

Isolation And Characterisation of Indian Liquorice Seeds by Uv-Vis Spectroscopy, Ft-Ir, Rp-Hplc, And Gc-Ms

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Abstract: Abrus precatorius plant is commonly called Indian liquorice, Rosary pea, Jequirity bean, crab'eye, and Gunja. The methanol crude extract CMME was prepared from the dried powdered Abrus precatorius seeds by the maceration method for 7 days. The compounds from the crude extract CMME were isolated by column chromatography using silica gel 60-120 mesh as the adsorbent. The isolated compounds are further purified by Thin Layer Chromatography. The compound that has the same RF value is combined. A total of three compounds are isolated from CMME and are named CMME I, CMME II, and CMME III. The first-eluted, second-eluted, and third-eluted compounds are CMME I, CMME II, and CMME III respectively. The RF value of the isolated compounds was determined by using a small TLC chamber and methanol as solvent. The spot was determined by the Iodine chamber method. The RF values of CMME I, CMME II, and CMME III were found to be 0.92, 0.74, and 0.67 respectively. The isolated compounds were characterized by UV-VIS spectroscopy, FT-IR, RP-HPLC, and GC-MS. The λ_{max} of the crude extract CMME was found to be 346.20nm. The λ_{max} of the separated compounds CMME I, CMME II, and CMME III was found to be 355.45nm, 318.17nm, and 298.20nm correspondingly by using solvent methanol as blank. The absorption frequencies from $4000-667\text{ cm}^{-1}$ were used for the structure elucidation, ($4000\text{ cm}^{-1}-1300\text{ cm}^{-1}$) was used as the high-frequency region and ($1300\text{ cm}^{-1}-667\text{ cm}^{-1}$) was used as the fingerprint region for the interpretation of CMME I, CMME II and CMME III by FTIR spectroscopy. In the RP-HPLC chromatogram 1.226min, 2.038min and 8.963 min is the retention time for three isolates CMME I, CMME II & CMME III correspondingly. The peak present at the m/z ratio 281.0 of the GC-MS spectra of compound CMME I indicate the presence of (4, 6-O- Benzylidene) methyl-alpha D-glucopyranoside, molecular formula C₁₄H₁₈O₆. The peak present at the m/z ratio 222 and 429.1 of the GC-MS spectra of compound II indicates the presence of isoflavone base and the presence of Malonyl Hexose respectively. So the presence of the peaks at the m/z of 222 and 429.1 of the GC-MS spectra of compound CMME II indicate the presence of Isoflavone base 20-O-malonyl Hex. The peak present at the m/z ratio 175.0 of the GC-MS spectra of compound CMME III indicates the presence of Trigonelline hydrochloride, molecular formula C₇H₈CINO₂. The retention time of the CMME I, CMME II, and CMME III was found to be 16.969, 17.253, and 17.310 min respectively.

Keywords: UV-VIS spectroscopy, FT-IR, RP-HPLC, GC-MS.

Introduction

The WHO (World Health Organization) described therapeutics from plants as herbal preparations prepared by extraction, fractionation, purification, concentration, or other physical or biological procedures which may generate nutritional or medical constituents for immediate consumption or as a basis for herbal products [1]. A few creators characterized Medicinal plants as plants possessing dynamic fixings utilized to fix infection or ease torment [2]. Therapeutic plants have been utilized for quite a long time as solutions for human infections and deal with another wellspring of organically dynamic substance compounds as antimicrobial specialists [3]. In the entire world, India is the most perceived country for flavors and customary therapeutics; these are having a more scope of physiological and pharmacological features [4]. Numerous plants have been utilized in customary therapeutic for a long time. Such plants ought to certify as medicinal plants. The spot of restorative plants in forestalling normal illness is additionally examined under the five centre standards of the Primary Health Care (PHC) approach. Medicinal plants assume an important place in illness avoidance and their advancement and utilization qualify for all current anticipation methods [5]. The term therapeutic plants incorporate various types of plants utilized in herbalism and a part of these plants have a medicinal effect [6]. Medicinal plants are the most extravagant bio resource of therapeutics in the conventional arrangement of drugs and they are moreover answerable for various tones, flavors, and smells of the plant. They likewise play as therapeutics [7]. The plant kingdom is a treasured source of potential therapeutics and in recent years there has been growing awareness about the essence of therapeutic plants [8]. One of the points of therapeutic plant research is the isolation and distinguishing evidence of usually happening substances. Compound investigation of concentrates from plant material assumes a focal place being advanced and modernization of natural therapeutics [9]. Therapeutic plants are utilized for healing as well as for curing human ailments because of the presence of phytochemical compounds [10]. Therapeutic plants are currently in impressive essential view because of their exceptional properties as a huge wellspring of remedial phytochemicals that might prompt the development of new therapeutics. Most of the phytochemicals from plant origins like phenolics and flavonoids emphatically influence well-being and ailment counteraction [11]. Plants consist of various dynamic mixtures like alkaloids, steroids, tannins, glycosides, unpredictable oils, fixed oils, pitches, phenols, and flavonoids which are gathered in their specific places, for instance, leaves, blossoms, bark, seeds, natural products, root, and so forth. The functional restorative impacts of plant materials ordinarily result from the mix of these auxiliary things [12]. Countless phytochemicals having a place with some substance classes have been shown to have inhibitory consequences for a broad

range of microorganisms in vitro [13]. Plant items have been essential for phytomedicines since time prehistoric. This can be acquired from barks, leaves, blossoms, roots, natural products, and seeds [14]. Phytochemicals have been separated and derived from organic products. Phytochemicals are primary and secondary constituents. Chlorophyll, proteins, and common sugars are included in primary compounds and secondary constituents have terpenoids, alkaloids, and phenolic constituents [15]. Among dynamic phytochemicals are phenolic substances, or polyphenols, results of the auxiliary digestion encompass one of the most numerous and commonly disseminated gatherings of constituents in the plant [16]. The chemical compounds that are exhibited in herbal materials have shown a wide range of uses in the control of numerous ailments including challenging ailments/conditions such as HIV/AIDS, cancer, sickle cell disease, malaria, and other infectious illness as well as non-infectious illness such as diabetes, obesity, infertility, and so on.

2. Materials and Methods

2.1 Collection and Identification of Sample

The *Abrus precatorius* seeds are purchased from the Meenakshi sundharanar shop present in the Rajapalayam and identified and authenticated by the botany department of Ayya Nadar Janaki Ammal College, Savakasi. The seeds are washed with distilled water and dried in the absence of sunlight. The dried seeds are converted into coarse powder by using a mechanical grinder and then stored in an air-tight container.

2.2 Method of Preparation of Sample

60 grams of the powdered *Abrus precatorius* seeds were taken in a 1000 ml volumetric flask previously washed with methanol. 600 ml of the methanol was added and then shaken well by closing the lid and then allowed to cold maceration for 7 days. During the process of maceration, the volumetric flask was shaken several times to get a better extraction. After 7 days the extracted solvent is filtered through Whatman filter paper no 1 and evaporated at room temperature. Finally, the crude extracts are dried under a vacuum. The methanol crude extract of *Abrus* seeds was named CMME. The three isolates were isolated from the crude extract CMME of *Abrus precatorius* seeds by column chromatography using silica gel 60-120 mesh as the adsorbent and methanol as the mobile phase. The separated compounds are more purified by TLC. The compounds that have the similar RF value are mixed together. The separated constituents were named CMME I, CMME II, and CMME III.

2.3 Determination of RF Value:

The retardation factor (RF) value is defined as the ratio between the distance travelled by solute and the distance travelled by solvent. Unit is cm. The RF value should be 0 - 1.

Procedure:

10 ml of the methanol was taken as a mobile phase in the three different small TLC chambers. The chamber was closed and allowed to chamber saturation for 30 min. The 10 cm length and 3.3 cm breath TLC glass plate were taken. The TLC plate was prepared by pouring method from the slurry of the Silica gel G and distilled water. After preparation of the TLC plate, it was activated in the hot air oven for 30 min at 105°C to remove the moisture present in the stationary phase. The eluted compound CMME I, CMME II, and CMME III was taken in the three separate capillary tubes and applied as a small spot on the three separate TLC plates at 2 cm above from the base of the plate and allowed to dry in the air. The three TLC plate was kept inside the three different small TLC chamber containing methanol as the mobile phase. The solvent was allowed to travel the $\frac{3}{4}$ distance of the plate and then the plate was removed from the TLC chamber and dried in the air and then kept in the Iodine chamber. Three different colored spots were obtained for the three eluted compounds. The RF value was calculated by measuring the distance travelled by the solute and the distance travelled by the solvent in cm. The result was presented in table 1. The structure of the small TLC chamber and TLC plates was present in the fig 1 and 2 correspondingly. The RF value was determined by the given below formula.

RF value = Distance travelled by the solute / Distance travelled by the solvent.

2.4 Determination of Isolated compounds by UV-VIS spectroscopy

Instrument Name: Double Beam UV-VIS spectrophotometer

Model: UV-1800

Company Name: Shimadzu

Solvent: Methanol

Sample cell: Quartz

Path length: 1 cm.

The λ_{\max} of the crude extract CMME and the isolated compounds CMME I, CMME II, and CMME III was determined by selecting the scan range of 200 nm to 600 nm and methanol as the solvent. The Quartz sample cell of path length 1cm was used to hold the sample. The crude extract and the isolated compounds have the λ_{\max} at the UV radiation wavelength range (200nm to 400nm). The UV-VIS spectra of compound CMME, CMME I, CMME II, and CMME III were present in fig 3.

2.5 Determination of Isolated compounds by FT-IR spectroscopy

The methanol-dissolved liquid form of CMME, CMME I, CMME II, and CMME III is used for the determination by FT-IR spectroscopy. A few drops of the liquid sample of CMME, CMME I, CMME II, and CMME III were placed separately on the Zinc Selenide crystal and then the IR spectrum was recorded in the wave number range between 4000-667 cm^{-1} . The absorption frequencies from 4000-667 cm^{-1} were used for the structure elucidation, (4000 cm^{-1} -1300 cm^{-1}) was used as the high-frequency region and (1300 cm^{-1} – 667 cm^{-1}) was used as the fingerprint region for the interpretation of CMME I, CMME II and CMME III by FTIR spectroscopy. The FT-IR spectra of CMME I, CMME II, CMME III, and Blank Methanol were present in figs 4, 5, 6, and 7 correspondingly.

2.6 Determination of Isolated compounds by RP-HPLC

Procedure

10 mg of the sample CMME, CMME I, CMME II, and CMME III was taken and dissolved in 10 ml of the HPLC grade methanol and then filtered through the Whatman filter paper no 1. From this filtrate 1ml was taken and made up to volume 10 ml with HPLC

grade methanol; it will produce a concentration of 100µg/ml. The 100µg/ml of the solution was injected by using a rheodyne injector. The HPLC spectra were recorded by using 70 % 0.05M potassium phosphate monobasic buffer pH 5 with 1mKOH and 30% Acetonitrile as the mobile phase and by using the column C18, 250 X 4.6 mm, 5micron, at the flow rate of 1ml/min at ambient temperature using HPLC Shimadzu lc 2010cht system and UV - VIS detector and isocratic pump at a wavelength 235nm. The HPLC chromatogram for Blank methanol, CMME, CMME I, CMME II, and CMME III fig 8, 9, 10, 11, and 12 correspondingly.

2.7. Determination of Isolated compounds by GC-MS

A gas chromatography 6890 equipped with an electronically controlled split less injection port and coupled with a single quadrupole inert mass selective detector 5973 with an electron impact ionization chamber was used for the GC-MS analysis. GC separation was performed on a DB-624 capillary column (60m X 0.25mm X 1.4µm). Helium was the carrier gas with a constant pressure of 25.6 psi. About 1µl of the sample solution was injected in split less mode at 260°C. The initial temperature of the oven was 150°C and ramped with a rate of 10°C per minute until achieving 260°C. The temperature was held at 260°C for not less than 25 minutes. Mass spectrometric parameters were set with electron impact ionization energy of 69.9eV, ion source temperature of 230°C, and MS quadrupole temperature of 150°C. The MS system was commonly arranged in selective ion monitoring (SIM) mode. The low boiling solvent methanol was taken in three different GC vials up to the mark 1.5ml and then one drop of the isolated compounds CMME I, CMME II, and CMME III was added to the GC vial containing methanol solvent separately. Cap the vial and invert it once or twice to dissolve the sample. The gas chromatography was run. The GC-MS spectra of the isolated compounds CMME I, CMME II and CMME III was recorded and present in fig.13, 14, and 15 correspondingly. The GC-MS spectra of compounds CMME I, CMME II, and CMME III are used for the interpretation of the molecular weight and the structure of the compound. The structure of compounds CMME I, CMME II, and CMME III are present in figs 16, 17, and 18 correspondingly.

3. Results and Discussions

The crude extract of the *Abrus precatorius* seeds was prepared by maceration for 7 days by using methanol as solvent. The compounds from the crude extract CMME were isolated by column chromatography using silica gel 60-120 mesh as the adsorbent. The isolated compounds are further purified by Thin Layer Chromatography. The compound that has the same RF value is combined. The RF value of the isolated compounds was determined by using a small TLC chamber, with methanol as solvent. The spot was determined by the Iodine chamber method. The RF values of CMME I, CMME II, and CMME III were found to be 0.92, 0.74, and 0.67 respectively. The λ_{max} of the crude extract CMME was found to be 346.20nm. The λ_{max} of the separated compounds CMME I, CMME II, and CMME III was found to be 355.45nm, 318.17nm, and 298.20nm correspondingly by using solvent methanol as blank.

In the FT-IR spectra of CMME I, the compound CMME I have the peaks at the following wave numbers 1116.78 cm^{-1} (C-O stretching), 1415.75 cm^{-1} (OH-bending), 1450.47 cm^{-1} (occurrence of aromatic ring), 2831.50 cm^{-1} (Carbon Hydrogen-stretching), 2943.37 cm^{-1} (CH-stretching-alkyl), No absorption in the region 1900 – 1600 cm^{-1} indicates the absence of carbonyl group, 3329.14 cm^{-1} (OH-stretching),

In the FT-IR spectra of CMME II, the compound CMME II has peaks at the following wave numbers 1114 cm^{-1} (C-O stretching), 1415.75 cm^{-1} (OH-bending), and 1452.40 cm^{-1} (occurrence of aromatic ring), 2831.50 cm^{-1} (CH-stretching), 2943.37 cm^{-1} (CH-stretching-alkyl), 3329 cm^{-1} (OH-stretching).

In the FT-IR spectra of CMME III, the compound CMME III has the peaks at the following wave numbers 1114 cm^{-1} (C-O stretching), 1415.75 cm^{-1} (OH-bending), 1450.47 cm^{-1} (occurrence of aromatic ring), 2362.80 cm^{-1} (OH-stretching), 2831.50 cm^{-1} (CH-stretching), 2943.37 cm^{-1} (CH-stretching-alkyl), 3329.14 cm^{-1} (OH-stretching).

The RP-HPLC chromatogram of CMME indicates that it contains three major compounds. The retention time of CMME I, CMME II, and CMME III was found to be 1.226, 2.038, and 8.963 minutes correspondingly.

The peak present at the m/z ratio 281.0 of the GC-MS spectra of compound CMME I indicate the presence of (4, 6-O- Benzylidene) methyl-alpha D-glucopyranoside, molecular formula C₁₄H₁₈O₆.

The peak present at the m/z ratio 222 and 429.1 of the GC-MS spectra of compound II indicates the presence of isoflavone base and the presence of malonyl hexose respectively. So the presence of the peaks at the m/z of 222 and 429.1 of the GC-MS spectra of compound CMME II indicate the presence of Isoflavone base 20-O-malonyl Hex.

The peak present at the m/z ratio 175.0 of the GC-MS spectra of compound CMME III indicates the presence of trigonelline hydrochloride, molecular formula C₇H₈ClNO₂.

Table: 1 Determination of RF value

| Compounds | Distance Travelled by Solute (cm) | Distance Travelled by Solvent (cm) | RF Value (cm) | RF value Average (cm) |
|-----------|-----------------------------------|------------------------------------|---------------|-----------------------|
| CMME I | 5.5 | 6 | 0.92 | 0.92 |
| | 5.5 | 6 | 0.92 | |
| CMME II | 4.5 | 6 | 0.75 | 0.74 |
| | 4.5 | 6 | 0.75 | |
| CMME III | 4 | 6 | 0.67 | 0.67 |
| | 4 | 6 | 0.67 | |



Fig: 1 Small TLC Chamber and Iodine chamber

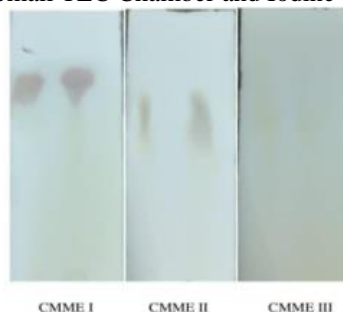


Fig: 2 Determination of RF Value

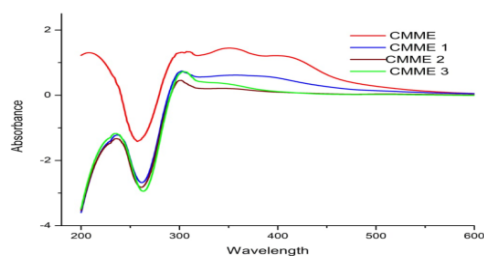


Fig: 3 UV-VIS spectra of CMME, CMME I, CMME II & CMME III.

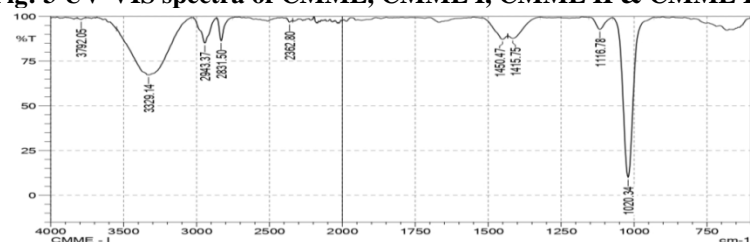


Fig: 4 FTIR spectra for sample CMME-I

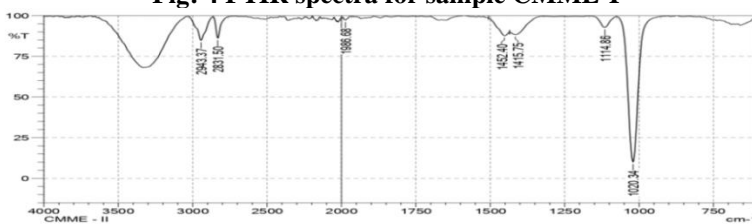


Fig: 5 FTIR spectra for sample CMME-II

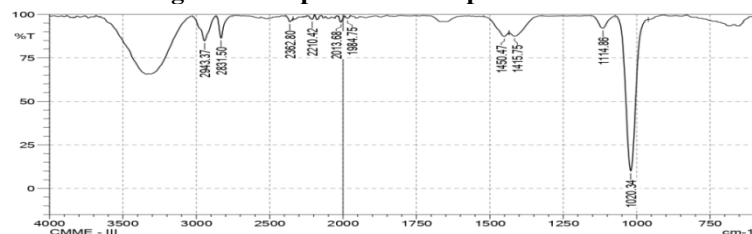


Fig: 6 FTIR spectra for sample CMME-III

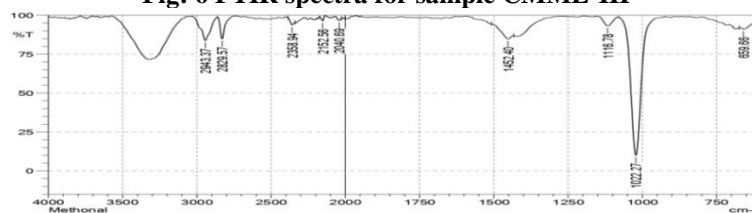


Fig: 7 FTIR spectra for Blank Methanol

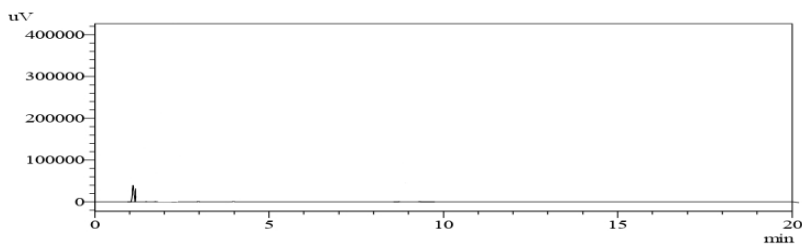


Fig: 8 HPLC Chromatogram of Blank Methanol

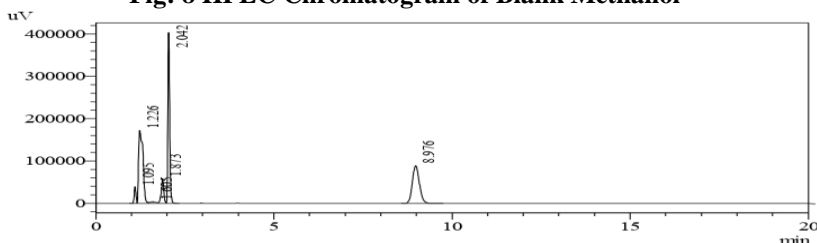


Fig: 9 RP-HPLC chromatogram for CMME

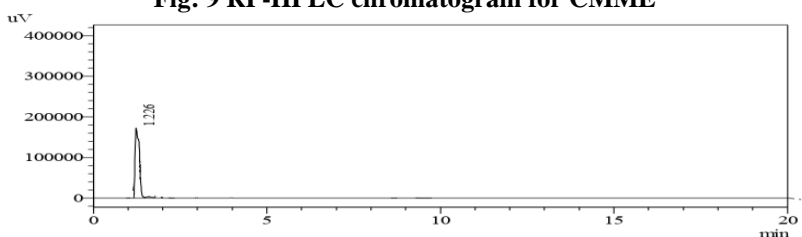


Fig: 10 RP-HPLC chromatogram for CMME I

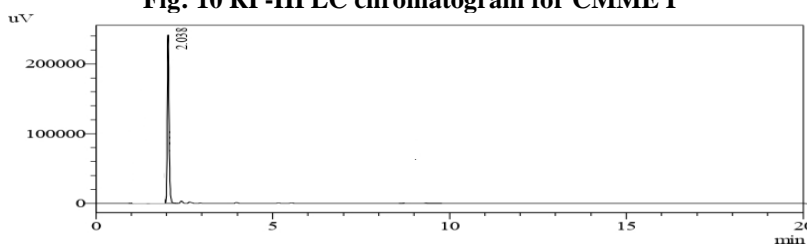


Fig: 11 RP-HPLC chromatogram for CMME II

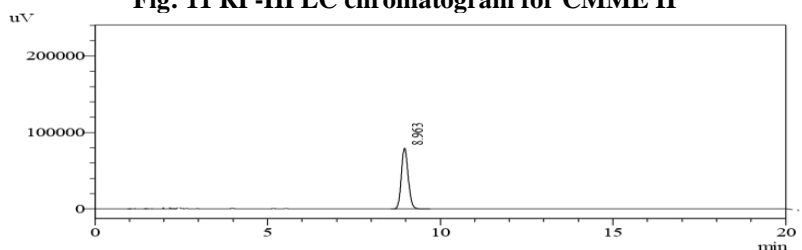


Fig: 12 RP-HPLC chromatogram for CMME III

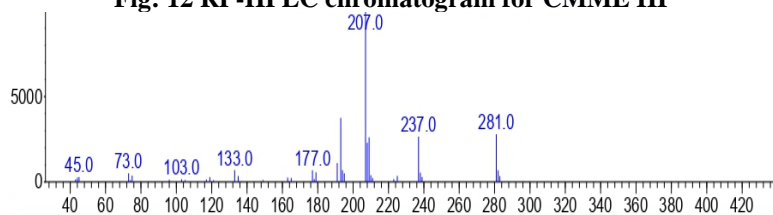


Fig: 13 GC-MS spectra of compound CMME-I

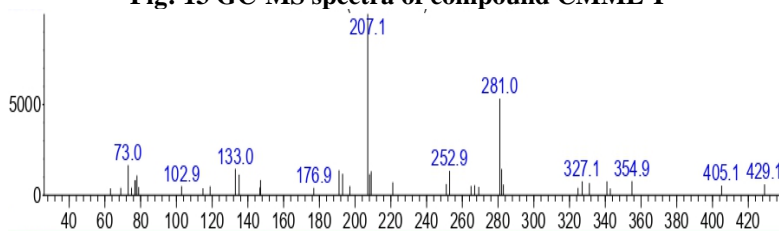


Fig: 14 GC-MS spectra of compound CMME II

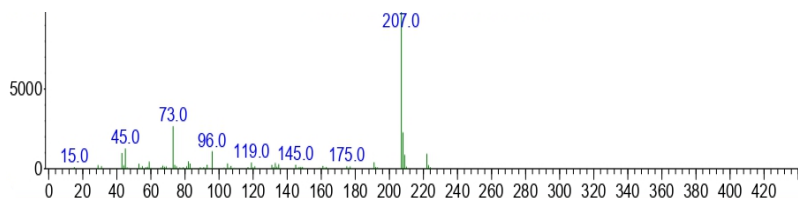


Fig: 15 GC-MS spectra of CMME III

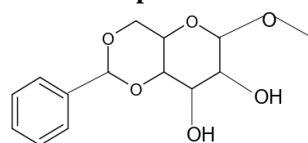


Fig: 16 Structure of compound I

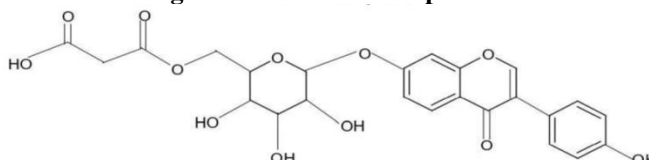


Fig: 17 Structure of Compound II

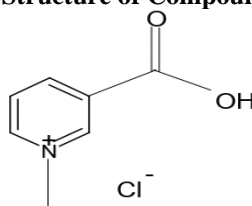


Fig: 18 Structure of compound III

Conclusion

The RF values of CMME I, CMME II, and CMME III were found to be 0.92, 0.74, and 0.67 respectively. The CMME and the isolated compounds CMME I, CMME II & CMME III were characterized by UV-VIS spectroscopy, FT-IR, RP-HPLC, and GC-MS. The CMME and all the isolated constituents have the absorption maximum at the UV radiation wavelength range like 200 nm-400 nm was found by Double beam UV-VIS spectroscopy utilizing methanol as a solvent. The absorption maximum of the crude extract CMME was found to be 346.20 nm. The absorption maximum (λ_{max}) of the separated compounds CMME I, CMME II, and CMME III was found to be 355.45 nm, 318.17 nm, and 298.20 nm respectively by utilizing methanol as blank. The absorption frequencies from 4000-667 cm^{-1} were used for the structure elucidation, (4000 cm^{-1} -1300 cm^{-1}) was used as the high-frequency region and (1300 cm^{-1} – 667 cm^{-1}) was used as the fingerprint region for the interpretation of CMME I, CMME II and CMME III by FTIR spectroscopy. The qualitative analysis was done on the methanol extract of the Abrus seeds and its isolated compounds by RP-HPLC. The RP-HPLC chromatogram obtained from the CMME consists of three peaks indicating that it contains three major constituents. The three isolates CMME I, CMME II & CMME III has the retention time of 1.226, 2.038 and 8.963 min correspondingly. The peak present at the m/z ratio 281 of the GC-MS spectra of compound CMME I indicate the presence of (4, 6-O- Benzylidene) methyl- α D-glucopyranoside, molecular formula $C_{14}H_{18}O_6$. The peak present at the m/z ratio 222 and 429.1 of the GC-MS spectra of compound II indicates the presence of isoflavone base and the existence of Malonyl Hexose respectively. So the existence of the peaks at the m/z of 222 and 429.1 of the GC-MS spectra of compound CMME II reveal the existence of Isoflavone base 20-O-malonyl Hex. The peak present at the m/z ratio 175.0 of the GC-MS spectra of compound CMME III indicates the existence of Trigonelline hydrochloride, molecular formula $C_7H_8ClNO_2$. The retention time of the CMME I, CMME II, and CMME III was found to be 16.969, 17.253, and 17.310 min respectively.

DECLARATION OF INTEREST

The author describes no conflicts of interest. The authors solely are liable for the content and writing of this article.

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