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Isolation and characterization of three isolates of *Abrus precatorius* seeds by LC-MS, ¹HNMR, And ¹³CNMR

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Abstract

The three isolates were isolated from the methanolic extract of *Abrus precatorius* seeds by column chromatography using silica gel 60-120 mesh as the adsorbent and methanol as the mobile phase. The isolated compounds are further purified by TLC. The compounds that have the same RF value are combined. The crude extract was named CMME. The isolated compounds named CMME I, CMME II, and CMME III are first-eluted, second, eluted, and third-eluted compounds respectively. The isolated compounds were characterized by LC-MS, ¹H NMR, and ¹³C NMR. The compound I was found to be (4,6-O-Benzylidene) methyl- α -D-glucopyranoside, m/z 282.3923, molecular formula C₁₄H₁₈O₆. The compound II was found to be Isoflavone base + 20, O-Malonyl Hex, m/z 502.42801, molecular formula C₂₄H₂₂O₁₂. The compound III was found to be 3-Carboxy-1-methyl pyridinium chloride, m/z 173.6, molecular formula C₇H₈CINO₂.

Keywords: Column chromatography; LC-MS; ¹H NMR; ¹³C NMR

1. Introduction

Plant extracts and their primary and secondary metabolites have main medicinal activity in the cure of many human ailments. *Abrus* seeds are utilized in the therapy of diabetes and chronic nephritis. The white colour *Abrus* seeds are utilized to synthesize oil that is used as an aphrodisiac. The hot water extraction of the *Abrus* seeds is used as an antifertility agent, abortifacient, and to stop conception by being taken orally. [1] India is one of the biggest manufacturers of herbs and herbal products. The huge assets of vegetables and therapeutic plants have been utilized regularly for the therapy of different ailments [2]. Medicinal plants are accepted to be a main source of new chemical substances with significant medicinal efficacy. As per WHO longer than 21000 plants are utilized as therapeutic plants. [3] *Abrus precatorius* is conventionally utilized in the therapy of tetanus, scratches, sores, and wounds produced by animals to stop rabies. Hot water extracts of dried leaves and roots are used for eye diseases.[4] Seeds are taken inwardly in the emotions of the nervous system and their paste is introduced locally in sciatica, stiffness of shoulder joints, paralysis, and eye diseases.[5] According to World Health Organization (W.H.O.) more than 85% of the populations in Sub-Sahara Africa, along with Nigeria still base on herbal conventional therapeutics for their healthcare requirements.[6] *Abrus precatorius* consists of antiseptic and – anti-inflammatory activity. It is used in the therapy of wounds. [7] The medicinal benefit of herbals has been well documented by the old Indian and Chinese systems of medicines[8] *Abrus precatorius* is declared as the richest origin of distinctive natural products for the development of therapeutics against different ailments and as well as for the preparation of industrial products.[9] The aerial parts of the *Abrus precatorius* plants are utilized for the therapy of leucorrhoea, gonorrhoea diarrhea, and dysentery.[10] *Abrus precatorius* is largely utilized in traditional medicine for the cure of various illnesses.[11] In west tropical Africa, the leaves of *Abrus precatorius* are utilized to sweeten foods and are also utilized as therapeutics for stomach problems and also utilized to cure fever, cough, and cold. [12]. The seeds are used for tuberculosis and painful swellings. The leaves

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are used in Nigeria for the therapy of many ailments including malaria, typhoid, cough, respiratory tract infections, and hepatitis.[13]. Plants have a huge numerous of organic & inorganic substances and a thorough knowledge of these substances is much important to realize the effect of drugs and the mechanism of action of the drug. [14]. in shorter than a quarter of a century, diabetes has become a public health issue in developing countries. *Abrus precatorius* has antidiabetic activity. [15]. *Abrus precatorius* L is also called as Ratti in hindi and Gunja in Sanskrit. [16]. *Abrus precatorius* plant has been used as a therapeutic agent from extremely ancient times not merely in this subcontinent but also China and other prehistoric culture. In specific tribal regions, people chew the leaf of *Abrus precatorius* for the alleviation of mouth ulcers. [17]. Various types of secondary compounds have been separated from this species, including alkaloids, steroids, and other triterpenoids, isoflavanoquinones, anthocyanins, starch, tannin, protein, flavonoids, phenolic compounds, fixed oil, and amino acid.[18]. Abrin of the seeds is responsible for anticancer activity. Seed Kernel consists of abrectorine and glycosides desmethoxycentaureidin 7-O-rutinoside, 8-C-glucosylscutellarine 6, 7-dimethyl ether, 2-O-apioside flavones C-glycoside, alkaloids methyl ester of N-N dimethyltryptophan metho cation and precatorine. [19]. *Abrus precatorius* plant contains different types of amino acids like Serine, Alanine, Valine, Choline, and methyl ester. [20].The flowers of the *Abrus precatorius* plant appear in the winter and the fruits of the *Abrus precatorius* plant ripen in late summer. The fruit pod contains 3-8 shiny hard seeds 6-7 mm in diameter.[21]. The roots, leaves, and seeds are utilized for therapeutic purposes in Ayurveda, Siddha, and Unani.[22]. Around 80,000 species of plants are used for curing numerous ailments. Most pharmaceutical companies exhibit attentiveness to green medicine because of its safety. [23]. *Abrus precatorius* possesses an essential role in the cure of conjunctivitis in numerous places of the world. [24]. In herbal medicine, the paste of seed is applied topically for skin ailments, and leaves are utilized as replacements for licorice, which is examined for biliousness, leukoderma, itching, and other skin ailments. Roots were utilized as a diuretic. [25]. The roots of *Abrus precatorius* have been utilized since the early 19th century as a replacement for licorice root, the origin of the sweet glycyrrhizin.[26]. Gujabhatra Rasa and Mahalaxminarayana Taila have been broadly utilized in the cure of Vata Vyadhi. [27]. Abrin, Saponins, and saponenols are examined to be utilized as an antifertility agents. [28]. Flavonoids are extensively dispersed in plants and have been described to use several biological effects, as well as, antioxidant, free radicals scavenging capacities anti-inflammatory, and anticancer activity. [29]. Leaves of the *Abrus precatorius* were utilized to cure mouth cancer in Malaysia. [30]. The Birth of the *Abrus precatorius* plant is in Southeast Asia. [31]. Roots consist of an excessive amount of glycyrrhizin than the leaf. [32]. *Abrus* means beautiful or graceful. It is utilized to represent the appearance of the seed. [33]. Chewing of the root is utilized for the treatment of snake bites. [34]. *Abrus precatorius* seeds have been described as having a poisonous effect on the kidney, liver, heart, spleen, intestine, and lungs. [35]. It is encouraged commonly on other plants or a fence. [36]. *Abrus precatorius* is an alternative to *Glycyrrhiza glabra*. [37]. Because of the content's medicinal value medicinal plants have been of age lengthy treatment for human ailments. [38]. *Abrus precatorius* seeds have a constant weight of 1/10 of a gram. They were utilized as common weights for weighing gold and silver in previous times.[39].It was described that as small as 0.00015% of poison per body can produce death in humans.[40]. *Abrus precatorius* contains choline and other biological substances having medicinal value. [41]. In addition to other medicinal uses, the Ayurvedic pharmacopeia of India has suggested the use of seeds for baldness.[42]. *Abrus precatorius* seeds contain polyphenolic compounds and thus could have medicinal value. [43]. *Abrus precatorius* plant has been utilized in Hindu therapeutics from ancient times in china and other earliest culture. [44]. Therapeutic plants work as a large origin of antioxidant compounds that are not injurious, economical, and freely obtainable. [45]. *Abrus precatorius* seeds were utilized in the therapy of fractures in veterinary medicine. [46]. *Abrus precatorius* is growing only in shady locations. [47]. Plants are the essential origin of biologically active molecules for medicine detection. [48]. *Abrus precatorius* plant extract was created to reduce the amount of hepatitis B virus developed in the bloodstream. [49]. *Abrus precatorius* extracts have been accepting awareness as antitumor agents as it has been revealed that numerous phytochemicals from *Abrus precatorius* have the characteristic to induce cell death in numerous types of cancers. [50]. A property of plant life is the manufacture of a huge number of natural compounds frequently called secondary metabolites. [51]. Crude herbs are utilized as medicines in various countries of the world and hence take up a fundamental portion of numerous drugs worldwide. [52]. Ayurveda prescribes the direction of gunja in ailments like Indralupta, Sotha, Kemi, Kustha, Kandru, Prameha, etc., after being used with particular Sodhana procedures. [53]. *Abrus precatorius* is one of the 60 plants utilized by a conventional therapist in Tanzania for the therapy of epilepsy. [54]. *Abrus precatorius* seeds were used to reduce elevated blood pressure and mitigate acute headaches. [55]. *Abrus precatorius* is a pea family. [56]. Essential oils obtained from plants have been authenticated safe as natural antioxidants, and little so far promoted as digestive improvers along with in elimination of various worst ailments.[57]. Medicinal plants are a role group of human society to battle ailments, from the birth of advancement. [58]. *Abrus precatorius* seeds have special red background along with black like the eyes of a chicken. Hence, the seeds are called chicken eye pearl (Ji mu zhu) in Fujian, Guangdong, and Taiwan, and at the same time in Guangxi, they are also popularly known as monkey eye. [59]. *Abrus precatorius* has been utilized by Nupes in the Bida emirate of Niger state, Nigeria, for the cure of considered acute and chronic cases of malaria mainly when the use of orthodox antimalarials potency has declined.[60]. The greatest toxic portion of the plant is the seed.[61]. *Abrus precatorius* plant is feasibly utilized in the cure of immune-composed diseased states in animals. [62]. *Abrus precatorius*-derived lectins have been mostly utilized in curing numerous tumors. [63]. Ayurvedha physicians profitably utilized

Abrus precatorius in the cure of ailments after suitable purification. [64].Crushed seeds are utilized as cattle toxic which counterfeit viper bite. [65].Triglyceride was established in the peduncle, and at the same time, stigmaterol and β -sitosterol were established in the seeds. [66]. Impurity is any ingredient of the drug product that is not a chemical substance. [67].*Abrus precatorius* is generally called a saga-saga. [68].*Abrus precatorius* seeds consist of significant HIV-1 PR inhibitory action. [69]. Native traditional herbal specialists utilize aqueous infusion or extracts (cold or hot) of leaf, seed, and root of *A.precatorius* for the therapy of intestinal diseases that could be of bacterial, viral, or protozoan origins.[70]. *A. precatorius* was first reported as a medicinal plant by William Boericke in the Homoeopathic Materia Medica entitled Jequirity. *Abrus precatorius* seeds extract can be accepted orally to treat malaria.[71]. Natural antioxidants available in food and other biological materials have devoted substantial interest because of their assumed safety and prospective nutritional and therapeutic effects. Because the extensive and expensive evaluation of food additives is compulsory to meet safety standards, synthetic antioxidants have commonly been removed from many food applications. The growing interest in the exploration of natural replacements for synthetic antioxidants has led to the antioxidant assessment of several plant sources, especially spices and herbs. A large number of plants have been showing as possible origins of natural antioxidants including tocopherol, vitamin C, carotenoids, and phenolic compounds which are answerable for the maintenance of health and preservation from coronary heart diseases and cancer. At the current time, medicinal plants as a rich source of natural bioactive components are given priority to study their antioxidant activity and explore their utilization in the treatment of diabetes mellitus, dyslipidemia, and cardiovascular diseases.[72]. Major constituents of more than 50% of all the drugs in clinical use are natural products and their derivatives. [73]. It has 45000 plant species of which 15000-20000 medicinal plants retain proven therapeutic value (Krishna Kumar, 1996). [74]. *Abrus precatorius* seeds are slightly smaller than ordinary peas; ovoid and scarlet with a black spot around the hilum. The root is woody, tortuous, and much branched, with a sweet taste [75]. *Abrus precatorius* leaf is effective in wound healing, infections with acne sores, or boils and wounds. It also helps in getting rid of itching and other skin-related problems. The plant kingdom synthesizes diverse active compounds which are valuable in the treatment and control of many diseases. These compounds are principally secondary metabolites. [76]. It is endemic in the tropics and generally known as the Rosary bean. It is known as Otuobiribiri (Igbo-Ohafia), Idon zakara (Hausa), Oju ologbo (Yoruba) in Nigeria. The roots of *A. precatorius* possess proteins, glycosides, phenolic compounds, fatty acids, fatty acid esters, anthocyanins, and minerals. [77]. The leaves are glabrous with long internodes. It has slender branches with a cylindrical wrinkled stem with a smooth textured brown bark (Hara and Williams, 1979; Fernando, 1988). Its roots are deeply and tenaciously difficult to be eradicated. It increases in population size following a fire (Holm et al., 1991). [78]. Plants have been extensively used to treat various diseases. The practice of using plants as a source of medicine could be traced back as far back as the beginning of human civilization. The earliest mention of the use of plants to treat diseases in Hindu culture is found in –Rigveda which was written between 4500 -1600 BC. [79]. Innumerable research has been reported in various parts of *Abrus precatorius* L. using different extracts for its pharmacological and therapeutic application (Attal et al., 2010;Rajaram and Janardhanan, 1992; and Pandey, 1994). Furthermore, the plant is been a valuable source of natural products for the development of medicine against various diseases and industrial products (Lebri et al., 2015). Different active components of plant tissue can be separated using appropriate solvents (Njila et al., 2017; Amita and Shalini 2014). Despite many studies having been reported on this plant species, the climatic and geographic conditions also impose a distribution of highly active components. [80]. The origin place of renewed systems of indigenous medicine like Siddha, ayurvedha, and unani is India. The medicines that were traditionally used were prepared from only one plant.The activity of medicine depends on the proper parts of plant use and its biological effect which in turn depends on the presence of the required quantity dose and nature of secondary metabolite in a raw drug material. The different chemical constituents are detected in crude dry powder of various medicinal plants, while different parts extracted like leaf, stem, fruits, and root was screened for phytochemical constituents by FT-IR spectroscopic analysis technique. The different active functional groups of chemical components in various extracts of medicinal plants were detected using the spectroscopic method.[81]. Medicinal plants are vital biological sources whose parts (leaves, seeds, stems, roots, fruits, foliage, etc.) extracts, decoctions, infusions, and powders are used in the treatment of various diseases of humans, plants, and animals. Plant extracts are highly efficient against microbial infections. It is estimated that over 70,000 plant species, from lichens to tall trees, have been utilized at one time or another for medicinal purposes. Secondary metabolites such as alkaloids, tannins, flavonoids, and phenolic compounds are rich in plants which have been found in-vitro to have antimicrobial properties. [82]. One of the endangered plant species of the flora of Saudi Arabia is *Abrus precatorius*. In the Al Baha region of Saudi Arabia, Al-Khulaidi et al. recognized that the frequency % and the density per hectare of *Abrus precatorius* were 0.31 and 0.05, respectively. Furthermore,*Abrus precatorius* is classified as a non-endemic-endangered species in Jabal Fayfa, southwest of Saudi Arabia.[83]

2. Material and methods

2.1. Collection of sample and Identification

The *Abrus precatorius* sample for this research work was obtained from the forest present in Srivilliputtur. The seeds are identified and authenticated by the Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi. The seeds are washed with water and dried at room temperature in the absence of sunlight, and after drying it was uniformly grounded using a mechanical grinder to make a coarse powder. The powdered material was stored in an airtight container.

2.2. Method of preparation of sample

1000 ml of the round bottom flask was taken and washed with methanol and allowed to dry. After drying 60 grams of coarse powder of *Abrus precatorius* seeds was taken in the round bottom flask and 600 ml of methanol was added and shaken well and allowed to cold maceration for 7 days. The round bottom flask was shaken several times during the process to get better extraction, then filtered through Whatman filter paper no 1 and evaporated at room temperature. The three isolates were isolated from the methanolic extract of *Abrus precatorius* seeds by column chromatography using silica gel 60-120 mesh as the adsorbent and methanol as the mobile phase. The isolated compounds are further purified by TLC. The compounds that have the same R_F value are combined. The crude extract was named CMME. The isolated compounds were named CMME I, CMME II, and CMME III.

2.3. Procedure

2.3.1. Determination of Isolated compound by LC-MS

The isolated compounds CMME I, CMME II, and CMME III were mixed separately in the HPLC grade methanol and then determined by the LC-MS instrument by injecting each sample separately by positive ion mode. The scanning was done from 100 m/z to 1000 m/z at a column temperature of 60°C and flow rate of 0.5 ml/min. The line spectra were obtained which are used for the interpretation of the compounds. The line spectra for sample CMME I, CMME II and CMME III was present in fig 1, 2 and 3 respectively.

2.3.2. Determination of Isolated compounds by ¹H NMR

The isolated compounds CMME I, CMME II, and CMME III were mixed separately with the Deuterated water (D₂O) and determined by the ¹H NMR spectrometer at 400 MHz. The ¹H NMR spectra were obtained separately for CMME I, CMME II, and CMME III and are used for the interpretation of the number of protons in the compounds. The ¹H NMR spectra for compound CMME I, CMME II, and CMME III were present in the fig 4, 6 and 8 respectively. The ¹H NMR interpretation of sample CMME I, CMME II and CMME III are present in the table 1, 2 and 3 respectively.

2.3.3. Determination of Isolated compounds by ¹³C NMR

The isolated compounds CMME I, CMME II, and CMME III were mixed separately with the Deuterated water (D₂O) and determined by the ¹³C NMR spectrometer at 400 MHz. The ¹³C NMR spectra were obtained separately for CMME I, CMME II, and CMME III and are used for the interpretation of the number of carbons in the compounds. The ¹³C NMR spectra for compound CMME I, CMME II, and CMME III were present in the fig 11, 13 and 15 respectively. The ¹³C NMR interpretation of sample CMME I, CMME II and CMME III are present in the table 4, 5 and 6 respectively.

3. Result and Discussion

In the LC-MS spectra of the compound, I the peak present at the m/e 282.3931 indicates the presence of compound Methyl (4,6-O-benzylidene) – alpha – D – glucopyranoside, In the LC-MS spectra of compound II the peak present at m/z 502.4272 indicates the presence of Isoflavone base + 20-O-Malonyl Hex. In the LC-MS spectra of compound III the peak present at m/z 173.6196 indicates the presence of trigonelline hydrochloride and the fragment peak present at m/z 137.0880 indicates the presence of trigonelline.

Interpretation of the isolated compounds CMME I, CMME II, and CMME III by ¹H NMR confirms the presence of 18H, 22H, and 8H in compound I, compound 2, and compound 3 respectively. The peak due to proton present in the carboxylic acid group of the second compound is not present, because it reacts with the deuterated water and forms the HDO.

Interpretation of isolated compounds CMME I, CMME II, and CMME III by ^{13}C NMR confirms the presence of 14C, 24C, and 7C in compound I, compound 2, and compound 3 respectively.

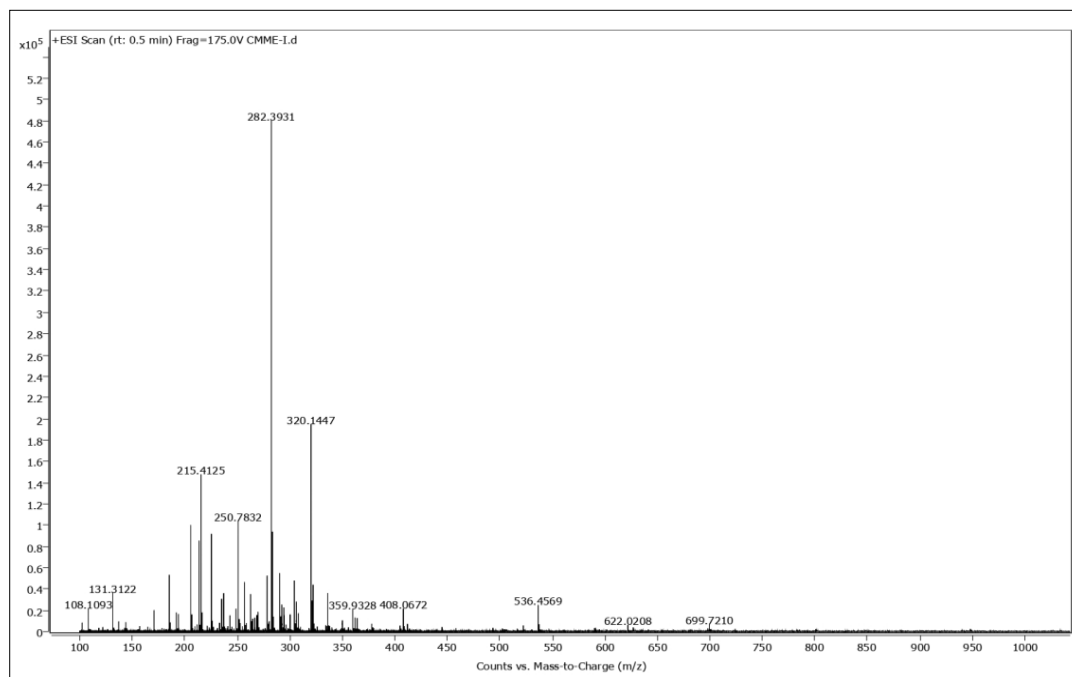


Figure 1 LCMS Spectra for sample CMME I

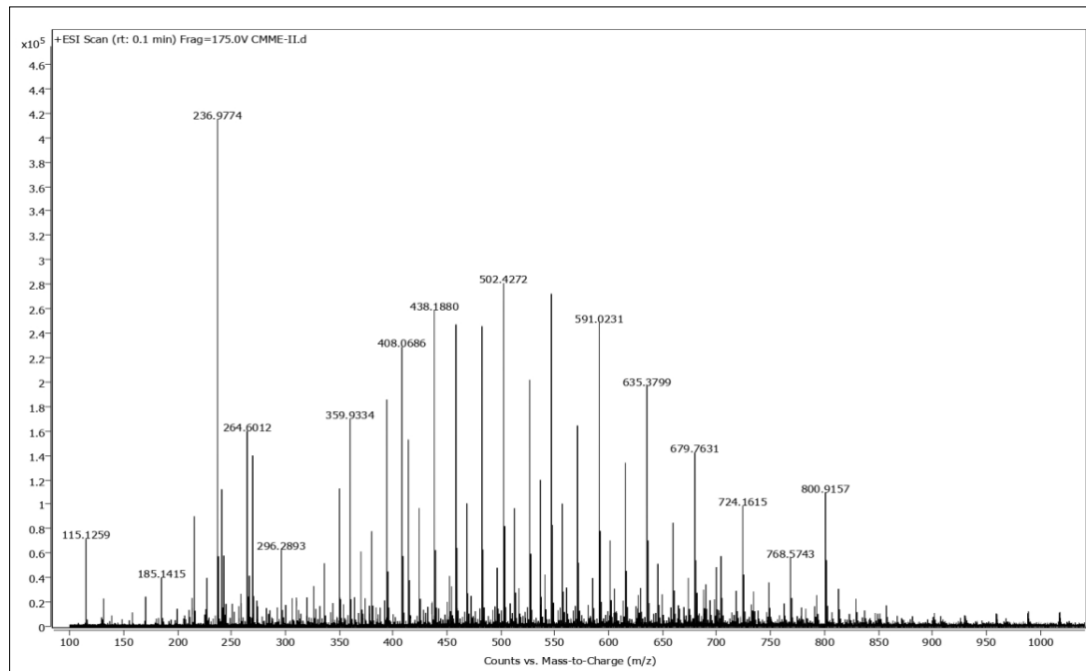


Figure 2 LCMS Spectra for sample CMME II

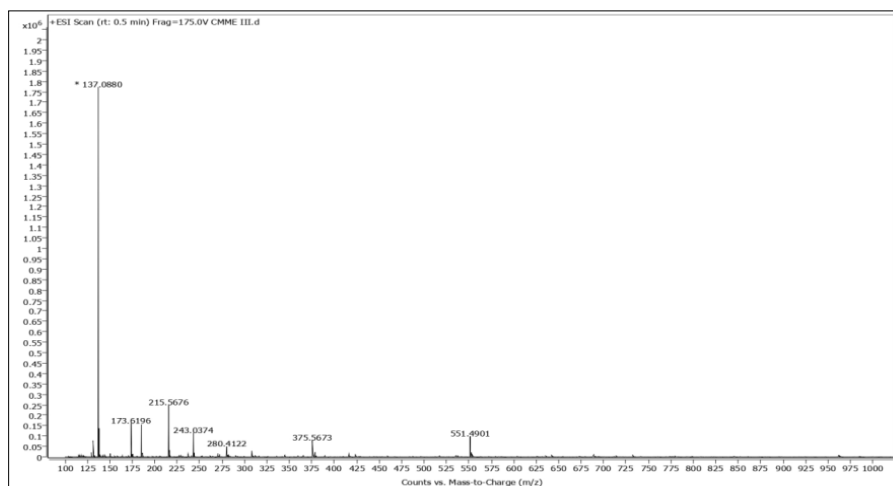


Figure 3 LCMS Spectra for sample CMME III

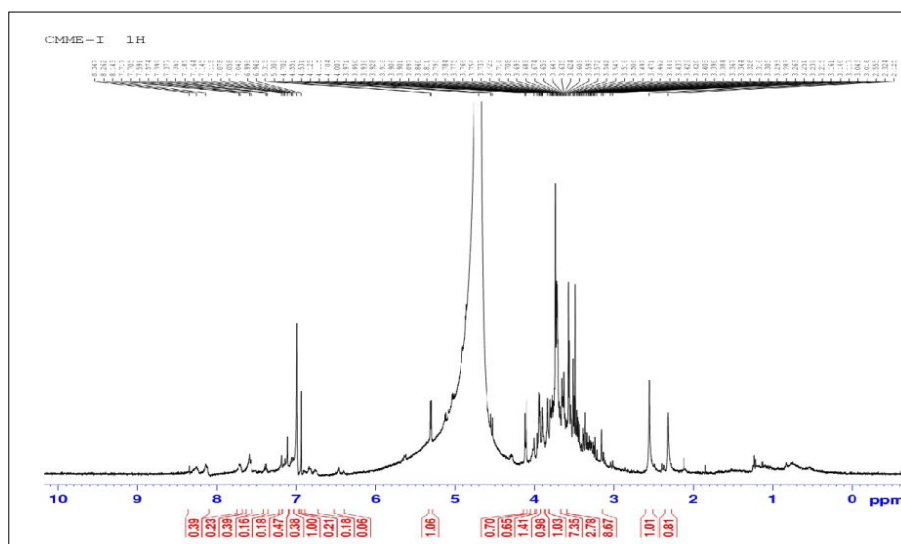


Figure 4 ^1H NMR spectra of CMME I

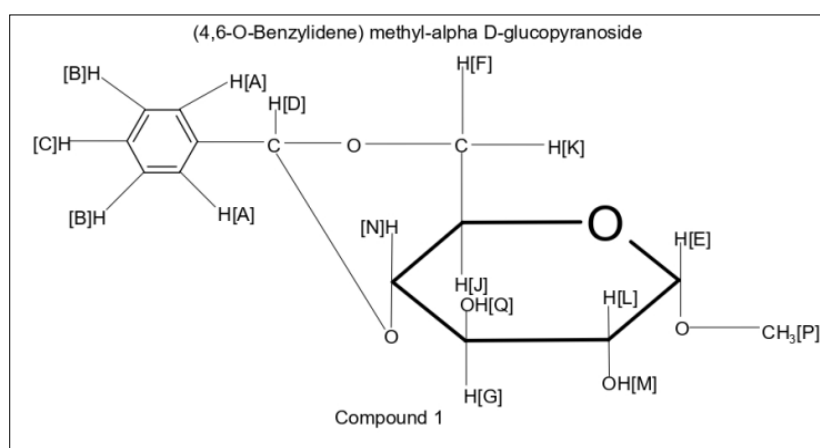
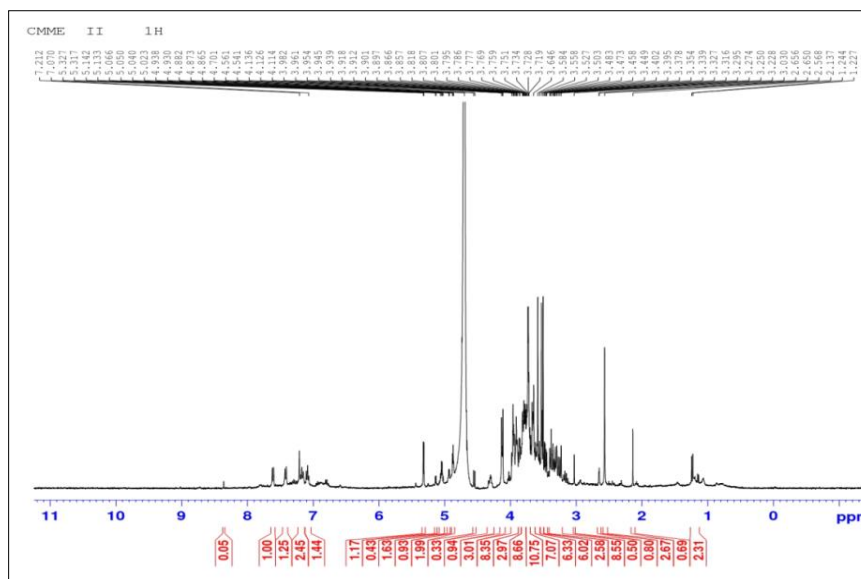


Figure 5 Structure of compound I

Table 1 ^1H NMR Interpretation of compound I

Assign	Chemical Shift (ppm)	No of protons	Multiplicity	J (Hz)
A	7.717	2	dddd	8.04,1.35,1.160,0.5
B	7.399	2	dddd	8.04,7.36,1.57,0.5
C	7.365	1	tt	7.36,1.35
D	5.315	1	-	-
E	4.531	1	d	2.62
F	4.125	1	dd	14.53,2.37
G	4.007	1	dd	3.64,3.51
J	3.811	1	td	3.16,2.37
K	4.115	1	dd	14.53,3.20
L	3.406	1	dd	3.51,2.62
M	2.324	1	s	-
N	3.897	1	dd	3.64,3.12
P	3.344	3	s	-
Q	2.125	1	s	-

**Figure 6** ^1H NMR spectra for CMME II

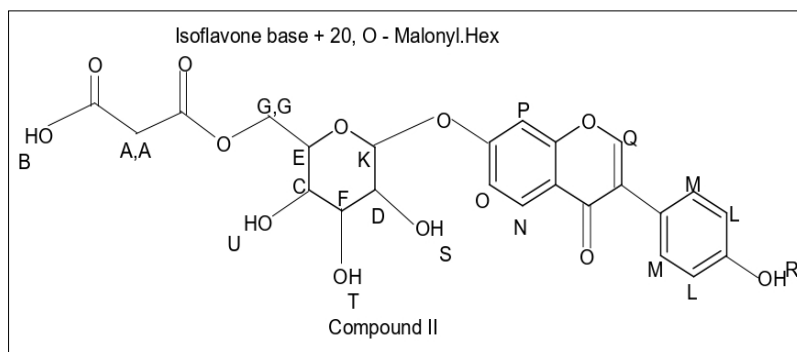


Figure 7 Structure of compound II

Table 2 H^1 NMR Interpretation of compound II

Assign	Chemical Shift (ppm)	No of protons	Multiplicity	J (Hz)
A	2.137	2	tt	5.05,4.80
C	3.030	1	dd	3.45,2.69
D	3.228	1	dd	3.45,2.69
E	3.483	1	td	4.57,2.69
F	3.646	1	t	3.45
G	3.818	2	d	4.57
K	4.882	1	d	2.69
L	3.857	2	d	4.57
M	7.212	2	dddd	8.30,1.93,1.32,0.45
N	7.171	1	dd	7.86,0.49
O	7.086	1	dd	7.87,2.39
P	7.070	1	dd	2.39,0.47
Q	7.105	1	-	-
R	5.27	1	-	-
S	3.91	1	-	-
T	2.63	1	-	-
U	3.91	1	-	-

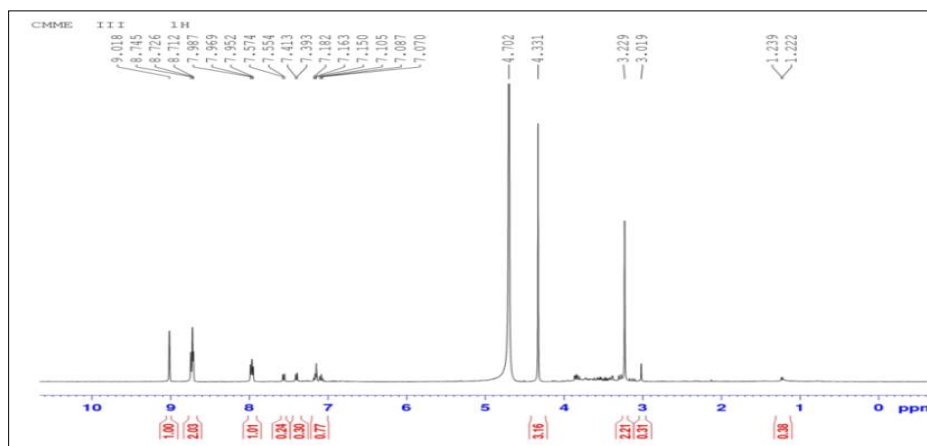


Figure 8 H1 NMR spectra for compound CMME III

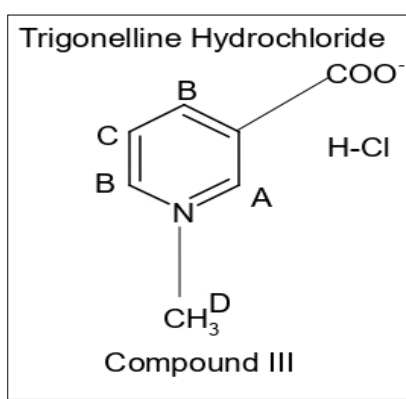


Figure 9 Structure of compound III

Table 3 Chemical shifts for sample CMME III

Assign	Chemical Shift (ppm)	No of protons	Multiplicity	J (Hz)
A	9.018	1	ddd	1.89,1.55, 0.47
B	8.726	2	ddd	7.92,1.89,1.55
C	7.969	1	ddd	7.92,4.66,0.54
D	4.331	3	s	-

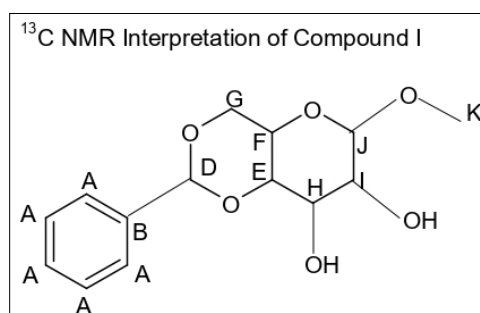


Figure 10 ¹³C NMR Interpretation of CMME I

Table 4 Interpretation of Compound I by ^{13}C NMR

Assign	Chemical Shift (ppm)	No of Carbon atoms
A	144.36	5C, s
B	137.91	1C, s
D	103.42	1C, s
E	74.10	1C, s
F	62.32	1C, s
G	68.9	1C, s
H	73.93	1C, s
I	74.20	1C, s
J	103.62	1C, s
K	52.41	1C, s

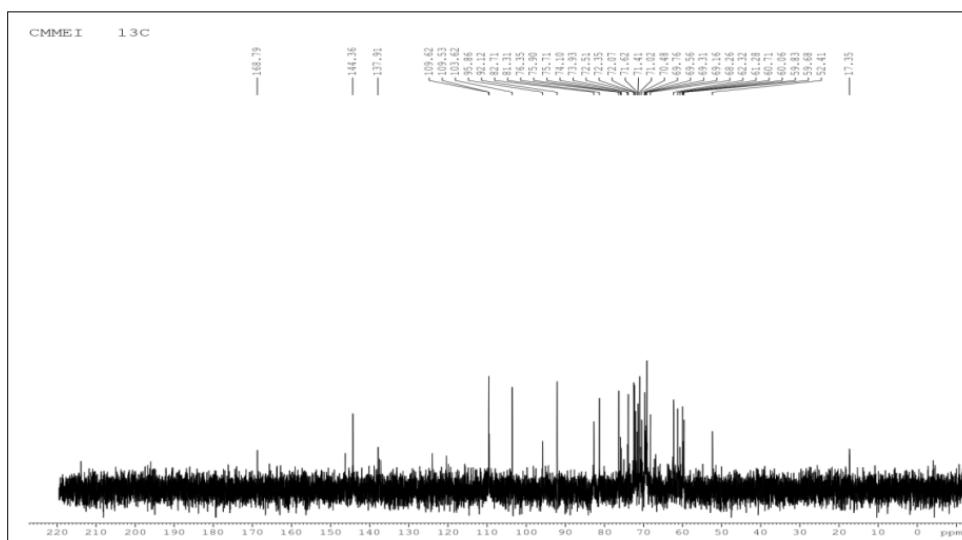


Figure 11 ^{13}C NMR spectra for CMME I

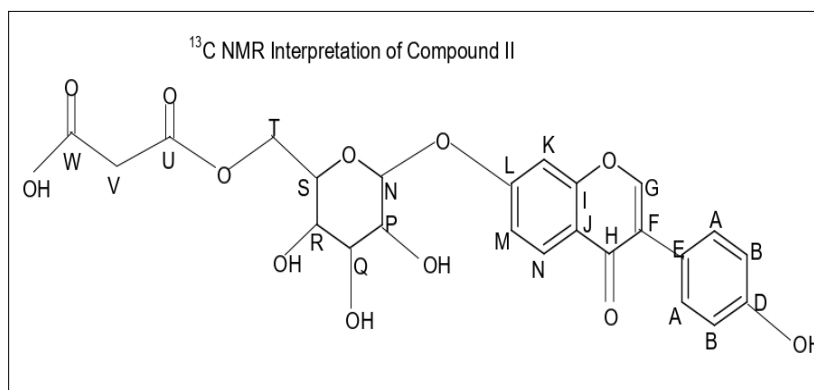
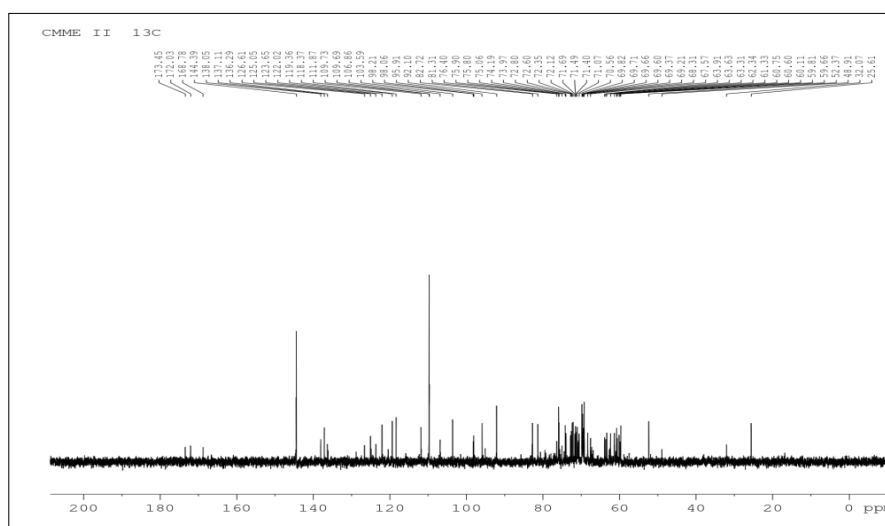


Figure 12 ^{13}C Interpretation of CMME II

Table 5 Interpretation of Compound II by ^{13}C NMR

Assign	Chemical shift (ppm)	No of Carbon atoms
A	136.29	2C, 1s
B	118.37	2C, 1s
D	172.03	1C,1s
E	136.29	1C,1s
F	126.61	1C,1s
G	144.39	1C,1s
H	48.92	1C,1s
I	168.78	1C,1s
J	123.65	1C,1s
K	98.21	1C,1s
L	173.45	1C,1s
M	118.37	1C,1s
N	103.59	1C,1s
P	74.06	1C,1s
Q	76.4	1C,1s
R	73.94	1C,1s
S	72.8	1C,1s
T	68.32	1C,1s
U	67.57	1C,1s
V	32.07	1C,1s
X	59.66	1C,1s

**Figure 13** ^{13}C NMR spectra for CMME II

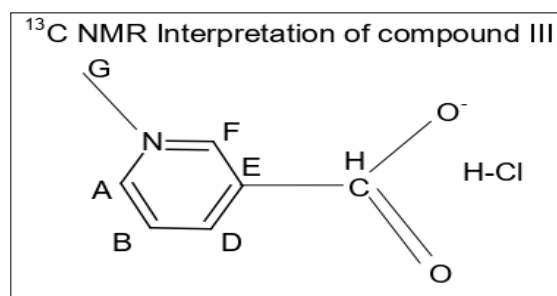


Figure 14 ¹³C Interpretation of CMME III

Table 6 Interpretation of Compound III by ¹³C NMR

Assign	Chemical shift (ppm)	No of Carbon atoms
A	48.16	1 C, s
B	122.06	1 C, s
D	127.52	1 C, s
E	125.11	1 C, s
F	144.63	1 C, s
G	32.09	1 C, s
H	106.93	1 C, s

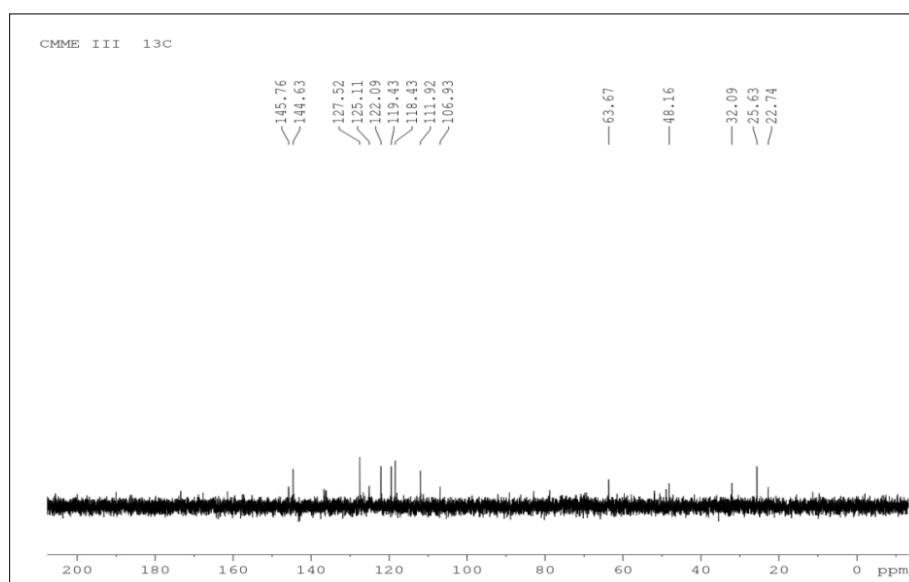


Figure 15 ¹³C NMR spectra for CMME III

4. Conclusion

Three major compounds were isolated from the methanolic crude extract of *Abrus precatorius* seeds by column chromatography using silica gel 60-120 mesh as the adsorbent and methanol as the mobile phase. The three isolated compounds are characterized by LC-MS, ¹H NMR spectroscopy, and ¹³C NMR spectroscopy. The compound I was found to be (4,6-O-Benzylidene) methyl- α -D-glucopyranoside, m/z 282.3923, molecular formula C₁₄H₁₈O₆. The compound II was found to be Isoflavone base + 20, O-Malonyl Hex, m/z 502.42801, molecular formula C₂₄H₂₂O₁₂. The compound III was found to be 3-Carboxy-1-methyl pyridinium chloride, m/z 173.6, molecular formula C₇H₈ClN₂O₂.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare that we have no conflict of interest. We alone are responsible for the content and writing of this article.

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