposed structure of compound TN-2 more closely related toLupeol. Depending on the structure to confirm the exact chemical shift of the compound TN-2 with Lupeol needs MS and 2D data. 4.4.3. Characterization of Compound –TN3 Compound TN3was isolated as white crystal from dichloromethane: methanol (1:1 ratio) extract. Structural elucidation of this compound was not due to lack of spectroscopic data like 13C NMR and DEPT spectrum. Only spectroscopic data of UV-vis, IR and 1HNMR spectrum are available and according to the UV spectrum (appendix-11) shows strong absorption pick between 230nm-260nm shows the presence of $\pi \rightarrow \pi^*$ transition of C=C double bond or C=O bond and $n \rightarrow \sigma^*$ transition. This might confirm the presence of conjugation or the presence of carbonyl in the structure. The IR spectrum (appendix-12) shows the presence of OH functionality with strong absorption at about 3490 cm^{-1}, aliphatic -CH strong stretching at about 2900 cm-1, aromatic =C-H stretching at about 2450 cm-1and C-O stretching at about 1100 cm^{1}.

The proton NMR spectrum (appendix-12) shows aromatic signals between δH 7.23 and 8.30. Moreover there are singlet signals δH 2.1, 1.4, probably methyl signals, δH 4.1 and 4.5 might be methoxy signals, multiples at δH 0.8-1.0, multiples between δH 4.15-4.2. Due to lack of spectral data of 13C NMR and DEPT spectrum compound TN3 is not fully characterized.

Conclusion And Recommendation

Conclusion

Teclea nobilis is a Rutaceae plant widely distributed in many African societies and used as medicinal plant for instance for treatment of gonorrhea,for curing fever also its leaves used as analgesics in Ethiopia.Phytochemical screening of the roots extract of Teclea nobilisrevealed that the presence of, alkaloids,terpenoids, flavonoids, phenolics, glycosides and tannins. Steroidsand saponnins are absent in the root of Teclea nobilis in all crude extracts (n-hexane, dichloromethane,dichloromethane: Methanol 1:1 ratio and Methanol). In this study, three compoundsTN1, TN2and TN3 were isolated from the dichloromethane: ethanol (1:1) extract of root of Tecleanobilis.Moreover, the structural elucidations of TN1 (39),TN2 (40) were done by using spectroscopic methods NMR, IR and UV-Vis.to confirm the exact chemical structure of compounds Ms and 2D data needed for further studies in the future.

Recommendation

- The present study started with 500g of the plant material but only with limited amount of solvent due to financialconstraints, andhence, futurephytochemical work is recommended starting with higher amount of the plant material in addition toavailability of solvent with further optimization of extraction yield.
- Two compounds, one quinoline alkaloid (TN1) and triterpinoid (TN2) were isolated from the root extract of Teclea nobilis in the present study. These compounds are known to posses' antimicrobial activities of different strains based on literature references. However, due to shortage of time, we couldn't conduct the bioassay of the isolated compounds as well as the crude extract. 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 analyze the crude extract of the root the plant, further study is recommended on other parts of the plant such as aerial parts, leaf and fruits. Finally, the crude TLC showed still coupled of unidentified compounds that we have missed during the chromatographic separation. However, due to shortage of time we couldn't continue of these minor constituents and we recommend a continuation of the work using high-tech separation techniques such as R-HPLC [30].

Acknowledgment

Above all, my priceless and prime veneration is attached to the Almighty God for He granted me strength and courage, good health and potency with his handful blessing to complete this thesis.I would like to express my profound gratitude to my advisor Dr.LegesseAdaneand co-advisor Desalegne Bekele for their consistent supervision and dedication in guiding my study and thesis writing.I extend my deepestthanks and appreciation in advance to Mr. DagneAddisu, Israel Alemayehuand Mr. Haftom W/ Rufaellecturers at Hawassa university(Chemistry Department), for

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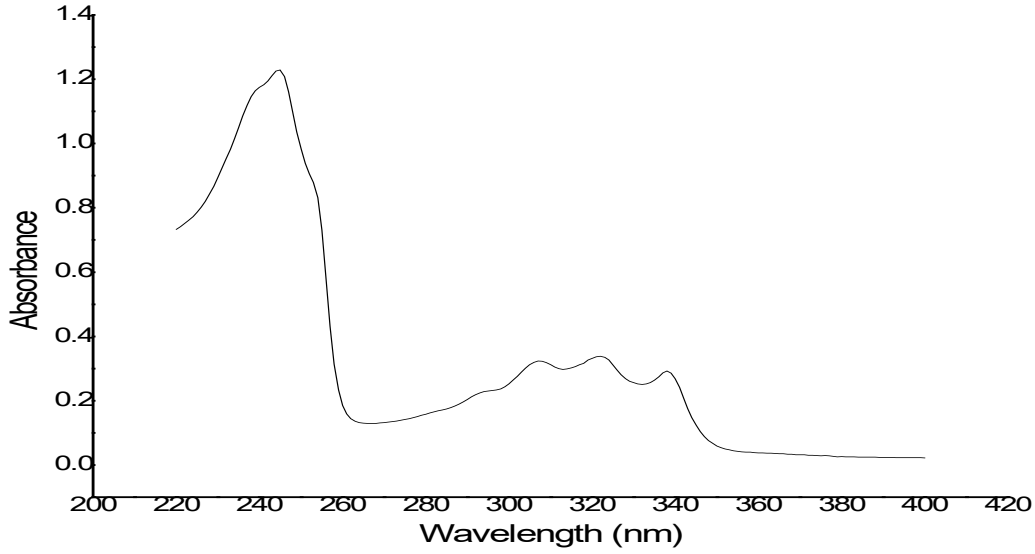
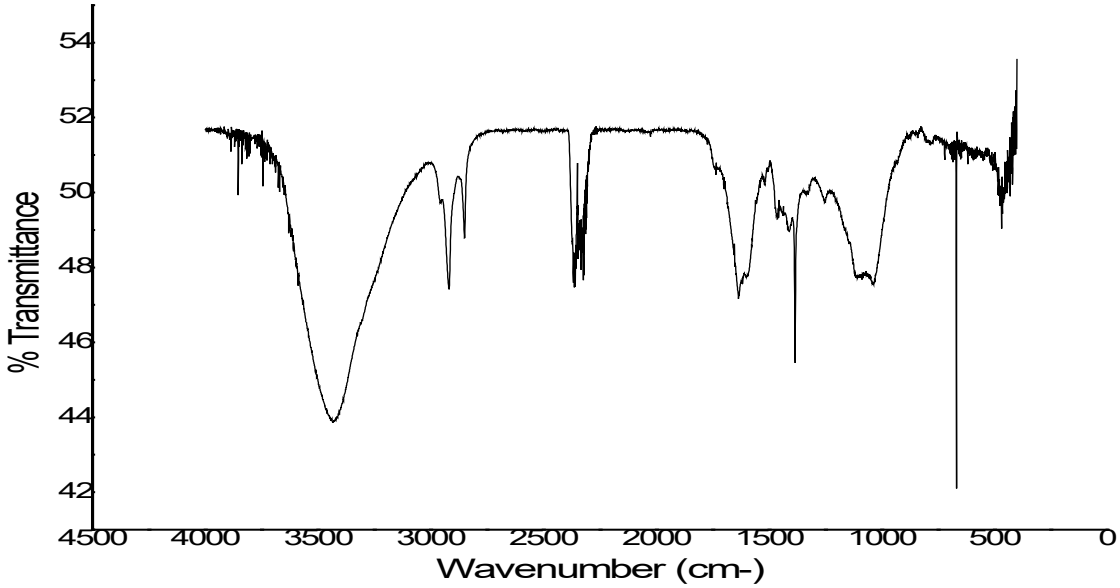
References

1. Phillipson, J. D. (2001). Phytochemistry and medicinal plants. Phytochemistry, 56(3), 237-243.
2. MacFoy, C. A. (1992). Biologically active natural products of plant origin. Conservation biology: a training manual for biological diversity and genetic resources., 189-194.
3. Okpuzor, J., Adebesin, O., Ogbunugafor, H., & Amadi, I. (2021). The potential of medicinal plants in sickle cell disease control: A review. International Journal of Biomedical and Health Sciences, 4(2).
4. Mustaffa, F., Indurkar, J., Ismail, S., Mordi, M. N., Ramanathan, S., & Mansor, S. M. (2010). Analgesic activity, toxicity study and phytochemical screening of standardized Cinnomomum iners leaves methanolic extract. Pharmacognosy research, 2(2), 76.
5. World Health Organization. (2001). WHO global strategy for containment of antimicrobial resistance (No. WHO/CDS/CSR/DRS/2001.2). World Health Organization.
6. Aliero, A. A., & Ibrahim, A. D. (2012). Antibiotic resistance and the prospects of medicinal plants in the treatment of salmonellosis. In Salmonella-A Diversified Superbug. IntechOpen.
7. Singh, R. (2015). Medicinal Plants: Rev. J. Plant Sci. Special Issue: Med Plants. 50-55.
8. Samuelsson, G. (2004). Drugs of natural origin: a textbook of pharmacognosy, 5thedition swedish Pharm. Press, Stockholm.
9. Newman, D. J., Cragg, G. M., & Snader, K. M. (2000). The influence of natural products upon drug discovery. Natural product reports, 17(3), 215-234.
10. Vivero, J. L., Kelbessa, E., & Demissew, S. (2006). Progress on the red list of plants of Ethiopia and Eritrea: Conservation and biogeography of endemic flowering taxa. Taxonomy and ecology of African plants, their conservation and sustainable use. Royal Botanic Gardens, Kew, Richmond, 761-778.
11. Edwards, S. (2001). The ecology and conservation status of medicinal plants in Ethiopia: what do we know?.
12. Abiy, Y.; Ermias, D. (1988). Alkaloids of Teclea nobilis Phytochem. 27, 651 - 653.
13. Al-Rehaily, A. J., Ahmad, M. S., Mossa, J. S., & Muhammad, I. (2002). New Axane and Oppositane Sesquiterpenes from Teclea n obilis. Journal of natural products, 65(9), 1374-1376.
14. Beentje, H. J. (1994). Kenya Trees, Shrubs and Lianas National Museums of Kenya. 722pp., figs, maps.
15. Lwande, W., Gebreyesus, T., Chapya, A., Macfoy, C., Hassanali, A., & Okech, M. (1983). 9-Acridone insect antifeedant alkaloids from Teclea trichocarpa bark. International Journal of Tropical Insect Science, 4(4), 393-395.
16. Mascolo, N., Pinto, A., Capasso, F., Yenesew, A., & Dagne, E. (1988). Antipyretic and analgesic studies of the ethanalic extract of Teclea nobilis delile. Phytotherapy Research, 2(3), 154-156.
17. Watt, J. M., & Breyer-Brandwijk, M. G. (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal., (Edn 2).
18. Yenesew, A., & Dagne, E. (1988). Alkaloids of Teclea nobilis. Phytochemistry, 27(2), 651-653.
19. Mwangi, E. S. K., Keriko, J. M., Machocho, A. K., Wanyonyi, A. W., Malebo, H. M., Chhabra, S. C., & Tarus, P. K. (2010). Antiprotozoal activity and cytotoxicity of metabolites from leaves of Teclea trichocarpa. J Med Plants Res, 4(9), 726-31.
20. Erasto, P., Omolo, J., Hamilton, C., & d Koning, C. (2013). Antimycobacterial quinoline alkaloid from the root wood of teclea amaniensis engl. Scientific Journal of Microbiology, 2(12), 214-219.
21. Tarus, P. K., Coombes, P. H., Crouch, N. R., Mulholland, D. A., & Moodley, B. (2005). Furoquinoline alkaloids from the southern African Rutaceae Teclea natalensis. Phytochemistry, 66(6), 703-706.
22. Onyancha, E. M., Tarus, P. K., Machocho, A. K., & Chhabra, S. C. (2014). Phytochemical and antimicrobial studies of Teclea nobilis Del. used in traditional medicine in Kenya. The Journal of Kenya Chemical Society Volume 8: Issue, 8(1), 89.
23. Njeru, D. N. (2015). Phytochemical investigation of the stem bark and the leaves of Teclea simplicifolia for analgesic activity (Doctoral dissertation, University of Nairobi).
24. Coombes, P. H., Mwangi, E. M., Peters, B. K., Crouch, N. R., & Mulholland, D. A. (2009). The cyclobuta [b] quinoline alkaloid cyclomegistine from Teclea gerrardii I. Verd. (Toddalioideae: Rutaceae). Biochemical Systematics and Ecology, 37(4), 494-496.
25. Al- Rehaily, A.J. (2001) Chemical and biological evaluation of the essential oils of Teclea nobilis leaf. Pak. J. bio, 2,166-168.
26. Muriithi, M. W., Abraham, W. R., Addae-Kyereme, J., Scowen, I., Croft, S. L., Gitu, P. M., ... & Wright, C. W. (2002). Isolation and in Vitro Antiplasmodial Activities of Alkaloids from Teclea t richocarpa: In vivo Antimalarial Activity and X-ray Crystal Structure of Normelicopicine. Journal of Natural products, 65(7), 956-959.
27. Prashant, T., Bimlesh, K., Mandeep, K., Gurpreet, K., & Harleen, K. (2011). Phytochemical screening and extraction: A review. Internationale pharmaceutica sciencia, 1(1), 98-106.
28. Ganesh, S., & Vennila, J. J. (2011). Phytochemical analysis of Acanthus ilicifolius and Avicennia officinalis by GC-MS. Research Journal of Phytochemistry, 5(1), 60-65.
29. Igoli, O. J., & Gray, I. A. (2008). Friedelanone and other triterpenoids from Hymenocardia acida. International Journal of Physical Sciences, 3(6), 156-158.

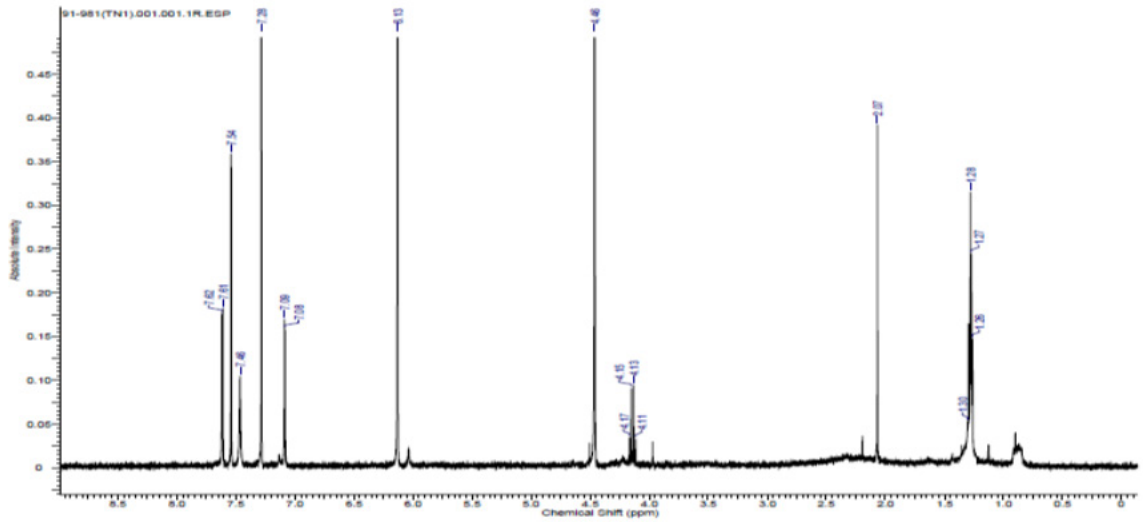
APPENDICES



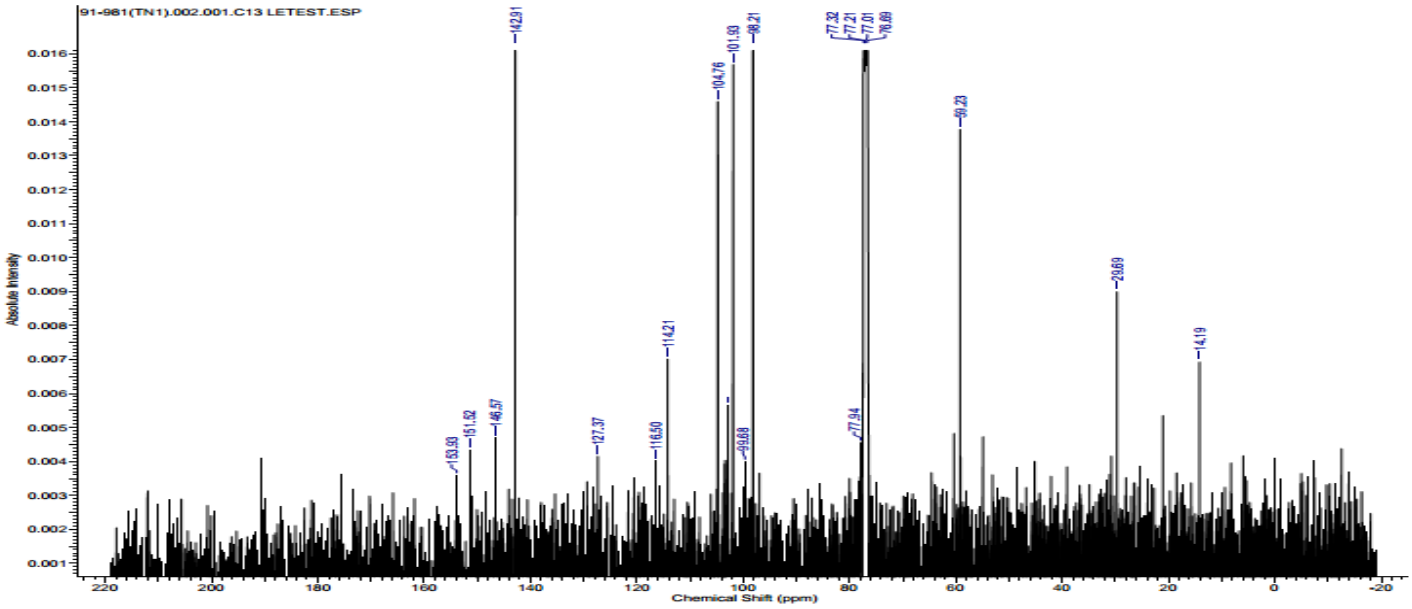
Appendix 1: The UV spectrum of compound-TN1.



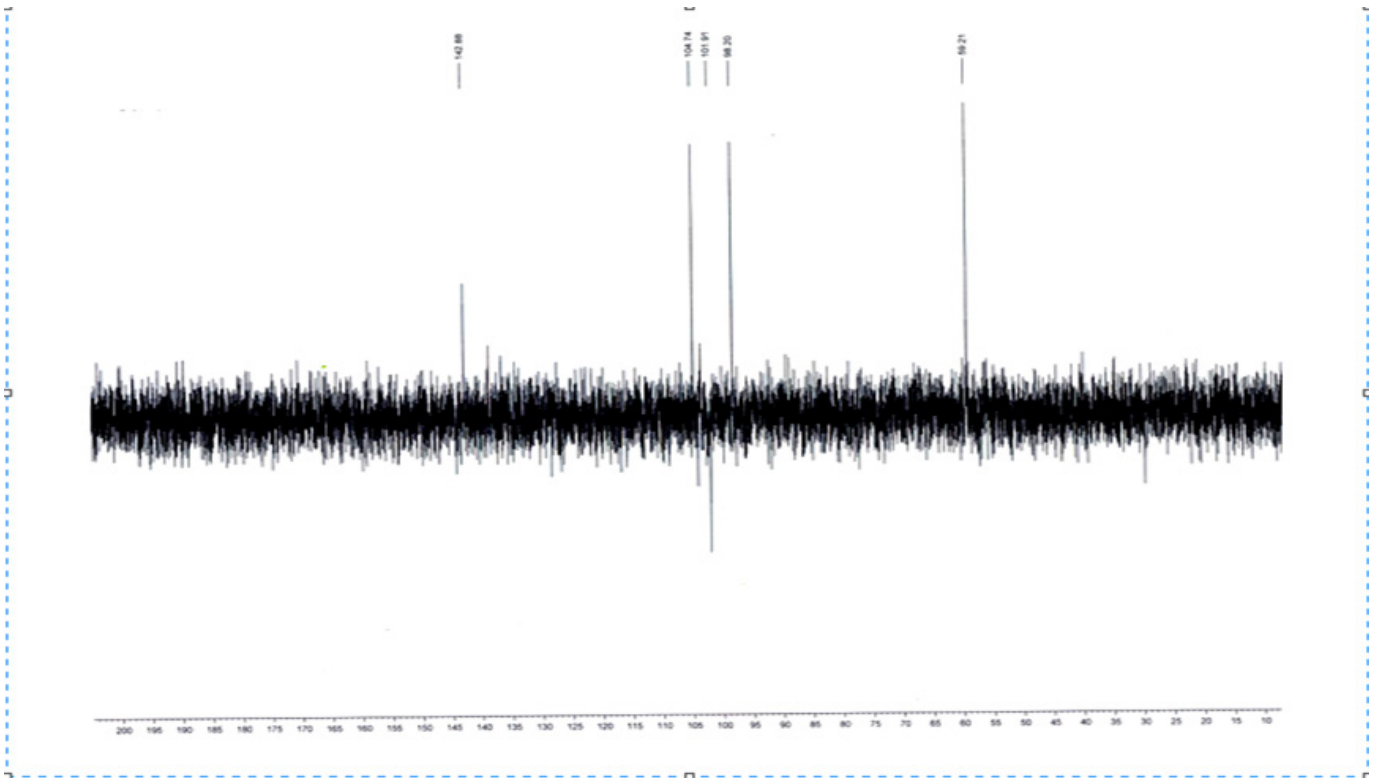
Appendix 2: The FTIR spectrum of compound-TN1



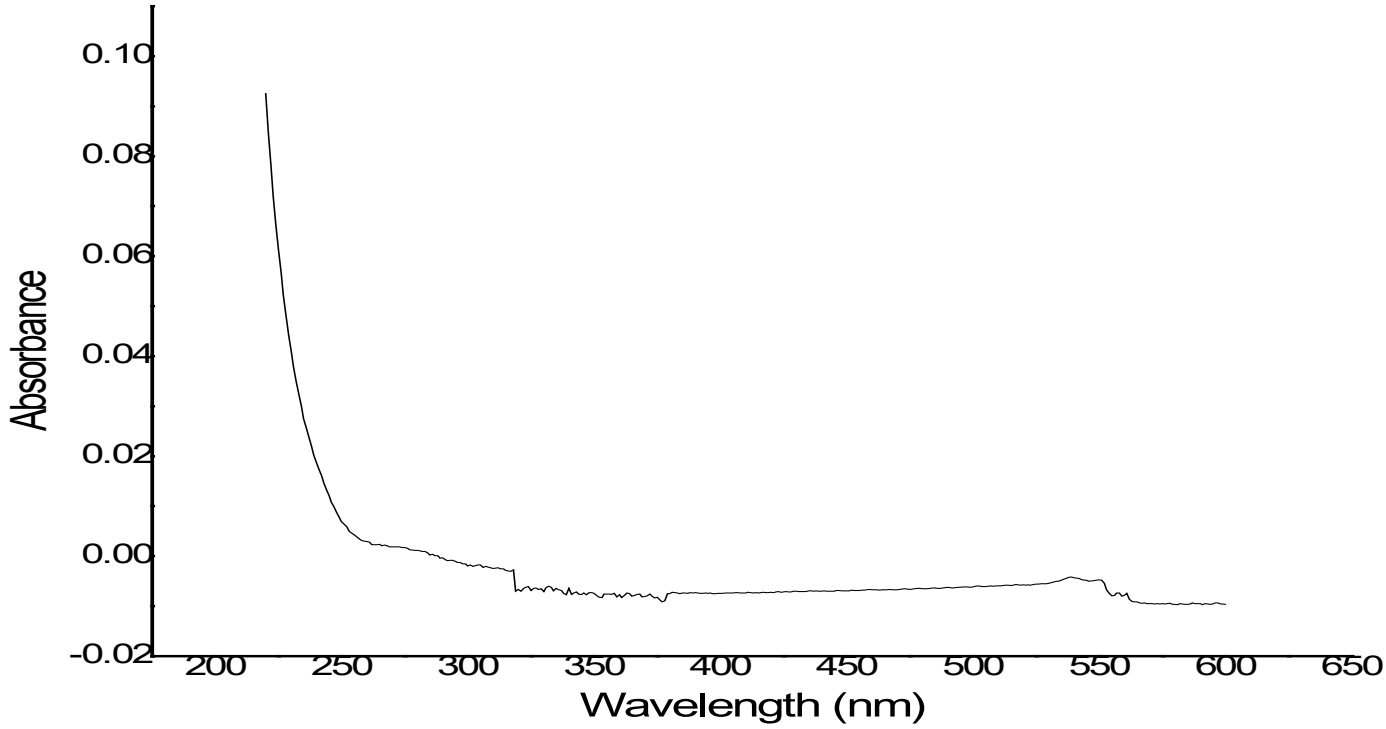
Appendix 3: The [1]HNMR spectrum of compound-TN1



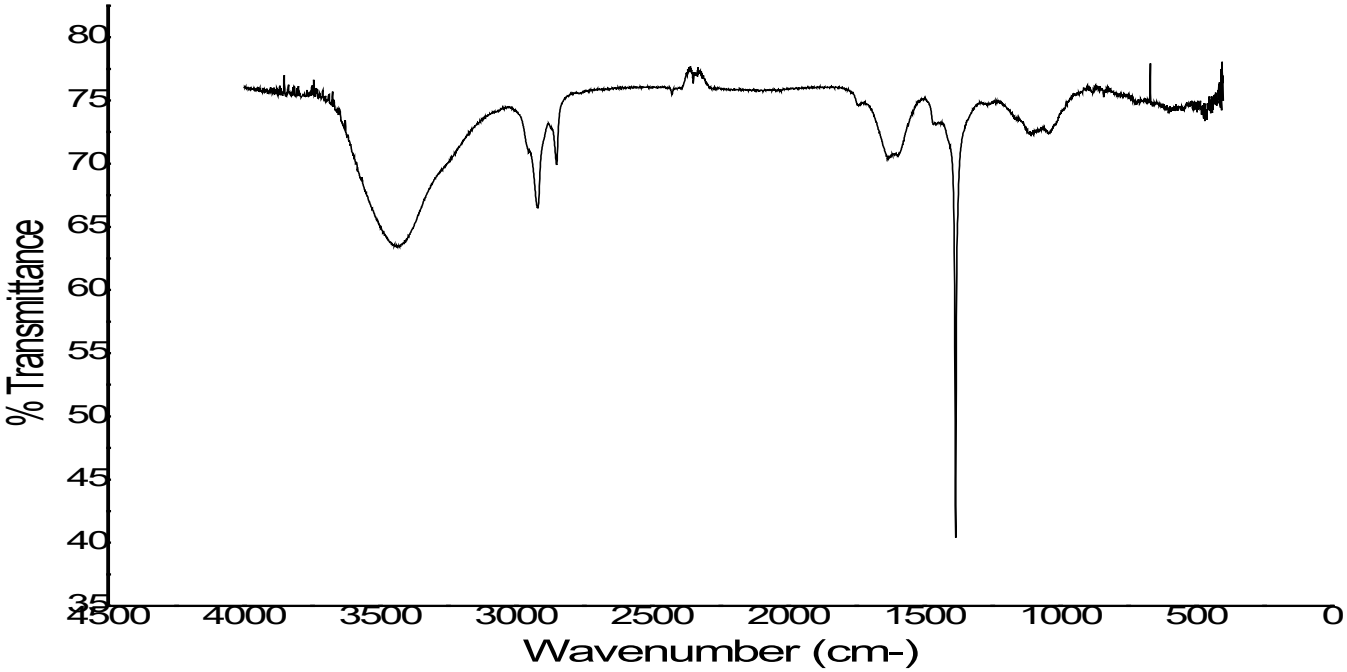
Appendix 4: The [13]CNMR spectrum of compound-TN1



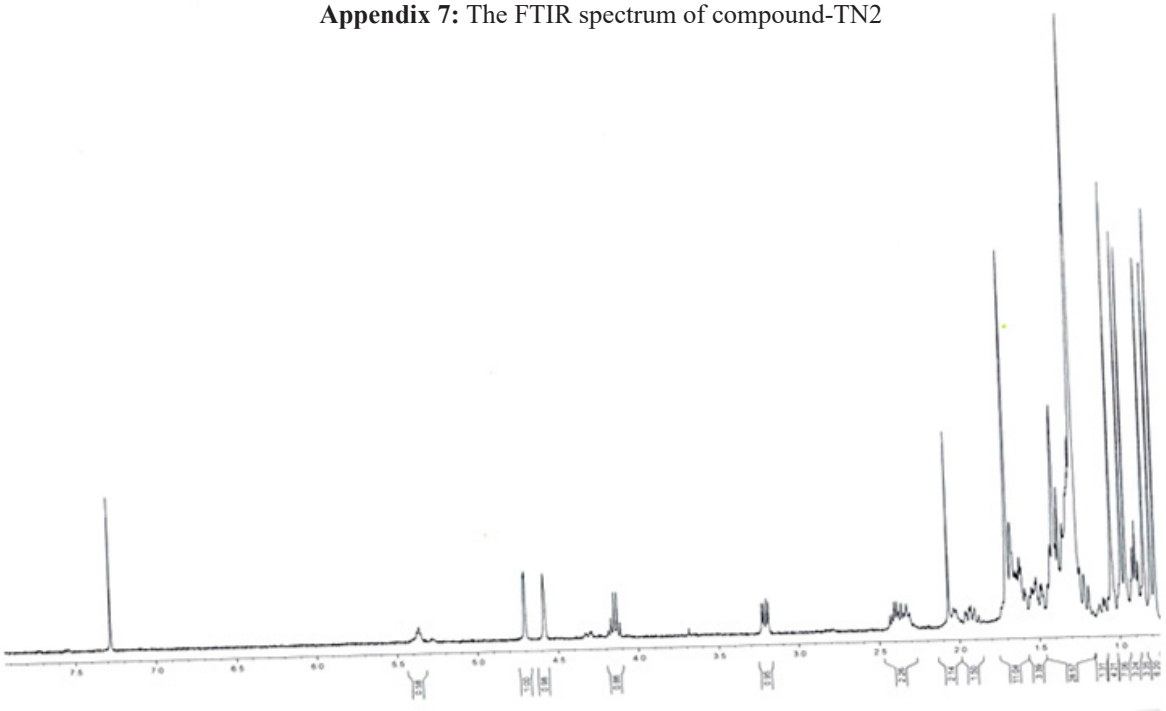
Appendix 5: DEPT-135 of compound TN1



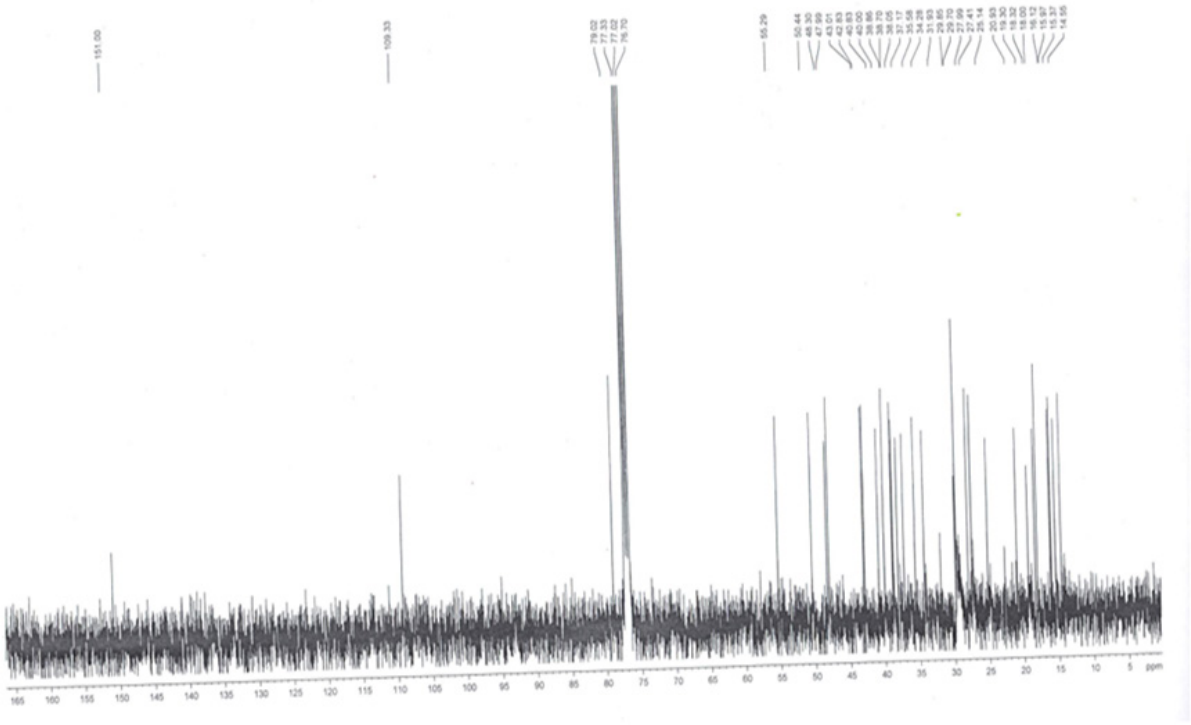
Appendix 6: The UV spectrum of compound-TN2



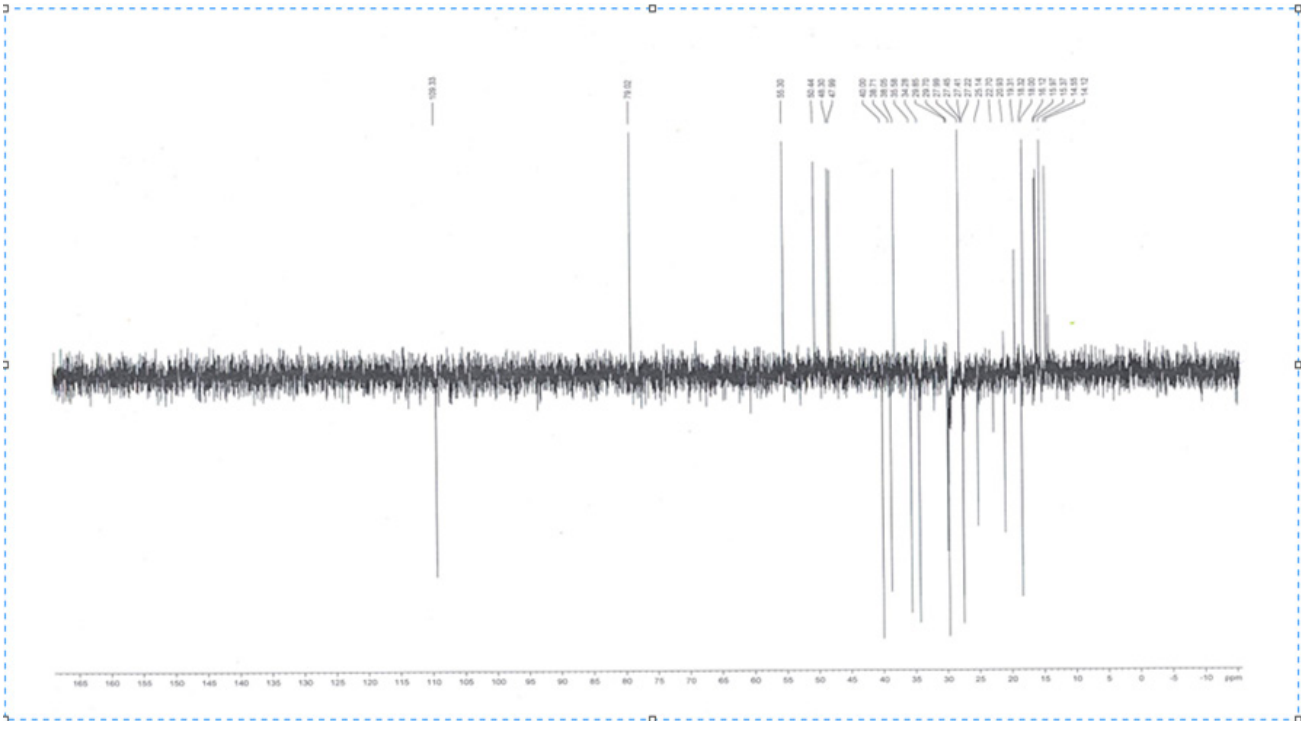
Appendix 7: The FTIR spectrum of compound-TN2



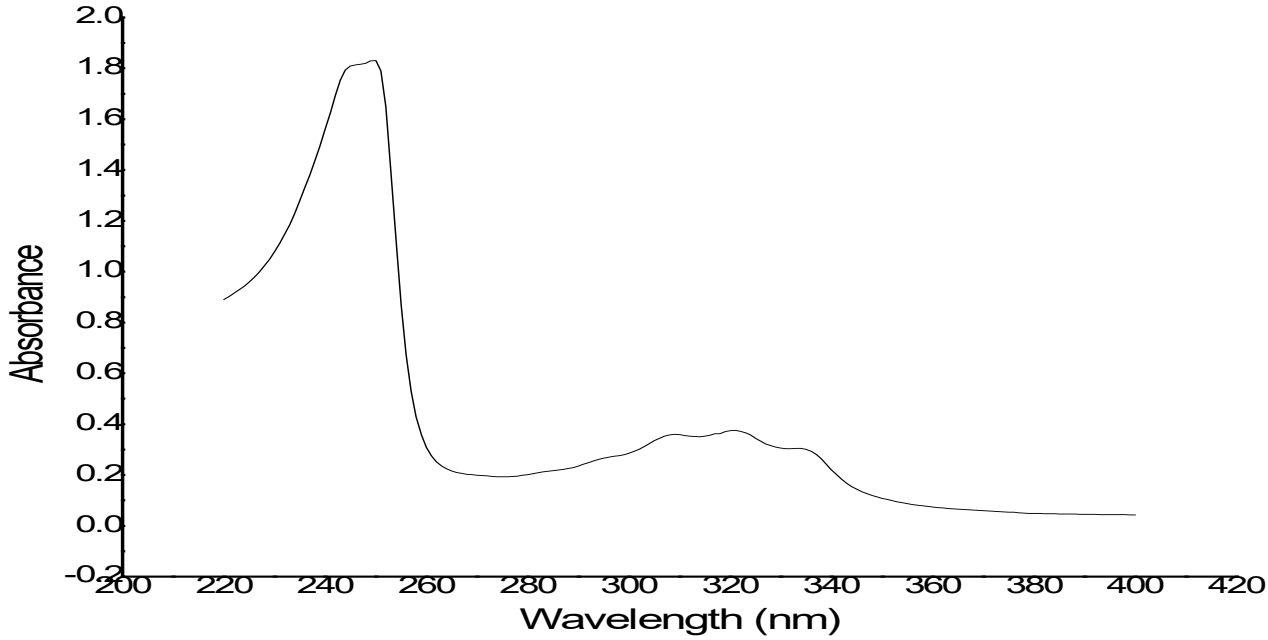
Appendix 8: The ^{1}HNMR spectrum of compound-TN2



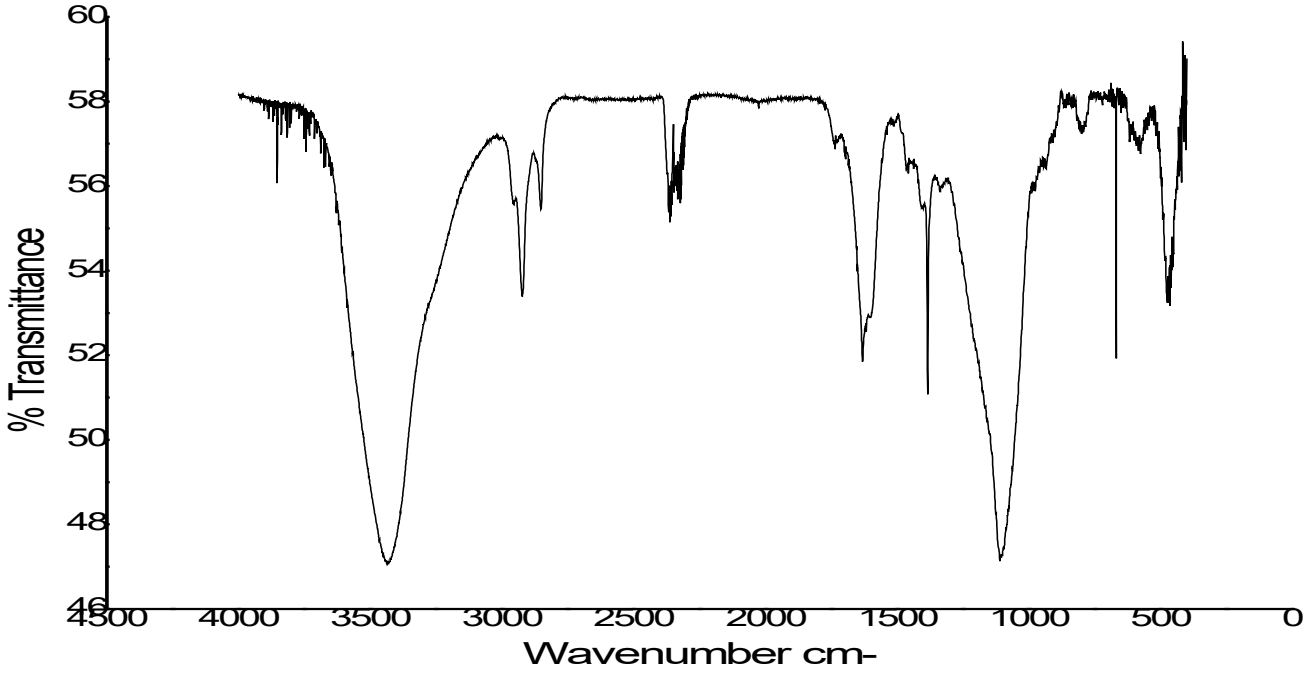
Appendix 9: The ^{13}CNMR spectrum of compound-TN2



Appendix 10: DEPT-135 of compound-TN2



Appendix 11: The UV spectrum of compound-TN3



Appendix 12: The FTIR spectrum of compound-TN3

Appendix 13: The 1HNMR spectrum of compound-TN3