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Novel investigation on *in-vitro* anti-diabetic and volatile profile of bioactive compounds present in methanolic extract of *Ficus krishnae*

Amarvani P Kanjikar, ArunaL H, Ramesh L Londonkar*

Department of Biotechnology, Biopharmaceutical and Nanobiotechnology Laboratory, Gulbarga University, Kalburagi, India.

Abstract : *Ficus krishnae* is one of the medically important plant belonging to the family *Moraceae*. It has been used extensively by ayurvedic practitioner in India to treatvarious ailments such as ulcers, vomiting, fever, inflammations, leprosy, syphilis, biliousness, dysentery and inflammation of liver. The present study aims that theinvitro anti-diabetic screening and bioactive components of *Ficus krishnae* stem bark extract of the plant have been evaluated by using GC-MS. The in vitro alpha-amylase inhibitory study was performed using different concentration of extract and compared with a standard drug. The results reveal that, there was a dose dependent increase in percentage inhibitory activity against these intestinal enzymes by methanol extract. Our findings revealed that methanol extract and acarbose have showed an efficient anti-diabetic activityi.e85.48% and 75.06% respectively. The chemical compounds of the methanol extract of *Ficus krishnae* were investigated using Perkin-Elmer Gas chromatography-Mass spectrometry. GC-MS analysis of methanol extract of *Ficus krishnae* shows the existence of 42compounds with valuable biological activities. This is the first report of identification of active constituents from the stem bark of *Ficus krishnae*..
Keyword : GC-MS, *Ficus krishnae*, soxhlet extract, anti-diabetic, alpha amylase.

Introduction

Medicinal plants are known for the alternative therapeutic agent for the treatment of various healths related problems with safer and potential activity. People from different areas have used particular medical plant parts to treat the health problems. Wide range of medicinal plants and their extracts have shown significant medicinal property like anti-oxidant, anti-diabetic, anti-inflammatory, anti-cancer, antimicrobial and immunomodulatory effects [1, 2,3].Diabetes mellitus (DM) is chronical metabolic syndrome in which the deficiency or insensitivity of insulin causes glucose to accumulate in blood,because ofnon-functional intestinal enzymes like α-amylase and α-glucosidase. These are very important for carbohydrate degradation and glucose absorption for the balance of glucose level in blood[4, 5]. The numbers of diabetes mellitus cases areincreasing day by day in worldwide. In 2000, the world health organization has estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030[6]. The most common treatment for diabetes includes oral antidiabetic drugs, insulin injection and management through diet. Oral hypoglycemic agents have effective glycemic control but has side effect such as liver disorder, flatulence, renal tumor etc[7, 8]. *Ficus krishnae* is known as Makkhann Katori in Hindi and Krishna fig or Krishna's butter cup in English. It is native to India and also found in tropical Africa and Sri Lanka[9]. The plant known to have medicinal values to cure the ailments likeulcers, vomiting, fever, inflammations,

leprosy,syphilis, biliousness, dysentery, diabetes and inflammation of liverect[10, 11]. The stem bark of the plant acts as a reservoir for bioactive compounds like phenols, flavonoids andother phytochemical contents.

Material and methods:

Collection of plant materials:

Stem bark of *Ficus krishnae* was collected by DevDevvana botanical garden of Bidar district, Karnataka. The plant is duly identified by Department of Botany, Gulbarga University Kalaburagi, Karnataka, India. The stem bark was allowed to dry in shade for two to four weeks. After drying, the bark was grinded into finely powder and stored in airtight container. The air dried bark powder (100 g) was successively extracted by soxhlet extraction with methanol. The extracts were dried and stored in a sterile container for further use.

Evaluation of *in vitro* anti-diabetic activity

Inhibition of alpha amylase enzyme assay

A total of 500 µl of test samples and standard drug (50 - 1000 mg/ml) were added to 500 µl of 0.20 mM phosphatebuffer (pH 6.9) containing α-amylase (0.5 mg/ml) solution and were incubated at 25 °C for 10 min. After these, 500µl of a 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reactionmixtures were then incubated at 25 °C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic(DNS) acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to roomtemperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measuredat 540 nm (13). Acarbose was used as a standard drug for assay. The control samples represent 100 % enzyme activityand were prepared without any plant extract. Each test was performed three times and the mean absorption was calculated the percentage ofα- amylase inhibition.

% Inhibition was calculated according to the formula:

$$\% \text{ Inhibition} = \frac{A_{540} \text{ control} - A_{540} \text{sample}}{A_{540} \text{ control}} \times 100$$

Gas Chromatography-Mass Spectrum Analysis:

2µl of methanol bark extract from *Ficus krishnae* was used for GC-MS analysis[14]. These extracts were dissolved in HPLC grade methanol and subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused with silica 50 m x 0.25 mm I.D. Analysis conditions were 20 min. at 100°C, 3 min at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300°C. Runtime was 30minutes.

Identification of components:

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns[15]. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST Library. Prediction of bioactivity of compound is done based on Dr. Duke's Phytochemical and Ethnobotanical Databases (Dr. Duke Database, 2017). The relative percentage amount of each phyto-component was calculated by comparing its average peak area to the totalarea.The name, molecular weight, molecular formula and the structure of the components of test materials were recorded.

Results and Discussion:

Evaluation of *in vitro* anti-diabetic activity

Inhibition of alpha amylase enzyme assay

Alpha amylase is hydrolysis enzyme that acts on alpha-bonds of large linked polysachharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha bonds of polysaccharides that prevent to degradation of mono and disaccharides[16].Fig.1 revealed that methanol extract of *F.krishnae* has showed a significant inhibition activity of α-amylase enzyme, when compared to standard acarbose. The inhibitory activity increases with increase in concentration of methanol extract and acarbose. At the minimum concentration of 50µg/ml of F.krishnae methanol extract and acarbose have showed 59.36% and 36.80% respectively. At maximum concentration of 1000 µg/ml both methanol and acarbose have showed 85.48% and 75.06% respectively. Whereas F. *glomerata* aqueous extracts of gum showed82.76 % at concentration of 1000µg/ml[17].

Table-1: Bioactive compounds detected in methanol stem bark extract of *Ficus krishnae.*

Sl. No:	RT	Compound Name	Molecular formula	Molecular weight	Peak area %
1	19.19	Dibutyl phthalate	$C1_6H_{22}O_4$	278.34	0.24
2	24.37	Lanosta8,24-dien-3-one	$C_{30}H_{48}O$	424.713	0.70
3	24.91	á-Amyrin	$C_{30}H_{50}O$	426.7264	7.45
4	25.90	Cholest-4-en-3-one	$C_{27}H_{44}O$	384.64	2.09
5	25.90	Pregn-4-en-3-one,20-hydroxy	$C_{22}H_{33}O_2$	316.48	2.09
6	25.90	Ergosta-5,24(28)dien-3-ol, (3á)	$C_{28}H_{46}O$	398.664	2.09
7	26.37	Lup-20(29)en-3-one	$C_{30}H_{48}O$	424.70	15.91
8	26.58	1-Heptatriacotanol	$C_{37}H_{76}O$	536.99	5.33
9	27.29	Lupeol	$C_{30}H_{50}O$	426.729	5.33
10	27.29	9,19-Cyclo-9á-lanostane-3á, 25-diol	$C_{30}H_{52}O_2$	-	5.33
11	28.98	4-Methylenecycloartan-3-one	$C_{31}H_{50}O$	440.756	2.22
12	28.98	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl,acetate, (3á,4à,5à)	$C_{32}H_{52}O_2$	468.7541	2.22
13	28.98	9,19-Cyclolanostan-3-ol, 24-methylene, (3á)	$C_{31}H_{52}O$	440.744	2.22
14	29.48	Stigmast-4-en-3-one	$C_{29}H_{48}O$	410	5.87
15	29.48	Testosterone cypionate	$C_{27}H_{40}O_3$	412 7.99	5.87
16	29.48	Cholest-4-en-3-one	$C_{27}H_{44}O$	382	5.87
17	29.93	12-Oleanen-3-yl acetate, (3à)	$C_{32}H_{52}O$	468.766	11.85
18	29.93	á-Amyrintrimethylsilyl ether	$C_{27}H_{44}O$	-	11.85
19	31.75	Lup-20(29)en-3-ol,acetate, (3á)	$C_{32}H_{52}O_2$	468.766	42.62
20	33.16	Lup20-(29)-en-28-oic acid, 3-hydroxy, methyl ester, (3á)	$C_{31}H_{50}O_3$	470.7371	1.09
21	33.16	13,14-Epoxyoleanan-3-ol,acetate	$C_{31}H_{50}O_3$	-	1.09

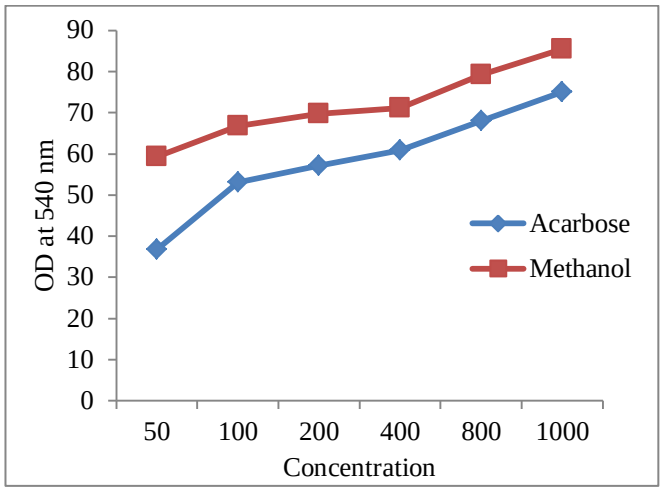

Figure-1: in *vitro* alpha amylase inhibition by acarbose and methanol extract of Ficus krishnae

GC- MS analysis

GC-MS is one of the method to identify the bioactive constituents of long chain hydrocarbons, acids, alcohols, alkaloids, steroids, nitro and amino compounds etc[18]. In the present investigation, 42 bioactive compounds have been identified from methanol extract of Ficus krishnae stem bark by GC-MS analysis as shows in Figure-1. The active principles with their Retention time (RT), molecular formula, molecular weight and peak area in percentage are presented in Table-1.

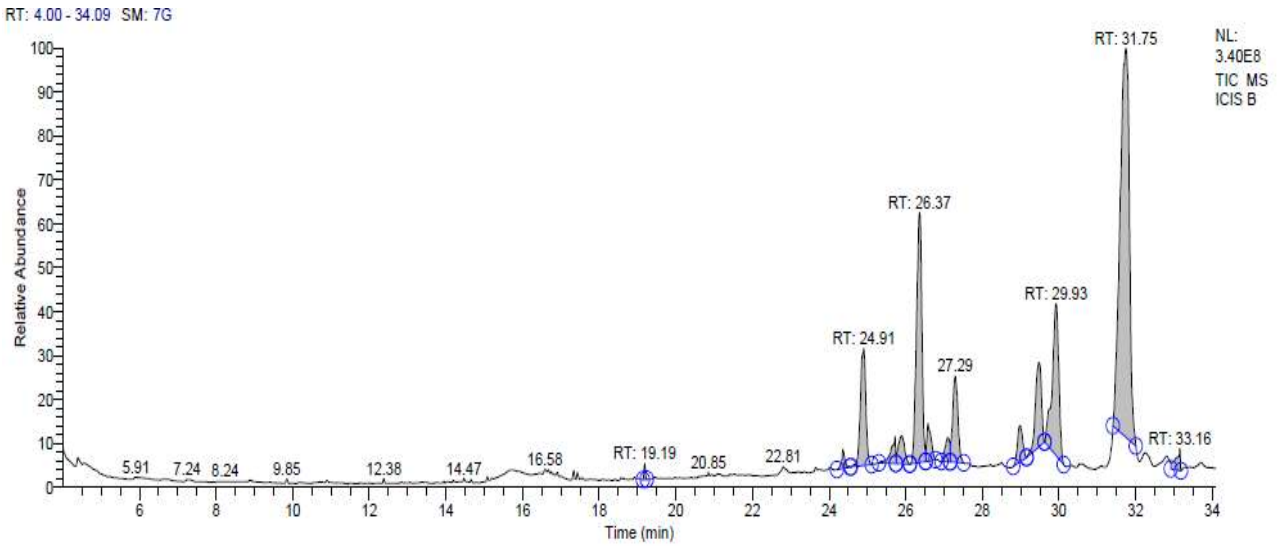

Figure- 2: GC-MS Chromatogram of the methanol extract of *Ficus krishnae* stem bark.

F. krishnae is an folkal medicinal plant, but thereare no sufficient phytochemicalanalyses has been carried out on it. Table-2 lists the major phytocompounds and their biological activities obtained through Dr. Duke's Phytochemical and Ethnobotanical Databases. Thus this type of GC-MS analysis is the first steptowards understanding the nature of activeprinciples present in this medicinal plant and will be helpful for further detailed study. The mass spectra and structuresof these bioactive compounds are presented in Figures 2, 3, 4, 5,6,7 and 8.

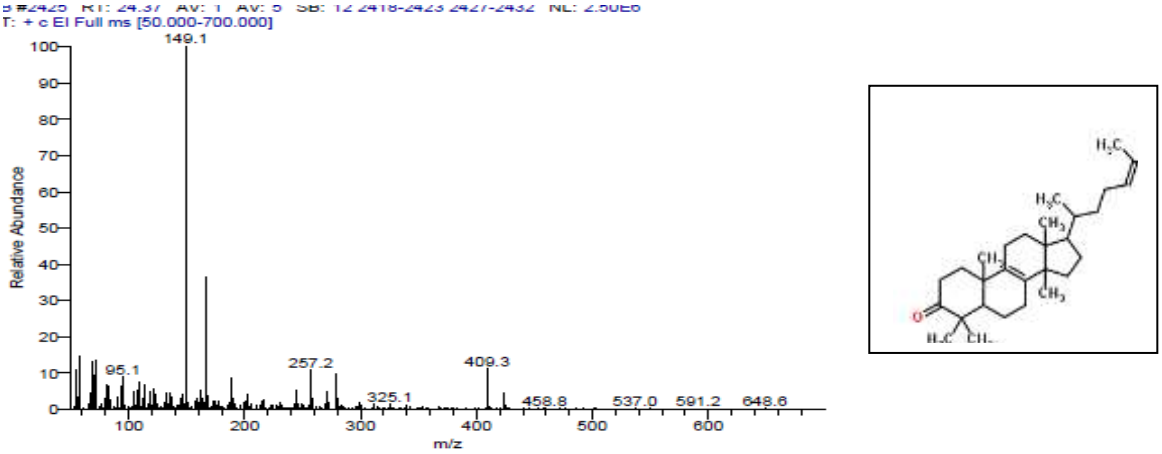

Figure-2: Mass spectrum and structure ofLanosta-8,24-dien-3-one

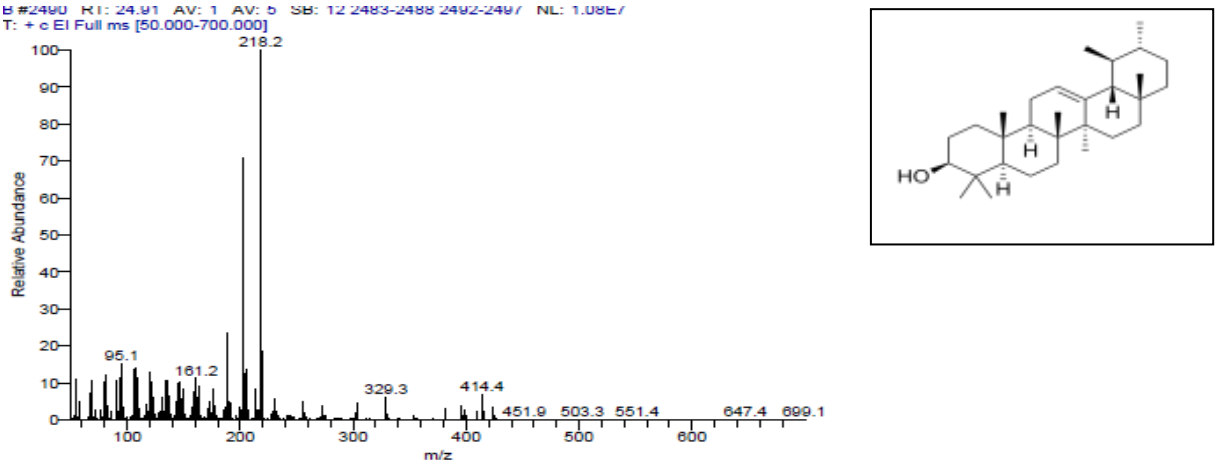

Figure-3: Mass spectrum and structure ofá-Amyrin.

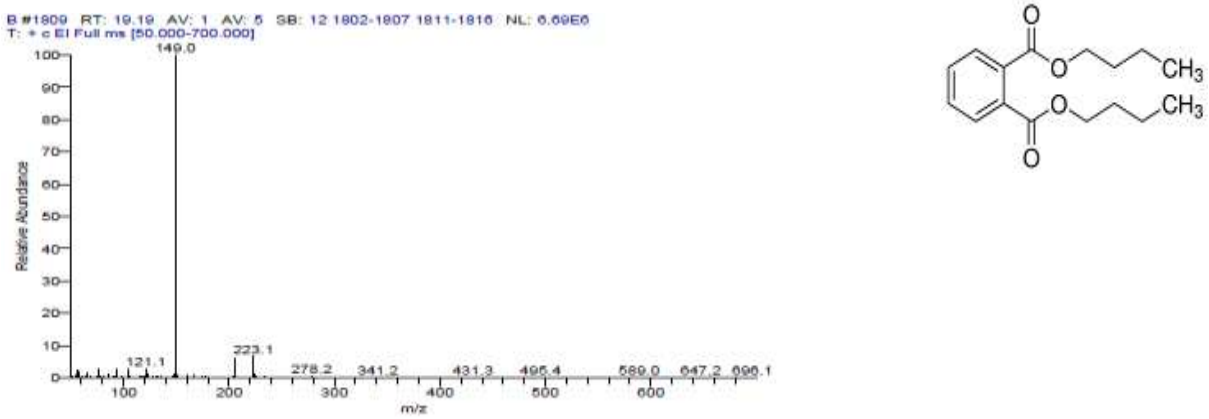

Figure-4: Mass spectrum and structure of Dibutyl phthalate.

Table-2: Biological activity of phyto-components identified in the methanol bark extract of *Ficus krishnae*.

Compound name	Compound nature	Biological activity
Dibutyl phthalate	Oily liquid	Ectoparasiticide
Lanosta-8,24-dien-3-one	Tetracyclic terpenols	Treatment of tumors, inflammation and/or pain.
á-Amyrin	Triterpenes	Management of malaria, ulcer, rhematic pain, toothache, and inflammatory disorders
Lup-20(29)en-3-one	Triterpenes	Antioxidant activity
1-Heptatriacotanol	-	Antibacterial activity
Lupeol	Triterpenoids	Antiprotozoal, antimicrobial, anti-inflammatory, antitumor and chemopreventive
4-Methylenecycloartan-3-one	Triterpenoid	Anti-bacterial
9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl,acetate, (3á,4à,5à)	-	Anti-microbial. Antioxidant
9,19-Cyclolanostan-3-ol, 24-methylene, (3á)	Triterpenes	colic, cure head or chest colds, and relieve cough
Stigmast-4-en-3-one	organic compound	Antidiabetc
Testosterone cypionate	Hormone	Treat hypogonadism in male
Cholest-4-en-3-one	Cholestenone	As intestinal metabolite of cholesterol
12-Oleanen-3-yl acetate, (3à)	Terpene	Antioxidant and anticancer
á-Amyrintrimethylsilyl ether	Triterpene	Anticancer Antimicrobial Antiinflammatory

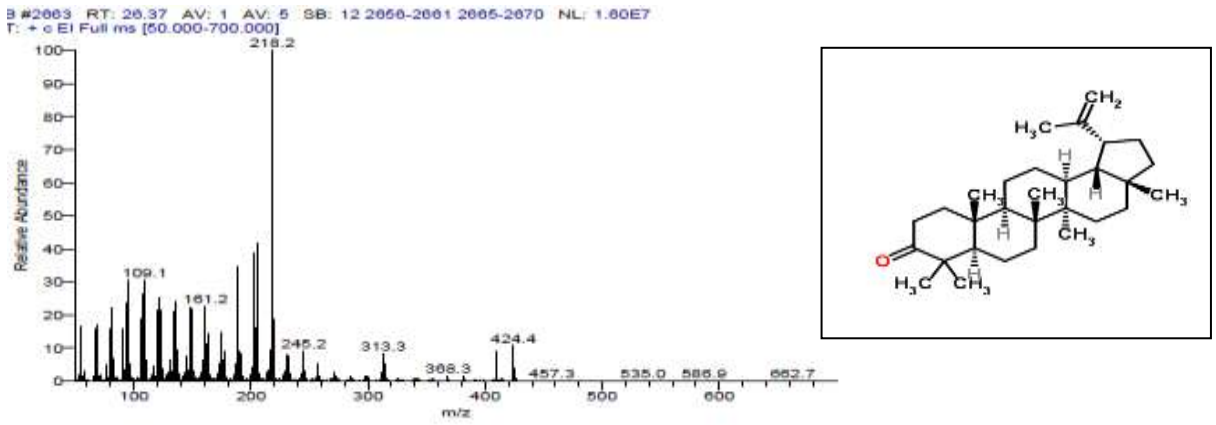

Figure-5: Mass spectrum and structure ofLup-20(29)en-3-one

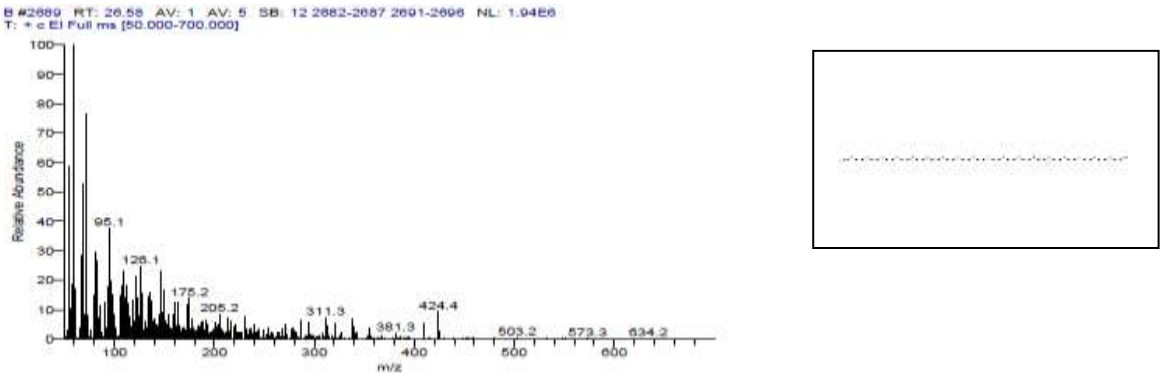

Figure-6: Mass spectrum and structure ofLup-20(29)en-3-one

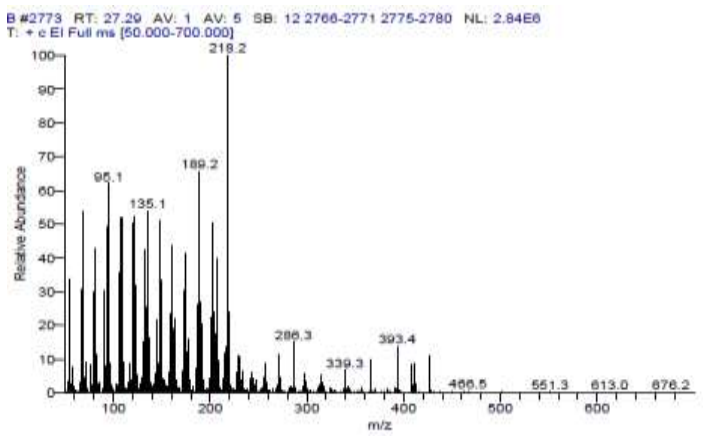

Figure-7: Mass spectrum and structure ofLupeol

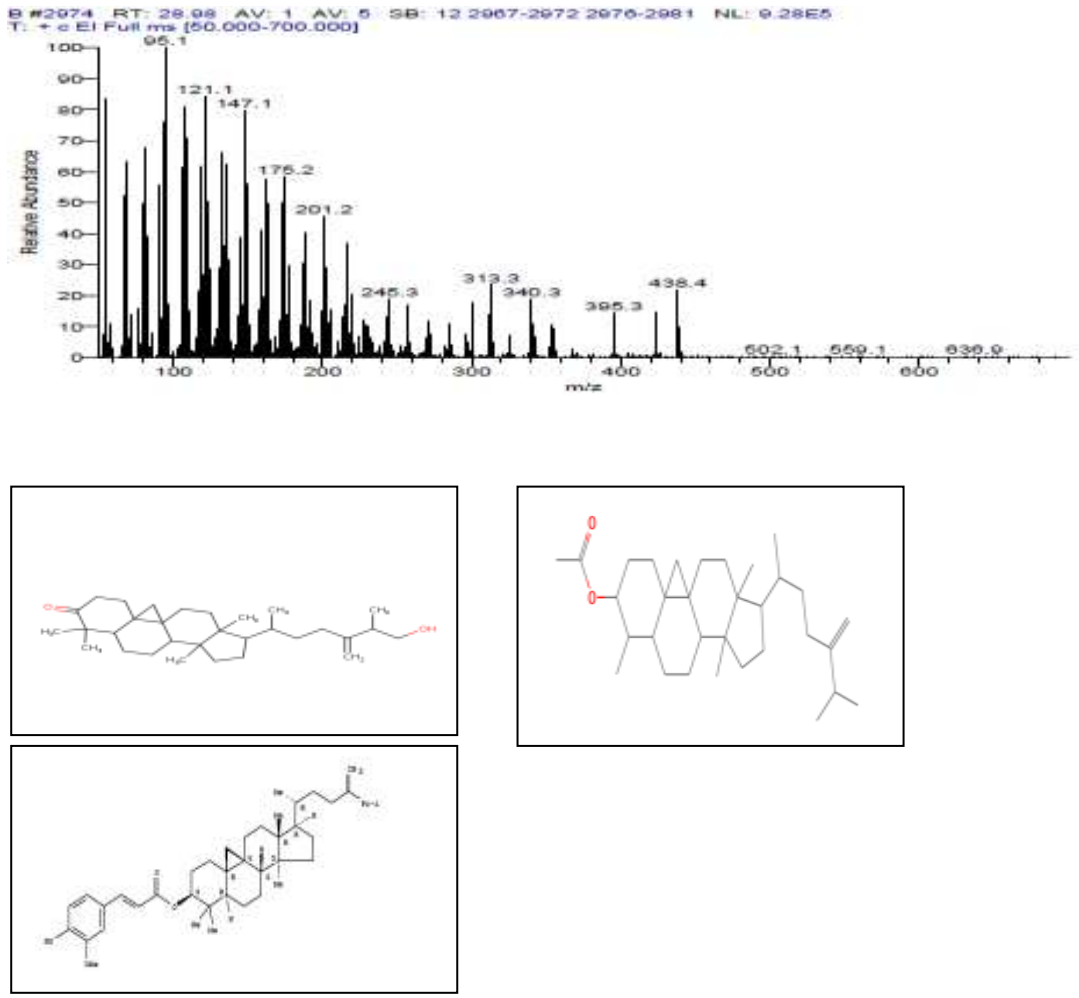

Figure-8: Mass spectrum and structure of4-Methylenecycloartan-3-one, 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl,acetate, (3á,4à,5à) and 9,19-Cyclolanostan-3-ol, 24-methylene, (3á)

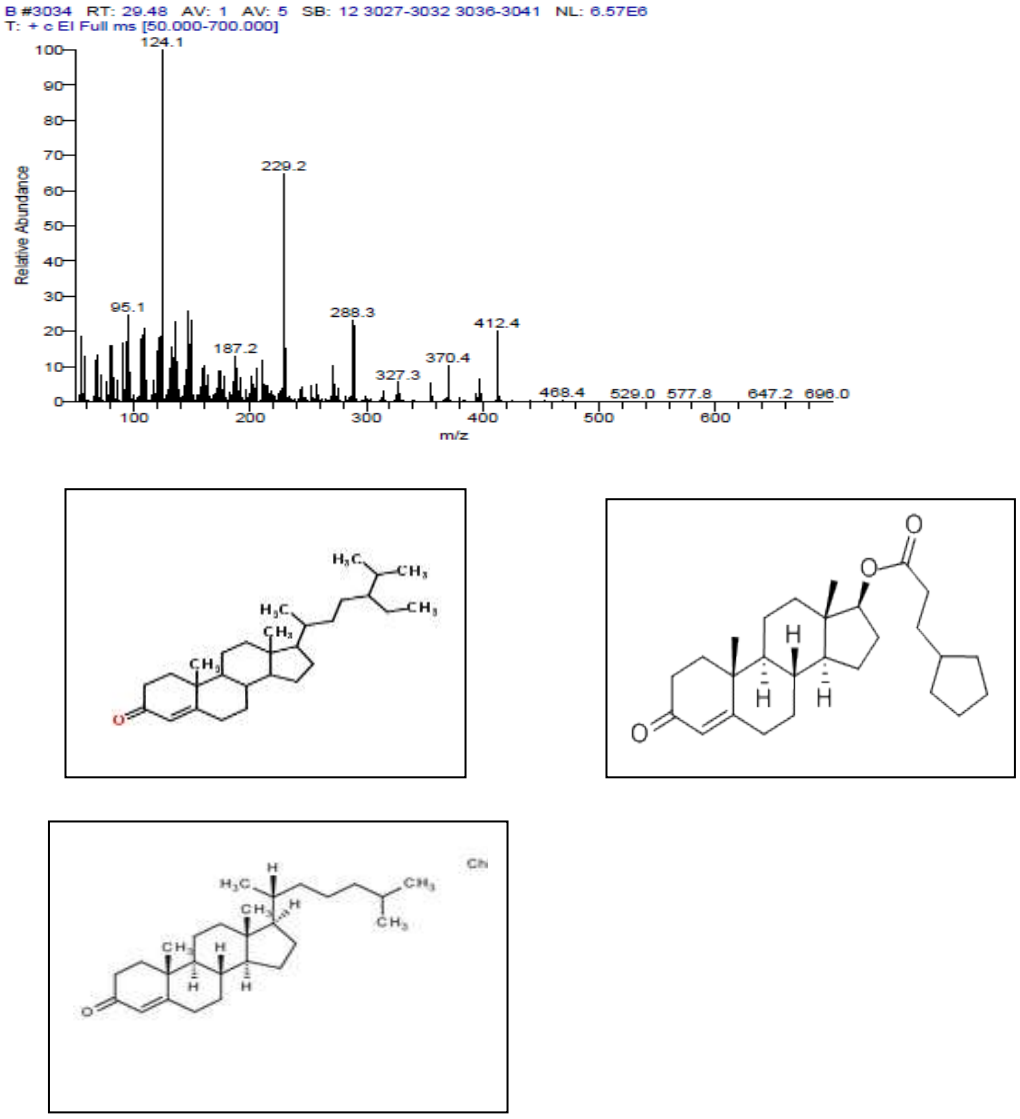

Figure-9: Mass spectrum and structure ofStigmast-4-en-3-one, Testosterone cypionate and Cholest-4-en-3-one.

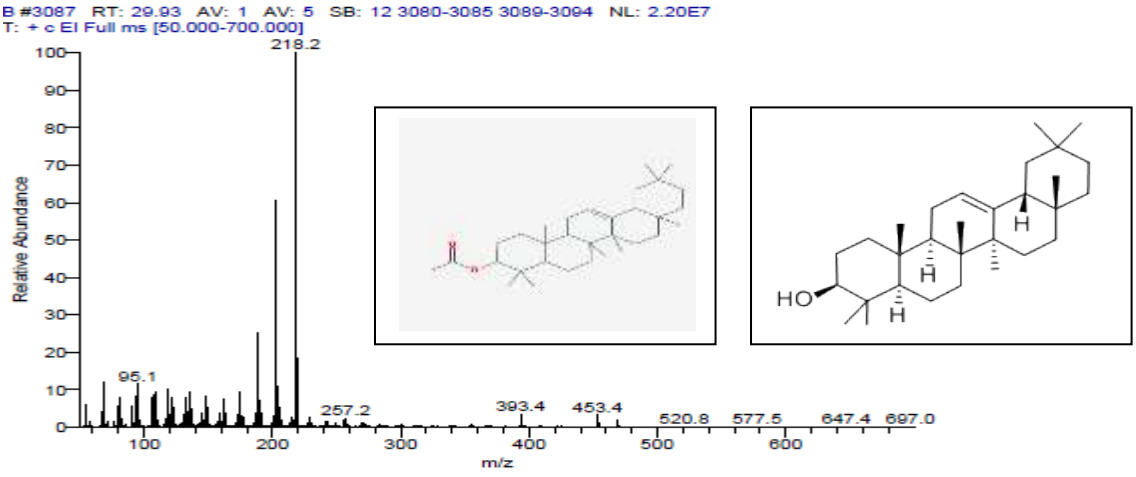

Figure-10: Mass spectrum and structure ofStigmast-4-en-3-one, Testosterone cypionate and Cholest-4-en-3-one.

Conclusion:

In this present investigation forty two bioactive compound have been identified from methanolic extract of *Ficus krishnae* by Gas chromatogram-Mass spectrometry (GC-MS) analysis.The presence of various bioactive compounds in this plant are responsible for the pharmaceutical properties like anti- diabetic property which has showed a good inhibition of alpha-amylase enzyme. Though, further studies will require for the isolation of pure compounds their bioactivity and toxicity profile.

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Authors' contributors: The authors of this study claim sole responsibility for the concepts included herein. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no competing interests.

Author details: 1 Department of Biotechnology Gulbarga University Kalaburagi-585106, Karnataka, India.

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