ADDIS ABABA UNIVERSITY
DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION ON THE STEM BARK OF SENNA FLORIBUNDA

GRADUATE PROJECT
(CHEM. 774)

BY: TSEGAYE GIRMA

JULY, 2006

PHYTOCHEMICAL INVESTIGATION ON THE STEM BARK OF SENNA FLORIBUNDA

A GRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY ADDIS ABABA UNIVERSITY

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Dedicated

to

My mother, My brothers and My sisters,

for their unfailing affection, encouragement and positive contributions to my education.

DECLARATION

This project is my original work except where due reference has been made in the acknowledgments. This work has not been submitted for a degree in any other University.

Signature --------------------

Date ----------------

This project has been submitted for examination with my approval as university advisor.

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ABSTRACT

Phytochemical investigation on the stem bark of Senna floribunda.

By Tsegaye Girma

Advisor: Dr. Gizachew Alemayehu

The dichloromethane: methanol (1:1) extract of Senna floribunda afforded two bianthraquinones: floribundone-1(11) and 5, 7'- physcion – fallacinol (15) along with three common anthraquinones: chrysophanol (5), physcion (8) and emodin (3). Several un-characterized pigments were also isolated. The compounds are identified on the basis of their color reactions and spectroscopic data.

1. Introduction

1.1. General

Phytochemicals, as the word implies, are the individual chemicals from which plants are made [1]. Phytochemical studies of plants are of great importance in developing drugs. They are useful in the study of chemotaxonomy and plant biodiversity as well as in documenting knowledge.

Natural product chemistry is one of the most remarkable of all the sciences; it is usually regarded by the layman as one of the most abstruse and remote from everyday life and thought [2]. The term natural product is commonly reserved for those organic compounds of natural origin that are unique to one organism, or common to a small number of closely related organisms [3]. This term is typically used to refer to an organic compound of limited distribution in nature (often called secondary metabolites) [4]. Secondary metabolites are found in only specific organisms, or groups of organisms, and are an expression of the individuality of species [5]. The primary metabolism of all organisms, even those which are genetically very distant, is similar [6]. In fact, the distinction between primary and secondary metabolism is merely artificial; the concept is a useful simplification of the complex phenomena, which occur in vivo. Natural products can also be classified based on their chemical structure, physiological activity, taxonomy, and biogenesis [7].

There are three principal starting materials. ('or building blocks') for secondary metabolism [3]:

(a) shikimic acid, the precursor of many aromatic compounds including aromatic amino acids, cinnamic acids, and certain polyphenols;

(b) amino acids, leading to alkaloids, and peptide antibiotics including the penicillins and cephalosporins;

(c) acetate, precursor of polyacetylenes, prostaglandins, macrocyclic antibiotics, polyphenols, and the isoprenoids terpenes, steroids, and carotenoids, via two entirely separate biosynthetic pathways.

Man has used natural products since the dawn of time. The preparation of foodstuffs, coloring matters, fibers, toxins, medicines and stimulants are examples of activities as old as mankind [8]. Medicinal plants are known to provide a rich source of raw materials for traditional medicine in Africa, Asia and other parts of developing world, particularly those living in villages.

The World Health Organization (WHO 1991) stated that about 80 % of the people in developing countries still rely on traditional plant derived medicines, mainly due to their lower price [9].
Herbal remedies have been used for centuries but more recently the compounds that are active have been identified and this has enabled them to be extracted and purified. Synthetic organic chemists have then been able to produce the molecules in vitro and so produce them on larger scales [10].
The isolation of natural products that have biological activity toward organisms has several advantages, for instance, it permits the structural determination of bioactive compounds that may enable the production of synthetic material, incorporation of structural modifications, and a rationalization of mechanisms of action.

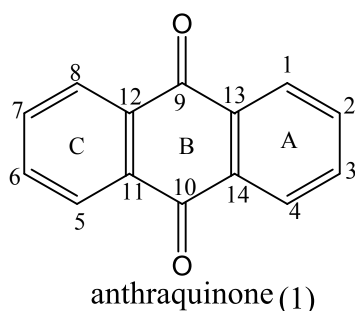
1.2. Medicinal Uses of Senna species

Natural products have been a major source of drugs for centuries. With more than 25% of the pharmaceuticals in use today derived from natural products, interest in natural product research remains strong [4].
Several species of Senna have important medicinal properties and they have been the subjects of several pharmacological investigations. They are used in the treatment of sexually transmitted diseases, skin diseases and are sources of the well-known Senna purgative. Some of them are also useful as appetizer and some others as a remedy for eye ailment. Many of them are found to possess insecticidal properties and some exhibit antibiotic properties. For instances, S. occidentalis is used as an ascaricide, to relieve abdominal pains and against malaria. Its leaf and root parts are particularly useful for Snake-bite, as pain-killer and for anthelimintic purposes.
In Gabon, the leaf decoction of S. hirsuta is drunk to care for hepatic disease. The leaves are also used to treat coughs, as sedatives and analgesics [11]. In East Africa, S. sieberiana pods are used as emmenogogue (an agent that induce menstrual flow), anthelmintic and laxative and the roots for the treatment of diarrhea, dysentery and vomiting. S. didimobotrya leaves are used in herbal medicine as a remedy for ringworm infection. S. septemtrionalis is used for warding off snakes [11].
If your bowels are hopelessly blocked up, and nothing else seems to work, then Senna will surely clean you out fast. It is the herbal equivalent of dynamite and will remove even the most impacted feces within a matter of hours, if adequate amounts are taken [12].

2. Anthraquinones

2.1. Chemical investigation

Anthraquinones are the largest group of quinones [8]. The quinones typically form strongly colored pigments covering the entire visible spectrum. Typically, however, they are found in the internal regions of the plant and thus don't impart a color to the exterior of the plant [1]. Anthraquinones are widely distributed in nature, occurring in both free and glycosidic forms, the latter being more common [13].

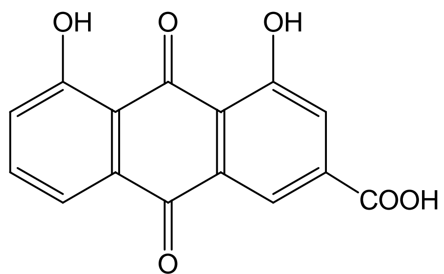


anthraquinone (1)

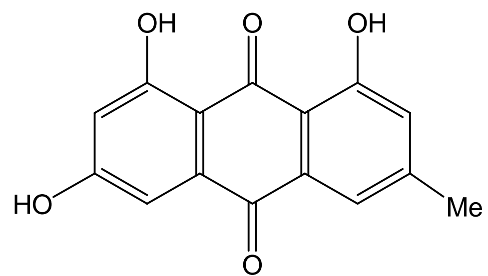
The anthraquinones occur widely in plants but in only a few animals. These brilliantly colored compounds have found wide application as dyes and as chemical indicators of acidity or alkalinity [14].

Plants containing anthraquinones have been used for millennia as dyestuffs and purgatives [12]. This important commercial dual function led to an early isolation and characterization of the active principles, which were shown to be derived from, or related to, the substance anthraquinone.

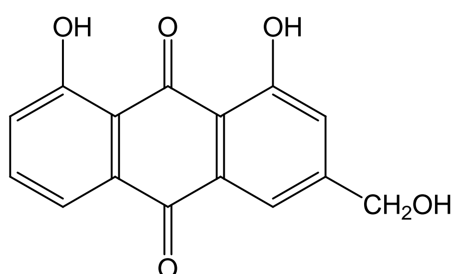
There are a great many varieties of anthraquinone derivatives, found in several plant families. They all share the same basic molecular configuration. They tend to be found in the form of their glycosides (the aglycone combined with one or more sugar molecules) which, because of the variety of possible sugars, increases the range even further. Direct anthraquinone derivatives include the following aglycones: Rhein (2) from Rheum, Rumex and Cassia spp., Emodin (3) from Rhamnus spp., Aloe-emodin (4) from Rheum and Cassia spp. Chrysophanol (5) from Rheum and Rumex spp.



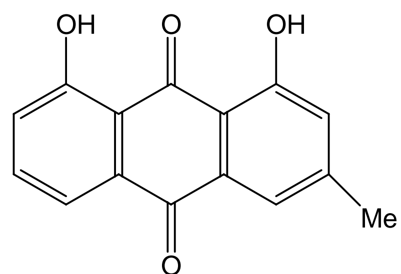
Rhein (2)



Emodin (3)



Aloe-emodin (4)



Chrysophanol (5)

2.2. Identification of Anthraquinones

2.2.1. Color reactions

Color reactions are useful particularly at the beginning of an investigation when crude extracts or even natural tissue may yield information of value. Very little material is required and the reaction may be carried, by spraying chromatograms [15].

Formation of pink, red or violate color with aqueous sodium hydroxide or ammonia indicates the presence of quinones [16]. Anthraquinones can be distinguished from benzoquinones and naphthaquinones as they usually give red solutions on reduction in alkaline solution (zinc or dithionite in aqueous sodium hydroxide) [15].

The orientation of hydroxyl groups of hydroxyanthraquinones can be predicted by observing the characteristics colors given when they are treated with methanolic magnesium acetate [17].

2.2.2. UV-Vis spectra

Anthraquinones consist almost entirely of polyhydroxy or alkoxy derivatives, and the influence of these substituents dominates the spectra [15]. Anthraquinones show intense benzenoid absorption at 240-260 nm, medium absorption at 320-330 nm, a strong quinonoid E.T. band at 270 – 290 nm accompanied by a weak quinonoid absorption at *ca* 405 nm.

The UV-Vis spectra of 1, 8-dihydroxyanthraquinones show a peak at 430-450 nm and those of 1, 4-dihydroxyanthraquinones exhibit absorption at 470-500 nm. This is also reflected in the color of anthraquinones, where 1, 8-dihydroxyanthraquinones are yellow or orange and 1, 4-dihydroxy-anthraquinones are red. Additional alpha hydroxylation results in a further red shift of the long wave length absorption [15].

2.2.3. IR- spectra

The carbonyl frequencies of quinones are useful diagnostic aids in structure determination and have been studied extensively. The carbonyl absorption of 9, 10-anthraquinones with no alpha hydroxyl group falls at around 1678 cm^{-1}. The stretching vibration of anthraquinone, having positions 9 and 10 in keto form, shows a second carbonyl band at lower frequency if it contains hydroxyl group in the alpha position due to conjugation and chelation. From the study of several

anthraquinones, Briggs and co-workers, found the correlation between the carbonyl frequency range and the number of alpha hydroxyl groups as shown in the Table 1.

Table 1 Carbonyl frequencies of hydroxyanthraquinones [18]

Number of α-OH groups	υ_{CO} (Nujol) cm^{-1}
None	1678-1653
1	1675-1647 and 1637-1621
2 (1,4- and 1,5-)	1645-1608
2 (1,8-)	1678-1661 and 1626-1616
3	1616-1592
4	1592-1572

2.2.4. NMR-Spectra

PNMR and CNMR spectra are powerful tools in the structural elucidation of anthraquinones. Analysis of chemical shifts and splitting patterns of anthraquinones give useful information for structural assignment.

In 9, 10-anthraquinone the α and β protons give multiplets centered at 8.07 and 7.67 ppm, respectively and are modified by substitution. Chelated α-hydroxyl groups at positions 1, 4, 5 and 8 are easily distinguished by their appearance at very low field (δ 12-14 ppm).

Information about orientation of substituents around the aromatic ring system can be obtained from the chemical shift positions of aromatic protons. Since many quinones are phenolic, calculation of theoretical chemical shifts by the use of shielding parameters compiled [19] for phenolic compounds can be used to predict the chemical shift of the aromatic protons and hence orientation of substituents.

Splitting patterns and coupling constants are also useful aids for the determination of orientation of aromatic substituents by NMR [20]. Coupling constants show wide variations depending on bond angles and bond hybridization. However, aromatic coupling constants are almost constant. Ortho coupling constant (Jo) is usually around 7-9 Hz, meta coupling constant (Jm) is 2-3 Hz and para coupling constant (Jp) is about 1Hz.

Any single aromatic proton may exhibit only one of seven possible first-order splitting patterns, as depicted in Table 2.

Table 2 Peak multiplicity nomenclature [20]

ortho	broad doublet
di-ortho	broad triplet
meta	narrow doublet
di-meta	narrow triplet
ortho-meta	doublet of doublets
diortho-meta	triplet of doublets
ortho-dimeta	doublet of triplets

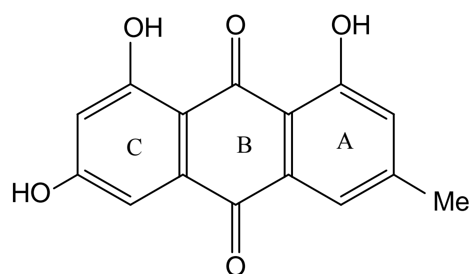
Anthraquinones of the chrysophanol (5) type show ortho-meta and di-ortho splitting patterns for the protons at position 5 or 7 and 6, respectively. They also show the meta multiplicity for the protons at position 2 and 4, where the signals are broadened by the allylic coupling with the methyl protons at position 3. Emodin (3) or physcion (8) type anthraquinones show only the meta coupling patterns. The other splitting patterns mentioned in Table 2 are not common in anthraquinones.

The CNMR spectra of several anthraquinones and naphthoquinones have been documented in the literature [21, 22].

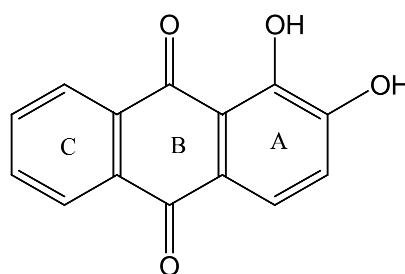
2.3. Biosynthesis of Anthraquinones

Quinones can be formed from phenolic systems generated by either the acetate or shikimate pathways [5]. Anthracenes, mostly at the quinone oxidation level are commonly found in microorganisms, plants, and lower animals [6]. Such compounds are mainly of polyketide origin and are derived from the cyclization of an octaketide chain, (e.g. Endocrocin (6), Scheme 1) [5]. The biosynthesis of anthraquinones in fungi has been studied extensively, and the involvement of the polyketide pathway has been established. In higher plants, chrysophanol (5) and emodin (3) have been shown to be polyketide derived [23].

Anthraquinones are categorized depending on how one or both A and C rings bear particular substituents such as OH, OCH_3, CH_3, etc. Those Anthraquinones with substituents on both A and C rings, such as emodin (3) are derived via the acetate-malonate pathway. Many other natural anthraquinones structures, such as alizarin (9), are formed by a more elaborate sequence involving shikimate and isoprene unit [5]**.** Such structures do not contain the characteristic *meta* oxygenation pattern, and often have oxygenation in only one aromatic ring.



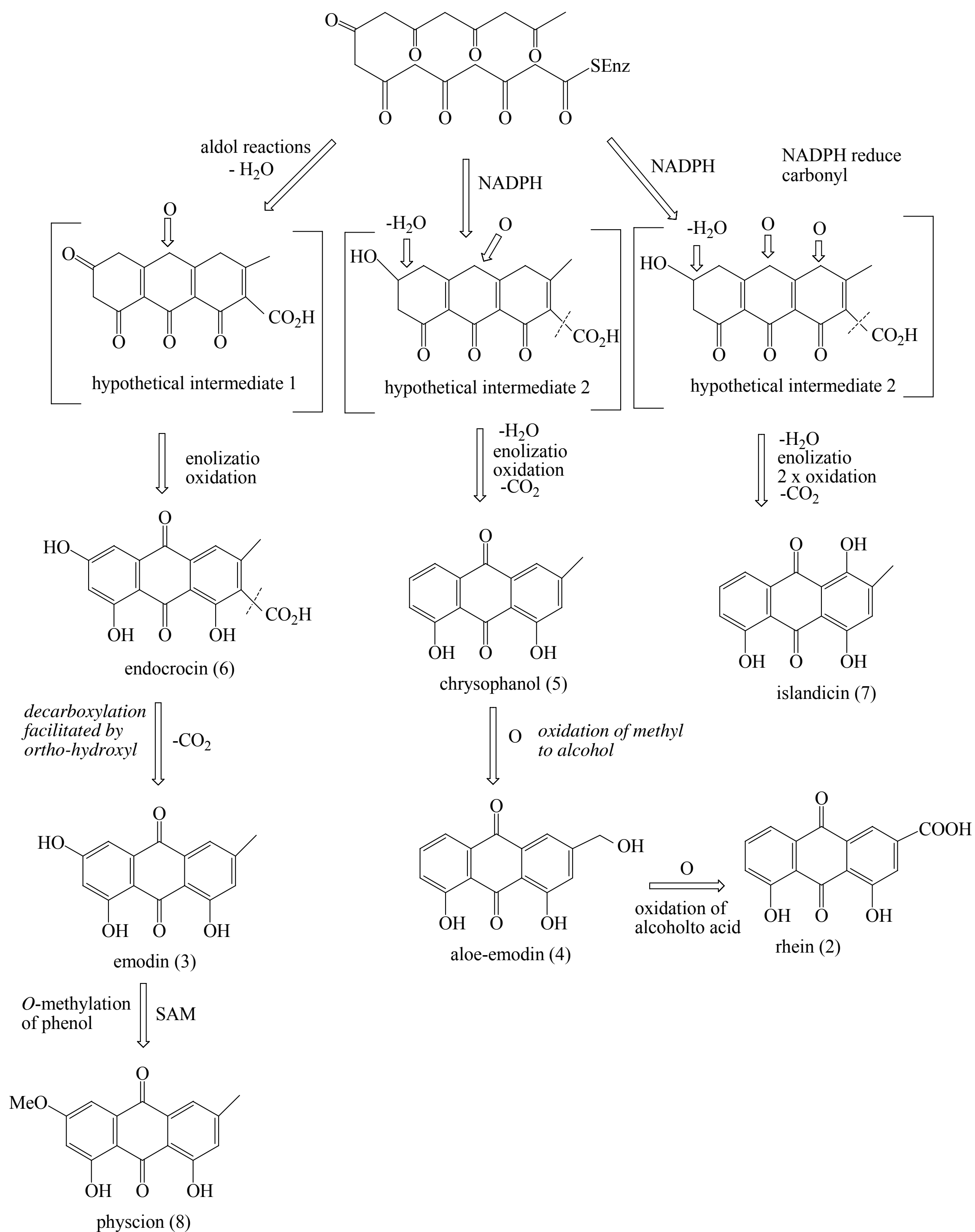
Emodin (3)



Alizarin (9)

Most anthraquinones of Leguminosae have substituents on both rings; therefore, the most plausible biogenetic route of them appears to be polyketide origin.

Scheme 1: Biosynthesis of anthraquinones via the acetate pathway.



Endocrocin (6), found in species of *penicillium* and *Aspergillus* fungi, is formed by folding a polyketide containing eight C_2 units to form the periphery of the carbon skeleton [5].

Three aldol-type condensations would give a hypothetical intermediate 1, and except for a crucial carbonyl oxygen in the centre ring, endocrocin results by enolization reaction, one of which involves the Vinylogous enolization

Emodin (3), a metabolite of some *penicillium* species, but also found in higher plants, eg. *Rhamnus* and *Rumex* species would appear to be formed from endocrocin by a simple decarboxylation reaction. *O*-methylation of emodin would then lead to physcion (8).

In chrysophanol (5), aloe-emodin (4), and rhein (2), the same oxygen function is lost by reduction as in islandicin (7), and decarboxylation also occurs. The three compounds are interrelated by a sequential oxidation of the methyl in chrysophanol to a hydroxymethyl in aloe-emodin, and a carboxyl in rhein.

3. Senna Floribunda

3.1. Botanical Background

The family leguminosae comprises some 748 genera and 19,700 species widely distributed over most of the world [11]**.** The genus Senna, one of the subgenera of Cassia, in the family Laeguminosae is known to have about 240 species distributed through out the tropics and subtropics [24]. In Ethiopia, there are eighteen species belonging to the genus Senna. These are: S. petersiana, S. septemtrionalis, S. singueana, S. baccarinii, S. occidentalis, S. sophera, S. obtusifolia, S. siamea, S. didymobotrya, S. rusplii, S. longinacemosa, S. ellisae, S. truncata, S. italica, S. holosericea, S. multiglandulosa, S. alexandriana, and S. bicapsularies. They are rich sources of anthraquinones, flavonoids, and anthraquinone and flavonoid glycosides [25].

Figure 1 Senna floribunda

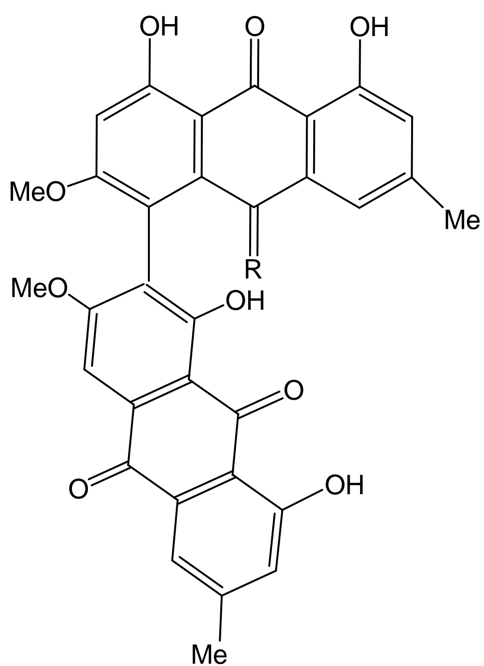
Senna floribunda (C. floribunda, C. laevigata, senna septemptrionalis) is a free flowering, bushy, evergreen, medium size shrub that may scramble up to 3 m, compound leaves, leaflet tips pointed, with large heads of yellow flowers resembling butterflies over many months in autumn through spring [26, 27]. It is native to Mexico and southward to Costa Rica widely cultivated for ornament and medicinal use and widely naturalized [28]. It is widely distributed in highlands of Ethiopia (1700-2400 m) which grows in Shewa, Arsi, Hararghe, Illubabor, Kaffa and Sidamo [24].

3.2. Secondary metabolites from Senna Floribunda

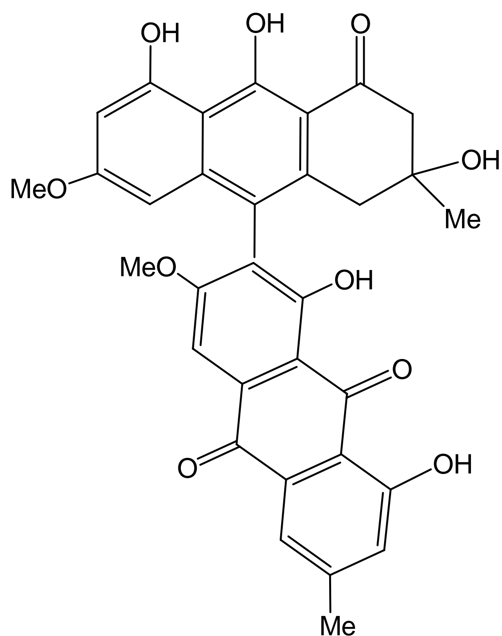
Phytochemical study on the genus senna has led to the isolation and characterization of different classes of secondary metabolites. Polysaccharides, alkaloids, flavonoids, anthraquinones, glycosides of flavonoids and anthraquinones and dimers of anthraquinones have been isolated from the different senna species. Anthraquinones are the most abundant class of metabolites reported with chrysophanol, physcion and emodin as the common anthraquinones.

Table 3 Some anthraquinones and their derivatives isolated from Senna floribunda.

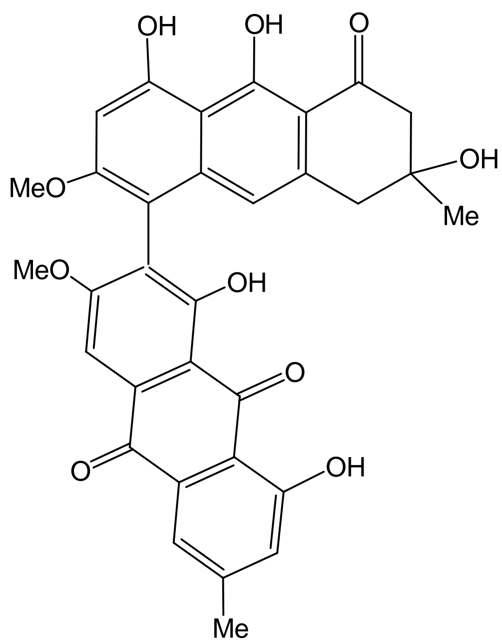
No	Name of compound	Structure	Parts from which the compounds are isolated	References
1	Chrysophanol	5	Leaves, pods	29, 30
2	Physcion	8	Leaves, roots	29, 31
3	Emodin	3	Leaves, roots	31
4	Torosachrysone	10	pods	32
5	Floribundone-1	11	Leaves	29
6	Floribundone-2	12	Leaves	29
7	Torosanin-9’,10’-quinone	14	Pods	32
8	Anhydrophlegmacin-9’,10’-quinone	13	Leaves	33
9	5,7’-Physcion-fallacinol	15	Pods	32, 36
10	Physcion-8-O-galactoside		Roots	31
11	Physcion-8-O-digalactoside		Pods	30



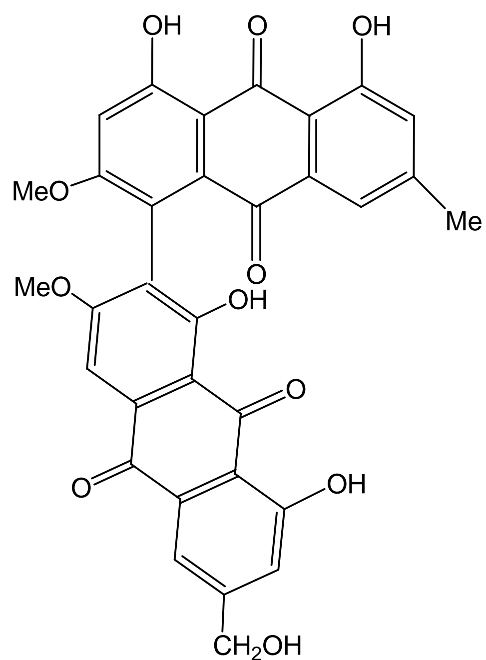
R = O, Floribudone-1 (11)
R = H_2, Floribundone - 2 (12)



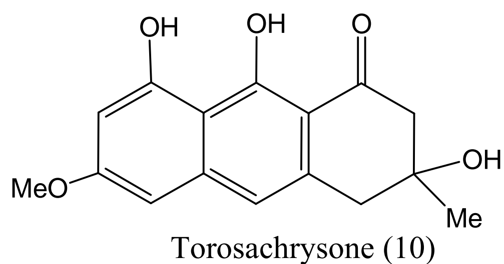
Anhydrophlegmacin -9', 10' - quinone (13)



Torosanin-9',10'-quinone (14)



5,7'-Physcion-fallacinol (15)



Torosachrysone (10)

4. Objective

This project work aimed at phytochemical studies of the stem bark of Senna floribunda. That is, to isolate and characterize anthraquinones from the stem bark of senna floribunda.

5. RESULTS AND DISCUSSION

5.1. General

The bark of Senna floribunda was extracted with dichloromethane and methanol mixture of 1:1 ratio. The brown gummy organic extract was subjected to column chromatography on silica gel followed by gel filtration using sephadex LH-20 and prep. TLC. This study generated five anthraquinones, two of which are dimeric, that is, floribundone-1 (11) and 5, 7'- physcion-fallacinol (15). The common anthraquinones are chrysophanol, physcion and emodin. Further more, on the basis of color reaction on TLC; un-characterized anthraquinone and flavonoids were isolated. The scheme of extraction is shown below:

Scheme 2 Extraction of the plant material

S.floribunda bark (235 g)
- dried in air
- grounded
- soaked with 500 ml of 5% HOAc
- dried in air for a week
- extracted with 500 ml of MeOH:CH_2Cl_2 (1:1) ratio.

extract (a)

marc
- soaked with 200 ml of MeOH: CH_2Cl_2 (1:1) for 22 hrs. at r.t.

extract (b)

marc
-soaked with 300 ml of MeOH:CH_2Cl_2 (1:1) for 24 hrs.

extract (c)
- extracts a, b, & c were combined
- the solvent removed under reduced presure.

brown gummy residue (a)

marc
- soaked with the recuvered solvent for 72 hrs.

extract
- added into the residue (a)
- the solvent removed under reduced pressure.

Brown gummy residue (1) (39.6 g)

marc
- soaked with 250 ml MeOH for 20 hrs.

extract
-the solvent removed under reduced pressure.

Brown gummy residue (2) (4.4 g)

marc
-discarded

5.2. Isolation and purification of Anthraquinones from the Dichloromethane: Methanol (1:1) Extract

Small portion of the organic extract (residue **1**) was dissolved in $CHCl_3$ and when developed on TLC, sprayed with 5% methanolic KOH, have shown characteristic color change which indicate the presence of anthraquinones.

20 g of the organic extract (residue **1)** was adsorbed on 30 g of silica gel and charged on to a column packed with 150 g of silica gel (impregnated with 5% oxalic acid) using $CHCl_3$ as a solvent (see Scheme 2). The column was eluted with the following solvent systems:

Table 4 Solvent systems used for isolation.

No.	Solvent system	Fractions collected	Volume collected in (ml)
I	$CHCl_3$ (100%)	1	150
II	$CHCl_3$ (100%)	2-4	100 each
III	$CHCl_3$: EtOAc (9:1)	5-7	"
IV	$CHCl_3$: EtOAc (4:1)	8-11	"
V	$CHCl_3$: EtOAc (2:1)	12-22	"
VI	$CHCl_3$: EtOAc (1:1)	23-26	"
VII	EtOAc (100%)	27-32	"
VIII	MeOH (100%)	33	150

Fraction 2 was applied on column (packed with silica gel) using petroleum ether and eluted with petroleum: ethyl acetate (9:1, 4:1, and 100:0); then, applied on chromatotron using pet. Ether: chloroform: acetone (4ml: 1ml: 3drps.). Four fractions were collected, the 2nd fraction was applied on Prep. TLC using petroleum ether: chloroform: acetone (4ml: 1ml: 3drps.) to yield two bands. The top band gave 2.3 mg of chrysophanol.

Fraction 3 and 4 were combined and loaded on sephadex LH-20, using $CHCl_3$: MeOH (2:1) and chromatographed on column (silica gel) with the solvent pet. Ether: EtOAc (10:1) to afford the common anthraquinone physcion.

Fractions 5, 6, and 7 were combined, loaded on sephadex LH-20 column and eluted with $CHCl_3$: MeOH (2:1) as solvent. Then, chromatographed on silica gel column with pet.ether: EtOAc (4:1) and prep. TLC using pet. ether: EtOAc (4:1) to give the dimeric anthracene derivative floribundone-1.

The combined fractions 10 and 11 were first loaded on sephadex LH-20 and eluted with $CHCl_3$: MeOH (2:1) as solvent, then applied on column (silica gel) using the solvent pet. ether: EtOAc (4: 1) to give the common anthraquinone emodin.

Fractions 12 and 13 were combined on the basis of their TLC similarity, loaded on sephadex LH-20 and eluted with $CHCl_3$: MeOH (2:1). Applied on chromatotron using CH_2Cl_2: MeOH (5ml: 4 drps) and then, on prep. TLC and eluted with CH_2Cl_2: MeOH (5ml: 4 drps) to yield 1.6 mg of the bianthraquinone 5, 7'- physcion-fallacinol.

The combined fractions 17-19 were loaded on sephadex LH-20 and eluted with solvent $CHCl_3$: MeOH ratio (2:1) and applied on column (silica gel) using the solvent $CHCl_3$: MeOH (9: 1). Then, applied on sephadex LH-20 and eluted with solvent $CHCl_3$: MeOH ratio (1:1) to give unidentified flavonoids and anthraquinone (on TLC, upon spraying with 5% methanolic KOH, showed characteristic yellow and red colors for flavonoids and anthraquinones, respectively).

Scheme 3: Isolation and purification

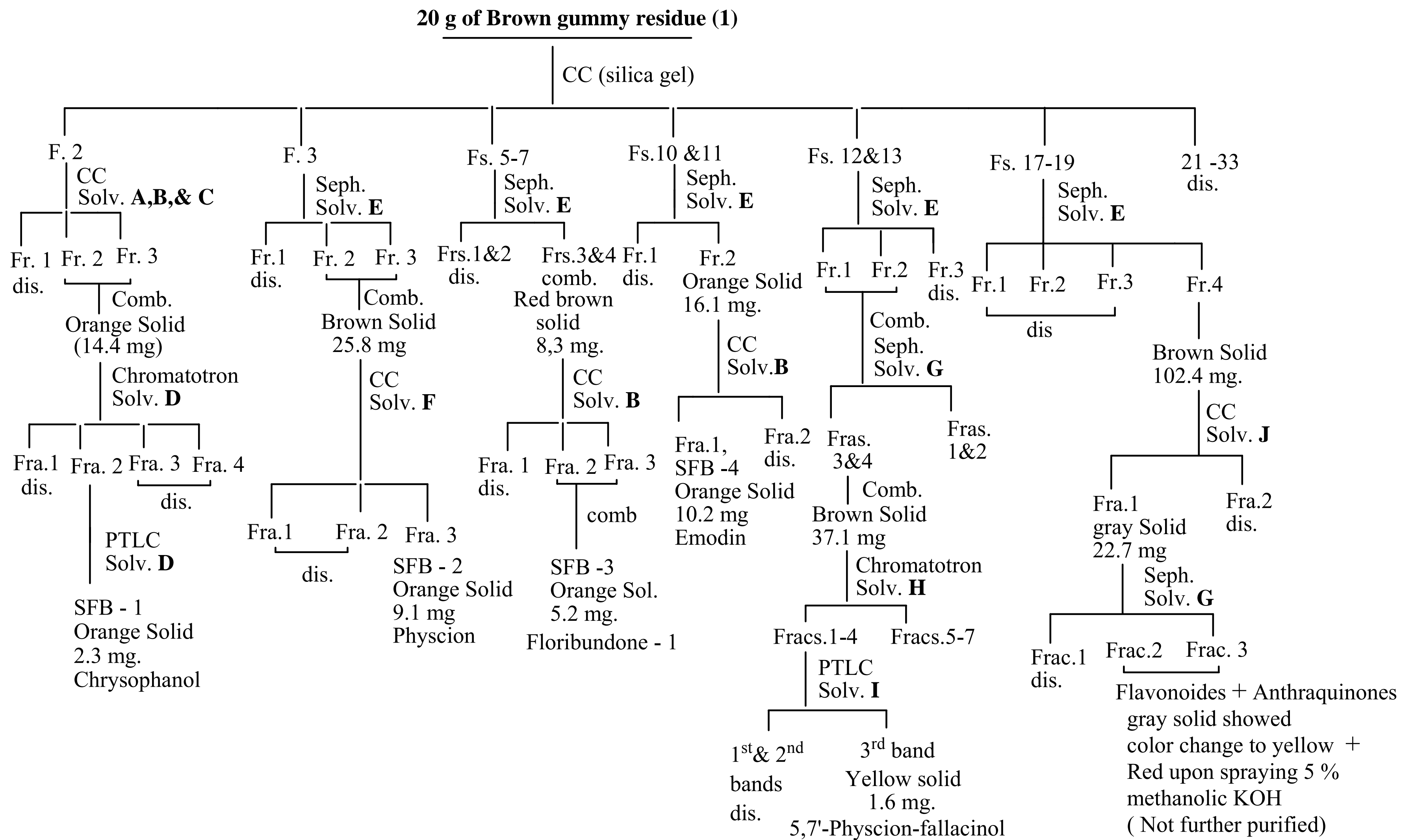
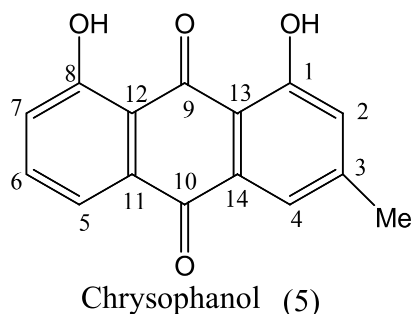


Table 5 Solvent systems used for purification.

No	Solvent system	Ratio
A	Petroleum ether: Ethyl acetate	9:1
B	Petroleum ether: Ethyl acetate	4:1
C	Ethyl acetate	100%
D	Petroleum ether: Ethyl acetate: Acetone	4ml: 1ml: 3drps.
E	Chloroform: Methanol	2:1
F	Petroleum ether: Ethyl acetate	10:1
G	Chloroform: Methanol	1:1
H	Dichloromethane: Methanol	5ml: 4drps.
I	Chloroform: Methanol	5ml: 5drps.
J	Chloroform: Methanol	9:1

5.2.1. Chrysophanol (5)

Compound **5** was obtained as an orange solid that showed a color change to violate on TLC plate upon spraying with 5 % methanolic KOH. It was identified as chrysophanol by comparing its ^{1}H NMR spectral data with those reported in the literature [11] for the same compound. Compound **5** displayed UV absorption at λ_{max} 259, 289 and 433 nm, characteristic of anthraquinones [15].
The IR showed bands at 1676 and 1628 cm^{-1} corresponding to the un-chelated and chelated carbonyl carbons, respectively. The ^{1}H NMR spectrum indicated the presence of two cheated hydroxyl groups (δ 12.01 &12.12), five aromatic groups (δ 7.11, 7.29, 7.65, 7.67, & 7.83), and one aromatic methyl group (δ 2.47). The ^{1}H NMR data of **5** displayed spin system for three adjacent aromatic protons, in which the lowest field doublet of doublet was assigned to the C-5 proton and high field aromatic broad singlet for the C-2 proton. Its melting point is 196-198 ^{0}C, which is in agreement with the reported value for chrysophanol (196 ^{0}C) [15].



Chrysophanol (5)

Table 6 Comparison of the observed ^{1}H NMR spectral data with the reported value of chrysophanol.

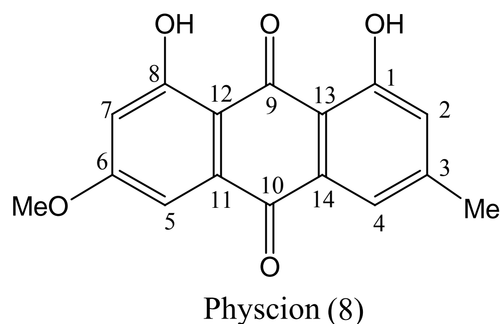
H	Observed data (400 MHz, $CDCl_3$) (δ in ppm)	Reported data (600 MHz, $CDCl_3$) [11] (δ in ppm)
1-OH	12.01 s	12.00 s
2-H	7.11 br s	7.09 br s
3-Me	2.47 s	2.48 s
4-H	7.66 br s	7.64 br s
5-H	7.83 dd, J=1.2, 8Hz	7.81 dd, J=1.1, 7.5 Hz
6-H	7.67 t, J=6, 10 Hz	7.67 t, J=8.3, 7.9 Hz
7-H	7.29 dd, J=1.2, 8 Hz	7.29 dd, J=1.2, 8.3 Hz
8-OH	12.12 s	12.11 s

5.2.2. Physcion (8)

Compound **8** was an orange pigment that turned to red on a TLC plate upon spraying with 5% KOH in methanolic solution, which is a characteristic color change for hydroxyanthraquinones [15]. Compound **8** displayed UV absorption at λ_{max} 265, 289 and 439 nm, characteristic of anthraquinones [15]. The IR showed bands at 1676 and 1630 cm^{-1} corresponding to the un-chelated and chelated carbonyl carbons, respectively. The ^1H NMR showed two chelated hydroxyl resonances at δ 12.30 and δ 12.11, two broad singlet at δ 7.09 and δ 7.63, assigned to the protons at C-2 and C- 4 respectively, two protons at δ 6.69 and δ 7.37 representing the

protons at C-7 and C- 5 respectively, a methoxy protons at δ 3.93, and a methyl group attached to aromatic ring resonates at δ 2.47.

The ^{13}C NMR and DEPT spectral data of 8 (see Table 8), showed the presence of 16 carbons, absence of methylene groups, the presence of four aromatic C-H groups resonating at (δ 124.48, 121.26, 108.18 and 106.78), one methoxy group at δ 56.05 and one methyl group at δ 22.14, and ten quaternary carbons. The ^{13}C data is consistent with that reported for physcion in the literature [34, 37].



Physcion (8)

Table 7 Comparison of the observed ^{1}H NMR spectral data with the reported value of physcion.

H	Observed data (400 MHz, $CDCl_3$) (δ in ppm)	Reported data (100 MHz, $CDCl_3$).[11] (δ in ppm)
1-OH	12.28 s	12.26 s
2-H	7.07 s	7.04
3-Me	2.44	2.45
4-H	7.61 s	7.57
5-H	7.35 brs	7.32
6-OMe	3.93	3.92
7-H	6.67 brs	6.60
8-OH	12.08 s	12.05 s

.

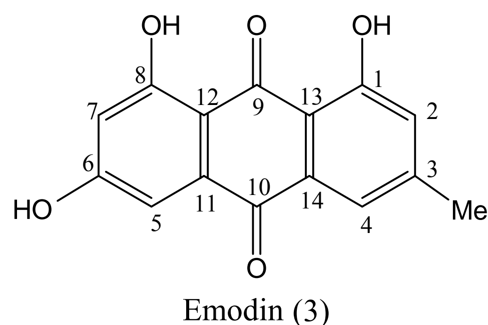
Table 8 ^{13}C (100 MHz) and DEPT spectral data of physcion in $CDCl_3$.

Position of Carbons	^{13}C data (δ in ppm)	DEPT 135 data (δ in ppm)	^{13}C Lit. Data ($CDCl_3$). (δ in ppm) [37]	Remarks
CH_3	22.13	22.13	22.2	CH_3
OCH_3	56.06	56.06	56.1	OCH_3
C-7	106.78	106.78	106.8	C-H
C-5	108.18	108.18	108.2	C-H
C-12 and C-13	113.69	__	113.2	Quaternary
C-4	121.26	121.26	121.3	C-H
C-2	124.48	124.48	124.5	C-H
C-14	133.23	__	133.3	Quaternary
C-11	135.27	__	135.3	Quaternary
C-3	148.42	__	148.5	Quaternary
C-8	162.52	__	162.5	Quaternary
C-1	165.20	__	165.2	Quaternary
C-6	166.56	__	166.6	Quaternary
C-10	181.95	__	182.0	Quaternary
C-9	190.79	__	190.8	Quaternary

5.2.3. Emodin (3)

Compound **3** was obtained as an orange solid. It was identified as emodin by comparing its ^{1}H NMR spectral data with that reported in the literature [11]. Compound **3** displayed UV absorption at λ_{max} 265, 289 and 442 nm, characteristic of anthraquinones [15]. The IR showed bands at 1670 and 1625 cm^{-1} corresponding to the un-chelated and chelated carbonyl carbons, respectively. The ^{1}H NMR shows two chelated hydroxyl protons resonating at δ 12.04 and δ 12.16, two meta coupled doublets at δ 6.65 and δ 7.24, two meta coupled broad singlet signals at δ 7.12 and 7.55, and one methyl group at δ 2.46 are characteristic of emodin.

The ^{13}C NMR spectrum data of 3 (see Table 10), displayed 15 carbon resonance and the DEPT spectral data showed the absence of methylene groups, the presence of one methyl group at δ 21.10, four aromatic C-H protons at (δ 124.03, 120.58, 108.75, and 107.97), and ten quaternary carbons is consistent with emodin structure.



Emodin (3)

Table 9 Comparison of the observed ^1H NMR spectral data with the reported value of emodin.

H	Observed data (400 MHz, acetone-d_6) (δ in ppm)	Reported data (600 MHz, acetone-d_6). (δ in ppm) [11]
1-OH	12.04 s	12.09 s
2-H	7.12 br s	7.16 br s
3-Me	2.46 s	2.48 s
4-H	7.55 br s	7.59 br s
5-H	7.24 d, J=1.6 Hz	7.27 d, J=2.4 Hz
6-OH	—	—
7-H	6.65 d, J=1.6 Hz	6.68 d, J=2.4 Hz
8-OH	12.16 s	12.21 s

Table 10 ^{13}C (100 MHz) and DEPT spectral data of emodin in acetone-d_6.

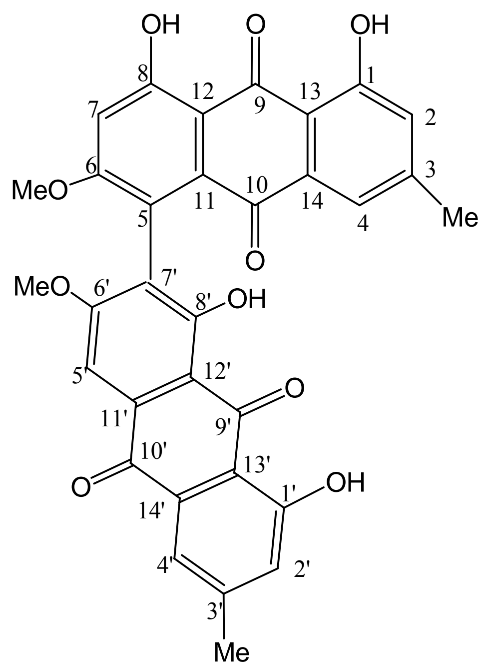
Position Of Carbons	^{13}C data (δ in ppm)	DEPT 135 Data (δ in ppm)	^{13}C Lit. data ($CDCl_3$ + MeOH – d_4). [37] (δ in ppm)	Remarks
CH_3	21.10	21.10	20.9	CH_3
C-7	107.97	107.97	107.6	C-H
C-5	108.74	108.74	108.7	C-H
C-12	109.58	__	109.0	Quaternary
C-13	113.55	__	113.1	Quaternary
C-4	120.58	120.58	120.3	C-H
C-2	124.03	124.03	123.6	C-H
C-14	133.31	__	132.7	Quaternary
C-11	135.70	__	134.8	Quaternary
C-3	148.63	__	147.5	Quaternary
C-1	162.39	__	161.6	Quaternary
C-8	165.37	__	164.7	Quaternary
C-6	165.41	__	165.6	Quaternary
C-10	181.18	__	181.9	Quaternary
C-9	190.79	__	189.8	Quaternary

5.2.4. Floribundone -1 (11)

Compound **11** was obtained as an orange solid. Compound **11** displayed UV absorption at $\lambda_{max:}$ 283 and 445 nm, characteristic of anthraquinones [15].

The IR showed bands at 1677 and 1626 cm^{-1} corresponding to the un-chelated and chelated carbonyl carbons, respectively. The ^{1}H NMR spectrum of **11** indicated the presence of four chelated hydroxyl groups (δ 13.05, 12.38, 12.12, 12.07), six aromatic C-H protons (δ 7.67, 7.57, 7.43, 7.10, 7.06, 6.83), two methoxy groups (δ 3.90, 3.85) and two aromatic methyl groups (δ 2.49, 2.38) (see Table 11).

The ^{13}C spectral data showed the presence of four carbonyl carbons resonating at (δ 191.34, 191.11, 182.58, and 182.22), aromatic carbons at (δ 103.68-166.13) (see Table 12), two methoxy groups at (δ 56.60 and δ 56.47), and two aromatic methyl groups at (δ22.16 and δ 22.09). Further more, the DEPT spectral data (see Table 12), indicated the presence of 22 quaternary carbons, six aromatic C-H groups resonating at (δ 124.44, 123.87, 121.2 (two), 104.62, and 103.68), two methoxy (δ 56.00and δ 56.48) and two aromatic methyl groups (δ 22.17 and δ 22.10).



Floribudone-1 (11)

Table 11 Comparison of the observed ^{1}H NMR spectral data with the reported value of floribundone -1

H	Observed data (400 MHz, $CDCl_3$) (δ in ppm)	Reported data (400 MHz, $CDCl_3$).[33] (δ in ppm)
1-OH	12.12[a] s	12.10[a] s
2-H	7.06 br s	7.04 d, J=2
3-Me	2.38 s	2.35 s
4-H	7.43 br s	7.42 d, J=2
5-H	—	—
6-OMe	3.85[b] s	3.82[b] s
7-H	6.83 s	6.83 s
8-OH	12.07[a] s	12.05[a] s
1'-OH	12.28[a] s	12.20[a] s
2'-H	7.10 br s	7.06 d, J=2
3'-Me	2.49 s	2.45 s
4'-H	7.69 br s	7.67 d, J=2
5'-H	7.57 s	7.57 s
6'-OMe	3.90[b] s	3.85[b] s
7'-H	—	—
8'-OH	13.05 s	13.10 s

Signals with the same superscript may be interchanged

Table 12 ^{13}C (100 MHz) and DEPT spectral data of floribundone-1 in $CDCl_3$.

Position of Carbons	^{13}C data (δ in ppm)	DEPT 135 Data (δ in ppm)	Remarks
3-CH_3	22.10	22.10	CH_3
3'-CH_3	22.16	22.16	CH_3
6[a]-MeO	56.47	56.47	MeO

Cont'd to Table 12.

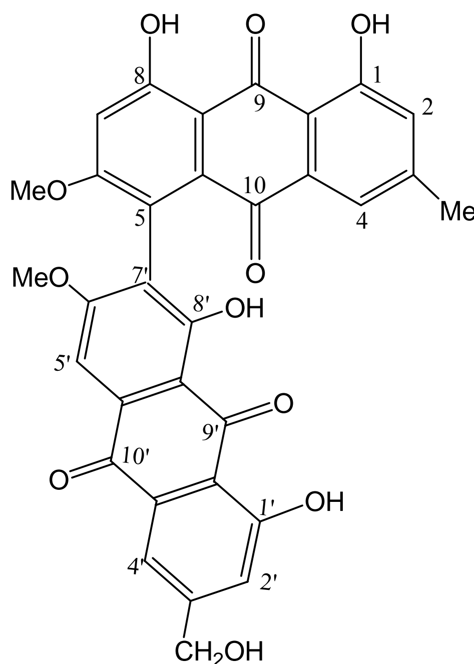
6'[a]-MeO	56.60	56.60	MeO
7-C	103.68	103.6	C-H
5'-C	104.62	104.62	C-H
7'-C	110.58	__	Quaternary
5-C	111.03	__	Quaternary
12-C	113.48	__	Quaternary
12'-C	113.81	__	Quaternary
4-C	117.43	117.43	C-H
4'-C	120.16	120.16	C-H
13-C	121.20	__	Quaternary
13'-C	121.20	__	Quaternary
2-C	123.87	123.87	C-H
2'-C	124.43	124.43	C-H
14-C	132.45	__	Quaternary
14'-C	133.34	__	Quaternary
11'-C	133.84	__	Quaternary
11-C	134.27	__	Quaternary
3-C	148.35	__	Quaternary
3'-C	148.43	__	Quaternary
1[b]-C	161.23	__	Quaternary
8[b]-C	162.12	__	Quaternary
1'[b]-C	162.50	__	Quaternary
8'-C	163.38	__	Quaternary
6[c]-C	164.72	__	Quaternary
6'[c]-C	166.13	__	Quaternary
10-C	182.22	__	Quaternary
10'-C	182.58	__	Quaternary
9-C	191.11	__	Quaternary
9'-C	191.34	__	Quaternary

Signals with the same superscript may be interchanged

5.2.5. 5, 7'-Physcion – fallacinol (15)

It is a yellow solid that turned to red on a TLC plate, upon spraying with 5% KOH in methanolic solution, which is characteristic for hydroxyanthraquinones [15]. This compound was previously reported from the pod of S. floribunda [36] and from the stem bark of the same species [35]. The UV-Vis spectrum of 15 displayed absorption maxima at 241, 283 and 445 nm consistent with the presence of a quinonoid chromophore. The ^{1}H NMR spectrum indicates the presence of four chelated hydroxyl groups (δ 13.03 s, 12.22 s, 12.17 s, and 12.06 s), six aromatic protons (δ7.28 d, 7.57 s, 7.42 d, 7.33 d, 7.05 brs, and 6.82 s), two aromatic methoxy groups (δ 3.89 s and 3.83 s), one aromatic methyl group (δ 2.36) and a signal at δ 4.48 s attributed to a–CH_2OH group.

The above data is in agreement with the reported data in the literatures [32] for the dimeric anthraquinone 5, 7'- physcion – fallacinol (15). The two meta coupled proton signals at δ 7.82 d and 7.33 d are assigned to 4'-H and 2'-H, respectively. The other meta coupled signals at δ 7.42 d and 7.05 brs are assigned to 4-H and 2-H, respectively. The two singlet signals at δ 7.57 s and 6.82 s could be assigned to 5'-H and 7-H, respectively.



5,7'-Physcion-fallacinol (15)

Table 13 Comparison of the observed ^{1}H NMR spectral data with the reported value of 5,7'- physcion – fallacinol (15).

H	**Observed data** (400 MHz, $CDCl_3$) (ppm)	**Reported data** (300 MHz, $CDCl_3$) [36] (ppm)
1-OH	12.06^{a} s	12.09^{a} s
2-H	7.05 brs	7.05 br d, J=2
3-Me	2.36 s	2.36 s
4-H	7.42 d, J = 1.2 Hz	7.42 br d, J=2
5-H	—	—
6-OMe	3.83^{b} s	3.84^{b} s
7-H	6.82 s	6.82 s
8-OH	12.22^{a} s	12.25^{a} s
1'-OH	12.17^{a} s	12.20^{a} s
2'-H	7.33 d, J = 1.2 Hz	7.34 br d, J=2
3'-CH_2OH	4.84 s	4.85 s
4'-H	7.82 d, J = 0.8 Hz	7.82 br d, J=2
5'-H	7.57 s	7.57 s
6'-OMe	3.89^{b} s	3.89^{b} s
7'-H	—	—
8'-OH	13.03 s	13.06 s

Signals with the same superscript may be interchanged

6. Conclusion

Five anthraquinones were isolated and characterized from the stem bark of Senna floribunda; two of these, floribundone-1 and 5, 7'- physcion - fallacinol are dimeric anthraquinones. The others are the common anthraquinones: chrysophanol, physcion and emodin. Except emodin (10.2 mg) and physcion (9.1 mg) which were isolated relatively in appreciable amount the other compounds were obtained in very small quantities ranging between 1.6 mg (5, 7'- physcion - fallacinol) to 5.2 mg (floribundone-1). Since the solubility of anthraquinones in $CDCl_3$ is very low, the PNMR and CNMR spectra are highly interfered by the signals of very little impurities.

This study yielded a number of unidentified anthraquinones and flavonoids, more polar than 5, 7'- physcion – fallacinol, which may contain sugar moiety and their structures can be elucidated if more quantity of the compounds are obtained.

7. Expermental

7.1. General

^{1}H NMR spectra were recorded on a Bruker Advance at 400 MHz and the ^{13}C NMR were measured at 100 MHz. The ultraviolet and visible (UV-Vis) spectra were taken on GENESY'S 2PC UV-Vis scanning spectrometer in the range 200-1000 cm^{-1}. Infrared (IR) absorptions were measured as KBr pellets on Perkin-Elmer BX Infrared spectrometer in the range 4000-400 cm^{-1}. Melting points were recorded using Thomas HOOVER Capillary melting point apparatus.
Analytical thin layer chromatograms were run on ready made 0.2 mm thick layer of Merck silica gel 60 F_{254} coated on aluminum foil. Observing in UV light and spraying with 5% methanolic KOH solution detected spots on the chromatograms. Preparative thin layer chromatograms were run on 0.5 mm and 1 mm thick layer Merck silica gel 60 $HF_{254+366}$. Column chromatography was conducted using different sizes of columns packed with silica gel, particle size 0.032 -0.063 mm.

7.2. Plant material

The stem bark of Senna floribunda were collected along the Addis Ababa –Ambo road, *ca* 80 Km west of Addis Ababa in November 2005.Voucher specimens are deposited in the National Herbarium, Addis Ababa University (Voucher no.: Tsegaye 1).

7.3. Extraction and isolation

The dried and ground stem bark (235 g) was soaked in 5% acetic acid (0.5L) and allowed to dry by air for a week. Then the dried sample was successively extracted with 1L of MeOH: CH_2Cl_2 (1:1) after it has been soaked for 24 hrs. The solvent was removed under reduced pressure at 45 ^{0}C to yield 39.6 g of brown gummy extract.
20 g of organic extract was adsorbed on 30 g of silica gel and charged on to column packed with 150 g silica gel (impregnated with 5% oxalic acid) using $CHCl_3$. The column was eluted using the following solvent systems: chloroform (100%): fractions 1-4, chloroform: ethyl acetate (9: 1): fractions 5-7, chloroform: ethyl acetate (4: 1): fractions 8-11, chloroform: ethyl acetate (2: 1): fractions 12-22, chloroform: ethyl acetate (1: 1): fractions 23-26, ethyl acetate (100%): fractions 27-32, methanol (100%): fraction 33.

Fraction 2 was applied on TLC and showed two colors (violate and pink) after spraying with 5% methanolic KOH. This fraction was applied on column (packed with silica gel) using petroleum ether. Three fractions were collected and the solvent systems used to elute the column were petroleum ether: ethyl acetate (9: 1) fraction 1, petroleum ether: ethyl acetate (4: 1) fraction 2, ethyl acetate (100%) fraction 3. Fractions 2 and 3 were pulled together and applied on chromatotron using pet. Ether: chloroform: acetone (4ml: 1ml: 3drps.) and four fractions were collected. The 2nd fraction was applied on PTLC using petroleum ether: chloroform: acetone (4ml: 1ml: 3drps.) to yield two bands. The top band gave 2.3 mg of chrysophanol.

Fractions 3 and 4 showed similar components and were combined together. The combined fraction was loaded on sephadex LH-20 column and eluted with $CHCl_3$: MeOH (2:1) to yield 3 fractions. Fractions 2 and 3 were combined. The combined fraction (25.8 mg) was applied on column (15 g silica gel) using petroleum ether: ethyl acetate (10: 1) and three fractions were collected. The 3rd fraction gave 9.1 mg physcion.

Fractions 5-7 were combined on the basis of TLC similarity loaded on sephadex LH-20 and eluted with $CHCl_3$: MeOH (2:1) to yield four fractions. The 3rd and 4th fractions were pulled together, applied on a silica gel column and eluted with petroleum ether: ethyl acetate (4: 1) to give 5.2 mg floribundone-1.

TLC examination showed that fractions 10 and 11 contained similar components. They were combined and loaded on sephadex LH-20 and eluted with $CHCl_3$: MeOH (2:1). Two fractions were collected and the 2nd fraction was applied on column chromatography (20 g silica gel) using petroleum ether: ethyl acetate (4: 1) to yield 9.4 mg of emodin.

Fractions 12 and 13 were combined on the basis of their TLC similarity, loaded on sephadex LH-20 and eluted with $CHCl_3$: MeOH (2:1). Three fractions were collected and fractions 1 and 2 combined, re-applied on sephadex LH-20 and eluted with $CHCl_3$: MeOH (1:1). Four fractions were collected, the 3rd and 4th fractions combined (37.1 mg) and applied on chromatotron using CH_2Cl_2: MeOH (5ml: 4 drps) and seven fractions were collected. Fractions 1-4 were combined, applied on prep. TLC and the 3rd band was eluted with CH_2Cl_2: MeOH (5ml: 4 drps) to yield 1.6 mg 5, 7’- physcion-fallacinol.

7.4. Spectral data

Chrysophanol (5): Orange solid, m.p. 196-197°C (lit.[15] m.p. 196°C); R_f 0.397 in pet. Ether: EtAOAc (9:1); UV-Vis λ_{max} ($CHCl_3$) nm: 259, 289, and 433. IR υ_{max} (KBr) cm^{-1}: 3436, 2921, 2851, 1735, 1677, 1606, 1628, 1475, 1453, 1374, 1271, 1208, 1164, 1008,754. ^{1}H-NMR ($CDCl_3$) δ: 2.47 (3H, s, CH_3), 7.11 (1H, br s, H-2), 7.29 (1H, dd, J= 1.2 and 8.0 Hz, H-7), 7.65 (1H, br s, H-4), 7.67 (1H, t, H-6), 7.83 (1H, dd, J=1,2 and 8.0 Hz, H-5), 12.01 (1H, s, -OH), and 12.12 (1H, s, -OH).

Physcion (8): Orange solid, m.p. 208-209°C (lit. [15] 207°C); R_f 0.248 in pet. Ether: EtAOAc (10:1); UV-Vis λ_{max} ($CHCl_3$) nm: 265, 289, and 439. IR υ_{max} (KBr) cm^{-1}: 2923, 1676, 1630, 1570, 1479, 1387, 1368, 1325, 1297, 1273, 1255, 1229, 1164, 1103 1036, 850,757. ^{1}H-NMR ($CDCl_3$) δ: 2.44 (3H, s, CH_3), 3.93 (3H, s, $-OCH_3$), 6.67 (1H, s, H-7), 7.07 (1H, s, H-2), 7.35 (1H, s, H-5), 7.61 (1H, s, H-4), 12.08 (1H, s, -OH), and 12.28 (1H, s, -OH);
^{13}C-NMR ($CDCl_3$) δ: 22.13 (CH_3), 56.06 ($-OCH_3$), 106.78 (C-7), 108.18 (C-5), 113.69 (C-12 and C-13), 121.26 (C-4), 124.48 (C-2), 133.23 (C-14), 135.27 (C-11), 148.42 (C-3), 162.52 (C-8), 165.20 (C-1), 166.56 (C-6), 181.95 (C-10), and 190.79 (C-9).

Emodin (3): Orange solid, m.p. 263-265°C (lit. [15] m.p. 264-265°C); R_f 0.275 in pet. Ether: EtAOAc (4:1); UV-Vis λ_{max} ($CHCl_3$) nm: 265, 289, and 442. IR υ_{max} (KBr) cm^{-1}: 3480, 2924, 1671, 1626, 1479, 1388, 1340, 1227, 1174, 1080,768. ^{1}H-NMR (acetone-d_{6},) δ: 2.48 (3H, s, CH_3), 6.65 (1H, d, J= 1.6 Hz, H-7), 7.12 (1H, br s, H-2), 7.24 (1H, d, J= 1.6 Hz, H-5), 7.55 (1H, br s, H-4), 12.04 (1H, s, -OH), and 12.16 (1H, s, -OH); 13C-NMR (acetone-d_6) δ:21.10 ($-CH_3$), 107.97 (C-7), 108.74 (C-5), 109.58 (C-12), 113.55 (s, C-13), 120.58 (C-4), 124.03 (C-2), 133.31 (C-14), 135.70 (C-11), 148.63 (C-3), 162.39 (C-1), 165.37 (C-8), 165.41 (C-6), 181.18 (C-10), and 190.79 (C-9).

Floribundone-1 (11): Orange solid, m.p.258-260 °C; R_f 0.333 in pet. Ether: EtAOAc (4:1); UV-Vis λ_{max} ($CHCl_3$) nm: 283 and 445. IR υ_{max} (KBr) cm^{-1}: 2922, 2851, 1734, 1678, 1627, 1559, 1560, 1469, 1429, 1386, 1316, 1274, 1225, 1130, 1075, 879, 765, and 754. ^{1}H-NMR ($CDCl_3$) δ: 2.38 (3H, s, -Me), 2.48 (3H, s, -Me'), 3.85 (3H, s, -OMe), 3.90 (3H, s, -OMe'), 6.83 (1H, s, H-7), 7.06 (1H, br s, H-2), 7.10 (1H, br s, H-2'), 7.43 (1H, br s, H-4), 7.57 (1H, s, H-5'), 7.69 (1H, br s, H-4'), 12.07 (1H, s, -OH), 12.12 (1H, s, -OH), 12.28 (1H, s, -OH), and 13.05 (1H, s, -OH); 13C-NMR ($CDCl_3$) δ: 22.10 ($-CH_3$), 22.16 (-'CH_3), 56.47 ($-OCH_3$), 56.60 (-OC'H_3), 103.68 (C-7), 104.62 (C-7'), 110.58 (C-7'), 111.03 (C-5'), 113.48 (C-12), 113.81 (C-12'), 117.43 (C-4), 120.16 (C-4'), 121.20 (C-13 and C-13'), 123.87 (C-2), 124.43 (C-2'),132.45(C-12),133.34 (C-12'), 133.84 (C-14'),134.27 (C-14), 148.35 (C-3), 148.43(C-3'),161.23(C-1), 162.12 (C-8), 162.50 (C-1'), 163.38(C-8'),164.72 (C-6), 166.137 (C-6'), 182.22 (C-10), 182.58 (C-10'), 191.11(C-9) and 191.34 (C-9').

5,7'- Physcion - fallacinol (15): Yellow solid, m.p.120-124 °C; R_f 0.460 in $CHCl_3$: MeOH (5 ml:5 dps); UV-Vis λ_{max} ($CHCl_3$) nm: 241, 283 and 445. ^{1}H-NMR ($CDCl_3$) δ: 2.36 (3H, s, -Me), 3.83 (3H, s, -OMe), 3.89 (3H, s, -OMe'), 4.84 (2H, s, 3'-CH_2OH), 6.82 (1H, s, H-7), 7.05 (1H, br s, H-2), 7.33 (1H, d, J=1.2 Hz, H-2'), 7.42 (1H, d, J=1.2 Hz, H-4), 7.57 (1H, s, H-5'), 7.82 (1H, d,J=0.8 Hz, H-4'),12.06 (1H, s, -OH), 12.17 (1H, s, -OH), 12.22 (1H, s, -OH),and 13.03 (1H, s, -OH);

8. References

[1]. Kaufman, B. … [et al.]. (1999) *Natural products from plants*, New York: by CRC press LCC. pp: 1, 23.

[2]. TODD, A. (1960) *Natural Product Chemistry - Retrospect and prospect*, Chemistry of Natural products international symposium, Australia: p.359

[3]. Mann, J. (1887) *Secondary Metabolism*, 2nd ed. Oxford University Press. pp: 1, 7-8.

[4]. Williams, A. D. and Lemke, L. T. (2002) *Foye's Principle of Medicinal Chemistry*, 5th ed. Philadelphia: Lippincott Williams & Wilkins. pp: 24-25.

[5]. Dewick, P. M. (2004) *Medicinal Natural Products,* A *Biosynthetic Approach*, 2nd ed. England: John Wiley & Sons Ltd. pp: 8, 63, 164.

[6]. Manitto, P. (1981) *Biosynthesis of Natural Products*, England: Ellis Horwood Ltd. pp: 10, 195.

[7]. Natori, S. (1974) *Natural products chemistry*, Tokyo: Kodansha Scientific LTD, vol. 1. p: 2

[8]. Kurt, B. and Torssell, G. (1997) *Natural product chemistry*, 2nd ed. Sweden Stockholm: pp: 15,

[9]. Ogwal, E. N. K. (1994) *The Biodiversity of African Plants, proceedings XIVth AETFAT Congress 22-27 August Wageningen*, The Netherlands: Kluwer Academic Publishers. pp: 768.

[10]. http://members.aol.com/MrDJReed/private/Plant2met.htm.

[11]. Muhanji, C. M. Sc. Thesis, (2001) University of Botswana, Department of Chemistry, Botswana.

[12]. http://www.herbs2000.com/miss/anthraquinones.htm

[13]. http://www3.interscience.wiley.com/
G. W. Francis *, D. W. Aksnes, Ø. Holt, Magnetic Resonance in Chemistry Volume 36, Issue 10, Pages 769 – 772

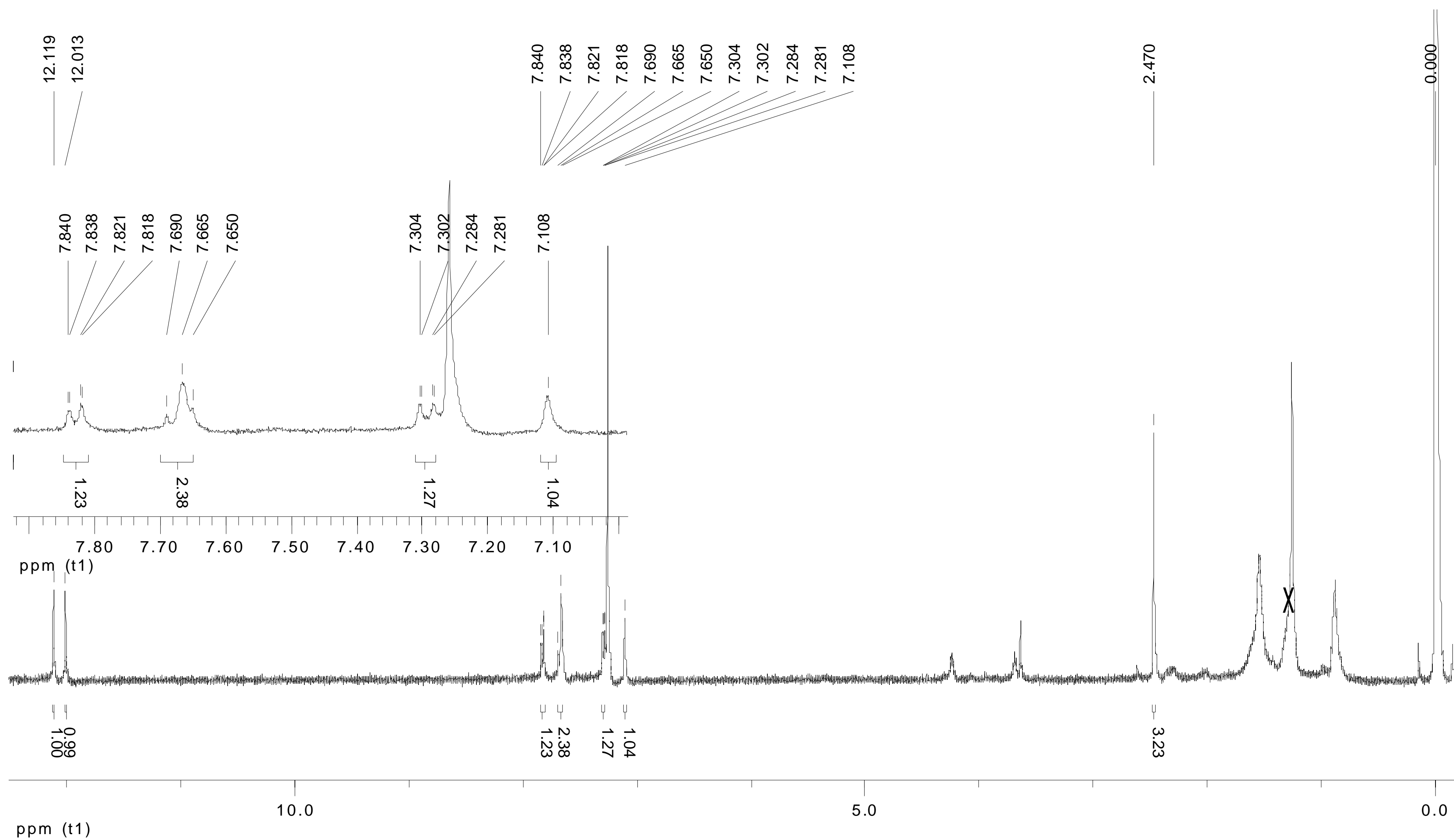
[14]. *Encyclopædia Britannica* from Encyclopædia Britannica Premium Service. <http://wwwa.britannica.com/eb/article-25388>

[15]. Thomson, R.H. (1971) *Naturally Occurring Quinones*, 2nd ed. London: Champman & Hall LTd. pp. 40, 57-64

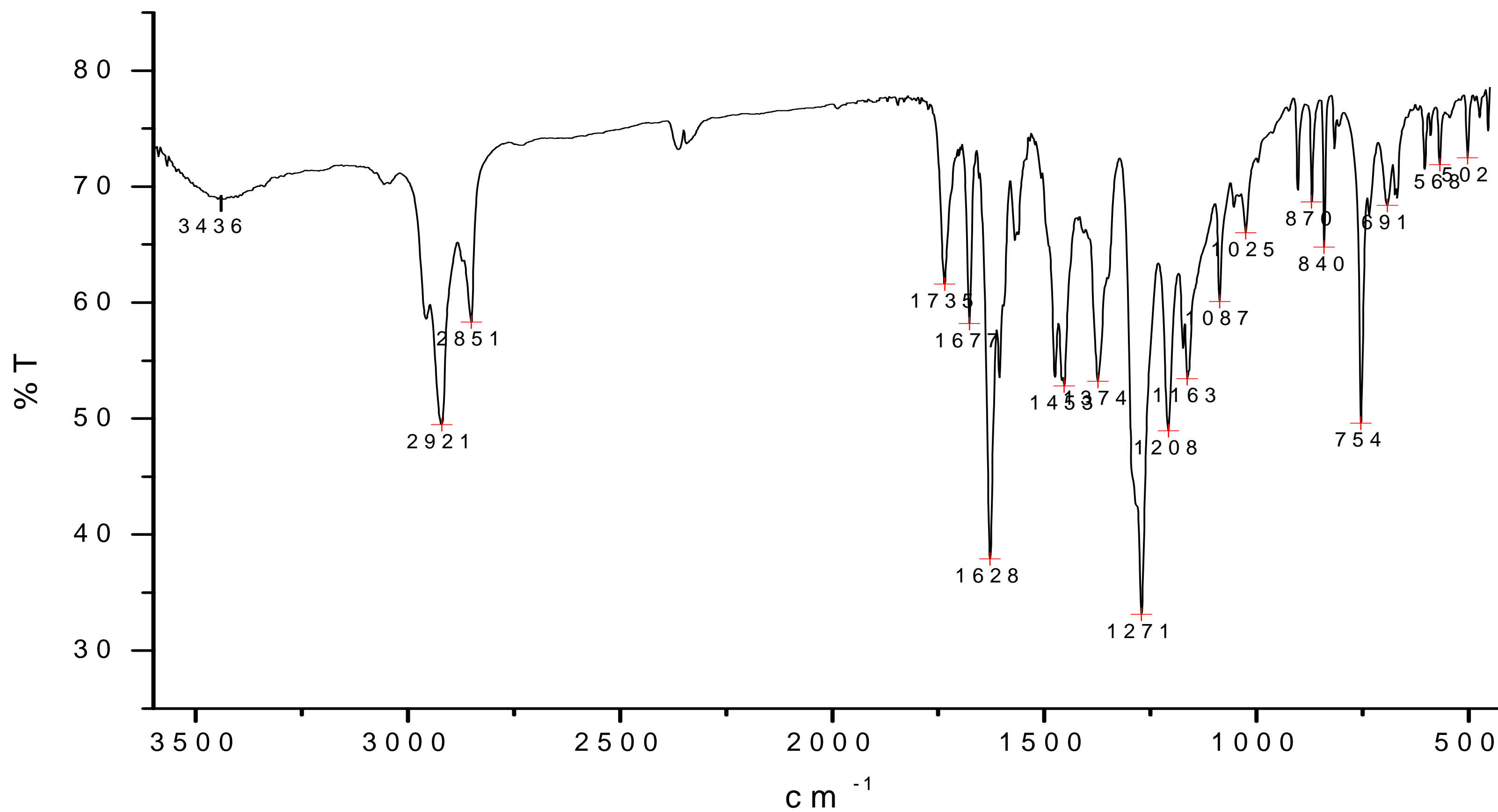
[16]. Trease, G. E. and Evans, W. C. (1978) *Pharmacognosy 11th Edition,* London: Bailleric Tindal, pp. 373-377.
[17]. Shibata, S.; Takido, M. and Tanaka, O. (1950) J. *Am. Chem. Soc*., 72, 2789.
[18]. Bloom, H. and Briggs, L. H. and Cleveley, B. (1959) J. *Chem. Soc.,* P. 178.
[19]. Ballantine, J. A. and Pillinger, C. T. (1967) *Tetrahedron*, 23, 1691.
[20]. Zanger, M. (1972) *Org. Mag. Res.,* 4, 1.
[21]. Hofle, G. (1977) *Tetrahydron*, 33, 1963.
[22]. Berger, Y. and Castonguay, A. (1978) *Org. Mag. Res.,* 11,375.
[23]. Natori, S. (1975) *Natural product chemistry*, vol. 2,
[24]. Thulin, M. (1989) in *Flora of Ethiopia,* (Hedberg, I. and Edwards, S., eds). Vol. 3, National Herbarium, Addis Ababa University, Addis Ababa.
[25]. Singh, J.; Tiwari, R.D. (1982) *Phytochemistry*, 21, 1832.
[26]. http://toptropicals.com/catalog/uid/SENNA_FLORIBUNDA.htm
[27]. http://www.bfns.org.au/index.php?c=3&w=29
[28]. www.hear.org/Pier/species/senna_septemtrionalis.htm
[29]. Alemayehu, G.; Abegaz, B.; Snatzke, G. and Duddeck, H**.** (1988) *Phytochemistry*, 27, 3255.
[30]. Tiwari, R. D. and Singh, J. (1979) *Phytochemistry*, 18, 2, 347.
[31]. Tiwari, R.D.; Tiwari, A. R. and Singh, J. (1980) *Phytochemistry*, 19, 6, 1253.
[32]. Woldeyesus, B., M.Sc. Thesis, (1996) Addis Ababa University, Department of Chemistry, Addis Ababa.
[33]. Abegaz, B.; Bezabeh, M.; Alemayehu, G. and Duddeck, H**.** (1994) *phytochemistry*, 5, 465.
[34]. Kitanaka, S. and Takido, M. (1982) *Phytochemistry*, 21, 2103.
[35]. Adane, L., M.Sc. Thesis, (1999) Addis Ababa University, Department of Chemistry, Addis Ababa.
[36]. Alemayehu, G.; Woldeyesus, B.; Abegaz, B.M. (1997) *Bull. Chem. Soc. Ethiop.,* 11, 1, 25-29.
[37]. Meselhy, R. M. (2003) *Molecules,* 8, 614-621.

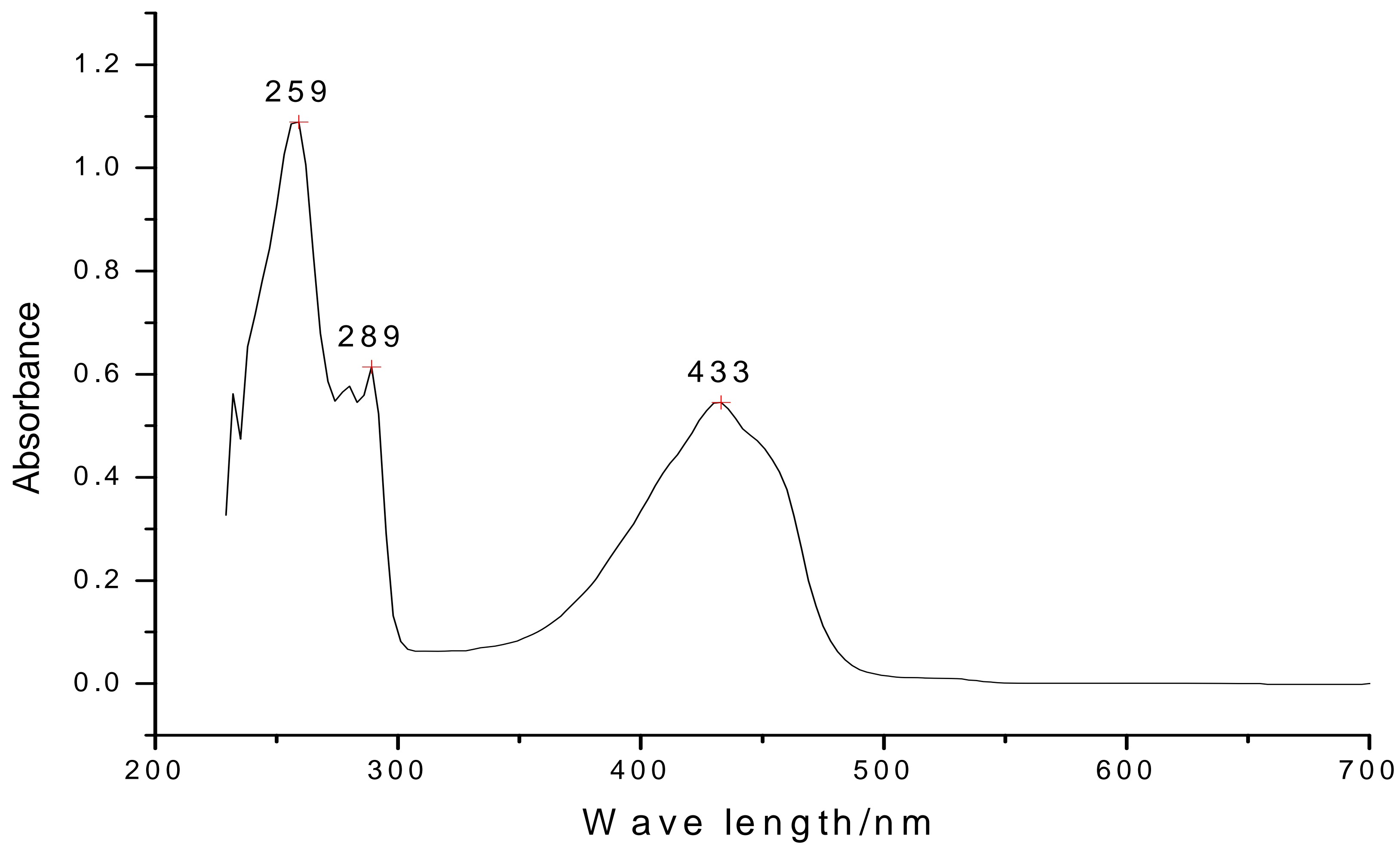
9. Appendices

Appenix 1.0 HNMR of Chrysophanol

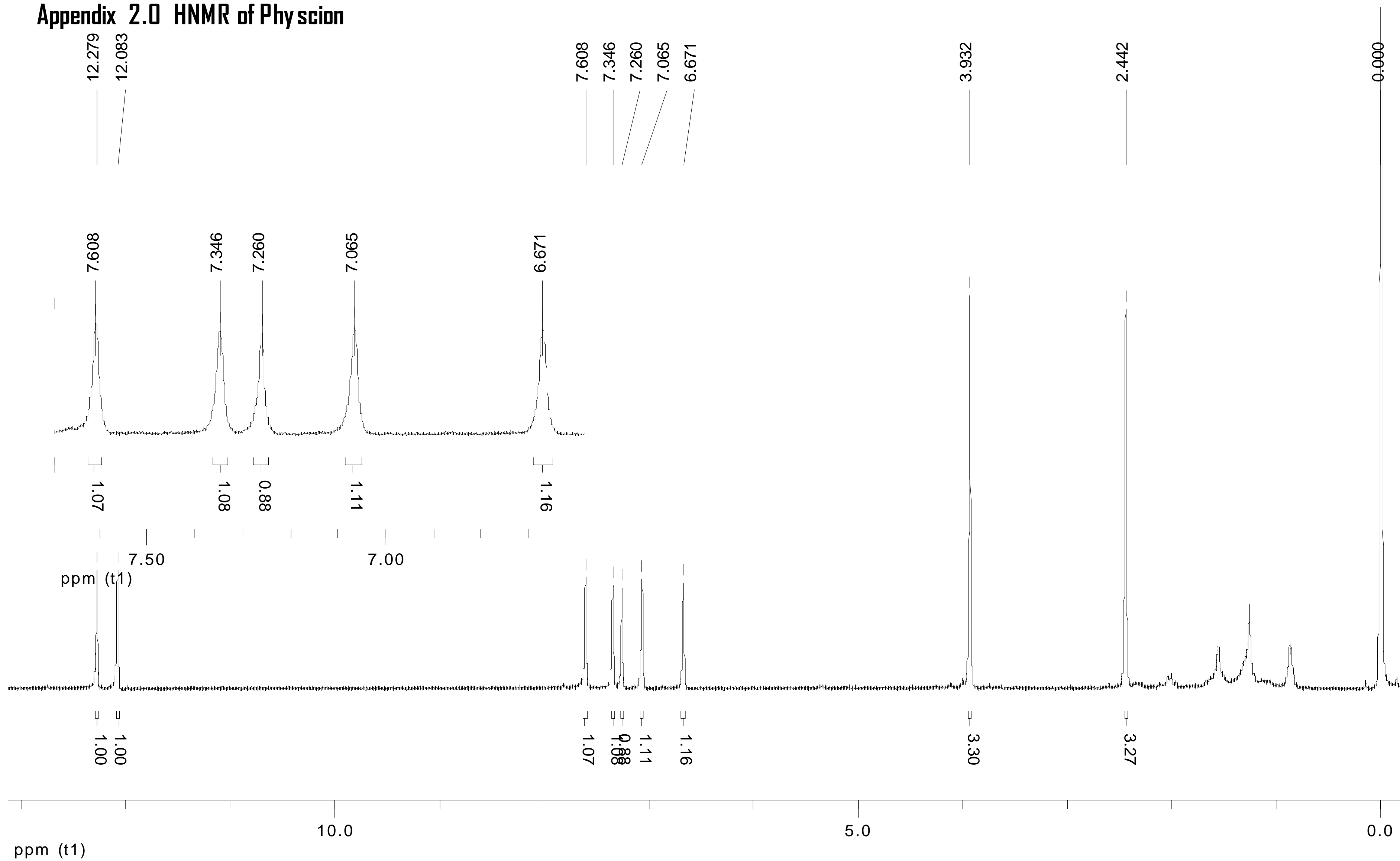


Appendix 1.1 IR of Chrysophanol

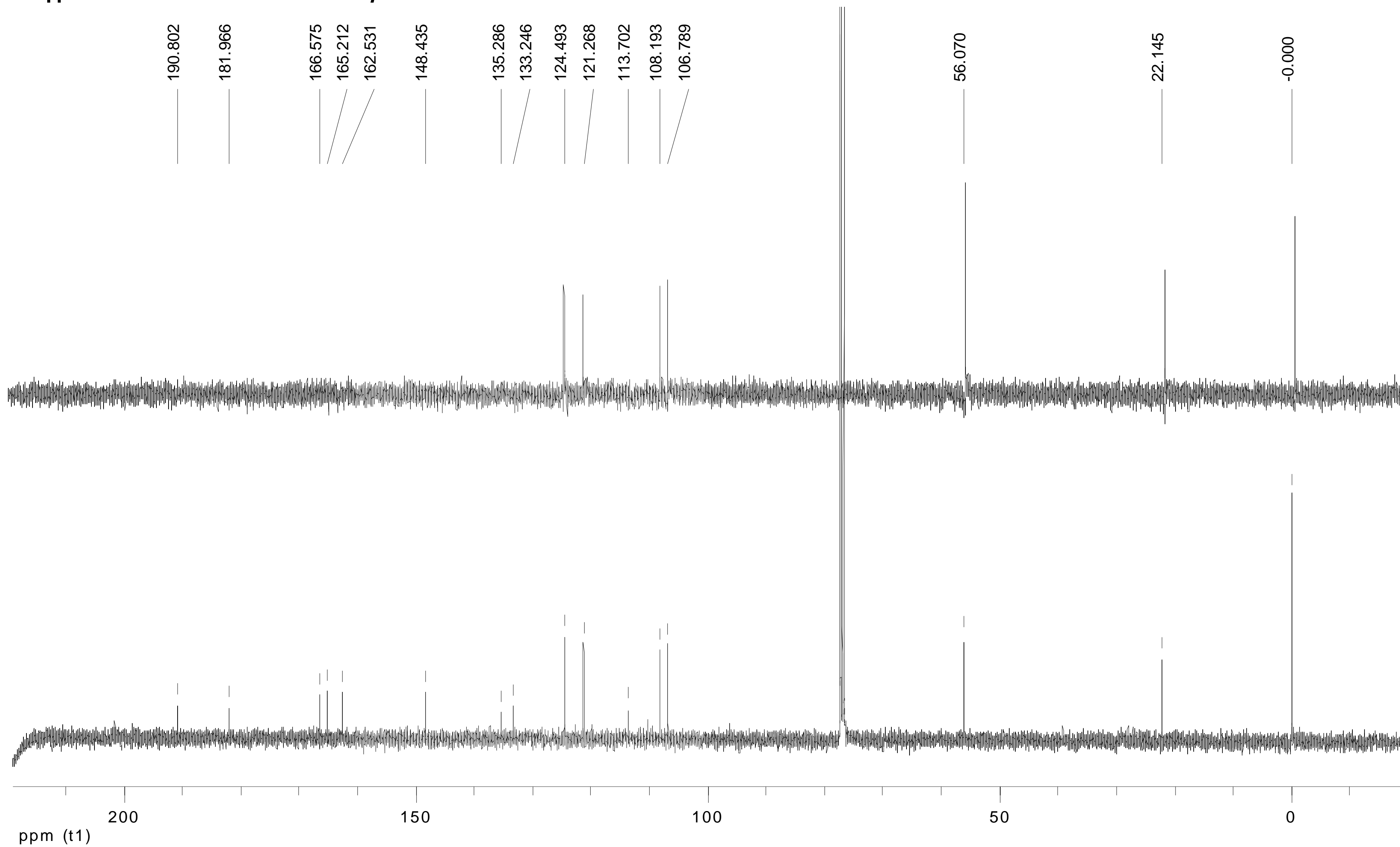



Appendix 1.2 UV-Vis of Chrysophanol
259
289
433
Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
200
300
400
500
600
700
Wave length/nm

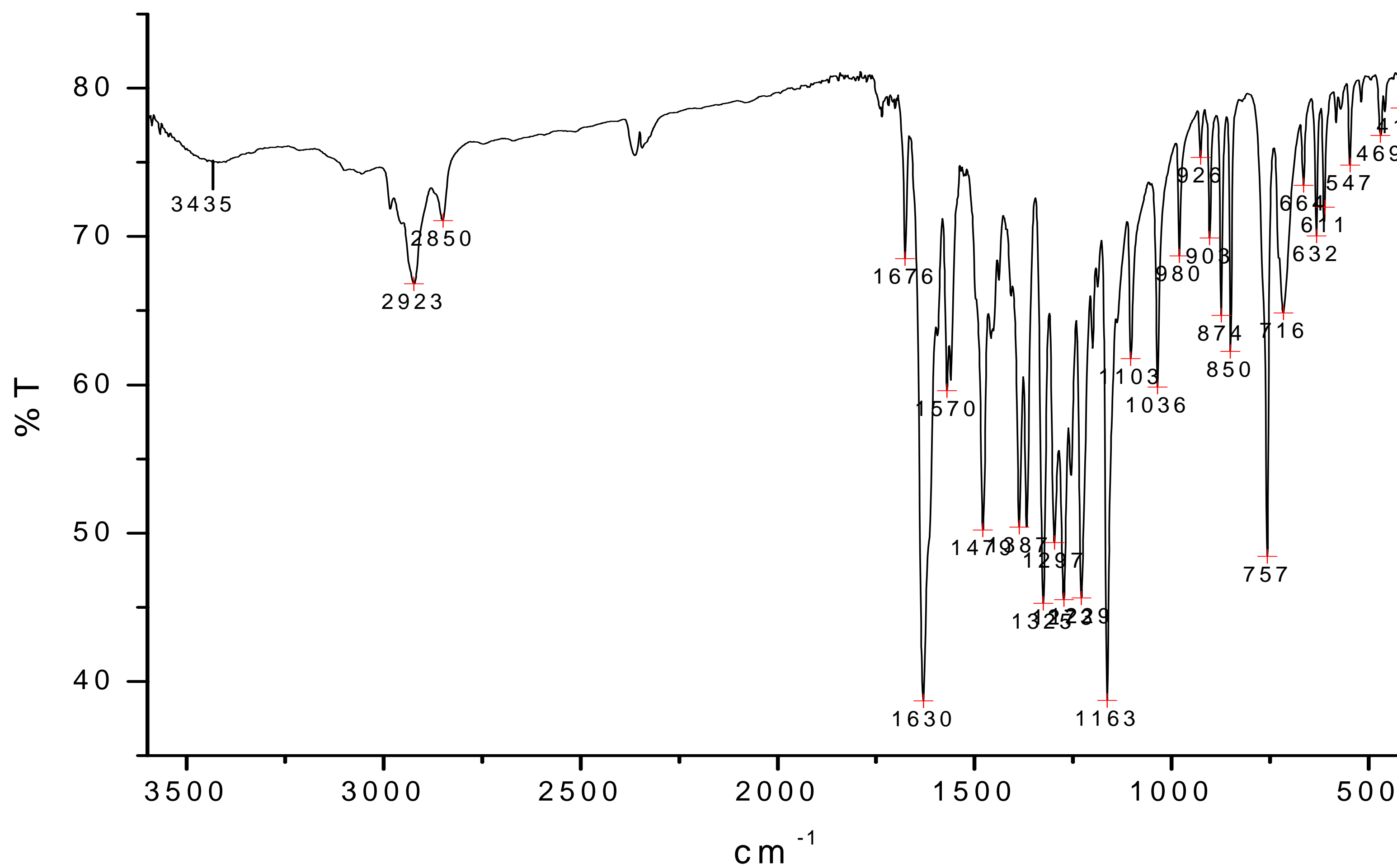
Appendix 2.0 HNMR of Physcion



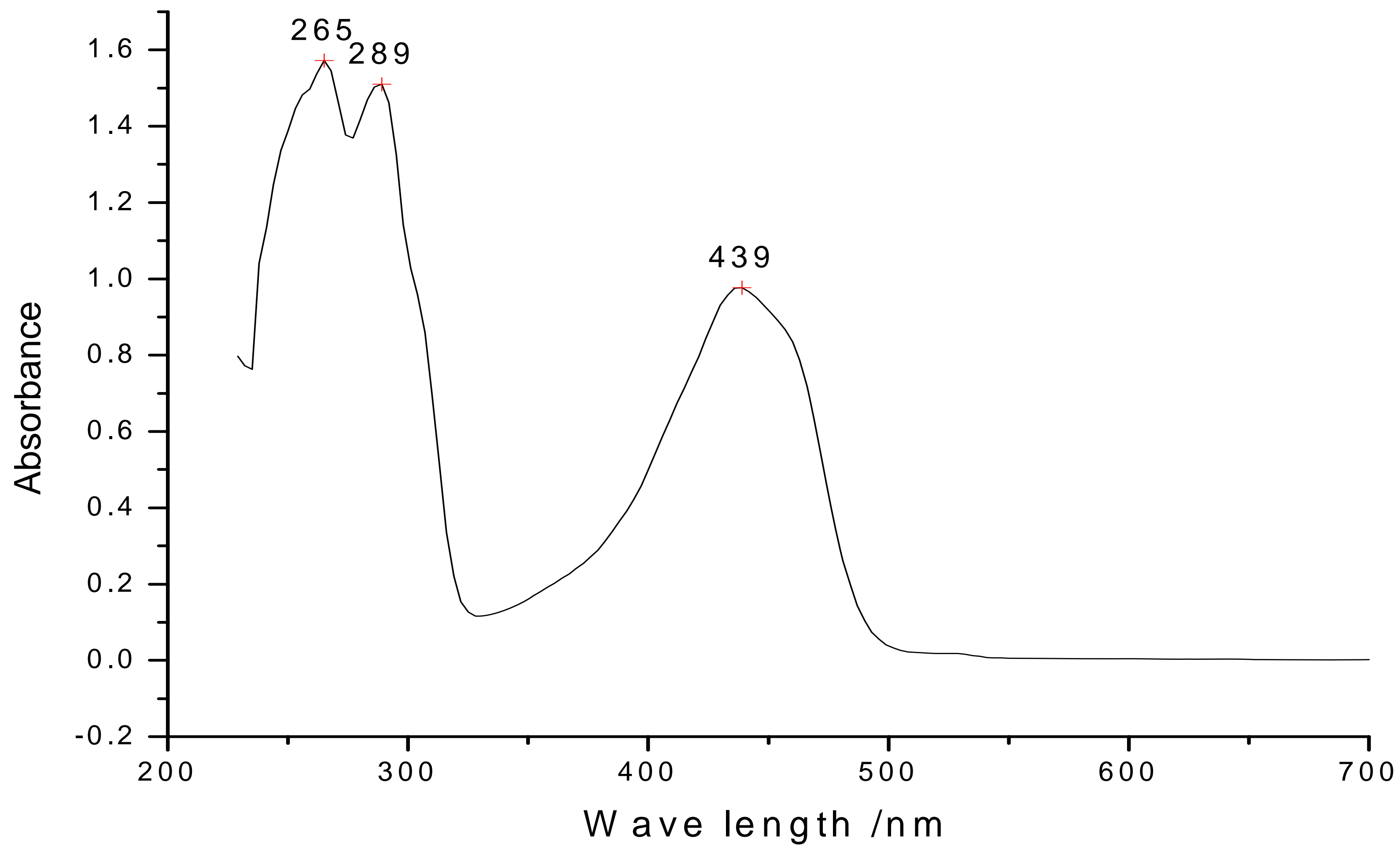
Appendix 2.1 13C and DEPT of Physcion



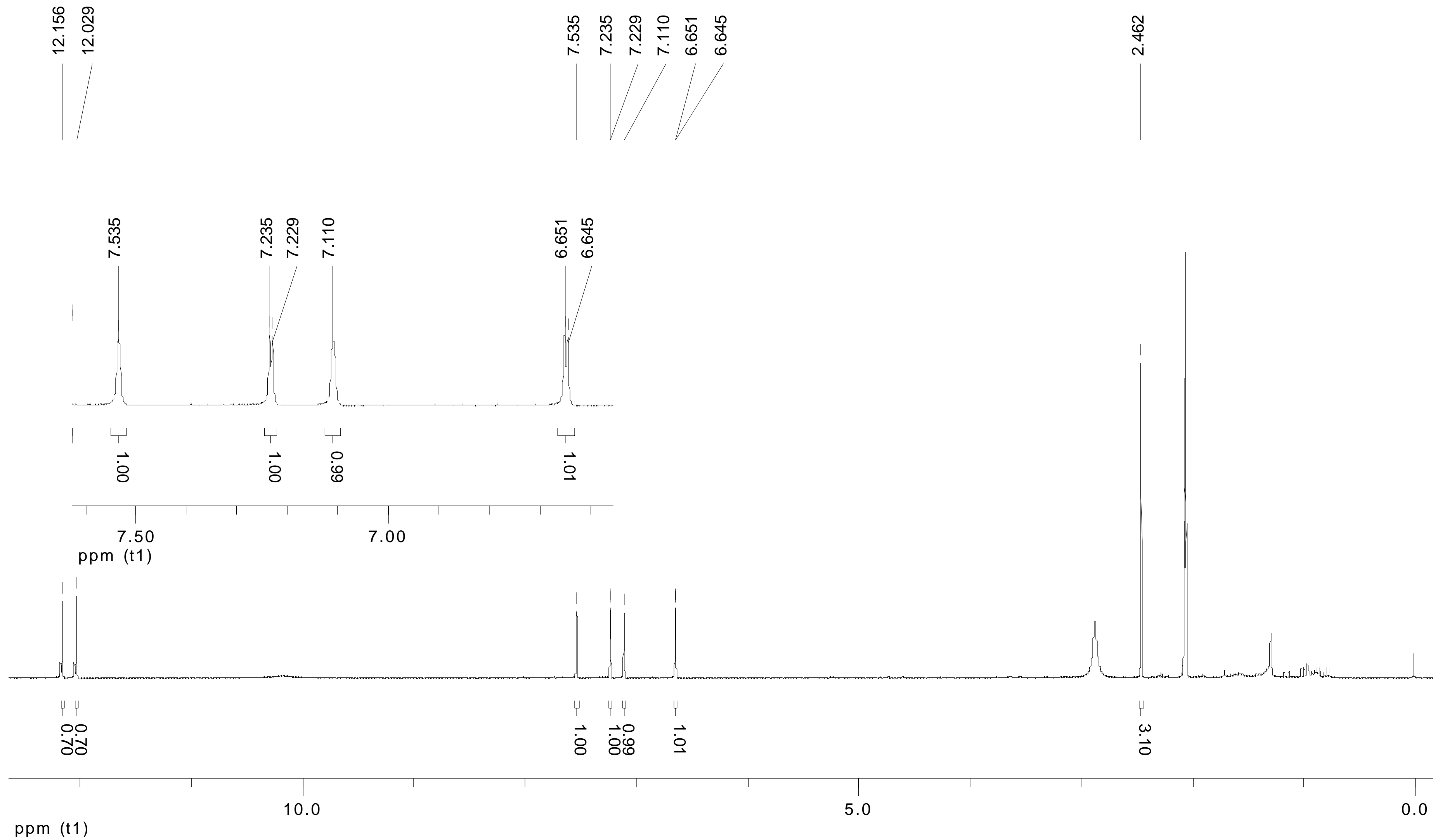
Appendix 2.2 IR of Physcion



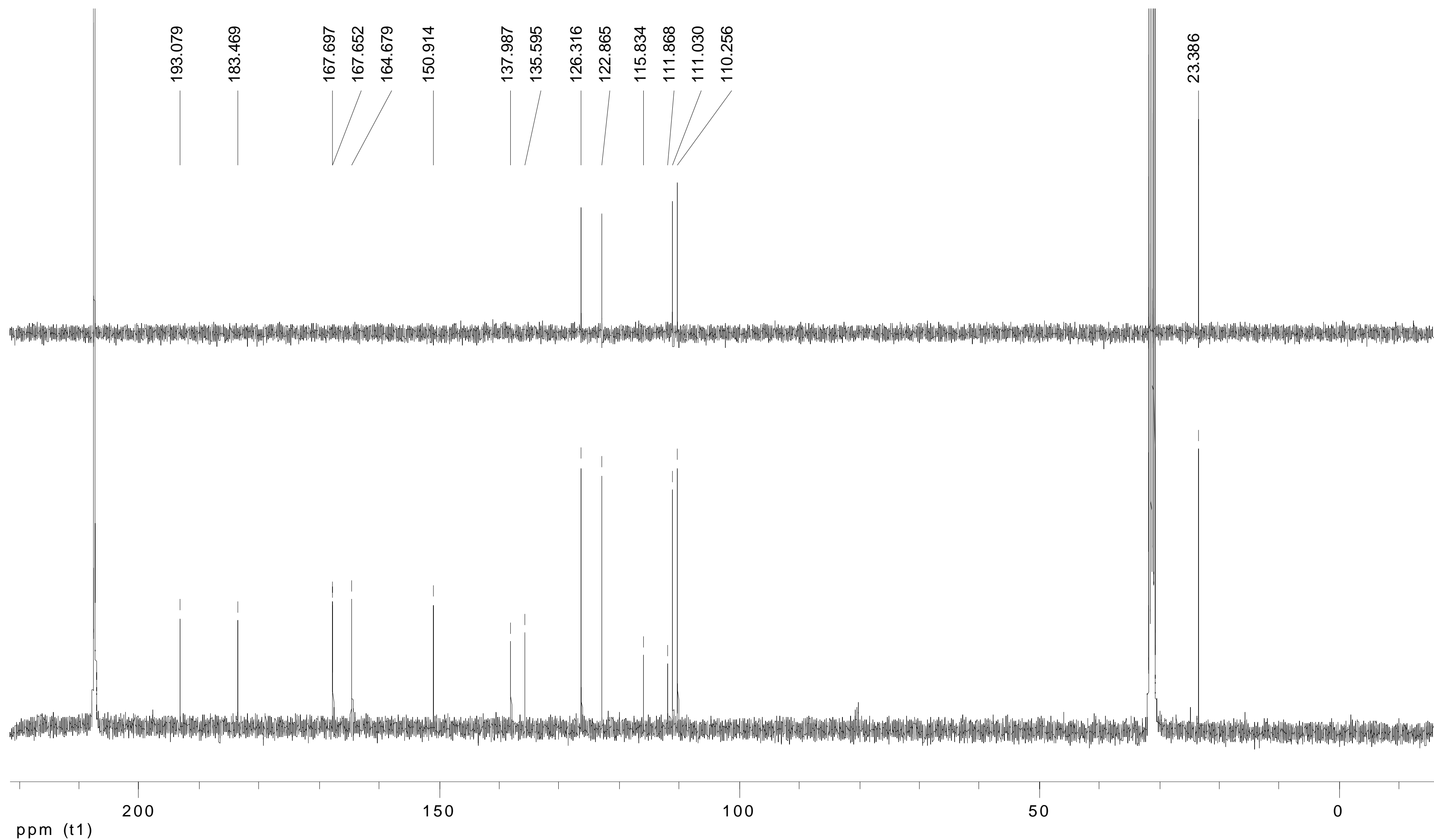
Appendix 2.3 UV-Vis of Physcion



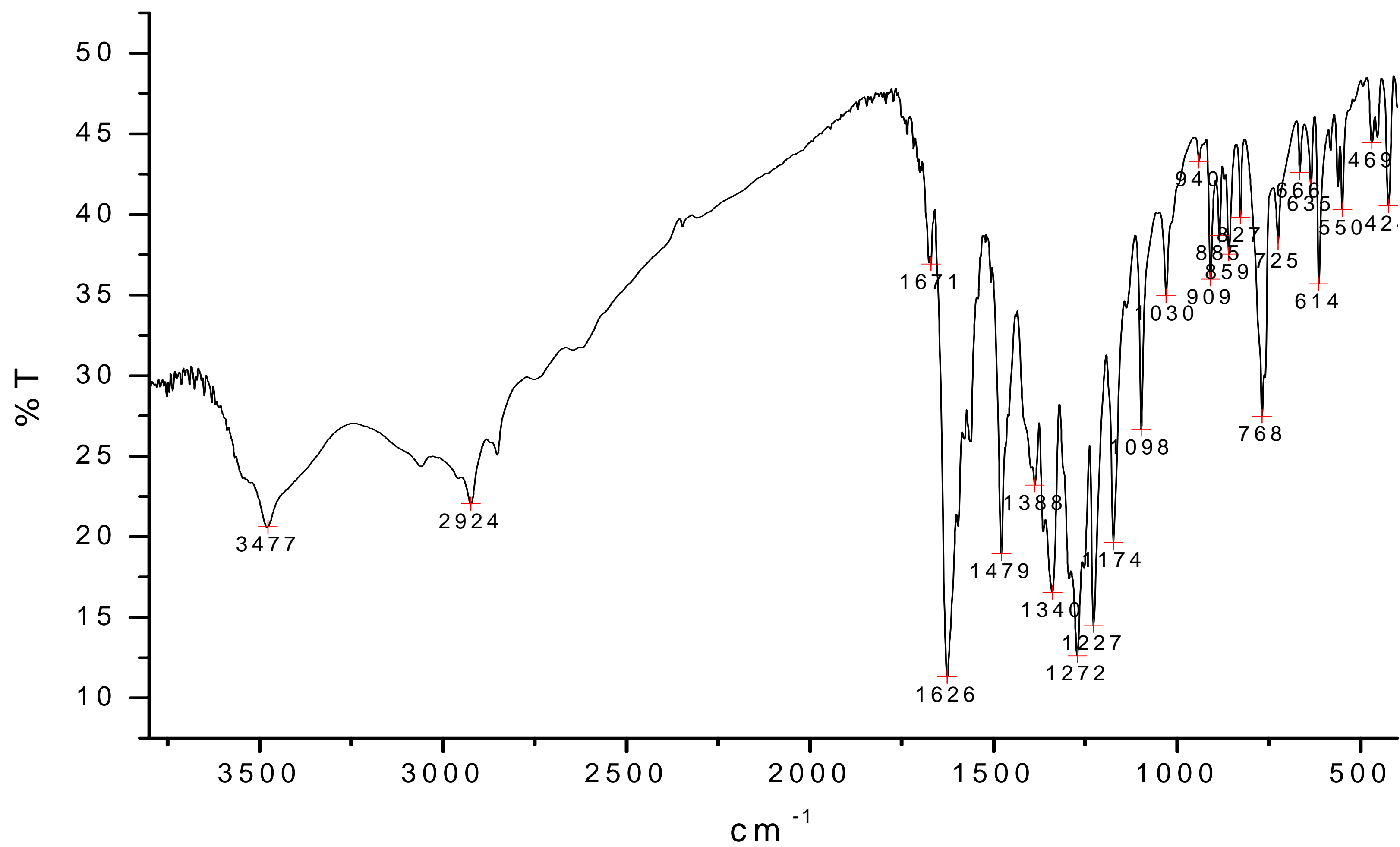
Appendix 3.0 HNMR of Emodin



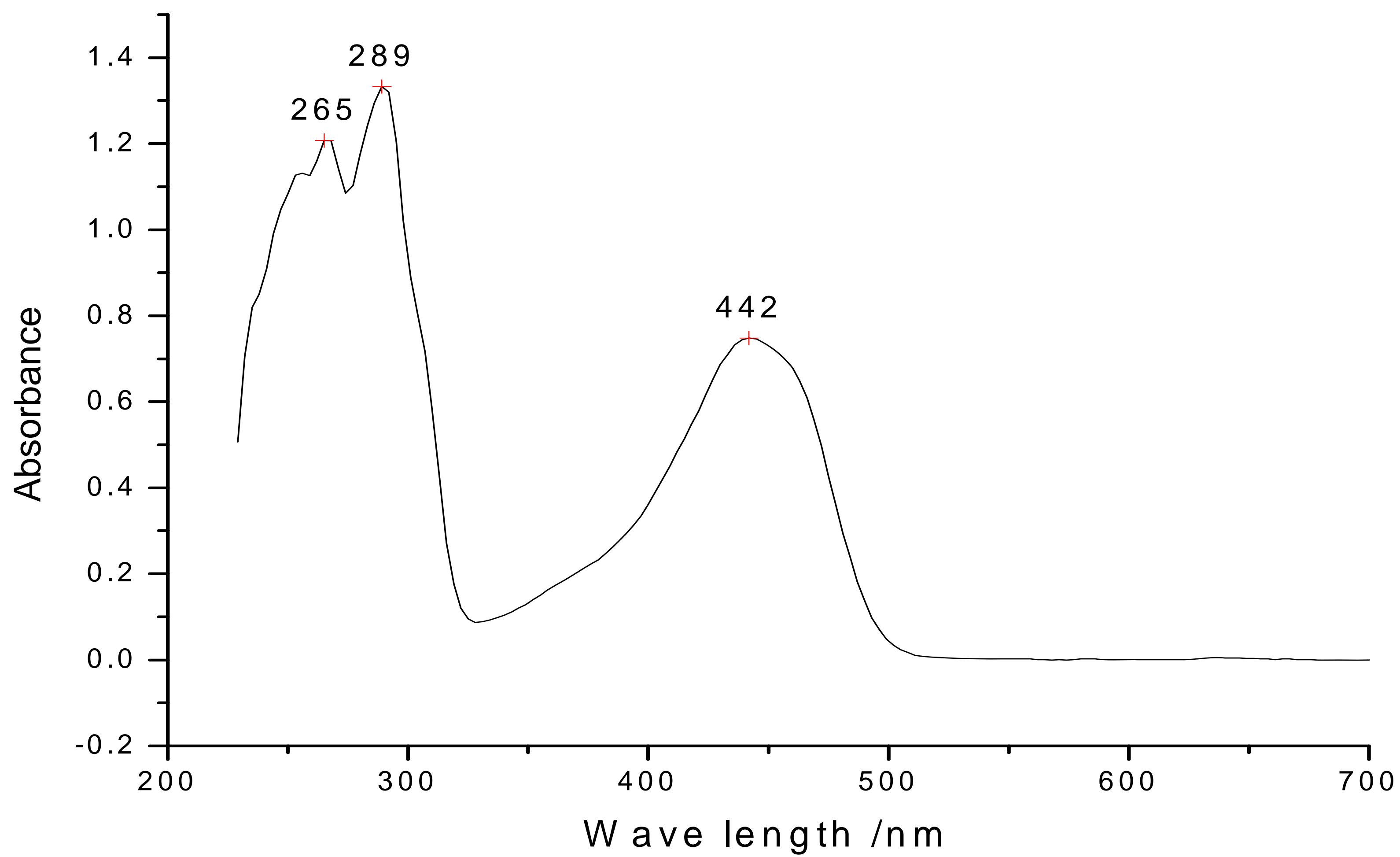
Appendix 3.1 13C and DEPT of Emodin



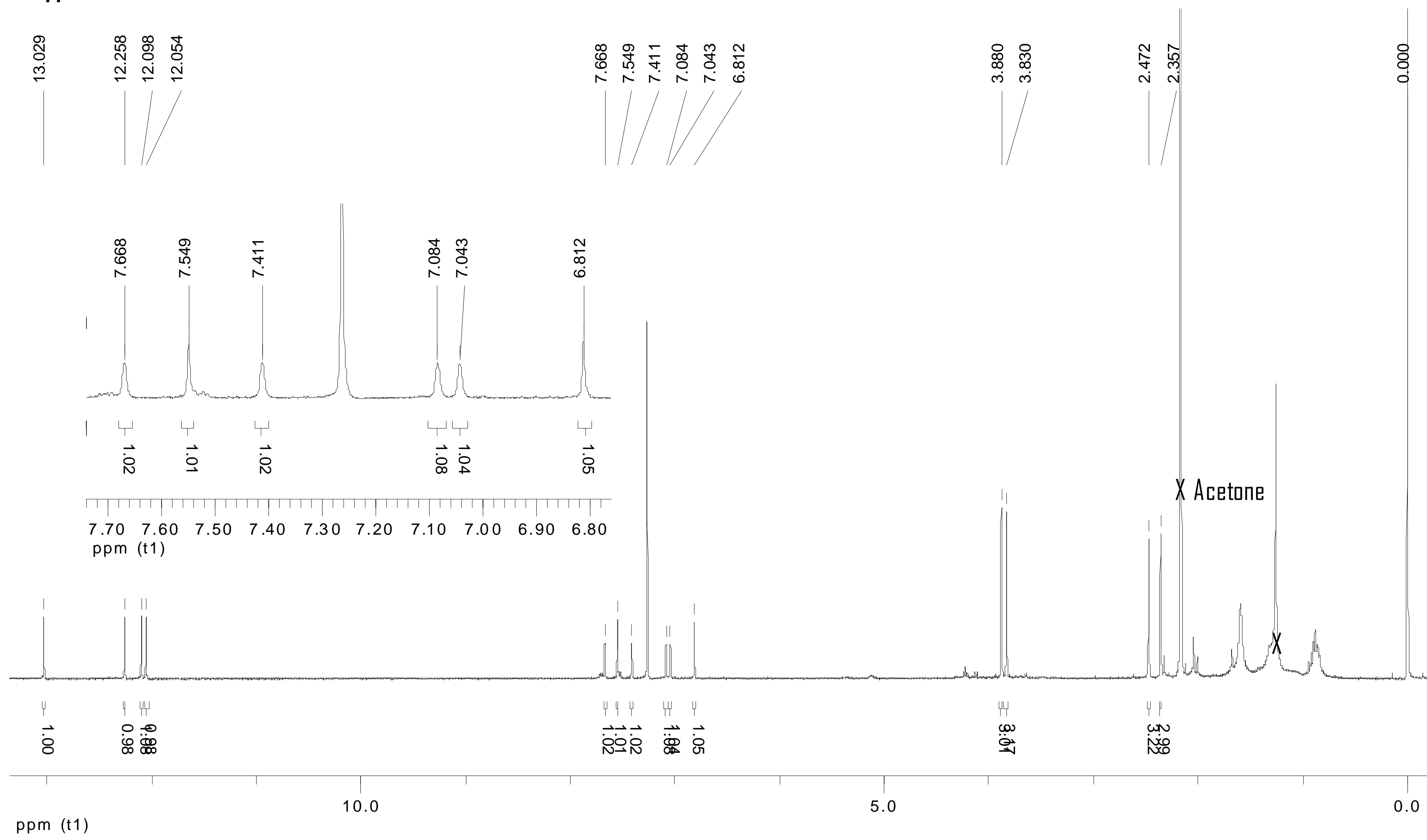
Appendix 3.2 IR of Emodin



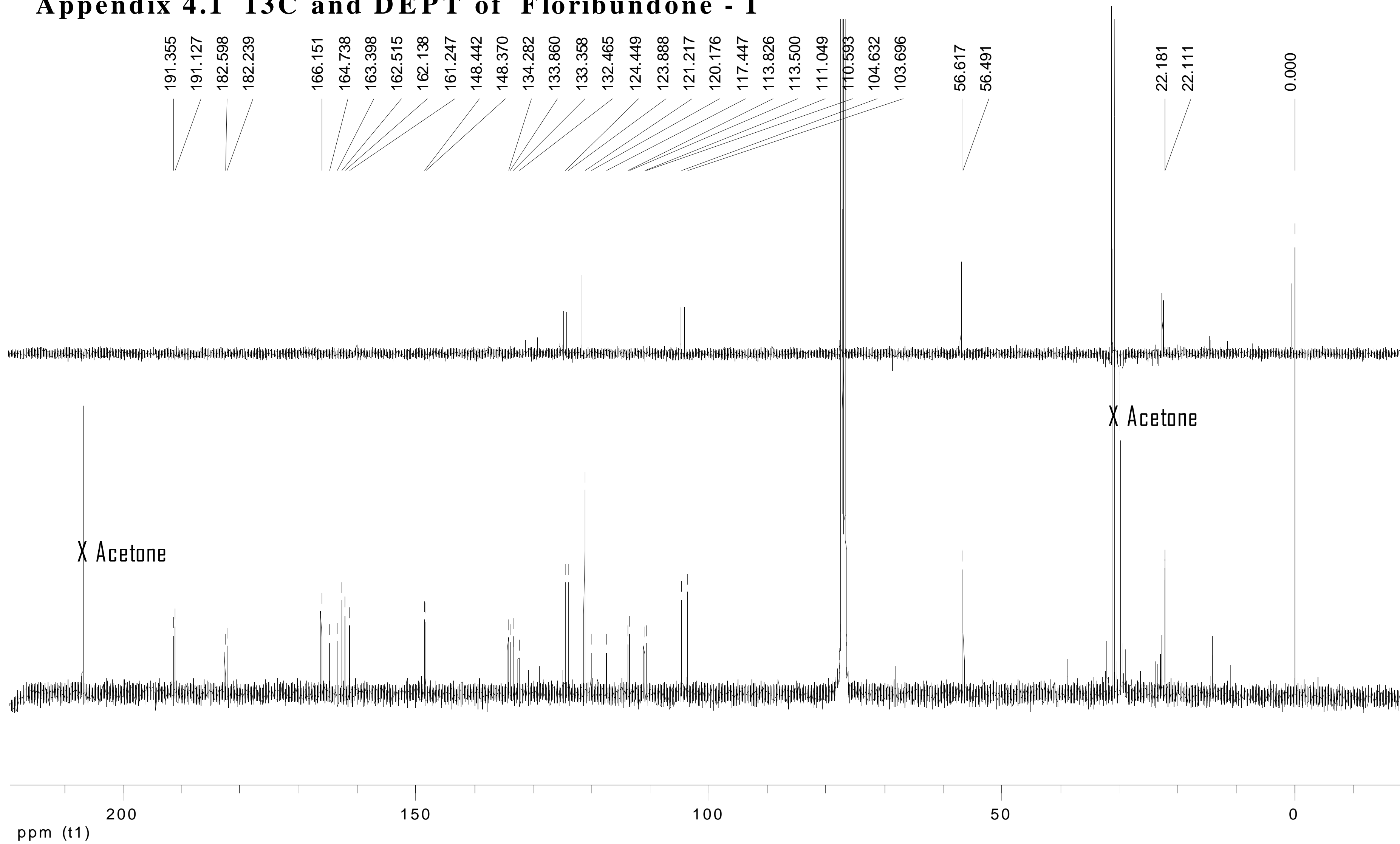
Appendix 3.3 UV-Vis of Emodin



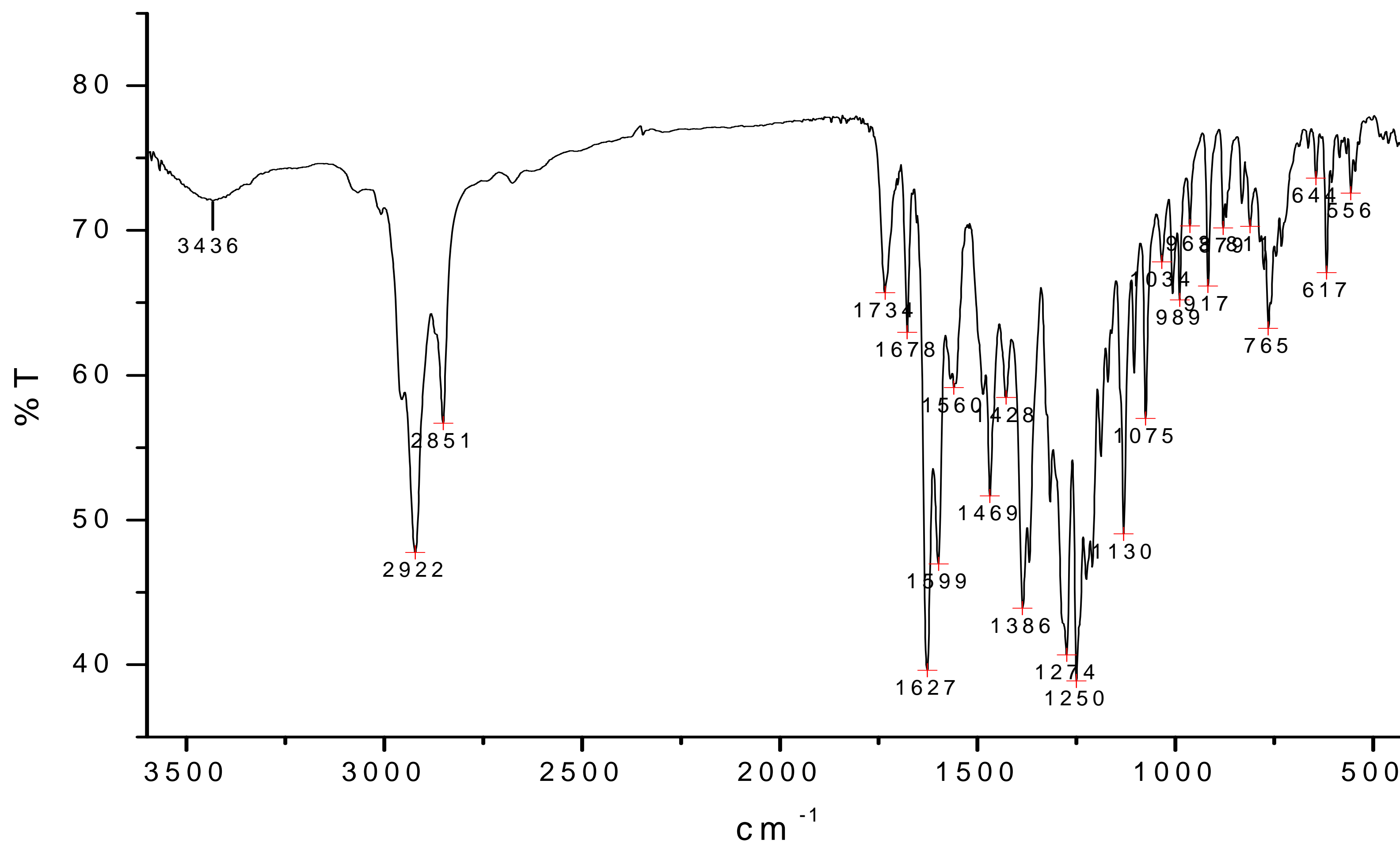
Appendix 4.0 HNMR of Floribundone - 1

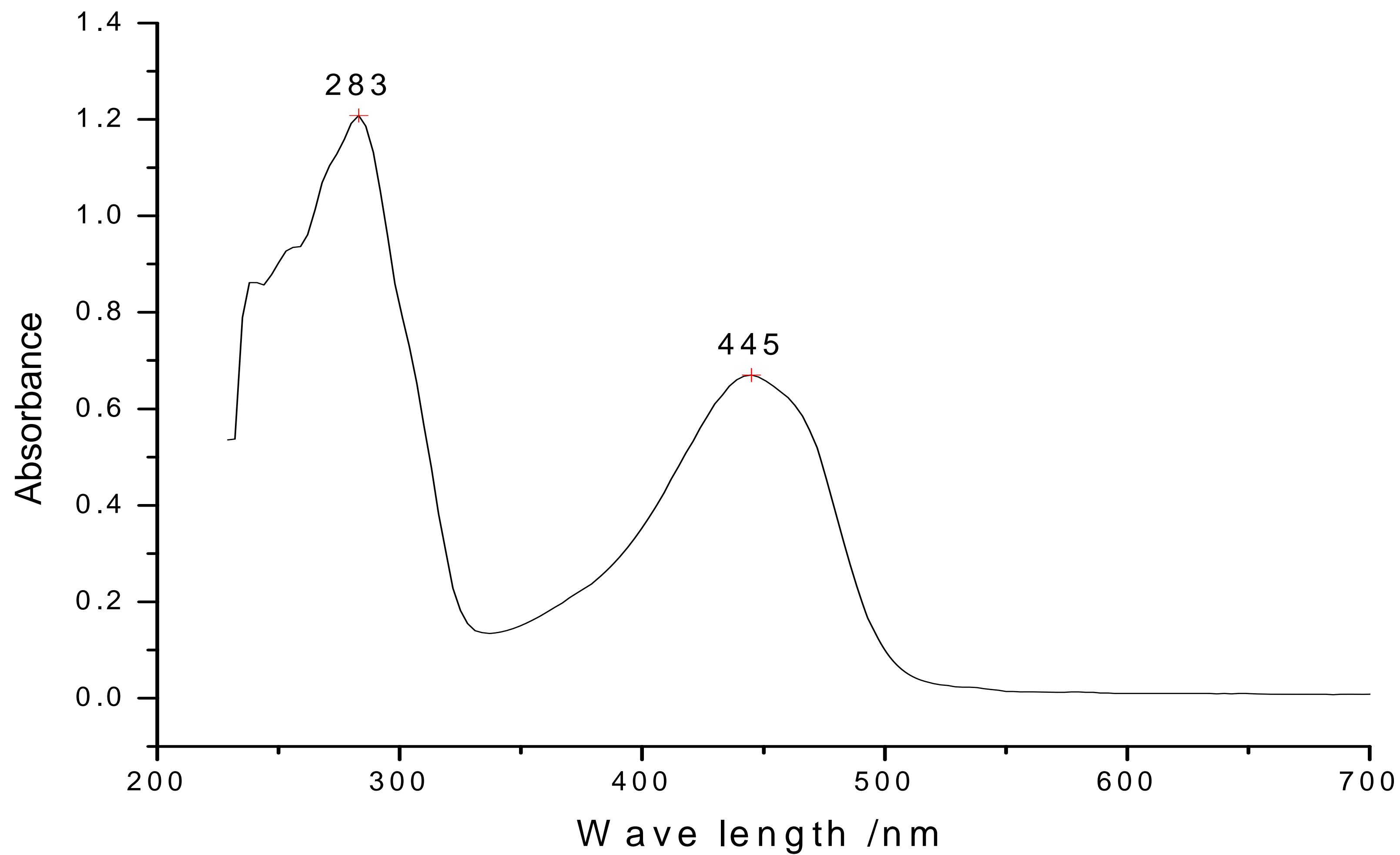


Appendix 4.1 13C and DEPT of Floribundone - 1

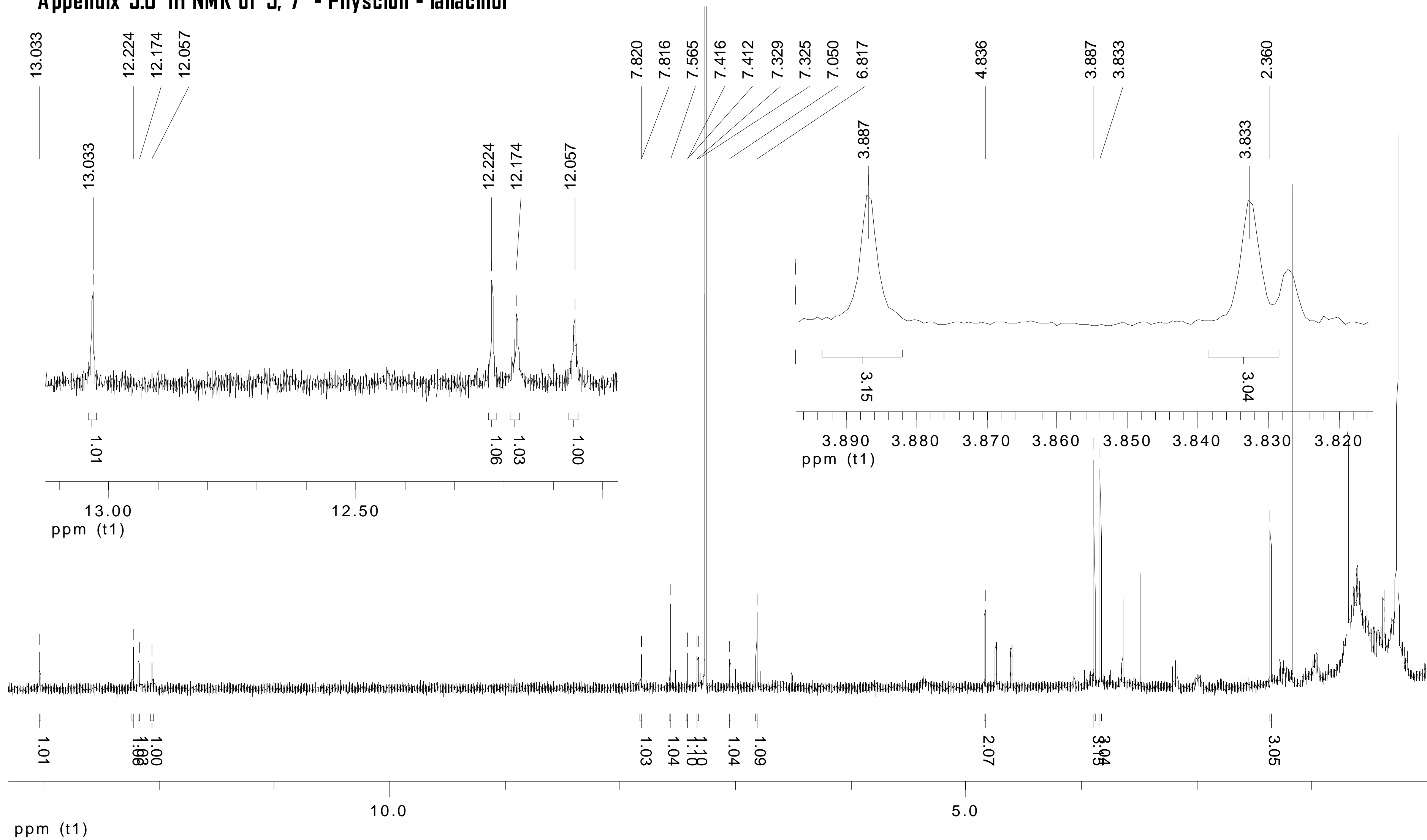


Appendix 4.2 IR of Floribundone-1

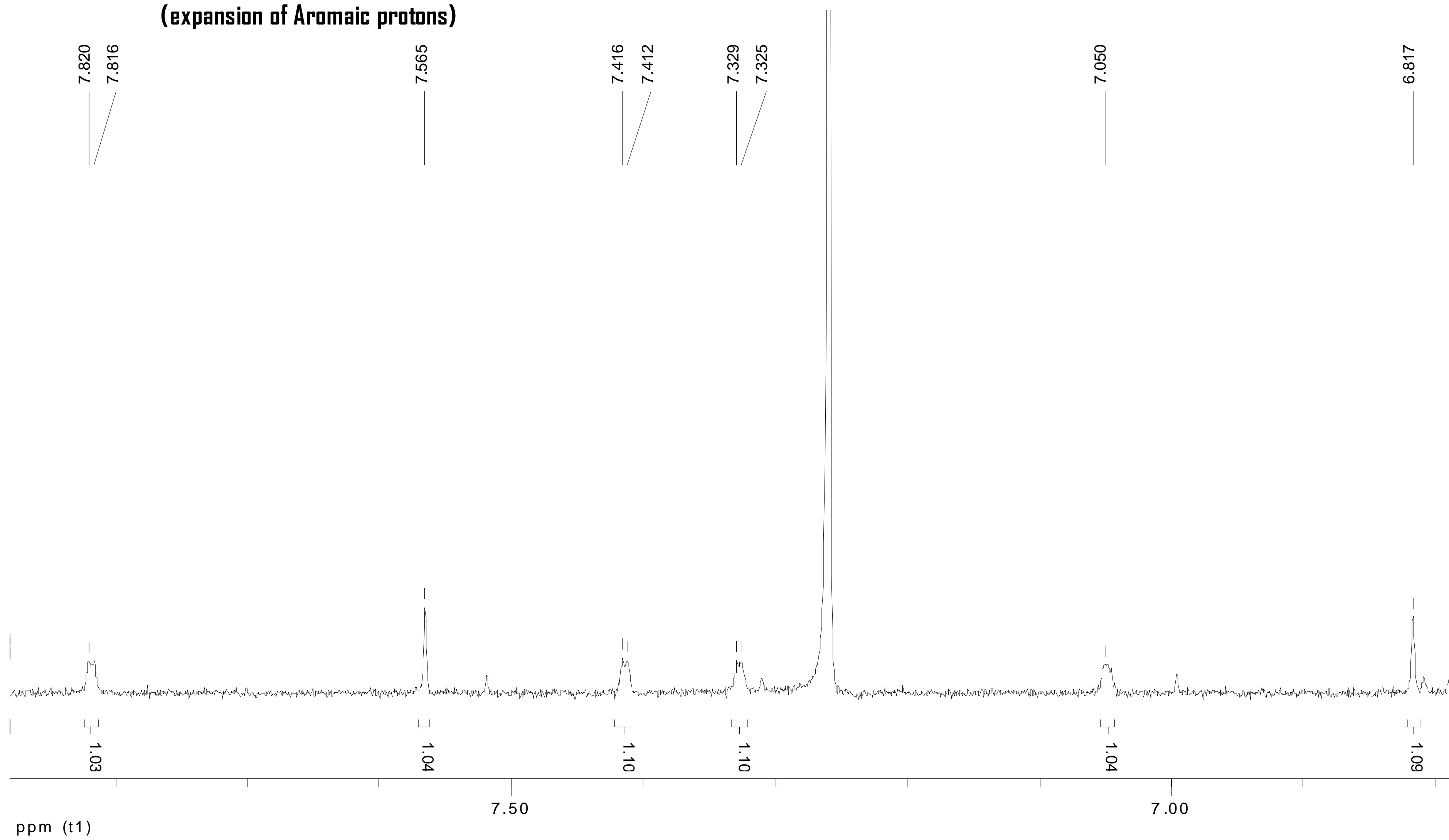


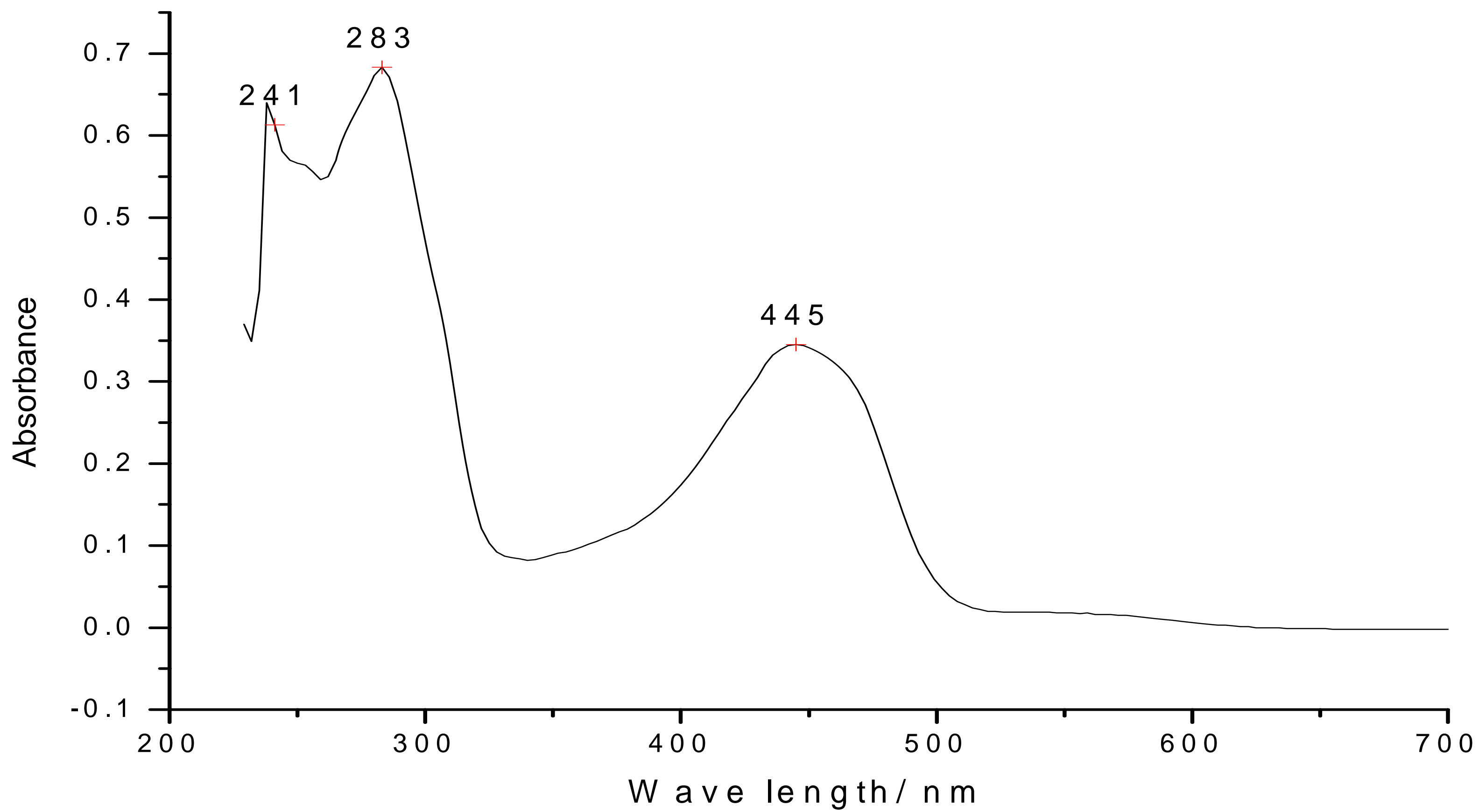

Appendix 4.3 UV-Vis of Floribundone-1
283
445
Absorbance
Wave length /nm
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
200
300
400
500
600
700

Appendix 5.0 1H NMR of 5, 7' - Physcion - fallacinol



Appendix 5.0.1 1H NMR of 5, 7' - Phy scion-fallacinol (expansion of Aromaic protons)




Appendix 5.1 UV-Vis of 5,7'-Physcion-fallacinol
283
241
445
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
200
300
400
500
600
700
Wave length/ nm