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Phytochemicals from *Beilschmiedia anacardioides* and Their Biological Significance

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1. Introduction

Medicinal plants provide a vast array of raw materials for primary health care in Africa and other countries of the world. The World Health Organization (W.H.O) estimates that about 80% of Africans living in the continent have resort to traditional medical practitioners and the use of traditional medicine for the treatment of their diverse ailments. This practice has a considerable importance within the economic and cultural milieu of Africa.

It is estimated that less than 10% of the world's genetic resources have been studied seriously as sources of medicines. Yet from this small fraction, humanity has reaped enormous benefits.

The search for bioactive plant natural products from higher plants is gathering momentum, as they have potential to provide new lead compounds or to be of use directly. There is an increasing sense of urgency about this search due to the destruction of natural resources. With regards to these plants, it has been estimated that 25-30 million hectares of the world's rainforests are lost each year. The crucial problem already expressed by several scientists then is how to search efficiently and rapidly for bioactive components from the vast number of unstudied plants. Part of the solution is to narrow down the search-selection.

Six approaches to the selection of plant materials for study exist: the locally random, the taxonomic, the ethnobotanic, the phytochemical, the information based, and serendipity. The ethnomedical approach appears to be the method of choice for natural product chemists working in Africa and other developing countries. In this method only plants used in traditional medicine are collected.

Very little attention has been paid to *Beilshmiedia* species. Previous studies concern trees and herbs of *Beilshmiedia* species, with the aim of cultivating herbs containing the same endiandric acid derivatives as trees. Other studies led to a patent on interesting synthesis of endiandric acid derivatives.

In our own search for prospective pharmacological products from ethnobotanic data, we have been looking at some traditional medicines whose therapeutic efficiency is scientifically established towards biomedical analyses of patients on treatment in a specialized clinic. We have selected a traditional medicine based on one plant *Beilshmiedia* *anacardioides* (Lauraceae), for its proven efficiency on genital infections and rheumatisms through clinical research. No phytochemical studies of *Beilshmiedia anacardioides* are however to our knowledge available in the literature. We propose that phytochemists looking for novel bioactive natural products should investigate the medicinal plants whose therapeutic efficiency has been established through clinical research on African medicine.

The genus *Beilschmiedia* comprises about 200 species widely distributed in the intertropical region (Fouilloy, 1974). *B. anacardioides* stem bark is used in the Western Province of Cameroon to cure uterine tumours (Tchouala, 2001). Some other species of the genus Beilschmiedia are used in traditional medicine in Africa for the treatment of several ailments (Tchouala, 2001; Iwu, 1993). Previous phytochemical investigations of plants of the genus *Beilschmiedia* reported the presence of bio-active lignans (Chen et al., 2006; Chen et al., 2007), flavonoids (Harbone et al., 1969), triterpenoids (Chen et al., 2006); tetracyclic endiandric acid (Bandaranayake et al., 1981; Banfield et al., 1994) and alkaloids (Clezy et al., 1966; Kitagawa et al., 1993; Chouna et al., 2011).

We have initiated a systematic phytochemical investigation of the extracts of *Beilshmiedia anacardioides* as well as the antibacterial activity of the eight new compounds isolated, towards five strains of microbes, namely *Bacillus subtilis*, *Micrococcus luteus*, *Streptococcus faecalis*, *Pseudomonas palida*, and *Escherichia coli*.

The methods used for the isolation of the compounds were mainly column chromatography and preparative TLC. The structures of all compounds were elucidated by means of modern spectroscopic techniques such as 1D-NMR (^{1}H-NMR, ^{13}C-NMR with DEPT experiments), and 2D-NMR (^{1}H-^{1}H-COSY, HMQC,HMBC,NOESY), MS , IR and X-Ray spectroscopies.

The antibacterial activities of the new compounds were examined using the dilution technique with respect to the zone of inhibition (ZI) and minimum inhibitory concentration (MIC).

We report here the results we have so far obtained and published in three renowned scientific journals (Chouna et al.,2009; 2010; 2011).

2. Study of the ethnomedical preparation

The ethnomedical preparation is a decoction.The decoction is prepared as follows: Boil about 80 g dry stem bark powder in 3 litres of water for 15 minutes. Filter when lukewarm. Drink a glass twice daily for ten days.

A treatment for fibromes could last about two to three months, depending on the patient's age.

3. Study setting

Cameroon is a bridge between Central Africa and West Africa, humid Africa and dry sahelian Africa, French speaking and English speaking Africa (French and English are official languages). The country is open to the Gulf of Guinea in his south-west border. Lake Chad is at the extreme –North border. A country of 475.442 square kilometers, Cameroon is borded in the west by Nigeria, on the east by Chad and the Central African Republic, and on the south by Congo, Gabon, and Equatorial Guinea.

The Bamoun are a Bantu people living in the west Region of Cameroon. They number more than half a million. They have a rich cultural Heritage, including famous traditional Healers. Sultan Njoya wrote a book on Bamoun traditional medicine. Important Bamoun towns are Foumban, Foumbot, Koutaba, Massangam, Magba, Malantouen. Among important villages are Mahoua, Manki 1 and Manki 2, where the plant *Beilshmiedia anacardioides* was collected.

3.1 Generalities on *Beilschmiedia* anacardioides

B. anacardioides is found in Central Africa, especially in Cameroon, Tchad and Gabon. In Cameroon, this species is found in the Adamaoua and the West Region (Eyog et al., 2006; Fouilloy, 1974). It is synonymous with *B. ngriki* and *B. Jacques-felixii* and it is commonly named *ntseum* (in Bamoun language) in the Noun subdivision of the West region of Cameroon (Eyog et al., 2006; Fouilloy, 1974; Tchouala, 2001).

3.2 Uses of *Beilschmiedia* species in traditional medicine

B. anacardioides stem bark is used in the Noun sub-division of the West Region of Cameroon to treat uterine tumours, rubella, rheumatisms, bacterial and fungal infections (Tchoula, 2001). Seeds are used as spices (Eyog et al., 2006). *B. lancilimba* is used in the same region to cure skin bacterial infections (Tchouala, 2001). *B. manii* is used to treat dysentery and headache. It is also used as an appetite stimulant (Iwu, 1993).

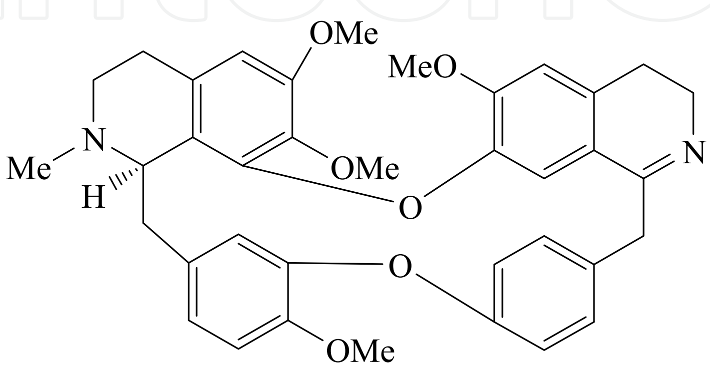
4. Phytochemistry of plant constituents of *Beilshmiedia* species

A review of the literature revealed that no phytochemial studies have been carried out on *Beilshmiedia anacardioides* prior to the initiation of our study. The various phytochemical and pharmacological studies performed and reported in the literature on the beilshmiedia genus are discussed below.

4.1 Alkaloids isolated from the *Beilschmiedia* genus

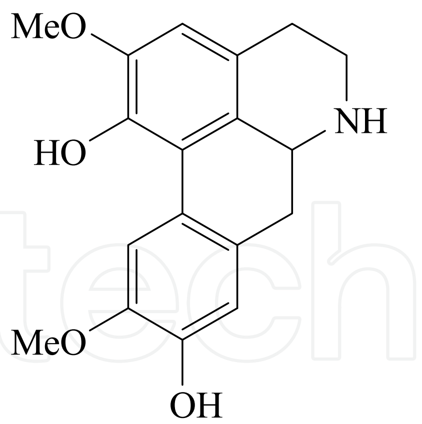
Very few alkaloids have been isolated from the *Beilschmiedia* genus.

Structure and name **Source and references**



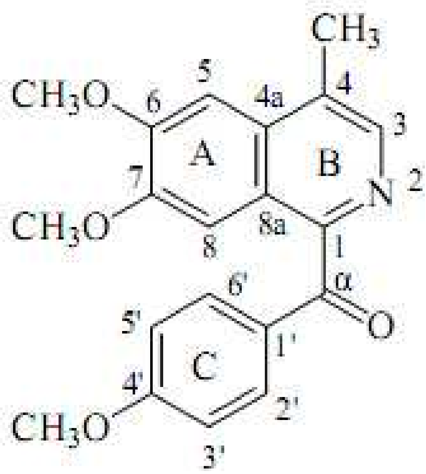
9: Dehatrine

Wood of *B. madang* (Kitagawa et al., 1993)



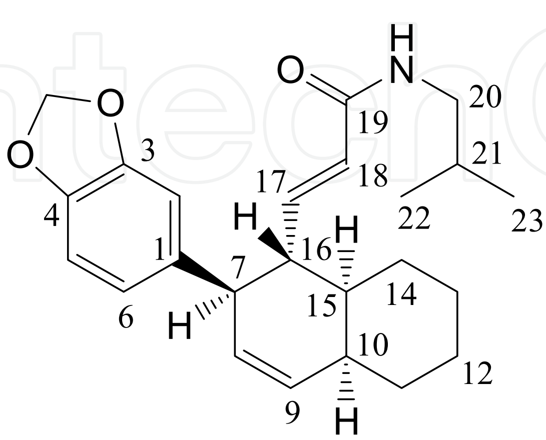
Stem bark of *B. elliptica* (Clezy et al., 1966)

10: Laurelliptine



Leaves of *B. Brevipes* (Pudjiastuti et al.,2010)

11: (6,7-Diméthoxy-4-methylisoquinolinyl)-(4′-methoxyphenyl)-methanone



Stem bark of *B. Obscura* (Lenta et al.,2011)

12: Obscurine

Table 1. Structure of some alkaloids isolated from the *Beilschmiedia* genus

Pharmacological importance of alkaloids isolated from the Beilschmiedia genus

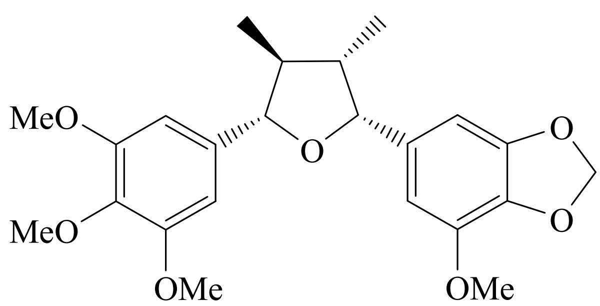
A bisbenzylisoquinoline alkaloid dehatrine (**9**) isolated from the wood of *B. madang*, exhibited potent inhibitory activity (IC_{50} value of 0.017 μM) against the proliferation of the malaria pathogen *P. falciparum* (Kitagawa et al., 1993). Paulo and coworkers (1992) demonstrated the antimicrobial properties of laurelliptine (**10**).

4.2 Phenolic and phenolic derived compounds

4.2.1 Lignans and neolignans

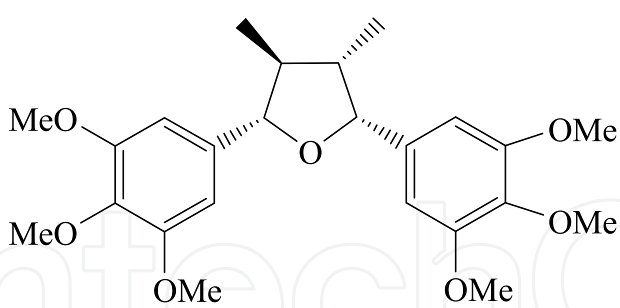
Lignans and neolignans and flavonoids are the main phenolic compounds encountered in the *Beilschmiedia* genus.

Structure and name | **Source and references**



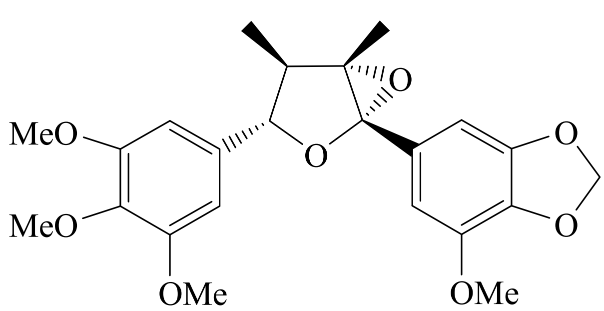
13: Beilschmin A

Stem of *B. tsangii* (Chen et al., 2006)



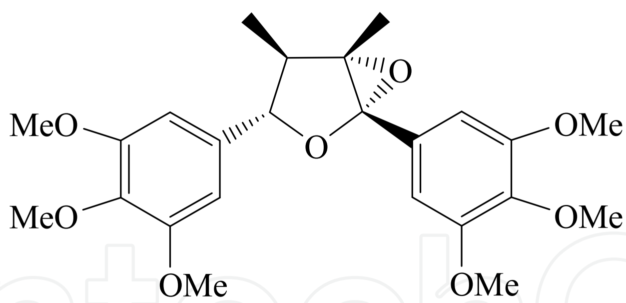
14: Beilschmin B

Stem of *B. tsangii* (Chen et al., 2006)



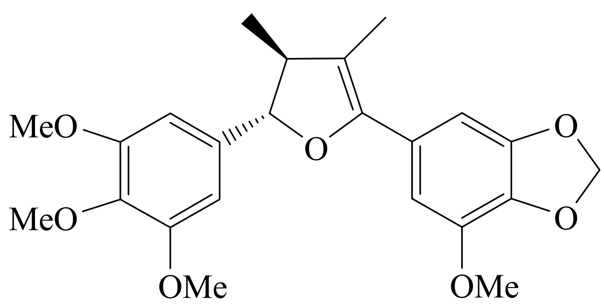
15: 4α,5α,-Epoxybeilschmin A

Leaves of *B. tsangii* (Chen et al., 2007)



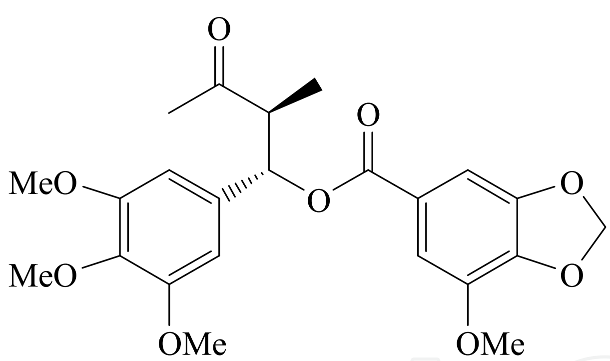
Stem of *B. tsangii*
(Chen et al., 2006)

16: 4α,5α,-Epoxybeilschmin B



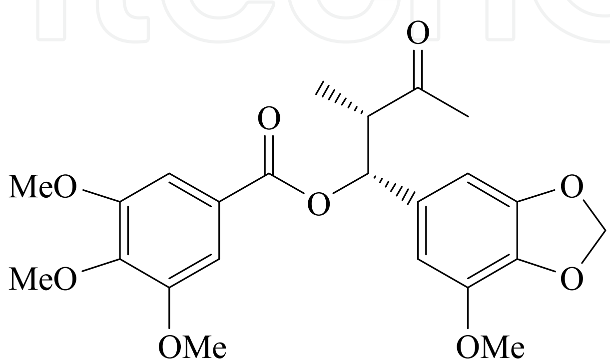
Stem of *B. tsangii*
(Chen et al., 2006)

17: Beilschmin C



Stem of *B. tsangii*
(Chen et al., 2006)

18: Tsangin A



Stem of *B. tsangii*
(Chen et al., 2006)

19: Tsangin B

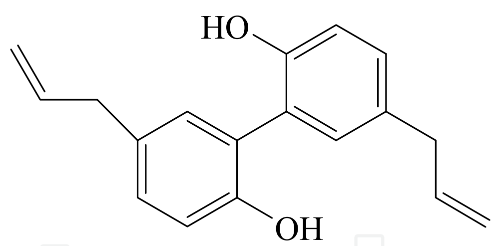
 **20**: Magnolol	Leaf of *B. volckii* (Banfield et al., 1994)

Table 2. Structure of some lignans and neolignans isolated from *Beilschmiedia* genus

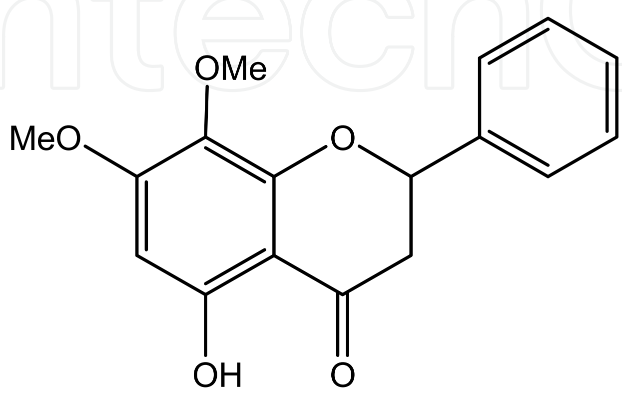
Pharmacological importance of lignans and neolignans isolated from the Beilschmiedia genus

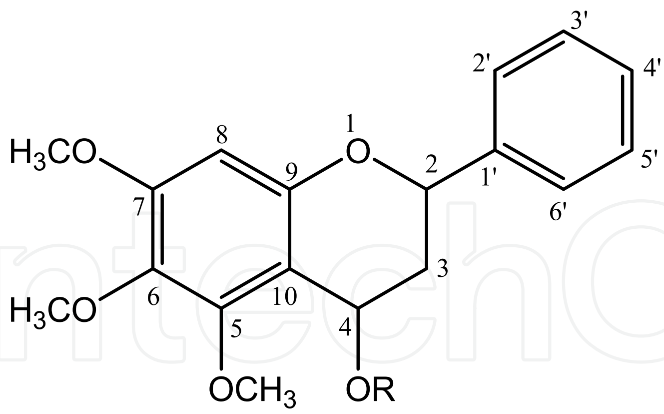
Tetrahydrofuran-type lignans beilschmin A (**13**) and B (**14**), dihydrofuran-type lignan beilschmin C (**17**) together with tsangin A (**18**) and B (**19**) were found cytotoxic (IC_{50} value below 4 μg/mL) in P-388 and/or HT-29 cell lines *in vitro* (Chen et al., 2006). In addition, beilschmin A (**13**) and B (**14)** exhibited potent antitubercular activity (MIC values of 2.5 and 7.5 μg/mL, respectively) against *Mycobacterium tuberculosis* 90-221387 in *vitro* (Chen et al., 2007). A neolignan, magnolol (**20**) displayed wide biological properties, mainly cytotoxic (Li et al., 2007), antidepressant (Li et al., 2007), antimicrobial (Park et al., 2004) and anti-inflammatory (Lee et al., 2005).

4.2.2 Some flavonoids isolated from *Beilschmiedia* genus

Pharmacological importance of flavonoids isolated from the Beilschmiedia genus

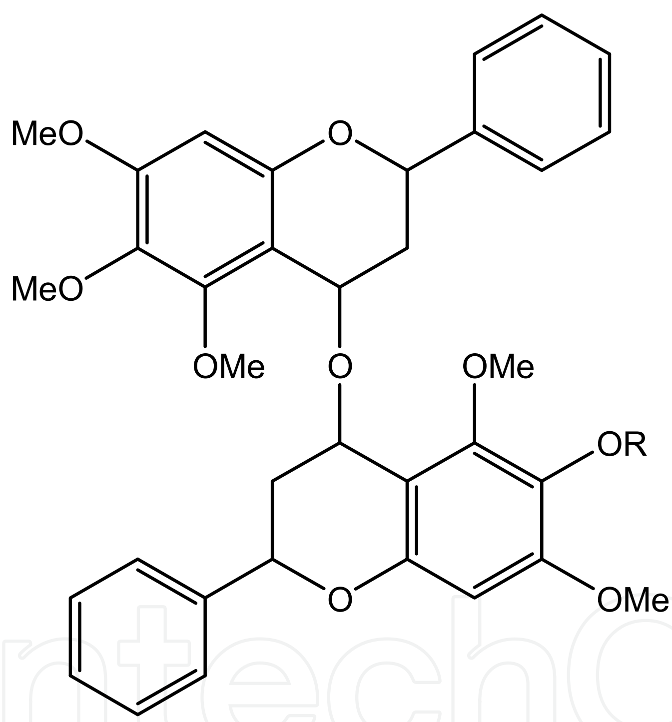
Lenta and coworkers (2009), evaluated the antibacterial activities of the extract and flavonoids isolated from the stem of *B. zenkeri*, in vitro against three strains of microbes, *pseudomonas agarici, Bacillus subtilis,* and *streptococus minor.* Their activities were moderate compare to reference drugs ampicillin and gentamicin. (2S,4R)-5,6,7-trimethoxyflavan-4-ol (**22a**) exhibited the best potency against *S. minor* (IC_{50} of 197.5 µM) (Lenta et al., 2009).

Structure and name	Source and references
 **21**: 5-Hydroxy-7,8-dimethoxyflavanone	Stem of *B. zenkeri* (Lenta et al., 2009)



Stem of *B. zenkeri* (Lenta et al., 2009)

22a: R = H: (2S,4R)-5,6,7-trimethoxyflavan-4-ol
22b: R = CH3: (2S,4R)-4,5,6,7-trimethoxyflavan



Stem of *B. zenkeri* (Lenta et al., 2009)

23a: R = CH3: Beilschmieflavonoid A
23b: R = H: Beilschmieflavonoid B

Table 3. Structure of flavonoids isolated from the *Beilschmiedia* genus

4.2.3 Other phenolic and phenolic derived compounds from the *Beilschmiedia* genus and their pharmacological importance

Vanillin (**21a**) and 4-hydroxybenzaldehyde (**24b**) were isolated from *Beilschmiedia tsangii* (Chen et al., 2006). Both compounds were reported to exhibit analgesic, anti-inflammatory and antifungal activities (Lee et al., 2005; Lee et al., 2006; Fitzgerald et al., 2005).

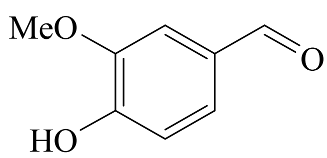
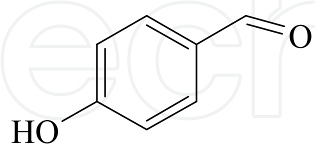
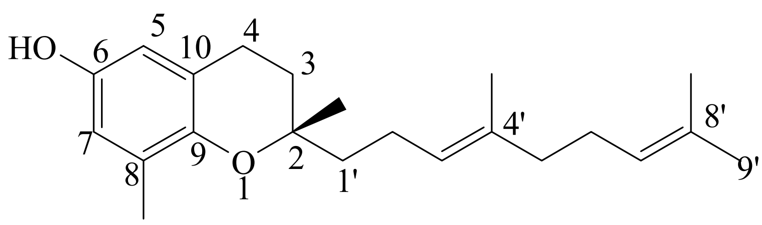
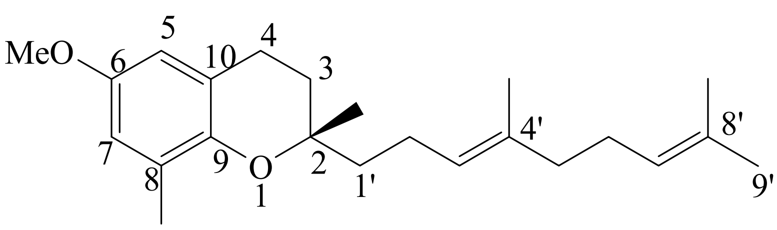
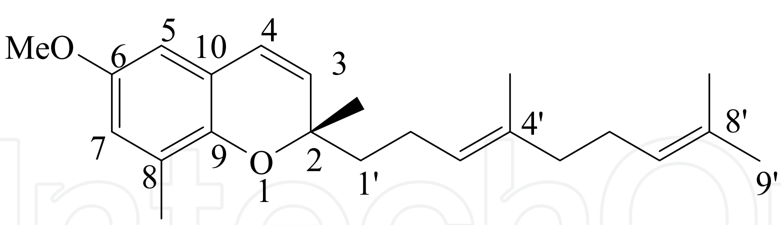
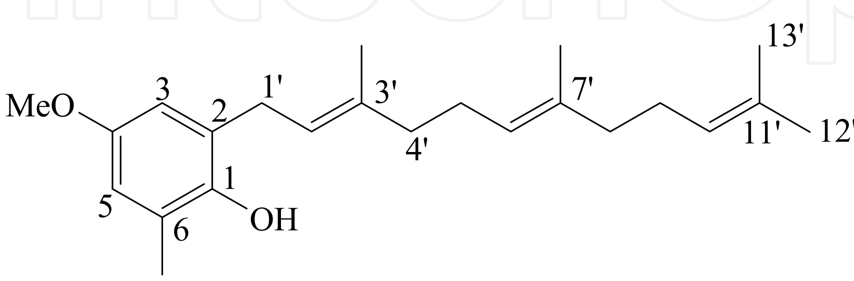
Structure and name	Source and references
 **24a**: Vanillin	Stem of *B. tsangii* (Chen et al., 2006)
 **24b**: 4-hydroxybenzaldehyde	Stem of *B. tsangii* (Chen et al., 2006)
 **25a**: Oligandrol	Bark of *B. oligandra* (Banfield et al., 1994)
 **25b**: Oligandrol methyl ether	Root of *B. erytrhophloia* (Yang et al., 2008)
 **25c**: 3,4-Dehydrooligandrol methyl ether	Root of *B. erytrhophloia* (Yang et al., 2008)
 **26**: Farnesylol	Root of *B. erytrhophloia* (Yang et al., 2008)

Table 4. Structure of other phenolic and phenolic derived compounds isolated from the *Beilschmiedia* genus

4.3 Endiandric acids

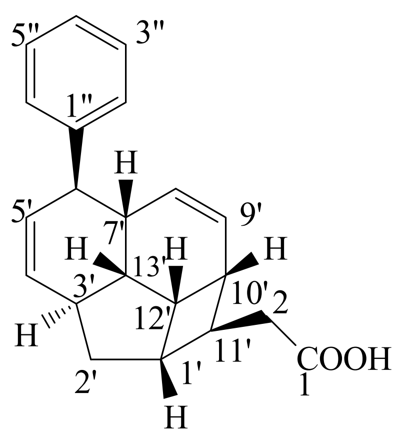
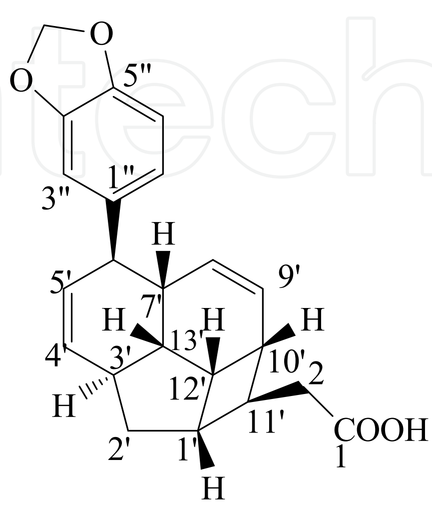
Endiandric acids are a rare class of secondary tetracyclic metabolites generally encountered in *Beilschmiedia* and *Endiandra* species of the Lauraceae family. Endiandric acids are products of electrocyclic cyclization of naturally occurring polyketides (Bandanarayake et al., 1980).

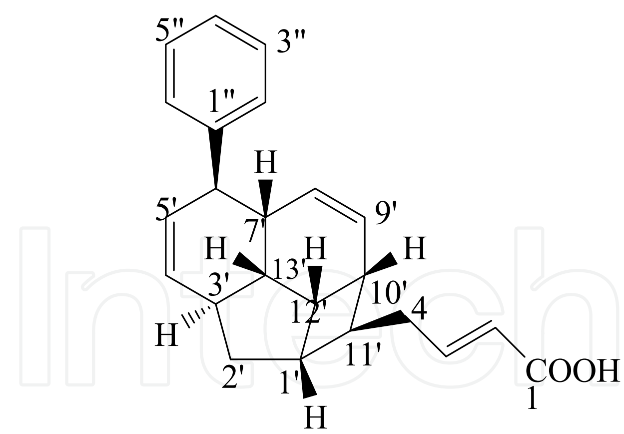
4.3.1 Some endiandric acids previously isolated

Pharmacological importance of the endiandric acids

Very few pharmacological studies have been done in this class of metabolites. Endiandric acid H (**41**) is used for the manufacture of medication, in particular for the treatment of asthmatic disorders or concomitant inflammatory symptoms of asthma (Eder et al., 2004).

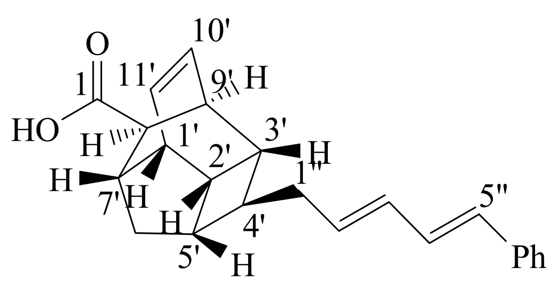
Erytrophloin C (**34**) exhibited antitubercular activity (MIC value of 50 μg/mL) against *Mycobacterium tuberculosis* H37Rv in *vitro* (Yang et al., 2009).

Structure and name	Source and references
 **27**: Endiandric acid A	Leaves of *Endiandra entrorsa* and *Endiandra oligandra* (Bandaranayake et al., 1981) Bark of *B. oligandra* (Banfield et al., 1994)
 **28**: Methylenedioxyendiandric acid A	Leaves of *Endiandra entrorsa* (Banfield et al., 1994) and Stem bark of *B. manii* (Mpetga, 2005)



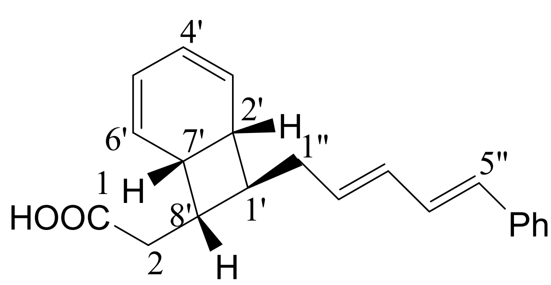
29: Endiandric acid B

Leaves of *Endiandra entrorsa* (Bandaranayake et al., 1982)
Barks and leaves of *Endiandra jonesii* (Banfield et al., 1994)



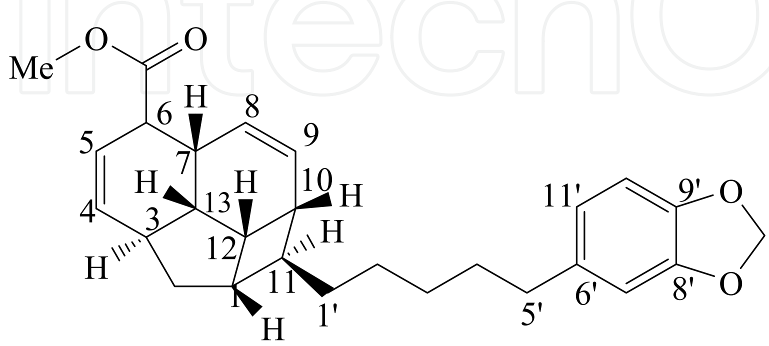
30: Endiandric acid C

Leaves of *Endiandra entrorsa* (Bandaranayake et al., 1982)
Bark and leaves of *Endiandra jonesii* (Banfield et al., 1994)



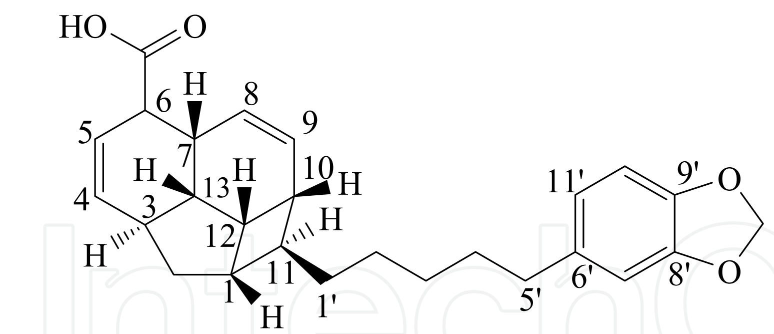
31: Endiandric acid D

Leaves of *Endiandra entrorsa* (Banfield et al., 1983)



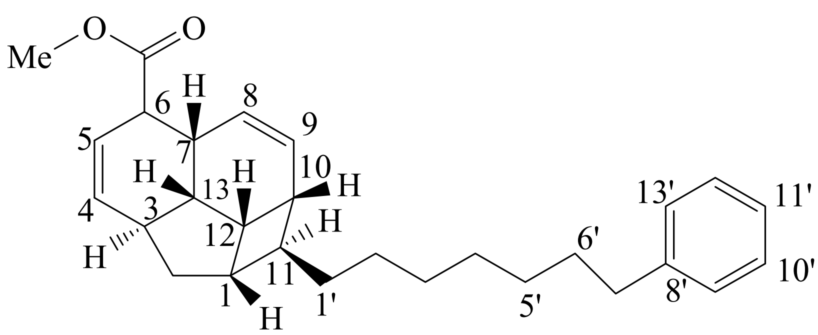
32: Erytrophloin A

Root of *B. erytrhophloia* (Yang et al., 2009)



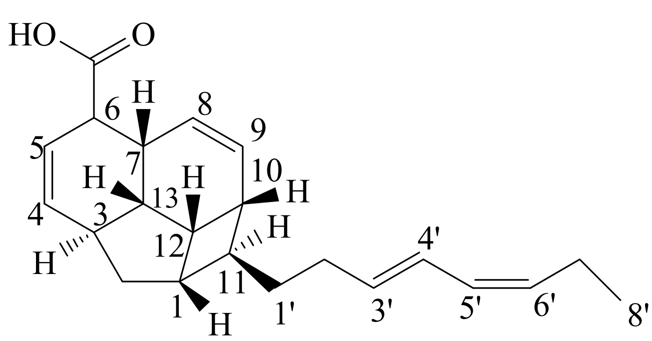
33: Erytrophloin F

Root of *B. erytrhophloia* (Yang et al., 2009)



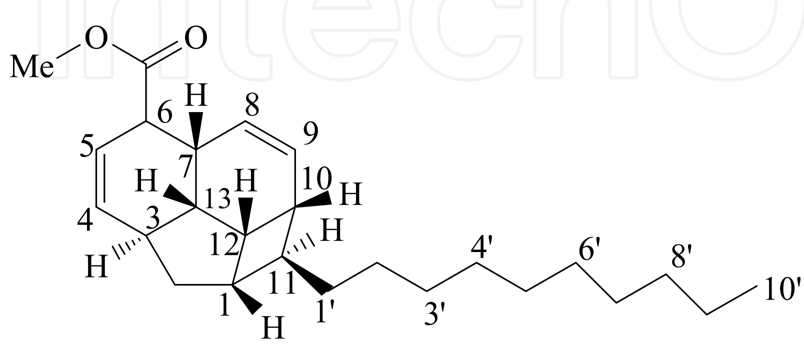
34: Erytrophloin C

Root of *B. erytrhophloia* (Yang et al., 2009)



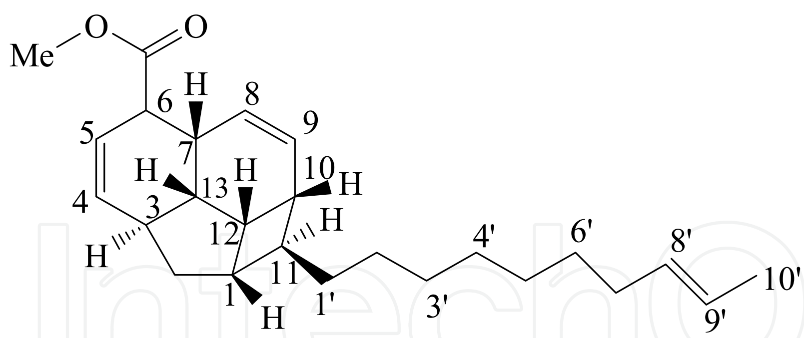
35: Erytrophloin E

Root of *B. erytrhophloia* (Yang et al., 2009)



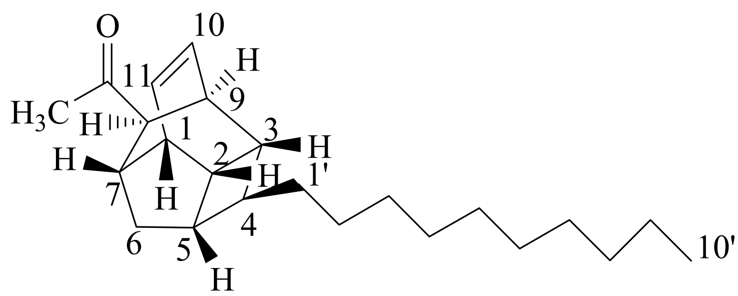
36: Erytrophloin B

Root of *B. erytrhophloia* (Yang et al., 2009)



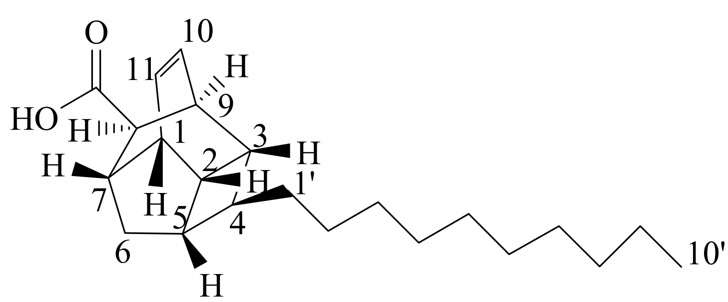
Root of *B. erytrhophloia* (Yang et al., 2009)

37: Erytrophloin D



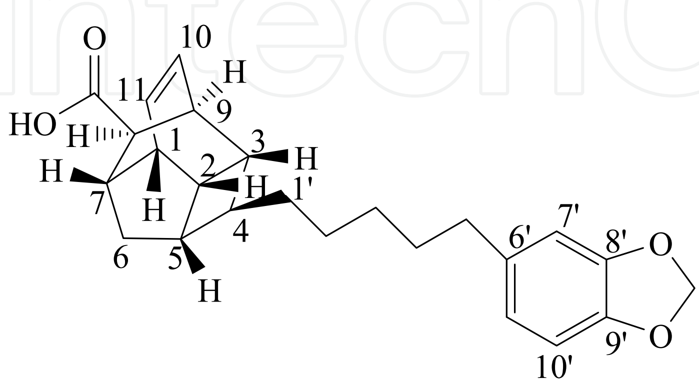
Root of *B. erytrhophloia* (Yang et al., 2009)

38: Beilcyclone



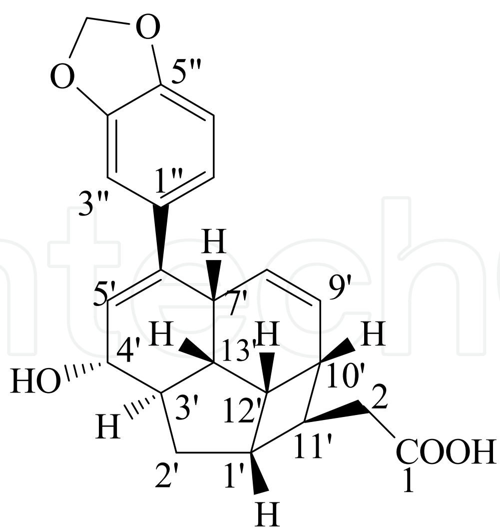
Root of *B. erytrhophloia* (Yang et al., 2008)

39: Endiandric acid J



Root of *B. erytrhophloia* (Yang et al., 2008)

40: Endiandric acid I



Stem of B. fulva (Eder et al., 2004)

41: Endiandric acid H

Table 5. Structure of some endiandric acids previously isolated

5. Results of our own studies

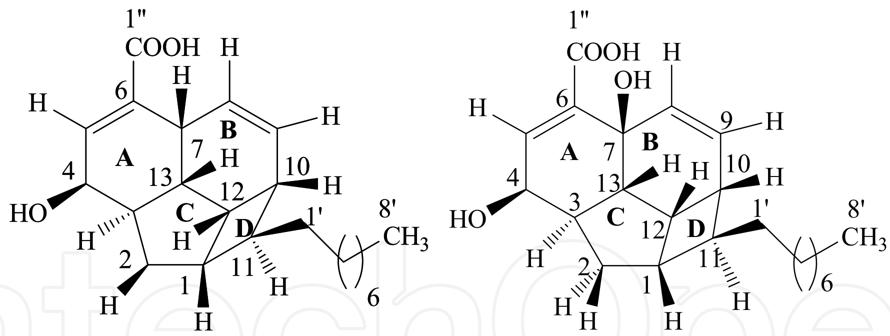
We have initiated a systematic phytochemical investigation of the extracts of *Beilshmiedia anacardioides* and have so far obtained the following results which have led to three publications in renowned scientific journals (Chouna et al.,2009; 2010; 2011).

Air-dried and ground stem bark of *B. anacardioides* was extracted successively at room temperature with MeOH. The methanol extract was re-extracted in turn with CH_2Cl_2 and EtOAc. These extracts were concentrated to dryness under reduced pressure.

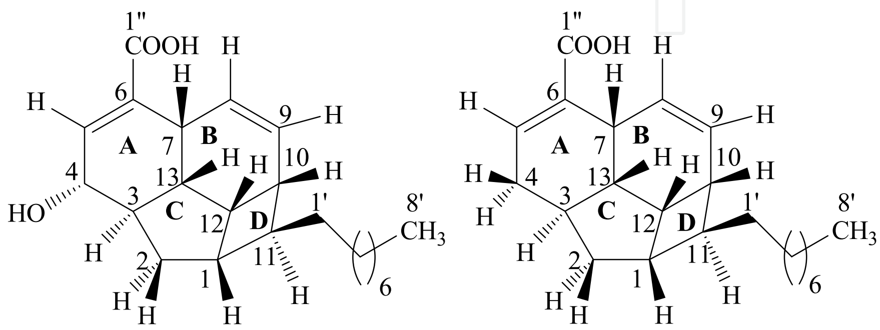
The CH_2Cl_2 extract was submitted to repeated column chromatography on silica gel, yielding beilschmiedic acids A (**1**), B (**2**) and C (**3**) and the known β-sitosterol (Chouna et al.,2009) .

Further Successive purifications by column chromatography over silica gel and preparative TLC afforded three new endiandric acid derivatives: beilschmiedic acids D(**4**) and E(**5**), and Beilshmiedin (**8**) (Chouna et al.,2010), together with the known compounds bisabolene (Mossa et al 1992; Barrero et al. 1990), and tricosanoic acid (Erdemoglu et al., 2008).

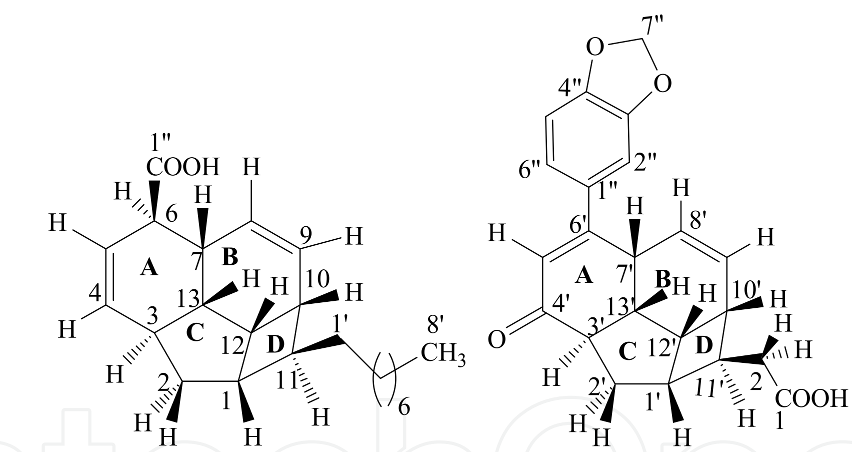
The ethyl acetate soluble part of the MeOH extract of the stem bark of *B. anacardioides* was fractionated by column chromatography over silica gel. Successive purifications by column chromatography and preparative TLC afforded two new endiandric acid derivatives: beilschmiedic acids F(**6**) and G(**7**) Chouna et al.,2011, along with the known constituents beilschmiedic acid A(**1**), beilschmiedic acid C(**3**) [6] and sitosterol-3-*O*-*β*-*D*-glucopyranoside (Chouna et al.,2011).



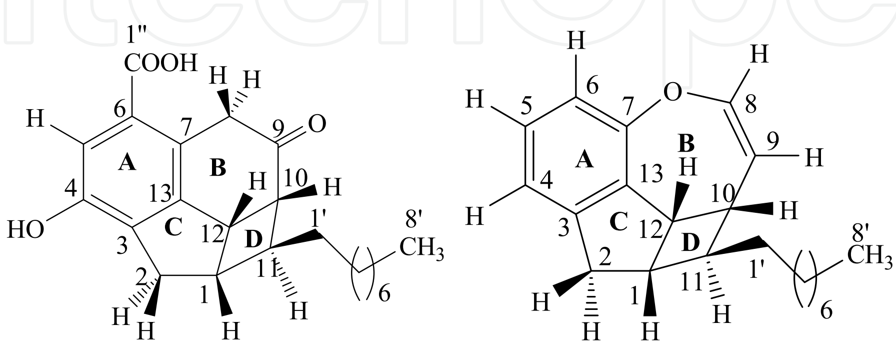
Beilshmiedic acid A (**1)** Beilshmiedic acid B (**2)**



Beilshmiedic acid C (**3)** Beilshmiedic acid D (**4)**



Beilshmiedic acid E (**5)** Beilshmiedic acid F (**6)**



Beilshmiedic acid G (**7)** Beilshmiedin (**8**)

Scheme 1. endiandric acid derivatives from *Beilshmiedia anacardioides.*

6. Biological activity and the significance of some compounds

Our preliminary antibacterial studies on the new endiandric acid derivatives have yielded chemical entities that have been shown to possess significant activities (Chouna et al., 2009) .

Antibacterial assay on some compounds isolated from *B.anacardioides*

Compounds **1**-**8** were tested in vitro for their antibacterial activity against *Bacillus subtilis*, *Streptococcus ferus*, *Streptococcus minor*, *Micrococcus luteus*, *Escherichia coli*, and *Pseudomonas agarici*, using the dilution technique.

The ZI (Table 1) and MIC (Table 2) obtained for these compounds indicated that they possessed strong to weak antibacterial activity against *gram* positive bacteria.

Beilshmiedic acid C (**3)** demonstrated the best potency against *B. subtilis* and *M. luteus*, compared to the reference drug ampicillin. The MIC values (Table 2) of Beilshmiedic acids B(**2)**, C (**3)** and G(**7)**, against *B. subtilis* and Beilshmiedic acid C (**3)** against *M. luteus* were found to be greater than that of standard drug ampicillin, indicating that this series of compounds might be possible candidates as antibacterial drugs.

None of the tested compounds was active against *Gram* negative *P. palida* and *E. Coli*. Therefore, they might be well tolerated as antibiotics even for long term treatments.

Compound tested	*B. subtilis*	*M. luteus*	*S. faecalis*	*S. minor*	*S. ferus*	*P. palida*	*E. coli*
1	15	12	14	n.t.	n.t.	-	-
2	16	15	15	n.t.	n.t.	-	-
3	13	30	18	n.t.	n.t.	-	-
4	10	n.t.	n.t.	10	-	-	-
5	12	n.t.	n.t.	-	12	-	-
6	10	n.t.	n.t.	-	-	-	-
7	20	15	16	-	-	-	-
8	10	n.t.	n.t.	-	-	-	-
Ampicillin	29	26	25	22	23	-	-

(-) inactive, n.t. (not tested)

Table 6. Antibacterial activity (Zone of inhibition of compounds in mm) of compounds **1-8** (500μg/mL) against *B. Subtilis, M. luteus, S. faecalis, S. minor, S. ferus*, *P. palida* and *E. Coli.*

Compound tested	*B. subtilis*	*M. luteus*	*S. faecalis*	*S. minor*	*S. ferus*
1	181.60	173.60	363.30	n.t.	n.t.
2	11.30	347.20	45.30	n.t.	n.t.
3	5.60	< 0.70	22.70	n.t.	n.t.
4	381.00	n.t.	n.t.	190.50	-
5	381.00	n.t.	n.t.	190.50	-
6	343.40	n.t.	n.t.	-	-
7	87.78	10.95	87.78	-	-
8	422.20	n.t.	n.t.	-	-
Ampicillin	89.5	1.95	3.9	1.05	5.25

(-) inactive, n.t. (not tested)

Table 7. Antibacterial activity (MIC in μM) of compounds **1-8** against *B. Subtilis*, *M. luteus*, *S. faecalis*, *S. minor*, *S. ferus*, *P. palida* and *E. coli*.

Beilshmiedic acid C (**3)** was more active than Beilshmiedic acid D (**4)** . The enhanced activity may be due to the additional hydroxyl group at C-4 position in Beilshmiedic acid C (**3)** . Beilshmiedic acid B(**2)** which possesses one hydroxyl group more than Beilshmiedic acid A (**1)** and Beilshmiedic acid C (**3)** was less active. Beilshmiedic acid A (**1)** was more active than Beilshmiedic acid C (**3)** . They are epimers at C-4 position; the modification of the configuration at this position influences significantly the activity.

Based on the skeletal features, it is difficult at this stage to define the contribution of the different functional groups with respect to the activity. The mechanism of action of this class of metabolites on these strains is not yet known. Further investigations will help to establish the mode of action of this particular skeleton. These interesting results highlight the potency of this rare class of metabolites that might be investigated for the search of new antibacterial drugs.

7. Conclusion

In our hypothesis we proposed that phytochemists looking for novel bioactive natural products should investigate the medicinal plants whose therapeutic efficiency has been established through clinical research on African medicine.

We suggested that Natural products from *Beilshmiedia anacardioides* may play a role in treating genital infections due to *B-subtilis* and *M. luteus*, and rheumatisms due *Streptococcus ferus* and *S. minor*. The biological activities of some of the constituents isolated in our studies, Beilshmiedic acid C presented above, more than lend support to this suggestion.

It is certain that as more and more data become available from the phytochemical and biological analysis of the constituents of therapeutic efficient medicinal plants selected after clinical research, the role of these plants in the treatment of diseases will become more defined. Thus African Traditional medicine will gain universal status.

8. Acknowledgments

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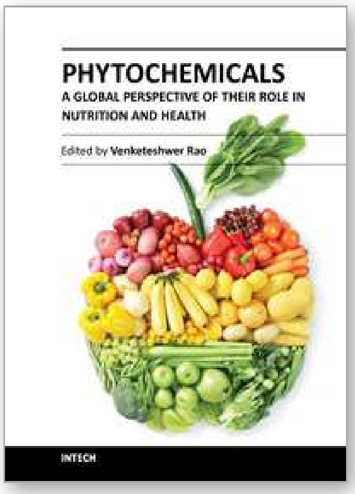

Phytochemicals - A Global Perspective of Their Role in Nutrition and Health
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