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ISOLATION OF CARVONE AND PHELLANDRENE FROM *Murraya koenigii* AND STUDY OF THEIR ANTIOXIDANT ACTIVITY

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The plant *Murraya koenigii* (L.) Spreng., belonging to the family Rutaceae, is native to India and is now distributed in most of southern Asia. The Plant was identified by the Botanical Survey of India (No. BSI/SRC/5/23/2011-12/Tech-100). The leaves of this plant, called curry leaves, are commonly used for flavouring southern Asian dishes. The Murraya species has also been used in traditional medicine in eastern Asia. Previous studies on the Murraya species include reports of coumarins, terpenoids, and many investigations on carbazole alkaloids [1–4]. The leaves of *Murraya koenigii* are also used as a herb in ayurvedic medicine. Their properties include antidiabetic [5] and hepatoprotective effects [6]. Studies on carbazoles isolated from *Murraya koenigii* leaves have shown that it exhibits antioxidant and antimicrobial activity [7–9].

This study reports on the isolation and investigation of the antioxidant activity of carvone and phellandrene isolated from *Murraya koenigii*. These two bioactive compounds are simple in structure and were characterized by FT-IR, ^{1}H NMR, GC, and GC-MS. These simple bioactive compounds can be used in food supplements and in food ingredients as natural antioxidants.

The *Murraya koenigii* leaves (curry leaves) were washed in flowing water and its roots were removed. After dehydration, the leaves and stems were ground and passed through an 80-mesh screen to obtain a uniform powder [9]. The powdered materials were subjected to solvent extraction in a Soxhlet-type apparatus at 80°C using ethanol, resulting in *Murraya koenigii* leaf extract [10].

In the UV spectrum of the ethanol extract of *Murraya koenigii* leaf, eight peaks are observed at 671, 610, 510, 490, 417, 298, 263.4 and 223.8 nm, and they correspond to eight different compounds. Out of these eight compounds, we were interested in studying only the two compounds whose absorption peaks were at 223.8 nm and 263.4 nm.

The extract was separated using a column packed with silica gel, with hexane as the eluent initially. The polarity of the solvent was increased step up step using a mixture of hexane and acetone. The first two fractions of the extract were obtained using the difference in polarity of the eluent. The first compound isolated (hexane 100%) was analyzed by UV spectrophotometry, which gave an absorption maximum of 223 nm. Column chromatography was again employed by increasing the polarity of the solvent mixture (hexane–acetone 95:5 v/v) when the second compound was isolated. The second compound was analyzed using the UV spectrophotometer, which gave an absorption λ_{max} of 263.4 nm.

The ethanol extract of compounds **1** and **2** was tested in a GC, and both compounds gave a single peak, confirming the presence of a single compound. For the first compound the retention time was 8.53 min, with 99.465% purity. For the second compound the retention time was 5.916 min, with 97.44% purity. The GC study confirmed the presence of these compounds as individual single compounds. The IR study reveals the following: IR spectrum (ν) compound **1**: 1760–1690 (C=O), 1259, 1126 (C-C-str), 1058, 1028 (C=C) cm^{-1}.

IR spectrum (ν) compound **2**: 1058, 927.79 (C=C-mono subs.), 1126 (C-C-str) cm^{-1}.

In the ^{1}H NMR spectrum of compound **1**, the peaks appearing at the δ 1.37 and 1.6 range indicate the presence of the CH_2 group in the molecule. The peak appearing at 3.93 shows the presence of a C-C linkage between the five- membered ring and the six-membered ring. Therefore it is concluded that compound **1** also has the same type of linkage.

In the ^{1}H NMR spectrum of compound **2** the peaks in the range of nearly δ 1.50 clearly showed the presence of the CH group in the molecule. The peak around the 2.15 range indicates the presence of the CH group of an aliphatic molecule.

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TABLE 1. DPPH Radical Scavenging Activity of Carvone (**1**) and Phellandrene (**2**)

Concentration of drug, μg/mL	**1**	**2**
	% DPPH radical scavenging, ± SEM	
0.5	24.67 ± 0.005	29.68 ± 0.005
1	28.67 ± 0.005	30.33 ± 0.005
2.5	34.34 ± 0.005	36.91 ± 0.005
5	39.04 ± 0.005	42.16 ± 0.005
10	46.87 ± 0.00	47.97 ± 0.005
20	56.65 ± 0.005	50.13 ± 0.005

IC_{50}: carvone – 12.3 μg/mL, phellandrene – 19.1 μg/mL.

There is a peak around 3.93 in the spectrum, which indicates the presence of a C-C linkage between the five- membered ring and a six-membered ring. Therefore compound **2** under study should also have the same type of linkage. Finally, the spectra show the presence of a major peak at 7.32, which is due to the presence of an aromatic ring in the compound.

In the GC-MS studies a good sharp peak is obtained. A major peak with *m/z* value 150 was observed. The molecular weight of carvone is 150.2. From the above GC-MS data, we conclude that the structure of the isolated compound is carvone (**1**).

In the GC-MS studies a good sharp peak with *m/z* value 136.2 was observed. The molecular weight of phellandrene is also 136.237. So according to GC-MS, the major peak is confirmed to be that of phellandrene. Thus, based on spectral analysis, the isolated compounds were identified as carvone and phellandrene (**2**).

Evaluation of Antioxidant Activity by *in vitro* Method. Stechiometrically, depending on the number of electrons taken up, DPPH was used to determine the proton radical scavenging action of extracts of the leaves of *Murraya koenigii* because it possesses a proton free radical and shows a characteristic absorbance at 517 nm. From the present results, it may be postulated that extracts of the leaves of *Murraya koenigii* reduce the radical to the corresponding hydrazine when the radical reacts with hydrogen donors in antioxidant extracts. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined from the decrease in their absorbance at 517 nm induced by antioxidants. The IC_{50} values were found to be 12.3 μg/mL for carvone and 19.1 μg/mL for phellandrene (Table 1).

Thus, the study confirms the value of plants used in traditional Asian food preparation, which could be of considerable interest in the development of new compounds with medicinal value.

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