ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

PHYTOCHMICAL INVESTIGATION ON THE LEAVES OF *SENNA SOPHERA*

MENGISTU WOLDEMARIAM

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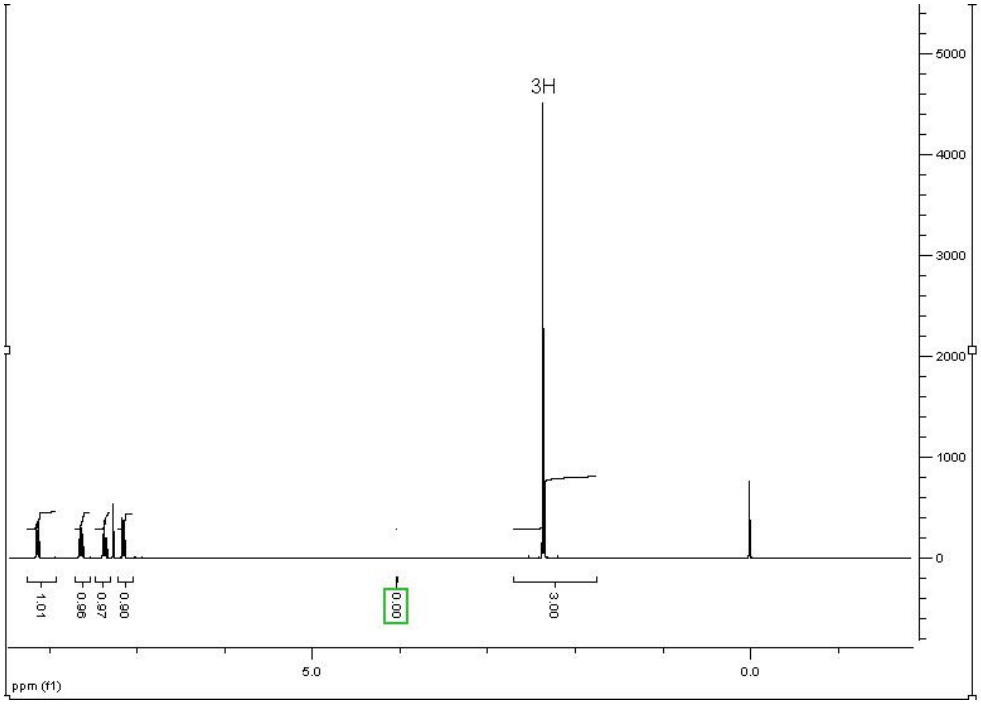
ABSTRACT

The chloroform extract of *Senna sophera* leaves, after repeated chromatography separation have yielded three bianthraquinones. Three of these were identified to be floribundone-1(**52**),10-hydroxfloribundone-2 (**V**) and 5,7'-physcion-fallacinol (**74**). To the best of our knowledge, there is no prior report on the isolation of 10-hydroxyfloribundone-2 from any *Senna* species. 5, 7'-physcion-fallacinol reported for the first time from the plant and floribundone-1 was isolated earlier from the plant.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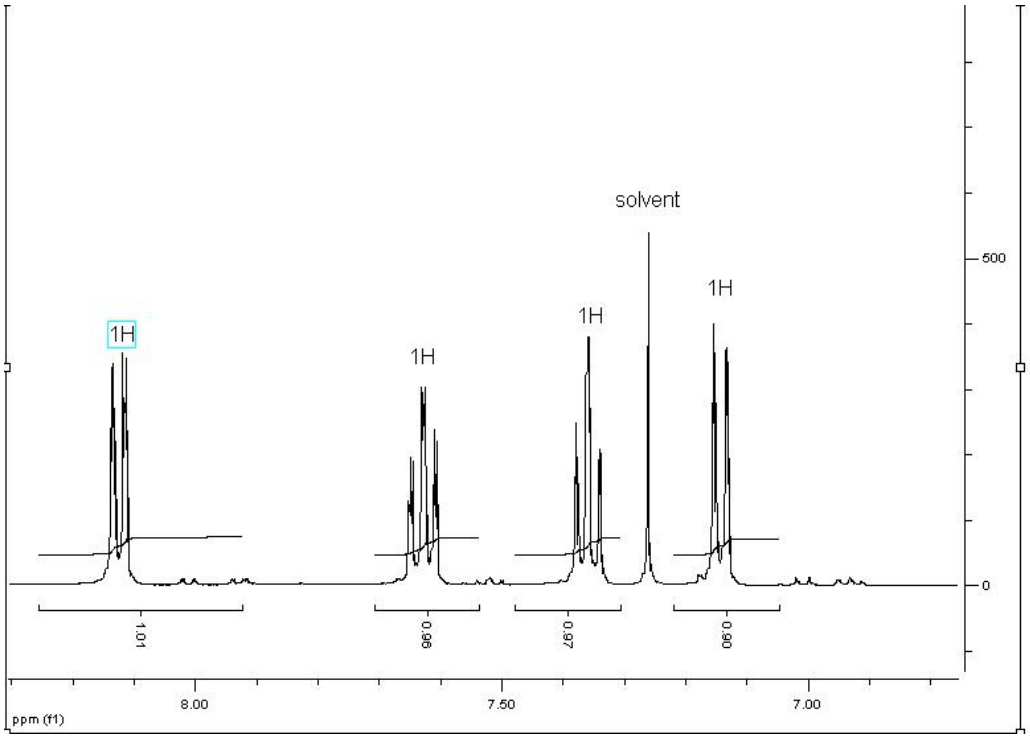
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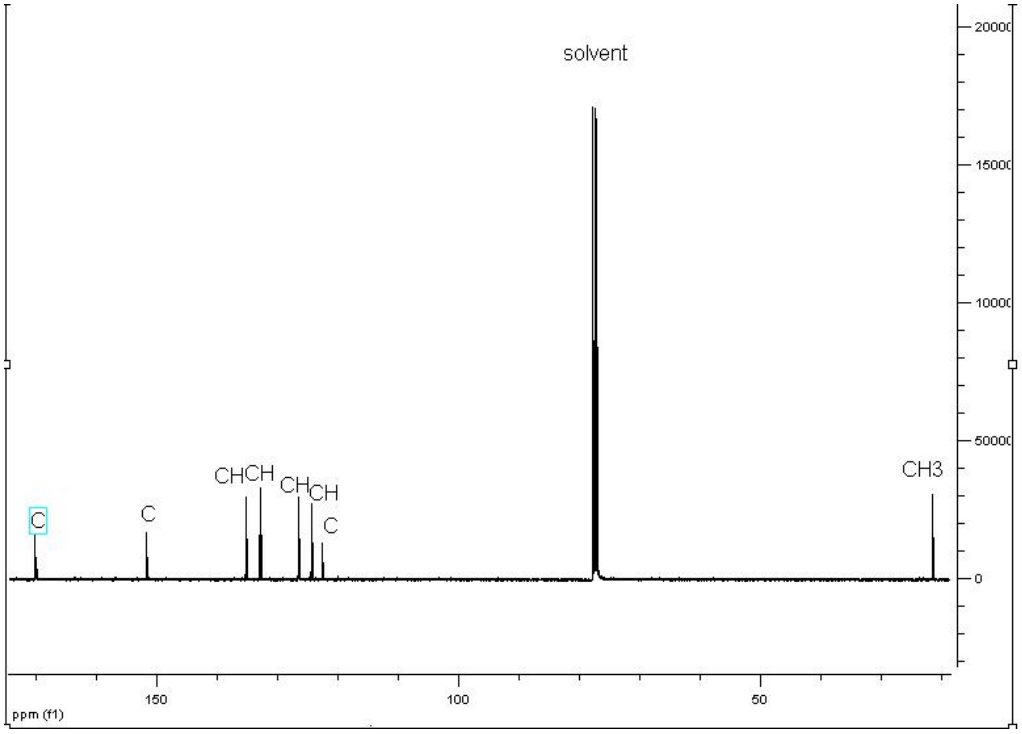
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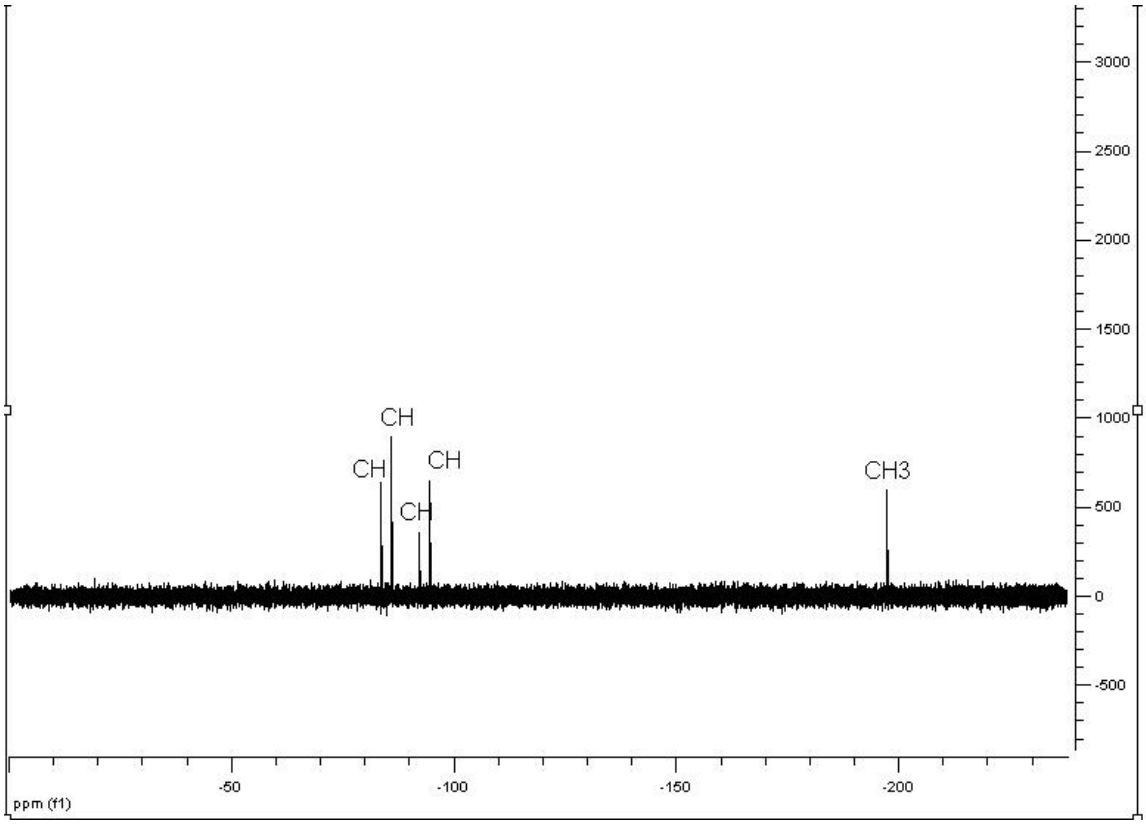
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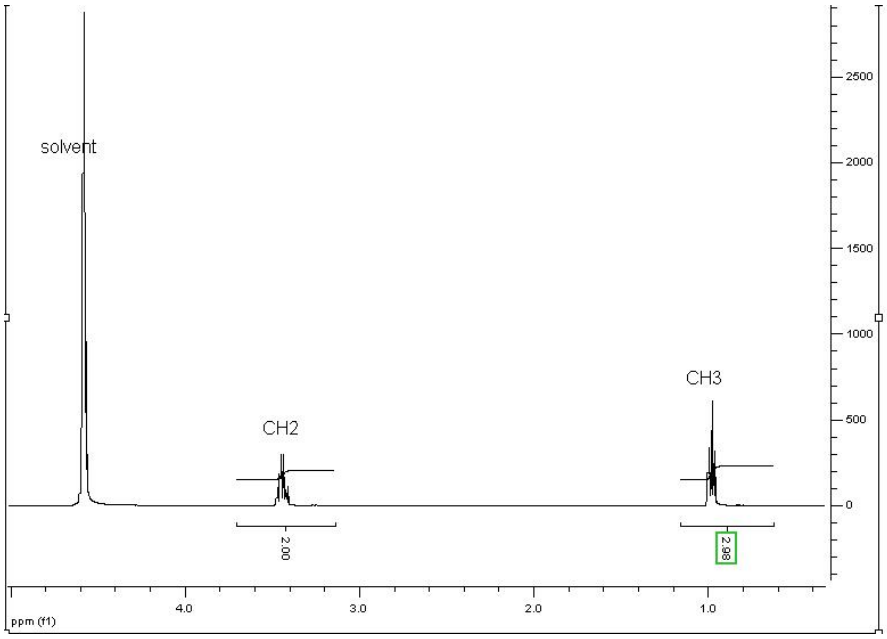
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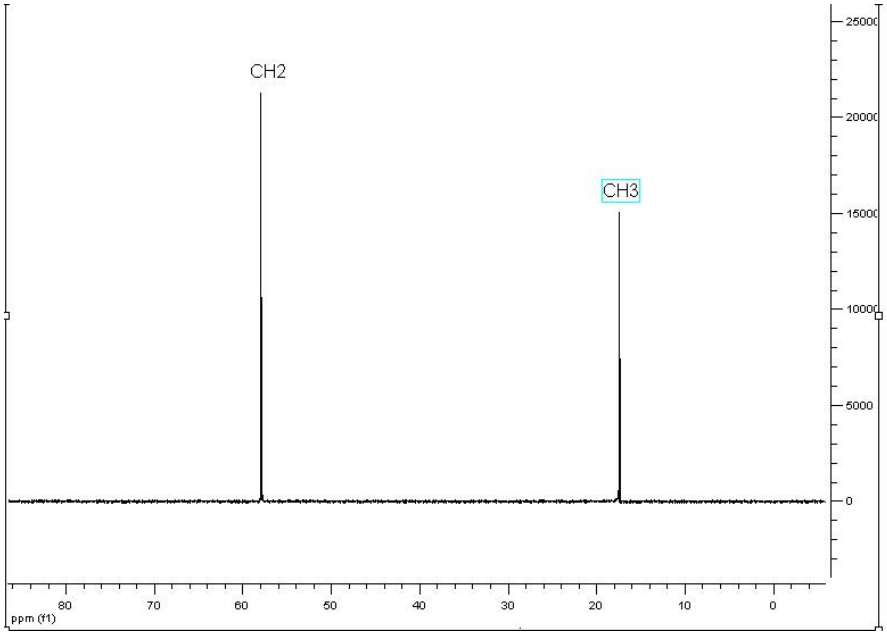
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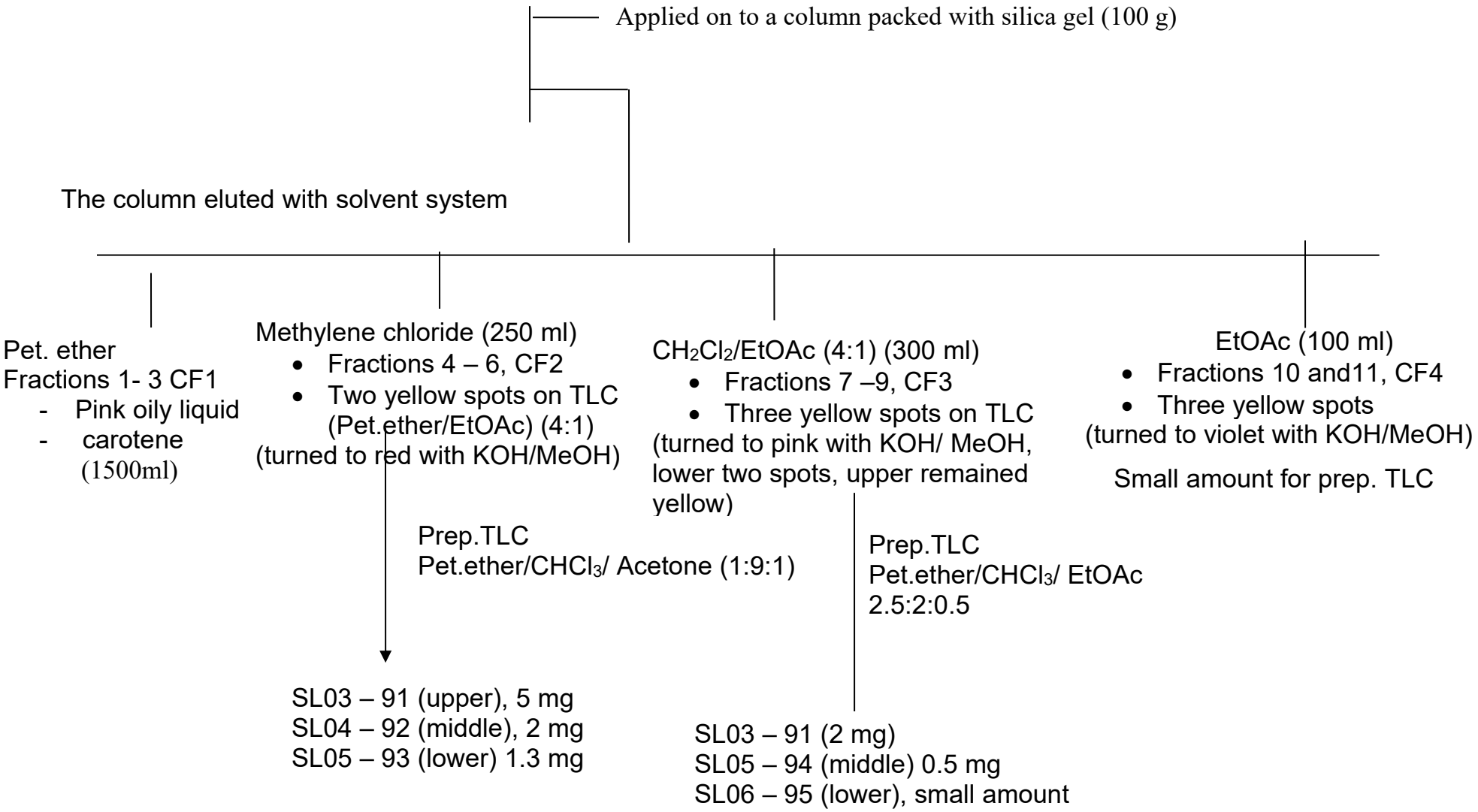
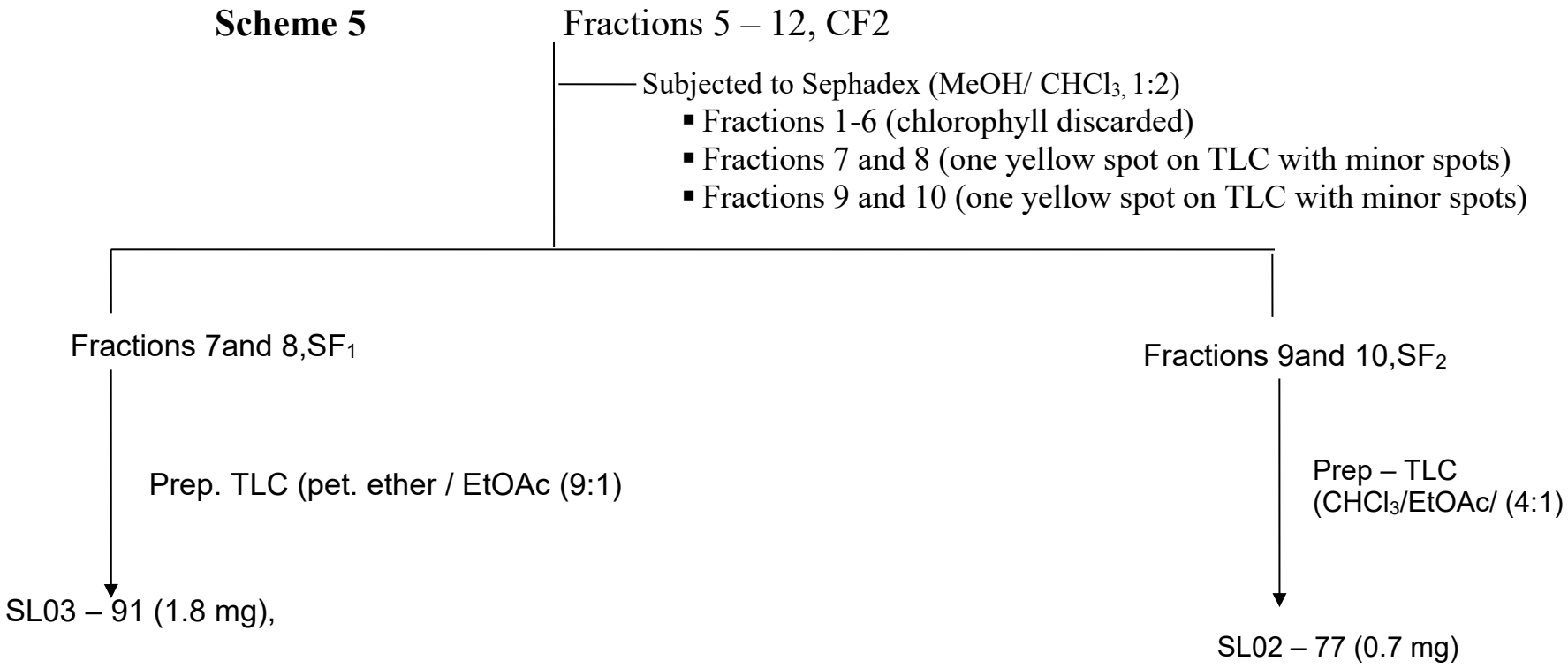
Scheme 4 Fractions 1-4 (10 g), CF



Scheme 5


Fractions 5 – 12, CF2
Subjected to Sephadex (MeOH/ CHCl3, 1:2)
Fractions 1-6 (chlorophyll discarded)
Fractions 7 and 8 (one yellow spot on TLC with minor spots)
Fractions 9 and 10 (one yellow spot on TLC with minor spots)
Fractions 7and 8,SF1
Fractions 9and 10,SF2
Prep. TLC (pet. ether / EtOAc (9:1)
Prep – TLC
(CHCl3/EtOAc/ (4:1)
SL03 – 91 (1.8 mg),
SL02 – 77 (0.7 mg)

Research into the chemical and biological properties of natural products over the past two centuries has not only yielded drugs for the treatment of many human ailments, but has provided the stimulus for the development of modern synthetic organic chemistry, and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents.[6]

Of the 119 plant derived drugs commonly in use, 74% were discovered as a result of chemical studies directed at the isolation of the active constituents of plants used in traditional medicine.[3] Examples include reserpine for the treatment of cardiac arrhythmias, vincamine as a vasodilator, vinblastine and vincristine as anti-tumor agents.[4] The effect of quercetin (common in *Senna* species) on the infectivity and replication of HSV-I (herpes simplex virus type-I), Polio virus type-I, Parainfluenza virus type-3 and respiratory syncytial virus (RSV) has been studied in cell culture and observed that quercetin caused a concentration- dependent reduction in the infectivity of each virus and, in addition, that intracellular replication of virus was reduced.[5] The cardiac glycosides from *Digitalis purpurea L*, the antihypertensive agent and traquilliser, reserpine, from the East Indian snakeroot, *Rauvolfia serpentina (L).* Bentham ex kurz, the antimalarial agent, quinine, from *Cinchona* species, and the analgesics, codeine and morphine, from *Papaver samniferum L*.[1] The finding of alkaloids from *Catharanthus roseus* with a specific effect in the human bone marrow led to phytochemical and pharmacological research, which provided modern medicine with vinblastin and vincristin, which are used against leukaemia in childern and against Hodgkin's lymphonia.[8]

Of the estimated 250,000 currently known higher plant species, 5-15% have been systematically investigated for the presence of bioactive compounds.[10] The fraction submitted to biological and pharmacological screening is even lower. Approximately 60% of the anti-tumor and anti-infective agents that are commercially available or in late stages of clinical trials today are of natural product origin.[7] Of the 52 hypertension drugs, 52% are synthetics and 48% are

synthetics modeled on natural products parents. Of the 87 approved anticancer drugs, 62% are of natural origin or are modeled on natural products parents.[10]

1.3 *Senna species*

The genus *Senna* which belongs to the Leguminosae family has about 240 species mainly found in the tropical and subtropical zones of the world. There are 38 species of *Senna* in Africa of which 18 are in Ethiopia.[11] These are,
S alexanderiana, S. baccarinii, S. bicapsularies, S. didymobotrya, S. ellisae, S. holosericea S. italica, S. longriacemosa, S. multigladulosa, S. obtusifolia, S. occidentalis, S. petersiane, S. ruspolii, S. septemtrionalis, S. siamea, S. singueana, S. sophera.

People living in the village of Africa, Asia and other parts of the developing world resort to traditional medicines for the continued maintenance of their health. [4,12,13] In this context, several species of *Senna,* having important medicinal properties, are also used as vital resort. They are used in the treatment of sexually transmitted diseases, skin diseases and are source of the well known *Senna* purgative. Some of them are also useful as appetizer.[14] Many of them are found to possess insecticidal properties and some exhibit antibiotic properties.
The importance of *Senna* species as a drug is compiled in Table 1.

Table 1. Ethno medical Application of *Senna*

***Senna* Species**	**Parts of the plant used**	**Bioactivities**
S. hirsuta	- Leaves	- Hepatic diseases, coughs and used as sedatives and analgesics. (Gabon).[15] - Used to treat psoriasis, eczema and constipation.[16]
S. alata	- Leaves	• Diarrhea (Botswana).[11] • For stomach pain, jaundice and worms (Cameroon). [21,24]
S. italica	- Roots - Leaves	• For digestive disorders and to free the placenta (Namibia) • For treatment of fever (Ethiopia)[17]
S. singueana	- Leaves and fruit - Leaves and roots	• For gonorrhea, bilharzias, heart burn, wounds, snake-bites and syphilis (Tanzania). [15, 20, 24]

***Senna* Species**	**Parts of the plant**	**Bioactivities**
S. tora	Seeds	- Improve vision (China).[18]
S.occidentalis	Seeds	- Mild purgative and a tonic (Japan and China). [18,19,20,21]
		- Ascaricide, to relieve abdominal pains,
	Leaves and roots	- Remedy for (abdominal pain, snake-bite and kidney troubles) anathematic against round worm, edema, fevers, malaria, antidote stomachache and pain-killer. [11,15,20,23]
		- Laxatives, for diarrhea, dysentery and vomiting.[24]
S.sieberiana	Pods and roots	
		- Remedy for ringworm infection.[25]
S. didimobotrya	Leaves	- Anti-malarial, purgative, for the treatment of (gonorrhea, cattle skin diseases, and backaches), and as appetizer. [14]
	root and stem bark	- Purgative and as an ingredient in fever remedies
S. acutifolia	Leaves and roots	- For the treatment of skin disease. [19]
S. surattensis	Fruits	- For warding off snakes. [19]
S. septemtrionalis	Leaves and roots	- Laxatives, as remedy for skin diseases and acute bronchitis.[11,26]
S. sophera	Seeds and roots	- Remedy for stomach trouble, for quickening of birth and antibiotic. [11,15,24]
S. obtusifolia	Aerial parts	

S.petersiana	Root and leaves	- For gonorrhea, haematuria, sterility, skin disease, coughs, syphilis, stomachache and as a purgative. - Promote menstruation and as purgative.[24]
S.septemtrionalis	Fruits	- Purgative. [11,24]
S. alexandrina	Roots, bark, and Leaves	- Stomach complaints in children. [15]
S. bicapsularis	Roots	

1.4 Pharmacological action

The biologically active compounds from the genus *Senna* are the purgative drugs, sennosides, which are glycosides of the anthranol dimers.[28] The leaves and fruit of *Senna* are effective both orally and rectally, and are counted among the most reliable and the strongest purgatives. Fresh leaves and fruit but especially seeds have a severe irritating effect. To avoid colic, severe irritation of the bladder and stimulation of the uterus up to abortion, only dried leaves and fruit or pods are used in the form of bathing.[16] The strong but slow acting purgative effect is attributed to anthraquinones, they are large intestine purgatives and cause fluid secretions in the intestinal lumen. 1,5-Dihydroxy-3-methoxy-7-methyl-anthraquinone was found to be non-toxic in human clinical trial. [29]

Singueanol-I (**96***)* exhibits papaverine-like antispasmodic activity. Torosachrysone (**1**) germichrysone (**5**)**,** singueanol-I and singueanol-II (**79**) inhibit the growth of gram-positive bacteria.[30] Taniguchi *et al found that* the 60% methanol extract of the root bark of S. *singueana* inactive against *Escherechia coli, Bacillus subtilis, Saccharomyces cereviseae* and *Penicillium crustosum.*[31]

Antifungal activity of some component of S. *alata* flowers was examined against five fungi. The crude methanolic extract and the partially purified fractions were both active against standard strains of *Aspergillus niger, Geotricum candidum,* and *Candida utilis,* and local isolates of *Aspergillus brevipes* and *Penicillium* species, at different concentrations. The partially purified S. *alata* extracts exhibited a relatively high antifungal activity against mycelia growth with total suppression of sporulation for four days at a concentration of 2mg/ml, while preventing growth after the seventh day. The crude methanolic extract lost its activity after 48 hours.[32] Phytochemical studies on S. *skinneri* and S. *wislizeni* resulted in the isolation of rutin, quercetin, 5,7-dimethoxyquercetin and 5,7-dimethoxyrutin. These flavonoids showed mutagenic activity in *Salmonella* thy*phimurium.*[33] Quercetin inhibits the growth of bacteria, fungi and yeast and posses a potent antiviral activity. It shows strong inhibitory effects against the malarial organism, *Plasmodium falciparum* with IC_{50} = 6.4µg/ml after 48 hours.[16]

2. ANTHRAQUINONE

2.1 Chemical investigation

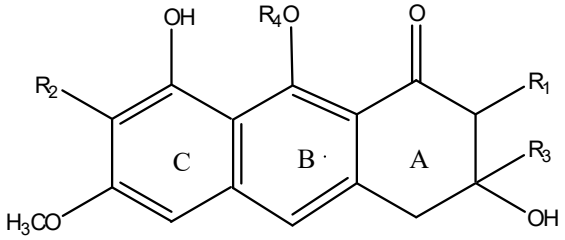
Phytochemical study on the genus *Senna* has led to the isolation and characterization of different classes of secondary metabolites. Anthraquinones constitute an important class of compounds with important biological properties. These compounds elaborated both by higher and lower plants, are also one of the most well known naturally occurring pigments. They range in color from yellow to orange to red. Anthraquinones and related compounds in higher plants are located in all parts of the plant, including root, heartwood, bark, leaves, seeds, flowers and often occur as glycosides. [21] A search through the Dictionary of Natural products shows that there are 409 compounds isolated from the *senna species.*[22]

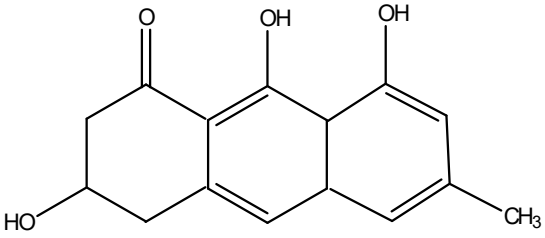
2.2 ANTHRAQUINONES FROM *SENNA*

2.2.1 Pre-anthraquinones

Five pre-anthraquinones have been reported from various S*enna* species. It is assumed that the preanthraquinones are the biosynthetic precursors to the corresponding anthraquinones.[28] They include torosachrysone (**1**) from *S.singueana* root bark,[34] invitro cultures of *S. didymobotrya*[35] and the leaves of *S.didymobotrya*.[36] Torosachrysone (**1**) was first isolated from the seeds of *S.* torosa.[37]7-Methyltorosachryson (**2**) occurs in the tissue cultures of *S.occidentalis*,[38] germitorosone (**3**) and germitorosone-9-methylether (**4)** in *S.torosa*[39] and tissue cultures of *S. occiddentalis*[38] and germichrysone (**5**) in the root bark of S. *singueana*[34] and invitro cultures of *S.didymobotrya*. [3]

Table 2 Pre-anthraquinone from *Senna*





Germichrysone

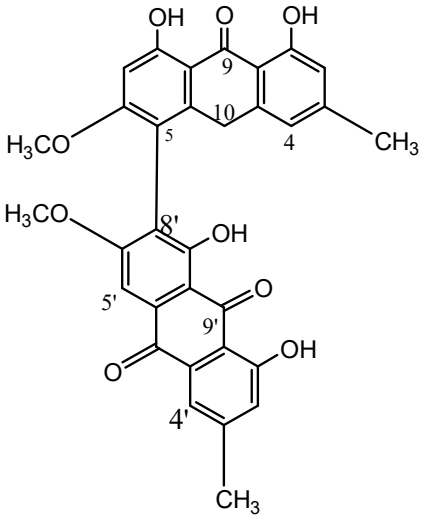
	R_1	R_2	R_3	R_4
1. Torosachrysone	H	H	H	H
2. 7-Methyltorosachrysone	H	Me	H	H
3. Germitorosone	OH	Me	H	H
4. Germitorosone-9-Methyl ether	OH	Me	Me	OMe
5. Germichrysone				

2.2.2 ANTHRAQUINONES

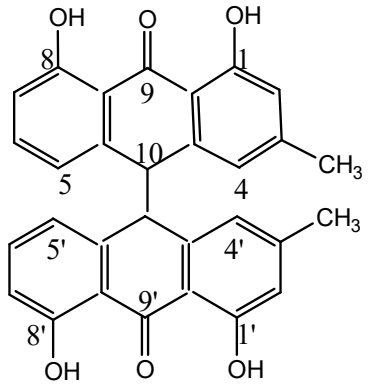
More than 25 *Senna* species studied are known to contain anthraquinones. The identified anthraquinones, with different number of hydroxyl groups and substitution patterns are documented in the literature.[40,41] Numerous dimeric anthraquinones have been isolated and all the dimeric anthraquinones so far reported have only C – C linkage in between the two units. For the past two decades many anthraquinones were isolated from the *Senna* species of Ethiopia. Some of them are:-

The chemical work done on the leaves of *S. floribunda* resulted in the isolation and identification of new bianthraquinones 5, 7'-biphyscion (floribundone-1) (**52**) 5, 7'-(Physcion-anthrone)-physcion (**53**) along with the monomers emodin (**8**) and physcion (**9**)**.** [42] Phytochemical studies on the leaves of *S.didymobotrya* afforded the monomeric anthraquinones chrysophonol (**7**), Physcion, aloe-emodin (**11**), fallacinol (**12**), rhein (**13**), Parietenic acid (**14**) and torosachrysone (**1**).[36]

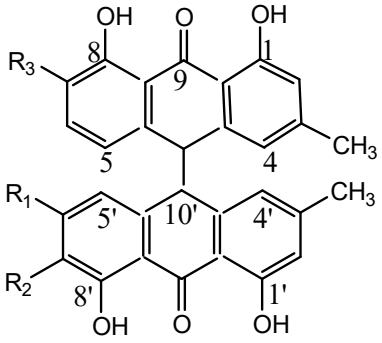
Abegaz et al. isolated bianthraquinones chrysophanol-10,10-bianthrone (**54**) chrysophanol-physcion bianthrone (**55**), isophyscion bianthrone (**56**), 10-(chrysophanonl-7'-yl)-10-hydroxy-chrysophanol-9-anthrone (**57**) and the monomers nataloe-emodine (**10**), chrysophanonl and physcion from *S. longiracemosa*. [43]



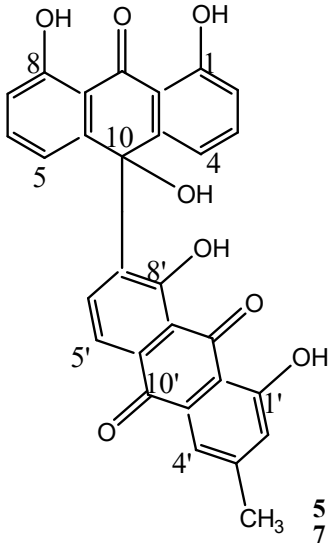
53 5,7;-Physcionanthrone-physcion



54 Chrysophanol-10,10'-bianthrone



	R_1	R_2	R_3	
55	OMe	H	H	Chrysophanol-physcion bianthrone
56	H	OMe	OMe	Isophyscion bianthrone

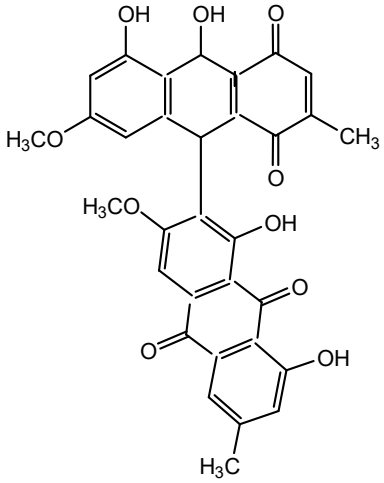


57

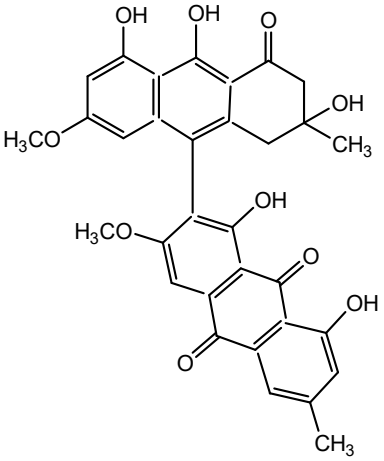
G. Alemayehu et al. isolated a novel bianthraquinone presengulone (**58**) along with sengulone (**59**), isosengulone (**60**), anhydrophlegmacin-9,10-quinone A_2 and

B_2 (**61**), 5,7'-physcion-physcion anthrone (**62**), 5,7'-Physcion anthrone-physcion and the monomers xanthorin (**15**) and chrysophanol from the seeds of *S. sophera.*[44] Sengulone and isosengulone were first reported to occur in S. multiglandulosa by Abegaz et al. [45,46]

Phytochemical studies on the pods of *Senna* septemtrionalis afford a new bianthraquinone 5,7'-physcion-fallacinol (**93**) along with chrysophanol, physcion, emodin, torosachrysone, floribundone-1 and torosanin-9, 10-quinone (**80**).[47]

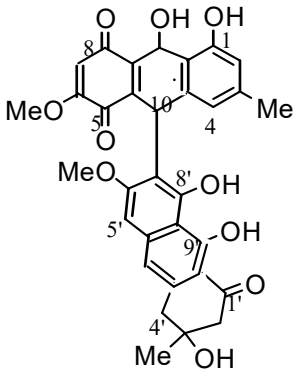


60 Isosengulone

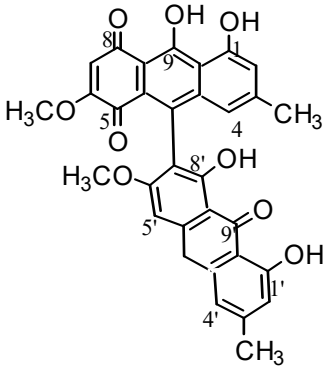


61

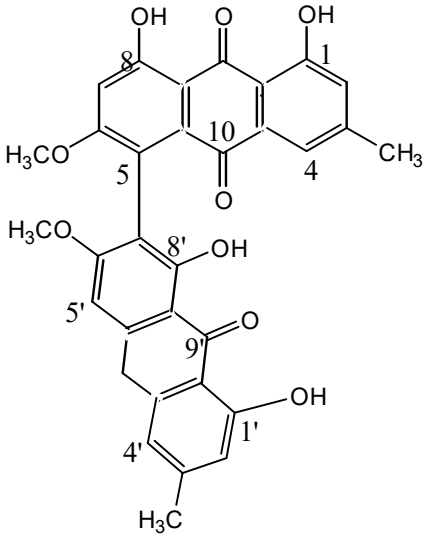
Anhydrophlegmacin-9,10-quinone A_2 and B_2



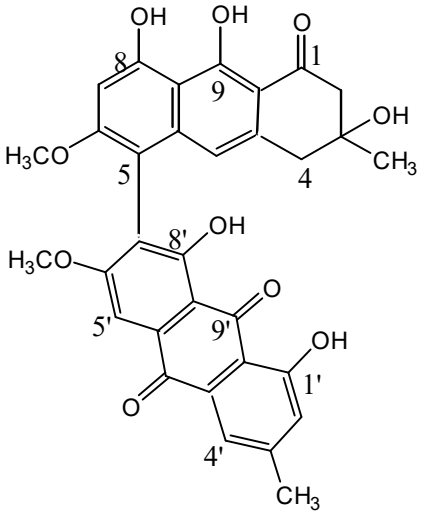
58 Presengulone



59 Sengulone

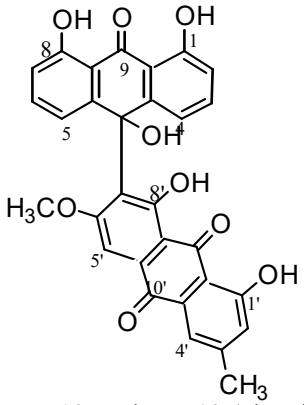


5,7'-Physcion-physcion anthrone

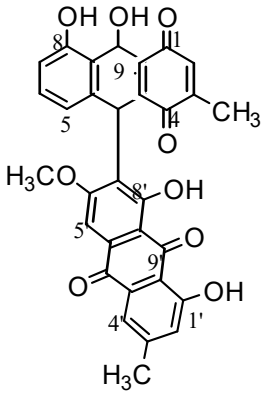


80 Torosanin-9,10-quinone

Studies on the pods of S.didymobotrya afforded two new bianthraquinones, 10-hydroxy-10-(Physcion-7'- yl)-chrysophanol anthrone (**94**) and 5,10-dihydroxy-2- methyl-9-(physcion-7'-yl)-1,4-anthraquinone (**95**) and common anthraquinones emodin, chrysophanol, physcion, and knipholone (**IV**).[58] The known monomeric and dimeric anthraquinone isolated from the *Senna species* are given in Table 3, 4, 5 and 6

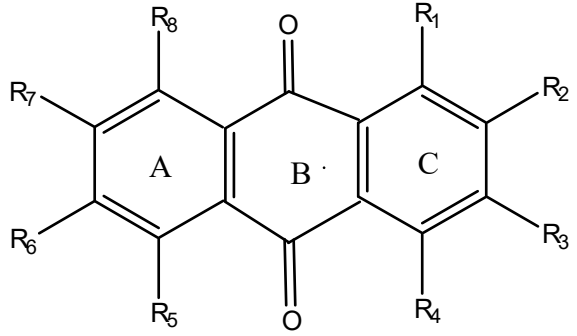


94 10-Hydroxy-10-(physcion-7'-yl)-chrysophanol anthrone



95 5,10-Dihydroxy-2-methyl-9-(physcion-7'-yl)-1,4- anthraquinone

Table 3. Anthraquinones of the genus *Senna* (Monomeric)



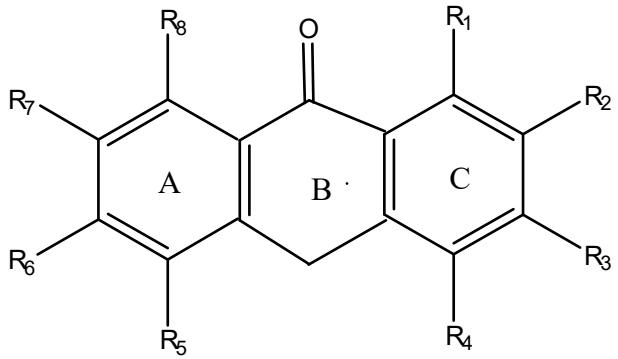
Name		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Source
1,8 – dihydroxy anthraquinone	**6**	OH	H	H	H	H	H	H	OH	
Chrysophanol	**7**	OH	H	Me	H	H	H	H	OH	*Senna spp.*[40]
Emodin	**8**	OH	H	Me	H	H	OH	H	OH	*Senna spp.*[40]
Physcion	**9**	OH	H	Me	H	H	OMe	H	OH	*Senna spp.*[40]
Nataloe - emodin	**10**	OH	H	Me	H	H	H	OH	OH	*S. longeracemosa.*[51]
Aloe – emodin	**11**	OH	H	CH_2OH	H	H	H	H	OH	*Senna spp.*[29]
Fallacinol	**12**	OH	H	CH_2OH	H	H	OMe	H	OH	*S. didymobotrya.*[51]
Rhein	**13**	OH	H	COOH	H	H	H	H	OH	*Senna spp.* [29]
Parietinic acid	**14**	OH	H	COOH	H	H	OMe	H	OH	*S. didymobotrya.*[51]
Xanthorin	**15**	OH	H	Me	H	OH	OMe	H	OH	*S.occidentalis.*[37,50]

Obtusifolin	**16**	OMe	OH	Me	H	H	H	H	OH	*Senna spp.* [40]
Aurantio-obtusin	**17**	OMe	OH	Me	H	H	OH	OMe	OH	*S. obtusifolia.* [37]
Obtusin	**18**	OMe	OH	Me	H	H	OMe	OMe	OH	*S. obtusifolia.* [37]
Chryso-obtusin	**19**	OMe	OH	Me	H	H	OMe	OMe	OMe	*S. obtusifolia.* [37]
Rubiadin	**20**	OH	Me	OH	H	H	H	H	H	*S. mulijuga.* [41]
Damnacanthal	**21**	OMe	CHO	OH	H	H	H	H	H	*S. alata.* [41]
Isochrysophanol	**22**	OH	Me	H	H	H	H	H	OH	*S. alata.* [41]
Chrysophanol-8-methyl ether	**23**	OH	H	Me	H	H	H	H	OMe	*S. speciosa.* [41]
8- Hydroxy rubiadin	**24**	OH	Me	OH	H	H	H	H	OH	*S. spectobilis.* [41]
Questin	**25**	OH	H	Me	H	H	OH	H	OMe	*S. melanoxylon* [41]
Emodin-6,8-dimethyl ether	**26**	OH	H	Me	H	H	OMe	H	OMe	*S. obtusifolia* [41]
Citreorosein -6,8- dimethyl ether	**27**	OH	H	CH_2OH	H	H	OMe	H	OMe	*S. melanoxylon* [41]
Islandicin	**28**	OH	H	Me	OH	H	H	H	OH	*S. occidentalis* [41]
Helmihosporin	**29**	OH	H	Me	H	OH	H	H	OH	*S. occidentalis* [41]
7- Hydroxyemodin-6,8-dimethyl ether	**30**	OH	H	Me	H	H	OMe	OH	OMe	*S. melanoxylon* [41]
5 - Hydroxyemodin	**31**	OH	H	Me	H	OH	OH	H	OH	*S. javanica* [41]
1,4,5- Trihydroxy-7-methoxy-3-methyl-anthraquinone	**32**	OH	H	Me	OH	OH	H	OMe	H	*S. occidentalis* [41]

Name		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Source
1,3,5-Trihydroxy-8-methoxy-2-methyl-anthraquinone	**33**	OH	Me	OH	H	OH	H	H	OMe	*S. alata.* [41]
1,3,8-Trihydroxy-6-methoxy-2-methyl-anthraquinone	**34**	OH	Me	OH	H	H	OMe	H	OH	*S. multijuga.* [41]
1,8-Dihydroxy-3,6 dimethoxy-2-methyl-7-Vinylanthraquinone	**35**	OH	Me	OMe	H	H	OMe	CH =CH_2	OH	*S. sophera.* [41]
1,3-Dihydroxy-6,8-dimethoxy-2-methyl-anthraquinone	**36**	OH	Me	OH	H	H	OMe	H	OMe	*S. multijuga.* [41]
2,7-Dihydroxy-emodin-8-methylether	**37**	OH	OH	Me	H	H	OH	OH	OMe	*S. melanoxylon.* [41]
2,7-Dihydroxy- emodin-6, 8-dimethylether	**38**	OH	OH	Me	H	H	OMe	OH	OMe	*S. sophera.* [53]
1-De-O-methyl-chryso-obtusin	**39**	OH	OH	Me	H	H	OMe	OMe	OMe	*S. obtusifolia.* [41]
1-De-O-methyl-Obtusin	**40**	OH	OH	Me	H	H	OH	OMe	OMe	*S. obtusifolia.* [41]
1-De-O-methyl-aurantio-obtusin	**41**	OH	OH	Me	H	H	OH	OMe	OH	*S. obtusifolia.* [41]
1,3,5 -Trihydroxy-6,7-dimethoxy-2-methyl-anthraquinone	**42**	OH	Me	OH	H	OH	OMe	OMe	OH	*S. tora.* [41]

Name		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Source
1,8-Dihydroxy-3,5,7-trimethoxy-2-methyl- anthraquinone	**43**	OH	Me	OMe	H	OMe	H	OMe	H	*S. renigera* [41]
1,3- Dihydroxy-5,7,8 trimethoxy-2-methyl- anthrnquinone	**44**	OH	Me	OH	H	OMe	H	OMe	OMe	*S. sophera.*[41]
1,3,5,8-Tetrahydroxy-6,7- dimethoxy-2-methyl anthraquinone	**45**	OH	Me	OH	H	OH	OMe	OMe	OH	*S. renigera.* [54]
Sopheranin	**46**	OH	Me	OH	H	H	OH	$CH = CH_2$	OH	*S. sophera.* [48]
Parietinic acid ester	**47**	OH	H	COOMe	H	H	OMe	H	OH	*S. didymobotrya.* [48]
Rhein methyl ester	**48**	OH	H	Me	H	H	H	OH	OH	*S. ongeracemosa.* [48]
1,3,6,7,8-pentahydroxy-4-methoxy-2-methyl anthraquinone	**49**	OH	Me	OH	OMe	H	OH	OH	OH	*S. didymobotrya.*[51]

Table 4. Anthrones of the genus *Senna*



Name		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Source
Chrysophanol-9-anthrone	**50**	OH	H	Me	H	H	H	H	OH	*S. siamea.*[40]
Physcion-9-anthrone	**51**	OH	H	Me	H	H	OMe	H	OH	*S. torosa.*[41]

Table 5. Bianthraquinone of the genus *Senna*

Name		Source
5,7'-biphyscion (floribundone - 1)	**52**	*S. floribunda*. [42]
5,7'-physcion-anthrone -physcion	**53**	*S. floribunda*. [42]
Chrysophanol-10,10'-bianthrone	**54**	*S. longiracemosa*. [43]
Chrysophanol-physcion-10,10'-bianthrone (palmidin D)	**55**	*S. longiracemosa*. [43]
Isophyscion bianthrone	**56**	*S. longiracemosa*. [43]
10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone	**57**	*S. longiracemosa*. [43]
Presengulone	**58**	*S. sophera*. [44]
Sengulone	**59**	*S. multiglandulosa*. [45,46]
Isosengulone	**60**	*S. multiglandulosa*. [45,46]
Anhydrophlegmacin-9,10-quinone A_2 and B_2	**61**	*S. sophera*. [44]
5,7'-Biphyscionanthrone	**62**	*S. sophera*. [44]
Cassiamin C (2,2' - bichysophanol)	**63**	*S. siamea*. [50]

Name		Source
Cassiamin A (2,2'-Chrysophanol-emodin)	**64**	*S. occidentalis*. [50]
Aloe-emodin-10,10'-bianthrone	**65**	*S. occidentalis*. [50]
Palamidin D (Chrysophanol-physcion-10,10'-bianthrone)	**66**	*S. longiracemosa*. [50]
Cassiamin B (2,2'-biemodin)	**67**	*S. siamea.*[40]
Chrysophanol-10,10'-bianthrone	**68**	*S. garrttiana.*[40]
Siamianin (4,4'-bichrysophonol)	**69**	*S. occidentalis*. [41]
Chrysophanol-9,9'-bianthrone	**70**	*Senna spp.*[51]
Phlegmacines A_2 and B_2 (7,10'-bitorosa-chrysone)	**71**	*S. torosa* [52]
Anhydrophlegmacin B_2 (7,10'-physcion anthrone-torosachrysone)	**72**	*S. torosa* [52]
Torosanin (7,5'-physcionanthrone-torosa-chrysone)	**73**	*S. torosa* [52]
Floribundone-2-(7, 5'-physcionanthrone-physcion)	**74**	*S. septemtrionalis* [52]
Chrysophanol-isophyscion-10,10'-bianthrone	**75**	*S. longiracemosa.* [43]
10-Hydroxy-5,7'-(Chrysophanol anthrone) chrysophanol	**76**	*S. longiracemosa.* [43]

Name		Source
Siamiadin (4,4'-Chrysophanol-emodin)	**76**	*S. siamea*.[50]
Cassianin	**77**	*S. siamea*.[50]
Singueanol -II	**78**	*S. siamea*.[50]
Torosanin-9',10'-quinone	**79**	*S. multiglandulosa*.[45]
10,10'-Bichrysophanol	**80**	*S. longiracemososa*.[43]
Physcion-10,10'-bianthrone	**81**	*S. ongiracemososa*.[43]
10,10-Chrysphonol-physcion	**82**	*S. longiracemososa*.[43]
Rheidin **B** (rhein-Chrysophanol bianthrone)	**83**	*S. alexanderiana*.[55]
Palmidin **C** (emodin-chrysophanol bianthrone)	**84**	*S. alexanderiana*.[55]
Palmidin **B** (aloe-emodin-chrysophanol-bianthrone)	**85**	*S. alexanderiana*.[55]
Rheidin **A** (rhein-emodin-bianthrone)	**86**	*S. alexanderiana*.[55]
Emodi -bianthrone	**87**	*S. Alexanderiana*.
Palmidin **A** (aloe-emodin-emodin bianthrone)	**88**	*S. alexanderiana*.

Name		Source
Occidentalol-I	**90**	*S. occidentalis.* [56]
Occidentalol-II	**91**	*S. occidentalis.* [56]
Singueanol-III	**92**	*S. occidentalis.* [56]
5, 7'-physcion-fallacinol	**93**	*S. septemtrionalis.* [47]
10-Hydroxy-10-(Physion-7'-yl)- chrysophanol anthrone	**94**	*S. didymobotrya.* [58]
5,10-dihydroxy-2-methyl-9-(Physcion-7' - yl)-1,4-anthraquinone	**95**	*S. didymobotrya.* [58]
Singueanol-I	**96**	*S. siamea.* [50]

2.2.3. ANTHRAQUINONE GLYCOSIDES

The presence of sugar moiety in anthraquinone and also the type of sugar, although, presumably has no direct activity of its own, is paramount for the enhancement of pharmacological activity of the aglycone[57]. Anthraquinones are prone to metabolic detoxification.The presence of the sugar group confers a resistance on the glycosides such that it withstands this detoxification mechanism of the body.[57] Anthraquinone glycosides of the genus *Senna* are given in Table 6.

Table 6. Anthranoid glycosides of the genus *Senna*

Name		Source
Aloe-emodin-8-mono-β-D-glucoside	**97**	*Senna spp*. [40]
Rhein-8-mono-β-D-glucoside	**98**	*S. occustifolia.*[40]
Sennosides **A** and **B** (Birhein-9-anthrone-8,8' -diglucoside)	**99**	*S. alexanderiana.* [40]
Sennosoides **C** and **D** (Aloe-emodin-rhein-bianthrone-8,8'- diglucooside**)**	**100**	*S. angustifolia.* [40] *S. alexanderiana.* [59,60]
Chrysophonol-1-O-β-gentiobiside	**101**	*S. tora.*[41]
Cassialin (10-hydroxy-10-C-D-glucosylchrysophanol-anthrone)	**102**	*S. gerrettiana.*[61]
Physcion-1-O-β-D-glucoside	**103**	*Senna. spp.*[40]
Physcion-1-glucosylrhamnoside	**104**	*S. tora.*[41]
Physcion-8-gentiobioside	**105**	*S. laevigata.*[41]
Physcion-8-galactoside	**106**	*S. tora.*[41]
Physcion-8-digalactoside	**107**	*S. laevigata.*[41]
5-Hydroxydiemodin-8-O-rhamonside	**108**	*S. lavanica.*[41]

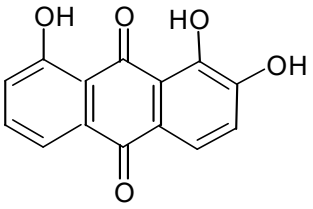
1,3,5-Trihydroxy-8-methoxy-2-methylanthraquinone-3-O-rutinoside	**109**	*S. multijuga.*[41]
1,3-Dihydroxy-6,8-dimethoxy-2-methyl-anthraquinone-3-O-α-L(-) rhamnopyranoside	**110**	*S. renigera.*[41]
1-Hydroxy-8-methoxy-2-methyl anthraquinone-3-O-α-L-(-) rhamnopyranoside	**111**	*S. renigera.*[41]
Physcion-8-O-α-L-xylopyronoside	**112**	*S. marginata.*[62]
Emodin-8-O-α-L-arabinopyranoside	**113**	*S. marginata.*[62]
Emodin 8-O-sophoroside	**114**	*S. alexanderiana.* [63]
Aloe-emodin dianthrone 8,8'-di-O-glucoside	**115**	*S. alexanderiana.* [63]
Physcion 8-O-galactose	**116**	*S. septemtrionalis.* [65]
Rhien-8-diglucoside	**117**	*S. alexanderiana.* [64]
Rhien anthrone-8-glucoside	**118**	*S. alexanderiana.* [64]
Aloe-emodin glucoside	**119**	*S. alexanderiana.* [64]
Chrysophanic acid glycoside	**120**	*S. alexanderiana.* [67]
Physcion 1-β-D-glucopyranoside	**121**	*S. occidentalis.* [68]
Sennoside A[1]	**122**	*S. alexandrina.* [68]

Aloe – emodin anthrone monoglucoside	**123**	*S. alexanderiana.* [69]
Aloe – emodin anthrone diglucoside	**124**	*S. alexanderiana.* [69]
Emodin athrone monoglucoside	125	*S. alexanderiana.* [69]
Chrysophanol anthrone diglucoside	**126**	*S. alexanderiana.* [69]
Physcion anthrone diglucoside	**127**	*S. alexanderiana*.[69]
Rhien anthrone diglucoside	**128**	*S. alexanderiana*.[69]
Obtusifolin mono-β -glucoside	**129**	*S. obtusifolia*.[70]
Aurantioobtusin-6-monoglucoside	**130**	*S.obtusifolia*.[70]

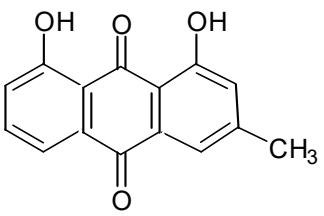
2.1.3. Biosynthesis of anthraquinones

Generally the biosynthesis of anthraquinones is believed to follow two routes, the acetate-malonate pathway and the shikimate-mevalonate pathway. [71] Based on their structure and biosynthesis, anthraquinones are either the emodin-type or the alizarin type. It appears that, at least in higher plants, those anthraquinones with substituents on both benzenoid A and C rings, follow the acetate-malonate biogenetic route, and are the emodin type. Anthraquinones of the alizarin-type are, totally devoid of substituents on one benzenoid ring and they arise by the shikimate-mevalonate pathway. However, the situation in lower organisms is not clear cut. Since pachybasin (**III**) which ought to be derived from shikimate, does infact arise via the acetate-malonate pathway.[5]

Anthraquinones of the Leguminosae have substituents on both rings with the exception of rubiadin (**20**) and damnacanthal (**21**). The most plausible biogenetic route for anthraquinones in the Leguminosae, therefore, appears to be of the polyketide origin. This is supported by labeling experiment which showed that, in higher plants, the biosynthesis of the emodin type anthraquinones proceed through the acetate-malonate pathway.[72] Most of the acetate derived anthraquinones examined so far are formed by cyclization of an octaketide chain. Other steps in the biosynthesis of these anthraquinones involve decorboxylation, reduction and aromatization of the cyclized polyketide. Anthrones are biosynthetic intermediates which lead to anthraquinone structures and the oxidative coupling of anthraquinones leads to dimeric anthraquinones. The biosynthetic pathway which leads to emodin 8 and endocrocin is given in scheme 1.[73,7]

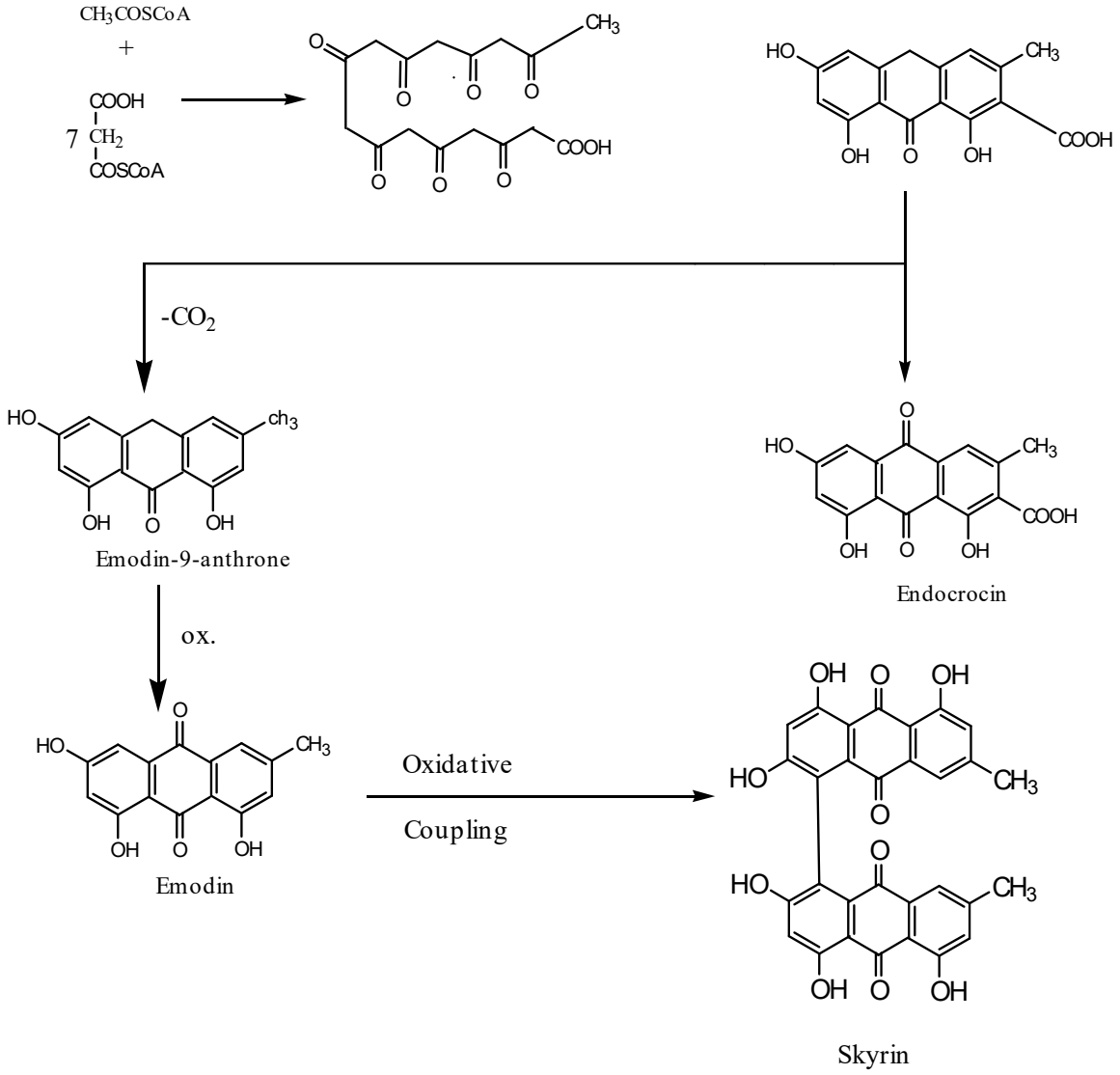


Alizarin



Pachybasin **III**

Scheme 1. Biosynthesis by polyketiede pathway.[73,74]



Anthraquinones of the emodin type take their start at carbon-oxygen skeleton generated from joining of acetate units in head to tail linkage, which then undergo subsequent structural modifications either before or after cyclization. These modifications may be principally the intoduction and removal of oxygen alkylation (notably with methyl), glycosidation, and dimerization.

The co-occurrence of torosachrysone (**1**), physcion (**9**) and physcion anthrone (**51**) with phlegmacines (**71**), anhydrophlegmacin-9-10-quinone (**72**) and

torosanin (**73**) in *S.torosa* suggests that, similar biogenetic route, which involves the conversion of pre-anthraquinoes to anthraquinones.[75,76] Experimental evidence indicated that, in the seedlings of *S. torosa*, germichrysone (**5**) is not derived from anthraquinone but it is a product of denovo biosynthesis. From this, it is possible to infer that, in plants the reduction of anthraquinones to pre-anthraquinones is least likely to occur.[75]

Anthraquinones can arise from either a single polyketide chain or form more than one polyketide chain. The active form of the first C_2 unit is acetyl CoA, while malonyl CoA, formed by carboxylation of acetyl CoA, is used for subsequent C_2 units.This utilization of two different units by the enzyme accounts for the different distribution of radioactivity in polyketide molecules obtained after incorporation of labelled malonic acid.

3. IDENTIFICATION OF ANTHRAQUINONES

3.1 Color reaction

Color reactions are useful particularly at the beginning of an investigation where crude extract or even tissues may yield information of value. The maceration of powdered plant material with organic solvents followed by filtration and addition of aqueous ammonia or sodium hydroxide, leads to the formation of pink, red or violet color, if hydroxy anthraquinones are present.[77]

The orientation of hydroxyl groups of hydroxyl anthraquinones can also be predicted by the color change observed in alkaline solution or when they are treated with methanolic magnesium acetate.[78] Anthracne derivatives containing two hydroxyl groups in 1,3 or 1,8 positions give generally an orange – red or pink color when treated with 0.5% methanolic magnesium acetate or 5% methanolic KOH, those in 1,4 positions give a purple color and those in 1,2 positions produce a violet color.[78]Anthraquinones are generally yellow, orange or brown colored solid. On silica gel layer, anthraquinones fluoresce brown yellow to red in

long wave UV light, after being sprayed with alkali solution, they exhibit a more intense yellow, orange or red fluorescence in long wave UV light.[79]

3.2 Chemical properties

Since the solubility of anthraquinones is low in the common NMR solvents such as $CDCl_3$, their solubility can be increased by conversion of the hydroxyl groups to the acetate units.This can be achieved by adding acetic anhydride and pyridine reagent and keeping the reaction mixture for 2 days at room temp..[80]

Reductive cleavage, with the use of sodium dithionite ($Na_2 S_2 O_4$) occurs readily in those dimmers where the monomeric groups are linked ortho to the hydroxyl groups while with little or no promptness in those linked para to the hydroxyl groups.[81,82,83] Anthrraquinones containing free carboxylic acid group in an organic solvent can be separated from the others by extraction with sodium bicarbonate solution.

SPECTRAL PROPERTIES

3.3.1 Ultraviolate/visible spectroscopy

Since most of the anthraguinone pigments are polyhydroxy or alkoxy derivatives, their UV-Vis spectra are dominated by the influence of these substituents. When a carbonyl group is conjugated with an ethylenic linkage both the (π-π*) and (n-π*) bands undergo bathochromic shift to the region 220 -260 nm and 310-330 nm respectively. The conjugation of a double bond with the (π) system of the carbonyl group lowers the energy difference between the (π) and (π*) orbitals. Anthraquinones show intense benzenoid absorptions at 240 -260 nm, medium absorptions at 320-330 nm, a strong quinonoid electron transfer band at 270 – 290 nm accompanied by a weak quinonoid absorption bond at 405 nm. The pattern in the ultraviolet region is not seriously affected by substitution. On the other hand the number of alpha hydroxyl groups influences absorption in the

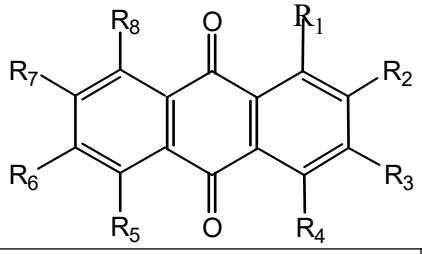
visible region. The influence of beta hydroxyls is much weaker except when adjacent to an alpha hydroxyl.

Hydroxyl groups in position 2,6 or 7 give rise to stronger red shift bands than those with hydroxyls in 1,4 or 5 position probably because in the former a hydroxyl group is para to one of the carbonyl group.[84] The UV-Vis spectra of 1,8-dihydroxy anthraquinones show a peak at 430-450 nm and those of 1,4-dihydroxy anthraquinones exhibit absorption at 470-500nm. 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 absorption. Therefore, 1,4,5-trihydroxyanthraquinones show maxima in the range 485-530nm and 1,4,5,8-tetrahydroxy compounds show absorption in the 540-560nm region.[85 - 87]

3.3.2 Infrared spectroscopy

The carbonyl stretching vibrational absorption region frequencies are important in indicating the presence of the fundamental anthraquinone unit and the hydroxylation pattern. Anthraquinones with no alpha hydroxyl groups have single carbonyl absorptions occurring between 1678 and 1653 cm^{-1}. A second carbonyl band at lower frequency can be observed if the anthraquinone contains a hydroxyl group in the (α)-position due to chelation and conjugation.[88] In the hydroxyl region of the IR spectra, if a hydroxyl group at the beta position is present on the anthraquinone nucleus, a sharp hydroxyl streching band will be apparent between 3600 and 3150 cm^{-1}. Alpha hydroxylanthraquinones show a broad and weak absorption band centered at approximately 3468cm^{1} corresponding to the stretching frequency of a chelated hydroxyl band.[88]

Table 7. Carbonyl absorption frequencies of hydroxy anthraquinonon



Anthraquinones	Carbonyl frequencies(cm^{-1})
No alpha OH (R_1,R_4,R_5 and $R_8 \neq$ OH)	1678 – 1653
R_1,R_4,R_5 and R_8 = OH	1675 – 1647 (The unchelated) 1637 – 1621 (The chelated)
R_1=R_4=OH or R_5 = R_8 =OH	1645 – 1608 (chelated)
R_1=R_8 =OH	1678 – 1661 (The unchelated, C -10) 1626 – 1616 (The chelated, C - 9)
R_1=R_4 =R_5 =OH	1616 – 1592 (chelated)

3.3.3 Nuclear magnetic resonance spectroscopy

NMR spectroscopy is powerful tool in the structural elucidation of anthraquinones. Analysis of chemical shifts and splitting patterns of anthraquinones give useful information in structural assignment and determination of orientation of substituents. In 9,10-anthraquinone the α and β-protons give multiplets centered at 8.1and 7.7ppm, respectively and are modified by substitution. Hydroxyl groups at position1, 4, 5, and 8 are easily distinguished by their appearance at unusually low field resonance between 11 and 14ppm, a shift accounted by the chelation of hydroxyl groups with 9,10-keto groups while for hydroxyl groups at position 8 and 9 low field resonance between 14 -17 ppm which is accounted for chelation of 9-OH with 8-OH and l-keto group of 1,4-antqraquinone. Information about orientation of substituents around the aromatic ring system can be obtained from

the chemical shift positions of aromatic protons. In this connection, since many quinones are phenolic, calculation of theoretical chemical shifts by the use of shielding parameters compiled for phenolic compounds can be used to predict the chemical shift of the aromatic protons and hence orientation of substituents.[91] Substituent shielding values are believed to be the net result of contributions from resonance, inductive, steric and magnetic anisotropy effects.

Splitting patterns and coupling constants have also diagnostic value for the determination of orientation of substituents. Coupling constants show wide variations depending on bond angles and bond hybridization. However, aromatic coupling constants are almost constant.**Ortho** coupling constant **(J*o*)** is usually around 7-9 Hz, **meta** coupling constant **(J_m)** is 2-3 Hz and **para** coupling constant **(J*p*)** is 1Hz. For all practical purposes, coupling with a Para proton is very small and therefore only ortho and meta coupling are considered. Zanger has pointed out that any single aromatic proton may exhibit only one of the seven possible first order splitting patterns (Table 8).[92]

Table 8. Peak Multiplicity First – Order Splitting Patterns

Coupling	Splitting Patterns
ortho	broad doublet
di-ortho	broad triplet
meta	narrow doublet
di-meta	narrow triplet
Ortho-meta	double of doublets
Diortho-meta	triplet of doublets
Ortho-dimeta	double of triplets

Anthraquinones of the chrysophanol (**7**) types show ortho-meta and di-ortho splitting patterns for the protons at positions 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 (**8**), or physcion (**9**) type anthraquinones show only the meta coopling patterns. The other splitting patterns mentioned in Table 8 are not common in anthraquinones.

Steglich and Losel studied the chemical shift differences for the corresponding aromatic protons in the ^{1}H-NMR spectra of per-acetylated and per-trimethyl-silylated anthraquinones and found the acetylation shift data as useful parameters in determinging the positions of O-alkyl or O-glycosyl substituents in 1,8-dihydroxy anthraquinone derivatives. These finding are based on the well known acetylation shifts in phenols where ortho protons are shifted by – 0.25 ppm and para protons by – 0.22 ppm whereas meta protons remain unaffected. The presence of many hydroxyl groups leads to an additive effect.[93]

The spin-spin couplings between ^{13}C and protons (two and three – bond) reported by Weigert etal. for benzene derivatives have been used for peak assignment of the carbon atoms in anthraquinones and naphthoquinones. The coupling constants are:

$^{1}J_{C-H}$ (one bond coupling) = 159Hz

$^{3}J_{C-H}$ (three bond coupling, vicinal) = 5-10 Hz

$^{2}J_{C-H}$ (two bond coupling, germinal) = 1-2 Hz

$^{4}J_{C-H}$ (four bond coupling) = 1-2 Hz

4. *SENNA SOPHERA*

Senna sophera is one of the 18 *Senna* species occurring in Ethiopia.[11] It is well known for its medicinal value in the traditional health delivery system of India. [71] *S.sophera* is an annual herb, shrub or shrublet 0.5 - 2m high. Leaves 10 - 25 cm long with a sessile clavate or cylindrical and linear pods. It is found in Asia, rare in east Africa[72]. It was reported in Ethiopia from Afar region and Harer region near Asebe Teferi along the high way.[11]

Previous phytochemical investigation on the various parts of the plant including roots, flowers, and pods were reported. A novel anthraquinone named

sopheranin (**46**) was isolated from the heart wood along with chrysophanol (**7**), emodin (**8**) and physcion (**9**) **.** Two anthraquinones were isolated from its root bark and characterized as 1,8-dihydroxy-3,6-dimethoxy-2-methyl-7-vinylanthraquinone (**35**) and 1,3-dihydroxy-5,7,8- trimethoxy-2-methylanthraquinon (**44**).[73] From its seeds, a new hydroanthracene derivative named presengulone (**58**) was isolated, together with physcion (**9**), physcion-10,10'-bianthrone (**82**), floribundone-1 (**52**), isosengulone (**60**), sengulone (**59**) and anhydrophlegmacin-9,10- quinons A_2 and B_2 (**61**).

41. OBJECTIVE

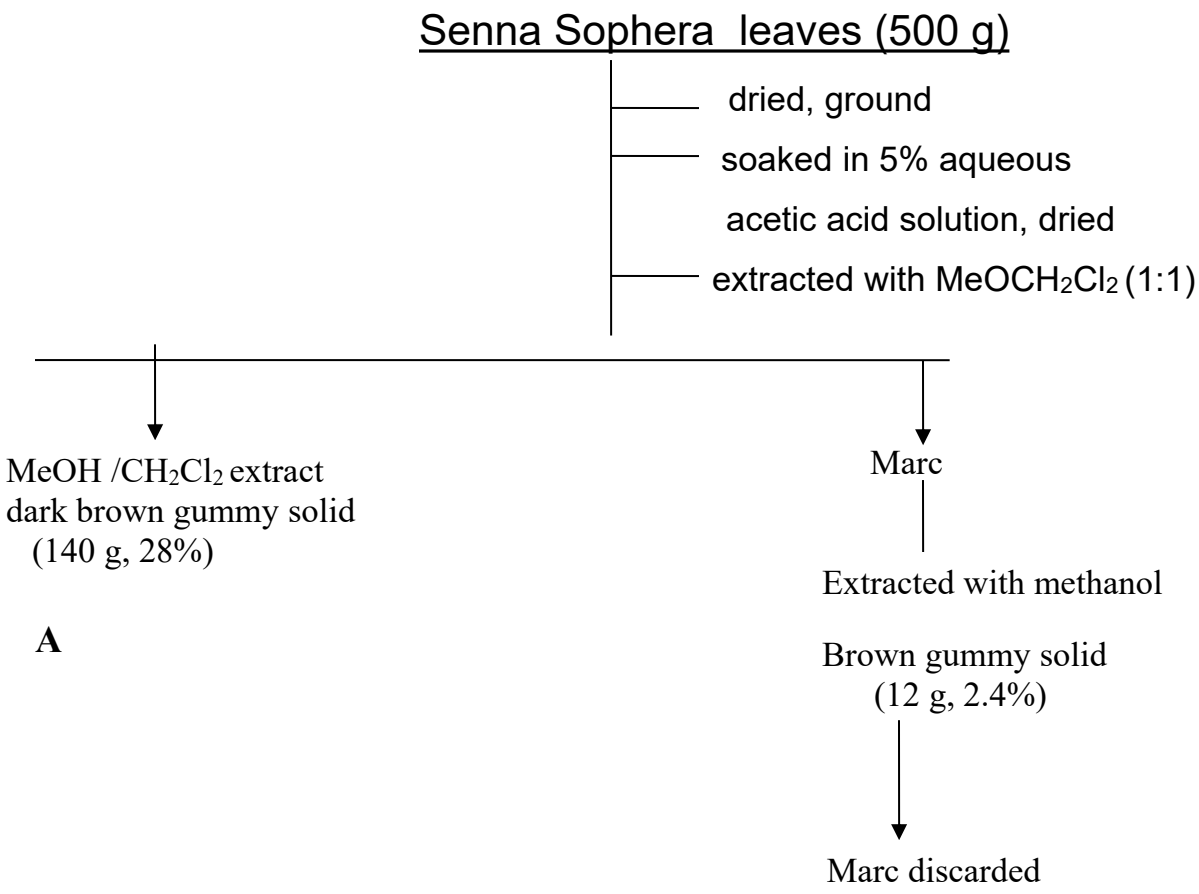
This research work aims at extending further earlier studies. The objective of this work is extending earlier studies by isolation and characterization, of anthraquinones, that may have chemotaxonomical or medicinal importance, from the leaves of *Senna sophera*.

5.0 RESULTS AND DISCUSSION

Study in the course of this work, of the leaves of *Senna sophera* resulted in the isolation of three anthraquinoes. The structure of the anthraquinones were derived on the basis of color reaction, ^{l}H-NMR (including 2D-NMR), UV, IR and comparison with literature data.

The powdered leaves of *Senna sophera* were extracted first with methylene-chloride-methanol mixture of 1:1 ratio and then with methanol (Scheme 2).

Scheme 2: Extraction and Isolation



The methylene chloride-methanol extract when developed on TLC and sprayed with 5% methanolic KOH solution showed characteristic color change from yellow to red that indicates the presence of hydroxy anthraquinones.

50g of the CH_2Cl_2 /MeOH extract was absorbed on 30 g silica gel and charged on to a column packed with silica gel (5% oxalic acid impregnated) using chloroform. The column was eluted using chloroform, chloroform /ethyl acetate and ethyl acetate solvent system. Fractions 1-4 (chloroform) 400 ml, fractions 5-9 (4:1 chloroform/ethyl acetate) 750 ml, fractions 10-12 (3:2 chloroform/ethyl acetate) 750 ml and fractions 13-16 (ethyl acetate) 1200 ml were collected together (Scheme 3).

Based on TLC examination fractions 1-4, CF1 (10 g) contained large amount of fat and showed more than two antraquinones on TLC examination. Further chromatography on column (Silica gel), separation on Sephadex and prep. TLC (Silica gel) gave pigments SL0I-78, SL03-9, SL04-92 and SL 05-93 (Scheme 4).

The chloroform/ethyl acetate fractions 5-12, CF2 (5 g) were combined together based on TLC examination and they contained yellow pigment which changed to violet after being sprayed with 5% methanolic KOH solution. Fraction E was subjected to sephadex (LH-20), silica gel column chromatography followed by prep. TLC (silica gel) to give three pigments SL0I-78, SL03-91 and SL02-77 (Scheme 5).

The major problem of the study was the failure to get enough amount of pigments SL0I-78, SL02-77, SL04-92, SL05-93 and others from 90 g of the previously CH_2Cl_2 / MeOH extracted and even, the same sample of 500 g dried leaves. This may be explained due to the instability of anthraquinones when they are exposed to air for long period of time in the, isolated compound, extracted or the plant material form. It was observed that the purely isolated pigment showed more than one spots on TLC test with the same solvent system, after it stayed in a solution or solid form in air.

5.1 Floribundone-1 (SL03- 91)

Compound SL03-91 was obtained as an orange solid with a melting point of greater than 300^0c. It is homogenous on TLC (R_f 0.24, silica gel, pet. ether/ EtOAc, 9:1, R_f 0. 63, silica gel, $CHCl_3$/Pet. ether/EtOAc, 7:2.5:0.5 and R_f 0.93, silica gel $CHCl_3$/EtOAc, 9:1). It showed color change from yellow into red when sprayed with 5% methanolic KOH solution on TLC test. This is a characteristic color change for hydroxy anthraquinones. Compound SL03-91 displayed UV spectrum absorption maxima at 443, 360, 276 and 236 nm suggesting a quinonoid chromophore. The IR spectrum showed absorption bands due to hydroxyl groups (3448 cm^{-1}), a chelated carbonyl group (1627 cm^{-1}) and broad strong C – O stretching at (1095cm^{-1}).

The ^{1}H-NMR spectrum of SL03-91 revealed the presence of the following groups: four chelated hydroxyl groups, six aromatic protons, two aromatic methoxyl groups and two aromatic methyl groups. A monomeric anthraquinone unit could not accomodate all the above substituents, and hence, this compound is proposed to be a bianthraquinone.

The chemical shifts of SL03-91 were suggestive of the presence of physcion **(9**) moieties (Table 9). This was further established by alkaline sodium dithionite reductive cleavage of SL03-91 which produced physcion. The structure of the produced physcion was identified by ^{l}HNMR, and ^{13}C-NMR (including 2D) spectroscopy.

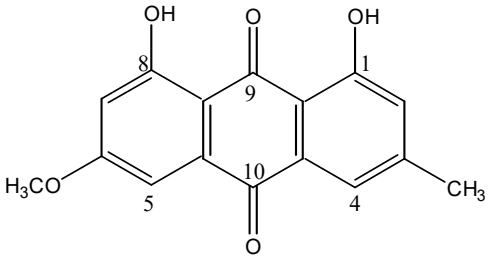
Comparison of the ^{l}H-NMR of SL03-91 and that of physcion revealed the presence of two sharp singlets at δ 7.6(1H) and 6.8(1H) in the spectrum of SL03-91 where as the corresponding signals in the spectrum of physcion appear as doublets at δ 7.4(1H) and 6.7(1H). These meta coupled signals (J = 2.5) at 7.4 and 6.7 are assignable to H-5 and H-7 of physcion respectively. The result is consistent with a 5,7' linked bianthraquinone structure, which gives rise to the appearance of two sharp singlets for the protons at position 7 (δ 6.8) and 5'(δ 7.6) of the two linked physcion moieties.

Table 9. ^{1}H-NMR data of physcion 8 and floribundone-1 (SL03-91)

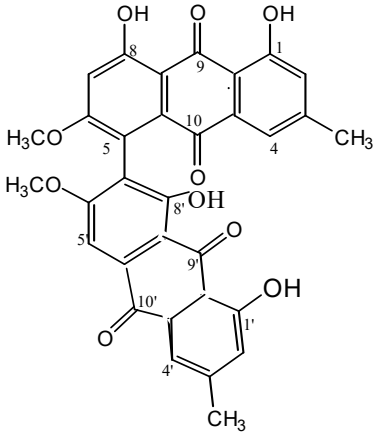
(400 MHz, $CDCl_3$)

Assignment	9 δ (ppm)	SLO3 – 91 δ (PPm)
1 – OH	12.2	12.0
2 – H	7.1	7.0
3 – CH_3	2.4	2.3
4 – H	7.6	7.5
5 – H	7.3	c
6 – OCH_3	3.8	3.7
7- H	6.7	6.8
8'-OH	12.4	12.1
1'-OH	-	12.3
2'-H	-	7.1
3'-CH_3	-	2.4
4'-H	-	7.7
5'-H	-	7.6
7'	-	c
8'-OH		13.1
3'-OCH_3		3.8

c. anthraquinonyl linkage



9 Physcion



52 Floribundone-1
(SL03-91)

From the COSY ($^1H \rightarrow ^1H$) correlated spectrum of SL03-91, there are cross peaks between the protons at δ 7.5 and δ 7.0 and the aromatic methyl at δ 2.3, that could be assigned to H-4 and H-2, respectively and between the protons at δ 7.7 and δ 7.1 and the aromatic methyl at δ 2.4, which are assignable to, H-4' and H-2' respectively. The difference between the COSY ($^1H \rightarrow ^1H$) of SL03-91 and that of physcion (**9**) is the presence of cross peak between the protons at δ 7.3(H-5) and δ 6.7(H-7) of physcion. This means that the anthraquinonyl linkage is 5,7' which is consistent with the absence of signal attributable to H-5 and H-7' (see Table 9). The HMBC spectrum recorded for (**9**) showed a long range correlation with the proton at δ 7.3 (H-5) and the carbon at δ 121.7 (C-4) and the proton at δ 7.6 (H-4) correlated with the carbon at δ 108.6 (C-5). This would be a ^5J-correlation, which is unexpected. These and other HMBC correlations observed for the compounds SL03-91 and (**9**) are represented in Figure 3 and Table 12.

Further more, the DEPT (Distortion less Enhancement by Polarization Transfer) spectrum of SL03-91 showed four methyl signals at δ 22.6. 22.7, 56.9 and 57.0 and five C-H signals at δ 124.8, 124.3, 121.6, 105.0 and 104.7.The intensity of the C-H signal at δ 121.6 is much higher than any of the other C-H signals, probably representing two C-H signals.

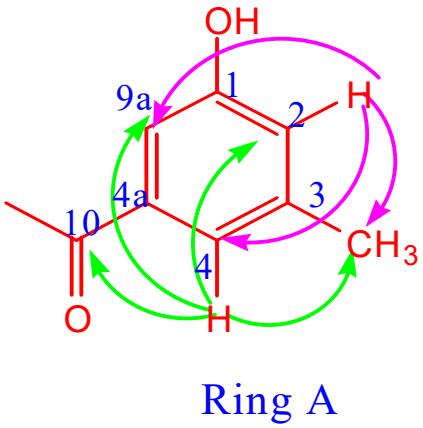
Table 10. HSQC spectral data of SL03-91

C	^{13}C-NMR δ (ppm)	^{1}H-NMRδ (ppm)
1	162.5	O- H,12.0 [a]
2	124.3	1H, 7.0
4	121.6	1H, 7.4
7	105.0	1H, 6.8
8	166.5	O- H, 12.3 [a]
3 – CH_3	22.6	3H, 2.3
6 – OCH_3	56.9	3H, 3.8
1’	162.9	O-H, 12.1 [a]
2’	124.8	1H, 7.1
4’	121.6	1H, 7.6
5’	104.7	1H, 7.6
8’	161.6	OH, 13.1 [a]
3’– CH_3	22.7	2.4
6’ – OCH_3	57.0	3.8

a. Carbon and proton correlation from HMBC

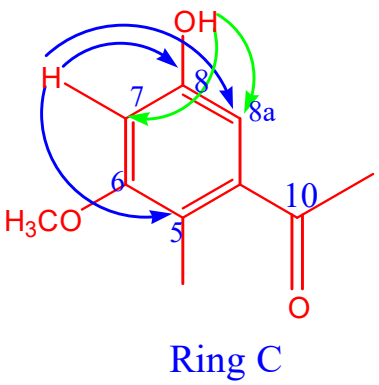
In the HMBC spectrum of SL03-91, pertinent correlations observed were between the aromatic proton at δ 7.0 (H-2) with the methyl at δ 22.5 (3-CH_3), the carbon at δ 121.7 (C-4), the oxygenated quaternary carbon at δ 162.5 (C-1) and a quaternary carbon at δ 113.9 (C-9a). The proton at δ 7.64 (H-4) correlated with the methyl at δ 22.5 (3-CH_3), the carbon at δ 124.3 (C-2), the quaternary carbon at δ 113.9 (C-9a) and the carbonyl carbon at δ 182.7 (C-10). The hydroxyl proton at δ 12.0 (1-OH) correlated with the oxygenated quaternary carbon at δ 162.5 (C-1), the quaternary carbon at δ 113.9 (C-9a) and the carbon at δ 113.9 (C-2).The methyl protons at δ 2.3 (3-CH_3) showed correlation with the quaternary carbon at δ 148.8 (C-3), the carbon at δ 121.6 (C-4). This observation together with the COSY (^{1}H → ^{1}H) correlation of H-2 meta coupled with H-4 and their HSQC correlation with C-2 (δ 124.3) and C-4 (δ 121.6) respectively, led to the structure of ring A and partial skeleton of ring B (Figure 1).

Likewise, there are correlations between the proton at δ 6.8 (H-7) with the carbon at δ 120.6 (C-5), with the quaternary carbon at δ 110.0 (C-8a) and the oxygenated quaternary carbon at δ 166.5(C-8). The methoxyl protons at δ 3.7 (6-OCH_3) correlated with the oxygenated quaternary carbon at δ 165.0 (C-6). The hydroxyl proton at δ 12.3 (8-OH) correlated with the oxygenated quaternary carbon at δ 166.5 (C-8), the quaternary carbon at δ 111.0 (C-8a) and the carbon at δ 105.0 (C-7). Absence of aromatic proton at C-5 (^{1}H-NMR and 2D-NMR) suggestive of, the position is the anthraquinonyl linkage of the compound. Together with the HSQC correlation of H-7 (δ 6.8) with the carbon at δ 105.0 (C-7), led to the structure of ring C and partial skeleton of ring B. The suggested structure of ring A and C together with the HMBC correlations are indicated in Figure 1.

HSQC^r	HMBC^s	Partial skeleton
$^2C \rightarrow H^2$	$H^2 \rightarrow C^1$, $H^2 \rightarrow CH_3$ $H^2 \rightarrow C^4$, $H^2 \rightarrow C^{9a}$	 Ring A
CH_3	$CH_3 \rightarrow C^2$, $CH_3 \rightarrow C^3$ $CH_3 \rightarrow C^4$	
$C^4 \rightarrow H^4$	$H^4 \rightarrow C^2$, $H^4 \rightarrow {}^3CH_3$ $H^4 \rightarrow C^{9a}$ $H^4 \rightarrow C^{10}$	
	$OH^1 \rightarrow C^1$, $OH^1 \rightarrow C^2$ $OH^1 \rightarrow C^{9a}$	

(a)

$C^7 \rightarrow H^7$	$H^7 \rightarrow C^5$ $H^7 \rightarrow C^8$ $H^7 \rightarrow C^{8a}$
OCH_3	$OCH_3 \rightarrow C^6$
	$OH^8 \rightarrow C^7$ $OH^8 \rightarrow C^8$ $OH^8 \rightarrow C^{8a}$



(b)

Figure 1: Suggested partial structure for physcion (**9**) moiety of SL03-91 with selected HMBC correlations.

The correlation between the carbonyl carbon at δ 182.7 (C-10) with that of the proton at δ 7.4 (H-4) and the down field chemical shift value of the two hydroxyl-groups at δ 12.0 (1-OH) and δ 12.3 (8-OH) which are chelated with the carbonyl carbon at δ 191.7 (C-9), indicated that the partial skeleton of ring B is aligned on both ring A and C. Application of the ring ABC system to the preceding structural data permits the structure of physcion moiety (**9**) of SL03-91 (Figure 2).

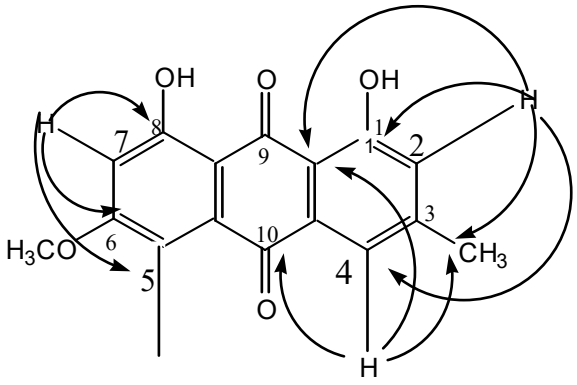


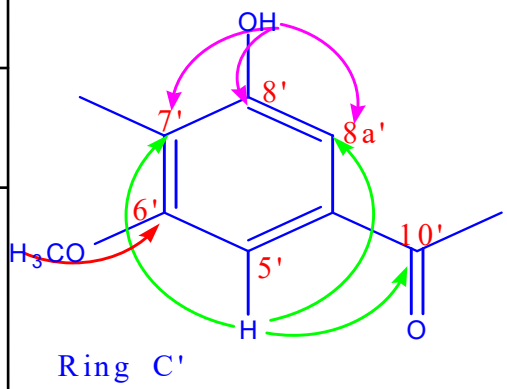
Figure 2: Structure with selected HMBC of physcion (**9**) moiety of SL03-91.

Similarly, the proton at δ 7.6 (H-5') correlated with carbon at δ 118.0 (C-7'), quaternary carbon at δ 111.4 (C-8a') and the carbonyl carbon at δ 182.4 (C-10'). The hydroxyl proton at δ 13.1 (8'-OH) correlated with the carbon at δ 118.0 (C-7'), the oxygenated quaternary carbon at δ 161.6 (C-8') and the quaternary carbon at δ 111.4 (C-8a'). Together with the HSQC correlation of H-5' (δ 7.6) with the carbon

at δ 104.7 (C-5'), led to the structure of ring C' and the partial skeleton of ring B'. Absence of a proton at C-7 (^{1}H-NMR and 2D-NMR) indicate this position is the anthraquinonly linkage of SL03-91 (Figure 3).

The proton at δ 7.1 (H-2') correlated with the carbons at δ 121.6 (C-4'), δ 22.7 (3'-CH_3) and the quaternary carbon at δ 111.8 (C-8a'). The proton at δ 7.7 (H-4') correlated with the carbons at δ 124.8 (C-2'), δ 22.7 (3'-CH_3), δ 114.2 (C-9a') and the carbonyl carbon at δ 182.4 (C-10').The methyl protons at δ 2.4 (3'-CH_3) showed correlations with the carbon at δ 124.8 (C-2'), δ 121.6 (C-4') and the quaternary carbon at δ 148.8 (C-3'). The hydroxyl proton at δ 12.1 (1'-OH) correlated with the carbons at δ 124.8 (C-2'), δ 162.9 (C-1') and the quaternary carbon at δ 114.2 (C-9a'). These observations together with the COSY (^{1}H-^{1}H) correlation of H-2' meta coupled with H-7' and HSQC correlation of H-2' (δ 7.1) with C-2' (δ 124.8) and H-4' (δ 7.7) with C-4' (δ 121.6), led to structure ring C' and partial skeleton of ring B'. The suggested structure of ring A' and C' are indicated in Figure 3.

$C^{5'}\rightarrow H^{5'}$	$H^{5'}\rightarrow C^{7'}$ $H^{5'}\rightarrow C^{8a'}$ $H^{5'}\rightarrow C^{10'}$
6'-OCH_3	$OCH_3\rightarrow C^{6'}$
	$OH^{8'}\rightarrow C^{7'}$ OH^{8} $\rightarrow C^{8'}$ $OH^{8'}\rightarrow C^{8a'}$



(c)

HSQC[r]	HMBC[s]
$C^{2'}\rightarrow H^{2'}$	$H^{2'}\rightarrow C^{1'}$ $H^{2'}\rightarrow {}^{3'}CH_3$ $H^{2'}\rightarrow C^{4'}$, $H^{2'}\rightarrow C^{9a'}$

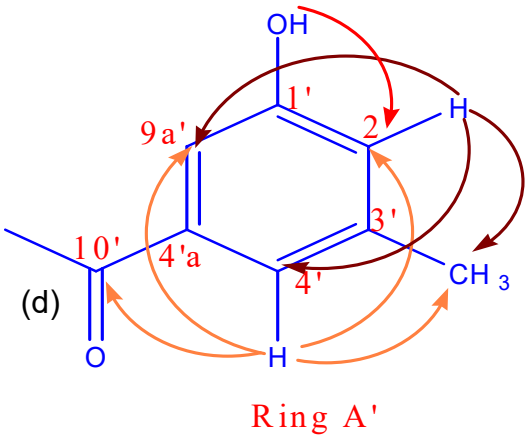


Figure 3: The partial structure of physcion moiety (9') of SL03-91 with HMBC correlation.

Likewise, the correlations between the carbonyl carbon at δ 182.4 (C-10) with that of the protons at δ 7.7 (H-4') and 7.6 (H-5') indicated that the partial skeleton of ring B' is aligned on both ring A' and C'. The downfield chemical shift values of the two hydroxyl groups at δ 12.1 and 13.1 suggestive of chelation with the carbonyl carbon at δ 191.2 (C-9') of ring C'. Application of the ABC system to the preceding structural data permits the proposed structure of physcion (**9'**) of compound SL03-91. Structure with selected HMBC of physcion (**9**') given in Figure 4.

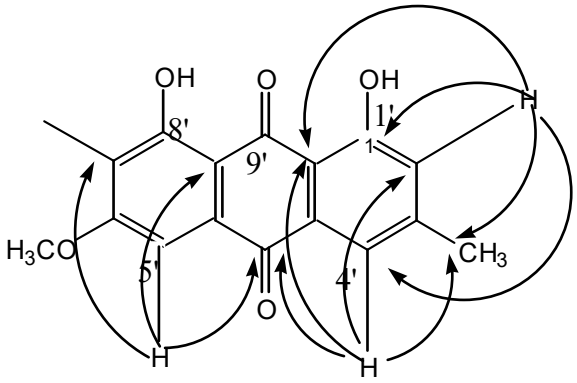
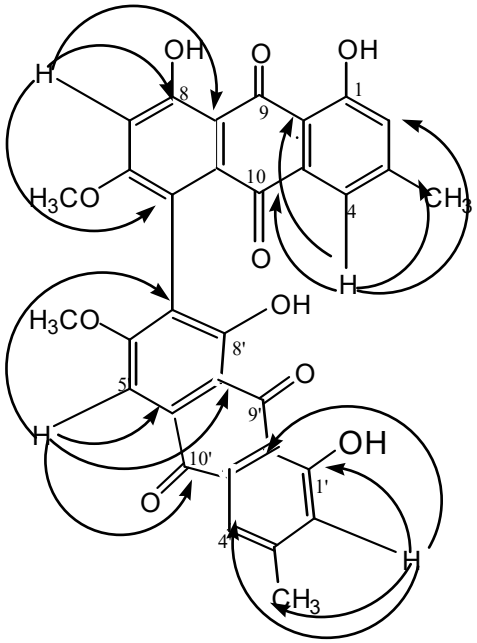


Figure 4: Strucure with selected HMBC of physcion moiety (**9**')

Since there are no assignable chemical shift values for protons at C-5 and C-7', these position are the anthraquinonly linkage of the two physcion moiety of SL0391. The structure of SL03-91 with selected HMBC is given in Figure 5



52
Floribundone-1
(SL03-91)

Figure 5: Structure of SL03-91 with selected HMBC corelation.

Reductive cleavage of SL03-91 with an alkaline solution of sodium dithionite resulted physcion.The structure of physcion was identified mainly on the basis of its ^{1}H-NMR and ^{13}C-NMR spectroscopic data as well as similarity on R_f values with authentic sample.

The compound (SL03-91) forms a tetra-acetate with acetic anhydride in pyridine. The ^{1}H-NMR of floribundone-1 tetraacetete showed six aromatic protons, two aromatic methoxyl groups, two aromatic methyl groups and four acetyl methyl groups. The IR absorption spectrum showed strong stretching band at 1775cm^{-1} which corresponds to the carbonyl stretching band of oxygen conjugated ester and absorption band at 1673 due to unchelated carbonyl stretching band. Also the yellow color of the compound disappeared and showed negative responsed to methanolic KOH solution. Based on the above data SL03-91 was identified to

be floribundone-1 which was providing reported from the leaves of *S. muliglandulosa* and *S. septemtrionalis* and from the seeds of *S. sophera* by Alemayehu et al. [36, 51]

Table 12. HMBC and HSQC correlation for SL03-91.

δ H	SL03 -91 HSQC	HMBC
6.8(7-H)	105.0	111.0, 120.6, 165, 166.5
7.0(2-H)	124.3	22.5, 121.0, 162.5
7.1(2'-H)	124.8	22.7, 114.2, 121.6, 162.9
7.4(4-H)	121.6	22.5, 113.9, 124.3, 182.6
7.6(5'-H)	104.1	111.4, 118, 134.7, 163.8, 182.5
7.7(4'-H)	121.6	22.7,114.2,124.8 182.5
3.7(6-OCH_3)	56.9	165.0
3.8(6'-OCH_3)	57.0	163.8
2.4(3'-CH_3)	22.7	121.6,124.8, 148.84
2.3(3-CH_3)	22.5	121.6, 124.3, 148.75
12(1-OH)	162.5	114.2, 124.8, 162.9
12.1(1'-OH)	162.9	113.9, 124.3, 162.5
12.3(8-OH)	166.53	111.4,118, 161.6
13(8'-OH)	161.63	105, 111.0, 166.5

Table 13. ^{1}H-NMR and ^{13}C-NMR spectral data of physcion

H δ (ppm)	^{13}C δ (ppm)	^{13}C δ (ppm)
12.2, 1- OH	162.9 (C-1)	148.8 (C-3)
7.1, 2 – H	124.9 (C-2)	167.0 (C-6)
2.4, 3 – CH3	22.5 (3-CH_3)	191.2 (C-9)
7.6, 4 – H	121.7 (C-4)	182.4 (C-10)
7.3, 5 – H	108.6 (C-5)	114. 1 (C-9a)
3.8, 6 – OCH3	56.5 (6-OCH_3)	110.7 (C-8a)
6.7, 7 – H	107.2 (C-7)	133.6 (C-4a)
12.4, 8 - OH	165.6 (8-OH)	135. 7 (C -10a)

5.2 Pigment SL05-93

Compound SL05-93 is a yellow solid of melting point above 300^{o}c. It showed a color change from yellow to red when the TLC plate was sprayed with methanolic KOH solution. This is a characteristic color change for hydroxy anthraquinones. It is homogeneous on TLC (R_f 0.18, silica gel $CHCl_3$ /Pet..ether/EtOAc, 7:2.5:0.5,R_f 0.64 silica gel, $CHCl_3$/EtOAc, 9:1). The IR spectrum showed absorption bands due to a hydroxyl group (3400cm^{-1}) and a chelated carbonyl group (1626cm^{-1}). The UV spectrum absorption maxima, at 240,270,370 and 440 nm suggested a quinonid chromophore in the compound.

The ^{1}H-NMR spectrum of SL05-92, revealed the presence of the following groups: four chelated hydroxyl groups, Six aromatic protons, two aromatic methoxyl groups, two aromatic methyl groups and one benzyne proton (H-10). A monomeric anthraquinone unit could not accommodate all the above substituents, and hence, this compound is proposed to be a bianthraquinone.

The chemical shifts of SL05-93 were suggestive of the presence of physcion (**9**) and 10-hydroxyphyscion-9-anthrone moieties (Table 14). This was established by comparison of the ^{1}H-NMR spectroscopic data with that of the reported values.[51] The chemical shift of the physcion moiety of SL05-93 closely resemble

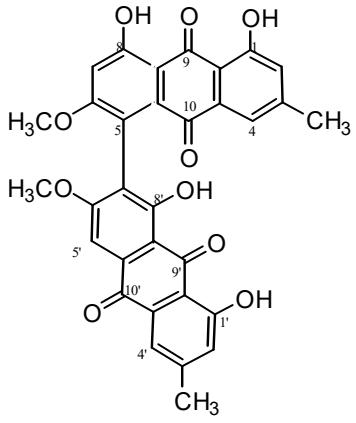
that of physcion except for the absence of the signal attributed to H-7'. The signals at δ 7.1, 7.6 and 7.7 (see Table 14) are assigned to H-2', H-5' and H-4',respectively of the physcion moiety. The fact that there is no signal attributable to H-7' and that the signal of H-5' (δ 7.6) appears as a sharp singlet, led to the conclusion that the 10-hydroxyphyscion-9-anthrone is linked to position C-7' of the physcion skeleton. The ^{1}H-NMR spectrum of SL05-93 resembles that of the reported values of floribundone-1 (SL03-91). A close comparison of the spectra, however, revealed that the two spectra have important differences. The ^{1}H-NMR spectrum of SL03-91 showed down field resonating proton at δ 7.4 that could be assigned to H-4 while the spectrum of SL05-93 showed a resonance of δ 6.6 assignable to H-4 which is shifted upfield by – 0.8 as compared to the corresponding signal in SL03-91. A possible cause for this shift is the anisotropic effect of C-10 carbonyl on H-4 of SL03-91. From the co-occurrence of SL05-93 and SL03-91 it can be presumed that SL05-93 is a biogenetic precursor, which upon oxidation is converted to SL03-91. The signals at δ 6.6, 6.7 and 7.0 are assigned to H-4, H-7 and H-2, respectively, of the 10-hydroxyphyscion-9-anthrone moiety. The signal attributed to H-4 is relatively upfield, indicating the anthrone nuture of the physcion skeleton. In addition there is a resonance at δ 5.2 which corresponds to the benzyne proton of H-10 . Since there are no signals that can be assigned to the H-5, it was assumed that the linkage is at this position of the physcion-9-anthrone moiety. The chemical shifts for the protons of SL05-93 were assigned using the data for physcion (**9**), floribundone-1(SL03-91) and folribundone-2 (**74**). It follows that the anthraquinonyl linkage is 5,7' and the proposed structure is shown below.

Table 14: ^{1}H-NMR spectral data for the compounds SL05-93(400MHz,$CDCl_3$) Floribundone-1 (**52**) (400MHz,$CDCl_3$) and Floribundone-2(**74**) (400MHz,$CDCl_3$)

H	SLO5-93 δ (ppm)	**52** δ (ppm)	**74** δ (ppm)
1 -OH	11.9	11.9	12.0
2- H	7.0[b]	7.0	6.8
3 –CH_3	2.3	2.3	2.3
4 - H	6.6	7.4	6.6
5	c	c	c
6 -OMe	3.8	3.8	3.8
7-H	6.7	6.8	6.6
8 - OH	12.3	12.3	12.3
10 - H	5.2		3.8 – 3.9
1' - OH	12.0	12.1	12.2
2' - H	7.1[b]	7.1	7.1
3' – CH_3	2.4	2.4	2.4
4' - H	7.7	7.7	7.7
5'- H	7.6	7.6	7.6
6'- OCH_3	3.8	3.8	3.8
7'	c	c	c
8'-OH	12.9	13.0	13.1

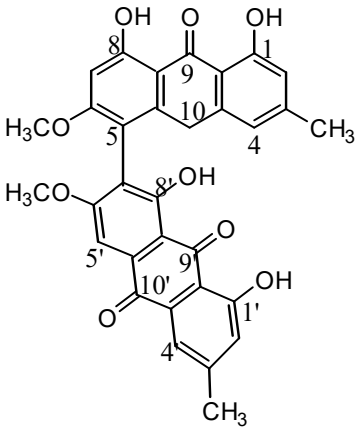
c. Anthraquinonyl linkage

b. Not distinctly observed, probably overlapped by the $CDCl_3$ signal

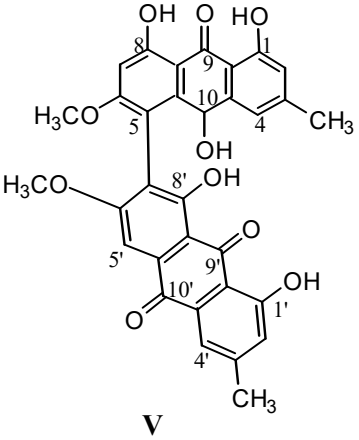


52 Floribundone-1

(SL03-91)



74 Floribundone-2



V

10-Hydroxyfloribundone-2

(SL05-93)

5.3 Pigment SL04-92

Compund SL04-92 is a yellow solid of melting point above 300^0C. It is homogeneous on TLC (R_f O.37, silica gel, $CHCl_3$ /Pet ether/ EtOAc, 7.2;2.5;0.5, R_f 0.8 silica gel, $CHCl_3$/EtOAc,9.1). It showed a color change from yellow to pink when the TLC plate was sprayed with methanolic KOH solution. This is a characteristic color change for hydroxy anthraquinones. The IR spectrum showed absorption bands at 3400,1665 and 1626 cm^{-1},for a hydroxyl, a non-chelated quinonoid carbonyl and chelated carbonyl groups, respectively. The UV spectrum absorption maxima, at 240,278,365 and 455 nm suggested a quinonoid chromophore in the compound.

The ^{1}H-NMR spectrum of SL04-92, revealed the presence of the following groups: four chelated hydroxyl groups, six aromatic protons, two methoxy groups, one aromatic methyl group and one CH_2OH group which can be accommodated on a bianthraquinone skeleton. The chemical shifts of SL04-92 were suggestive of the presence of physcion (**9**) and fallancinol (**12**) moieties (Table 15). This was established by comparison of the ^{1}H-NMR spectroscopic data with that of the reported values.[41,47] The chemical shifts of the physcion moiety of SL04-92 closely resemble that of physcion except for the absence of the signal attributed to H-5. The signals at δ 6.5, 6.8 and 7.3 (Table 15) are assigned to H-7 H-2 and

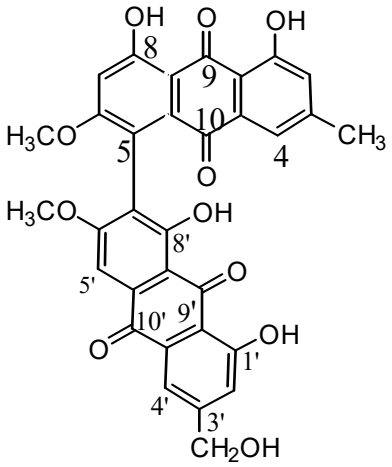
H-4, respectively of the physcion moiety. Since there are no signals that can be assigned to the physcion proton at C-5, it was assumed that the coupling is at this position of the physcion moiety. The signals at δ 7.2,7.6 and 7.7 are assigned to H- 2',H – 5' and H – 4', respectively of the fallacinol moiety.

Since there are no signals attributable to H-7', the physcion moiety linked to positio C-7' of the fallancinol skeleton. ^{1}H-NMR spectrum of SL04-92 resembles that of the reported values of 5,7'-physcion-fallancinol (**93**) the chemical shifts for the protons of SL04-92 were assigned using the data for physcion (**9**) and 5,7' physcion-fallacinol (**93**). It follows that the anthraquinonyl linkage is 5,7' and the proposed structure is shown below.

Table 14. ^{1}H-NMR spectral data for compounds SL04-92 (400MHz, $CDCl_3$) **93** (300MHz, $CDCl_3$) and **9** (400 MHz,$CDCl_3$)

H	SL04-93 δ (ppm)	**93** δ (ppm)	**9** δ (ppm)
1 - OH	12.1	12.1	12.2
2 - H	6.8	7.0	7.0
3 – CH_3	2.3	2.3	2.4
4 - H	7.3[b]	7.4	7.6
5 - H	c	c	7.3
6 – OCH_3	3.8	3.8	3.8
7 - H	6.5	6.6	6.8
8 - OH	12.0	12.0	12.4
1'- OH	12.7	12.6	
2' - H	7.2[b]	7.3	
3' – CH_2OH	5.0	4.9	
4'- H	7.7	7.7	
5'- H	7.6	7.6	
6'- OCH_3	3.9	3.9	
7'	c	c	
8'- OH	12.9	13	

b. not distinctly observed, probably overlapped by the $CDCl_3$ signals.
c. Anthraquinonyl linkage



93 5,7'-Physcionfallancinol

(SLO4-92)

6. EXPERIMENTAL

6.1 General

^{1}H-NMR spectra were measured on a Bruker Avance 400 Spectrometer at 400 MHz. ^{13}C-NMR spectra were measured on a Bruker Avance 400 Spectrometer at 100 MHz. The ultraviolet and visible (UV-Vis) spectra were taken on Spectronic Genesys^{TM} 2PC UV-Vis Scanning Spectrometer. Infrared (IR) absorptions were measured on a Perkin Elmer System FT-IR Spectrometer as KBr pellets. Analytical thin layer chromatograms were run on ready made 0.25 mm thick layer of Merck silica gel 60 F_{254} coated on aluminum foil. Spots on the chromatograms were detected by observing in UV light and spraying with methanolic KOH solution (prepared by dissolving KOH pellets (5g) in methanol (95 ml) to make 5% solution) and vanillin- sulphuric acid spray. Preparative thin layer chromatograms were run on 1mm thick layer Merck silica gel 60 PF_{254} coated on 20x20 glass plates. Flash chromatography and column chromatography were conducted using different sizes of columns packed with Merck silica gel 60, particle size 0.063 - 0.200mm(70 -230mesh ASTM).

6.2 Plant Material

Senna sophera leaves were collected from near Asebe Teferi along the high way on October 14, 2004. The plant was identified by Dr. Ensermu Kelbesa and its voucher specimen deposited at the National Herbarium, A.A.U with a Voucher number of 77622.

6.3 Extraction and Isolation

500 g of dried and ground leaves were soaked in 5% acetic acid (2 L), dried and powdered.The dried leaves extracted three times using methylene chloride- methanol (1:1) mixture for 24 hours. The crude extract was concentrated by Rota vapor at 40^0c to yield 140 g (28%) of dark brown gummy solid. The marc was further extracted using methanol for 24 hours. The weight of the methanol extract was 12 g (2.4%). The extracted tested for anthraquinone. The methylene chloride-methanol extract showed the presence of anthraquinones (two spots on TLC showed a color change from yellow to red after being sprayed with methanolic KOH).

50 g of the extract was absorbed on 30 g of silica gel and charged on to a column packed with silica gel (5% Oxalic acid impregenated). The column was eluted using the following solvent systems: chloroform: fractions 1-4(100 mlx4), chloroform/EtOAc (4:1): fractions 5-9(150 mlx5), chloroform/EtOAc (3:2): fractions 10-12(250 mlx3) and EtOAc: fractions 13-16(300 mlx4).

Fractions 1-4 (CF) 10 g contained large amount of fat (pink color), and showed more than two anthraquinones on TLC examination. Fractions 5-12(E), (9.1 g) black solid, showed two characteristic spots for anthraquinones on TLC when sprayed with methonolic KOH solution, the color changed from yellow to red. Fractions 13-16(D) did not show presence of anthraquinones upon TLC examination.

The combined fractions 5-12 E (9.1 g) were subjected to sparation on sephadex resulting in the collection of 10 fractions (700 ml). Fractions 1-6 contained only green pigment and were discarded. Fractions 7-10 showed two major yellow

spots which were labelled as SL03-91 and SL02-77 and other trace yellow spots on TLC.

The combined fractions 7 and 8 SF1 were applied on prep. TLC using pet.ether/EtOAc(9:1) resulting in the isolation of SL03-91 (1.8 mg), and fractions 9 and 10 SF2 were chromatographed on prep. TLC using chloroform/EtOAc (4:1) resulting the isolation of SL02-77 (0.7 mg) and small amount of SL03-91.

The combined fractions 1- 4 (CF) 10 g, were applied on to a column packed with 100gm of silica gel using the following solvent: Pet. ether (Fractions 1-3,CF1), methylenechloride (Fractions 4 - 6 , CF2) CH_2Cl_2/EtOAc, 4:1 (Fractions 7-9,CF3) and EtOAc (Fractions 10 and 11, CF4). Fractions 1-3 CF1 (150 ml) contained only fat and were discarded. Fractions 4-6 CF2 (250 ml) showed two yellow spots on TLC (changed to red when sprayed with KOH/ MeOH), fractions 7-9 CF3 (300 ml) showed three major yellow spots (the lower two spots turned to red when sprayed with KOH/ MeOH while the upper remained yellow) and fractions 10 and 11 CF4 (100 ml) showed three major yellow spots which turned to violet color when sprayed with KOH/ MeOH solution.

Fractions 4-6 CF2 was applied on prep. TLC using pet. ether /$CHCl_3$/acetone (1:9:1) resulting the isolation of SL03-91 5 mg, SL04-92 2 mg and SL05-93 1.5 mg. Fractions 7-9 CF3 were chromatographed on prep. TLC using pet.ether/ $CHCl_3$/EtOAc (2.5:2:0.5) resulting SL03-91 (2mg), SL05-93 (0.5mg) and SL06-95 (lower) small amount. The combined fractions 10 and 11 CF4 were small amount for further prep. TLC separation and reserved.

As it was described in the result and discussion part, from ^{1}H-NMR and IR data the isolated pigments are all dimeric anthraquinones. To increase the quantity and the quality of the isolated pigments, the reserved 90 g methylenechloride-methanol crude extracted was fractionated with the same procedure as before.

90 g of the methylenechloride-methanol extract was absorbed on 50gm of silicagel and charged to a column packed with 350 g of silicagel (5% oxalic acid

impregnated) using chloroform. The column was eluted using the following solvent systems: chloroform: fractions 1-8 F1 100 ml x 8, chloroform/EtOAc (4:1): fractions 9-12 F2 100 ml x 4 and EtOAc: fractions 13 and 14 F3 200 ml x 2. Fractions 1 and 2, f1 contain large amount of fat, showed a single homogenous yellow spot but turned to red and violet when sprayed with 5% methanolic KOH solution on TLC examination. Fraction 3-8 f2 also showed a single homogenous yellow spot but turned to red and violet when sprayed with 5% methanolic KOH solution. Fractions 9-12 showed yellow spots on TLC before and after sprayed with KOH/MeOH, no anthraquinone. Fractions 13 and14 do not contained anthraquinone.

The combined fractions 1 and 2 f1 were applied on to a column packed with 100 g of silicagel using pet. ether. The column eluted with pet. ether: fractions 1-3 (25 mlx3) X_1, methylenechloride: fractions 4-6 (25 mlx3) X_2 and CH_2Cl_2/EtOAc(4:1) fractions 7-9(25 mlx3) X_3 were collected. Fractions 1-3 contained only fat and were discarded.

Fractions 4-6 X_2 showed a single homogenous yellow spot on TLC but turned into red and violet color when sprayed with 5 % methonolic KOH solution. Fractions 7-9 X_3 showed yellow spots on TLC which remained yellow after sprayed with 5% methonolic KOH solution.

The combined fractions 4-6 (X_2) applied on prep. TLC using chloroform/pet. ether (2:1) to give two bands. The top band turned out to be SL0l-78 (trace amaount) and the lower band was SL03 -91 (Floribundone -1) 5 mg.

The combined fractions 3-8 f2 were subjected to separation on sephadex resulting the collection of 30 fractions. Fractions 1-18 contained only green pigment and were discarded. Fractions 19-25 contained yellow pigment and TLC examination showed yellow spots which remained yellow after sprayed with KOH/MeOH solution. Fractions 26-30 were tested on TLC and showed a single homogeneous yellow spot which turned to red and violet when sprayed with 5% methanolic KOH solution. These fractions were SL01-78 and SL03-91 and were separated with prep. TLC using chloroform/pet. ether (2.1) resulting 3mg SL03 -91 (Floribundone-1) and small amaount of SL01-78.

7.4 Characterization of the pigments of *Senna sophers.*

Floribundone-1 (SL03-91). An orange solid, λ max 443, 360, 276, and 236 nm. IR KBr pellets, 3400, 1627.7 and 1095 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.9 (lH,s,1-OH), 7.0 (1H,s,2-H), 7.4(1H,d,4-H), 6.8(1H,s,7-H), 2.3(3H,s,3-CH_3) 3.7(3H,s,6-OCH_3),12.3(1H,s,8-OH), 12.1(1H,s,1'-OH), 7.1(1H,s,2'-H) 7.7(1H,s,4'-H), 7.6(1H,d,5'-H), 2.4(3H,s,3'-CH_3), 3.8(3H,s,6'-OCH_3) and 13.05(1H,s,8'-OH) .^{13}C-NMR (100 MHz,$CDCl_3$) δ 162.5(C-1), 124.3(C-5), 148.8(C-3), 121.6(C-4), 120.6(C-5), 165.0(C-6), 105.0(C-7), 166.5(C-8), 191.7(C-9), 182.7(C-10), 22,6(3-CH_3), 56.9(6-CH_3), 134.6(C-4a), 111.0(C-8a), 113.9(C-9a), 133.7(C-10a), 162.9(C-1'), 124.8(C-2'), 148.8(C-3'), 121.6(C-4'), 104.7(C-5'), 163.8(C-6'), 118(C-7'), 161.6(C-8'), 191.5(C-9'), 182.5(C-10'), 22.7(3'-CH_3), 57.0(6'-OCH_3), 134.0(C-4a'), 111.4(C-8a'), 114.2(C-9a') and 134.7(C-10a').

Reductive cleavage of floribundone -1 (SL03-91): To a solution of SL03-91 (5 mg) in 4 ml of an aqueous NaOH(5%), sodium dithionite,$Na_2S_2O_4$(2O mg) was added and left for 2 hours. The solution was then acidified and extracted with $CHCl_3$, dried and evaporated to give only Physcion(TLC test,^{1}H-NMR and ^{13}C-NMR, See Table13).

Esterification of floribundone-1(SL03-91): Pyridine (2 ml) and acetic anhydride (2 ml) were added to the SL03-91(5 mg) in a round bottom flask. The sample was left to stand at room temp, for 48 hours while stirring. Methanol (5 ml) was then added to the sample to react with the excess acetic anhydride and to pet.ether (4x100 ml) was added successively to remove pyridine. After each addition, the solvent was evaporated off on the Rota Vapor and dried to give floribundone-1 tetra-acetate (TLC test, color reaction negative to KOH /MeOH solution). The ^{1}H-NMR indicates six aromatic protons δ (7.8 brs, 7.7 s, 7.6 s, 7.1 s, 7.0 s and 6.8 s), two aromatic methoxyl groups at δ (2.4 and 2.3), four acetyl methyl groups at δ (2.4, 2.3, 2.17 and 2.0).

**5$_1$7^1-Physcion-fallancinol (SL04-92**): A yellow solid, λ max 455, 365,278 and 240. IR KBr Pellet 3400 and 1626cm^{-1}. ^{1}H-NMR δ 12.1 (1 - OH), 6.8 (2- H), 7.3 (4 - H), 6.5 (7 - H), 12.0 (8 -OH), 3.8 (6-OCH_3), 2.3 (3-CH_3), 12.7 (1'-OH), 7.2 (2' - H), 7.7(4'-H), 7.6 (5' - H), 12.9 (8'- OH), 3.9 (6'-OCH_3) and 5.0 (3' - CH_2OH).

10-Hydroxyfloribundone-2 (SL05-93): A yellow solid, λmax 440, 370, 270 and 240. IR KBr Pellet 3400, and 1626 cm $^{-1}$. ^{1}H-NMR δ 11.9 (1- OH), 7.0 (2 - H), 6.6 (4 -H) 6.7 (7-H), 5.2 (10 - H), 12.3 (8- OH), 3.8 (6-OCH_3), 2.3 (3 – CH_3), 12.O (1' - OH), 7.1 (2' - H), 7.7 (4'- H), 7.6 (5'- H), 3.8 (6'-OCH_3) and 12. 9(8'-OH).

CONCLUSION AND RECOMMENDATION

Three compounds were isolated and characterized from the leaves of *Senna sophera*. Floribundone-1 is a known compound, which was isolated previously from its seeds. 5,7'-physcion-fallacinol is common while there is no prior report on the isolation of 10-hydroxyfloribundone-2.These two compounds are identified by comparing their ^{1}H-NMR spectrum for similar compounds in the literature. The structure of floribundone-1 is identified by spectroscopic and chemical methods. Except floribundone-1, which was isolated in appreciable amount (25mg), the other compounds were obtained in small quantities 2 mg (SL04-92) and 3mg (SL05-93). The study yielded more than three unidentified anthraquinones (color reaction test) whose structure can be elucidated in the future.

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Appendices