COMPARATIVE CHEMICAL STUDIES ON THE LEAVES OF SOME ALOE SPECIES OF ETHIOPIA

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ABSTRACT

Comparative Chemical Studies on the Leaves of Some *Aloe* Species of Ethiopia

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Chemical investigations on the leaves of *A. berhana* Reynolds, *A. rivae* Baker, *A. megalacantha* Baker and *A. pulcherima* ined. resulted in the isolation of a total of nine compounds, the structures of which were established by spectroscopic means as well as chemical degradation to known compounds and comparison, in some cases, with authentic samples. Chrysophanol and β-Sitosterol were isolated from all four species. Furthermore, *A. berhana* afforded a novel pre-anthraquinone (tentatively given the trivial name berhanone), protocatechuic acid (a biologically important aromatic acid rare from a natural source), barbaloin (the major active constituent of the officially recognised commercial aloes responsible for the use of the plant as purgative) and aloe-emodin. *A. rivae* and *A. megalacantha* yielded aloe-emodin, berhanone, barbaloin and aloinoside (a constituent of the commercially important Cape and Socotrine aloes). *A. pulcherima* afforded nataloin (major constituent of the commercial Natal aloes) and 7-hydroxybarbaloin (the characteristic substance of the commercially important Curacao aloes in the European Pharmacopoeia).

A further comparative chemical screening on the presence of the commercially important compound, barbaloin, in the leaves of some *Aloe* species by means of HPLC and TLC analyses revealed that this glucoside is the major constituent of *A. berhana* Reynolds, *A. rivae* Baker, *A. megalacantha* Baker, *A. calidophylla* Reynolds, *A. secundiflora* Engler, *A. mcloughlinii* christian, and *A. otalensis* Baker. Barbaloin was, however, not detected in the leaves of *A. pulcherima*.

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1. INTRODUCTION

1.1 The Genus *Aloe*

The family Liliaceae, classified under the order Liliiflorae, is one of the widely distributed families of flowering plants. It Consists of over 250 genera comprising 3700 species, mostly perennial herbs with rhizome or bulb[1].

However, a major revision of superorders, orders and families within the monocotyledons has been proposed by Dahlgren and Clifford[2]. The proposed scheme gave greater weight to cryptic characters including phytochemical information and is now gaining wide acceptance. According to this scheme most of the genera earlier grouped in the family Liliaceae are now classified under other families. Accordingly the genera *Aloe*, *Bulbine*, *Kniphofia* ... etc are now categorized under the Asphodelaceae. Only the cultivated genera such as *Fritillaria* and *Lilium* are still under Liliaceae. However, concerning the genus *Aloe* there are still queries such as on whether it should be put in a separate family of its own namely Aloaceae or should be kept as proposed in the Asphodelaceae[3].

The genus Aloe includes herbs, shrubs and trees bearing spikes of white, yellow or red flowers. The leaves are fleshy, strongly culticularized and are usually prickly at the margins[1]. Aloes are indigenous to East and South Africa but have been introduced in the West Indies (where they are extensively cultivated) and into tropical countries and fluorished even in the countries boardering Mediterranean[4]. According to Mabberley[5] the genus Aloe consists of over 360 species found over most of Africa, Southern Arabia and Madagascar.

1.2 Traditional Medicinal Uses of Aloe Species

The term aloe is from the Arabic word allaeh meaning a shining bitter substance[6]. The gel and dried leaf exudates of Aloe species have been used medicinally in ancient times by Egyptians, Assyrians and Mediterranean Civilizations[7]. These species have enjoyed wide folkloric usage since then and are also now popularly used as medicines in many societies of the developed as well as the less developed world. Some of the numerous important traditional medicinal uses of some Aloe species are given in Table 1.

Table 1 Important Traditional Medicinal Uses of Some *Aloe* Species

Species	Used in the treatment of	Ref.
A. excelsa A. Berger	Veneral sores, asthma and abdominal pains	8
A. saponaria Haw	boils	5
A. vera Linne	burns, abrasions, and other skin irritations, injuries and wounds,	
	x-ray and atomic radiation burns	6
	ring worm and acute x-ray burns	9
A. ferox Miller	ophthalmia and syphilis, and scab on sheeps	10
A. marlothii Berger	tape worm infestation	10
A. mutabilis Pillans	x-ray burns	10
A. variegata L.	haemorrhoids	10
A. longibracteata Pole Evans	sores	10
A. macracantha Bax	cleaning out the intestine after taking a purgative medicine, feverish colds, stomach troubles	10
A. arborescens Mill	parturition in women and sick calf as a drench	10

Species	Used in the treatment of	Ref.
A. castanea Schonl	the ash of leaves insect repellent for stored grain	10
Other Unidentified Aloe species	painful menstruation associated with sterility, ophthalmia, abortifacient, cancer, anthelmintic, fish poison, skin ulcers, constipation, tooth pain, chronic conjunctivitie, blepharitis ... etc.	10

1.3 Commercial Importance of *Aloe* Species

1.3.1 Medicinal Action and Uses of the Drug Aloe

Pharmacologically the term aloe covers a variety of products obtained from fleshy leaves of the plant. The bitter leaf exudates of latex of some *Aloe* species are commercially important sources of the laxative aloe drug[11]. Exudates of some *Aloe* species are also important trade items in the cosmetic industry. They have been used as additives in shampoos, shaving and skin care creams topical medication for treatment of burns and constituents of many other co metic preparations. Partial purification of the crude aloe drug yields a yellow powder known commercially as "aloin", which has been used as purgative for centuries, from which the active constituent barbaloin could be isolated[12,13]. In recent literature the two names barbaloin and aloin have become interchangeable.

The drug aloe is one of the safest and best warm and stimulating purgatives to persons of sedentary habits and phlegmatic constitution. Its action is exerted mainly on the large intestine, for which reason it is also used as vermifuges[4,6,9]. The drug aloe is an ingredient of compound benzoin tincture and cathartic. Benzoin tincture when taken internally acts as an expectorant and antiseptic and diuretic properties[1,6].

The usual cathartic does of aloin has been taken to he 15 mg[6].

1.3.2 Commercially Important Sources of the Drug Aloe

The chief varieties of aloes that are officially recognised for medicinal uses are Curecao or Barbados, Cape and Socotrine aloes obtained from different geographical sources and species[4,13].

Curacao aloes is obtained from A. chinensis Staud and A. vera Linn. Aloe barbadensis Miller (or A. vera Linn) is a native of northern Africa but was introduced into the Barbados islands in the seventeenth century. Aloe chinensis, a variety of A. vera was introduced from China in 1817. The drug has been prepared extensively on the Dutch islands of Curacao, Arubia and Bonaire. In 1976 the principal areas of production were Venezuela, Curacao and South Africa. Curacao aloes possesses the nauseous and bitter taste that is characteristic of all aloes and a disagreable penetrating odour. It is the most importnat form occurring in the United State of America. Analyses have revealed that Curacao aloes is superior to Cape aloes.[6]

Cape aloes is prepared in Cape colony from A. ferox Miller and hybrids of this species with A. africana Miller

purgative properties than any other variety of the drug and is preferred to other varieties on the continent, but is chiefly employed in this country for veterinary purposes only, tough for this purpose, the Curacao aloes is as a rule preferred[4]. The drug has a very characteristic, sour odour (the so-called rhubarb or apple-tart odour), which is particularly noticeable if one breathes on the drug before smelling[1]. Large quantities of the drug were exported from Cape Town and Mossel Bay.

Socotrine aloes is prepared from the leaves of A. perryi Baker, a species in the islands of Socotra. When it is prepared it is commonly poured into goat skins, which are then packed into cases. It may be distinguished principally from Curacao aloes by its different odour.

The major constituent of the above three commercial varieties of aloes is the anthraquinone C-glucoside, barbaloin. The other variety of commercial aloes which does not contain barbaloin is Natal aloes. The source of this variety is not yet definitely ascertained[4,14]. Natal aloes contains as chief active principles the anthraquinone C-glucosides nataloin and homonataloin. According to Beaumont et al.[15] the commercial drug was said to be obtained from a chemivar of A. marlothii

Berger or from a chemivar of *A. spectabilis* Reyn. In general barbaloin and homonataloin are never present together in the same exudate[16].

1.4 Secondary Metabolites of *Aloe*

Chemical studies on some members of the genus *Aloe* have so far resulted in the isolation and characterization of about 55 secondary metabolites.

The secondary metabolites like anthraquinones, anthrones, chromones and their glycosides have been obtained from many *Aloe* species and therefore could be considered as a common feature of the genus. However pre-anthraquinones and their glucosides and bianthraquinoids have so far been reported from only one species namely *A. saponaria* Haw. Sulfur containing benzoyl derivative, a coumaric acid ester and a naphthoic acid lactone derivative have been reported from *A. pluridens*, *A. ferox* and *A. saponaria*, respectively. The only two species that have been investigated for their phytosterols and triterpene composition are *A. arborescens* and *A. barbadensis*.

Bioactive extracts have also been reported from some *Aloe* species. These include antifungal properties against *Aspergillus niger*

1.5 *Aloe* Species of Ethiopia

1.5.1 Taxonomy and Traditional Medicinal Uses

The recent taxonomic study of the Ethiopian *Aloe* species was that of Reynolds[20]. Reynolds recorded about 37 *Aloe* species, 12 of which were described by him from previously undercollected areas of Central, Nothern and Eastern Ethiopia. Reynolds indicated that his work was not complete and predicted that several new species may be discovered from some African countries including Ethiopia. Based on field observations and herbarium studies, a preliminary taxonomic investigation of 17 *Aloe* species is now in progress. This study revealed two new taxa named as *A. pulcherima* and *A. gilberti*[21].

Aloe berhana Reynolds is an endemic species which occurs near Debre Berhan, North Shoa Administrative Region[22]. Some of the *Aloe* species described by Reynolds[20] were named after their place of location eg. *A. adigratana*, *A. massawana*, *A. yavellana* ... etc. or after the discoverers eg. *A. jacksonii*, *A. schelpei* ...etc. A particular type of *Aloe* locally known as *sete eret*, characterized by the lack of spines on the leaf margins, is commonly prescribed as medicine in many parts of shoa. This plant has turned one to be a hitherto undescribed species named as *A. pulcherima*[21].

Other species used in folk medicine of this country to treat various ailments include A. trichosantha Berger and A. megalacantha Baker to cure intestinal complaints.

Having an intensely bitter taste, Aloe species in many parts of the countrysides are used to discourage children from biting their fingernails and interrupt breast-feeding.

1.5.2 Prior Work on Some Aloe Species

The only report available in the literature on the constituents of Aloe species of Ethiopia is by Croom and Reynolds[16]. In the course of their chemical investigation of barbaloin levels in the exudates from the leaves of 68 species of Aloe, they have included about 13 Aloe species which are known to occur in Ethiopia. Plants of Aloe species used in the analyses were collected from the wild and grown in glasshouses at at the Royal Botanic Gardens, Kew, England. The method used to estimate the amount of barbaloin in exudates from leaves of Aloe species was separation of barbaloin in the crude extracts by HPLC followed by integration of UV absorption of the peak at a characteristic wavelength (375 nm).

Under the solvent conditions they used, the two diastereomers of barbaloin were not separated so that, the barbaloin peak was estimated as a single entity. As was mentioned in section 1.3 of this manuscript, barbaloin is the purgative principle of the commonly available aloes of commerce. The name aloin was given to a crude material, amorphous or crystalline according to the degree of purification, from which barbaloin could be isolated.

The exudates from most species contain between 10-20% although a few concentrations of around 30% were found. The level in the leaf was usually around 1.1% of the dry weight in plants in glasshouses at Kew although some species were found to contain up to 5%. The partial results of their investigation[16] is given in Table 2. Section I shows levels of barbaloin in Ethiopian Aloe species, Section II in aloes of commerce and Section III in Aloe species of potential commercial importance.

Table 2 Barbaloin Levels of Some Ethiopian *Aloe* Species and Some Important Commercial Aloes

Plant	Barbaloin % dry wt of exudate	Barbaloin % dry wt of leaf
Section I		
A. trichosantha Berger	29.9	5.4
A. calidophylla G. Reyn	28.9	3.8
A. adigratana C. Reyn	21.2	2.6
A. rivae Baker	20.2	0.4
A. harlana C. Reyn.	17.7	0.6
A. secundiflora Engler	17.4	3.7
A. massawana C. Reyn.	17.3	1.1
A. schelpei C. Reyn	17.1	1.1
A. megalacantha Baker	13.8	2.4
A. retrospiciens G. Reyn et Bally	12.0	0.9
A. elegans Tod	11.0	5.5
A. mcloughlinii Christian	7.5-31.29[a]	0.9-2.1[a]
A. gilberti inad.	5.4	0.4
Section II		
A. ferox Miller	9.7	1.3
A. africana Miller	11.6	0.7
A. barbadensis Miller	17.3	0.9
A. perryi Baker	23.5	2.4
Section III		
A. turkanensis Christian	30.8	6.6
A. classenii G. Reyn	29.4	5.4

a = range of levels in individuals from different localities

Based on their results obtained, Groom and Reynolds have pointed out that Cape aloes of commerce derived from A. ferox miller harvested in the Wild is seen to be not the best choice because chemivars giving different yields occur and the plants are slow-growing and without side shoots so that propagation is difficult. Aloe perryi Baker the source of Socotrine aloes, said to be finer than Cape aloes, was found to have a high concentration of barbaloin (25.5%) in the exudates. In addition to the above comment, they have suggested that A. classenii G. Reyn and A. turkanensis Christian would be suitable choices because as well as having high concentration of the compound in the exudate, they form clumps and are readily propagated by offsets.

Inspection of Table 2 shows that the level of barbaloin in some species is interestingly comparable with those of the commercial aloes and even much better in some cases notably the first three entries. These promising results encourage rigorous analyses on Aloe species of Ethiopia in the search for best choices of sources of barbaloin. Once this is done, the remaining task would then be practicing extensive cultivation of best candidates of Ethiopian Aloe species to fulfill the demand for barbalin, provided that they are fast-growing and readily propagating.

1.6 The Objective of the Present Study

The genus Aloe is widespread in Eastern and Southern Africa extending to Sudan, but in Asia is only known from South-West Arabia[23]. The genus is known to possess important traditional medicinal uses and is also commercially important. Leaf exudates of a few species of Aloe have been used for centuries for their purgative activity, which has shown to be due to anthraquinone derivatives notably barbaloin and which is still listed in many national Pharmacopoeias[3]. As a result of these, the cultivation of Aloe plants is now rapidly becoming world-wide.

To date about 55 secondary metabolities have been isolated and characterized from some members of the genus Aloe. Since the number of species so far examined covers only a small percentage of the 360 or so recorded Aloe species the above number of compounds is more likely to rise.

The main objective of the present study is to investigate the chemical constituents of some Aloe species of Ethiopia. To our knowledge, Aloe species of Ethiopia have not yet been subjected to detailed systematic phytochemical or pharmacological study except for the chemical screening work of Groom and Reynolds[16]. Groom and Reynolds examined the level of the commercially important compound, (barbaloin) in the exudates of about 13 Aloe species which are known to occur in Ethiopia.

The list includes A. rivae, A. megalacantha, A. secundiflora, A. mcloughlinii and A. calidophylla. Whereas, the leaves of A. berhana, A. otalensis and A. pulcherima have so far not been investigated at all.

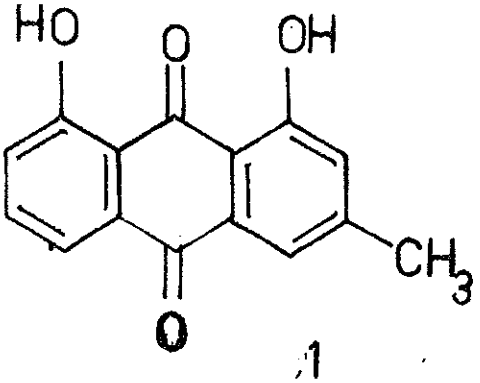
The present study covers phytochemical studies on the leaves of A. berhana, A. rivae, A. megalacantha and A. pulcherima for which large scale collections were available. In addition to this, screening work on the presence of the commercially important compound (barbaloin) have been performed in the leaves of the above species and four additional namely A. calidophylla, A. secundiflora, A. mcloughlinii and A. otalensis by means of HPLC and TLC methods. An attempt will made to compare the major constituents of these eight species with the commercially recognised important Aloe species of the world. Furthermore, the present study demonstrates the need for a collaboration between botanists and chemists in light of the taxonomic work in progress on Aloe species of Ethiopia.

2.2 Elucidation of Structures of Compounds

2.2.1 Chrysophanol (1)

This compound was obtained from A. berhana (both extraction methods) as well as the remaining three species

The ^1H NMR spectrum of this orange - yellow pigment revealed the presence of two singlets in the chelated hydroxy region, five aromatic protons (ABC type and two broad singlets) and one aromatic methyl group. These signals together with the appearance of orange colour upon treatment with methanolic $Mg(OAc)_2$ suggested 1,8-dihydroxyanthraquinone nucleus[24]. In accordance with the above facts, the IR spectrum has also diplayed absorption bands due to hydroxyl (3420 cm^{-1}), a non-chelated quinone carbonyl (1675 cm^{-1}) and a chelated carbonyl (1630 cm^{-1}) group



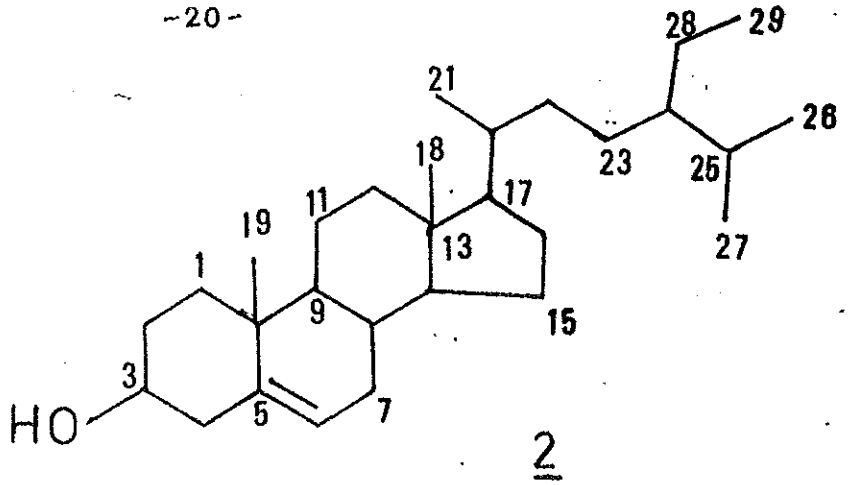
1

The combined evidence presented above establish 1 as the structure of this anthraquinone, which was also further confirmed by co-TLC and mmp comparisons with authentic specimen. Chrysophanol is one of the most widely distributed anthraquinones of higher plants[25] but its occurrence in Aloe species has been reported only from one species namely A. saponaria[26].

2.2.2 β -Sitosterol (2)

This compound was isolated from all four species.

A dark purple colour upon treatment with vanillin-sulphuric acid spray reagent was observed for this compound suggesting terpenoid or sterol skeleton[27]. The high resolution EIMS of this compound revealed a molecular ion at m/z 414 consistent with the molecular formula $C_{29}H_{50}O$ further indicating triterpenoid or sterol nucleus[28,29]. The ^{13}C NMR spectrum showed 27 signals for 29 carbons and in the DEPT spectrum five CH_3, eleven CH_2, nine CH and two quaternary carbon signals were observed. Particularly noteworthy are the last three downfield carbon resonances at δ 141.2, 121.7 and 72.1, which are characteristic feature of Δ^5 - phytosterols[30]. These are assignable to carbon resonances of C - 5, 6 and 3 respectively[31,32]. Accordingly, the H-6 signal was discernable at δ 5.4 appearing as a multiplet in the ^1H NMR spectrum. Comparison of the ^1H NMR spectrum with spectra of 42 phytosterols[33] has revealed the presence of an ethyl side chain at position 24 of this phytosterol. In accordance with the above facts, the MS of this phytosterol showed a relevant ion at m/z 329, observed for Δ^5 - phytosterols only, whose genesis must involved complex rearrangement reaction[34,35].



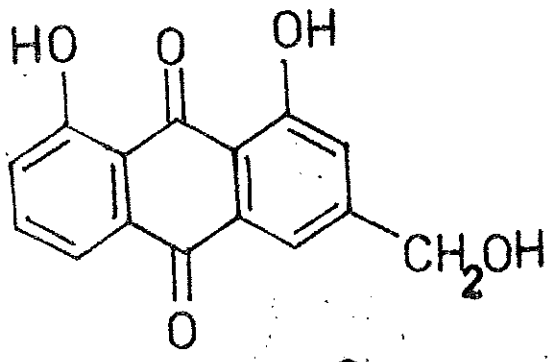
2

On the basis of the above discussed spectra data this phytosterol was identified as β - Sitosterol (2). Further confirmation of this assignment was derived from co-TLC and mmp comparison with authentic sample. The ^{13}C NMR data of 2 closely agree with those of the sitosterol moieties of sitosterol 3-O-glycosides[31,32]. β - Sitosterol (2) is widely distributed in plants[30,36] and its occurrence in Aloe species has been reported only from two species[37,38].

2.2.3 Aloe-emodin (3)

This compound was obtained from A. berhana (Soxhlet ext), A. rivae and A. megalacantha.

This orange-yellow compound gave orange-red colour upon treatment with methanolic $Mg(OAc)_2$ suggesting 1,8-dihydroxy-anthraquinone skeleton[24]. The identification of the structure of this compound as 3, which was isolated in each case in small quantities, was only based on colour reactions with spray reagents, co-TLC and mmp comparisons with authentic sample.



3

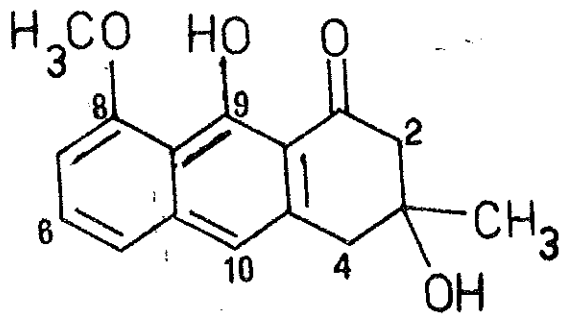
Aloe-emodin was obtained as orange-yellow needles from MeOH mp 219-220° (lit.[12] 223 - 4°). As the name implies it is present predominantly in Aloe species[12] and known to exhibit antileukemic properties[1].

2.2.4 Berhanone (4)

This compound was obtained in pure form from A. berhana (Soxhlet ext) and as an inseparable mixture from A. rivae and A. megalacantha.

The ^{1}H NMR spectrum of this compound revealed the presence of hydrogen bonded hydroxyl group appearing at δ 15.0, characteristic of 9 - OH of pre-anthraquinone skeleton[26,39]. Furthermore the spectrum displayed resonances attributable to three adjacent aromatic protons (δ 6.8, 7.2, 7.5), an isolated aromatic proton (7.0), an aromatic methoxyl (δ 4.0), an aliphatic methyl (δ 1.5) and two singlets (δ 2.8, 3.2) each integrating for two protons. The appearance of the methyl signal as a singlet together with multiplicities of the methylene protons required 3-OH substitution on the pre-anthraquinone nucleus. The 8-OMe

substitution was derived from coupling patterns and chemcial shift considerations of the four aromatic protons. The UV spectrum showed absorption bands at 216, 262, 293 and 370 nm which are comparable with the maxima of pre-anthraquinones[26,39].



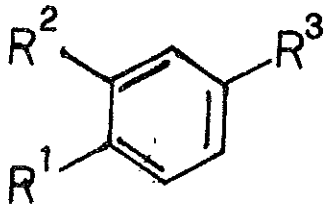
4

The above discussed spectral characteristics can only be accomodated by structure 4 for this greenish - yellow flourescing compound. This compound was found to be identical with authentic sample (co-TLC, co-HPLC, ^{1}H NMR) that has recently been isolated from the roots of A. berhana given the tentative trivial name berhanone[40]. The structure of berhanone was established on the basis of UV, IR, ^{1}H NMR, MS and chemical transformations into chrysophanol-8-methyl ether and chrysophanol. A search in the chemical literature revealed that 4 is a new natural product.

2.2.5 Protocatechuic Acid (7)

This compound was isolated only from A. berhana (both methods of extraction).

The HRMS of this compound showed an M^+ analysing for $C_7H_6O_4$ at m/z 154. The ^1H NMR spectrum displayed resonances for three aromatic protons typical of 1,2,4 - trisubstituted benzene ring. Its ^{13}C NMR spectrum revealed signals for 6 carbons resonating at δ 167.1, 149.1, 144.6, 121.7 116.6, and 115.0. The intensity of the signal at 121.7 is much higher than the others probably representing two carbons. The MS showed relevant peaks at m/z 137 $[M-OH]^+$ and 109 $[M-CO_2H]^+$, which are characteristic ions of aromatic acids[41]. The above data together with IR absorpotion bands for carbonyl group at 1700 cm^{-1} and hydroxyl groups 3300 - 3200 cm^{-1} suggested dihydroxybenzoic acid series. Three alternative structures can be assigned to this compound based on the above data. These are 2,4 - dihydroxybenzoic acid or resorcylic acid (5), 2,5 - dihydroxybenzoic acid or gentisic acid (6) and 3,4 - dihydroxybenzoic acid or protocatechuic acid (7).



5: $R^1 = CO_2H$, $R^2 = R^3 = OH$

6: $R^2 = CO_2H$, $R^1 = R^3 = OH$

7: $R^3 = CO_2H$, $R^1 = R^2 = OH$

These can be distinguished on the basis of ^{13}C chemical shift calculations of the aromatic carbons and MS fragmentation pattern.

Assuming additivity of substituent effects, ^{13}C chemical shift of an aromatic carbon "a" in a polysubstituted benzene can be predicted using the relationshp:

$$\delta_a = \delta^o + \sum_i S_{x,\,o,\,m\ or\ p\,;\,X_i}$$

Where δ a is the chemcial shift of carbon "a", δ^o is the chemcial shift of benzene (128.5 ppm), Sx, o, m or p is the shielding parameters of substituent Xi on the carbon or on the carbon ortho, meta or para to it and where the summation is carried over all the substituents on the ring.

The aromatic ^{13}C shielding parameters of OH and CO_2H substituents in the given order are +28.7, -12.8, +1.2, -8.5 and +1.9, +1.3, +0.2, +4.8 respectively.

The ^{13}C chemical shifts of 5, 6 and 7 can therefore be calculated by inserting the shielding parameters and chemical shift of benzene in the given equation and these values are given in Table 3.

Table 3 Calculated ^{13}C Chemical Shifts of 5, 6 and 7

C	5	6	7
1	109.1	118.8	123.1
2	159.7	259.0	118.2
3.	103.1	117.1	144.8
4	163.2	121.7	149.2
5	107.4	148.9	117.1
6	132.2	118.2	122.5

The ^{13}C chemical shifts of the carbonyl carbon of 5, 6 and 7 as reported by Scott[42] are 172.2, 172.1 and 169.4 respectively.

Comparison of these expected values with those chemcial shifts generated for this compound (167.1, 149.1, 14416, 121.7, 116.0 and 115.0) essentially ruled out the first two structures and hence structure 7 was assigned for this brown amorphous solid. This was also further substantiated by the lack of significant $[M - H_2O]^+$ ion in the MS of 7 expected from ortho- effect[41,43].

Protocatechuic acid (7), although it is rare from a natural sources, has been reported form Coffee pulp[44] and can be extracted from lignin[36]. The occurrence of 7 in the leeves of A. berhana reported here appeared to be the first record in the genus Aloe. This biologically important aromatic acid[42] is also commercially available and has got the price of 18.1$ for 25 g as listed in the Aldrich catalog[45]. Derivatives of 7 have been frequently used in the biosynthetic studies and syntheses of some pharmacologically important isoquinoline alkaloids[46,47].

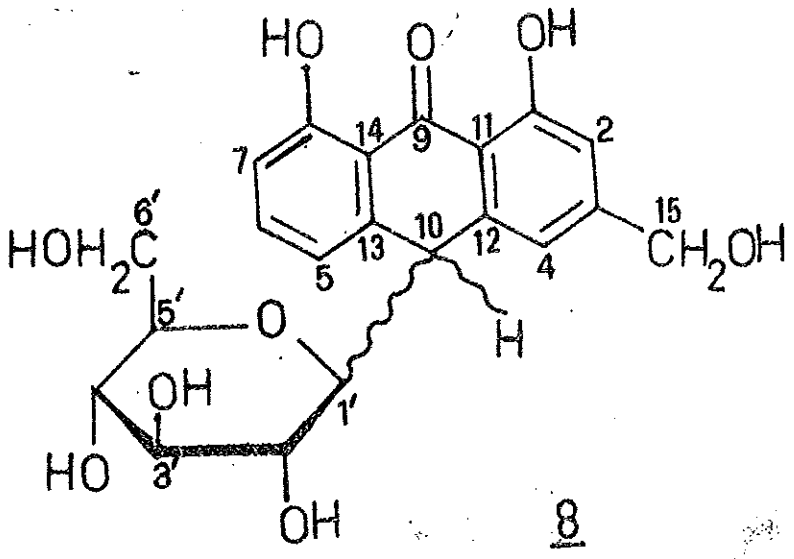
2.2.6 Barbaloin (8)

This commercially important compound was isolated from A. berhana (Soxhlet ext), A. rivae and A. megalacontha.

The ^{1}H NMR spectrum revealed resonances for five aromatic protons as two broad singlets and ABC type. Furthermore, the spectrum disclosed the presence of atleast two chelated hydroxyl groups, glycosyl protons and a multiplet at δ 4.6. The ^{13}C NMR spectrum showed the substitution pattern typical of aloe-emodin anthrone nucleus with a C - linked glycoside at C - 10. The ^{13}C chemical shifts such as δ 193.6 for C - 9 and 43.9 for C-10 together with the IR absorption band for a chelated carbonyl group at 1630 cm^{-1} were typical of the anthrone nucleus[48,49,50]. A positive borax test (greenish-yellow fluorescence) has

also been obtained which is characteristic of anthranols[1]. Resonances at δ 77.9 and 3.2 in the ^{13}C and ^{1}H NMR spectra were typical of C - 1 anomeric position of C - glucoside[50]. The other sites of linkage of anthrone and sugar units were ruled out from the fact that these linkages do not occur under biological conditions as verified by Rees[51] and Pigman and Horton[52]. The C - 10 glucoside nature of this compound was also derived from the fact that, when this compound was subjected to standard acid and alkaline hydrolysis, it did not resulted in the detection of any sugar like materials[52,53,54]. Oxidative hydrolysis by $FeCl_3$ gave glucose and a product which after treatment with base followed by acidification essentially yielded aloe-emodin (3) further indicating C-glucoside nature[1].

On the basis of the above discussed facts structure 8 was assigned to this compound and substantiated by co-TLC comparison with authentic sample.



8

Barbaloin (8) is the major active constituent of the commercially recognised aloes that is responsible for the use of the plant as purgative, still listed in many national Pharmacopoeias[3,12].

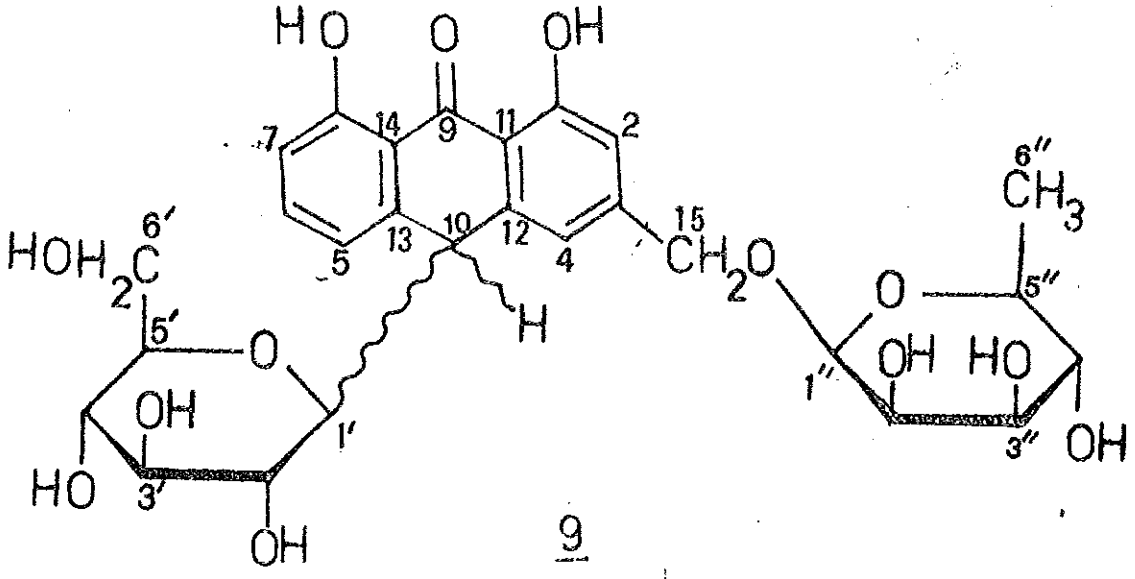
The ^{1}H NMR spectrum in the chelated hydroxy region and ^{13}C NMR chemcial shifts and patterns of 8 (Table 4 and 5) indicated that it occurs as a mixture of two stereoisomers of barbaloin in which the glucose unit is attached α or β at C - 10[49,50]. Comparable C - 10 isomers have been reported for homonataloin (24)[50]. Optical rotation measurement indicated $[\alpha]_D$ 0 (MeOH, c = 0.02) for 8. The values in the chemical literature are -8.3 (EtOH) and -10.4 (EtOAc) but do not distinguish to which isomer are these values reported for[36]. The ^{1}H NMR data (Table 4) as well as the UV and mp of 8 are compatible with those reported in the literatures[49,50,36].

2.2.7 Aloinoside (9)

This compound was obtained from A. rivae and A. megalacantha.

The ^{13}H NMR spectra of this compound revealed all the resonances attributable to barbaloin (8). In addition to these, the spectra showed signals such as 99.4 and 5.1

typical of C - 1 anomeric position of an O-glycoside[53,54]. Accordingly acid and alkaline hydrolysis of this compound have afforded rhamnose and a product identical to 8 confirming that it was an O-rhamnoside of barbaloin. The pronounced deshielding of the 3-CH_2 signal of barbaloin moiety of this compound (from 62.3 to 67.0) required placement of the rhamnose unit at the 3-CH_2O-position of barbaloin nucleus[48]. Further confirmation of this assignment was derived from $FeCl_3$ oxidative hydrolysis followed by treatment with base and subsequent acidification gave glucose and a product which after acid hydrolysis yielded rhamnose and aloe-emodin (3) in synthetically useful yields. Moreover, a greenish-yellow flourescence was also obtained for this compound when subjected to borax test[1].



9

On the basis of the above discussed spectral data and chemical transformations, this compound was characterized as barbaloin-15-O-rhamnoside or aloinoside (9) that has previously been isolated from two of the commercially important aloes namely Cape and Socotrine aloes[12]. The occurrence of 9 as mixture of two stereoisomers namely aloinoside A and B was also discernable from the spectroscopic data presented in Tables 4 and 5. This was also substantiated by optical rotation measurement in which case $[\alpha]_D$ + 75 (MeOH, c = 0.02) was obtained. The literature value for aloinoside B is $[\alpha]_D^{20}$ -45.3 (aq. dioxane)[12].

Table 4, ^1H NMR Data of 8 and 9

H	8	9
2	6.8 brs	6.8 brs
4	7.0 brs	7.0 brs
5	6.8 d (7.8)	6.9 d (7.8)
6	7.5 t (7.8)	7.4 t (7.8)
7	7.1 d (7.8)	7.1 d (7.8)
10	4.6 brs	4.6 brs
3 - CH_2	4.6 brs	4.8 brs
1, 8-OH	11.85,11.30,11.75	11.8 brs
1"	3.2 brs	3.3 brs
2' - 6'	2.5 - 3.4 m	2.5 - 3.4 m
1"	-	5.1 brs
2" - 5"	-	3.5 - 4.9 m
6"	-	1.2 d (4.5)

Spectra run in DMSO - d_6, J values in parentheses

Table 5 ^{13}C NMR Data of 8 and 9

C	8	9
1	160.7	160.8
2	119.8, 118.4[a]	118.0
3	151.8, 150.9[a]	145.3, 146.0[a]
4	115.3, 115.0[a,b]	115.3[b]
5	112.5, 112.7[a]	113.7
6	135.5, 134.7[a]	135.4
7	117.6, 116.1[a,b]	117.1[b]
8	160.7	160.8
9	193.6	193.2
10	43.9	43.9
11	117.0[c]	117.0[c]
12	145.2[d]	145.2[d]
13	141.5[d]	141.3[d]
14	115.0[c]	114.0[c]
15	62.3	67.0
1'	79.9	78.0
2'	70.4	70.4
3'	80.2	79.5
4'	70.1	70.1
5'	84.8	84.7
6'	61.5	61.7
1"	-	99.4
2"	-	70.8
3"	-	72.4
4"	-	69.5
5"	-	64.7
6"	-	17.1

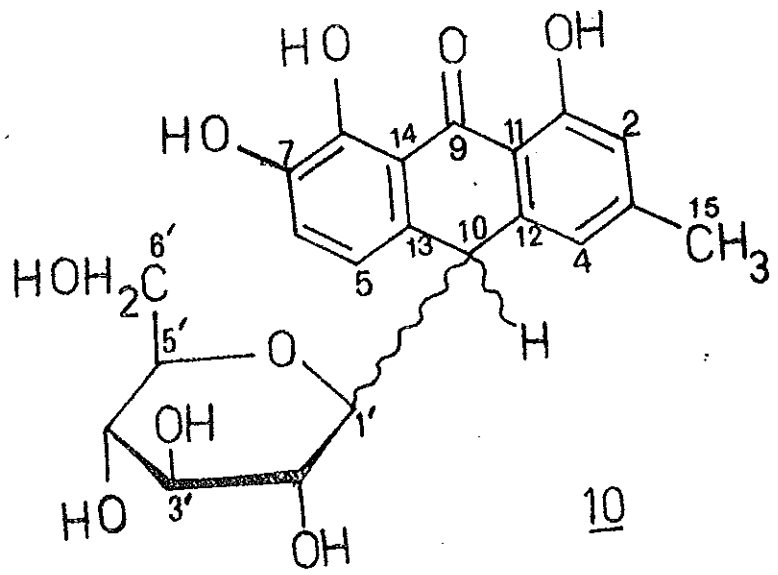
a = a pair of signals with equal intensity but lower than the others, indicative of diastereomeric mixture.

b,c,d = assignments with the same label may be interchangeable

2.2.8 Nataloin (10)

This orange amorphous solid was isolated from A. pulcherima. This compound gave voilet colour upon treatment with methanolic $Mg(OAc)_2$, indicating a 1,2-dihydroxyanthraquinone homolog [24]. The ^1H NMR spectrum disclosed the presence of three D_2O exchangeable phenolic hydroxyl groups (two H-bonded), four aromatic protons (two ortho - coupled AB$_q$ type and two broad singlets), an aromatic methyl group and glycosyl protons. The H - 1' anomeric position of this glycoside appearing at δ 3.2 suggested the C-glycoside nature [53,54]. The ^{13}C chemical shifts such as 193.6 and 43.6 assignable to C - 9 and 10, respectively, together with the signal at 79.7 attributable to C - 1' indicated the anthrone 10 - C - glucoside skeleton. The UV spectrum showed absorption maxima at 263, 273, 305 and 356 nm indicating the anthrone nucleus[50]. In accordance with the above spectroscopic data, acid and alkaline attempted hydrolysis did not also resulted in the detection of any sugar like materials[53,54]. The $FeCl_3$ oxidative hydrolysis, however, has afforded glucose an unidentified pigment owing to its unstability.

Based on the combined evidence presented above this compound was identified as nataloin (10), which is the major constituent of the commercial Natal aloes[4,14].



10

The ^{1}H and ^{13}C NMR data generated by Conner et al.[48] are in full agreement with those presented in Tables 6 and 7.

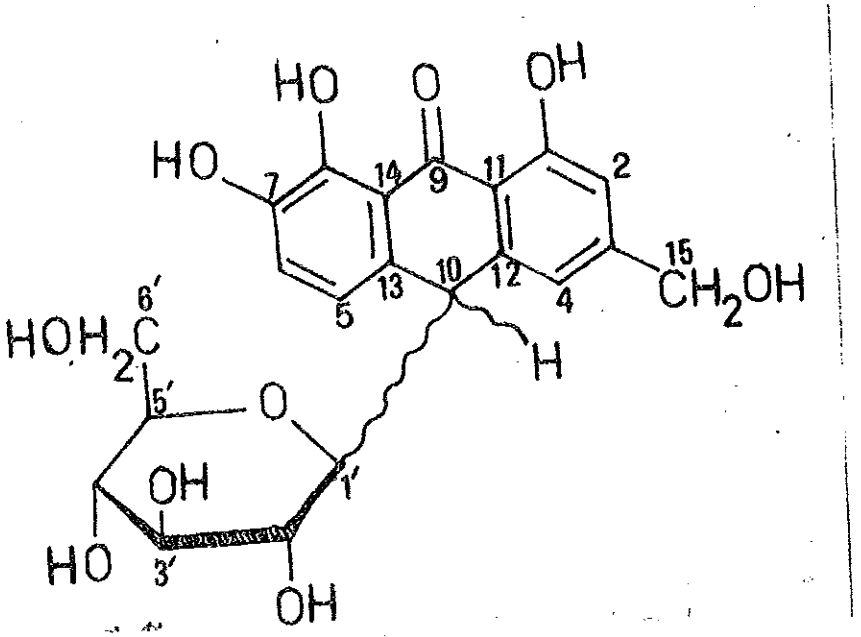
2.2.9 7-Hydroxybarbaloin (11)

This compound was isolated from A. pulcherima.

A violet colour upon treatment with methanolic $Mg(OAc)_2$ was observed for this yellow pigment suggesting 1,2-dihydroxyanthraquinone nucleus[24]. The IR spectrum showed absorption bands due to a chelated carbonyl group at 1625 cm^{-1} and 3730-3400 cm^{-1} attributable to hydroxyl groups, and the UV spectrum displayed absorption maxima at 268, 304 and 358 nm indicating the anthrone C-glycoside skeleton[48,50]. Furthermore, following the same spectroscopic arguments forwarded previously for 10, the anthrone 10-C-glycoside nature of this compound was established. The $FeCl_3$ oxidative hydrolysis has also afforded glucose and an unstable pigment. The only striking differences between the ^{1}H and ^{13}C NMR spectra of 10 and that of the yellow pigment under discussion were the presence of CH_3 signal at 2.4 and

21.3 in the spectra of 10, whereas there are no signals corresponding to these chemical shifts in the spectra of this yellow compound. The spectra, however, revealed signals at δ 4.8 and 62.4 assignable to a hydroxymethyl (CH_2OH) group.

The above spectroscopic and chemical degradation findings led to the identification of this compound as 7-hydroxy - barbaloin (11).



- 11

7-Hydroxybarbaloin has been reported to be the characteristic substance of the commercially important Curacao aloes in the European Pharmacopoeia III[55].

2.3 Chemcial Screening of Barbaloin in Eight *Aloe* Species by HPLC and TLC methods.

HPLC has been displacing progressively other chromatographic techniques for separation, identification and quantification of compounds[56,57]. The recently introduced HPLC system with a computer controlled photodiode array detector provides a high potential for the manipulation of recorded chromatographic and absorption spectral data[58]. This method has been employed to undertake screening of leaves of eight *Aloe* species of Ethiopia for the presence of the commercially important compound (barbaloin).

The areas or heights of peaks in the elution chromatogram are function of composition[59] and quantitative estimation is carried out by comparing with the standard peak. In this preliminary screening work identification of the peak due to barbaloin was made possible using the on-line UV spectra of sample aided by use of a wavescan compare software and also injecting a fixed amount fof the standard sample (barbaloin) which resulted in the enhancement of the barbaloin peak of the eluted sample chromatograph.

The eight *Aloe* species investigated for the presence of barbaloin are *A. berhana*, *A. rivae*, *A. megalacantha* *A. calidophylla*, *A. mcloughlinii*, *A. secundiflora*, *A. otalensis* and *A. pulcherima*.

2.4 Conclusion

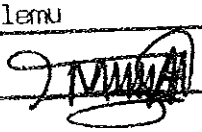
The four Aloe species studied in large scale namely A. berhana, A. rivae, A. megalacantha and A. pulcherima have proved to be rich in anthraquinone glycosides, which can be regarded as typical of the genus Aloe. Interestingly, the major constituents of the officially recognised commercial aloes notably barbaloin (8)(responsible for the purgative activity of Aloe species), aloenoside (9) (constituent of the Socortine and Cape aloes), nataloin (10) (major constituent of Natal aloes) and 7-hydroxybarbaloin (11) (the characteristic substance of Curaçao aloes in the European Pharmacopoeia) have been obtained in high yields. The other types of compounds isolated from all four species namely chrysophanol (1) and β - sitosterol (2) appeared to be the second and third record, respectively, in the genus Aloe. The biologically important aromatic acid, protocatechuic acid (7), which is rare from a natural source, is the first record for the genus and to our-knowledge the pre-anthraquinone, tentatively given the trivial name berhanone (4) is believed to be a new natural product.

In addition to these, two phenolic compounds were obtained in sufficient quantitities whose structure elucidations require generation of more spectroscopic data. Furthermore, a few trace phenolic compounds, giving charncteristic colour

DECLARATION

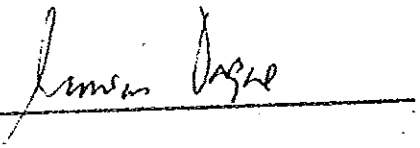
This thesis is my original work and has not been presented for a degree in any other university.

Name: Melaku Alemu

Signature: 

Date of submission: June, 1990

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

Ermias Dagne, Dr. 

reaction tests, have also been encountered in studies of all four species. It is our hope that, future work on large scale of each species will certainly culminate in the isolation and identification of these compounds.

Out of the eight studied species that particular type of Aloe notably A. pulcherima is characterized by the lack of spines at the leaf margins. The colour of the spines, their height and the space between two successive spines are among the many botanical characters used for taxonomic grouping in Aloe species. The remaining seven Aloe species collectively are known locally in Amharic as wonde eret, whereas A. pulcherima is known as sete eret. Our chemical studies also distinguishes A. pulcherima from the other seven Aloe species studied by the lack of barbaloin (8) and containing instead nataloin (10) and 7-hydroxybarbaloin (11).

It must be recalled that, the usefullness of chemistry in plant taxonomy has already been appreciated even by pre-Darwinian taxonomists[60,61].

One of the main taxonomic problems of the genus Aloe is the delimitation of boundaries between different species[16,62]. Various workers including Reynolds[62] Reynolds and Nicholls[63] and Beaumont et al.[64] have attempted to correlate chemical

relationship in the leaves of various Aloe species with taxonomic groupings. Compounds in the exudates from a number of shrubby species of Aloe have been saparated by TLC and defined as entities by their colour reactions with Fast Blue B[62,65]. Although the chemical structures of many of the compounds are largely unknown, the patterns of chromatographic zones have been used to support taxonomic conclusions in two multidisciplinary studies.

Their method seem to have some limitations in instances where the leaf exudate is lacking colour forming compounds, since the failure to detect an expected important compound doesnot necessarily mean that it is absent.

In conclusion, the structurally elucidated constituents of the presently studied Aloe species of Ethiopia would certainly contribute to the taxonomic work in progress on Ethiopian Aloe species as well as the genus Aloe ingeneral. The taxonomy of the genus Aloe still remains to be tentative[16,62] and our results can be used in the delineation of major groups or separating closely related taxa. The sum total of morpholgical, anatomical, cytological and biochemical information generated by Cutler et al.[66] together with the already proposed taxonomic groupings[16,62,64] (after being further substantiated by structurally known compounds) must be used effectively to settle some of the queries, All these aspects would therefore encourage further rigorous investigations on members of the genus Aloe with an intimate and effective multidisciplinary communications.

3. EXPERIMENTAL

3.1 Apparatus and Materials

Melting points were determined on Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were measured using KBr discs on Perkin-Elmer IR spectrophotometer model 727B. ^{1}H NMR spectra were recorded on Jeol FX 900 (90 MHz.) and ^{13}CNMR at 22.5 MHz. MS were recorded at 70ev. Optical rotations were measured on Perkin-Elmer 241 polarimeter. HPLC analyses were performed on an LkB HPLC system attached to an NIR PC6 model computer. UV spectra were obtained from the photodiode array detector with in the scanning range of 190 - 370 nm of the above HPLC System. Analytical TLC were run on Silica gel 60 F_{254} 0.2 mm thick layer, aluminium sheets (Merck). Preparative TLC (PTLC) were run on self-made ca. 2 mm glassplates. Column chromatography was performed on Silicagel 60 (Merck) and Silica gel 60 (Fluka) and Sephadex LH - 20. Paper chromatography was performed using Whatman No. 1 chromatographic paper.

3.2 Solvent Systems

The solvent systems used for chromatographic separations (TLC, PTLC, HPLC, PC and Sephadex LH - 20) are given in Table 8.

Table 8 Solvent Systems

Solvent System	Solvent Mixture and Ratio	
1A	petrol - EtOAc.	(95:5)
1B	petrol - EtOAc	(9:1)
1C	petrol - EtOAc	(7:3)
1D	petrol - EtOAc	(1:1)
2	EtOAc	
3A	EtOAc - MeOH	(9:1)
3B	EtOAc - MeOH	(2:1)
4A	benzene - petrol - EtOAc	(3:9:2)
4B	benzene - petrol - EtOAc	(3:4:3)
4C	benzene - petrol - EtOAc	(1:1:2)
5	EtOAc - MeOH - H_2O	(100:16:13)
6	EtOAc - EtOH - H_2O	(100:20:13)
7	$CHCl_3$ - MeOH -	(1:1)
8	MeOH	
9	n - BuOH - HOAc - H_2O	(4:1:5 upper phase)

3.3 Visualization Methods of Compounds and Sugars.

Compounds on TLC plates were visualized by fuming with ammonia vapour or Conc. H_2SO_4 or exposing to UV light (254 and 366nm) or spraying with 0.5% aq.

Fast Blue B salt followed by 0.1N NaOH solution or vanillin-sulphuric acid spray (0.5 g vanillin/50 ml conc. H_2SO_4) followed by heating at 100°C for 2 min to stabilize the colour [27] or 0.5% methanolic $Mg(OAc)_2$ or 5% ethanolic $FeCl_3$.

Sugars on chromatography paper were visualized according to Markham[54] and Stahl[27] by spraying with a mixture of aniline (0.9 ml), phthalic acid (1.66 g), n-BuOH(100 ml satd. with H_2O) followed by heating the chromatograms at 100°C for 2 min.

3.4 Plant Materials

The leaves of the eight Aloe species studied were collected from the following places, identified by Dr. Sebsebe Demissew and voucher specimens kept in the National Herbarium, Biology Department, Addis Ababa University.

a. Aloe berhana Reynolds, 121 km away from Addis Ababa on the road to Debre Berhan (Nothern Shoa Administrative Region), voucher No. Sebsebe 2209.

b. A. rivae Baker, Mega (Borena Adm. Region) 665 km from Addis Ababa, alt. 2201 m, Sebsebe 2201.

c. A. megalacantha Baker, Asba Teferi (Western Hararghe Adm. Region), 325 km from Addis Ababa, alt. 1830 m, Sebsebe 2282.

d. A. pulcherima ined., Debre Libanos (Norhtern Shoa Adm. Region), 100 km from Addis Ababa, alt. 2470 m, Sebsebe 2386.

e. A. calidophylla Reynolds, 675 km from Addis Ababa (or 10 km from b above towards Moyale) (Borena Adm. Region) Sebsebe 2198.

f. A. obtalensis Baker, 667 km from Addis Ababa on the road to Moyale (Borena Adm. Region) Sebsebe 2205.

g. A. secundiflora Engler, 17 km North of Moyale (Borena Adm. Region), Sebsebe 2196.

h. A. mcloughlinii Christian, 16 km NW of Negele (or 5 km from Bitata towards Negele) (Borena Adm. Region) alt. 1620 m, Sebsebe 2166.

3.5 Extraction and Isolation of Compounds

3.5.1 Aloe berhana Reynolds

Fresh leaves (1.5 g) were chopped into pieces and extd with EtOH at room temp. by percolation for 7 days. Removal of EtOH yielded on aq. oily residue which was successively extd with $CHCl_3$, EtOAc and n-BuOH. The $CHCl_3$ ext was subjected to CC over Silica gel 60 (100 g) and elution was carried out using petrol-EtOAc mixtures of increasing polarities. A total of 75 fractions each 100 ml were collected and analysed by TLC. Fraction 3 - 11 (eluted with solvent 1A) afforded compound 1 (20 mg) and fractions 17 - 28 (solvent 1C) yielded compound 2 (35 mg) upon crystallization from MeOH and Me_2CO respectively. The remaining fractions yielded

three phenolic unidentified compounds each less than 5 mg. The EtOAc and n-BuOH exts were separately passed through Sephadex LH - 20 column (Solvent 8) and purified on PTLC (Solvent 4C) to give 35 mg and 6 mg of compound 7 respectively.

The sun dried leaves of A. berhana were finely powdered (85 g) and extd with EtOH in a Soxhlet apparatus for 8 hrs. Removal of EtOH yielded an oily residue (5.2 g) which was chromatographed over Silica gel 60 (75 g). The CC was performed by eluting with petrol-EtOAc followed by EtOAc - MeOH mixtures of increasing polarities. A total of 35 fractions were collected, fractions 1 - 15 (each 50 ml), 16-25 (each 100 ml), 26-30 (each 200 ml) and 31-35 (each 300 ml). Fraction 2 (eluted with solvent 1C) yielded compound 1 and fractions 3-4 (solvent 1C) gave compound 2 (15 mg) upon recrystallization from MeOH and Me_2CO respectively. Fractions 7-8 (solvent 1C) gave trace of compound 3. Fractions 9-12 (solvent 1C) showed on TLC (solvent 4B) the presence of a greenish-yellow pigment which upon percolation through Sephadex: LH - 20 (solvent 7) followed by PTLC gave compound 4 (6 mg). Fractions 16-17 (solvent 1D) and 18 (solvent 2) were combined and passed through Sephadex LH - 20 (solvent 8) to give compound 7 (3 mg). Fractions 25-27 (solvent 3A) were

rechromatographed on CC to give compound 8 (20 mg) and fractions 28-32 (solvent 3A) afforded an unidentified orange amorphous solid (600 mg) which gave red-colour with Fast Blue B spray.

3.5.2 Aloe rivae Baker

Finely ground leaves (185 g) were extd with EtOH in a Soxhlet apparatus for 12 hrs. Removal of EtOH yielded a dark gummy residue (10 g) which was ubjected to CC over Silica gel 60 (90 g). Elution was carried out using petrol - EtOAc followed by EtOAc - MeOH mixtures of increasing polarities. A total of 50 fractions each 200 ml were collected. Fractions 3-4 (solvent 1B) yielded 1 and 5 - 10 (solvent 1B) gave unidentified white amorphous solid (30 mg, mp 100°.). Fractions 11 - 13 (solvent 1C) afforded compound 2 and 14 - 25 (solvent 1C) showed on TLC (solvent 4B) mixture of two pigments. This mixture was separated by means of PTLC to give trace of compound 3 and a greenish-yellow flouresceing compound 4 (8 mg, impure). Fractions 42-47 (solvent 3A) contained mainly one spot on TLC (solvent 5) which upon further purfication by means of column filtration (solvent 2) gave compound 8 (900 mg). The last two fractions 49-50 (solvent 3B) were recrystd from MeOH to give compound 9 (455 mg).

3.5.3 Aloe megalacantha Baker

The powdered leaves (200 g) were extd with EtOH in a Soxhlet apparatus for 12 hrs. Removal of EtOH yielded a gummy residue (18 g). This crude ext was chromatographed over Silica gel 60 (120 g) and eluted using petrol - EtOAc followed by EtOAc - MeOH mixtures of incresing polarities.

A total of 70 fractions each 200 ml were collected, fractions 1 - 15 (were eluted using solvent 1A), 16-20 (Solvent 1B), 30-37 (solvent 1C), 38-40 (solvent 1D), 41-50 (solvent 3B). TLC analysis of these fractions showed a similar patterns of major compounds as that of section 3.5.2 above and a detailed analysis employing analogous methods led to the isolations of further quantities of compounds 1 (3 mg), 2 (50 mg), 3 (2 mg), 4 (impure), 8 (650 mg) and 9 (255 mg).

3.5.4 Aloe pulcherima ined.

Finely ground leaves (300 g) were extd with EtOH in a Soxhlet apparatus for 12 hrs. Removal of EtOH gave an oily dark residue (28 g) which on TLC (solvent 5) showed the presence of atleast five spots of which the last two polar are major compounds. The crude ext was then subjected to CC over Silica gel 60 (200 g) and elution was carried out using petrol - EtOAc followed by EtOAc - MeOH mixtures of increasing polarities.

A total of 95 fradtions each 200 ml were collected and analysed by TLC. Fractions 5-7 (solvent 1A) gave compound 1 and 11 - 19 (solvent 1C) yielded compound 2. Fractions 38-62 (solvent 1D) gave two unidentified phenolic compounds, an amorphous solid (120 mg, mp 120°C) and 5 mg (oily). Fractions 81-84 (solvent 3A) contained mainly one spot on TLC (solvent 5) which were combined and passed through Sephadex LH - 20 (solvent 8) and further purified by PTLC gave compound 10 (510 mg). Analogous treatment of fractions 88-92 (solvent 3B) as fractions 81-84 however using solvent 6 afforded compound 11 (300 mg).

3.6 Borax Test

To the sample (1 mg) dissolved in H_2O (2 ml) was added borax ($Na_2B_4O_7 . 10H_2O$, sodium tetraborate, BDH) (10 mg). The mixture was heated on a water bath with gentle warming until it became homogeneous. The resulting clear solution was exposed to UV light 366 nm. There followed a greenish-yellow fluorescence, a characteristic test of anthranols[1].

3.7 Chemical Degradation Methods.

3.7.1 $FeCl_3$ Oxidative Hydrolysis

A solution of the sample (1 mg) and $FeCl_3$ (5 mg) in H_2O (5 ml) was heated under reflux on a water bath for 8 hrs. The resulting reaction mixture was cooled and extd with $CHCl_3$, dried over Na_2SO_4 and concd to give the expected aglycone (anthrone) and glucose[1].

The sugar D-glucose in all cases was detected by PC using solvent 9 and visualized as discussed in section 3.3. The anthrone was treated with 10% NaOH followed by neutralization with 5% HCl to give the corresponding anthraquinone.

3.7.2 Acid and alkaline hydrolysis

The glucoside (2 mg) was dissolved in 5 ml of 2 N HCl - MeOH (1:1) in a round bottom flask and heated under a condenser on a water bath for 1 hr. The solution was cooled and evaporated to dryness. The residue was diluted with H_2O and extd with EtOAc, dried and concd to give the aglycone. The resulting aq. layer was concd by evaporating on a water bath and analysed by PC using solvent 9 and visualized as discussed in section 3.3.

A solution of the glucoside (2 mg) and 4 N NaOH (5 ml) was refluxed for 2 hrs. The resulting mixture was neutralized with 2M HCl, extd with EtOAc to give the aglycone. The aq. layer after being concd on a water bath was analysed by PC and visualized as discussed above.

Hydrolysis was shown to have occurred in both cases for compound 9 only.

3.8 Physicochemical Data of Compounds

Chrysophanol (1)

Orange needles from MeOH, mp 193-195° (Lit.[26] 197) IR ν_{max}^{KBr} cm^{-1}: 3420, 1675, 1630, 1610, ^1H NMR ($CDCl_3$) δ : 2.3 (3H, s, CH_3), 7.0 (1H, dd, H - 7), 7.2 (1H, brs, H - 2), 7.3 (1H, dd, H - 6), 7.6 (1H, brs, H - 4), 7.7 (1H, dd, H - 5), 12.0, 12.1 (2x1H, 2xs, 1, 8 - OH).

β - Sitosterol (2)

White-needles from Me_2CO, mp 137° (Lit.[36] 136°) Found $[M]^+$ 414.3092, $C_{29}H_{50}O$ requires 414.3861. ^1H NMR ($CDCl_3$) δ : 0.7 (3H, s, Me - 18) 0.8 (6H, d, J = 6 Hz, Me - 26,27), 0.9 (3H, d, J = 6, Me - 29), 1.8 (3H, brs, Me - 21), 1.3 (3H, s, Me - 19) 0.95 m, 1.2 m, 1.4 - 2.4 m, 3.5 (2H, m, H - 7), 5.0 (2H, m, H - 3), 5.4 (1H, m, H - 6). ^{13}C NMR : 141.1 (C-5), 121.7 (C - 6), 72.1 (C - 3), 57.2 (C - 14) 56.6 (C - 17), 50.7 (C - 9),

Table 9 Colour Reactions and TLC Rf Values of Compounds

Compound	Rf	Solvent system	NH_4OH	conc. H_2SO_4	$Mg(OAC)_2$	Fast Blue B	$FeCl_3$
1	0.90	4B	Orange-red	Red	Orange	No change	Brown
2	0.79	4B	No change	Orange	No change	No change	No change
3	0.53	4B	Orange	Red	Orange-red	No change	Red-brown
4	0.40	4B	No change	Orange-red	Yellow	Red-brown	Green
7	0.63	5	No change	No change	No change	Brown	Dark-green
8	0.37	5	Yellow	Orange-yellow	Orange	No change	Black
9	0.22	5	No change	Orange	Orange	No change	Black
10	0.43	5	Yellow	Greenish-yellow	Violet	Violet	Black
11	0.35	5	Yellow	Greenish-yellow	Violet	Violet	Black

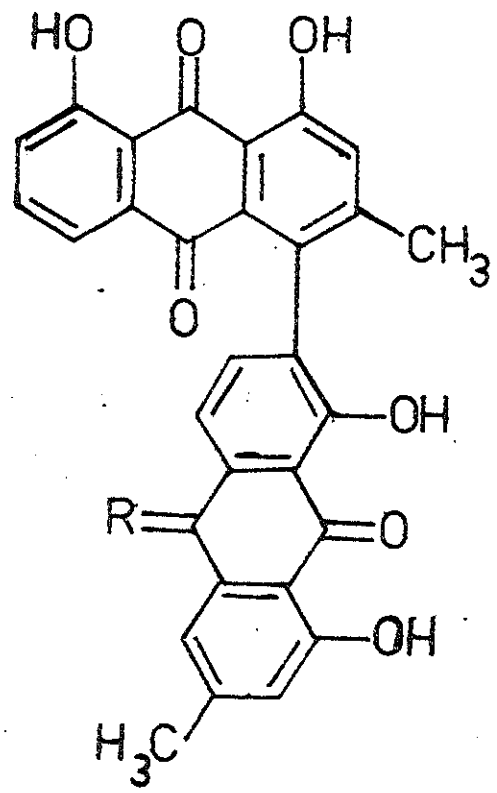
4. A P P E N D I C E S

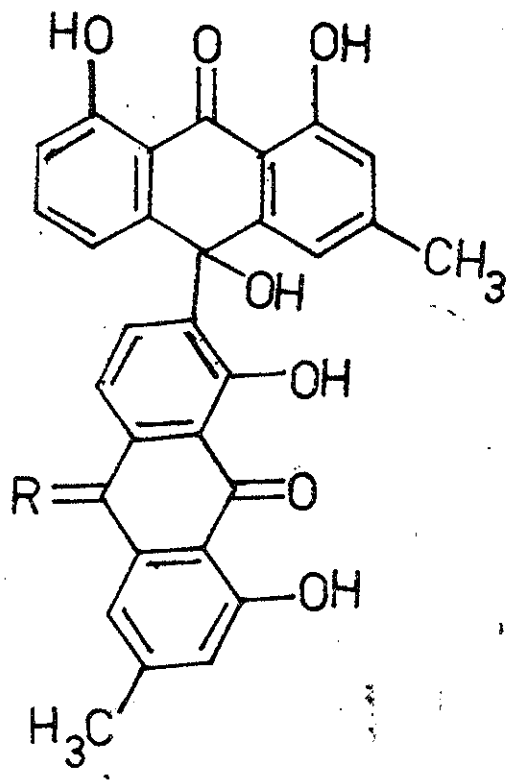
Number	Compound Name	Structure						Source	Ref.
		R^3	R^4	R^5	R^6	R^7	R^8		
1	Chrysophanol	Me	H	H	H	H	OH	A. saponaria	26
14	Desoxyerythrolaccin	OH	H	H	OH	H	Me	A. saponaria	39
15	1,5-Dihydroxy-3-hydroxymethyl anthraquinone	CH_2OH	H	OH	H	H	H	A. excelsa	8
16	Helminthosporin	H	OH	H	Me	H	OH	A. saponaria	26
17	Isoxanthorin	H	OH	OH	Me	H	OH	A. saponaria	26
18	Laccaic acid D methyl ester	OH	H	H	OH	COOMe	Me	A. saponaria	26
19	Nataloe-emodin	Me	H	H	H	OH	OH	A. nyeriensis	48
20	Nataloe-emodin-8-methyl ether	Me	H	H	H	OH	OMe	A. specrosa	25

Appendix 2. Anthraquinone Glycosides of Aloe

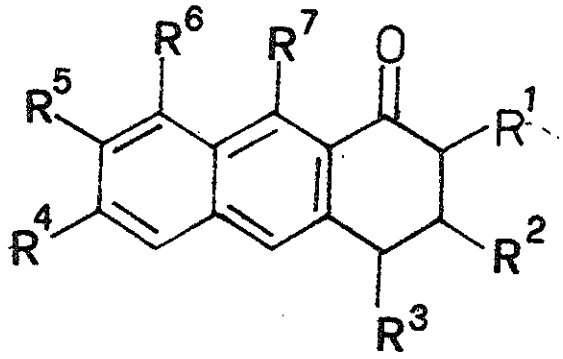
Number	Compound Name	Source	Ref.
8	Barbaloin or aloin	100 Aloe species	69
	(Aloe-emodin-9-anthrone-10-C-glucoside	A. arborescens	68
		68 Aloe species	16
		A. excelsa	8
	Two-diastereomers of barbaloin	A. arborescens	70
	(C-10 stereoisomers of barbaloin)	A. capensis	70
		A. ferox	70
		A. rabaiensis	49
21	Aloe-emodin-9-anthrone-10-C-rhamnoside	A. rabaiensis	49
22	Aloe - emodin-15-O-rhamnoside	A. rabaiensis	49
9	Aloinoside	Socotrine aloes	12
	(Barbaloin - 15 - O - rhamnoside)	Cape aloes	12

Appendix 3. Bianthraquinoids of Aloe

Number	Compound Name	Structure	Source	Ref.
				
25	(+) - Asphodelin	R=O	A. saponaria	71
26	Asphodelin - 9'- anthrone	$R=H_2$	A. saponaria	71

Number	Compound Name	Structure	Source	Ref.
				
27	1,1',8,8',10-pentahydroxy-3,3'-dimethyl - 10,7' — bianthracene (10'H, 10'H) - 9,9',10 - trione	R = O	A. saponaria	71
28	1,1',8,8', 10 -pentahydroxy- 3,3'-dimethyl - 10,7'- bianthracene (10'H, 10'H) - 9,9'-dione	$R = H_2$	A. saponaria.	71

Appendix 4 Pre-anthraquinones of Aloe

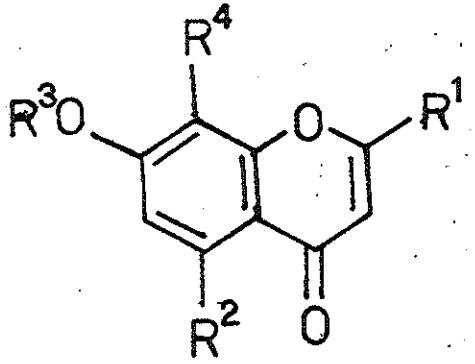


Number	Compound Name	R^1	R^2	R^3	R^4	R^5	R^6	R^7	Source	Ref.
29	Aloesaponol I	H	OH	H	OH	COOMe	Me	OH	A. saponaria	39
30	Aloesaponol II	H	OH	H	OH	H	Me	OH	A. saponaria	39
31	Aloesaponol III	H	H	OH	Me	H	OH	OH	A. saponaria	26
32	Aloesaponol IV	OMe	H	OH	Me	H	OH	OH	A. saponaria	26

Appendix 5 Pre - anthraquinone Glucosides of *Aloe*

Number	Compound Name		Source	Ref.
33	Aloesaponol I	6-O-β-D-glucopyranoside	*A. saponaria*	22
34	Aloesaponol II	6-O-β-D-glucopyranoside	*A. saponaria*	72
35	Aloesaponal III	8-O-β-D-glucopyranoside	*A. saponaria*	72
36	Aloesaponol IV	8-O-β-D-glucopyranoside	*A. saponaria*	73

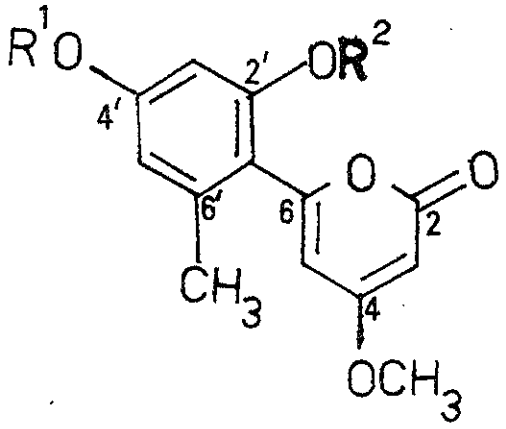
Appendix 6 : Chromones of *Aloe*

Number	Compound Name	Structure				Source	Ref.
							
		R^1	R^2	R^3	R^4		
37	Aloesone	CH_2Ac	Me	H	H	11 *Aloe* species	74
						34 *Aloe* species	75
38	Aloesine (Aloeresin B)	CH_2Ac	Me	H	D-glucose	19 *Aloe* species	76
						Aloe species	77
						9 *Aloe* species	78
						A. saponaria	72
						A. ferox	79
						A. excelsa	8
						A. cremnophila	50
						A. jacksonii	50

Number	Compound Name	Structure				Source
		R^1	R^2	R^3	R^4	
39	Aloeresin A	CH_2Ac	Me	H	2'-p-coumaroyl glucopyranoside	A. arborescens A. ferox A. cremnophila A. jacksonii
40	Iso-aloeresin A	CH_2Ac	Me	H	2'-p-coumaroyl glucopyranoside	A. barbadensis
41	Aloeresin C	CH_2Ac	Me	glucose	2'-p-coumaroyl glucopyranoside	A. ferox
42	Aloeresin D	CH_2-CHCH_3 (OH)	Me	Me	2'-p-coumaroyl glucopyraneside	A. ferox A. rabaiensis
43	2'-p-Methoxyl coumaroyl aloeresin	CH_2Ac	Me	H	2'-p-methoxycoumeroyl glucopyranoside	A excelsa
44	Rabaichromone	CH_2-CHCH_3 (OH)	Me	Me	2'-caffeoyl - glucopyranoside	A. rabaiensis

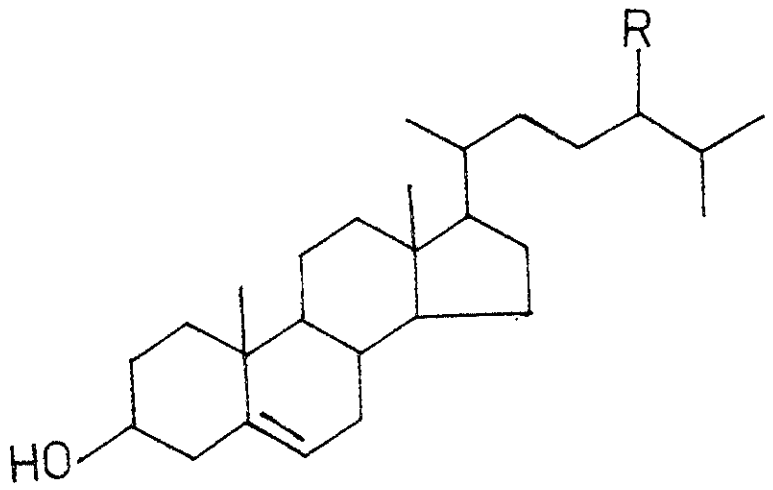
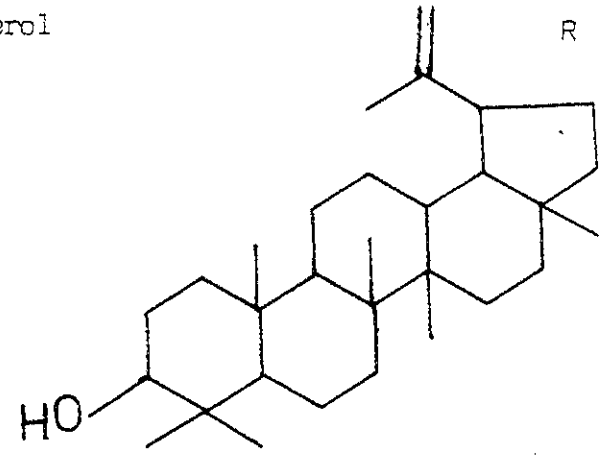
Number	Compound Name	Structure				source	Re
		R^1	R^2	R^3	R^4		
45	2'-O-Feruloyl aloesin	CH_2Ac	Me	H	2'- feruloyl - glucopyranoiside	A. arborescens	8
46	2'-O-Tigloyl aloesin	CH_2Ac	Me	H	2'-tigloyl - glucopyranoside	A. cremnophila	5
						A. jacksonii	5
47	Carboxyethenyl - 5-7-dihydroxy chromone	CH=CH-COOH	Me	H	H	A. cremnophila	5
48	Aloearbonaside [Methyl - 2 - (5 - methyl - 7 - hydroxy - 4 - O - D - glucopyranosyl) chromenylidene acetate]					Aloe species	8
						A. arborescens	8
						A. arborescens	8

Appendix 7. Phenyl Pyrone Derivatives of Aloe



Number	Compound Name	R^1	R^2	Source	Ref.
49	Aloenin Aglycone	H	H	A. nyeriensis	48
50	Aloenin	H	D-glucose	Aloe species	84
				A. arborescens	87
				A. arborescens	88
				A. saponaria	72
51	Aloenin-2''- p - coumaroyl ester	H	2''-O-coumaroyl - glucopyranoside	A. nyeriensis	48
52	Aloenin B	D-glucose	2'''-p - coumaroyl - glucopyranoside	A. ferox	89

Appendix 9. Phytosterols and Triterpene of Aloe

Number	Compound Name	Srtucture	Source	Ref.
				
56	Cholesterol	R = H	A. barbadensis	37
57	Campesterol	R = CH_3	A. barbadensis	37
2	β- Sitosterol	R = CH_2CH_3	A. arborescens	37
			A. arborescens	38
58	Lupeol		A. barbadensis	37

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