



## Phytochemical investigation of the roots of *Grewia microcos* Linn.

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### ABSTRACT

The present study reports the phytochemical investigation of the roots of *Grewia microcos* linn. Seven Phytoconstituents have been reported namely 9,12- octadecadienoic acid; Ursolic acid; Stigmasterol; 6,4-dihydroxy-3-propen chalcone; Dioctyl phthalate; N-methyl-6- $\beta$ -(1',3',5'-trienyl)-3- $\beta$ -methoxy-3- $\beta$ -methyl piperidine; and Dibutyl phthalate have been reported for the first time from the roots of *Grewia microcos*.

**Keywords:** *Grewia microcos*, Phytoconstituents, Ursolic acid, Dibutyl phthalate.

### INTRODUCTION

*Grewia microcos* is a small semi-deciduous tree, sometimes shrubby up to 50 feet high and 5 feet girth, belonging to the family Tiliaceae[1]. The plant is also known as *Microcos paniculata* [2]. It is found in North eastern parts of India, Western Ghats and Andaman islands. Flowers are in terminal panicles and auxiliary towards the apex, is the distinguishing character of this plant[3]. Boiled leaves along with turmeric and shell of snail are taken for the treatment of Jaundice. Traditionally it is used to improve digestion and is also for used for cold, hepatitis, diarrhea, heat stroke, dyspepsia, typhoid fever, syphilitic ulceration of the mouth, small pox, eczema and itches [4,5].

Literature survey revealed that, the stem bark of *Grewia microcos* contained a new alkaloid, N-methyl-6- $\beta$ -(deca-1',3',5'-trienyl)-3- $\beta$ -methoxy-2-methylpiperidine which showed good insecticidal activity against *Aedes aegypti* second instar larve[6,7]. Two new piperidine alkaloids microcosamine A and B were isolated from the leaves, showed significant larvicidal activity against *Culex quinquefasciatus*[8]. A new triterpene named methyl -3-o-p-hydroxy-cinnamoyloxy-2- $\alpha$ , 23-dihydroxyolean-12-en-28-oate, epicatechin, 3-trans-feruloyl masilinic acid and masilinic acid were identified from the stem bark[9]. Analgesic and cytotoxic activity of leaves extract were also reported[10].

### EXPERIMENTAL SECTION

All the melting points were recorded in Bio Technics India, Model No. BT2-38 melting point apparatus and were uncorrected. IR spectra of the compounds were recorded using KBr pellet method on Bruker  $\alpha$ -T Spectrophotometer, at National Facility for Clinical Trials, ISISM Chennai. <sup>1</sup>HNMR spectra and LC MS spectra of the compounds were taken on Bruker 500 MHz PMR Spectrophotometer using CDCl<sub>3</sub> as solvent and LC-MS Shimadzu LC 2020 at National Facility for Clinical Trials, ISISM Chennai. ESI-MS spectra were recorded using ESI-MS Expression CMS Advion at SynZeal Research Laboratory, Ahmedabad. TLC was carried out using Aluchrosep Silica Gel 60/UV<sub>254</sub> from S. D. Fine Chemicals Pvt. Ltd, Mumbai. Column Chromatography was carried out using glass column with stopcock, 30 x 600 mm from Merck Specialities Pvt. Ltd, Mumbai packed with Silica Gel (200-400 mesh) from Molychem, Mumbai. All the Chemicals and Reagents used were obtained in high purity either from S. D. Fine Chemicals Pvt. Ltd, Molychem and Chemport Pvt. Ltd, Mumbai.

**Authentication and Collection of the Plant Material**

The roots of *Grewia microcos* was collected from Dhavali, Ponda –Goa during the month of October 2012. It was authenticated by Prof. G.I. Hukkeri, Department of Botany, Dhempe College of Arts and Science, Miramar-Goa [3].

**Preparation of Ethanolic extract**

The roots were collected, washed and dried in shade. The dried roots were then powdered (500gm) and exhaustively extracted by maceration with ethanol (95%) for three days. After three days, the ethanolic layer was decanted off. The process was repeated thrice. The solvent from the total extract was distilled off using Rotary vacuum evaporator (Superfit) and then evaporated to dryness (45g) [11].

**Preliminary Phytochemical analysis**

A preliminary phytochemical screening was carried out using the ethanolic extract of the roots by employing the standard procedures[12, 13].

## 1. Alkaloids

• *Dragendroff's Test:*

To 2mg of the methanolic extract, 5ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1ml of Dragendroff's reagent was added. Formation of orange or orange-red precipitate indicated the presence of alkaloids.

• *Mayer's Test:*

To 2mg of the methanolic extract, a few drops of Mayer's reagent was added. Formation of white or pale yellow precipitate indicated the presence of alkaloids.

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• *Wagner's Test:*

To 2mg of the methanolic extract, 1ml of Hydrochloric acid was added along with few drops of Wagner's reagent. A yellow or brown precipitate indicated the presence of alkaloids.

• *Hager's Test:*

To 2mg of the methanolic extract, was taken in a test tube, a few drops of Hager's reagent was added. Formation of yellow precipitate confirmed the presence of alkaloids.

## 2. Carbohydrates

• *Molish's Test*

In a test tube containing 2ml of the extract, 2 drops of freshly prepared 20% alcoholic solution of  $\alpha$ -naphthol was added. 2ml of conc. Sulphuric acid was added so as to form a layer below the mixture. Red –violet ring appeared, indicating the presence of carbohydrates, which disappeared on the addition of excess of alkali.

• *Benedict's Test*

To 0.5ml of the extract, 5ml of Benedict's solution was added and boiled for 5 minutes. Formation of the brick red colored precipitate indicated the presence of carbohydrates.

• *Fehling's Test*

To 2ml of the extract, 1ml mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes. Formation of the brick red colored precipitate indicated the presence of carbohydrates.

## 3. Flavanoids

• *Shinoda Test*

In a test tube containing 0.5ml of the extract, 10 drops of Hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish, or brown color indicated the presence of flavonoids.

• *Lead acetate Test*

To 2ml of plant extract add 1ml of Lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

• *Vanillin HCl Test*

Vanillin HCl was added to the alcoholic solution of drug, formation of pink color shows the presence of flavonoids.

## 4. Triterpenoids &amp; Steroids

• *Liebermann –Burchard's*

2mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was added along the sides of the test tube. A brown colored ring is formed at the junction of two layers and the upper layer turns green which shows the presence indicated the presence of steroids and formation of deep red color indicates presence of triterpenoids.

• *Salkowshi Test*

Treat the extract with few drops of conc. Sulphuric acid, red color in the lower layer indicates presence of steroids and formation of yellow colored lower layer the indicates the presence of triterpenoids.

## 5. Tannins and Phenolic compounds

• To 1-2ml of the extract, few drops of 5% w/v  $\text{FeCl}_3$  solution were added. A green color indicated the presence of gallotannins, white brown color indicated the presence of pseudotannins.

• To 1-2ml of the extract add lead acetate was added. White precipitate indicated the presence of tannins and phenolic compounds.

## 6. Resins

1ml of the extract was dissolved in acetone and the solution was poured in distilled water. Turbidity indicated the presence of resins.

## 7. Proteins

• *Biuret's Test*

To 1ml of hot extract, 5-8 drops of 10% w/v of sodium hydroxide solution, followed by 1 to 2 drops of 3% w/v of copper sulphate solution were added. Formation of violet red color indicated the presence of proteins.

• *Millon's Test*

1ml of the extract was dissolved in 1ml of distilled water and 5-6 drops of millon's reagent were added. Formation of white precipitate, which turns red on heating, indicated the presence of proteins.

## 8. Glycosides

Determine free sugar content of the extract. Hydrolyze the extract with mineral acids (dil. HCl/dil.  $\text{H}_2\text{SO}_4$ ). Again determine the total sugar content of hydrolyzed extract. Increase in sugar content indicates the presence of glycosides in the extract.

## • Test for cardiac glycosides

i. *Baljet's test*

A thick section shows yellow to orange color with sodium picrate.

ii. *Legal's Test*

To aqueous or alcoholic extract, add 1ml pyridine and 1ml sodium nitropruside. Pink to red color appears.

## iii. Test for deoxysugars(killer –killani test)

To 2ml extract add glacial acetic acid, one drop 5%  $\text{FeCl}_3$  and conc.  $\text{H}_2\text{SO}_4$ . Reddish brown color appears at the junction of the two liquid layers and upper layer appears bluish green.

iv. *Liebermann's test (test for bufadienoloids)*

Mix 3ml extract with 3ml acetic anhydride. Heat and cool. Add few drops conc.  $\text{H}_2\text{SO}_4$ . Blue color appears.

## • Test For Anthraquinone Glycosides

i. *Borntrager's test for Anthraquinone glycosides*

To 3ml extract add 5ml 5% dil.  $\text{H}_2\text{SO}_4$ . Boil and filter. To cold filtrate add equal volume of benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer turns pink or red.

ii. *Modified Borntrager's test for C-glycosides*

To 3ml extract add 5ml of 5% dil. HCl. Heat for 5minutes in boiling water and cool. To cold filtrate add equal volume of benzene or organic solvent. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer turns pink or red.

## • Test For Saponins

i. *Foam test*

Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

• Test For Coumarin Glycosides

i. *Fluorescence test*

Take moistened powder in a test tube. Cover test tube with filter paper soaked in dilute NaOH. Keep in water bath. After some time expose filter paper to UV light. It shows yellowish-green fluorescence.

ii. *FeCl<sub>3</sub> Test*

To the concentrated alcoholic extract of drug few drops of alcoholic FeCl<sub>3</sub> solution was added. Formation of deep green color, which turned yellow on addition of conc. HNO<sub>3</sub> indicates the presence of coumarins.

9. Starch

0.01gms of Iodine and 0.075gms of KI were dissolved in 5ml of distilled water and 2-3ml of extract was added. Formation of blue color indicated the presence of starch.

### Isolation of constituents

The ethanolic extract (12 gms) was mixed with silica gel (2 gms). After mixing, the sample was loaded on column packed with 160 gms of silica –gel (240-400 Mesh ) prepared in Pet. Ether. The column was subjected to different solvent systems, starting with 100 % Pet. Ether (60-80<sup>0</sup>C) followed by graded mixtures of Pet. Ether (60-80<sup>0</sup>C) : CHCl<sub>3</sub> ( 95:5, 90:10, 80:20, 70:30, 60:40, 50:50); CHCl<sub>3</sub> 100% followed by graded mixtures of CHCl<sub>3</sub> : EtOAc. (95:5, 90:10, 80:20, 70:30, 60:40, 50:50); EtOAc. (100%) and finally with graded mixtures of EtOAc. : Methanol (99:1, 98:2, 97:3, 96:4, 95:5) [11].

The Elutions were monitored by TLC (Silica gel-G; visualization by UV 254nm, 366nm and Vanillin-Sulphuric acid spraying reagent heated at 110<sup>0</sup>C). Each time 10 ml elutes were collected and identical elutes were combined (TLC monitored) and concentrated to 5ml and kept aside. Elutions carried out with graded mixture of Pet. Ether(60-80<sup>0</sup>C) : CHCl<sub>3</sub> (80:20) resulted a single component on TLC (Pet. Ether(60-80<sup>0</sup>C) : CHCl<sub>3</sub> 80:20). After removing the solvent yellow oily liquid resulted, which was designated as Compound I (160mg).Elutions carried out with CHCl<sub>3</sub> 100% resulted a single component on on TLC (CHCl<sub>3</sub> 100%). After removing the solvent yellow powder resulted, which was designated as Compound II (100 mg).Elutions carried out with CHCl<sub>3</sub> : EtOAc. (80:20) resulted a single component on TLC (CHCl<sub>3</sub> : EtOAc. 80:20). After removing the solvent pale yellow crystalline powder resulted, which was designated as Compound III (110 mg).Elutions carried out with CHCl<sub>3</sub> : EtOAc. (50:50) resulted a single component on TLC (CHCl<sub>3</sub> : EtOAc. 50:50). After removing the solvent white powder resulted, which was designated as Compound IV (95 mg).Elutions carried out with EtOAc. : Methanol(99:1) resulted a single component on TLC (EtOAc. : Methanol 99:1). After removing the solvent yellow liquid resulted, which was designated as Compound V (125 mg). Elutions carried out with EtOAc. : Methanol(98:2) resulted a single component on TLC (EtOAc. : Methanol 98:2). After removing the solvent viscous brown liquid resulted, which was designated as Compound VI (130 mg). Elutions carried out with EtOAc. : Methanol(95:5) resulted a single component on TLC (EtOAc. : Methanol 95:5). After removing the solvent pale yellow liquid resulted, which was designated as Compound VII (140 mg).Elutions carried out with other graded mixtures resulted in brown resinous mass which were not processed further[11] .

## RESULTS AND DISCUSSION

Phytochemical screening of the ethanolic extract of *G.microcos* led to the presence of phytoconstituents like alkaloids, carbohydrates, flavonoids, steroids, triterpenoids, tannins, and saponins.

The chemical investigation led to the isolation of seven compounds from the ethanolic extract of the roots of *G. microcos*. The isolated compounds are 9,12- Octadecadienoic acid; Ursolic acid; Stigmasterol; 6,4-dihydroxy-3-propen chalcone; Dioctyl phthalate; N-methyl-6-β-(1',3',5'-trienyl)-3-β-methoxyl-3-β-methyl piperidine; &Dibutyl phthalate.

**Compound I (9, 12- Octadecadienoic Acid):** b.p. 229<sup>0</sup>C (lit. 229-230<sup>0</sup>C);IR (KBr): 3369.69 cm<sup>-1</sup> (br, OH), 2926.13 cm<sup>-1</sup> (C-H str. inCH<sub>3</sub>), 2858.69 cm<sup>-1</sup> (C-H str. in CH<sub>2</sub>), 1727.47 cm<sup>-1</sup> (C=O str. ofCOOH), 1458.23 cm<sup>-1</sup> (C-H def. in CH<sub>3</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 0.866 - δ 0.896 (m, 3H, H-18) terminal methyl group, δ 0.907 - δ 0.960 (m, 14H, H-4, 5, 6, 7, 15, 16, 17), δ 1.687 (s, 2H, H-3) , δ 2.287 (s, 4H, H-8, 14) δ 2.301 (s, 2H, H-2), δ 2.316 (s, 2H, H-11), δ 5.348 (d, 4H, H-9, 10, 12, 13) Vinylic proton. The ESI-MS spectrum displayed the molecular ion peak at m/z 280.5 corresponding to the molecular formula C<sub>18</sub>H<sub>32</sub>O<sub>2</sub>.

Table no. 1: Result of the qualitative tests for Phyto-constituents of the ethanolic extract of the roots of *G. microcos*.

Sr. No.	Tests	Inference
1.	<b>ALKALOIDS</b> a) Dragendroff's Test b) Hayer's Test c) Wagner's Test d) Mayer's Test	+ve +ve +ve +ve
2.	<b>GLYCOSIDES</b> Test for Cardiac Glycosides a) Baljet Test b) Legal Test c) Keller-Killani's Test Test for Anthraquinone Glycosides a) Borntrager's Test b) Modified Borntrager's Test	-ve -ve -ve -ve -ve
3.	<b>CARBOHYDRATES</b> a) Molish's Test b) Fehlings Test c) Benedicts Test	+ve +ve +ve
4.	<b>FLAVONOIDS</b> a) Shinoda's Test b) Vanillin HCl Test	+ve +ve
5.	<b>PROTEINS</b> a) Biuret's Test b) Millon's Test	-ve -ve
6.	<b>TANNINS</b>	+ve
7.	<b>RESINS</b>	-ve
8.	<b>SAPONINS</b>	+ve
9.	<b>TRITERPENOIDS</b> a) Libermann-Burchard's Test	+ve
10.	<b>STEROIDS</b> a) Libermann-Burchard's Test b) Salkowshi Reaction	+ve +ve
11.	<b>STARCH</b>	-ve

+ = Present , - = Absent

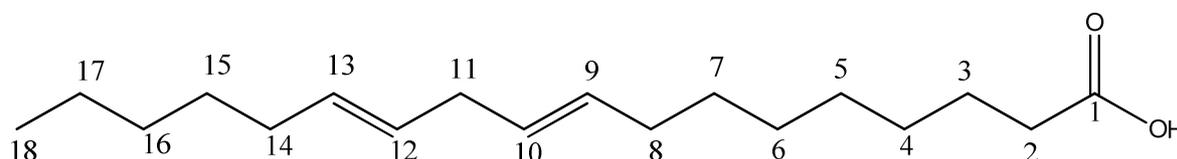


Fig. 1: 19,12-Octadecadienoic acid

**Compound II (Ursolic Acid):** m.p. 287<sup>0</sup>C. IR (KBr): 3429.35 cm<sup>-1</sup> (br, OH), 2926.03 cm<sup>-1</sup> (C-H str. in CH<sub>3</sub>), 2859.85 cm<sup>-1</sup> (C-H str. in CH<sub>2</sub>), 1728.33 cm<sup>-1</sup> (C=O str. of COOH), 1604.24 cm<sup>-1</sup> (C=C str), 1457.64 cm<sup>-1</sup> (C-H def. in CH<sub>3</sub>), 1379.11 cm<sup>-1</sup> (C-H def. in gem dimethyl). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) : δ 0.856 - δ 0.894 (m, 21H, 7x CH<sub>3</sub>), δ 1.259 - δ 1.705 (m, 18H, 9x CH<sub>2</sub>), δ 2.144 - δ 2.193 (m, 1H, OH), δ 2.301 - δ 2.377 (m, 1H, H-18), δ 2.435 - δ 2.594 (m, 4H, methine protons), δ 3.545 - δ 3.574 (m, 1H, H-3), δ 5.341 - (s, 1H, Vinylic proton). The LC-MS spectral data of the compound showed the molecular ion peak at m/z 456.20[M<sup>+</sup>] and the base peak m/z 248.10 along with a strong absorption peak at m/z 203.5 due to Retro-Diel-Alder fragmentation, typical for Δ<sup>12</sup>-oleanene or ursine triterpene with molecular formula C<sub>30</sub>H<sub>48</sub>O<sub>3</sub>[14].

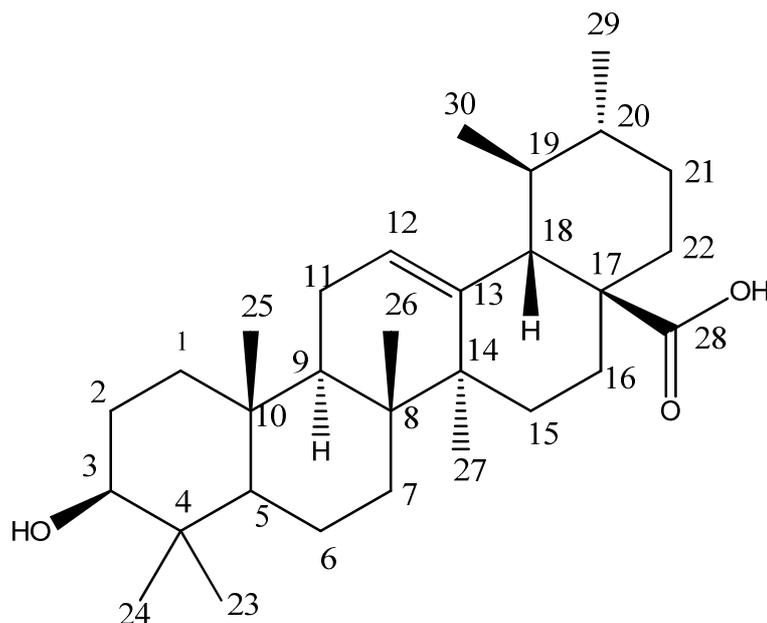


Fig. 2 Ursolic acid

Compound III (Stigmasterol): m.p. 167<sup>0</sup>C. IR (KBr): 3429.35 cm<sup>-1</sup> (br, OH), 2926.03 (C-H str. in CH<sub>2</sub>), 1604.24 cm<sup>-1</sup> (C=C str.), 1457.64 cm<sup>-1</sup> (C-H def. in CH<sub>3</sub>), 1072.76 cm<sup>-1</sup> (C-O str. in 2<sup>o</sup> alc.). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 0.831- δ 0.848 (t, 9H, H-18, 26, 27), δ 0.854 (s, 3H, H-29), δ 0.940 - δ 0.943 (d, 6H, H-19, 21), δ 1.254 - δ 1.728 (s, 18H, 9 x CH<sub>2</sub>, H- 1, 2, 4, 7, 11, 12, 15, 16, 28 and 7H, H-6, 8, 9, 14, 17, 20, 24, 25 methine protons), δ 2.014-δ 2.039 (m, 1H, OH-3), δ 3.657 (s, 1H, H-3), δ 5.338-δ 5.340 (t, 3H, H-6, 22, 23 Vinylic proton). The LC-MS spectrum showed the molecular ion peak at m/z 413.25 [M+H<sup>+</sup>] which was consistent with the molecular formula C<sub>29</sub>H<sub>48</sub>O [15].

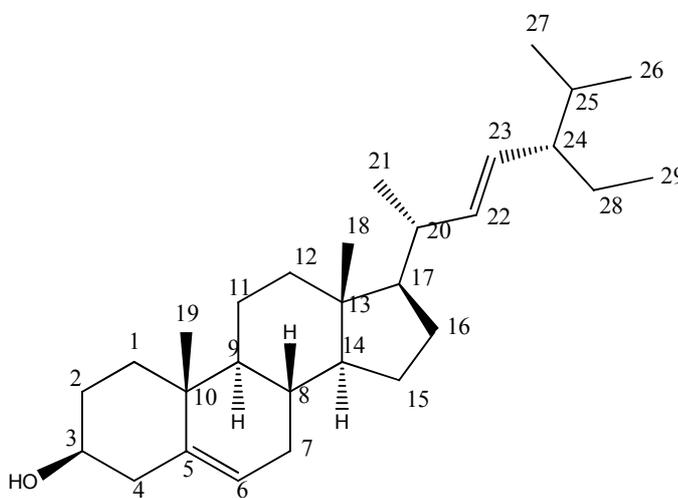


Fig. 3 Stigmasterol

**Compound IV (6, 4-dihydroxy-3-propen chalcone):** IR (KBr): 3408.15 cm<sup>-1</sup> (br, OH), 2926.21 cm<sup>-1</sup> (C-H str. Of CH<sub>3</sub>), 1728.06 cm<sup>-1</sup> (C=O str.), 1600.33 cm<sup>-1</sup> (C=C str. ), 1072.23 cm<sup>-1</sup> (C-O str.). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 1.201 - δ 1.296 (m, 3H, H-9'), δ 4.289 (s, 1H, H-8'), δ 4.316 (s, 1H, H-7'), δ 5.007 (d, 2H, OH-4', 6), δ 6.683 (t, 1H, H-5), δ 6.712 (t, 1H, H-4), δ 7.161 - δ 7.193 (m, 2H, H-3, H-5'), δ 7.505 - δ 7.519 (m, 2H, H-2', 6'), δ 7.692 (s, 1H, H-2), δ 7.703 (t, 2H, H-∞, β). The ESI-MS spectrum of the compound showed the presence of molecular ion peak at m/z 280.2 [M<sup>+</sup>] which was consistent with the molecular formula C<sub>18</sub>H<sub>16</sub>O<sub>1</sub>. The peak obtained at 279.2 was due to proton migration. The compound under goes α- cleavage from carbonyl to produce a base peak at 149.1 (C<sub>9</sub>H<sub>9</sub>O<sub>2</sub>)[16].



**Compound AS VII (Dibutyl phthalate):** b.p. 337<sup>0</sup>C. IR (KBr): 2926.13 cm<sup>-1</sup> (C-H str. in CH<sub>3</sub>), 2858.69 cm<sup>-1</sup> (C-H str. in CH<sub>2</sub>), 1727.47 cm<sup>-1</sup> (C=O str.), 1604.24 cm<sup>-1</sup> (C=Cstr.), 1073.09 cm<sup>-1</sup> (C-O str.), 744.44 cm<sup>-1</sup> (C-H def. in aromatic ring). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 0.866 - δ 0.960 (m, 6H, H-4', H-4''), δ 1.258 (s, 4H, H-3', H-3''), δ 1.718 (s, 4H, H-2', H-2''), δ 4.306 (t, 4H, H-1', H-1''), δ 7.514 - δ 7.525 (t, 2H, H-4, H-5), δ 7.704 - δ 7.772 (dd, 2H, H-3, H-6). The ESI-MS spectra showed molecular ion peak at m/z 279.4 [M+H]<sup>+</sup> in positive ion mode which was consistent with the molecular formula C<sub>16</sub>H<sub>22</sub>O<sub>4</sub>. The mass spectrum showed other fragments at 167.2 and 149.2 which are considered characteristic of alkyl phthalates. The fragment ion at 223.4 suggested that the alkyl phthalate is Dibutyl phthalate. The base peak at 149.0 is due to the protonated phthalic anhydride (C<sub>8</sub>H<sub>5</sub>O<sub>3</sub>) [18].

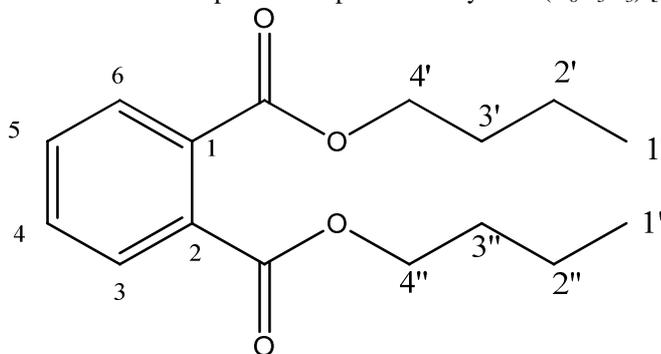


Fig. 7: Dibutyl phthalate

### CONCLUSION

The chemical investigation led to the isolation of seven compounds from the ethanolic extract of the roots of *G. microcos*. The isolated compounds are 9,12- Octadecadienoic acid; Ursolic acid; Stigmasterol; 6,4-dihydroxy-3-propen chalcone; Dioctyl phthalate; N-methyl-6-β-(1',3',5'-trienyl)-3-β-methoxyl-3-β-methyl piperidine; & Dibutyl phthalate. All the compounds named above are isolated for the first time from the roots of *G. microcos*.

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