ABSTRACT
The utility of crude drug was due to its therapeutically active constituents. The phytochemicals with pharmacological activity were extracted from a variety of herbs. Several herbs can help to provide some protection against cancer and many other chronic diseases and it stimulate the immune system. The aim of the present study is to isolate coumarins form the plant Ceropegia juncea (Roxb.). Coumarin derivatives has wide therapeutic properties and is reported by many researchers from different plants belongs to different families. Coumarin has been known to have antitumor activity (Soine, 1964; Borges et al., 1999; Sever and Dewick, 2002). Coumarin and its derivatives have long been recognized for their pharmacological activities including anticancer, antithrombosis, antiviral, and anticarcinogenic activities (Matern and O’Kennedy, 1997; Parameswaran et al., 1999). In Ayurvedic and traditional systems of medicine, Ceropegia juncea (Roxb.) is known for its medicinal properties and is used in various formulations for the treatment of various diseases. The plant is believed to be a source of coumarin derivatives, and some reports (Endress and Bruyns, 2000) have indicated the presence of coumarins in the plant. Ceropegia juncea (Roxb.) has been used in Ayurvedic medicine for several centuries and is considered to be a valuable source of bioactive compounds. The present study aims to isolate and identify coumarin derivatives from the methanol extract of Ceropegia juncea (Roxb.).

KEYWORDS: Ceropegia juncea (Roxb.).

INTRODUCTION

Many of the plants were investigated for their bio-active molecules. Based on literature data very few works were done on this particular plant Ceropegia juncea (Roxb.) belongs to the family Asclepiadoideae. The plant is otherwise known as Soma plant which is used in the spiritual rituals called Yaga especially Somayagam in India (Sudha Karayil et al., 2011). The plant Soma has wide variety of Therapeutic uses in Ayurvedic system of medicine. Phytochemical analysis of the Ceropegia juncea (Roxb.) was done and the revealed the presence of alkaloids, steroids, flavanoids phenyl proponoids, glycosides, fatty acids, carotenoids, tannins, anthocyanin and anthracene glycosides, coumarins etc. in methanol and hexane extract. Ceropegia juncea (Roxb.) has been known to be a valuable source of bioactive compounds. The present study aims to isolate and identify coumarin derivatives from the methanol extract of Ceropegia juncea (Roxb.). Ceropegia juncea (Roxb.) is an important medicinal plant belonging to the family Asclepiadoideae. It is well known ethnomedicinal and ethnomedicalally important plant. The secondary metabolites are considered as prospective sources of new natural drugs, antibiotics, insecticides and herbicides, drugs, flavouring agents etc. (Croteau and Lewis, 2000; Dewick, 2002). Ceropegia juncea (Roxb.) has been known to be a valuable source of bioactive compounds. The present study aims to isolate and identify coumarin derivatives from the methanol extract of Ceropegia juncea (Roxb.).
Coumarins are a few examples that have been reported (Irena Kostova, 2005). Kofinas et al. (1998) isolated seven coumarins from aerial parts of Tordylium apulum and established their structures by spectroscopic means. There are very few reports regarding the presence of coumarin compounds in the family Asclepiadaceae and Apocynaceae on a novel 4-methyl Coumarin was isolated and reported by Sudha Karayil et al., (2014).

MATERIALS AND METHODS
The plant was collected at Kollengode hills, Western Ghats, Kerala, India. Shade dried whole plant and the powder is subjected to extraction by Soxhlet by using methanol and hexane. The coumarin is isolated from Column chromatography and HPTLC. The crude coumarin obtained is elucidated for its structure by Spectral studies.

1. Nuclear Magnetic Resonance (NMR) Spectroscopy
2. Infra-red Spectroscopy (IR)
3. Mass Spectrometry (MASS)

**Coumarin Extraction**
The crude sample was extracted thrice in 500ml petroleum ether by refluxing for 5 hours. The sample was treated with ceramic filter and the filtrate was evaporated by rotary evaporator at 40°C under reduced pressure. The sample was extracted into the following solvent mixture in the ratio of Ethyl acetate: Pet Ether: Methanol: water 5: 5: 7: 3. A pale yellow color crud was obtained. It was dried at room temperature.

**HPLC Quantification of Coumarin**
Coumarin was analyzed by Reverse phase HPLC on C-18 column (250×4.6mm 5μ). The HPLC was flushed with 100% methanol and changed to the required mobile phase. Benzopyrone (Sigma, 99% HPLC purified) standard sample was procured from Sigma Chemicals. The run time of Coumarin was 24 minutes. For every five runs, the HPLC was re-standardized using the Coumarin standard. The HPLC parameters were set following with standard methods with appropriate modifications. Mobile phase condition for HPLC: methanol–acetonitrile–phosphate buffer (PH 4.8; 1mM) (22.5:15:62.5, v/v/ν). Stationary phase: RP-C18 (250×4.6mm/5μ) Flow rate: 0.5ml per minute Detection range: 274 nm-365nm.

**Spectral studies**
For spectral studies the fractionated samples were dissolved to a concentration of 10 ppm and used for analysis. The structures of the compounds were established by spectral data and by comparison with the literature data. All the compounds were isolated by chromatographic techniques. The structural elucidation of these compounds was based on spectroscopic data, especially IR, 1H-NMR and 13C-NMR, Mass, 2D COSY, HMBC, HSQC.

**RESULTS AND DISCUSSION**
The two coumarin compounds obtained is
1. (E)-2-cyano-3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-N-(4-methylbenzyl) acrylamide
2. 4-methyl-8-(2-oxo-2-p-tolyethyl)-2H-Chromen-2-one

**Structure of Coumarin-1**
Ionization Mass spectrometry indicated the molecular ion peak m/z = 360. High Resolution Chemical Ionization Mass spectroscopy (HRCIMS) and High Resolution Electron Impact Mass spectroscopy (HREIMS) supported the molecular formula C_{21}H_{16}N_{2}O_{2} for the coumarin. The molecular ion fragment was observed at m/z 345. The major fragment ions at m/z 320, 283.1, 179, 165, 100. The number of protons from integration of the H¹ NMR spectrum and carbons from C¹³ NMR matched with number of protons and carbons presented by the molecular formula. Mass spectrum data supports the molecular data.

**IR Analysis**
The wave number indicates 948 C-N bonds, 1062 represent C-O, 1199 indicates C-C bonds wave number 1118 indicates the carbon bond between C17-C22 in IR spectra. 2924 indicates C-H bond and 3052 indicates N-H, 3430 indicates the presence of O-H bonds. 2866 represents the presence of C=C bonds. 17130 indicates C-O bond.

**C¹³ NMR Analysis**
The chemical shift δ value 160.2, 160.4, 160.5 indicates the presence of C1, C3, C12, δ value of 116.4, 116.8 and 117.3 indicates the presence of C10, C13. δ, 116.4, 116.8 and 117.3 indicates the C14 to C15 carbons. The 1520.6 indicates C11, 20.2 indicates the C21. CNMR around 120 values indicates the position of Carbon 22.

**H¹ NMR Analysis**
The chemical shift δ 1.82 indicates the H (proton) on C21. δ 7.14, 7.16, 7.19 ascribed to the protons around C14-C19 carbons and C2-C3 carbons respectively. The 4.3, 5.8 indicate the proton on C10 and C13. In 7.63 Values indicating the Hydrogens on C22, 2D –gCOSY, g HMBC, gHSQC and data also supports the structure.
Structure of Coumarin-2

Ionization Mass spectrometry indicated the molecular ion peak m/z = 360. High Resolution Chemical Ionization Mass spectrometry (HRCIMS) and High Resolution Electron Impact Mass spectrometry (HREIMS) supported the molecular formula C_{18}H_{16}O_{3} for the coumarin. The molecular ion fragment was observed at m/z 293. The major fragment ions at m/z 275, 230, 188, 172,128, 100. The number of protons from integration in the H^{1} NMR spectrum and carbons from C^{13} NMR matched with number of protons and carbons presented by the molecular formula.

IR Analysis

The Wave number 1680 indicates C=O, 2860 indicates C≡C, 2924 indicates C-H bond and 1199 indicates C-C bonds in IR spectra. 1082 represents C=O, 2860 indicates C≡C, 2924 indicates C-H bond and 1199 indicates C-C bonds in IR spectra. 1082 represents C=O, 2860 indicates C≡C, 2924 indicates C-H bond and 1199 indicates C-C bonds in IR spectra. 1082 represents C=O, 2860 indicates C≡C, 2924 indicates C-H bond and 1199 indicates C-C bonds in IR spectra.

C^{13} NMR Analysis

The chemical shift ascribed to C_1, C_5, C_{11} resonated at \( \delta \) 160, 161,162, \( \delta \) value 119.0, 120, 121, 122, indicates C_{12}-C_{17} and C_{19} carbons and \( \delta \) 141, 142, 143 shows C_{4}-C_{9} and \( \delta \) 202 indicates C_{18} carbons.

H^{1} NMR Analysis

The chemical shift \( \delta \) value of 1.5 indicates the presence of H on C_{10}; \( \delta \) 5.2-5.8 indicates H on C_{10} and H on C_{2}; \( \delta \) value 7.0 indicates the presence of H on C_{12}-C_{17}, \( \delta \) 7.2 shows protons around C_{4}-C_{9} and \( \delta \) 8.3 shows the presence of H on C_{10},C_{19}.2D –gCOSY, g HMBC, gHSQC, data also supports the present structure.

The structures of these two coumarin compounds were established from the above spectral analysis. From the literature data, it was assumed that these compounds isolated were not reported by any investigator earlier and might be the first and new compounds.

After extraction the elutants were analyzed. Based on the spectral data two new coumarin compounds were isolated. All the spectral studies supported the structures of coumarin. There was only one earlier report on the presence of coumarins in *Ceropegia juncea* (Roxb.). But there are several reports on Coumarins in different medicinal plants. The compounds include 7-hydroxy coumarins and 4-methyl coumarins with cyano, keto, benzene, methyl functional groups. The hydroxyl coumarins are typical phenolic compounds and therefore act as potent metal chelators and free radical scavengers (Irena Kostova, 2005). Similar results have been in different medicinal plants and with different Coumarin structures. As per the literature, the following Coumarins are the new compounds reported first in the present test plant *Ceropegia juncea* (Roxb.). The nomenclature used for chemical compound is in accordance with the nomenclature rules formulated by IUPAC.

REFERENCE


