Journal of Medicinal Plants Research Vol. 5(9), pp. 1756-1761, 4 May, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

*Full Length Research Paper*

# Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica*, Linn.

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Accepted 4 February, 2011

***Plumbago zeylanica*** **is a medicinal plant widely used in forklore medicine in Africa and Asia for the management of ailments such as parasitic diseases, scabies, ulcers, piles, diarrhoea, skin diseases, leprosy, fever or malaria, rheumatism and intestinal parasites. The present study was carried out to identify the phytochemical components present in the ethanolic root extract of the plant by phytochemical screening methods and GC-MS analysis. The phytochemical screening showed the presence of alkaloids, tannins, steroids, flavonoids, saponins, anthraquinones, cardiac-glycosides, phlobatinnins and carbohydrates. The gas chromatography-mass spectrometry (GC-MS) analysis also identified the presence of phytochemical components like phenolics (Phenol, 2,4-bis(1,1-dimethylethyl) (RT: 6.796), Cyclopentadecane (RT: 7.305), fatty acids/methyl esters, Feroprofen (RT: 9.602), 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane (RT: 8.253), Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro- (RT: 10.162), 2H-Indol-2-one,1-(2,6-dichlorophenyl)-1,3-dihydro- (RT: 11.191), 2-Methyl-7-phenylindole (RT: 14.540), 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4,5,6,7-tetrahydro-, isopropyl ester (11.528), and Anthranilic acid, 3-chloro-N-(o-chlorophenyl)- (RT: 12.443).**

**Key words**: *Plumbago zeylanica*, gas chromatography, mass spectrometry, phytochemical screening.

## INTRODUCTION

The use of plants in ethnomedicine is increasing around the world. The World Health Organization (WHO) has reported that approximately 80% of the world's population currently uses herbal medicines as teas, decocts or extracts with easily accessible liquids such as water, milk or alcohol (Julsing et al., 2007; Kalidass et al., 2010). In Nigeria, the use of herbs in traditional medicine is widely accepted as a significant instrument of health care. Herbal drugs are widely prescribed, even when their biological components are not known, as a result of their effectiveness, fewer side effects and relatively low cost (Kumar et al., 2009).

*Plumbago zeylanica* Linn. belongs to the family Plumbaginaceae. *P. zeylanica* is a branched evergreen shrub reaching up to 2 m. It is distributed throughout the tropical and subtropical countries of the world. Four species of the genus *Plumbago* have been reported. They are *Plumbago indica, Plumbago rosea, Plumbago capensis,* and *P. zeylanica* (Arunachalam et al., 2010). *P. zeylanica* is known by different names in different parts of the world. Such names include *white leadwort* or *ceylon leadwort* (in English), *bleiwurz or zahnkraut* (in German), *chitrak or chitramol* (in India), *ensain or enkin* (in Arabia),

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*sanza* (in Swahili), and *inabiri* among the Yoruba (in South- West Nigeria). The whole plant, roots, powder of the root, leaves and stem-bark are widely used as medicinal herbs throughout Asia and Africa. In traditional herbal medicine, the root part of *P. zeylanica* is used for treatment of different ailments such as parasitic diseases, scabies and ulcers (Olagunju et al., 2006), piles, diarrhoea, skin diseases and leprosy (Uma et al., 1999), fever or malaria, rheumatism, intestinal parasites, anemia due to 'stagnant blood', internal and external trauma, toxic swelling and furunculous scabies (Olagunju et al., 2006; Jeyachandran et al., 2009; Jiangsu New Medical College, 1979; Dai et al., 2004).

The pharmacological studies of various root extracts of *P. zeylanica* have shown that the plant has antimicrobial (Ahmad et al., 2000), antioxidant and anti-inflammatory (Oyedapo, 1996; Raimi and Oyedapo, 2009), antiplamodial (Simonsen et al., 2001), antihyperglycemic (Olagunju et al., 1999), antifungal (Mehmood et al., 1999), hypolipidaemic and antiatherosclerotic (Sharma et al., 1991), and central nervous system stimulatory, cytotoxic and anti-insecticidal properties (Arunachalam et al., 2010). Several active compounds previously identified and isolated from the root of *P. zeylanica* include five naphthoquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone), five coumarins (seselin, 5-methoxyseselin, suberosin, xanthyletin and xanthoxyletin), two plumbagic acid glucosides (3´-*O*-β-glucopyranosyl plumbagic acid and 3´-*O*-β-glucopyranosyl plumbagic acid methyl ester) (Lin et al., 2003), neoisoshinanolone and 1-epineo-isoshinanolone (Jetty et al., 2010). In this study, further investigation was carried out to identify other active constituents present in the ethanolic root extract of the plant by phytochemical screening and Gas chromatography-mass spectrometry (GC-MS) analytical methods.

## MATERIALS AND METHODS

### Plant material

The root parts of *P. zeylanica* was collected in the second week of August, 2008 at Babajakan Village, Osun State, Nigeria through Professor O. O. Oyedapo of the Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Plant identification and authentification had been done earlier by Olagunju et al. (2006) and voucher specimen with code QC 488 had been deposited at the Ife herbarium.

### Preparation of extract

The roots were separated from the stalks and thoroughly but gently rinsed with tap water thrice to remove sands. These were then oven dried completely at 27 to 30°C for 1 week. The dried roots were ground to fine powder using a local grinder.

150 g of the powdered root of *P. zeylanica* was exhaustively extracted in 800 ml 99.9% (absolute) ethanol with soxhlet extractor and then concentrated using rotary vacuum evaporator. 10.0 ml extract was measured out from the concentrate for phytochemical screening while the remaining extract was evaporated to complete dryness at 35°C given a dark-brown color solid residue [yield: 3.14% ($^{w}/_{w}$)]. The dried extract was stored in airtight container and placed in refrigerator.

### Phytochemical screening

The extract was subjected to preliminary phytochemical screening to test for the presence or absence of phytochemical constituents using the methods described below by Evans (1996).

### Alkaloids

1.0 ml extract was stirred with 5 ml dil. Hydrochloric acid on a steam bath, filtered and 1.0 ml of each Dragendoff's/Mayer's/Wagner's reagents was added to 1.0 ml separate portions of filtrate. A cloudy orange-red/slightly yellow/turbid brown color indicates the presence of alkaloids.

### Tannins

1.0 ml extract was stirred with 1.0 ml Ferric chloride. A greenish black precipitate indicates the presence of tannins; 1.0 ml extract was also stirred with 1.0 ml bromine water. A reddish brown turbid color indicates the presence of tannins.

### Cardiac-glycoside (Legal's test):

1.0 ml extract was dissolved in 5.0 ml pyridine, 2 drops 2% Sodium Nitroprusside and 2 drops 20% NaOH were added. A deep red color faded to brown indicates presence of cardenolide.

### Kedde's test

1.0 ml extract was mixed with 1.0 ml 2% 3,5-Dinitro-benzoic acid in methanol and 1.0 ml aqueous NaOH. A violet color precipitate indicates lactone ring present in cardenolide.

### Steroids (Liebermann-Burchard's test)

0.5 g extract dissolved in 2.0 ml acetic anhydride, cooled in ice and 1.0 ml conc. $H_2SO_4$ was carefully added. A blue-green ring indicates the presence of steroids.

### Salkowski's test

0.5 g extract was dissolved in 2.0 ml chloroform and 1.0 ml conc. $H_2SO_4$ was carefully added. A reddish brown color indicates the presence of steroids.

### Flavonoids (Ferric chloride test)

0.2 ml extract was added to 10% $FeCl_3$ and mixture was shaken. A wooly brownish precipitate indicates the presence of flavonoids.

### Lead acetate test

0.2 ml extract was added to 0.2 ml 10% lead acetate and gently shaken. A dirty brownish precipitate indicates the presence of flavonoids.

**Table 1.** Results of phytochemical screening of ethanolic root extract of *P. zeylanica*

| Phytochemicals | Result |
|---|---|
| Alkaloids | + |
| Tannins | + |
| Cardiac-glycosides | + |
| Steroids | + |
| Flavonoids | + |
| Saponins | + |
| Anthraquinones | + |
| Phlobatinnins | + |
| Carbohydrates | + |

+ = present.

**Sodium hydroxide test**

0.2 ml dil. NaOH was added to 0.2 ml extract, gently shaken. A dirty yellowish brown precipitate indicates the presence of flavonoids.

**Saponins (Frothing test)**

0.2 ml extract was mixed with 5.0 ml distilled water, shaken for 20 min. Persistence of foams indicates presence of saponins.

**Anthraquinones (Borntrager's test)**

2.0 g extract in 10 ml ethanol, steamed for 5 min and filtered, 2.0 ml filtrate was added to 2.0 ml chloroform, shaken thoroughly, chloroform layer was taken off and 5.0 ml distilled water added which was shaken with 5.0 ml dilute ammonia solution. A red color in ammonia upper phase indicates the presence of anthraquinones.

**Phlobatinnins test**

1.0 ml extract was boiled in 2.0 ml 1% aqueous HCl. A red precipitate indicates the presence of phlobatinnins.

**Carbohydrate tests**

1.0 ml extract was added to 2.0 ml Fehling's solution and boiled for 5 min. A red precipitate indicates the presence of reducing sugars; 1.0 ml extract was added to 2.0 ml Barfoed' reagent and boiled for 1 min. A red precipitate indicates the presence of reducing monosaccharides; 1.0 ml extract was added to 1.0 ml Molisch's reagent and 1.0 ml conc. $H_2SO_4$ was carefully added. A reddish ring indicates the presence of carbohydrates.

**Gas chromatography-mass spectrometry analysis**

The GC-MS analysis was carried out using a Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with a flame ionization detector and Hewlett Packard 7683 series injector, MS transfer line temperature of 250°C. The GC was equipped with a fused silica capillary column- HP-5MS (30 x 0.25 mm), film thickness 1.0 µm. The oven temperature was held at 50°C for 5 min holding time and raised from 50 to 250°C at a rate of 2°C /min, employing helium gas (99.999%) as a carrier gas at a constant flow rate of 22 cm/s. 1.0 micron of extract (1 mg dissolved in 1 ml absolute alcohol), at a split ratio of 1:30 was injected. MS analysis was carried out on Agilent Technology Network Mass Spectrometer (Model 5973 series) coupled to Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with NIST08 Library software database. Mass spectra were taken at 70 eV/200°C, scanning rate of 1 scan/s.

Identification of compounds was conducted using the database of NIST08 Library. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Library.

## RESULTS AND DISCUSSION

Table 1 shows the results of the phytochemical screening of the ethanolic root extract of *P. zeylanica*. This reveals the presence of alkaloids, tannins, steroids, flavonoids, saponins, anthraquinones, phlobatinnins, cardiac-glycosides and carbohydrates. The results of the GC-MS analysis identified the various compounds present in the plant (Figure 1 and Table 2). Figure 1 shows the gas chromatogram of the extract which shows 24 distinct peaks identified by GC-MS while the compounds identified through the NIST08 L. database are listed in Table 2. The major compound present in the ethanolic root extract of *P. zeylanica* as identified by GC-MS was Phenol, 2,4-bis(1,1-dimethylethyl) with RT: 6.796% of Total: 54.62 and quality: 96 (Table 2). Mass spectrum of Phenol, 2,4-bis(1,1-dimethylethyl) was shown in Figure 2. The GC-MS spectrum gives the structure of the compound (Figure 3), molecular formula as $C_{14}H_{22}O$, molecular weight as 206 and biological activity as antioxidant (Table 3). Other components also identified in the root of *P. zeylanica* are Cyclopentadecane (RT: 7.305), Fenoprofen (RT: 9.602), Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6.7-tetrahydro- (RT: 10.162), Anthranilic acid, 3-chloro-N-(O-chlorophenyl)-(RT: 12.443), 2-Methyl-7-phenylindole (RT: 14.540) and Docosanoic acid, methyl ester (RT: 13.277) (Figure 1).

It has been reported that many medicinal plants are rich in varieties of secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids (Lewis and Ausubel, 2006; Adekunle and Adekunle, 2009). Reports have it that secondary plant metabolites exert a wide range of biological activities on physiological systems (Olagunju et al., 2006). Kumar et al. (2010) also reported the activities of some phyto-components with compound nature of flavonoids, palmitic acid (hexadecanoic acid, ethyl ester and n-hexadecaonoic acid), unsaturated fatty acid and linolenic (docosatetraenoic acid and octadecatrienoic acid) as antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary. It is therefore not unlikely that these phytochemicals found in *P. zeylanica* may play major roles in the reported biological activities and pharmacological properties of the plant.

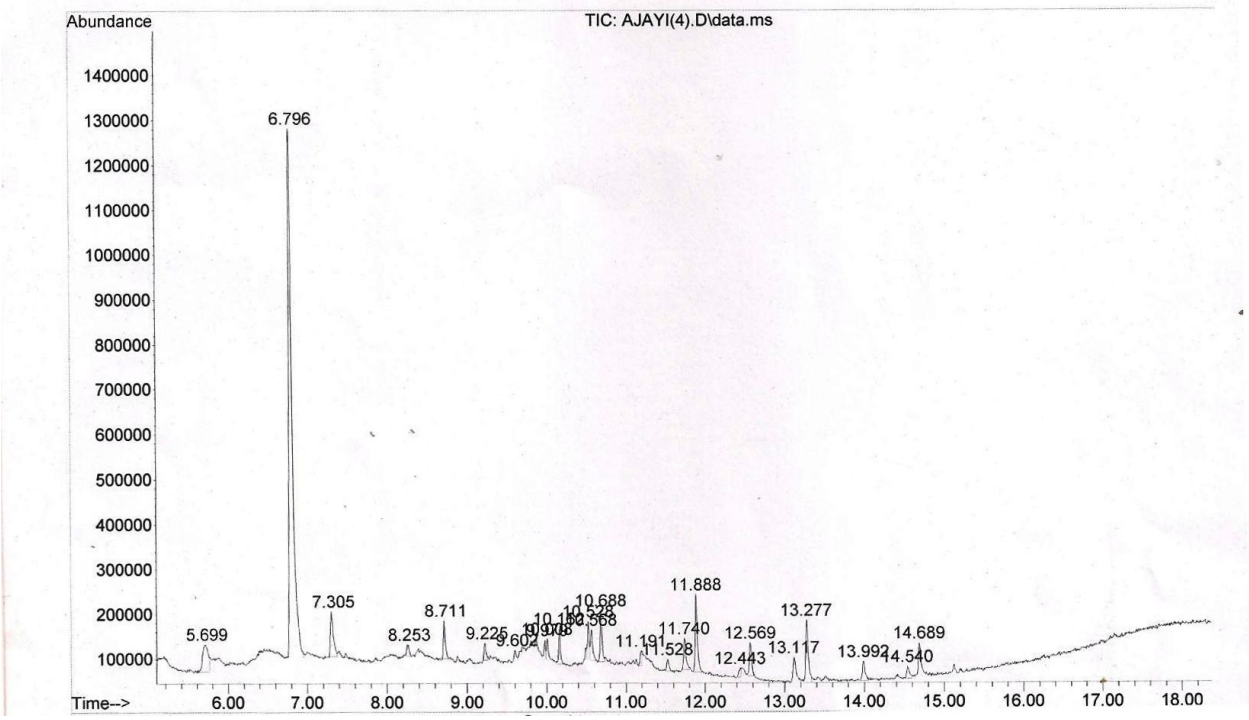


**Figure 1.**GC-MS chromatogram of ethanolic root extract of *P. zeylanica.* Peak 2 with retention time of 6.796 was identified as phenol,2,4-bis(1,1-dimethylethyl) and as the major phyto-component of the plant while other peaks were of the various phyto-components present.

**Table 2.** Phytochemical components identified in the ethanolic root extract of *P. zeylanica* by GC-MS showing their RT, % of Total and quality of compounds.

| PK | RT | Identified compound | MSD | % of total | Qual |
|---|---|---|---|---|---|
| 1 | 5.699 | 1-Tetradecene | 56562 001120-36-1 | 4.602 | 95 |
| 2 | 6.796 | Phenol,2,4-bis(1,1-dimethylethyl) | 63980 000096-76-4 | 54.623 | 96 |
| 3 | 7.305 | Cyclopentadecane | 67239 000295-48-7 | 3.748 | 97 |
| 4 | 8.253 | 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy) tetrasiloxane | 206266 038147-00-1 | 0.750 | 38 |
| 5 | 8.711 | 1-Octadecene | 99556 000112-88-9 | 2.106 | 99 |
| 6 | 9.225 | Hexadecanoic acid, methyl ester | 113682 000112-39-0 | 0.942 | 96 |
| 7 | 9.602 | Fenoprofen | 91701 031879-05-7 | 0.633 | 52 |
| 8 | 9.979 | 3-Eicosene, (E)- | 121344 074685-33-9 | 0.650 | 98 |
| 9 | 10.008 | Methyl 7-methylhexadecanoate | 124582 018720-85-9 | 1.037 | 64 |
| 10 | 10.162 | Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro- | 141172 1000310-86-5 | 1.647 | 64 |
| 11 | 10.528 | 9-Octadecenoic acid, methyl ester (E)- | 133723 001937-62-8 | 1.909 | 99 |
| 12 | 10.568 | Methyl 11-Octadecenoate | 133684 1000336-35-8 | 1.939 | 99 |
| 13 | 10.688 | Octadecenoic acid, methyl ester | 135389 000112-61-8 | 2.783 | 99 |
| 14 | 11.191 | 2H-Indo-2-one, 1-(2,6-dichlorophenyl)-1,3-dihydro- | 118782 015362-40-0 | 0.653 | 99 |
| 15 | 11.528 | 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester | 174606 1000316-17-5 | 0.773 | 22 |
| 16 | 11.740 | cis-11-Eicosenoic acd, methyl ester | 154536 1000333-63-8 | 2.069 | 99 |
| 17 | 11.888 | Eicosanoic acid, methyl ester | 156024 001120-28-1 | 4.689 | 98 |
| 18 | 12.443 | Anthranilic acid, 3-chloro-N-(o-chlorophenyl)- | 121641 099513-96-9 | 0.581 | 62 |
| 19 | 12.569 | Heneicosanoic acid, methyl ester | 165667 006064-90-0 | 2.095 | 99 |
| 20 | 13.117 | 13-Docosenoic acid, methyl ester, (Z)- | 172949 001120-34-9 | 2.369 | 97 |

**Table 2.** Contd.

| | | | | | |
|---|---|---|---|---|---|
| 21 | 13.277 | Docosanoic acid, methyl ester | 174189 000929-77-1 | 4.519 | 99 |
| 22 | 13.992 | Tricosanoic acid, methyl ester | 181855 002433-97-8 | 1.612 | 99 |
| 23 | 14.540 | 2-Methyl-7-phenylindole | 64822 001140-08-5 | 1.029 | 86 |
| 24 | 14.689 | Tetracosanoic acid, methyl ester | 188588 002442-49-1 | 2.242 | 99 |

PK=Peak, RT=Retention time, MSD=Mass spectrum data, Qual=Quality.

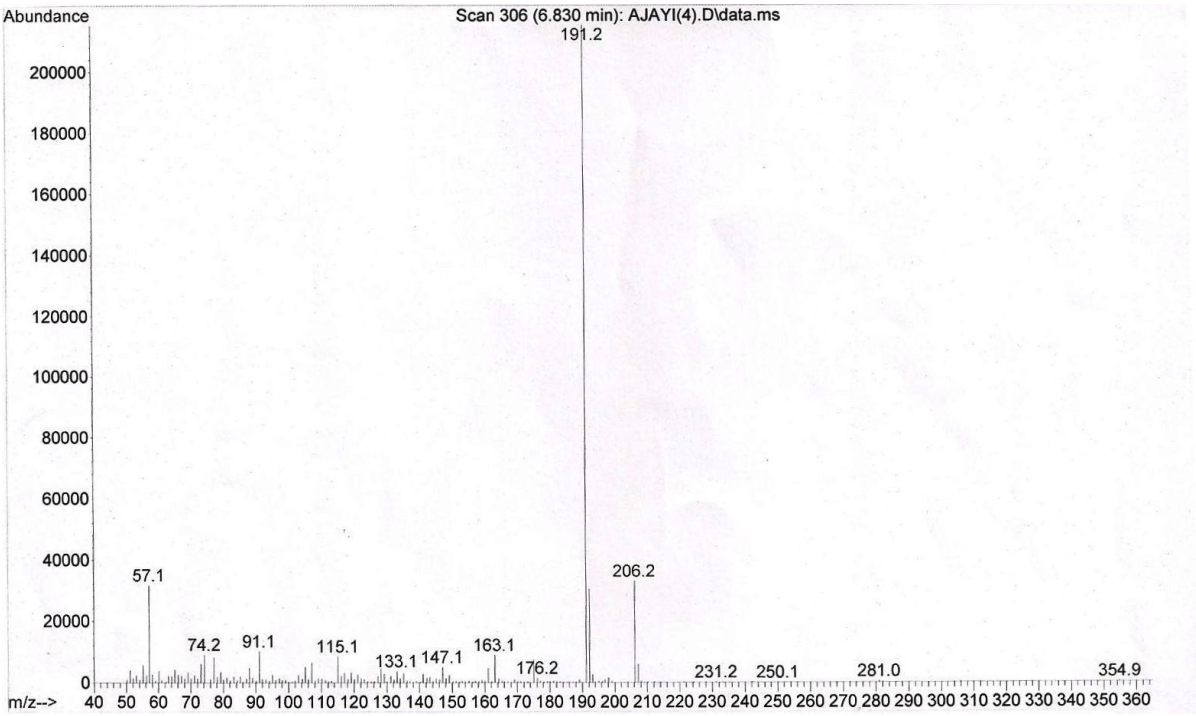


**Figure 2.** GC-MS mass spectrum of phenol, 2,4-bis(1,1-dimethylethyl) indicating abundant ions m/z 191.2.

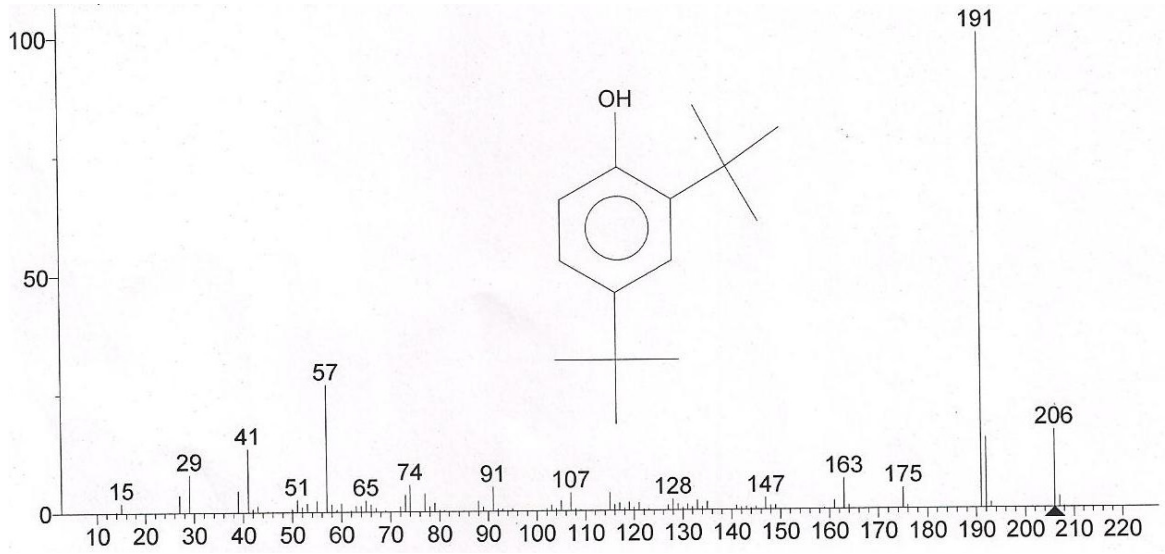


**Figure 3**. GC-MS mass spectrum and molecular structure of phenol, 2,4-bis(1,1-dimethylethyl).

**Table 3.** GC-MS indicated properties of phenol, 2,4-bis(1,1-dimethylethyl).

| Properties | Given as |
|---|---|
| Molecular formula | $C_{14}H_{22}O$ |
| Molecular weight | 206 |
| Biological activity | Antioxidant |

Phenolic compounds have also been known as antioxidant agents, which act as free radical terminators and have shown medicinal activity as well as exhibiting physiological functions. It was reported that compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of most plants (Omale and Okafor, 2007). The presence of these phytochemicals in *P. zeylanica* root is a significant finding in this present study.

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