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(RESEARCH ARTICLE)

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Identification and isolation of phytochemical constituents from the *Syzygium cumini (L.) Skeels*

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Abstract

The development of various drugs from medicinal plants are based on its phytochemical and pharmacological approaches for curing different diseases, therefore, various plants are recognized in the pharmaceutical industries for their broad structural diversity and wide range of pharmacological activities. These phytochemicals compounds are used as sources of direct medicinal agents or can serve as a raw material base for elaboration of more complex semi-synthetic chemical compounds. Since there is very minimal or no side effects, the need of looking inwards to search for herbal medicinal plants with the aim of validating the medicinal use and subsequently isolation and characterization of compounds becomes important which can be added to the potential list of drugs. The present study deals with the extraction of active compounds from the seeds of *Jambolan* plant and qualitative analysis of the phytochemicals using GCMS. The major constituents identified in the extract were gamma-sitosterol, cholest-4-ene-3-one, humulene, caryophyllene, alpha-santalol, campesterol, globulol, eicosane, azulene, trans-sesquisabinene, diosgenin, heneicosane, beta tocopherol, carotol etc. Many other compounds were also identified as considered as low level. Out of which gamma-sitosterol is the main phytochemical having functional group of OH, which may be responsible for reducing the level of glucose in blood in curing diabetes. This preliminary study gives an idea to isolate the major active constituents present in the seed of the plant and also helps to develop potential pharmacologically active compounds in therapeutic use of various ailments including diabetes.

Keywords: Syzygium; *Jambolan*; Insulin; Hyperglycemia; Flavonoids; Myrcetin

1. Introduction

When glucose concentrations in blood plasma increases by 50–100 mg/dL for as little as 24 hours can cause down regulation of the glucose transport system in the body can significantly increasing the resistance of insulin. Over time, insulin resistance peaks and then plateaus as increases in plasma insulin compensate to maintain the glycemic state. The inadequate production of insulin or if the cells do not respond properly to insulin or both can lead to high blood sugar which can experience poly urea (frequent urination), increasingly thirsty (Polydipsia) and hungry (Polyphage) [1]. Diabetes which can be of Type 2 Diabetes, Pre-Diabetes, and the Metabolic Syndrome Fasting hepatic glucose production is increased in both obese and no obese diabetic patients, compared with normal individuals and those with impaired glucose tolerance that have not met the criteria for diabetes. This increase in hepatic glucose output, owing to increases in glycogenolysis and gluconeogenesis, results in fasting hyperglycemia in type 2 diabetic patients. At some point, usually approximately 10 years after insulin resistance and hyperinsulinemia develop, postprandial hyperglycemia begins to develop, resulting from β-cell dysfunction and/or depletion. Postprandial hyperglycemia is characterized by a delay in first-phase insulin release and blunted second-phase output. This first-phase response plays an important role in the suppression of hepatic glucose production. This progressive deterioration leads to fasting hyperglycemia

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when insulin levels begin to decline although insulin resistance remains elevated. The progressive nature of the disease and the progressive lack of glycemic control are predominantly caused by this ongoing deterioration of β-cell function with subsequent decreased production of insulin.

The evaluation of the antioxidant activity, screening the phytogenic chemical compounds and also to access the alkaloids present in E. intermedia for the treatment of asthma and bronchitis have been studied [2] using High Performance Liquid Chromatography (HPLC) and observed for further isolation of therapeutically active substance. The phytochemicals are divided into primary metabolites which include proteins, carbohydrate, chlorophylls etc. and secondary metabolites are the steroids, terpenoids, flavanoids, alkaloids, glycosides and tannins [3]. These bioactive components exhibit various medicinal importance and may be responsible for curing or reducing the harmful effects of the diseases. Therefore, the qualitative screening of these phytochemicals will help to know about the variety of chemical compound present in the plant material and the quantitative screening will lead to extract, isolate, purify and identify the bioactive compound for medicinal aspect to human being [4-5]. The study on the antidiabetic and antioxidant of methanol extract of *M. oleifera pods* (MOMtE) has been carried out with the diabetic rats by treating 150/300mg/kg MOMtE for 21 days and concluded that the MOMtE exhibiting significant level of on antidiabetic and antioxidant activity [6]. Isolation, identification of the various phytoconstituents from the *Rostellularia diffusa* and the Henna leaves (*Lawsonia inermis L.*) has been for carried out further research sourcing the pharmaceutical significance[7-8]. The same type of work was also carried out from *Pistia stratiotes L., Eichhornia crassipes* (Mart) with various solvents and showed different types of low molecular compounds by GCMS, from the root nodules of *Vigna mungo L* [9-10] and And *rographis paniculata* [11],from this study unknown bioactive compounds have been identified qualitatively by using the method [12].Phytochemical constituents in Curcuma *Caesia Roxb (*Black Turmeric) extracted with methanol and identified using GSMS and FTIR which revealed the presences of tannins, terpenoids, flavonoids, alkaloid, phenol phytosterol qoinones and saponins and observed Curcuma Caesia Roxb as an herbal alternative for various disease [13]. Medicinal roles of tannins, flavonoids, saponins and steroids extracted with methanol from Carissa spinarum have been identified using GCMSto understand the role of the plant as a medicine [14]. Various phytochemical constituents, steroids, saponins, myrcetin, cholesterol and beta sitosterol, have been extracted from *K. Pinnta* and *Acalypha indica* using chloroform and analysed with HPLC and GCMS [15-16]. Hydrocarbons, Carbohydrates, Fatty acids, Fatty acid ester, Alcoholic compounds, Alkaloids, Ketones and Alkenes have been extracted *Alseodaphane semecarpifolia Nees* (Lauraceae) with alcohol and identified using GCMS and suggested these phytochemicals may help in the protection against the incurable diseases [17]. The species of *Solena amplexicauils* and *Costus spicatus* were extracted with methanol and ethanol respectively and analysed using GCMS for the determination of 35 compounds comparing with library of National Institute of Standards and Technology (NIST) [18-20].In another study, GCMS has been used to analyse 24 bioactive phytochemical compounds extracted from With an obtusifolia for the purpose of their identification [21]. Accordingly, a number of research papers have been published in different journals by maximally using GCMS and FTIR and in minimum using HPLC for the identification of various phytochemical constituents which are responsible for curing various diseases. In the present study the authors have taken the powder from the seeds of *Jamun* for the purpose of extracting the phytochemical constituents for their identification and also analysed the functional groups, which are responsible for decreasing the level of glucose in the diabetic patients, using GCMS and FTIR [22].

2. Material and methods

2.1. Plant Material

The ripped fruits were collected directly from the *Jambolan* tree and stripped off the pulp and washed the seed repeatedly with running and sterile distilled water and dried in the sun light. The scales on the seed were removed and again dried well till it attains easy brittleness to make a fine powder. The seed was coarsely powdered with an agate motor and stored in the well tightened glass bottle and stored in the room temperature in order to prevent the spoilage of the powder sample for further analysis.

2.2. Extraction

Each one gram of dried finely ground seed powder was used to extract for 72hr.with different solvents such as Acetone, methanol, hexane, dichloromethane, ethanol, ethyl acetate and petroleum ether and kept for extraction using the Soxhlet apparatus. The extract was repeated and filtered using 42 No. Whatman filter paper and collected as a filtrate for further analysis using Thin layer Chromatography for constituent separation and Gas Chromatography Mass Spectrometry (GCMS)) for identification of the various components contained in the filtrate. The extract was chemically analysed for the presence of various heavy atoms such as potassium, Zink, Iron, Magnesium etc. However, heavy atoms are not present in the extract as detailed in the Table 1.

2.3. Thin Layer Chromatography

The filtrate/extract from different solvents were spotted on the TLC plate, (TLC silica gel 60 F254 Aluminium sheet 20X20cm), and kept the plate under various solvent system and observed the separation of different compounds present in the sample. Three different combinations of solvent system were prepared and used to separate the compounds as

S. No.	Solvent System
1	100% Methanol
2	Acetone and Methanol (50:50)
3	Hexene: Benzene: Ethanol: Acetic Acid (6:2:1:1)

Detailed below:

Finally, the TLC plates, thus, having separated the components were visualized under the UV light of short and long wavelength and their Rf value was calculated for different separation bands, thus, observed.

Table 1 Detection of various elements in the compound is as detailed

S. No.	Elements	Detected/Not detected
1	Sodium (Na^{+})	Not detected (-)
2	Potassium(K^{+})	
3	Zinc (Zn^{2+})	
4	Magnesium(Mg^{2+})	
5	Cadmium (Cd^{2+})	
6	Copper(Cu^{2+})	
7	Iron (Fe^{2+})	
8	Manganese(Mn^{2+})	
9	Calcium(Ca^{2+})	
10	Chromium (Cr^{3+})	

2.4. Gas Chromatography Mass Spectrometry (GCMS)

Table 2 Details of Gas Chromatography Mass Spectrometry

Programming Methods	Gas Chromatography Mass Spectrometry	
	Method I	**Method II**
Carrier gas	He	He
SPL programming		
Temperature	280 ^{0}C	250 ^{0}C
Injection mode	Splitless	Splitless
Sampling Time	1.00 min	1.00 min
Column Flow	1.33 ml/min	1.20 ml/min
Column programming		
Initial temperature	100 ^{0}C	100 ^{0}C
Hold Time	2 min	2 min

Rate	10 ^{0}C/min	4 ^{0}C/min
Final Temperature	300 ^{0}C	280 ^{0}C
Hold Time	5 min	30 min
MS Programming		
Ion source Temperature	300 ^{0}C	250 ^{0}C
Interface Temperature	300 ^{0}C	300 ^{0}C

The extract from different solvents was analyzed under GCMS using different temperature and column programming. The parameters for running the sample in the GCMS are as given in Methods I and II respectively.

3. Results and discussion

Table 3 Preliminary phytochemical analysis of Jamun Seed (- ve, not present) and (+ ve, present)

S. No	Phytochemicals	Chemical tests	Results
1	Alkaloids	Mayer's test	-
		Wagner's test	-
2	Flavonoids	Lead acetate test	+
		Alkaline test	+
3	Terpenoids	Salkowski test	-
4	Phenol's test	Ferric chloride	+
		Lead acetate test	+
5	Steroids	Liebermann-Burchard test	-
6	Arthroquinone	Borntrager's test	-
7	Saponin	Froth test	-
8	Tannin	Bromine water test	-
9	Glycoside test	Steroidnal aglycone part test	+
10	Proteins & Amino acids	Biuret test	-
		Ninhydrin test	-
11	Carbohydrates	Molish test	-
12	Oils & Resins	Filter paper test	-

Different bands were found after visualizing the TLC plate under UV light and also noticed that 100% methanol gives best result as solvent system.

Table 4 Results of TLC under Short/long UV wavelength

Solvent System I		
Extract	**Short UV(254 nm)**	**Long UV(365nm)**
Acetone		
Ethyl acetate		

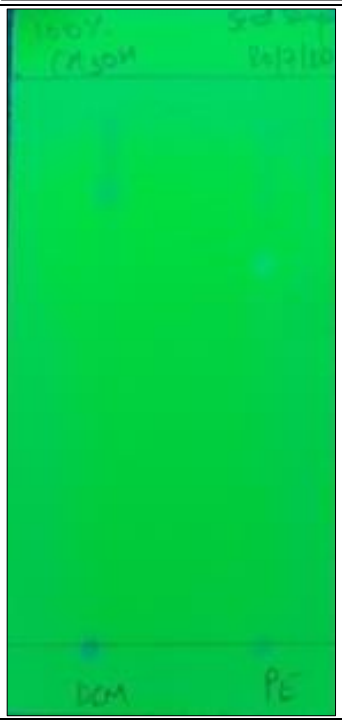
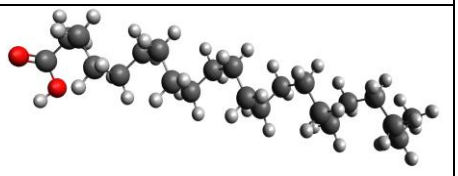
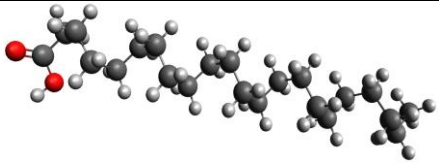
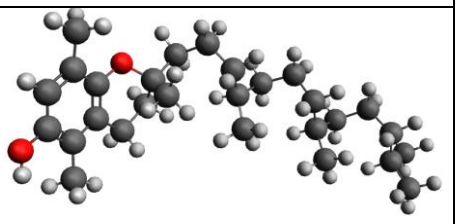
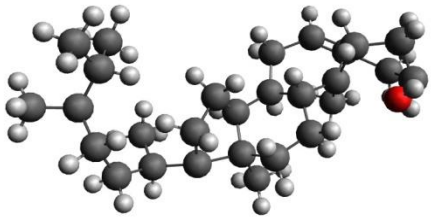
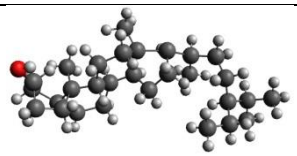
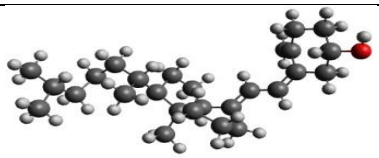
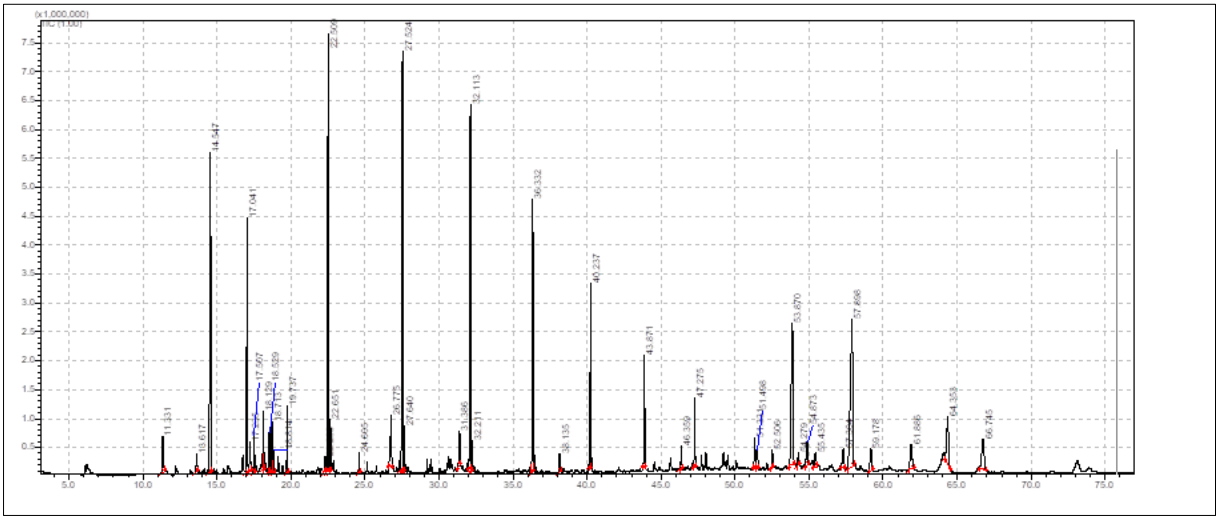
Ethanol& Acetone		
Dichloromethane (DCM)& Petroleum Ether		

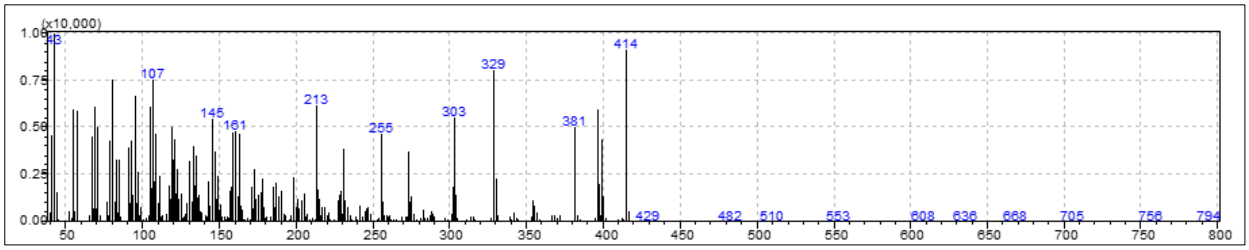
Table 5 Different phytochemicals were extracted using different solvents through method II, which was identified by GCMS

S. N	Solvent	Phytochemical Compound Identified by GCMS	Molecular Formula	Molecular Weight (g/mol)	Area %	Retention Time in min	Molecule structure
1	Ethyl Acetate	Carotol	C15H26O	222.36	0.37	22.884	
		Behenic alcohol/Diosgenin	C27H42O3	414.630	1.63	36.301	
		Beta tocopherol	C28H48O2	416.7	0.61	47.723	
		Campesterol	C28H48O	400.691	0.74	51.509	
		Gamma.-sitosterol	C29H50O	414.707	15.07	53.851	
		Cholest-4-ene-3-one	C27H44O	384.638	19.05	57.858	

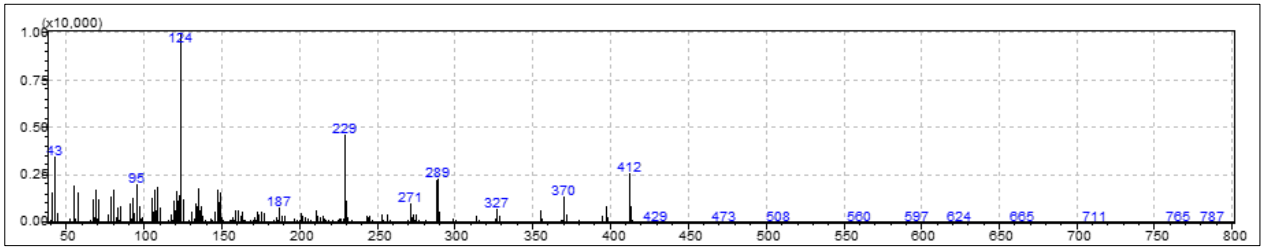


The mass spectrum of major important phytocomponents identified in the extract of the *Jamun seed* by GC-MS/

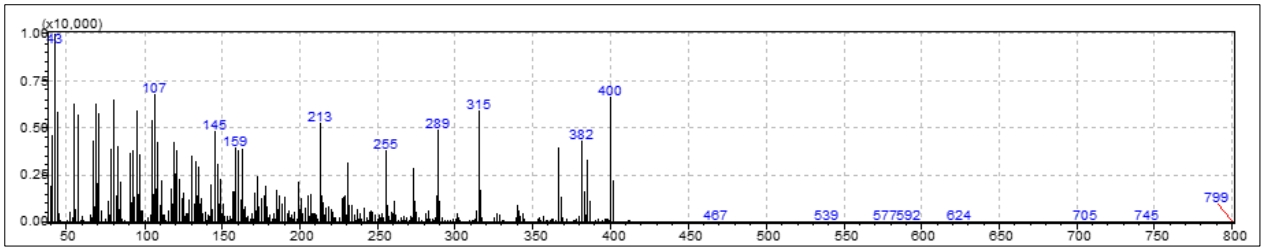
Gamma sistosterol



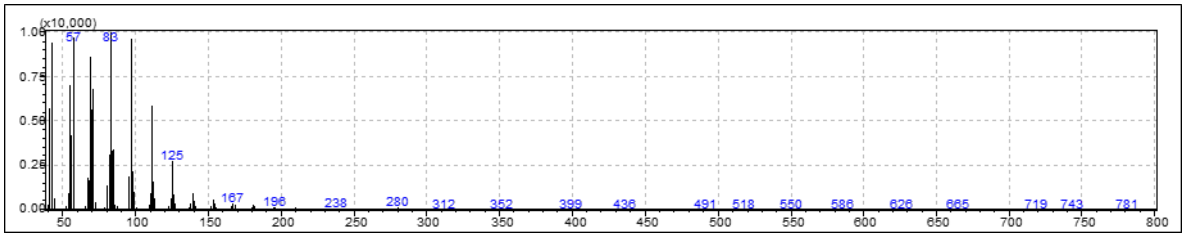
Cholest-4-en-3-one



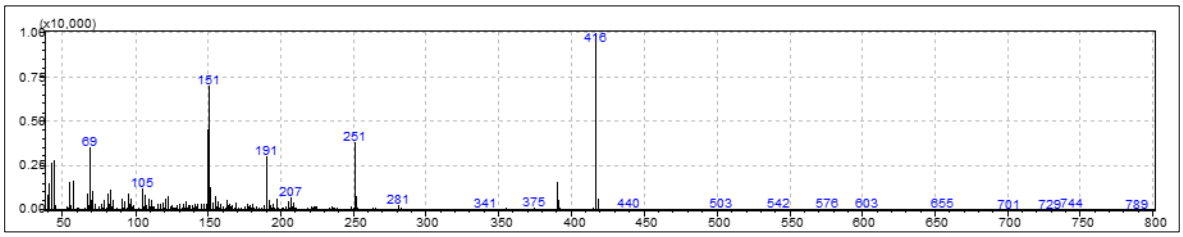
Campesterol



Diosgenin



beta.-Tocopherol



Carotol

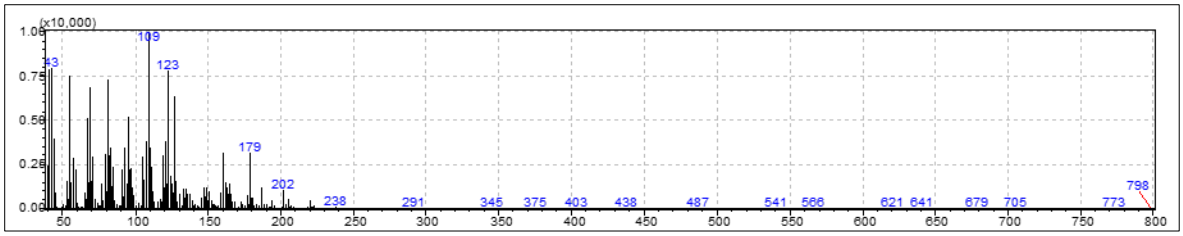


Figure 1 GC-MS analysis of constituents from the extracts of *Jamun* seed

4. Conclusion

The present work has been carried out to establish the various phytochemicals present in the *Jamun seed* seed using GCMS. It has been found that the major phytoconstituent i.e. gamma sistosterol resulted in decreases in glycated hemoglobin, serum glucose, and nitric oxide, with concomitant increases in serum insulin levels which could serve as important and has commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the innovative drugs. Hence, on the basis of observation under reference and from this study it could be concluded that the component, gamma sistosterol, present in the seed powder of *Jambolan* plant could be playing major role in decrease of glucose level in the blood. This primary information will facilitate in conducting further studies on discovery of bioactive constituents, resolve of their efficacy by *in vivo* studies and demonstration of their safety and efficacy in clinical trials.

Compliance with ethical standards

Acknowledgments

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Authors' contributions

Munusamy Baskar subject was initiated and supervised the whole work till the finalization of the manuscript. Akanksha Singh and Bharti Arya analysed the sample, collection of references and manuscript preparation.

Disclosure of Conflict of interest

The authors declare no conflict of interest.

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