Antidiabetic Potential of *Ficus glomerata* Roots with a Special Emphasis on Estimation of Bioactive Compounds by a Novel Validated HPTLC Technique

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**ABSTRACT**

**Background:** The data presented in this article for *Ficus glomerata* Linn. belonging to family Moraceae which is commonly found all over India. This study aimed towards the development and validation of high-performance thin-layer chromatography (HPTLC) method for simultaneous estimation of lupeol and quercetin from *Ficus glomerata* and correlate with its antidiabetic potential. **Methods:** The various fractions of ethanolic extract of *Ficus glomerata* root were prepared. The HPTLC analysis of quercetin and lupeol which are the important phytoconstituents responsible for various pharmacological actions was carried out at 525 nm. ICH guidelines were followed to validate this method for accuracy, precision and repeatability. **Results:** The linearity range of quercetin and lupeol were obtained as 400-2400 ng/spot and 1000-5000 ng/spot respectively. Percent drug content was highest in diethyl ether fraction (quercetin 2531.8 ng and lupeol 1400 ng). The limit of detection value (LOD) obtained for quercetin and lupeol was 3.0793 and 3.1645 ng and the limit of quantification (LOQ) was 9.3314 and 9.5895 ng respectively. This method developed was accurate, precise and simple has shown higher resolution from other phytoconstituents present in the fractions. The method can be very effectively applied for analyzing the quality of herbal material and formulations containing *Ficus glomerata*. Antidiabetic activity of various fractions of ethanolic extract of *Ficus glomerata* roots was studied on alloxan-induced diabetic rats. Treatment with fractions was continued for 11 days. The effect of the fractions on glucose was analyzed. Diabetic rats treated with diethyl ether fraction exhibited a significant (p<0.05) decrease in glucose levels, indicating the potential use of *Ficus glomerata* in diabetes mellitus. **Conclusion:** As per the ICH guidelines, the HPTLC method used for simultaneous estimation of lupeol and quercetin was accurate, precise and specific. The method used for phytochemical standardization of various fractions of ethanolic extract of the roots of *Ficus glomerata* and correlated with its antidiabetic activity. **Key words:** *Ficus glomerata*, HPTLC, Lupeol, Quercetin, Alloxan, Antidiabetic activity.

**INTRODUCTION**

Diabetes Mellitus (DM) is a dreadful metabolic disorder featured by enhanced blood glucose levels occurring due to marked impairment in metabolic processes due to defects in either secretion of insulin or response or both. Insulin resistance, hyperglycemia and relative insulin deficiency are the major clinical manifestations observed in patients of both Type 1 and Type 2 forms of DM. As of 2020, the worldwide prevalence of diabetes has been increasing constantly and about 500 million people are suffering from DM. The pathologic indication of DM especially Type 2 DM comprehends both macrovascular and microvascular complications. The chronicity of hyperglycemia results in injury to organ systems mainly the eyes, kidneys, nerves and heart.
The majority of the patients with Type 2 DM die of renal diseases and complications in the cardiovascular system due to hyperlipidemia, obesity, atherosclerosis and hypertension induced by Type 2 DM. Insulin and oral hypoglycemic agents along with lifestyle and dietary modifications are the main strategies for management of Type 2 DM. Insulin plays a significant role in Type 2 DM when the blood glucose levels cannot be maintained by exercise, diet, weight loss and oral medicine treatments. Oral hypoglycemic agents such as biguanides, alpha-glucosidase inhibitors, sulphonylureas and thiazolidinediones act by attenuating metabolic disturbances such as inadequate insulin secretion from the pancreas and insulin resistance. Although these drugs remain as the mainstay of diabetes management but these drugs are associated with several untoward effects resulting in morbidities and life-threatening consequences. Hence a need arises to search for a novel antidiabetic agent having greater efficacy towards amelioration of diabetes and associated complications with fewer side effects.

In the past few years, research regarding biofriendly, eco-friendly and relatively safer plant-based medicines from the traditional system of medicine has gained a lot of interest. WHO has listed more than 21,000 plants with important medicinal properties. India is said to be the botanical garden of the world and is considered a mine rich in medicinal plants. Around 2500 medicinal species possessing a variety of pharmacological actions are available in India, from which more than 150 species are commercially used in the development of newer drugs.

The *Ficus glomerata* (*F. glomerata*) has been reported for various pharmacological properties and their role as excipients in pharmaceuticals. As mentioned in the Ayurvedic system of medicine, roots of *Ficus glomerata* are used for the treatment of hydrophobia. Its stem bark is used as an antidiuretic agent, galactagogue and is helpful in the treatment of gynecological disorders. The fruits are active against menorrhagia, leprosy, intestinal worms, urinary tract infections, burns, blood disorders and dry cough. The leaves are useful in the treatment of piles, bronchitis and bowel syndrome reported in the Unani System of Medicine. The latex portion is applied externally to the wounds for reducing pain, edema and inflammation. It is also used in children to reduce dysentery and diarrhoea.

The various phytoconstituents have been isolated from *F. glomerata* such as bergapten, bergaptol, β-Sitosterol, Stigmasterol, lanosterol, β-sitosterol-d-glucoside (phytosterolin), lupen-3-one and vitamin k. The bark is reported to contain saponin, wax, tannin, lupeol, lupeol acetate, β-sitosterol and leucocyanidin ceryl behenate. The leaves have shown the presence of tannic acid, arginine, alanine, tryptophan, tyrosine, proline, serine, valine, leucine, aspartic acid, glycine, methionine, n-nonacosane, isoleucine, campesterol, α- amyrin, isofucosterol, hexa-cosanol, lupeol, stigmasterol, n-octacosan and threonine. These biologically active phytoconstituents isolated from this plant have been reported to possess several important therapeutic potentials.

High performance thin layer chromatography (HPTLC) is considered as the most applicable and widely used analytical method as it has many benefits such as reliability. This method is based upon the quantitative estimation of various phytoconstituents and is considered a cost-effective technique. It has been reported as a very effective technique as it is economical, sample throughput is high and sample clean-up required is minimum. The important benefit of HPTLC is considered as the reduction in the time of analysis. Similar to that of gas chromatography (GC) and high performance liquid chromatography (HPLC) techniques, HPTLC is also preferred for the development of chromatographic fingerprint methods which can be applicable for identification of complex phytoconstituents of herbal extracts and its data analysis system including experimental conditions can be optimized.

Phytoconstituents of *F. glomerata* such lupeol and quercetin has been isolated and quantified using HPTLC technique. Qualitative and quantitative determination of phytoconstituents such as lupeol, stigmasterol, quercetin, kaempferol, gallic acid, curcumin, kaempferol and lupeol has been carried out either individually or together simultaneously using HPTLC technique in several studies in past. Reverse phase- HPTLC (RP-HPTLC) technique (using RP-18 F\textsubscript{254} TLC plates with the dual run) has been applied for simultaneous estimation of chief flavonoids containing quercetin and apigenin from extracts has also been reported. Literature survey indicated that no particular method was developed in past for the simultaneous quantitation of quercetin and lupeol from various fractions of *F. glomerata* roots extract.

Considering all the aforementioned aspects, the present study was carried out in two parts with an aim to carry out simultaneous quantification of quercetin and lupeol, the major antidiabetic biomarkers of *F. glomerata* by a validated HPTLC method in the first part and determination of antidiabetic activity of different fractions of ethanolic extract of roots of *Ficus glomerata* for antidiabetic potential in the second part.
MATERIALS AND METHODS

Chemicals
Chemicals of AR grade used were procured from different manufacturers such as Fischer Inorganics, Reachem Ltd, NICE Chemicals Ltd, and Aromatic Ltd and Ranbaxy Fine Chemicals Ltd. Alloxan was procured from Ranbaxy Fine Chemicals Ltd.

Animals
Male Wistar albino rats weighing 150-250 gm were used in the current investigation. The animals were purchased from National Institute of Biosciences (NIB), Pune, Maharashtra and separately caged into various groups randomly for 7 days for acclimatization and maintained at standard environmental conditions including 25 ± 2°C temperature and relative humidity of 45 to 55% under 12 hr dark: 12 hr light cycle. They were provided with standard pelleted feed (Nutrivel lifesciences). Water and food were not provided for 16 hr ad libitum for animals to maintain fasting. The animals were kept in the laboratory 1 hr before start of the experiment. All the experiments were carried out between 12:00-16:00 hr. The animals were transferred to the experimental laboratory from the animal house one hour before the start of the experiment.

Ethical clearance
Experiments on animals were performed in compliance with guidelines of Institutional Animal Ethical Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Section 15 of the Prevention of Cruelty to Animals Act, 1960; Ministry of environment and forest (AWD), Government of India). IAEC of Modern College of Pharmacy (For Ladies), Moshi, Pune - 412015 approved the protocol (Proposal No.: 1036/PO/Re/S/CPCSEA/16-17/F1).

Collection of plant material, preparation and fractionation of extract
The plant material F. glomerata roots were collected from local areas of Pune. Procured plant was authenticated from the Botanical Survey of India, Pune; Maharashtra, India. Then further air-dried under a shed at room temperature and the coarsely powdered drug was further processed for extraction. Extraction was carried out using Soxhlet apparatus using ethyl alcohol (95%). The ethanolic extract was fractionated successively by liquid-liquid extraction with petroleum ether, diethyl ether, acetone, ethyl acetate, and n-butanol. Fractions were extracted in triplicate and concentrated using a rotary evaporator (Buchi R-114) and percentage yield was calculated.

Preparation of standard solutions
The standard solutions of quercetin (0.2 mg/ml) and lupeol (0.5 mg/ml) were prepared. Accurately weighed lupeol (5mg) and quercetin (2mg) was diluted in 10 ml of 80% (v/v) methanol. Stock solutions were further prepared by diluting with 80% (v/v) methanol to obtain standard solutions of concentration 1.0, 2.0, 3.0, 5.0 μg/ml for lupeol and 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 μg/ml for quercetin respectively.

Preparation of sample solutions of fractions
All samples were prepared by dissolving 50 mg fractions in 10 ml of petroleum ether, diethyl ether, ethyl acetate, acetone n-butanol respectively in the volumetric flask. After that, all solutions were sonicated for 30 min and filtered before being applied to HPTLC plate.

Instrument and chromatographic parameters for method development:
For performing HPTLC study, pre-coated silica gel 60F254 (aluminum sheet of 20 × 10 cm, Merck) was used. All the sample and standard solutions were dropped to plate with Linomat V automatic sample applicator (Camag, Switzerland) as a band. To provide the best resolution mobile phase toluene: methanol (9:1 % v/v) was used with 20 min of chamber saturation time. The plate was developed was at a distance of 80 mm in Camag twintrough chamber. The densitometer analysis was carried out by using TLC scanner 4 (Camag, Switzerland using WinCATS software). The detection wavelengths for lupeol and quercetin were found to be 525 nm, and 250 nm respectively. The vanillin sulphuric acid was used as derivatizing agent.

HPTLC method validation
According to International Conference on Harmonization (ICH) guidelines (IUPAC, 2002), validation of this HPTLC method was performed in with respect to its accuracy, precision, linearity, LOQ, LOD, specificity, repeatability and percentage recovery. The linearity test was carried out at various concentrations of the standard stock solution of quercetin and lupeol at wavelengths 250 nm and 525 nm corresponding to the concentration of 400ng-2400ng and 1000ng-5000ng respectively. The calibration curves for linearity were estimated for standards, based upon the graphs plotted as peak area versus concentration of each standard. The specificity of this method was estimated by performing a comparison of R values of standard quercetin, lupeol and for fractions. The
procedure was repeated for \( n = 6 \) times. The experiment was carried out to determine the percentage recovery and repeatability of the standard. The sensitivity of this method was carried out and confirmed based upon the limit of detection (LOD) and limit of quantitation (LOQ). The limits of detection (LOD) of quercetin and lupeol were estimated to be 3.3 \( \sigma / S \) while LOQ as 10 \( \sigma / S \). Accuracy and precision for diethyl ether fraction were confirmed by evaluating repeatability and percent drug recovery.\(^{39}\)

**Evaluation of the antidiabetic effect of fractions of ethanolic extract of roots of \( F. \) glomerata**

Experimental animals were grouped into seven groups with six animals in every group. Group I (normal control group) and Group II (diabetic control group) were administered with normal saline solution (1 ml/kg, p.o.). Groups III, IV, V, VI and VII (test groups) received respective fractions of ethanolic extract orally at doses of 150 mg/kg and Group VIII (standard group) received reference standard metformin at the dose of 50 mg/kg, p.o. For the induction of diabetes, a single intraperitoneal alloxan monohydrate at the dose of 140 mg/kg was administered to animals of all the groups except Group I.\(^{40}\) Alloxan was weighed according to the body weight of each animal, just before injection and dissolved in 0.2 ml saline (154 mM NaCl). After three days of alloxan administration, the levels of blood glucose were estimated and the rats with blood glucose levels higher than 140 mg/dl were selected for further study. Dosing with respective drugs was started after 72 hr of alloxan injection and continued further for 11 days. Collection of blood samples of all the rats was done through retro-orbital route on 0, 1 and 11 days to estimate fasting blood glucose levels using glucose oxidase-peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA).\(^{41}\)

**Statistical Analysis**

All results were expressed as mean ± SEM. For statistical analysis of glucose tolerance test one-way ANOVA followed by Dunnet’s test was used and for antidiabetic activity one-way ANOVA followed by Tukey’s Kramer multiple comparison test was used. Values of \( P < 0.05 \) were considered significant.

**RESULTS**

The alcoholic extract of roots of \( F. \) glomerata was fractionated in different solvents such as petroleum ether, diethyl ether, acetone, ethyl acetate and n- butanol. Diethyl ether fraction showed the highest yield of 1.08 % w/w (Table 1). The different mobile phase systems were tried for simultaneous determination of quercetin and lupeol using HPTLC technique. A system containing toluene:methanol (9:1 % v/v) was confirmed, as this mobile phase was found to give good separation with symmetric peaks, 250 nm for quercetin (R\(_f\) value: 0.14 ± 0.02) and 525 nm for lupeol (R\(_f\) value: 0.65 ± 0.02). The estimations showed that higher amounts of quercetin (2531.8 ng) and lupeol (1400 ng) were found in diethyl ether fraction as compared with other fractions. Petroleum ether fraction revealed 35.27 ng of quercetin and 410 ng of lupeol. The acetone and ethyl acetate fractions revealed the presence of lupeol 76 ng and 600 ng respectively while quercetin was not observed. The n-butanol fraction did not show the presence of quercetin and lupeol according to (Table 1).

Linearity range was observed in concentration range of 400 to 2400 ng/spot \((r=0.99196)\) for quercetin and 100 to 5000 ng/ spot \((r=0.99694)\).

Lowest amount of analyte which can be detected is considered as Limit of detection and limit of quantitation is termed to be the lowest amount of the standard that can be quantified and having precision and accuracy which is acceptable. The limit of detection and limit of quantification was determined as 3.0793, 9.3314 ng/spot for quercetin and 3.1645, 9.5895 ng/ spot for lupeol (Table 2).

The recovery studies and repeatability considered as validation parameters were performed for diethyl ether

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<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Solvent</th>
<th>Color and consistency</th>
<th>Percent w/w yield</th>
<th>Quercetin (ng)</th>
<th>Lupeol (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether</td>
<td>Brown, solid</td>
<td>0.85</td>
<td>35.37</td>
<td>410</td>
</tr>
<tr>
<td>2</td>
<td>Diethyl ether</td>
<td>Dark brown, semisolid</td>
<td>1.08</td>
<td>2531.8</td>
<td>1400</td>
</tr>
<tr>
<td>3</td>
<td>Acetone</td>
<td>Brown, semisolid</td>
<td>0.98</td>
<td>--</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl acetate</td>
<td>Brown, semisolid</td>
<td>0.87</td>
<td>--</td>
<td>600</td>
</tr>
<tr>
<td>5</td>
<td>n-Butanol</td>
<td>Yellowish brown, semisolid</td>
<td>0.5</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
fraction as it has shown higher amounts of quercetin and lupeol (Table 3).

The recovery studies and repeatability considered as validation parameters were performed for diethyl ether fraction at 80%, 100% and 120% levels as it has shown higher amounts of quercetin and lupeol (Table 3). Linearity plots of reference standards and estimation of diethyl ether fraction of *F. glomerata* root extracts are shown in Figure 1-3.

Results of antidiabetic study showed that different fractions like petroleum ether, diethyl ether and ethyl acetate exhibited antihyperglycemic activity while acetone and n-butanol fractions did not show any significant activity. According to the results mentioned in Table 4, diethyl ether fraction showed more significant effect on blood glucose levels.

**DISCUSSION**

For confirmation of the quality and identification of important traditional medicines, chromatographic fingerprint estimations have been proven to be a rational and effective practical approach. The HPTLC technique

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**Table 2: HPTLC analysis data of quercetin and lupeol.**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Quercetin</th>
<th>Lupeol</th>
</tr>
</thead>
<tbody>
<tr>
<td>R_f values</td>
<td>0.14 + 0.02</td>
<td>0.65 + 0.02</td>
</tr>
<tr>
<td>λ_max</td>
<td>250 nm</td>
<td>525 nm</td>
</tr>
<tr>
<td>Linearity range</td>
<td>400 ng-2400 ng</td>
<td>1000 ng-5000 ng</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = 798.521 + 3.291 x X</td>
<td>Y = 3054.867 + 1826.841 x X</td>
</tr>
<tr>
<td>Regression (r²)</td>
<td>0.99196</td>
<td>0.99694</td>
</tr>
<tr>
<td>LOD</td>
<td>3.0793 ng</td>
<td>3.1645 ng</td>
</tr>
<tr>
<td>LOQ</td>
<td>9.3314 ng</td>
<td>9.5895 ng</td>
</tr>
</tbody>
</table>

**Table 3: Validation parameters for diethyl ether fraction.**

<table>
<thead>
<tr>
<th>Phytoconstituents in diethyl ether fraction</th>
<th>Area</th>
<th>Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>RSD</td>
</tr>
<tr>
<td>Quercetin</td>
<td>106.832</td>
<td>4.188</td>
</tr>
<tr>
<td>Lupeol</td>
<td>283.916</td>
<td>3.463</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentage drug recovery from diethyl ether fraction.</th>
<th>Quercetin (% Recovery)</th>
<th>Lupeol (% Recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 %</td>
<td>101.10</td>
<td>97.10</td>
</tr>
<tr>
<td>100 %</td>
<td>103.53</td>
<td>100.59</td>
</tr>
<tr>
<td>120 %</td>
<td>105.52</td>
<td>99.84</td>
</tr>
</tbody>
</table>

**Table 4: Effect of various fractions of alcoholic extract of *Ficus glomerata* roots on blood glucose levels in alloxan-induced diabetic rats.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Groups</th>
<th>0 Day (mg/dl)</th>
<th>1st Day (mg/dl)</th>
<th>11th Day (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>79±4.51</td>
<td>78±3.78</td>
<td>78±4.18</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>259±9.53***</td>
<td>236±6.21</td>
<td>179±5.53</td>
</tr>
<tr>
<td>III</td>
<td>Metformin</td>
<td>250±7.61</td>
<td>160±5.03</td>
<td>97±4.12**</td>
</tr>
<tr>
<td>IV</td>
<td>Pet Ether</td>
<td>240±6.13</td>
<td>216±7.22</td>
<td>118±5.32**</td>
</tr>
<tr>
<td>V</td>
<td>Diethyl ether</td>
<td>237±6.35</td>
<td>190±6.01</td>
<td>95±4.10**</td>
</tr>
<tr>
<td>VI</td>
<td>Acetone</td>
<td>245±7.12</td>
<td>230±7.63</td>
<td>179±5.18</td>
</tr>
<tr>
<td>VII</td>
<td>Ethyl acetate</td>
<td>233±8.55</td>
<td>219±6.89</td>
<td>145±4.99*</td>
</tr>
<tr>
<td>VIII</td>
<td>n-Butanol</td>
<td>236±7.10</td>
<td>224±7.10</td>
<td>204±7.60</td>
</tr>
</tbody>
</table>
was employed for establishing particular identification parameters for drugs. The obtained fingerprint results can be then applied to conclude the presence or absence of markers of interest. An estimation of concentration and quality of major chemical constituents are important because a small deviation in a principle ingredient significantly affects their efficacy.

The results revealed that the other phytoconstituents present in the plant extracts did not show any interference with the peaks of quercetin and lupeol. So the method was determined as a specific one. The spectrum of standard compounds quercetin and lupeol were found to be exactly similar to the corresponding spots observed in the fractions of alcoholic extract of *F. glomerata* roots, indicating that there was no interference by any other phytoconstituents of the plant. The purity of peaks obtained was confirmed based upon comparison performed at three different levels such as peak start, peak apex and peak end positions.

The low relative standard deviation values obtained indicated that this developed method is precise and repeatable. Based upon good recovery values it can be confirmed that the method is devoid of any interference. This method was established and can be effectively used for the simultaneous detection of quercetin and lupeol present in various fractions of ethanolic extract of *F. glomerata* roots. So, herewith we have revealed HPTLC method which is new, simple and sensitive for the determination of important phytoconstituents found in *F. glomerata* roots. The validation parameters of this method were determined as per International Conference on Harmonization (ICH) guidelines.

Major populations worldwide have been affected by DM which is considered to be a major health problem. In several epidemiological and clinical studies, hyperglycemia has been documented as the principal cause of serious diabetic complications. For minimizing risk related to various microvascular and macrovascular complications, hyperglycemia should be reduced. The roots of *F. glomerata* contain high amounts of flavonoids and phenolic compounds which are effective for main therapeutic activities such as antioxidant, antidiabetic, anti-inflammatory actions, etc. Hence *F. glomerata* roots in the form of alcoholic extract were used in the present study.

In comparison with that of the pure isolated compound, the standardized fraction of an effective extract can be proved as therapeutically more potential, having less toxicity and economical as well. Keeping this in mind, the present study was targeted for evaluation of different fractions of ethanolic extracts of *F. glomerata* for antidiabetic potential against diabetes induced by alloxan to confirm the most active fraction of *F. glomerata* roots. Alloxan was administered intraperitoneally at a dose of 140 mg/kg, which was selected referring to previously published literature. Due to selective cytotoxic effect on pancreatic beta cells alloxan results in induction of hyperglycemia within a minimum of 72 hr after its intraperitoneal administration. Its cytotoxicity occurs by virtue of the formation of free radicals, proven in both in vitro and in vivo studies and can be considered as intracellular phenomena.

Antidiabetic activity observed may be related to insulinomimetic and antioxidant action and can be attributed to bioactive compounds such as quercetin and lupeol. These biomarkers have been already documented to possess potent antidiabetic effects in several studies.

The present study can be effectively implemented as the developed HPTLC method was found to be without any interferences and having good recovery values. For simultaneous estimation of quercetin and lupeol from various fractions of ethanolic extract of *F. glomerata* roots, this developed and validated method is found to be most effective and applicable. The method reliability was studied and determined by assessing different validation parameters as mentioned in the ICH guidelines. This HPTLC method was confirmed to be simple, sensitive, specific, precise and accurate for the identification and quantification of quercetin and lupeol. Thus, a developed and validated method can be applicable for standardization, checking quality, analysis and quantification of quercetin and lupeol in the roots of *Ficus gomerata*. Also, as discussed roots of *Ficus gomerata* possess important therapeutic potential in the treatment and management of various medical conditions and are found to contain a good concentration of these important phytoconstituents. So this method can be implemented for isolation and simultaneous estimation of the pharmacologically important phytoconstituents. The anti-diabetic activity was more significant in diethyl ether fraction rich in quercetin and lupeol as compared with other fractions of alcoholic extract of *F. glomerata* roots indicating the potent antidiabetic activity of diethyl ether fraction attributed to the presence of these antidiabetic biomarkers.

**CONCLUSION**

The roots of *F. glomerata* contain higher amounts of flavonoids and phenolic compounds which show main therapeutic activities like antioxidant, antidiabetic, anti-inflammatory actions, etc. The present investigation was conducted to study the most potential fraction of
**Ficus glomerata** root extracts. The anti-diabetic activity was more significant in diethyl ether fraction as compared with other fractions of alcoholic extract of *F. glomerata* roots. In this study, good recovery values were obtained which indicates that the developed HPTLC method is devoid of interferences. This method was effectively developed and validated for the analysis of simultaneous estimation of quercetin and lupeol from various fractions of ethanolic extract of *F. glomerata* roots. More amounts of quercetin and lupeol were estimated in the diethyl ether fraction which attributes to its antidiabetic activity. Further studies of isolation and formulation are required to get a potential antidiabetic drug from natural origin.

**ACKNOWLEDGEMENT**

Authors are indebted to Dr. Asha Thomas (Professor and Head of Department Pharmaceutical Chemistry) Dr. D. Y. Patil Unitech Society's Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research Sant Tukaram Nagar, Pimpri, Pune - 411 018 (MH) India her constant guidance, motivation and being supportive during this course of the investigation.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**ABBREVIATIONS**

HPTLC: High performance thin layer chromatography; *F. glomerata*: *Ficus glomerata*; LOD: Limit of detection; LOQ: Limit of quantification; DM: Diabetes Mellitus; WHO: World Health Organisation; GC: Gas chromatography; RP-HPTLC: Reverse phase- High performance thin layer chromatography; TLC: Thin layer chromatography; NIB: National Institute of biosciences; IAEC: Institutional Animal Ethical Committee; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; ICH: International Conference on Harmonization; Rf: Retention factor; NaCl: Sodium chloride; SEM: Standard error of mean; ANOVA: Analysis of variance; P: Probability; Ng: nanogram; Hr: Hour; Min: Minutes.

**REFERENCES**


**PICTORIAL ABSTRACT**

**SUMMARY**

- HPTLC analysis of the important phytoconstituents (quercetin and lupeol) responsible for antidiabetic activity was carried out (at 525 nm).
- Method developed was accurate, precise and simple
- Antidiabetic activity of various fractions of ethanolic extract of *Ficus glomerata* root was studied on alloxan-induced diabetic rats. Diabetic rats treated with diethyl ether fraction exhibited significant (p<0.05) decrease in glucose levels, indicating the potential use of *Ficus glomerata* in diabetes mellitus.
About Authors

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