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Isolation, Purification and Characterization of Arbutin from *Cleidion nitidum* (Muell. – Arg.) Thw. ex Kurz. (Euphorbiaceae)

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Abstract: ***The methanolic leaf extract of Cleidion nitidum (Muell. – Arg.) Thw. ex Kurz. (Family: Euphorbiaceae) was subjected to compound isolation and characterization.The extract was purified and isolated by column chromatography and thin layer chromatography (TLC). The isolated compound was then subjected to UV spectrum, FTIR for identification of functional groups and ^{1}H-NMR and ^{13}C-NMR for identification of protons and carbon atoms. ESI-MS was done to identify the molecular weight of the isolated compound. From the spectra obtained from FT-IR, ^{1}H-NMR, ^{13}C-NMR and ESI-MS, the isolated compound was found to be Arbutin (Hydroquinone β-D-glucopyranoside) with the molecular weight of 272.25***

Keywords: *Cleidion nitidum*, Euphorbiaceae, Arbutin.

1. Introduction

The plants are still important for the discovery of new drugs as provider of the drugs based on secondary compounds from plants. Many scientific research has been carried out on these plants and their secondary metabolites of medicinal importance i.e. alkaloids, flavonoids and terpenoids, etc. have been reported [1]. According to literature survey various phytoconstituents have been isolated from different plants [2-4]. *C. nitidum* belongs to Euphorbiaceae family. This genus comprises about 33 species, which are pantropical in Asia and the South West Pacific [5]. The taxa are represented as 3 species with 4 varieties in India [6], of which *C. nitidum* is distributed in Andaman and Nicobar Islands of Indian subcontinent. Recently it has been reported in Eastern Ghats of Peninsular India [7] and in Southern Western Ghats of Tamilnadu[8]. Two species of this genus *Cleidion viz C. javanicum* BC and *C. speciflorum* Merr.are used as medicine traditionally in Thailand and Philiphines. Several parts of the above two plant species have been employed as analgesic, antipyretic and diaphoretic [9]. Decoction of its leaves is reputed to cause abortion; where as a decoction of the bark is used for treatment of stomachic. Its seeds are used for treatment of constipation[10].The main objective of the present study is to isolate compound from *C.nitidum* leaf by extraction, column chromatography, TLC followed by characterization using UV, FT-IR, ESI-Mass spectrum, ^{1}H-NMR and ^{13}C-NMR spectra.

2. Experimental

2.1 Materials and Reagents

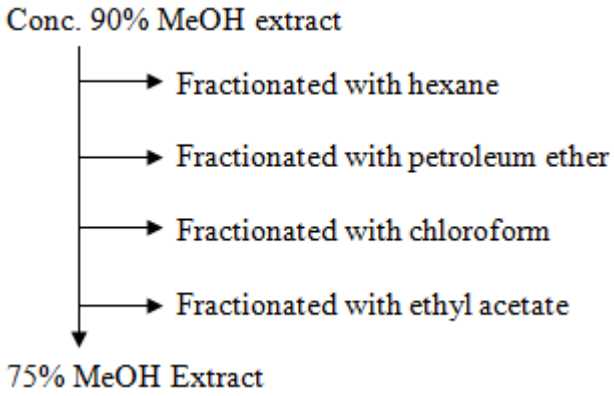
The fresh leaves of *C.nitidum* were collected from Thadagamalai Reserve Forest range, 8º18. 960'N - 77º29. 845'E, alt. ca. 750m (msl) of Kanyakumari Wildlife Sanctuary, Kanyakumari District in Southern Western Ghats, Tamilnadu, India. The plant specimens were collected and identified taxonomically with help of local flora [11-13]. The botanical identity was confirmed with authentic specifmens deposited at Madura College Herbarium, Madurai (Voucher specimen number S. Karuppusamy *et al.* 1583). A voucher specimen of this plant was deposited in the PG Department of Botany and Research Centre, S.T. Hindu College, Nagercoil, Kanyakumari District, Tamil Nadu, India.

Hexane, Petroleum ether, Chloroform, Ethyl acetate, Acetone, Methanol and Ethanol were of analytical grade procured from Merck. Column chromatography was performed on column (length 50 & diameter 150 mm), silica gel (60-120 mesh) and Merck TLC readymade sheets 20 x 20 cm.

The spectrophotometer systems used were Shimatzu UV spectrophotometer, Shimadzu spectrum 1 FT-IR spectrometer and ESI-MS analysis (TofSpec 2E MALDI time - of flight (TOF) Instrument (Micromass, Manchester, UK). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Brucker spectrometer using CDCl3 as solvent and TMS as internal standard. The observed chemical shifts were recorded in ppm and the coupling constants (J) were recorded in Hz.

2.2 Extraction

100g of shade dried leaf powder of *C.nitidum* were extracted with 90% methanol using soxhlet apparatus and concentrated for further using simple distillation method. The concentrated plant extract liquid fractionated with the solvents hexane, petroleum ether, Chloroform, Ethyl acetate. Altogether, 5 fractions were obtained and used for separation of pure isolates using column chromatography method.



2.3 Column chromatography

60-120 mesh size silica gel was dissolved in the low polarity solvent hexane and tightly packed in 50 X 150 mm glass column up to 100 mm height without air bubbles. Then the experimental extracts were loaded individual glass columns and fractionated with solvents hexane, petroleum ether, chloroform, ethyl acetate and methanol at various proportion of solvent mixture.

2.4 Screening of purity for column chromatography Fractions using TLC

15 ml of fractions were collected using each solvents and the collected fractions were screened for purity using thin layer chromatography (Merck TLC Readymade sheets 20 X 20 cm) with appropriate solvent systems (Petroleum ether : Hexane :Chloroform : Ethyl acetate Acetone : Methanol : Ethanol 7 : 1 : 0.5 :0.5: 0.5 : 0.5 : 0.5).

2.5 Preparative TLC

The closely mixture fractions was re-separated using PTLC. The mixture fractions were spotted on TLC for separation individual components and scraped using sterile needles and dissolved in methanol. Then centrifuged at 10,000 rpm. The supernatant was taken for further characterization like TLC, UV-VIS spectrophotometer, FTIR, ESI-MS, MS, ^{1}H-NMR, ^{13}C-NMR and structure elucidation.

3. Results

The ethyl acetate fraction was purified using ethyl acetate and methanol as eluent in the combination of 7: 3 by silica gel column chromatography (60-120 mesh). The isolated fractions 12 to 102 showed colourless substance and they are showed mixture of compounds along with major spots in screening of purity on TLC under iodine vapour visualization. Consequently, flash column was used to separate the pure compound with silica gel column (200 mesh). The mobile solvent is Petroleum ether : Hexane :Chloroform : Ethyl acetate Acetone : methanol : ethanol 7 : 1 : 0.5 : 0.5 : 0.5 : 0.5 : 0.5. The fractions 12 to 102 are show similar pattern on TLC under iodine vapour with few major spots. The PTLC carried out for one of the major spot and re-isolation by scraping, dissolving in pure methanol, followed by centrifuge at 10,000 rpm for 10 minutes. The supernatant was concentrated and screened for purity and appear single spot on TLC under iodine vapour. It was taken for further characterization. *Viz.,* TLC, UV scanning, FTIR/IR, MS/ESIMS/EIMS, ^{1}H-NMR, ^{13}C-NMR.

TLC
Colourless substance. Rf value was 0.73 (Fig. 1)

Figure 1: TLC of isolated compound in iodine chamber

UV-VIS
Λmax are 203, 223 and 282 nm (Fig. 2)

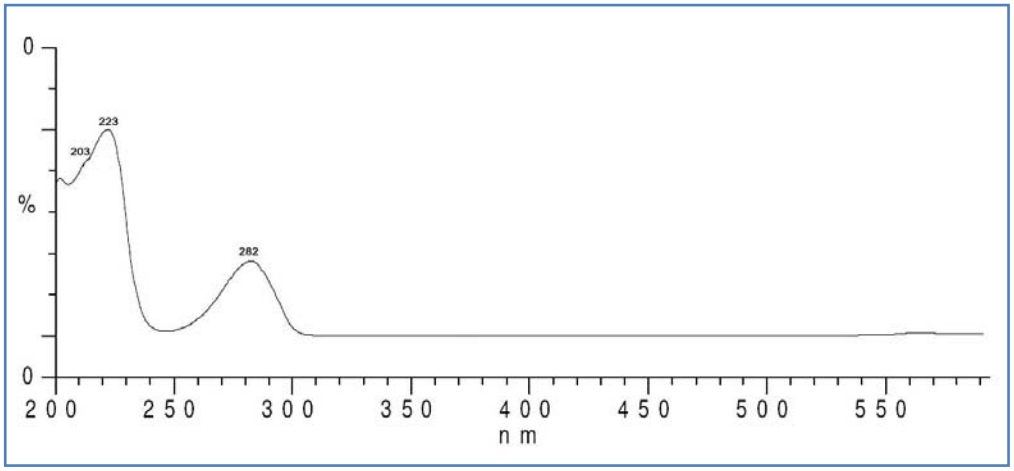


Figure 2: UV-VIS spectra of isolated compound

FT-IR Spectrum

The spectral analysis of the isolated substance shows broad band at 3477cm^{-1} to 3000 cm^{-1} due to OH stretching, bands at 2974, 2931, 2879 cm^{-1} observed to HC- H asymmetrical stretching, bands at 1512, 1458, 1429, 1379 and 1599 cm^{-1} assigned to -C-C- stretching, bands at 1265, 1222 cm^{-1} for –C=C- stretching and bands at 1039, 927, 615 cm^{-1} is observed C=C- mono subs (Fig. 3)

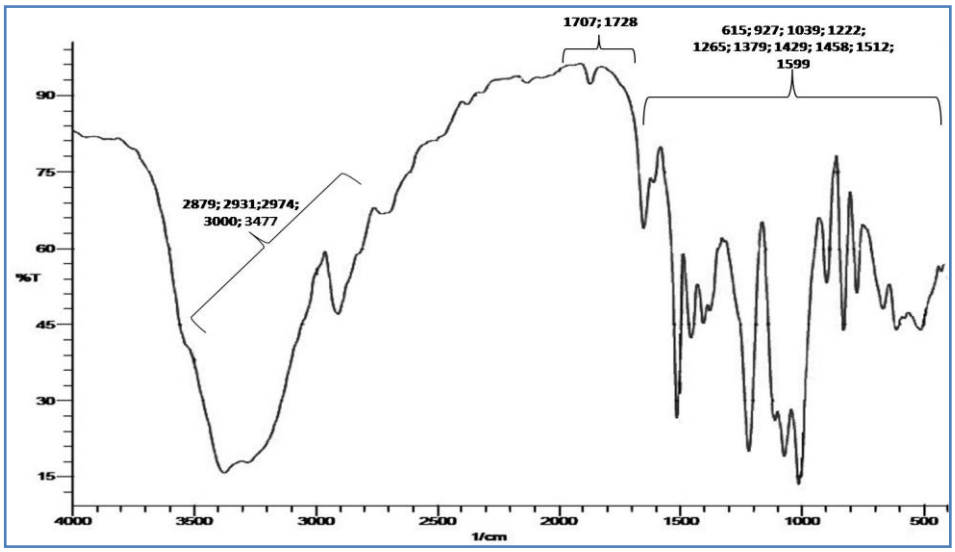


Figure 3: FT-IR characterization of isolated compound

ESI-Mass Spectrum:

Shows molecular weight 272.25 (Fig. 4)

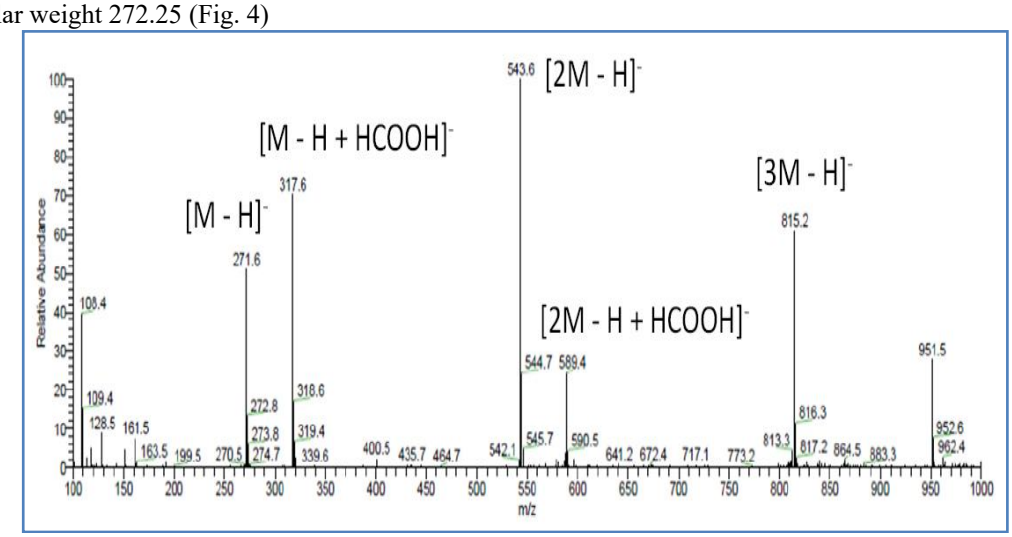


Figure 4: ESI-Mass Spectra of isolated compound

NMR

The 1H and 13C NMR spectra are exhibited an AA'BB' system [δH 6.90 (d, *J* = 9.0 Hz) and δC 119.1; δH 6.72 (d, *J* = 9.0 Hz) and δC 121.3] and a sugar moiety. From the HMBC spectrum of 26 (Figure 3.132), a correlation was noticed between the anomeric proton at δ4.82 (H-1') and the carbon (δ154.1) of the *para*-hydroxyphenol residue, establishing that the glucosidic bond is between C-4 and C-1'. The 1H and 13C NMR spectra were identical to those of arbutin. (Fig. 5&6).

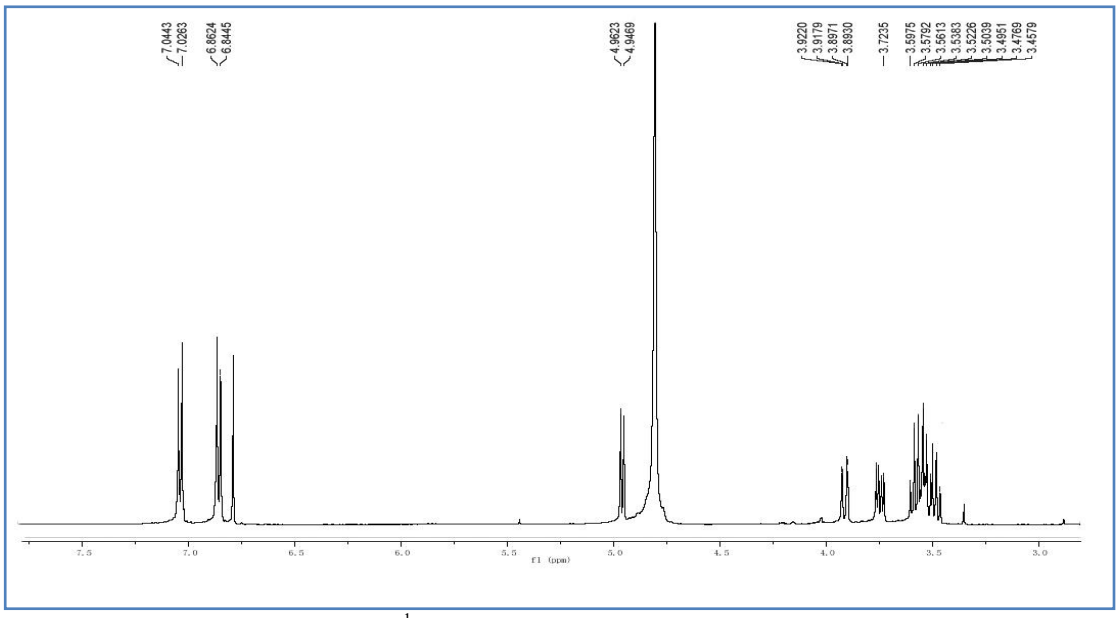
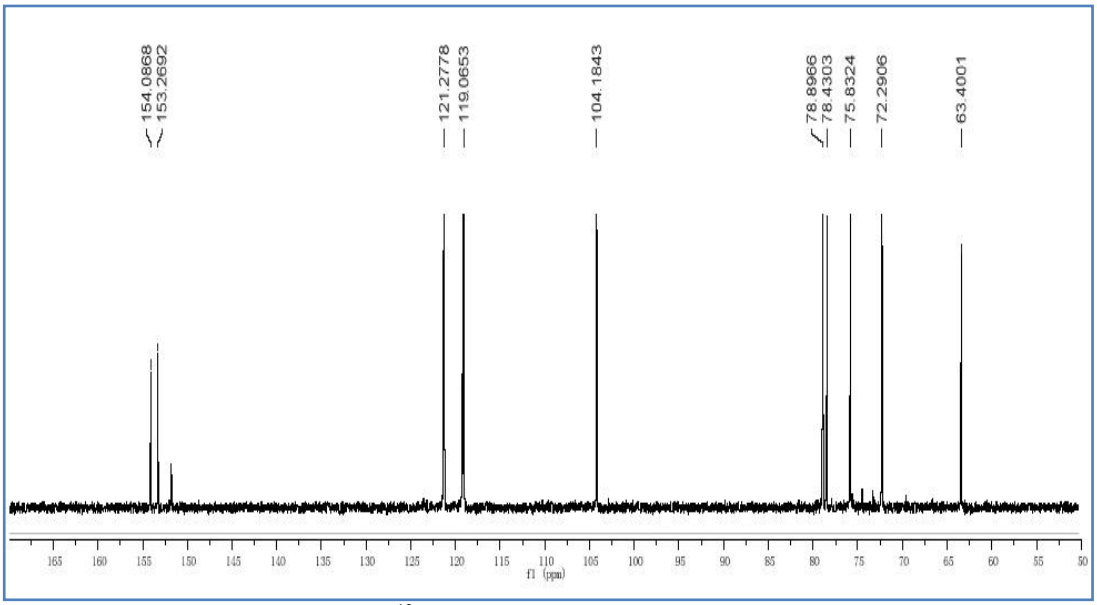


Figure 5: [1]H-NMR Spectra of isolated compound

[13]C-NMR

The observed atom positions and δH (mult., *J* in Hz) through spectral datas as followingly 2- CH- 7.04, d (9.0); 3- CH- 6.85, d (9.0); 5- CH- 6.85, d (9.0); 6- CH- 7.04, d (9.0); 1'- CH- 4.95, d (7.6); 2'- CH- 3.54, m*a;* 3'- CH- 3.58, m*a;* 4'- CH- 3.48, m*a;* 5'- CH- 3.54, m*a;* 6'- CH2-3.91, dd (12.3, 1.9) and 3.74, dd (12.3, 5.4) The observed atom positions and δC through spectral datas as followingly 1- qC-153.3; 2- CH-121.3; 3- CH-119.1; 4- qC-154.1; 5- CH-119.1; 6- CH-121.3; 1'- CH-104.2; 2'- CH-75.8; 3'- CH-78.9; 4'- CH-72.3; 5'- CH-78.4; 6'- CH2-63.4. (Fig. 6).



Figurre 6: [13]C-NMR Spectra of isolated compound

Through evaluation with the literature it was confirmed that the compound was Arbutin (Fig. 7).

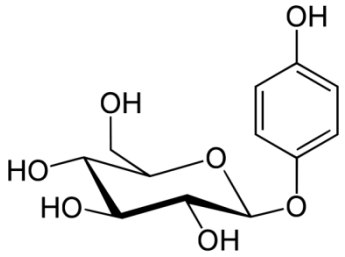


Figure 7: Structure of Arbutin isolated from leaf of *C. nitidum*

4. Discussion

Arbutin is a naturally occurring beta-D-glucophyranoside of hydroquinone [14] which was isolated from *C. nitidum* leaf extract. Significant amounts of arbutin levels were found in wheat products, pears, coffee, tea [15]. It is an inhibitor of melanin formation and a skin-lightering agent that is induced in compositions used for treating skin cancer [16,17]. This is effective in the topical treatment of various cutaneous hyperpigmentations characterized by hyper active melanocyte function. Arbutin could alleviate oxidative stress derived from the melanogenic pathway in the skin in addition to its function as a whitening agent in cosmetics[14,18]. In a study on healthy adults who were exposed to ultraviolet (UV) radiation followed by topical treatment with arbutin, hyperpigmentation was inhibited remarkably was reported by[19-21]. This compound inhibited UV-induced nuclear factor-kappa B activation in human keratinocytes [22] Arbutin significantly decrease the number, intensity and frequency of coughs[23]. This compound exhibited potent inhibitory effects on rat platelet aggregation induced by adenosine diphosphate (ADP) and collagen [24]. Arbutin decreasing phospholipase D, myeloperoxidase and elastase activity and suppress the onset and progression of inflammation [25]. It is used as an anti-infective for the urinary system as well as a diuretic [26]. Arbutin inhibited the growth of human colon carcinoma HCT-15 cells [27]. Toxicogenomics of A 375 human malignant melanoma cells treated with arbutin have been elucidated using DNA microarray. The interactions network of 14 differentially expressed proteins was found to be correlated with the downstream regulation of p53 tumour suppressor and cell apoptosis. These proteins were found to play important roles in the suppression of cancer development[28].

5. Conclusion

Arbutin is a glycosylated hydroquinone compound isolated from the leaf of *C.nitidum.* This compound inhibits the melanin formation in the skin and commercially used as a skin-lightering agent.

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