*SHORT REPORT*

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# Secondary Metabolites from *Jacaranda Mimosifolia* and *Kigelia Africana* (*Bignoniaceae)* and Their Anticandidal Activity

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**Abstract:** From the stem barks of *Jacaranda mimosifolia* benzoic acid (**1**), 1-naphthaleneacetic acid, 5-carboxy-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-[1S-(1α,2β,4aβ,8aα)] ( **2**), betulinic acid ( **3**)**,** lupeol (**4**) and ursolic acid (**5**) were isolated. Similarly, lapachol (**6**), dehydro-α-lapachone (**7**), 2- acetylfuro-1, 4-naphthoquinone (**8**), *p*-coumaric acid (**9**), caffeic acid (**10**), nonacosanoic acid, 2-(4-hydroxyphenyl)ethyl ester (**11**), *β*-sitosterol (**12**), kigelinol (**13**), oleanolic acid (**14**), *β*-friedelinol (**15**), pomolic acid (**16**), and kojic acid (**17**) were isolated from the stem barks of *Kigelia africana*. All the isolated compounds were characterized by using spectroscopic methods especially 1D and 2D NMR and ESI mass spectrometry and comparison with literature data. To the best of our knowledge, compounds **1**, **2**, **3** and **5**, and compounds **11**, **14**, **15** and **16** were isolated for the first time from *Jacaranda mimosifolia* and *Kigelia africana*, respectively. All these compounds were screened for anticandidal activity by agar diffusion method and microbroth dilution technique on four *Candida albicans* strains (ATCCL26, ATCC12C, ATCCP37039, and ATCCP37037). Among them, compounds **9, 10**, and **17** exhibited the highest anticandidal activity that varied between the microbial species (MIC= 0.01 ± 0.00 − 0.03 ± 0.00 mg/mL) on *C. albicans* ATCCL26, ATCCP37037, ATCCP37039 and ATCC12C strains. Compound **17** was likely the most active against the four *Candida albicans* strains (MIC= 0.01 ± 0.00 − 0.02 ± 0.00 mg/mL).

**Keywords:** Bignoniaceae; *Jacaranda mimosifolia*; *Kigelia africana;* anticandidal activity. 

## 1. Plant Source

*Kigelia africana* (Lam) Benth and *Jacaranda mimosifolia* D. Don belonging to *Bignoniaceae* family are widely distributed in tropical and subtropical areas of the world. These plants are useful in traditional medicine to cure many diseases. *J. mimosifolia* is used in different countries to cure wounds, ulcers, and serves as an astringent in diarrhoea and dysentery [1, 2] and *K. africana* is commonly used by African and Indian traditional healers to treat a wide range of skin ailments like, fungal infections, boils, psoriasis and eczema, and for the treatment of dysentery, wounds, lumbago, syphilis, and rheumatism [3, 4, 5].

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The stem barks of *J. mimosifolia* were collected at Melen (February 2010), while the stem barks of *K. africana* was collected at Dschang (November 2012), Centre and Western Regions of the Republic of Cameroon, respectively. These two plants were identified at the Cameroon National Herbarium (HNC) where voucher specimens of *J. mimosifolia* (N° 50081/HNC) and *K. africana* (N° 157/HNC) were deposited.

## 2. Previous Studies

Previous phytochemical studies revealed that irridoids [6], flavonoids [7], naphthoquinones [8], limonoids [9] and steroids [10] are the main secondary metabolites isolated from *K. africana*, while triterpenes [11], flavonoids [12], acetosides [12], quinones [13], phenylpropanoid derivatives [12, 14], fatty acid [15] and anthocyanins [16] have been reported from *J. mimosifolia.*

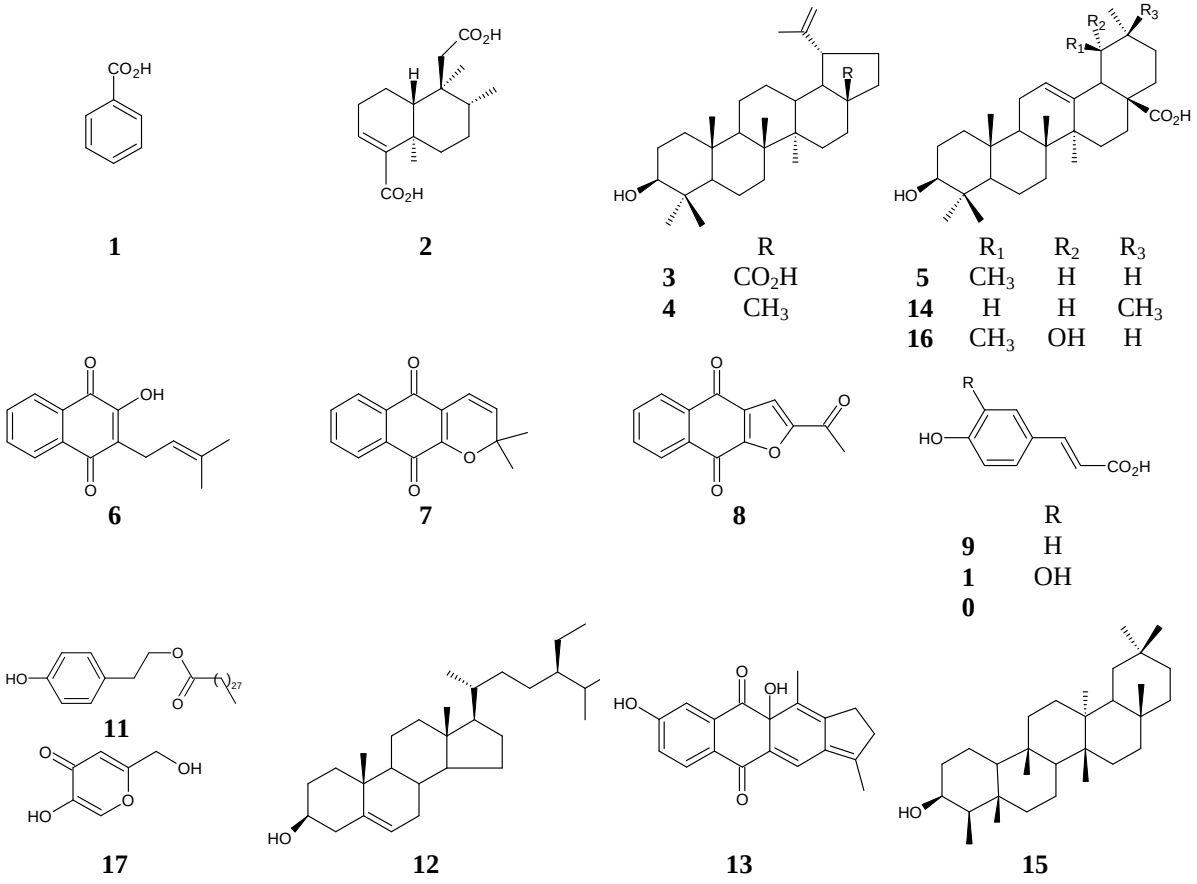
Antioxidant, cytotoxic, and antitrypanosomal activities of *K. africana* [17, 18, 5], and the antimicrobial activity of *J. mimosifolia* against *Bacillus cereus*, *Escherichia coli* and *Staphylococus aureus* was reported [19].

## 3. Present Study

The air-dried stem barks (2 kg) of *J. mimosifolia* were exhaustively macerated with 12 L mixture of $CH_2Cl_2$-MeOH (1:1) at room temperature for 72h. The macerate was filtered and evaporated under reduced pressure to afford a crude extract (300 g). Part of this extract (200 g) was treated with EtOAc to give after evaporation under reduce pressure 75 g of EtOAc extract. 70 g of this EtOAc extract was subjected to silica gel column chromatography and eluted with *n*-hexane, *n*-hexane-EtOAc and EtOAc, in order of increasing polarity yielding 5 major fractions. Fraction 1 was further purified by silica gel column chromatography and eluted with *n*-hexane-EtOAc (93:7) to give benzoic acid (**1**, 25 mg) [20]. Similarly, 1-naphthaleneacetic acid, 5-carboxy-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-[1S-(1α,2β,4aβ,8aα)] (**2**, 23 mg) [21] was isolated from fraction 2, after elution with a mixture of *n*-hexane-EtOAc (90:10), betulinic acid (**3**,15 mg) [20] was isolated from fraction 3 with *n*-hexane-EtOAc (75:25). Column chromatography of fraction 4 using *n*-hexane–EtOAc (70:30) as the eluent afforded lupeol (**4**, 15 mg) [11]. Similarly, CC of fraction 5, eluted with a mixture of *n*-hexane–EtOAc (60:40), afforded ursolic acid (**5**, 3 g) [22].

The air-dried stem barks (1.5 kg) of *K. africana* were extracted with MeOH by soxhelet apparatus. The extract was evaporated to dryness yielding 160 g of residue. Part of this extract (140 g) was treated with *n*-hexane-EtOAc (1:1), EtOAc and $H_2O$ to give 3 main fractions. Part of the *n*-hexane-EtOAc extract (20 g) was subjected to silica gel column chromatography and eluted with *n*-hexane, *n*-hexane-EtOAc and EtOAc, in order of increasing polarity yielding 4 major fractions. Fraction 1 was further separated by silica gel column chromatography and eluted with *n*-hexane-EtOAc (99:1) to give lapachol (**6,** 65 mg) [23, 24]. Similarly, dehydro-α-lapachone (7, 15 mg) [24] was purified from fraction 2, after elution with a mixture of *n*-hexane-EtOAc (98:2) and 2- acetylfuro-1,4-naphthoquinone (**8**, 20 mg) [25] was isolated from fraction 3 with *n*-hexane-EtOAc (90:10). Column chromatography of fraction 4 using *n*-hexane-EtOAc (55:45) as the eluent afforded *p*-coumaric acid (**9**, 30mg) [26] and caffeic acid (**10**, 29 mg) [26, 27]. Part of the EtOAc extract (25 g) was subjected to silica gel column chromatography and eluted with *n*-hexane, *n*-hexane–EtOAc, EtOAc, and EtOAc-MeOH in order of increasing polarity yielding 5 major fractions. Fraction 1 was further purified by silica gel column chromatography and eluted with *n*-hexane-EtOAc (95:5) to give nonacosanoic acid, 2-(4-hydroxyphenyl)ethyl ester (**11**, 25 mg) [28]. Similarly, *β*-sitosterol (**12**, 35 mg) [29] was purified from fraction 2, after elution with a mixture of *n*-hexane-EtOAc (90:10), and kigelinol (**13**, 25 mg) [30] and oleanolic acid (**14**, 35 mg) [22] were isolated from fraction 3 with *n*-hexane-EtOAc (80:20). Column chromatography of fraction 4 using *n*-hexane-EtOAc (75:25) as the eluent afforded *β*-friedelinol (**15**, 20 mg) [31]. Similarly, CC of fraction 5, eluted with a mixture of EtOAc-MeOH (95:5), gave pomolic acid (**16**, 15 mg) [32]. Part of the $H_2O$ extract (25 g) was subjected to silica gel column chromatography and eluted with EtOAc and EtOAc-MeOH in order of increasing polarity yielding 3 major fractions. Fraction 1 was further purified by silica gel column chromatography and eluted with EtOAc-MeOH (90:10) to give kojic acid (**17**, 100 mg) [33].

The structures of isolated compounds (**Figure 1**) were elucidated by means of spectroscopic experiments mainly 1D and 2D NMR and ESIMS / HRESIMS mass spectrometry and by comparison with literature data. To the best of our knowledge, compounds **1**, **2**, **3**, **5**, and compounds **11**, **14**, **15**, **16** are reported for the first time from *J. mimosifolia* and *K. africana*, respectively.




**1** **2**

| | R |
|---|---|
| **3** | $CO_2H$ |
| **4** | $CH_3$ |

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **5** | $CH_3$ | H | H |
| **14** | H | H | $CH_3$ |
| **16** | $CH_3$ | OH | H |

**6** **7** **8**

| | R |
|---|---|
| **9** | H |
| **10** | OH |

**11** **17** **12** **13** **15**

**Figure 1.** Chemical structures of compounds **1−5** isolated from *J. mimosifolia* and **6- 17** from *K. africana.*

*Anticandidal assessment:*

Four *Candida albicans* strains ATCCL26, ATCC12C, ATCCP37039, and ATCCP37037 were obtained from BEI Resources, NAID, and NIH. The disc-diffusion method was used to determine the inhibition zones of the tested compounds against the standard *C. albicans* strains [34].

The Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentrations (MFC) was determined according to National Committee for Clinical laboratory Standards (NCCLS) M27-A3 microdilution method [35].

The inhibition zone diameter values of the compounds are summarized in Table 1. Compounds **5, 10**, and **17** exhibited the highest inhibitions on *C. albicans* (ATCCL26, ATCCP37037, ATCCP37039 and ATCC12C strains). All compounds showed diameter zones ≥ 6 mm on the *C. albicans* ATCCL26. Compounds **2**, **3**, **4**, **8**, **9**, **10, 15,** and **17** showed diameter zones ≥ 6 mm on the four yeast strains while compounds **1** and **16** showed no inhibition zone diameter on *C. albicans* ATCC P37037, compound **13** showed no inhibition zone diameter on *C. albicans* ATCC P37039, and compounds **5** and **11** showed no inhibition zone diameter on *C. albicans* ATCC 12C. Compound **17** was likely the most active against the four *C. albicans* strains and is more active (9.0 ± 0.0 mm) than the antifungal reference Nystatin (8.0 ± 0.0 mm) on *C. albicans* ATCC P37039 and ATCC 12C.

The Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentrations (MFC) of the compounds are summarized in Tables 2 and 3. The MIC of the compounds tested ranged from 0.01 ± 0.00 − 0.83 ± 0.00 mg/mL with compound **17** having the best activity on the four *Candida albicans* strains. This activity (MIC= 0.01 ± 0.00 − 0.03 ± 0.00 mg/mL) is far better than that of the reference compound Nystatin (> 0.83 ± 0.00 mg/mL).

The anticandidal effects observed for these compounds may validate the use of *K. africana and J.mimosifolia* by many traditional healers to treat a wide range of infectious diseases like, fungal infections. However, the use of these compounds as anticandidal drugs needs further studies.

**Table 1.** Yeast inhibition zone diameters of compounds isolated from *J. mimosifolia* (**1-5**) and *K. africana* (**6-17**)

| Test Microorganism (*C. albicans*) | Inhibition zone diameter ± S.D[a](mm) Compounds | | | | | | | | | | | | | R.A[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1**[b] | **2**[b] | **3**[b] | **4**[b] | **5**[b] | **8**[c] | **9**[c] | **10**[c] | **11**[c] | **13**[c] | **15**[c] | **16**[c] | **17**[c] | Nyst |
| ATCCP37037 | 0.0±0.0 | 7.0±0.0 | 6.0±0.0 | 6.0±0.0 | 7.0±0.0 | 7.0±0.0 | 6.0±0.0 | 8.0±0.0 | 6.0±0.0 | 6.0±0.0 | 7.0±0.0 | 0.0±0.0 | 7.0±0.0 | 10.0±0.0 |
| ATCCP37039 | 8.0±0.0 | 8.0±0.0 | 6.0±0.0 | 6.0±0.0 | 7.0±0.0 | 7.0±0.0 | 7.0±0.0 | 7.0±0.0 | 6.0±0.0 | 0.0±0.0 | 8.0±0.0 | 6.0±0.0 | 9.0±0.0 | 8.0±0.0 |
| ATCCL26 | 7.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 10.0±0.0 | 6.0±0.0 | 7.0±0.0 | 7.0±0.0 | 6.0±0.0 | 6.0±0.0 | 7.0±0.0 | 7.0±0.0 | 7.0±0.0 | 10.0±0.0 |
| ATCC 12C | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 8.0±0.0 | 0.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 0.0±0.0 | 6.0±0.0 | 7.0±0.0 | 7.0±0.0 | 9.0±0.0 | 8.0±0.0 |

[a] Each experiment was performed three times, and the data were averaged ($n$ = 3). Values are means of three replication ± SD. [b] *J. mimosifolia* pure compounds: benzoic acid **1,** 1-naphthaleneacetic acid, 5-carboxy-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-[1S-(1α,2β,4aβ,8aα)] **2** , betulinic acid **3**, lupeol **4** , ursolic acid **5**; [c] *K. africana* pure compounds : lapachol **6,** dehydro-α-lapachone **7**, 2- acetylfuro-1, 4-naphthoquinone **8** , *p*-coumaric acid **9**, caffeic acid **10,** nonacosanoic acid, 2-(4-hydroxyphenyl)ethyl ester **11**, *β*-sitosterol **12**, kigelinol **13**, *β*-friedelinol **15**, pomolic acid **16** and kojic acid **17**, R.A: [d]RA (Reference antifungal: positive control) Nys: Nystatin.

**Table 2.** MIC value of pure compounds **3**, **5**, **9**, **10**, **11**, **15** and **17**

| Test Microorganism (*C. albicans*) | MIC ± S.D[a] (mg/mL) Compounds | | | | | | | R.A[d] |
|---|---|---|---|---|---|---|---|---|
| | **3**[b] | **5**[b] | **9**[c] | **10**[c] | **11**[c] | **15**[c] | **17**[c] | **Nys** |
| ATCCP37037 | >0.83±0.00 | >0.83±0.00 | 0.42±0.00 | 0.83±0.00 | >0.83±0.00 | >0.83±0.00 | 0.03±0.00 | >0.83±0.00 |
| ATCCP37039 | >0.83±0.00 | >0.83±0.00 | 0.21±0.00 | 0.83±0.00 | >0.83±0.00 | >0.83±0.00 | 0.01±0.00 | >0.83±0.00 |
| ATCCL26 | >0.83±0.00 | >0.83±0.00 | 0.05±0.00 | 0.41±0.00 | >0.83±0.00 | >0.83±0.00 | 0.01±0.00 | >0.83±0.00 |
| ATCC 12C | >0.83±0.00 | >0.83±0.00 | 0.10±0.00 | 0.83±0.00 | >0.83±0.00 | >0.83±0.00 | 0.01±0.00 | >0.83±0.00 |

[a] Each experiment was performed three times, and the data were averaged ($n$ = 3). Values are means of three replication ± SD. [b] *J. mimosifolia* pure compounds: betulinic acid **3**, ursolic acid **5**; [c] *K. africana* pure compounds *p*-coumaric acid **9**, caffeic acid **10,** nonacosanoic acid, 2-(4-hydroxyphenyl)ethyl ester **11**, *β*-friedelinol **15**, and kojic acid **17**; [d]R A (Reference antifungal: positive control) Nys: Nystatin.

**Table 3.** MFC value of pure compounds **3**, **5**, **9**, **10**, **11**, **15**, and **17**

| Test Microorganism (*C. albicans*) | MFC ± S.D[a] (mg/mL) Compounds | | | | | | | R.A[d] |
|---|---|---|---|---|---|---|---|---|
| | **3**[b] | **5**[b] | **9**[c] | **10**[c] | **11**[c] | **15**[c] | **17**[c] | **Nyst** |
| ATCCP37037 | >0.83±0.00 | >0.83±0.00 | >0.83±0.00 | 0.83±0.00 | >0.83±0.00 | >0.83±0.00 | 0.05±0.00 | >0.83±0.00 |
| ATCCP37039 | >0.83±0.00 | >0.83±0.00 | 0.10±0.00 | 0.83±0.00 | >0.83±0.00 | >0.83±0.00 | 0.10±0.00 | >0.83±0.00 |
| ATCCL26 | >0.83±0.00 | >0.83±0.00 | 0.83±0.00 | 0.83±0.00 | >0.83±0.00 | >0.83±0.00 | 0.03±0.00 | >0.83±0.00 |
| ATCC 12C | >0.83±0.00 | >0.83±0.00 | 0.83±0.00 | >0.83±0.00 | >0.83±0.00 | >0.83±0.00 | 0.10±0.00 | >0.83±0.00 |

[a] Each experiment was performed three times, and the data were averaged ($n$ = 3). Values are means of three replication ± SD. [b] *J. mimosifolia* pure compounds: betulinic acid **3**, ursolic acid **5**; [c] *K. africana* pure compounds *p*-coumaric acid **9**, caffeic acid **10,** nonacosanoic acid, 2-(4-hydroxyphenyl)ethyl ester **11**, *β*-friedelinol **15**, and kojic acid **17**; [d]R A (Reference antifungal: positive control) Nys: Nystatin.

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## Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

## References

[1] V. Gambaro, J.A. Garbarino, C. Galeffi, M. Nicoletti, I. Messana and G.B. Marini-Bettolo (1988). Constituents of *Jacaranda mimosaefolia*, *Rev. Latinoam. Quim.* **19**, 17– 19.

[2] G.H. Mahran, A.M. El-Fishawy, A.M.S. Hosny and A.M. Hilal (1991). Phytochemical and antimicrobial study of *Jacaranda mimosifolia* D. Don grown in Egypt, *Herb. Hung.* **30**, 98– 108.

[3] J. del Hoyo, A. Elliott and J. Sargatal (1997). Hand book of the Birds of the world (eds), *Lynx edicions* **4**, 415-420.

[4] M. Arbonnier (2009). Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. 3rd ed. Paris: MNHN Quæ Editions; p.192 ,

[5] P.J. Houghton, A. Photiou, S. Uddin, P. Shah, M. Browning, S.J. Jackson and S. Retsas (1994). Activity of extracts of *Kigelia pinnata* against melanoma and renal carcinoma cell lines, *Planta Med.***60**, 430-433.

[6] Y. G. Gouda, A. M. Abdel-Baky, F. M. Darwish, K. M. Mohamed, R. Kasai and K.Yamasaki (2003). Iridoids from *Kigelia pinnata* DC. fruits, *Phytochemistry* **63**, 887–892.

[7] S.M. El-Sayyad (1981). Flavonoids of the leaves and fruits of *Kigelia pinnata*, *Fitoterapia* 52,189–191.

[8] C.R. Weiss, S.V.K. Moideen, S.L. Croft, and P.J. Houghton (2000). Activity of extracts and isolated naphthoquinones from *Kigelia pinnata* against plasmodium falciparum, *J. Nat.Prod.* **63**,1306-1309.

[9] J. Bushra, R. Naheed, S. Muhammad, N. M. Akram, A. Maqsood, T.M. Nawaz, P. Gennaro, A. Muhammad, E.S. Abida, A. Irshad, A. Jabbar (2013). Isolation and characterization of limonoids from *Kigelia africana*. *Z. Naturforschung, B: J. Chem. Sc*. **68**, 1041-1048.

[10] M.R. Khan, S. M. Mlungwan (1999). γ-Sitosterol, a cytotoxic sterol from *Markhamia zanzibarica* and *Kigelia africana*. *Fitoterapia* **70**, 96-97.

[11] L. Prakash and G. Garg (1980). Chemical examination of the root barks of *Jacaranda mimosifolia* D. Don and *Tabebuia pentaphylla* (Linn) Hemsl, *Pharmazie.* **35**, 649.
[12] F.A. Moharram and M.S.A. Marzouk (2007). A novel phenylethanoid dimer and flavonoids from *Jacaranda mimosifolia, Z. Naturforsch B,* **62**, 1213-1220.
[13] A. Rana, S. Bhangalia and H.P. Singh (2013). A new phenylethanoid glucoside from *Jacaranda mimosifolia*, *Nat. Prod. Res.* **27**, 1167-1173.
[14] M. Salome, G.O. Kunert, M. Kaiser, R. Brun, R.A. Munoz, R. Bauer, W. Schuhly ( 2010). Jacaranone-Derived Glucosidic Esters from *Jacaranda glabra* and Their Activity against *Plasmodium falciparum* *J. Nat. Prod.* **73**, 553–556
[15] M.J. Chisholm, C.Y. Hopkins (1962). Isolation and structure of a new conjugated triene fatty acid. *J. Org. Chem* . **27**, 3137-9.
[16] M.E. Fernandez (1968). Anthocyanins from flowers of Jacaranda mimosifolia and Jacaranda semiserrata. Anales de la Asociacion Quimica Argentina 56, 135-137.
[17] M.T. Olaleye and J.B.T. Rocha (2008). Acetaminophen induced liver damage in mice: Effects of some medicinal plants on the oxidative defense system, *Exp. Toxicol. Pathol*. **59**, 319-327.
[18] M.T. Olalye and J.B.T. Rocha (2007). Commonly used tropical medicinal plants exhibit distinct in vitro antioxidant activities against hepatotoxins in rat liver, *Exp. Toxicol. Pathol*. **58**, 433-438.
[19] J.J. Rojas, V.J. Ochoa, S.A. Ocampo and J.F. Munoz (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections, *BMC Complementary and Alternative Medicine* **6**, 1-6.
[20] T. Getahun, P. Reneela and A. Dekebo (2012). Isolation and characterization of natural products from *Helinus mystacinus* (Rhamnaceae), *J. Chem. Pharm. Res.* **4**, 1756-1762.
[21] R. Aquino, M.L. Ciavatta, N. De Tommasi and E. Gacs-Baitz (1992). Tetranorditerpenes from *Detalium microcarpum*, *Phytochemistry* **31**, 1823-1825.
[22] Z. Guvenalp, N. Kilic, C. Kazaz,Y. Kaya and L.O. Demirezer (2006). Chemical Constituents of *Galium tortumense*, *Turk. J. Chem.* **30**, 515-523.
[23] Govindachari, S.J. Patankar, N. Viswanathan (1971). Isolation and structure of two new dihydroisocoumarins from *Kigelia pinnata*, *Phytochemistry*, **10**, 1603-1606.
[24] K.C. Joshi, L. Prakash, P. Singh (1973). Quinones and other constituents from *Tabebuia rosea*, *Phytochemistry*, **12**, 942–943.
[25] B.A. Dawson, M. Girard, D. Kindack, J. Fillion, D.V.C. Awang. (1989). $^{13}$C NMR of lapachol and some related naphthoquinones. *M. R. C.* **27,** 1176-1177.
[26] O.A. Binutu, K. Adesogan, J.I. Okogun (1997). Constituents of *Kigelia pinnata*, *Nig. J. Nat. Prod. Med*. 1-68.
[27] R. Dayal, T.R. Seshadri (1979). Chemical constituents of *Jacaranda mimosifolia* wood. *J. Ind. Chem. Soc.* 56, 1269-70.
[28] U. S. Kumar, A. K. Tiwari, S. V. Reddy, P. Aparna, R. J. Rao, A. Z. Ali, J. M. Rao (2005). Free-Radical-Scavenging and Xanthine Oxidase Inhibitory Constituents from *Stereospermum personatum*, *J. Nat. Prod*. **68**, 1615-1621.
[29] H.L. Holland, P.R. P. Diakow, G.J. Taylor (1978). 13C nuclear magnetic resonance spectra of some C-19-hydroxy, C-5,6 epoxy, C-24 ethyl, and C-19-norsteroids. *Can. J. Chem*. **56**, 3121-3127.
[30] D.N. Akunyili and P.J. Houghton (1993). Meroterpenoids and naphthoquinones from *Kigelia pinnata*, *Phytochemistry* **32**, 1015-1018.
[31] G.C.M. Salazar, G.D.F. Silva, L.P. Duarte, S.A. Vieira Filho and I.S. Lula (2000). Two epimeric friedelane triterpenes isolated from *Maytenus truncata* Reiss: $^{1}$H and $^{13}$C chemical shift assignments, *Magn. Reson. Chem.* **38**, 977–980.
[32] Tzong-Huei Lee, Shin-Hun Juang, Feng-Lin Hsu and Cheng-Yi Wu (2005). Triterpene acids from the leaves of *Planchonella duclitan* (Blanco) Bakhuizan, *J. Chin. Chem. Soc.* **52**, 1275-1280.
[33] D.C. Hurd, J.R. Sims and S. Trofimenko (1959). Anomalous reactions of kojic acid and related compounds with acrylonitrile and acrylic ester, *J. Am. Chem. Soc.* 81, 1684-1687
[34] NCCLS (2004). Method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline. NCCLS document M44-A. **24** [ISBN 1-56238-532-1]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.
[35] Clinical Laboratory Standard Institute (2008**).** Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard, third edition M27- A3.Wayne, PA: Clinical Laboratory Standard Institute.