



Pharmaco-chemical Characterization and Evaluation of *In Vitro* Antioxidant and Antidiabetic Activity of Ethanolic Flower Extract of *Clerodendrum paniculatum*

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ABSTRACT

Quantitative and qualitative analysis of different phytochemical components and antioxidant and antidiabetic activities of the extract of *Clerodendrum paniculatum* flower (CPF) were analyzed *in vitro*. Chromatographic identification of phytochemicals of *Clerodendrum paniculatum* flower was also identified by GC-MS analysis. To assess the biochemical features of CPF. Sequential solvent extraction of CPF was performed using solvents in increasing order of polarity (petroleum ether, chloroform, ethyl acetate, ethanol, and water) and solvent with maximum phytochemical profile was standardized for further analysis. Quantitative analysis of selected secondary metabolites like tannin, flavanoids, alkaloids, and phenols of the flower extract was done by UV spectrophotometer. *In vitro* antioxidant assays and *in vitro* antidiabetic efficacy of the flower extract were analyzed by respective *in vitro* assays. Chromatographic identification of phytochemicals in CPF was identified by using GC-MS analysis. Quantitative estimation revealed an accountable amount of secondary metabolites like phenols (47.66mg/g gallic acid equivalent), flavonoids (24mg/g quercetin equivalent), tannins (41mg/g catechine equivalent), and alkaloids (1.79mg/g of extracted plant material). Chromatographic analysis (GC-MS) also confirmed convincing bioactive compounds in the extract. From *in vitro* antioxidant and antidiabetic assay, the IC₅₀ value of the extract of CPF was measured and compared with standard, and from the results, it was evident that the extract had significant *in vitro* antioxidant and antidiabetic activity. From the above results, it can be confirmed that CPF has got pharmacologically significant phytoconstituents and therapeutically active ingredients, as evident in chromatographic analysis.



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INTRODUCTION

From ancient times herbs have been used in abundant endemic practices of medicines for a remedial idea. They are increasingly becoming prominent in modern society as a substitute for synthetic medicines. Herbal medicines are culturally more acceptable because they cause fewer side effects than some other commercial drugs (Carlson, 2002; Dey and De, 2015). Today, scientific attestation is ample to support the aid of herbal medicines. Herbal medicines are nothing but "green gold" of the earth. It gives protection to humans and animals and

contributes directly and indirectly towards environmental sustainability.

Of late, the availability of experimental data substantiates the proof for the involvement of free radicals in several pathophysiological states. Still, pieces of evidence are not enough to ascertain whether the ROS cause any consequence of pathology. This has led to increasing curiosity among scientists and researchers to evaluate the potential benefits of antioxidant therapy. Antioxidants are important radical scavengers and help reduce various health disorders, such as cancer, aging, cardiovascular disease, cataracts, diabetics, and brain dysfunction (Negi *et al.*, 2011). Antioxidants are being recognized as prophylactic and therapeutic agents that are pharmacologically active in the prevention of several diseases. Medicinal plants are the primary antioxidant sources in nature.

Natural products contain various important secondary metabolites such as tannin, phenols, alkaloids, flavonoids, terpenoids, anthocyanins, and fatty acids (Jain *et al.*, 2011). Antidiabetic (Ghorbani, 2017) and anti-inflammatory activities (Ambriz-Perez *et al.*, 2016; Haminiuk *et al.*, 2012) of phenolic compounds were well documented. Tannins are classes of biomolecules of polyphenolic nature. Tannins constitute a major class of phenolic compounds that exhibit remarkable biological activities like preventing oxidative stress and cellular damage and providing antihyperglycemic and antihyperlipidemic effects (Velayutham *et al.*, 2012). Radical scavenging activity (Hatano *et al.*, 1988) and anticancer activity (de Pascual-Teresa *et al.*, 2000) of flavonoids were proved. Alkaloids are cyclic organic compounds. Many phytochemical researchers proved the pharmacological activity of alkaloids and found it effective for the central nervous system (Yadav *et al.*, 2014). Flavonoids are a low molecular weight phenolic group of phytochemicals, and they exhibit strong antioxidant activity. Flavonoid compounds exhibit medicinal properties such as preventing cellular damage, providing anti-carcinogenic and anti-inflammatory activities (Galati, 2004), and improving hyperlipidemia and hyperglycemia (Jung *et al.*, 2006).

Clerodendrum paniculatum is commonly known as an ornamental flowering tree and one of the members of the Lamiaceae family. This genus has more than 580 species worldwide. *Clerodendrum paniculatum* leaves are used for the treatment of wounds (Vijayan and Gopakumar, 2015), anemia, liver complaints, blood purification (Sen *et al.*, 2016), and malaria (Iyama and Idu, 2015) and the roots have antityphoid activity (Shil and Choud-

hury, 2009). Some research showed the antioxidant property of *Clerodendrum paniculatum* roots (Arun *et al.*, 2011; John *et al.*, 2008). The ethanolic extract of *Clerodendrum paniculatum* leaf exhibited antihelmintic, antiaging, and antibacterial properties (Krishnan *et al.*, 2017) and *C.paniculatum* root extract has antimutagenic and anticancer activities (Sundaraganapathy, 2016; John *et al.*, 2010). Various phytoconstituents have been identified in *C.paniculatum*. As the species being less explored, it is selected for the present study.

MATERIALS AND METHODS

Sample collection

The study material of CPF was collected in late October 2018 from Wayanad district, north Kerala. They were identified in the Herbarium of Botany department, University of Calicut, Kerala. The voucher number is 148233 on 22.10.2018.

Qualitative Phytochemical studies

Acquired plant specimens were cleaned carefully by distilled water and dried under shade. The powdered sample was then subjected to extraction in different organic solvents sequentially in increasing order of polarity (petroleum ether, chloroform, ethyl acetate, ethanol, and water). Consecutive flower extract was then analyzed for characteristic phytochemical profiles including, alkaloids, flavonoids, phenols, tannins, steroids, cardioglycosides, carbohydrates, amino acids/proteins, saponins, oils/fats, and terpenoids, according to (Trease and Evans, 1978).

Estimation of secondary metabolites

Secondary metabolites are the richest source of drugs (Joseph *et al.* 2014). The total content of the secondary metabolites such as flavonoid (Chang *et al.*, 2002), alkaloid, tannin (Afify *et al.*, 2012), and phenol (Bhalodia *et al.*, 2011) was measured quantitatively according to the standard methods. For flavanoid estimation, quercetin was used as a reference standard, gallic acid was used for phenolic compound estimation, and tannic acid was used for the tannin estimation. The absorbance of test and standard solution for estimation flavonoid, phenolic, and tannin was read from the UV spectrophotometer. The result of the total content was expressed in terms of milligrams of reference standard equivalent per gram. The alkaloid content was measured from the dried residue.

Determination of antioxidant potential of CPF ethanolic extract

Different assays were used for determining free rad-

Table 1: Phytochemical screening of CPF

Solvent extraction	AL	FL	TP	AP	CH	CG	SA	OF	TN	ST
Petroleum ether	+	-	-	+	+	+	-	-	-	+
Chloroform	+	+	+	-	+	+	-	-	+	+
Ethyl acetate	-	+	+	-	+	+	+	+	-	+
Ethanol	+	+	+	-	+	+	+	+	+	+
Aqueous	-	+	+	-	+	+	-	-	+	+

AL-Alkaloids , CG-Cardioglycosides,SA-saponin , OF-Oils and Fats, TP-Tannin and Phenolic, TN-Terpenoids , FL- Flavanoids, AP-Aminoacids and Proteins, ST- Steroids, CH-Carbohydrates +-positive, - negative

Table 2: Estimation of Phytoconstituents in ethanolic extract of CPF

Phytoconstituents	Results
Total Phenol content (mg/g gallic acid equivalent)	47.66±0.47
Total Flavanoid content (mg/g quercetin equivalent)	24.00±0.94
Total Tannin content (mg/g catechin equivalent)	41.00±0.8
Total Alkaloid content (mg/g of extracted plant material)	1.79±0.23

(All results are expressed as mean ± SD for triplicate)

Table 3: IC₅₀ value of samples and standards

Antioxidant assay	Sample (IC ₅₀)	Standard(IC ₅₀)
DPPH radical scavenging assay	53.24±0.18	53.24±0.13
Nitric oxide radical scavenging assay	75.10±0.30	58.01±0.09
Hydroxyl radical scavenging assay	73.51±0.37	64.00±0.08
Hydrogen peroxide radical scavenging assay	70.99±0.16	67.57±0.42

(Results are expressed as Mean ±SD for triplicate)
Units- µg/ml

Table 4: IC₅₀ value of samples and standards in antidiabetic assay

Antidiabetic assay	Sample (IC ₅₀)	Standard(IC ₅₀)
α- amylase inhibition assay	70.96±0.77	47.96±0.77
β-glucosidase inhibition assay	67.33±0.12	46.68±0.25

Results are expressed as Mean ±SD for triplicate
units- µg/ml

Table 5: GC-MS profile of bioactive compounds present in CPF

S.No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	4.022	2-Oxepanone	C6H10O2	114	2.48
2	11.054	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H6O3	126	1.43
3	18.085	5-Hydroxy-9-Oxabicyclo[3.3.1]Nonan-2-One	C8H12O3	156	2.88
4	31.403	n-Hexadecanoic acid	C16H32O2	256	13.84
5	31.757	hexadecanoic acid, ethyl ester	C18H36O2	284	0.77
6	34.544	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	32.85
7	40.336	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330	2.08
8	43.436	Octadecanoic acid, 2,3-dihydroxypropyl es	C21H38O4	354	1.27
9	46.354	Eicosanoic Acid, 2-Hydroxy-1-(H	C23H46O4	386	2.05
10	48.399	gamma-Tocopherol	C28H48O2	416	0.21
11	49.891	.alpha.-Tocopherol-.beta.-D-mannoside	C35H60O7	592	3.91
12	52.435	Pregn-5-En-3-Ol, 20-Methyl-21-[3-	C29H46O	410	17.54

ical scavenging activity of ethanolic extract of CPF such as:

1. DPPH radical scavenging activity.
2. FRAP assay (Benzie and Strain, 1996).
3. Hydroxyl radical scavenging activity assay (Halliwell et al., 1987).
4. Nitric oxide radical scavenging activity (Green et al., 1982).
5. Hydrogen peroxide radical scavenging assay (Ruch et al., 1989).
6. Reducing power assay (Oyaizu, 1986).

The extract was dissolved in 95% of ethanol and the concentration was fixed at 1mg/1ml. From this

stock solution, appropriate dilution was carried out, and sample concentration was standardized to 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg. Ascorbic acid was used as the standard.

In vitro antidiabetic assays

α-amylase inhibition activity assay (Challa et al., 2011) and α-glucosidase inhibition activity assay (Tadera et al., 2006) were used to determine the *in vitro* antidiabetic efficacy of the ethanolic extract of CPF. Enzyme activity was calculated from the formula given below.

$$\% \text{ of enzyme inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

High Performance Thin Layer Chromatographic analysis (HPTLC)

It is a powerful analytical technique for phytochemi-

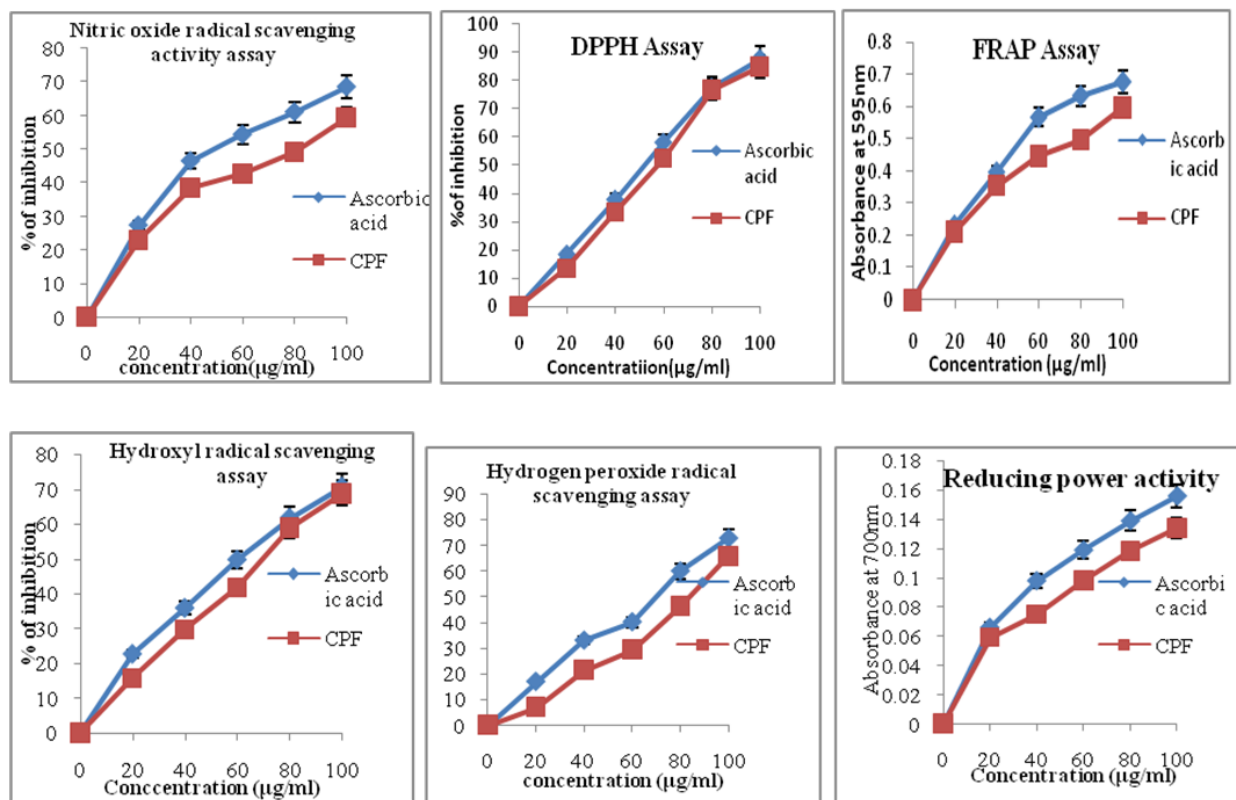


Figure 1: Antioxidant assays (results are expressed as Mean ± SD for triplicate)

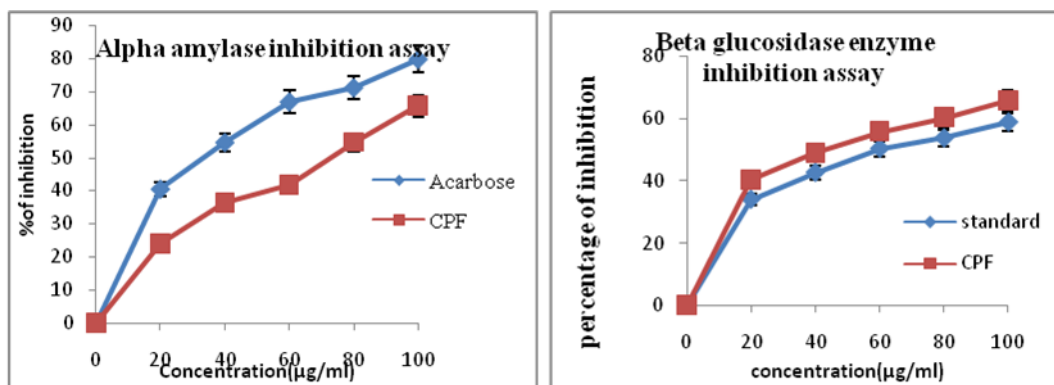


Figure 2: results are expressed as Mean ± SD for triplicate

cal documentation and one of the tools for assessing herbal drugs (Devaki, 2016).

Chromatographic separation of the sample was done on Merck TLC plates. 5 µl of the sample was applied to the TLC plates (6 mm width) by using 100 µl size of syringe and UNOMAT 5 instrument.

Scanning of the sample was done by TLC scanner 3 at 366 nm through fluorescence mode.

TLC plates were visualized under UV 366 nm and visible light.

Gas chromatography-Mass spectrometry Analysis

GC-MS was used for the organic compound identification and quantification. Sample analysis was performed using the Thermo GC-Trace ultra version. The equipment has a capillary standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 µm film. Helium was used as a carrier gas. The diluted samples of 2 µL were injected. The compound identification was based on comparison with Willey & Nist libraries.

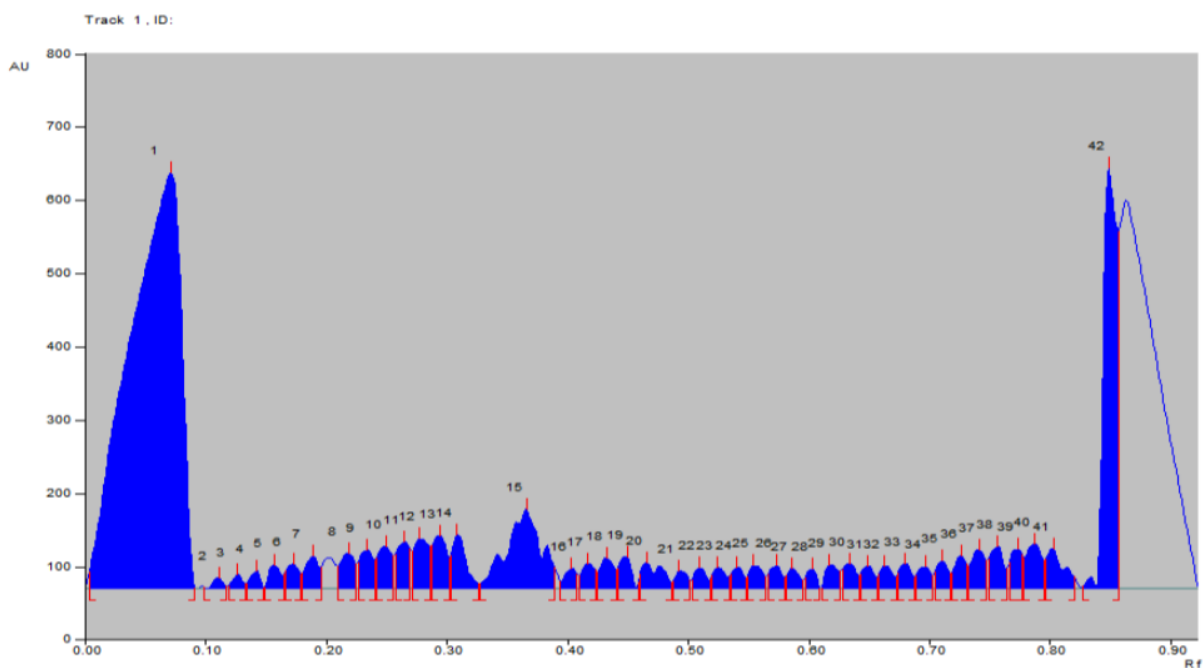


Figure 3: Densitogram of CPF

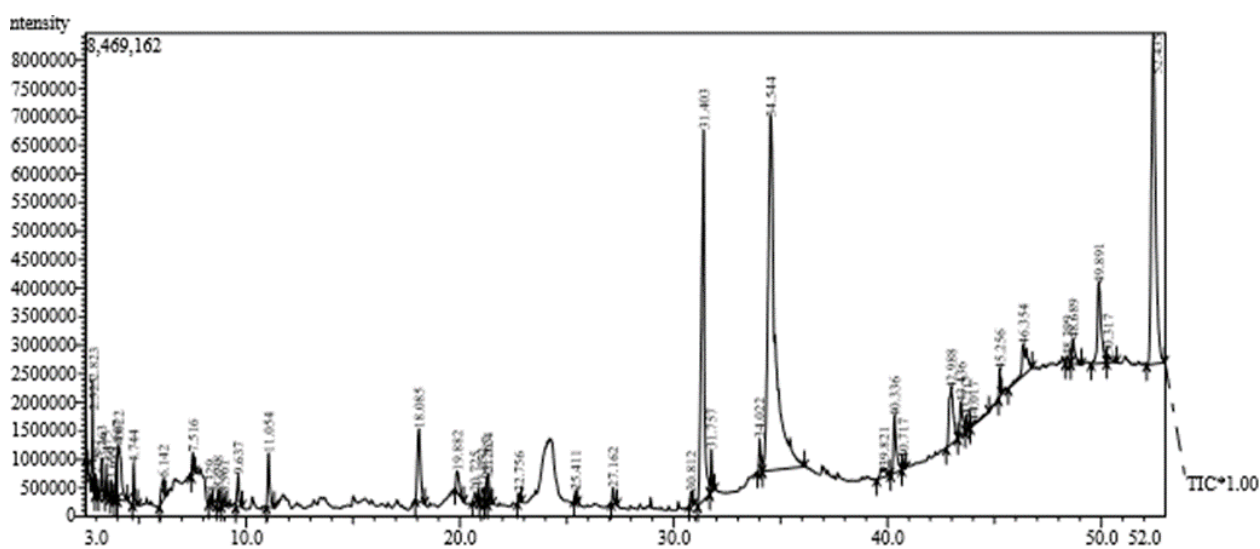


Figure 4: GC-MS chromatogram of ethanolic extract of CPF

The sample components were identified by using the data bank of mass spectra.

RESULTS AND DISCUSSION

Understanding phytochemical compounds of the species under concern is the initial step of pharmacological research. The qualitative phytochemical profile of the ethanolic flower extract of *Clerodendrum paniculatum* showed the presence of phytochemicals like alkaloids, flavonoids, steroids, glycosides, saponins, amino acids, carbohydrates, terpenoids, tannins, and phenols (Table 1). Data as per consecutive solvent extraction is evident that the ethanolic extract has a maximum phytochemical

profile.

Estimation of phytoconstituents in ethanolic CPF

The protective effect of secondary metabolites from medicinal herbals and their publicity in disease are inferred from the epidemiological values as well as experimental studies. The results are shown in Table 2. The total content of phenol, flavonoid, tannin, and alkaloid in the ethanolic flower extract was found to be 47.66 ± 0.47 (mg/g of gallic acid), 24.00 ± 0.94 (mg/g of quercetin), 41.00 ± 0.8 (mg/g of tannin), and 1.79 ± 0.23 (mg/g of extract), respectively. The results reveal a higher concentration of polyphenols.

Antioxidant activity determination

Accumulation of reactive oxygen species has been associated with chronic disease due to oxidative stress. The natural antioxidants can prevent this oxidative stress or neutralize the free radical formation. Antioxidant efficacy of the sample is concerning with phytoconstituent capable of protecting the oxygen system against the harmful effect of oxidative stress. In this study, the antioxidant capacity of extracts from CPF was assessed by different assays. Results of antioxidant assays and corresponding IC₅₀ values are shown in Figure 1 & Table 3. FRAP and reducing power assay also exhibit an increased percentage of scavenging with an increase in concentration (Figure 1). The present plant species under consideration has considerable antioxidant potential. The IC₅₀ value of radical scavenging assay compared with standard ascorbic acid proved that the CPF has significant radical scavenging activity. FRAP and reducing power assay further confirms the result. The effective antioxidant potential of the sample is attributed to the presence of potent bioactive phytoconstituents.

In vitro antidiabetic assays

Digestive inhibitors reduce carbohydrate and fat absorption. The enzyme inhibitory action of CPF was measured by Alpha-amylase inhibition assay and Alpha-glucosidase inhibition assay. Inhibition of α -amylase and α -glucosidase is the preliminary way to manage the hyperglycemia in Type 2 Diabetes. From the results of antidiabetic assays, it is evident that the CPF has significant antidiabetic potential compared with standard acarbose. The results of inhibition activity assays are shown in Figure 2 and corresponding IC₅₀ values in Table 4.

High Performance Thin Layer Chromatographic analysis

The ethanolic extract of CPF, when subjected to HPTLC profiling, revealed 42 compounds (Figure 3) at 366 nm with mobile phase Hexane: Ethyl acetate (8:2). Out of these polyvalent compounds with R_f values, 0.39, 0.80, 0.85, 0.76, 0.39, and 0.09 were more prominent peaks with area percentage of 5.92%, 2.16%, 12.83%, 2.06%, 3.86%, and 20.21%, respectively.

GC MS Analysis

The chromatographic profile of CPF revealed 44 peaks corresponding to 44 volatile phytoconstituents. GC-MS chromatogram is depicted in Figure 4. Among the 44 secondary metabolites, compounds having a comparatively higher peak area and having reports on pharmacological activity are depicted in Table 5. The main volatile compo-

nents of CPF were found as 2-oxepanone (2.48%), 5-hydroxy-9-oxabicyclo [3.3.1]nonan-2-one (2.88%), methyl 2-oxopropanoate (0.28%), n-hexadecanoic acid (13.84%), 9,12-octadecadienoic acid (z,z) (32.85%), propyleneglycol monooleate (3.16%), eicosanoic acid, 2-hydroxy-1 (2.05%), alpha-tocopherol-beta-d-mannoside (3.91%), pregn-5-en-3-ol, 20-methyl-21-(17.54%). Among these identified compounds having antioxidant, anti-inflammatory, antilipidemic, antidiabetic, and anticancer activities.

CONCLUSIONS

The phytochemical investigation of the CPF ethanolic extract shows that it contains significantly active secondary metabolites. The ethanolic extract of CPF exhibits significant *in vitro* free radical scavenging activity and *in vitro* antidiabetic activity. GC-MS and HPTLC analysis of CPF revealed the presence of pharmacologically active phytoconstituents. Based on these preliminary results, the ethanolic flower extract of *Clerodendrum paniculatum* is chosen for further *in vivo* studies. These aggregative findings pave the way for further exploration of the species for isolation of pharmacologically active and assessing antidiabetic potential by *in vitro* and *in vivo* models and consistent refinement into the molecular mechanism of action in the biological system. Preliminary biological evaluation of the present research reveals that the plant has significant medicinal properties.

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Conflict of Interest

The authors declare no conflict of interest.

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