

## **Isolation & Antimicrobial Screening of Ten Long Chain Aliphatic Compounds from *Psidium guajava* (Leaves)**

**B K Mehta<sup>1</sup>, Ritu Nigam\*<sup>1</sup>, Varsha Nigam<sup>1</sup> and Asheesh Singh<sup>2</sup>**

<sup>1</sup>*School of Studies in Chemistry and Biochemistry, Vikram University Ujjain, (MP), India*

<sup>2</sup>*Center for Research & Development, IPCA Laboratories, Sejavta Ratlam, (MP), India*

---

### **ABSTRACT**

For the millions of rural populations in countries of the developing world, diarrhoeal diseases continue to be the major cause of morbidity and mortality, with an estimated 1 billion episodes of illness and some 5 million or more deaths in children under 5 years (5-8). In such populations, preparations from herbs and plants remain the most common forms of treatment for diarrhoeal disease. Decoctions of the leaf, root or stem of the guava plant, *Psidium guajava* have been used as antidiarrhoeal therapy in many systems of traditional medicine in tropical countries. *Psidium guajava* Linn. is one of such medicinal plants. Belonging to the family Myrtaceae that is also used as a source of food. Ten new aliphatic compounds pentapentacont-17, 31-diol (**1**), 11-hydroxy-tricont-35-pentatriacontanoate (**2**), 34-octahexacontanol (**3**), heptatriacont-8-ol (**4**), 14,15-dimethyl (cyclopropyl)-9-ol-octadecyl-3-(4-hydroxyphenyl) propanoate (**5**), hexaeicosan-16-ol (**6**), pentatetracosan-10, 25-diol (**7**), untricontan-11, 19-diol (**8**), tricosan -17-ene-5-ol (**9**), and nonacosan-23-ene-3-ol (**10**) isolated from the ethanol extract of the leaves of *P. guajava*. All compounds exhibited moderate activity against *Staphylococcus aureus* and poor activity against *Shigella* spp and *Klebsiella pneumoniae*. The plant can be used for the formulation of oral antibacterial drugs to manage surgical, skin and soft tissue infections.

**Keywords :** *Psidium guajava*, leaves, Myrtaceae, aliphatic alcohol, antimicrobial activity.

---

### **INTRODUCTION**

*Psidium guajava*: (Myrtaceae), Commonly known as the poor man's apple of the Tropics has a long history of traditional use, much of which is being validated by scientific research.<sup>1</sup> The native home of the guava is all warm areas in the world especially tropical America, west India, Asia, Africa, and other subtropical countries. Guava is rich in tannins, phenols, flavanoids, essential oils, lectins, vitamins, fatty acids etc. Much of the guava's medicinal activity is attributed to these flavanoids. The flavanoids have demonstrated anti-bacterial activity<sup>1</sup>. Flowers are also mashed & applied to painful eye conditions such as sun strain, conjunctivitis or eye injuries. Commercially the fruit is consumed fresh or used in the making of Jams, Jellies, paste. Guava leaves are in the "Ducth-Pharmacopoea" for the treatment of Diarrhea. It is a medicinal plant used in various Ayurvedic preparations<sup>2</sup>. The different extracts of the plant have shown antibacterial activity, antimutagenic activity, antidiarrhoeal activity, antiamoebic activity, anticough activity, antihyperglycemic activity, antioxidant and anti-inflammatory activities<sup>3-5</sup>. The present work an assessment of the isolation, structural elucidation & antibacterial potential of leaves of Guava of ten new lipids (Figure1) isolated from leaves of *Psidium guajava* Linn.

## MATERIALS AND METHODS

**Plant material**

The leaves of *P. guajava* Linn were collected from the nearby area of Ujjain city, identified from IEMPS, Vikram University, Ujjain.

**General procedures**

<sup>1</sup>H NMR was recorded on the MHz Varian XL spectrometer and 400 MHz Bruker WM spectrometer, <sup>13</sup>C NMR spectra were recorded on Varian XL 75 MHz spectrometer. <sup>1</sup>H-<sup>1</sup>H COSY NMR was performed on the same spectrometer, using standard Varian pulse sequences. IR spectra were recorded in KBr discs on Perkin-Elmer-377 spectrometer, ESIMS on Jeol-JMS D 300 mass spectrometer. Chromatography was performed using alumina grade III for column and silica gel G for TLC. The purity of the compounds were checked by <sup>1</sup>H and <sup>13</sup>C NMR spectral analysis and TLC plate, revealed with vanillin (0.5 g) in H<sub>2</sub>SO<sub>4</sub>: EtOH(4:1).

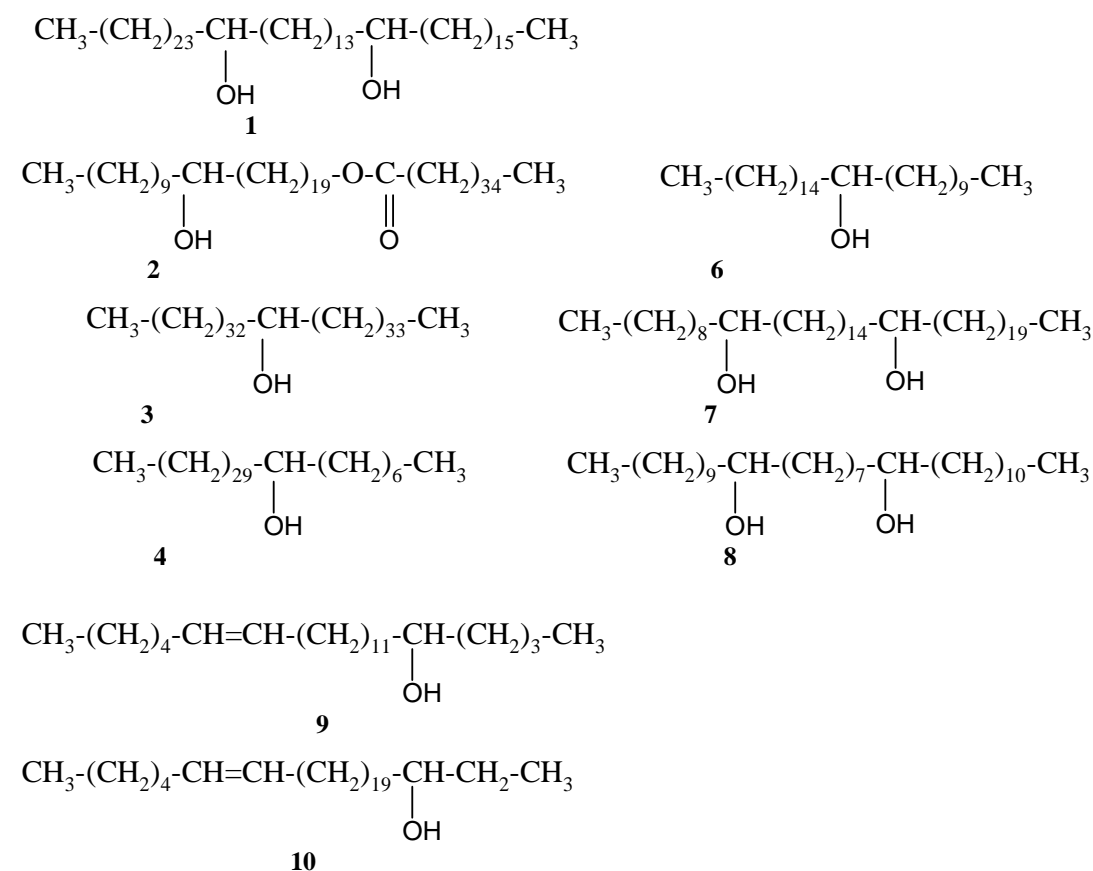


Figure-1 Chemical structures of isolated compounds 1-10

**Extraction and isolation of the constituents**

The leaves (8 kg) of *P. guajava* were shade dried, cleaned, coarse powdered and extracted with ethanol in soxhlet extractor for 72 h. The extract was concentrated by rotary evaporator to afford (3500 mL). Yielded (118 g) matter which was separated by repeated column chromatography on silica gel grade III. The column was eluted by gradient elution in increasing order of polarity. The fractions were collected in bulk and monitored by TLC. The residue (8.8 g) of benzene: acetone fraction was subjected to rechromatograph on silica gel on basis of increasing order of polarity of eluents. Fraction 8 (hexane: benzene, v/v 7:3) was purified and identified as compound (1), fraction 8 (benzene: methanol, v/v 9:1) was purified and identified as compound (2), fraction 9 (benzene: methanol, v/v 9:1) was purified and identified as compound (3), and compound (4) and fraction 10 (benzene: methanol, v/v 9:1) was

purified and identified as compound (5) respectively. The residue (5.4 g) of ethyl acetate fraction was subjected to rechromatograph on silica gel on basis of increasing order of polarity of eluents. Fraction 6-10 (hexane: benzene, v/v 2:3 and 3:2) were purified and identified as compounds (9) and (10) respectively. The residue (6.8 g) of ethanol fraction was subjected to rechromatograph on silica gel on basis of increasing order of polarity of eluents. Fraction 5-8 (hexane: benzene, v/v 7:3, 2:3 and 9:1) were purified and identified as compounds (6), (7), and (8) respectively. Presence of these acids were analyzed by IR, <sup>1</sup>H NMR and mass spectrometry, and compared with literature data.<sup>6-9</sup> chemical structure of isolated compounds were shown in figure 1.

### Screening of antimicrobial activity

The antimicrobial activity of the extracts was evaluated by the disc diffusion method described by BAUER *et al.*<sup>10-11</sup>. *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Shigella boydi*, *Salmonella typhimurium* and *Bacillus subtilis* were cultured on plates containing Nutrient agar, Cetrimide agar, SS agar and TSA agar respectively. The plates were incubated at 37°C for 24 hours. The results are being shown in Table-1.

**Table-1: Antimicrobial assays for the compounds 1-10**

Sr.No.	Antibacterial activity	1	2	3	4	5	6	7	8	9	10
1	<i>Staphylococcus aureus</i>	++	+++	++	++	++	+++	+++	++	+++	+++
2	<i>Shigella spp</i>	+	+	-	-	+	+	+	-	-	+
3	<i>Salmonella typhimurium</i>	-	-	-	-	-	-	-	-	-	+
4	<i>E. coli</i>	+	-	+	+	-	-	-	-	+	+
5	<i>Klebsiella pneumoniae</i>	+	-	+	+	-	+	-	+	-	+
6	<i>Pseudomonas aeruginosa</i>	-	-	-	-	+	-	-	-	+	-
7	<i>Bacillus subtilis</i>	+	-	+	-	-	+	-	-	-	-

Disc diameter = 4.0 mm; - = no activity; + = 6.8 - 8.0 mm; ++ = 9.0 - 11 mm; +++ = 12 - 14 mm; ++++ = 16 - 20 mm; +++++ = 18 - 24 mm. The standards are in the form of sterile Hi - Disc cartridges, each disc containing 10 mcg of the respective drug. For antifungal activity Amphotericin B was used as standard.

All compounds isolated from benzene: acetone fraction of *P. guajava* gave moderate activity against *Staphylococcus aureus*. All of compounds showed noticeable activity against *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. All compounds showed very poor activity and no activity against *Shigella spp*, *E. coli* and *Salmonella typhimurium*. The results are summarized in the following table (Table-1).

### Pentapentacont-17, 31-diol (1)

Mol formula: C<sub>55</sub>H<sub>112</sub>O<sub>2</sub>; MP: 90-92 °C; IR (KBr cm<sup>-1</sup>): 3458, 2918, 2849, 2360, 1469, 1166, 960, 719; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 0.88 (t, 6H, 2x -CH<sub>3</sub>), 1.5 (s, 2H, 2x -OH), 3.64 (t, 3H), 1.55 (t, 8H, 4x-CH<sub>2</sub>), 1.25 (94 H, s, 47x-CH<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>, TMS, PPM): 68.8, 66.0, 32.82, 31.84, 29.60, 29.36, 29.25, 19.9; ESIMS m/z: 804 [M<sup>+</sup>], 692, 550, 466, 436, 413, 393, 368, 337, 254, 115, 83.

### 11-hydroxy-tricont-35-pentatriacontanoate (2)

Mol formula: C<sub>66</sub>H<sub>132</sub>O<sub>3</sub>; MP: 197-198 °C; IR (KBr cm<sup>-1</sup>): 3467, 1730; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 4.03 (2H, t, -CH<sub>2</sub>-O-CO-, J 6.0 Hz), 2.30 (2H, t, -CH<sub>2</sub>-CO-O-, J 6.0 Hz), 1.56 (8H, m, 4x-CH<sub>2</sub>, β to ester group), 3.67 (m, 1H, -CHOH, J = 8.0 Hz), 1.25 (120H, s, 60x-CH<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>, TMS, PPM): 174.0, 68.06. ESIMS m/z: 972 [M<sup>+</sup>], 831, 804, 649, 551, 535, 496, 477, 437, 425, 323, 171, 141.

### 34-octahexacontanol (3)

Mol formula: C<sub>68</sub>H<sub>138</sub>O; MP: 155-156 °C; IR (KBr cm<sup>-1</sup>): 3454 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 3.67 (t, 3H, -CHOH). ESIMS m/z 970 [M<sup>+</sup>], 886, 826, 774, 773, 745, 731, 692, 641, 620, 589, 580, 559, 532, 495, 474, 437, 409, 390, 381, 353, 325, 279, 239, 225, 197, 196, 141, 84.

### Heptatriacont-8-ol (4)

Mol formula: C<sub>37</sub>H<sub>76</sub>O; MP: 160-161°C; IR (KBr cm<sup>-1</sup>): 3637, 2917, 239, 1463, 1376, 972, 719 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 0.85 (t, 6H, 2x -CH<sub>3</sub>, J = 7.5 Hz), 1.56 (brs, 1H, -OH), 3.67 (t, 3H, -CHOH), 1.26 (s, 68H, 34-CH<sub>2</sub>); ESIMS m/z: 536 [M<sup>+</sup>], 437, 353, 339, 311, 227, 199, 186 and 99.

### 14, 15-dimethyl (cyclopropyl)-9-ol-octadecacyl-3-(4-hydroxyphenyl) propanoate (5)

Mol formula: C<sub>30</sub>H<sub>50</sub>O<sub>4</sub>; MP 160 °C; IR (KBr cm<sup>-1</sup>): 3443, 2918, 2849, 1708, 1593, 1463, 1262, 1124, 937 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 0.91 (t, 3H, -CH<sub>3</sub>, J = 7.5 Hz), 1.53 (s, 1H, -OH), 3.57 (t, 1H, -CHOH, J = 8.0 Hz), 2.13 (t, 2H, -CH<sub>2</sub>-CO-O J = 7.0 Hz), 4.19 (t, 2H, -CH<sub>2</sub>-O-CO-), 6.86 (d, 2H, J = 7.1 Hz), 6.48 (d, 2H, J = 7.1 Hz),

2.66 (t, 2H,  $J = 6.6$  Hz), 1.25 (32H, s, 16x-CH<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>, TMS PPM): 174.0, 154.2, 137.8, 132.1, 123.5, 34.5, 32.0, 31.5, 29.8, 19.9. FAB  $m/z$ : 474 [M<sup>+</sup>], 431, 367, 307, 277, 207, 165, 121, 107 and 93.

#### **Hexaeicosan-16-ol (6)**

Mol formula: C<sub>26</sub>H<sub>54</sub>O; MP: 167-168 °C; IR (KBr cm<sup>-1</sup>): 3657, 2919, 2317, 1478, 1368, 986 and 719 cm<sup>-1</sup>. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 0.83 (t, 6H, 2x -CH<sub>3</sub>),

1.57(brs, 1H, OH), 3.68(t, 3H, -CHOH), 1.26 (brs rest of -CH<sub>2</sub>). EIMS  $m/z$ : 382 [M<sup>+</sup>], 365, 325, 297, 269, 242, 197, 169, 141, 99 and 57.

#### **Pentatetracosan-10, 25-diol (7)**

Mol formula: C<sub>45</sub>H<sub>92</sub>O<sub>2</sub>; MP: 171-173, IR (KBr cm<sup>-1</sup>): 3452, 2916, 2842, 1470, 1168, 960 and 716 cm<sup>-1</sup>. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 0.86(t, 6H, 2x -CH<sub>3</sub>), 1.5 (s, 2H, 2x -OH), 3.64 (t, 3H), 1.26(brs rest of -CH<sub>2</sub>). EIMS  $m/z$ : 664 [M<sup>+</sup>], 529, 485, 437, 413, 385, 343, 311, 301, 289, 247, 219, 202, 161, 159, and 149, 132.

#### **Untricontan-11, 19-diol (8)**

Mol formula: C<sub>30</sub>H<sub>62</sub>O<sub>2</sub>; MP: 210-211 °C; IR (KBr cm<sup>-1</sup>): 3534, 2914, 2843, 1489, 1170 and 716 cm<sup>-1</sup>. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 3.61(1H, t, -CHOH,  $J$  8.0 Hz), 5.04(4H, t, 2x -CH = CH); 0.86(6H, t, 2x -CH<sub>3</sub>), 1.56(1H, s, -OH); EIMS  $m/z$ : 454 [M<sup>+</sup>], 487, 415, 391, 375, 353, 331, 317, 316, 311, 301, 289, 279, 261, 247, 231, 221, 202, 187, 175, 172, 160, 148, 134, 126, 118, 116 and 113.

#### **Tricosan-17-ene-5-ol (9)**

Mol formula: C<sub>23</sub>H<sub>46</sub>O; MP 60 °C, IR (KBr cm<sup>-1</sup>): 3418, 2921, 2849, 1626, 1468, 1353, 1062, 767, 723 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 3.64 (1H, t, -CHOH,  $J$  8.0 Hz), 5.02 (4H, t, 2x -CH = CH); 0.88 (6H, t, 2x -CH<sub>3</sub>), 1.59 (1H, s, -OH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz, PPM): 118.0, 70.1, 31.9, 29.6 - 29.1 merged, 25.1, 22.2, 19.9, 14.1. FAB  $m/z$ : 338 [M<sup>+</sup>] (2.2), 293 (7.8), 281 (7.3), 231 (5.5), 195 (3.5), 153 (19.3), 97 (100), 83 (60.1), 57 (40.2).

#### **Nonacosan-23-ene-3-ol (10)**

Mol formula: C<sub>29</sub>H<sub>58</sub>O; MP: 60°C; IR (KBr cm<sup>-1</sup>): 3429, 2920, 2848, 1626, 1468, 1352, 1122, 1063, 767, 722 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>, TMS): 3.60(1H, m, -CHOH,  $J$  8.0 Hz), 5.02 (4H, t, 2x -CH = CH); 0.85(6H, t, 2x -CH<sub>3</sub>), 1.56(1H, s, -OH), 1.26(brs rest of -CH<sub>2</sub>). FAB MS  $m/z$ : 422 [M<sup>+</sup> - 2H] (2.2), 364 (7.8), 338 (7.3), 323 (5.5), 250 (3.5), 169 (19.3), 97 (100), 83 (60.1).

## RESULTS AND DISCUSSION

The compounds Pentapentacont-17,31-diol (**1**), 11-hydroxy-tricont-35-pentatriacontanoate (**2**), 34-octahexacontanol (**3**), heptatriacont-8-ol(**4**), 14,15-dimethyl(cyclopropyl)-9-ol-octadecyl-3-(4-hydroxyphenyl) propanoate (**5**), hexaeicosan-16-ol (**6**), pentatetracosan-10, 25-diol (**7**), untricontan-11, 19-diol (**8**), tricosan -17-ene-5-ol (**9**) and nonacosan-23-ene-3-ol (**10**) were identified as part of the lipid structures by analysis of IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR (DEPT) and mass spectral analysis and comparison with <sup>1</sup>H NMR and <sup>13</sup>C NMR data of literature.

The mass spectrum and elemental analysis of penta penta cont-17, 31-diol (**1**) indicated the molecular ion peak at  $m/z$  804 suggesting its molecular formula C<sub>55</sub>H<sub>112</sub>O<sub>2</sub>. IR spectrum showed absorption bands for hydroxyl group (3458 cm<sup>-1</sup>) and long chain aliphatic nature (1166, 719 cm<sup>-1</sup>). <sup>1</sup>H NMR spectrum showed a triplet at  $\delta$  0.88 for six protons was due to terminal methyl groups present at terminal position, while a triplet at  $\delta$  3.64 for three protons was due to carbinolic proton (-CHOH). The methylene protons adjacent to carbinolic (-CH<sub>2</sub>-CHOH) moiety were resonated as triplet at  $\delta$  2.36. A doublet at  $\delta$  1.55 was due to the presence of hydroxyl proton in hydroxyl group. Rests of the methylenes were resonated at  $\delta$  1.25 as an intense singlet.

The <sup>13</sup>C NMR spectrum showed the presence of terminal methyl carbon at 19.9 ppm. The hydroxyl carbons at C-17 and C-31 were resonated at 66.0 and 68.8 ppm respectively. The methylene carbons,  $\alpha$ - and  $\beta$ - to hydroxyl group were resonated at 32.82 and 31.84 ppm respectively and rest of the methylene carbons were resonated at 29.8 ppm. The coupling exhibited in the COSY spectrum between hydrogen at  $\delta$  2.36 and cross peak  $\delta$  1.5, showed connectivity between methylene and  $\beta$ -CH<sub>2</sub>- group in the molecule. The base peak at  $m/z$  413(-Na<sup>+</sup>) was due to  $\alpha$ -cleavage to the hydroxyl group (C-31) and abundant peak at  $m/z$  277(-Na<sup>+</sup>) was due to  $\alpha$ -cleavage to other hydroxyl

group (C-17). The other abundant peaks at  $m/z$  692, 561, 529, 498, 485, 441, 393, 301, 236, 149, 115 and 83 were in agreement with the proposed structure.

Agar diffusion technique was used for the screening of antimicrobial activity. The results are shown that the compounds 2, 6, 9 & 10 exhibited moderate activity against *Staphylococcus aureus* and compounds 1, 3, 4, 5, 7 and 8 poor activities against *shigella spp.* and *K. pneumoniae* (Table-1).

### CONCLUSION

In conclusion, the result obtained for this study shows that *P. guajava* leave extracts have antimicrobial activity against bacteria that commonly cause surgical wound, skin other soft tissue infections. Further characterizations of active components of *p. guajava* leaves for antibacterial potential of leaves. This study has provided a scientific basis on the use of crude extracts of guava in herbal medicine. Its full potential in the pharmaceutical Industries is however dependent on the full identification, purification and characterization of the biologically active components in the plant.

### Acknowledgments

Authors are grateful to RSIC, CDRI, Lucknow and RSIC; IIT Bombay, Mumbai for spectral analysis and UGC, New Delhi for financial assistance.

### REFERENCES

- [1] Jaiarj. P, Khoohaswan. P, Wongkrajang. Y, *et al. J. Ethnopharmacol.* **1999**, 67, 203-212.
- [2] Ismail. M, Minhas. P.S, Khanum. F, *et al. Int J of Res Pharma and Bio Sci.* **2012**, 3, 1-2.
- [3] Gnan. S. O, Demella, M. T, *J. Ethnopharmacol.* **1999**, 68, 103-108.
- [4] Matsuo. T, Hanamura. N, Shimoi. K, *et al. Phytochem.* **1994**, 36, 1027-1029.
- [5] Jinic. H, Hersh, T, *Physicians Guide to the Etiology and Treatment of Diarrhoea.* **1982**, 523-526.
- [6] Tona. L, Kambu. K, Ngimbi. N, *et al. J. Ethnopharmacol.* **1998**, 61, 57-65.
- [7] Jackmann. L. M, Sternhell. S, *Application of Nuclear Magnetic Resonance Spectroscopy in Chemistry*, (Pergmon Press, New York), 1969.
- [8] Bernhard. K, *Helv. Chim. Acta.* **1948**, 31, 977.
- [9] Crombie. L, *J. Chem. Soc.* **1962**, 2449.
- [10] Kabalka. G. W, *J. Org. Chem.* **1975**, 40, 1776.
- [11] Bauer. A.W, Kirby. W.M.M, Sherris. J.C, *et al. Amer. J. Clin. Path.* **1966**, 36: 493-496.