Bioactive metabolites from Aframomum species

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Bioactive metabolites from *Aframomum* species

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Abstract
Aframomum (Zingiberaceae) species is well represented in lowland rainforest and in many mountain areas. There are some fifty species of *Aframomum* in Africa, about half of them in Cameroon-Gabon region. In Cameroon they occur mostly in Southern and Central regions. Many species are widely used for medicinal, ethnodietary and spiritual purposes, prompting chemical investigation. The classes of compounds generally found in *Aframomum* species include diterpenoids, sesquitepenoids, arylalkanoids and flavonoids. Some extracts and compounds from this genus have been screened for biological activities. These include antifungal, cytotoxic, antibacterial, insect antifeedant, antiplasmodial, antihypercholesterolemic and antiviral activities.

I- INTRODUCTION

The Zingiberaceae constitute a family of terrestrial rhizomal herbs with over 1400 species distributed in over 50 genera.[1] They are mostly found in tropical areas (Asia and Africa).[2] In West and Central Africa plants of the Zingiberaceae family are wispread in humid forest regions. They are distributed among eight genera five of which are indigenous or endemic (*Aulotandra, Costus, Kampferia, Reneilmia* and *Aframomum*). The three others (*Pheaeomeri, Zingiber* and *Curcuma*) have been introduced.

II- THE GENUS *AFRAMOMUM* K. SCHUM

The genus *Aframomum* is represented in West and Central Africa by approximately 50 species that can be distinguished from the other genera by the generally large size. They are perennials and aromatics when any part of the plant is crushed.[3,4] They possess highly colored flowers. The peduncles are covered with sterile imbricated bracts. In Cameroon *Aframomum* species occur mostly in South-West, South, North-West, West and Central regions. From now more than 23 species have been identified[5] and voucher specimens are kept in the National Herbarium, Yaounde, Cameroon, or at Botanical Garden in Limbe, Cameroon. These include

1- *Aframomum alboviolaceum* Ridley
2- *Aframomum aulacocarpos* Pellegr Ex. J. koechlin
3- *Aframomum arundinaceum* (Oliv. & Hanb) K. Schum
4- *Aframomum Chlamidanthum* Loes & Midbraed
5- *Aframomum citratum* (Pereira) K. Schum
6- *Aframomum danielli* K. Schum
7- *Aframomum giganteum* (Oliv. & Hanb) K. Schum
8- *Aframomum handburyii* K. Schum
9- *Aframomum kayserianum* (Afzel) K. Schum
10- *Aframomum masuianum* (De Wild & Th. Dur) K. schum
11- *Aframomum letestuianum* Gagnepain

12- *Aframomum melegueta* (Roscoe) K. Schum
13- *Aframomum polyanthum* K. Schum
14- *Aframomum pruinosum Gagnepain*
15- *Aframomum sanguinum* (Sm) Hepper
16- *Aframomum strobilaceum* (Oliv. & Hanb) K. Schum
17- *Aframomum sulcatum* (Oliv. & Hanb) K. Schum
18- *Aframomum sceptrum*
19- *Aframomum subsericeum*
20- *Aframomum zambesiacum*
21- *Aframomum limbatum*
22- *Aframomum leptolepis*
23- *Aframomum flavum*

Many species are commonly used for ethnodietary, medicinal and spiritual purposes.

II-1. *Aframomum* used in ethnodietary preparations

An ethnodietary preparation is a dish eaten by a specific tribal or ethnic grouping. It is generally a soup and usually made up of several plant-derived ingredients. In Cameroon for example there is "Mbongo tsobi" a health food of the Bassa of the Littoral province, "Nah'poh", a yellow soup of the Bamilekes of the Western province, and "Ndombah" a special dish of the Betis of the Central province. .*Aframomum citratum* is the main ingredient of "Mbongo tsobi". *A. danielli*, *A. pruinosum* and *A. polyanthum* are the three *Aframomum* ingredients used in "Nah' poh" while in "Ndombah" we have *A. sulcatum*, *A. letestuianum* and *A. chlamydanthum*.

II-2. *Aframomum* as a source of ethnomedicinal and spiritual purposes

Many species of the genus *Aframomum* are used for ethnomedicinal and spiritual purposes (Table 1).

Table 1

SPECIES	ETHNOMEDICINAL USES
1. *A. alboviolaceum*	• Protection against evil spirits (whole plant) • Crushed seeds as catalysts in ethnomedicinal preperations
2. *A. aulacocarpos* Beti: "Mvonlo' Bassa: "Mbongo"	• Seeds: fevers, skin diseases • Abdominal pains
3. *A. chlamydanthium* Yambassa: "Cuefo- knang"	• Decoction of seeds used against infections and parasitic diseases of the abdomen. Also used as vermifuge.
4. *A. citratum*	• Leafy stems used as steam-bath against fevers and intercostals pains while seeds are masticated as a tonic and as an aphrodisiac.
5. *A daniellii* Beti: "Esson" Bangwa: "Besak" Foto: "Atso'o"	• Seeds used as laxative and anti-helmintic in Congo. • Fresh juice of rhizomes against body odor • Rhizomes against toothache • Crushed seeds against fungal head infections
6. *A. giganteum*	• Crushed leaves: cicatrisant effect on wounds

Beti: "Kombe" Bassa: "Liyambi"	• Leaves against cough and chest pain (Congo) • Fresh fruits as collyre against conjunctivitis • Rhizomes against toothache
7. *A. handburyi* Beti: "Eson befan" Bangwa: "Kem ndidi"	• Crushed seeds used as catalyst in ethnomedicinal preparations, • Seeds against sorcery, poisons and undiagnosed illnesses
8. *A. Kayserianum* Beti: "Eson-nlong" Bafia: "Kibouma"	• Decoction of seeds used against mumps (Bafia) • Rhizomes as vermifuge • Leave decoctions against dysmenorrhoea (Mali)
9. *A. letestuianum* and A. *pruinosum* Bangwa: "Sia-ndidi"	• Highly cultivated in the Western highlands and sold. • Seeds masticated for their tranquilizing effects • Seeds widely eaten by women who consider them to favor conception of male children • Used against female sterility
10. *A. melegueta* graines de paradis, poudre de Guinée Beti: "Ndong" Bamileke: "Sok-kwa" Douala: "Ndongo à mumda"	• Most highly utilized and widely cultivated • Leaf decoctions with *Manihot esculentus* treat fractures (Congo) • Seeds in dysmenorrhoea, broncho-pulmonary disorders, gastrointestinal problems (Mali) • Seeds to consolidate the frontanel of babies and female sterility (Benin) • Seeds against migraines and rhizomes as vermifuge (Gabon) • Seeds against hay fever, tonic and sexual asthenia • Most generally used as catalyst in ethnomedicinal preparations.
11. *A. sanguineum*	• Leaf decoctions against headaches, migraines, and vertigo • Rhizomes plus roots of *Annona senegalensis* to treat orchitis • Seeds decoctions decrease urine production (Anuria) and are thus given to young girls after circumcision and eaten by men before a heavy drinking party
12. *A sulcatum* Bayangam: "Tso-nko"	• Decoction of seeds against umbilical hernia and purgative (laxative)

II-3. Previous study on *Aframomum*

At the start of our phytochemical studies on *Aframomum* in 1979 only two reports were available on two species, *A. giganteum* and *A. melegueta*. The whole plant of *A. giganteum* yielded mostly flavonoids[6] while hydroxyphenyl alkanoids were reported from *A. melegueta*.[7]

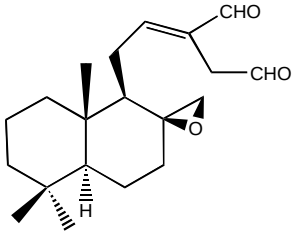
II-4. Our phytochemical and chemical contributions

The *Aframomum* species as well as other Zingiberaceae are best known for the production of labdane diterpenoids and flavonoids. Other classes of compounds encountered in the genus include sesquiterpenoids and arylalkanoids. Out of the species that have been chemically studied, at least eleven contain diterpenoids.

II-4-1. Diterpenoids

Aframodial (**1**), aulacocarpinolide (**2**), aulacocarpin A (**3**) and aulacocarpin B (**4**) have been isolated from *A. aulacocarpos*.[8,9] Aframodial (**1**) was isolated for the first time from *A. danielli*.[10] It has also been obtained from *A. polyanthum, A. masuianum, A. keyserianum*

(5g/kg dry weight of seeds),[9] *A. sulcatum,*[11] *A. longifolius,*[12] *A. arundinaceum*[13] and *A. latifolium*.[14] Compounds **3** and **4** were also found in the seeds of *A. escapum*.[15]



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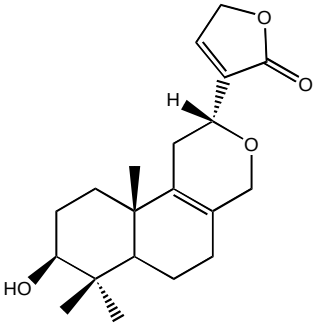

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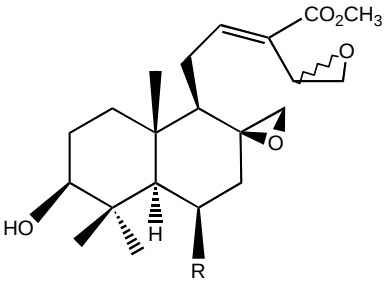
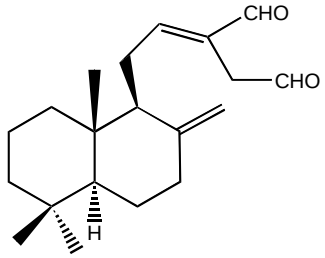


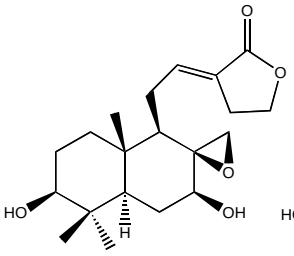
3 R = H

4 R = OH



5

Labda-8(17),12-dien-15,16-dial (**5**) has been found in *A. sceptrum,*[14] *A. longifolius*[16] and *A. danielli*.[17] Many other diterpenoids were isolated from *A. sceptrum*. These include 8β(17)-epoxy-3 β,7 β -dihydroxylabd-12(E)-en-16,15-olide (**6**), methyl 8 β(17)-epoxy-3 β,7 β,15-trihydroxylabd-12(E)-en-16-oate (**7**), 3 β,7 β,8 β,12ζ,17-pentahydroxylabdan-16,15-olide (**8**), coronarin B (**9**).[14]



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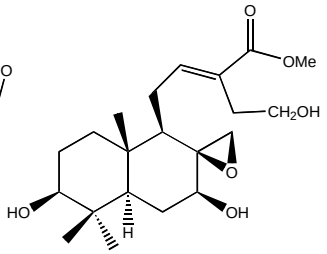

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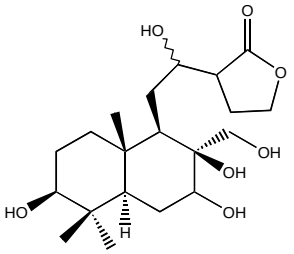

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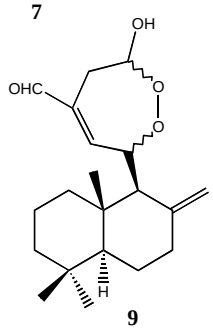
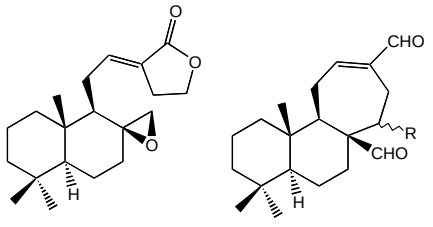


9

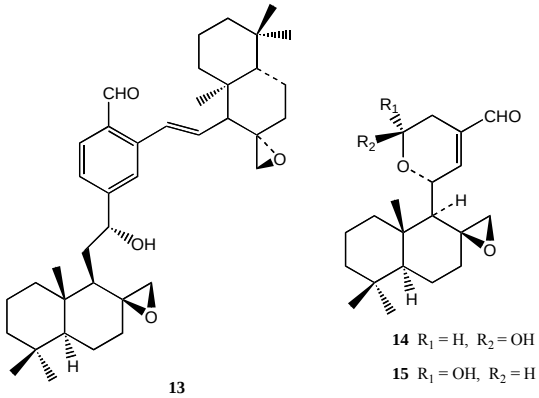
Galanolactone (**10**), galanal A (**11**) and B (**12**) were obtained from *A. sulcatum* and *A. latifolium*.[11,14] Other diterpenoids with 8(17)-epoxy moiety were isolated from *A. sulcatum*. These include a norbislabdane sulcanal (13), 12(E), 8β(17)-epoxy-11-hydroxy-12-labden-15,16-dial-11,15-hemiacetal **14** and **15**.[11] Galanolactone (**10**) was also found in *A. arundinaceum*.[13]



10

11 R = α-OH

12 R = β-OH

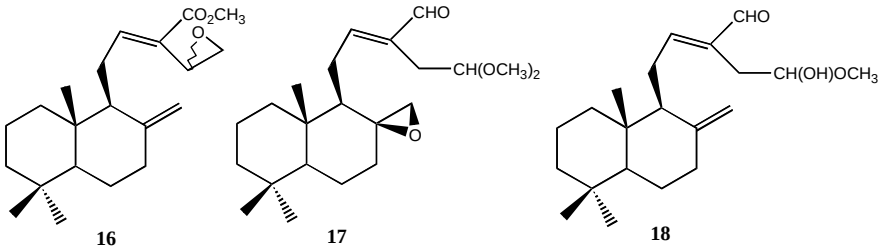


13

14 R_1 = H, R_2 = OH

15 R_1 = OH, R_2 = H

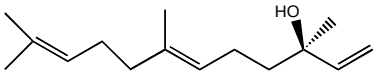
Further studies on *A. danielli*[17] and *A. arundinaceum*[13] yielded methyl 14,15-epoxy-8(17),12(E)-labdadiene-16-oate (**16**). An acetal, 8β(17)-epoxy-15,15-dimethoxylabd-12(E)-en-16-al (**17**) and an hemiacetal 15-hydroxy-15-methoxylabda-8(17),12(E)-dien-16-al (**18**) were isolated from *A. longifolius*.[12]



16 **17** **18**

II-4-2. Sesquiterpenoids

Sesquiterpenoids are rarely found in *Aframomum* species. Up to date only three sesquiterpenoid derivatives have been reported in these species, these are (+)-S-nerolidol (**19**) isolated from *A. sceptrum*[18] and *A. escapum*,[15] 6,7-epoxy-3(15)-caryophyllene (**20**) and (-)-α-bisabolol (**21**) found in *A arundinaceum*.[13]



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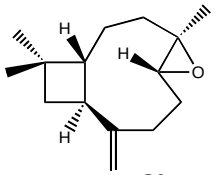

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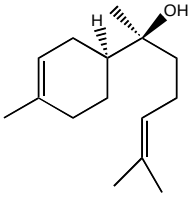
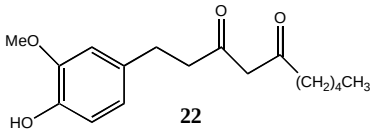


21

II-4-3. Arylalkanoids

Gingerdione (**22**), [6]-paradol (**23**), [6]-gingerol (**24**) and [6]-shogaol (**25**) have been isolated from *A. melegueta*.[19]



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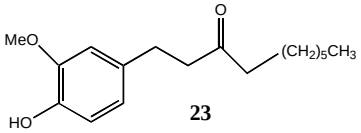

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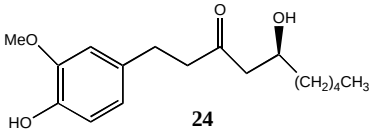

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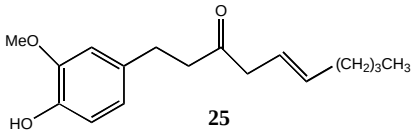
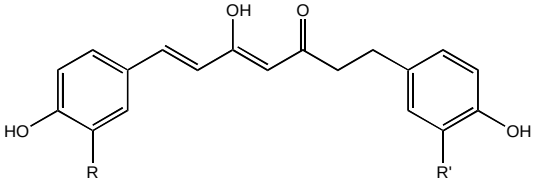


25

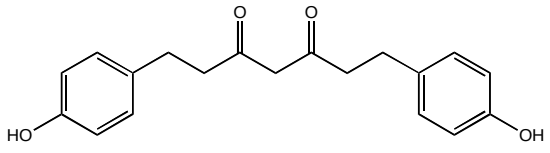
From *A. letestuianum*[20] four diarylheptanoids were isolated: (4Z,6E)-5-hydroxy-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one (**26**), letestuianin A (**27**), B (**28**) and C (**29**).



26 R = R' = H

27 R = H, R' = Me

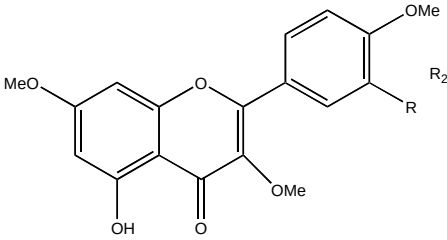
28 R = OMe, R' = H



29

II-4-4. Flavonoids

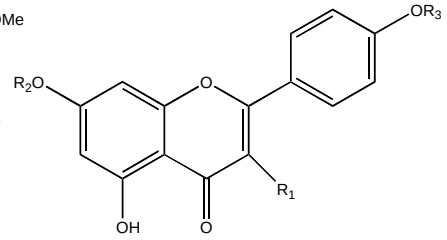
Flavonoids, as diterpenoids, are commonly found in *Aframomum* species. Both of them can be considered as chemotaxonomic markers of the genus. Eight flavonoids have been reported in five different species of the genus. Kaempferol-3,7,4'-trimethylether (**30**), quercetin-3,7,3',4'-tetramethylether (**31**), quercetin-3,7,4'-trimethylether (**32**) were isolated from *A. giganteum*.[6] 3-Acetoxy-5,7,4'-trihydroxyflavone (**33**) has been isolated from *A. letestuianum,*[20] *A. sceptrum,*[18] *A. pruinosum*[21] and *A. handburyii*.[22] 3-Acetoxy-5,4'-dihydroxy-7-methoxyflavone (**34**) and 3,5-dihydroxy-7,4'-dimethoxyflavanone (**35**) were found in *A. letestuianum, A. pruinosum* and *A. handburyii*.[20-22] 3-Acetoxy-5,7-dihydroxy-4'-methoxyflavone (**36**) was reported in *A. pruinosum* and *A. handburyii*.[21,22] Finally 3,5,7,4'-tetrahydroxyflavone (**37**) was obtained from *A. sceptrum*.[18]



30 R = H

31 R = OMe

32 R = OH



33 R_1 = OAc, R_2 = R_3 = H

34 R_1 = OAc , R_2 = Me, R_3 = H

35 R_1 = OH, R_2 = R_3 = Me

36 R_1 = OAc, R_2 = H, R_3 = Me

37 R_1 = OH, R_2 = R_3 = H

II-4. Biological activities of *Aframomum* extracts and *Aframomum* constituents

II-5-1. Biological activities of extracts

Many *Aframomum* species have been biologically screened. Bioassays of the extract of *A. danielli* revealed active growth inhibitors of *Salmonella enteriditis, Pseudomonas fragi, Pseudomonas flourescens, Proteus vulgaris, Streptococcus pyogens, Staphylococcus aureus, Aspergilus flavus, A. parasiticus, A. ochraceus* and *A. niger*.[23] The combined hexane and methanolic extracts of seeds of *A. melegueta* showed strong antifeedant activity against workers of *Reticulitermes speratus*.[19] Bioassays on the fruits and seeds have been tested against several bacterial and fungal strains.[24] The crude extracts revealed potent bactericidal activities against *Escherichia coli, Pseudomonas aeruginosa, Yersinia enterocolitica, Bacillus subtilis, Proteus vulgaris*, *Klebsiella pneumoniae* and *Serratia marcescens*, and fungicidal activities against *Candida albicans, Ttrichophyton mentagrophytes, Aspergilus niger*, *Botryodiplodis theobromae* and species of *Cladasporium cladasporiodes*.[24] Bioassays

of *Aframomum sanguineum* demonstrated that the flesh and seeds of the fruits contained powerful inhibitors of bacterial growth, acting against *Bacillus cerus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, while *A. milbraedii*, a specie sympatric with *A. sanguineum*, revealed no similar antibacterial agents.[25] Bioassay of the dichloromethane-methanol (1:1) extract of *A. longifolius* revealed potent antimicrobial activity against *Candida albicans, Cryptococcus neoformans, Staphylococcus aureus* and Methicillin resistant *S. aureus*, with IC_{50} of 20, 9, 40 and 25 μg/mL respectively.[12]

II-5-2. Biological activities of *Aframomum* constituents

Many labdane diterpenoids have been isolated from *Aframomum* species and screened for biological activities.

Aframodial (**1**), a diterpene dialdehyde, presents a broad spectrum of biological activities. Of particular significance is its antifungal activity.[9] The minimum inhibitory concentrations (MIC) of this compound against a variety of microorganisms are presented in table 2. Aframodial (**1**) particularly exhibited strong activity against *Saccharomyces cerevisae*, *Schizosaccharomyces pombe, Hansenula anomala,* and *Candida utilis*. **1** is lightly more active than the commercially available antifungal amphotericin B, which is one of the several antifungal drugs currently used to stop the evolution of numerous deep-seated mycoses (Candidiases, aspergillosis…) although its high toxicity limits its wide use. At a time systemic infections cause by filamentous fungi have become increasingly serious especially when the host's defense mechanism is weakened, these results suggest that aframodial (**1**) is a promising therapeutic antifungal agent. [9]

Table 2: Antimicrobial activity of aframodial (**1**) [9]

Microorganisms Tested	**MIC (μg/ml)**
Staphylococcus aureus NCTC 8530	12.5
Bacilus subtilis K-49	12.5
Escherichia coli IFO 3545	>100
Pseudomonas aeruginosa IAM 1007	>100
Saccharomyces cerevisiae IFO 0203	0.78
Schizosaccharomyces pombe IFO 0342	0.39
Hansenula anomala IFO 0136	1.56
Candida utilis ATCC 42402	1.56
Sclerotinia libertiana ss	3.13
Mucor mucedo IFO 7684	25
Rhizopus chinensis IFO 4745	12.5
Aspergillus niger ATCC 6275	12.5
Penicillium crustosum Thom	6.25

Aframodial also exhibits strong cytotoxic activity (ED_{50} 2.5 μg/mL) towards L 1210 cells[9] and towards KB cells.[16] Furthermore, studies by the Japanese workers have demonstrated the anti-hypercholesterolemic effect of aframodial.[26] The antibacterial[12] and antiplasmodial[14] activities of **1** have also been demonstrated.

Aulacocarpinolide (**2**), aulacocarpin A (**3**) and B (**4**) moderately inhibited the growth of the pathogenic bacterium *Bacillus subtilis* (MIC 25 μg/mL) [8]. These compounds were also weakly active against *Mucor miehei* (MIC 50 μg/mL). Aulacocarpinolide (**2**) and

aulacocarpin B (**4**) further showed cytotoxicity against L 1210 cells at a concentration of 12.5 μg/mL and 25 μg/mL respectively (ED_{50} values).[8]
Labda-8(17),12-dien-15,16-dial (**5**) and coronarin B (**9**) were found to have modest antiplasmodial activity[14] while 8β(17)-epoxy-3 β,7 β -dihydroxylabd-12(E)-en-16,15-olide (**6**) showed slight tripanosomial activity.[18]

Labdane diterpenoids with broad range of activities are characteristic of *Aframomum* species. Galanolactone (**10**), galanal A (**11**) and B (**12**), isolated from *A. sulcatum* and *A. latifolium*, have been demonstrated to possess antifungal, cytotoxic and antiplasmodial activities.[11,14]
Some arylalkanoids, **22-25**, isolated from *A. melegueta*, showed termite antifeedant activity. [6]-gingerol (**24**) and [6]-shogaol (**25**) exhibit the strongest antifeedant activity at 1000 ppm, corresponding to 8 μg/cm^2.[19] Shogaols and paradols showed strong antibacterial and antifungal proprieties at very low doses.[24]

The diarylheptanoids **26-29**, isolated for the first time from the seeds of *A. letestuianum*, were tested for growth inhibitory activity *in vitro* versus bloodstream forms of African trypanosomes. IC_{50} values in the range of 1-3 μg/mL were found for compounds **26** and **29**. Compound **27** was inactive (IC_{50}>100 μg/mL) for all isolates tested.[20] These compounds belong to the family of diarylheptanoids which are known to exhibit a broad range of potent biological activity that include anti-inflammatory, antihepatoxic, antifungal, antibacterial and antitumor promoting. This group of compounds has also recently been shown to have an inhibitory activity against nitric oxide production in activated murine macrophages.[27]
A. giganteum has been shown to contain the flavonoids quercetin and kaempferol, both possessing antibacterial activities which inhibits the growth of fungi and yeast. They also display potent antiviral responses and are anti-inflammatory.[25] Quercetin and its methylated derivatives (**31** and **32**) showed strong activity against polio type 1 and Coxsackie B4 viruses (*in vitro* and *in vivo*). Both quercetin and kaempferol inhibit the release of rat mast cell histamine, and are very efficient flavonol radical scavengers. Quercetin exerts growth inhibitory effects on several malignant cell lines *in vitro*. This compound is also an inhibitor of multidrug resistant human breast cancer cells.[28]

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