5. DISCUSSION

5.1. Antidiabetic medicinal plants

Medicinal plants and their uses for the treatment of various diseases are a common practice in rural and economically backward areas of the world. In a developing country like India, people still rely on rich knowledge of traditionally used medicinal plants or plant-based products for treating common ailments. In the present study, a survey was conducted among the Bodo tribe of Kokrajhar district to collect the putative antidiabetic medicinal plants used in the traditional medicine system. A total of 54 different villages were visited and one informant was interviewed from each village. A total of 54 traditional healers and knowledgeable individuals were interviewed. The study found that there is an orally transmitted knowledge system. Although ethnomedical knowledge is passed on uniformly, it was found that men generally possess and remember more traditional knowledge than women. Additionally, there is a belief in rural areas that a traditional healer is typically an older person, leading to a lack of trust in the ethnomedicine practiced by younger herbalists. Out of the 54 interviewees, only 15 informants (27.77%) responded positively and shared information about the antidiabetic medicinal plants. The present study also observed that the ethnomedicinal knowledge bearers were mostly aged individual, above 40 years of age. Similar findings were reported by Mussarat et al. (2014) in the Dera Ismail Khan District of Pakistan, where ethnomedicinal knowledge was found to be predominantly held by older individuals. Other studies have reported similar trends, indicating that knowledge of medicinal plant use is mostly confined to the older population in communities (Traore et al., 2013; Bartha et al., 2015; Nyahangare et al., 2015; Ahmad et al., 2016; Usha et al., 2016; Chitura et al., 2018). Regarding literacy, most of the informants were found to be literate, with 67% of the overall kavirajas and 80% of the kavirajas with antidiabetic knowledge being literate. Similar findings have been reported in other studies (Mussarat et al., 2014; Umair et al., 2017; Faruque et al., 2018). About 74% of the total informants were above 50 years old, with the remaining 26% in the age group of 40−50 years. Among antidiabetic healers, most were from the age group of 40-50 years, with fewer healers above 50 years old. This may be due to increased disease rates and modernization, prompting younger individuals to learn methods to cure newly emerging diseases. Antidiabetic healers were generally more literate compared to the overall kavirajas, possibly because the disease is more prevalent in town areas (Kokrajhar and Kachugaon blocks) where literacy rates are higher. There is also a higher incidence of diabetes mellitus due to increasing modernization and sedentary lifestyles, requiring healers to address this disease effectively. We observed that literate individuals continue to document ethnomedicinal knowledge and pass it on to the next generation. This suggests that despite their literacy, their belief in the efficacy of plant-based or Ayurvedic medicine in treating diseases remains strong, likely due to their recognition of the healing potential of these plants.

Contrary to our study, Mussarat et al. (2014) and Hassan et al. (2017) reported that most informants were illiterate. The majority of antidiabetic healers hailed from Kachugaon and Kokrajhar blocks, likely due to the higher number of villages in these areas compared to others. Additionally, these blocks are situated near a wildlife sanctuary (Chakrashila Wildlife Sanctuary in Kokrajhar block) and a reserve forest (Ultapani reserve forest in Kachugaon block), providing healers with more options for collecting medicinal plants. Our study revealed that males possess more ethnomedicinal knowledge than females, with 69% male and 31% female interviewees, and 73% male and 27% female antidiabetic healers. This difference may stem from the greater number of males in the district according to the Census 2011. Moreover, societal norms often place males as the heads of the family, granting them more opportunities for education and other pursuits. However, both male and female informants cited plants almost equally, consistent with findings by Fassil (2003), which showed no significant differences in medicinal plant knowledge between genders.

Our study found that literate informants cited approximately 4.6 plants, while illiterate informants cited five. This finding aligns with Umair et al. (2017), who also reported that illiterates possess more information than literate informants. Interestingly, literacy and gender did not appear to hinder the distribution of ethnomedicinal knowledge. However, when it comes to antidiabetic healers, literate informants cited around 4.16 plants, while illiterate ones cited 2. This difference may be because literate individuals can document their knowledge, aiding memory retention. Conversely, illiterate individuals may struggle to do so, potentially leading to knowledge loss and fewer citations.

The present study showed a high diversity of plants used for the treatment of diabetes. A total of 37 medicinal plant species belonging to 33 genera and 24 families were reported to be used as antidiabetic medicine. Families such as Apocynaceae (16.6%), Moraceae, Combretaceae, and Myrtaceae (12.5%), and Cucurbitaceae, Acanthaceae, Apiaceae, and Lamiaceae (8.3%) represented the highest numbers of species as compared to other families. This could probably be attributed to species richness and locally available plants in that area. Of the 24 plant families, the most cited and popular family was Apocynaceae (16.6%). Similar to the present study, Fatimi (2019) reported the Apocynaceae family to be the most dominant in the southern mainland of Yemen. This indicates a strong medicinal property of the Apocynanceae family and suggests that there is not much difference in knowledge of these plants among healers from different geographical regions. *Hodgsonia heteroclita* was found to be the most common medicinal plant cited (FC=0.46 IFC=0.40). The efficacy of the plant is also experimentally validated by Usha et al. (2017). Following *H. heteroclita*, *Andrographis paniculata* (FC=0.2 IFC=0.33) is the most cited plant by the traditional healers. The effectiveness of the plant was confirmed experimentally on diabetic rat model by Akhtar et al. (2016). This indicates that the healers thoroughly observed the medicinal properties of plants and their therapeutic potential, possibly testing them on themselves to understand their efficacy.

Trees (40.54%) are the most cited plant life-forms by the herbal healers to cure diabetes, followed by herbs (29.72%), shrubs (16.21%) and climbers (13.51%). Many such studies have reported similar use of traditionally used medicinal plants (Choudhury et al., 2015; Raj et al., 2018). Usage of big trees might be linked to their ability to withstand fluctuating temperatures, change in seasons, as the areas fall in subtropical regions and thus resulting their availability throughout the year. Usage of herbs, shrubs, and climbers in herbal medicine is seen in different survey reports of traditional medicine systems (Lawal et al., 2010; Otieno and Analo 2012; Rahman et al., 2014). Many plant parts were used in the preparation of traditional herbal medicines. The dominant plant part used in traditional formulations was leaves (51.35%). Similar to our finding, leaves have been the most used parts for preparing herbal remedies in other studies (Muthu et al., 2006; Tugume et al., 2016; Uzun and Koca, 2020). Leaves may be useful to the healers, because of their abundance and easily renewing capacity compared to other parts of the plants. Usage of leaves also causes less threat to the plant species thereby maintaining the overall status of the plant in that area.

The traditional medicines were prepared using different methods such as decoction, infusion, and raw consumption. Decoction involves grinding or making a paste of the plant sample, which is then either soaked or boiled and consumed. Infusion involves dipping plant parts in water, and the water is then consumed. Raw consumption of medicines refers to consuming the plant sample directly from natural sources without any processing. Based on the preparation methods, our survey found that decoction (54.05%) is the most commonly used method adopted by traditional healers. Similar to our study, many other studies have also observed that decoction is the most suitable herbal formulation method practiced by different traditional healers (Tshikalange et al., 2016; Chaachouay et al., 2022). A decoction may be useful for extracting the natural juices of plants, especially for harder substances, where consumption by infusion or raw may lead to less extraction of the important compounds. This method can lead healers to surmise that the plant is more effective in delivering medicinal properties to the diseased. Consumption of medicine in raw form or by infusion was also confirmed by Abouri et al. (2012) in a survey conducted in Tata Province of Morocco. Many other studies have also confirmed the use of medicinal plants in raw form, infusion, and decoction (Sargın et al., 2013; Canga et al., 2022).

We conducted a literature survey on all the plants cited by the kaviraja and found that 65% of these plants have one or more studies on their antidiabetic properties. This indicates that the healers have substantial knowledge about the plants and their uses. The scientific efficacy of the plants suggests that traditional healers possess significant knowledge about the disease and have observed the plants' effects over a long period. However, 35.13% of the plants and parts cited by the traditional healers were found to lack scientific literature, particularly on antidiabetic properties. This may be due to the varying availability of plants in different geographical regions. It also provides a new avenue for studying the efficacy of these plants in treating diabetic patients.

5.2. Phytochemical Analysis and Antioxidant Assays

Plants are a rich source of phytochemicals and antioxidants. Phytochemicals are natural bioactive compounds that can act as a defense system to combat many diseases (Vasanthi et al., 2012; Behl et al., 2021). Medicinal plants, therefore, are valuable due to their high phytochemical content, and understanding their medicinal value requires thorough phytochemical studies. In our study, 11 out of 37 medicinal plants were processed for their phytochemical content. The methanolic crude extract was prepared through the decoction process and used in the experiment. Phytochemicals were extracted from plants using various techniques such as infusion, decoction, percolation, and hot extraction (Soxhlet extraction) (Ammar et al., 2015; Padma et al., 2019). Among these, the most commonly used traditional method was decoction, which involves grinding dried plant parts into a powdered form and soaking them in a solvent to extract the phytochemicals (Castro et al., 2017).

The finer the particle size, the better the extraction outcome, likely due to enhanced penetration of the solvent into the particles (Hidayat and Wulandari, 2021). Various solvents such as hexane, diethyl ether, ethyl acetate, methanol, ethanol, chloroform, and water were used for preparing crude extracts by different researchers. (Alabri et al., 2014; Ojiako, 2014; Kebede et al., 2021). In a study done by Truong et al. (2019) methanol was identified as most effective solvent for extraction resulting in higher extraction yield. In our study, the methanolic crude extracts of all the plants were subjected to qualitative and quantitative analysis to determine the presence of phytocompounds. The analysis revealed the presence of proteins, carbohydrates, phenols, flavonoids, alkaloids, and glycosides in the methanolic extracts of different plants (Yadav et al., 2017; Kebede et al., 2021). Proteins and carbohydrates were found in all plant extracts, which also showed high phenol and flavonoid contents, similar to findings by Swargiary et al. (2016). Saponins were present in all plants except *Phlogacanthus thyrsiformis* and *Alstonia scholaris*. Saponins are important compounds that affect the immune system, help protect the body against cancers, and lower cholesterol and blood glucose levels (Shi et al., 2004). The absence of saponins in some plant extracts was also observed in a phytochemical screening by Yadav et al. (2014). Tannins were absent in selected plants such as *Alstonia scholaris, Rauvolfia tetraphylla*, and *Clerodendrum infortunatum*, while other tested plants contained tannins. Tannins have antibacterial, antidiabetic, and antioxidant properties (Haslam, 1996; Khanbabaee and Ree, 2001). This absence was similarly reported by Kubmawara et al. (2007) and Edeoga et al. (2005). All plants showed the presence of glycosides and alkaloids, recognized for their pharmacological and medicinal values (Jaramillo et al., 2016). The presence of these phytochemicals in the crude extracts suggests the potential medicinal properties of the plants.

In the present study, quantitative analysis was conducted on all 11 plants to determine the presence of crude protein, carbohydrate, phenolic, and flavonoid contents. The study revealed a high content of protein and carbohydrate in the tested plants, which are essential for maintaining and repairing the animal body and can also trigger the defense mechanism of organisms. Proteins are essential components of cells required for normal bodily functioning. Some amino acids, such as leucine, are known for their hypoglycemic properties, as demonstrated in a study by Fajans et al. (1969), where a combination of essential amino acids increased plasma insulin concentration in healthy and mildly diabetic patients. Proteins also play a crucial role in generating hormones and enzymes controlling various bodily activities like growth and repair. Our analysis revealed the highest carbohydrate and protein content in *Alstonia scholaris*, suggesting its potential nutritional value. Plant proteins bear structural resemblance to vertebrate protein kinase B, an enzyme crucial for insulin production, which underscores their significance in managing diabetes (Mahdi et al., 2020). Additionally, the considerable phenolic content across the tested plants, particularly in *Phlogacanthus thyrsiformis*, aligns with previous research highlighting its rich phenolic content and the potential benefits of polyphenols in disease prevention, including cardiovascular diseases and diabetes (Laitonjam et al., 2013; Poeaim et al., 2016; Montenegro-Landívar et al., 2021). Polyphenols, abundant in plants, contribute significantly to disease prevention, while flavonoids, another group of antioxidants, help reduce free radicals in the body. Natural flavonoids like diosmin normalize superoxide radicals produced due to stress and may aid in fighting diseased conditions (Mustafa et al., 2022). Our study identified *P. thyrsiformis* as having the highest flavonoid content, consistent with previous findings associating high phenolic content with high flavonoid content (Mahmood et al., 2012). Interestingly, our study found a higher phenol content than flavonoids in almost all plants, similar to findings by Mahmood et al. (2012). However, *Andrographis paniculata* exhibited less phenolic content than flavonoid content, contrasting with other findings. Similarly, Abifarin et al. (2019) reported higher values of flavonoid content compared to phenolic content in different solvent extracts of *Cucumis africanus* L.f.

The human beings are exposed to several pro-oxidants, as an attribute to aerobic life, which is responsible to damage significant biological molecule such as nucleic acids, protein, carbohydrates, and lipids altering the normal redox status of the body. Along with that, oxidation process or metabolic response of the body also produces free radicals such as superoxide radicals, singlet oxygen molecules, peroxyl ions, and hydroxyl ions. Free radicals are the atom or molecule containing unpaired electron in the outermost shell of the orbit, which is highly unstable and attack another molecule to stabilize itself. The attacked molecule thereby becomes a free radical itself. Because of their highly reactive nature, they undergo a cascade of reactions that finally attack the living cell damaging it (Phaniendra et al., 2015). Therefore, it is essential to limit or scavenge the formation of free radicals, which could otherwise lead to various metabolic disorders such as diabetic mellitus, neurodegenerative disorders, or cardiovascular diseases. Among the numerous defense strategies, organism has an in-built antioxidant system, to scavenge the dangerous free radicals. However, different pathological condition in an organism is closely associated with overproduction of free radicals, which could worsen the diseased condition (Vendemiale et al., 1999). Medicinal plants are packed with full of antioxidant molecule. Studies have shown a positive corelation between the medicinal property of the plant and its antioxidant system. To study the antioxidant activity of the plant five assays, such as DPPH, ABTS, TBARS, FRAP, and TAA were conducted. All the tested plant showed a good antioxidant potential indicating the medicinal property of the plants. In case of DPPH assay, the methanolic extract of *Phlogacanthus thyrsiformis* flower showed the highest activity (IC_{50} 23.34±0.33 µg/ml) followed by *Oroxylum indicum* (92.42±1.49 µg/ml). In a study conducted in Thailand, edible flowers are reported to have a good DPPH free radical scavenging activity. Similar to our study other parts *P. thyrsiformis* was also seen to possess a high antioxidant property (Ponneganti et al., 2022). In a study conducted in Orissa, the IC_{50} value of DPPH scavenging activity of *Oroxylum indicum* leaves extract was found almost similar to our value (Mishra et al., 2010). For TBARS and ABTS activity, *O. indicum* exhibit the strongest antioxidant potential among the tested plants. *O. indicum* leaves and other parts possess a strong antioxidant potential with similar studies (Sithisarn et al., 2016). Similarly, *Phlogacanthus thyrsiformis* was reported to show the highest antioxidant potential in FRAP and TAA activity. The amount of antioxidant present in the plant is linked to its phenolic and flavonoid content (Butsat and Siriamorpun, 2009).

5.3. Elemental Analysis

Metallic content plays a crucial role in our daily lives, with potential harm if it exceeds permissible levels. In our analysis, toxic heavy metals such as Pb and Cd were found to be below the WHO permissible limits (WHO, 1996), indicating that the plant extracts are safe for human consumption. Conversely, heavy metals like Pb, Cd, and Ni, while lacking any beneficial roles, are known to be toxic to health (Jaishankar et al., 2014). Exposure to Pb can lead to a range of health issues, from headaches and vomiting to severe complications like brain and kidney damage (Anal and Chase, 2016; Al-Fartusie and Mohssan, 2017). Studies have shown that Ni exposure induces glucagon release from the pancreas in rats, leading to hepatic glycogenolysis and hyperglycemia (Tikare et al., 2008). Our study revealed negligible amounts of toxic heavy metals in the tested plants, affirming their safety for medicinal purposes and diabetes treatment. Trace elements such as Cr, Zn, Cu, Fe, and Mg are essential for various biological activities and human health. These elements function as co-factors for many proteins and enzymes, particularly in glucose metabolism. For example, Cr plays a vital role in carbohydrate metabolism by modulating insulin function and enhancing glucose uptake by cells (Hua et al., 2012; Yakout et al., 2021). Among the 11 plants, *Ficus racemosa* exhibited the highest Cr concentration, indicating its potential as a valuable drug candidate for managing diabetes. Additionally, Mn, another essential element, boosts immunity, defends against free radicals, and helps control blood sugar levels (Hajra et al., 2016; Forte et al., 2013).

5.4. GC-MS Analysis

The GC-MS technique serves as a crucial tool for identifying and analyzing unknown phytocompounds in plants. Analysis of the methanolic crude extract of *Andrographis* *paniculata* unveiled eight probable compounds at retention times of 6.450, 7.774, 19.830, 21.509, 22.277, 22.620, 23.196, and 23.795. Previous studies by various researchers have reported numerous compounds with diverse biological activities from *A. paniculata* (Roy et al., 2010; Kalaivani et al., 2012; Rahman et al., 2014). Our study represents the first-time reporting of compounds not similar to those reported by other researchers. The differences in the compounds may be attributed to the geographic area of plant collection and the solvent used in the extraction process. Roy et al. (2010) reported the presence of many aromatic compounds, phenols, carboxylic acids, and esters from *A. paniculata* when analyzed using a GC-MS system. Six possible compounds were detected from *Alstonia scholaris*. Jayashree and Velraj (2019) reported 10 compounds from the aqueous extract of *A. scholaris* bark at different retention times. Conversely, Carranza et al. (2020) reported 12 compounds from the methanolic and dichloromethane extract of *A. scholaris,* including a compound not similar to our report. Our study revealed a compound, Androstan, known for its antimicrobial activity (Sabaani et al., 2019). Acetamide is known for its strong anti-inflammatory, antioxidant, and cytotoxic properties (Autore et al., 2010). Napthalene derivatives have been reported to possess antioxidant and antimicrobial properties (Fahad et al., 2021). Another compound, 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine, when interacting with monoamine oxidase and serotonin and M3 muscarinic acetylcholine esterase, could potentially exhibit good pharmacokinetic properties for diarrhea and depression (Nasrin et al., 2022). Conocarpan, found in different medicinal plants (Pessini et al., 2003; Felipe et al., 2006), is known for its bioactivity, including the induction of apoptotic cell death, as reported by Yang et al. (2019). These findings suggest that the phytochemicals reported from *A. scholaris* possess significant biological activity, making it an important candidate for drug consideration.

GC-MS analysis of *Clerodendrum infortunatum* revealed three possible compounds. Among the three compounds detected, Carbonic acid, 2-chloroethyl 2-pentyl ester was also identified in the ethanolic root extract of *Berberis baluchistanica* Arendt (Gul et al., 2022). Analysis of *Ficus racemosa* fruit extract via GC-MS revealed six possible compounds, all of which showed low toxicity levels and low oral bioavailability when studied through in-silico approaches (Swargiary et al., 2020). These compounds demonstrated a strong binding affinity with the alpha-glucosidase enzyme, indicating their potential for diabetes treatment. Anand et al. (2021) identified a compound from *Ficus racemosa* fruit extract through GC-MS analysis, which showed pancreatic lipase inhibition. Three compounds were identified through GC-MS analysis of *Hydrocotyle sibthorpioides*, none of which had literature regarding their biological activity. These compounds were reported for the first time in Hydrocotyle species. GC-MS analysis of *Lindernia crustacea* revealed ten compounds from the whole plant extract. All compounds were shown to have moderate to high absorption by the human intestine and exhibited a strong binding affinity with alpha-glucosidase and amylase enzymes. Notably, 1-(4-Hydroxybenzoyl)-6,7-dimethoxyisoquinoline demonstrated the strongest binding affinity. However, Manganese pentacarbonyl (2,3,3,4,4,5,5,6,6-nonafluoro-1-cyclohexen-1-yl)- and Thiophene, 3-methyl-5-octadecyl-2-pentadecyl- violated two properties of the Lipinski rule of five, suggesting they could be strong antihyperglycemic agents (Swargiary et al., 2022). From the GC-MS spectrum, five major compounds were revealed from the methanolic aerial part extract of *Musa balbisiana*. These compounds were reported for the first time from *M. balbisiana* corm extract, with no literature regarding their pharmacological properties. However, previous studies reported several esters' compounds from the fruit peel of Musa species, suggesting potential biological activities. Kumari et al. (2020) reported seven major phenolic compounds from the fruit pulp of *M. balbisiana*, indicating cardioprotective activity. Additionally, rutin was identified as the major compound in the leaves of *M. balbisiana*. Several phytosterols and triterpenes, known for their biological activities, were reported from the inflorescence extract of *M. balbisiana* (Revadigar et al., 2017). GC-MS analysis of *Oroxylum indicum* revealed the presence of seven possible compounds. Upon further analysis, the third compound violated the logP value, while the fourth compound failed to fulfill the molecular weight criteria of the Lipinski rule of five. However, the third compound showed the strongest α-glucosidase binding activity, indicating its potential as a α-glucosidase inhibitor. Various phytochemicals were reported from different parts of the plant, but none had literature regarding their biological activities (Swargiary and Daimari, 2020).

GC-MS analysis of *Paspalum fimbriatum* revealed five compounds, one of which was also reported in the methanolic stem extract of *Gynochthodes ridsdalei* Razafim. & B.Bremer and in the flower extract of *Cassia fistula* L. This compound showed antifungal, antibacterial, and antimicrobial properties. Similarly, GC-MS analysis of *Phlogacanthus thyrsiformis* revealed six possible compounds, none of which had literature regarding their biological activities. While several co-workers reported various phytocompounds from Phlogacanthus species, none matched our findings, indicating the possibility of novel chemicals and compounds useful in drug discovery. Finally, GC-MS analysis of *Ravoulfia tetraphylla* revealed four compounds, none of which had literature regarding their biological activities. Further analysis revealed that the third compound violated one law for logP value, while the first compound showed strong binding with alpha-glucosidase inhibitor. Similar studies by other researchers identified probable compounds through GC-MS analysis in different parts of the plant, some of which exhibited promising biological activities (Swargiary and Daimari, 2020).

5.5. Anti-hyperglycemic properties of crude extracts and the best fraction

Enzymes, crucial as biological catalysts, regulate product conversion rates in biological processes, including diabetes mellitus, characterized by uncontrolled or sluggish metabolism. Central to carbohydrate metabolism are α-amylase and α-glucosidase enzymes, located in saliva, pancreas, and intestinal brush border, inhibition of which is vital for managing Type-2 diabetes, effectively reducing postprandial hyperglycemia (Wani et al., 2015; Kazeem et al., 2015). The methanolic crude extracts of various plants demonstrated concentration-dependent inhibition of both enzyme activities, with *Ficus racemosa, Oroxylum indicum*, and *Hydrocotyle sibthorpioides* exhibiting superior α-amylase inhibition compared to acarbose, while *F. racemosa* and *Musa balbisiana* showed stronger α-glucosidase inhibition. Particularly, *F. racemosa* displayed robust inhibition for both enzymes, surpassing acarbose, indicating its potential as an effective inhibitor of carbohydrate hydrolyzing enzymes in vitro. Previous studies, like one by Ibrahim et al. (2014), highlighted the superior α-amylase and α-glucosidase inhibitory properties of *Khaya senegalensis* (Desr.) A. Juss. extracts compared to acarbose, with several other studies reporting various plants exhibiting better inhibitory properties than acarbose. Among the 11 plants studied, *F. racemosa* demonstrated the most significant inhibitory activity against both enzymes, consistent with previous reports highlighting its antidiabetic properties. Studies by Chaware et al. (2020) and Arunachalam et al.

(2018) showcased the antidiabetic potential of *Ficus racemosa* bark and *Ficus amplissima* Sm. extracts, respectively, with strong inhibition of α-amylase and α-glucosidase. Similar observations of potent inhibitory activity against these enzymes have been reported in various Ficus species. Moreover, *F. racemosa* exhibited favorable phytochemical content and antioxidant properties without any toxic elements in our study. Given these promising findings, *F. racemosa* emerges as a compelling candidate for further fractionation and subsequent in vivo experiments. Its potent inhibitory activity against key enzymes involved in diabetes management underscores its potential as a therapeutic agent for this metabolic disorder. Solvent fractionation of the methanolic crude extract of *F. racemosa* was conducted based on the polarity index, followed by analysis of the fractions for their phytochemical content and antioxidant activity. Recent scientific studies have highlighted a strong correlation between the phytochemical profile of plants and their potential to treat diseases (Lee and Bae, 2017). Our study found the highest protein content in the diethyl ether fraction of *F. racemosa*. Interestingly, this fraction exhibited superior inhibitory activity against both α-amylase and α-glucosidase enzymes compared to the other fractions, suggesting a relationship between protein content and enzyme activity. Similarly, the diethyl ether fractions also showed the highest phenol content, with enzyme activity being inhibited in a concentration-dependent manner. A study by Anjum and Tripathy (2019) fractionated the methanolic fruit extract of *Ficus palmata* Forssk. into four fractions: butanol, ethyl acetate, methanol, and water, where the methanolic extract demonstrated the strongest inhibitory property among the fractions. Additionally, antioxidant studies revealed better activity in the diethyl ether fractions. Given that phenolic compounds are known antioxidant molecules that scavenge free radicals, our study observed a positive correlation between phenolic content and antioxidant activity. In essence, the presence of phenolic compounds in the diethyl ether fraction may account for its antioxidant properties.

Natural products are considered safe and effective to use as a medicine. *F. racemosa* fruit has been widely used in Indian Ayurveda for the treatment of ailments. The diethyl ether fraction of *F. racemosa* was used for the toxicity study in albino rats. The present study did not see any signs of acute as well as sub-acute toxicity in plant-extract-treated groups. In a similar study, Panwar et al. (2010) also did not observe any acute and subacute toxicity of *F. racemosa* aqueous bark extract on albino mice and found safe up to a dose of 1000 mg/kg bw, while some physiological changes were seen in the kidney and liver. Along with the morphological and behavioural observations for 24 h, a 28-day chronic toxicity test was performed to see if there are any physiological changes.

5.6. Toxicity studies in rat models

A physiological and hematological study is essential for assessing an organism's overall health and detecting conditions like anemia and blood disorders, providing vital information on the body’s response to stress or injury (Lee and Bae, 2017). While white blood cells (WBCs) constitute only 1% of total blood components, they play a crucial role as the first line of defense against foreign invaders or threats to the body. Our study found no significant difference in WBC count compared to the control, indicating that the plant extract had a negligible negative effect on the immune system (Panwar et al., 2010). Lymphocytes are a very important component of WBC that is solely responsible for the production of antibodies. The increase in monocyte is essential as they ingest dead cells or bacteria. Lymphocytes and other components of the WBC showed no significant difference compared to the control group indicating that the plant extract has a negligible negative effect on the defense mechanism or immune system of the body. A significant increase in RBC count has been seen in all the FRDF-treated groups compared to the control. The increase of RBC in the treated rat groups might be due to the nutrient composition of the *F. racemosa* fruit, which is rich in iron as reported by Bhogaonkor et al. (2014). Iron is an important element that is required by the body to produce RBC and hemoglobin. An increase in iron intake increases RBC production in the bone marrow (Alleyne et al., 2008; Nagababu et al., 2008). In the present study, the increase in RBC and Hb was found within the normal range (Rasekh et al., 2012; Chakroun et al., 2016). Our study also revealed a significantly higher hematocrit in 100 and 200 mg FRDF-treated groups. In a similar study, Bagheri et al. (2015) reported 45% hematocrit content in the normal control group which indicates that though slight differences in hematocrit percentage in 100 and 200 mg doses from the control, the effect would not possibly cause severe damage to the organism body. RDW-CV percentage measures the variation in cell size and volume and RDW-SD measures the width of RBC distribution. The present study did not see any change in the RDW-SD. Lower RDW-SD might indicate a medical complication like anemia and higher RDW-SD might indicate liver, kidney, or heart disease. The findings indicate that up to 500 mg/kg bw, FRDF extract does not exert significant effects on overall organ health (Bagheri et al., 2015; Abdel Daim et al., 2016). While mean platelet volume (MPV) and platelet distribution width (PDW) remain within normal ranges and comparable to the control group. PLT and PCT levels are elevated, potentially influenced by the fruit's iron-rich composition, a known factor in increasing platelet counts (Bhageri et al., 2015; Abdel Daim et al., 2016). Despite the observed differences in platelet count compared to the control group, similar or higher platelet counts have been reported in other studies on animal models (Bagheri et al., 2015; Abdel Daim et al., 2016), indicating a lesser concern regarding these variations. ALT, ALP, and bilirubin are crucial markers reflecting liver function, with ALP, ALT, and AST showing variability in stressed organisms (Bolkent et al., 2008). This study found no significant discrepancies between control and plant extract-treated groups, consistent with findings by Poormoosavi et al. (2018), who observed similar ranges of ALT, ALP, and AST in normal rats. The extract's hepatoprotective effect is evidenced by the decrease in ALP concentration across all extract doses, suggesting the safety of FRDF extract up to 500 mg/kg bw. Conversely, decreased creatinine levels were noted in rat groups treated with 200 mg and 500 mg of FRDF, a phenomenon that may be attributed to aging or a low-protein diet.

HDL, known as "good" cholesterol, plays a crucial role in cholesterol metabolism by absorbing cholesterol from the bloodstream and facilitating its efflux from cells (Barter et al., 2004). Higher HDL levels enhance antioxidant properties and mitigate the risk of heart disease (Xepapadaki et al., 2020). In the 200 mg and 500 mg FRDF-treated rat groups, HDL concentrations were notably elevated compared to control groups, a finding consistent with the observations of Heidarian and Rafieian-Kopaei (2013). On the other hand, LDL, with its pivotal role in cholesterol transportation, poses a heightened risk of cholesterol residue accumulation with elevated levels (Brown et al., 1984). Hence, it is imperative for drugs to target cholesterol levels in organisms to mitigate associated side effects. Our study revealed decreased LDL levels in plasma among FRDF-treated animals, indicative of a potentially beneficial effect on cholesterol metabolism.

OGTT serves as a crucial examination to assess the body's response to elevated glucose levels (Islam et al., 2009). In our study, OGTT in normal rats aimed to evaluate the impact of plant extract on glucose levels. Inducing animals with STZ (50 mg/kg bw) results in the destruction of the Islet of Langerhans (Mythili et al., 2004), leading to increased blood glucose levels following glucose injection, a trend observed similarly in control rats by Islam et al. (2009). Conversely, treatment with FRDF extract resulted in reduced glucose concentrations, with the 200 mg FRDF-treated group exhibiting the most significant reduction among all groups. However, a slight increase in plasma glucose was noted in the 500 mg FRDF-treated group, potentially attributed to the higher carbohydrate content of *F. racemosa* fruits. Given that blood glucose levels decreased below the normal range in rats treated with 500 mg plant extract, it is plausible that the extract inhibited the K-channel of pancreatic β-cells, leading to enhanced insulin secretion regardless of plasma glucose concentration, akin to sulfonylurea class drugs (Costello et al., 2022). Additionally, the extract may inhibit the sodium-glucose co-transporter-2 (SGLT-2) in renal tubules, reducing glucose reabsorption and promoting urinary glucose excretion (Lioudaki et al., 2017). Moreover, it may act as an insulin analogue within the body.

5.7. Biochemical and histochemical analysis of antidiabetic effects of active fraction of plant

Hyperglycemia, whether stemming from deficient insulin production in Type 1 diabetes mellitus or inadequate insulin production coupled with insulin resistance in Type 2 diabetes mellitus, necessitates achieving normal blood glucose levels as the primary aim of current diabetes mellitus treatment. Exploring medicinal plants holds promise for future diabetologists in addressing this challenge. Diabetes induction involved a single intraperitoneal administration of 55 mg/kg bw STZ, elevating blood glucose levels to 400-500 mgdL-1, a method paralleled by Prakasam et al. (2003), who induced diabetes in rats with doses ranging from 40-60 mg/kg body weight. Similarly, Mythili et al. (2004) reported a mean blood glucose level of 376 mg/dL three days post 50 mg/kg

STZ induction, highlighting STZ's capacity for partial beta-cell destruction while leaving the exocrine part unaffected.

During the seven-day experimental period, minimal changes were noted in blood glucose levels across all five experimental groups. However, significant alterations emerged after the 14th, 21st, and 28th days of FRDF and glibenclamide treatment. Both 100 mg and 200 mg FRDF concentrations induced notable reductions in blood glucose compared to the diabetic control group, suggesting the plant's potential antihyperglycemic properties. Notably, the 200 mg FRDF-treated group exhibited a dramatic decrease in blood glucose levels, showing no significant difference ($P \leq 0.05$) compared to the normal control groups, underscoring FRDF's potent impact on blood glucose levels and validating its antihyperglycemic potential, a finding supported by Misbah et al. (2013) regarding the antihyperglycemic properties of Ficus species fruit fractions. The mechanism underlying FRDF's antihyperglycemic action may involve beta-cell regeneration, glucose absorption via GLUT-4 receptors, enhanced insulin activity, insulin release stimulation, or hepatic gluconeogenesis inhibition (Fosset et al., 1988; Gray et al., 2010). Given its comparable efficacy to glibenclamide, FRDF likely shares a similar mode of action. Notably, numerous studies affirm the antihyperglycemic properties of medicinal plants used in Ayurvedic medicine, emphasizing the importance of traditional medicine preservation for new therapeutic drug development (Raphael et al., 2002). Our study also observed a decline in rat body weight, consistent with diabetes-associated tissue protein breakdown (Sachan et al., 2006), echoing findings by Ahmed et al. (2005) showing diabetic rats experiencing up to a 27% decrease in body weight. Drug-treated groups exhibited a gradual blood glucose reduction, mirroring the slow decline in body weight. Elevated enzyme levels in diabetes may also contribute to decreased rat body weight.

Measurement of enzymes such as ALP, ALT, and AST are crucial, as alteration of such enzymes are indication for tissue damage and indicates the organism is in stress either through toxicants or through disease (Bolkent et al., 2008). In our study, it has been seen that serum ALP increases in diabetic control rats. Generally, elevated serum ALP level has also been observed in diabetic patients and may be associated with fatty infiltration in liver (Maxwell et al., 1986). Liver is the organ where in toxification of wide range of toxic chemical occurs. The elevated level of ALP in the serum might be due to the leakage of ALP enzymes from liver to the blood stream (Sachan et al., 2006). In our study both the dose of FRDF 100 mg/kg bw and 200 mg/kg bw showed a decrease ALP level when compared to diabetic control rats and gliblenclamide treated rats. Similar to our study, Gometi et al. (2014) also reported high ALP concentration in diabetic rats and decreased ALP concentration after the administration of drugs. Likewise, similar findings were also observed by Eltimamy et al. (2022). Lower ALP level in FRDF doses treated rats might indicate the lesser toxicity with better potential of healing than the standard chemical gliblenclamide. ALT is an important enzyme for the conversion of pyruvate to glucose which in turn leads to high glucose in the blood. Higher ALT level is often associated with metabolic syndrome, insulin resistance, liver damage and diabetes (Qian et al., 2015). Our study observed an increase in ALT activity in diabetic rats up to two-fold from the normal control rats. These findings align with numerous previous studies conducted by many researchers (Eidi et al., 2006; Alum, 2022) where ALT activity rises in diabetic rats. Our study also revealed that 200 mg/kg bw crude extract dose was found to be more effective in lowering the ALT activity showing better activity than the standard glibenclamide. Similar to our finding, Fagbohun et al. (2020), reported higher ALT in diabetic rats and lower in diabetic rats administered with the plant extracts. One cause of reduced blood glucose might be due to the restoration of ALT enzymes. The more the ALT, the more is the conversion of alanine to pyruvate and finally to glucose. So, if the ALT levels are low, there will no conversion of pyruvate from alanine and no glucose production through gluconeogenesis. It suggests the drug reduces blood glucose level by acting on ALT enzymes. Additionally, we can also say that, the drug might have the ability to suppress the gluconeogenesis process.

AST accompanying ALT is an important enzyme in the detection of liver damage. ALT level in diabetic rat groups increased upto two-fold, Diabetic Control, glibenclamide treated diabetic rats, 100 mg FRDF treated diabetic rats showed an elevated serum AST level, indicating alteration of liver enzymes. The concentration of hepatic enzymes increases in the bloodstream as the enzymes escape from the liver. In 200 mg treated groups the AST levels was found to be similar to normal control groups ($P \leq 05$) indicating that the drug has an effect on serum AST enzyme. Since the body constantly tries to remove oxidative stress from our body, there is a chance of an increase of antioxidant marker enzymes such as AST to elevate. Similar to our finding, Uyar and Abdulrahman (2020), also reported higher AST level in diabetic rats. These reductions of ALT, AST, and ALP enzymes by the plant extract suggest the hepatoprotective property of the plant. Estimation of lipid profile is necessary to know if there is any effect of drugs in cardiovascular system. Serum lipid profile to some extent measures the condition of the cardiac muscle. Serum triglyceride level for normal rats was found to 90.7 mg/dL. Similar to our study Ahmed et al. (2005) reported the normal control triglyceride level in the same range. Serum triglyceride level in diabetic rats increases up to 270 mg/dL which is almost three times the normal control rats. Upon administration of gliblenclamide and FRDF drugs, the triglyceride level decreases. Similar to our study, Ahmed et al. (2005) also reported increase in triglyceride level up to threefold than the normal control rats and decreases upon administration of drugs.

The normal range of total cholesterol is 74.25±19.51 mg/dL which increases on diabetic condition. Elevated total cholesterol increases the chance of heart diseases. Our study revealed decreased total cholesterol level in gliblenclamide and both the crude extracts treated rat groups. These is similar to the study conducted by Eidi et al. (2006) where cholesterol level increases from 70-110 mg/dL in diabetic rats and decreases in the gliblenclamide treated groups and when treated with the crude extract concentrations. A high VLDL and LDL level may also be associated with a higher risk for heart disease. Our study revealed a high VLDL and LDL in diabetic rat groups. This finding is similar to the findings of different researchers, where both the VLDL and LDL level increases in diabetic condition (Shamsi-Goushki et al., 2020; Wickramasinghe et al., 2022). Upon the administration of the drugs (especially, 200 mg FRDF treated rat groups), both the VLDL and LDL levels dropped and returned to normalcy showing no statistical difference with the normal control rats. These findings are similar to different works done by many researchers (Arafa et al., 2020; Mohammed and Kakey, 2020; Abu et al., 2023). Our study also revealed a low HDL level in diabetic groups when compared to the normal control group. However, upon the administration of the standard drugs, and FRDF (100 mg and 200 mg), the HDL level was seen to be restored which was supressed in diabetic condition. This finding is similar to the study conducted by Arafa et al. (2020). The improvement of lipid profile with the treatment of FRDF indicates the cardiogenic property of the plant. Moreover, rats treated with 200 mg FRDF has the better efficacy than the arts treated with standard drug, glibenclamide, suggesting that the plant extract may have fewer adverse effects especially on the cardiac system.

Our study revealed that the diabetic control group exhibited significantly higher GST activity in the liver compared to the normal control group, suggesting that the body might increase GST activity as a compensatory mechanism to counteract oxidative stress in diabetic conditions (Sharma et al., 2016). This finding aligns with Sheweita et al. (2002). We observed significant differences in GST activity between the diabetic control group and the diabetic rat groups treated with 100 mg and 200 mg of FRDF; GST activity increased under diabetic conditions but decreased following administration of standard drugs and plant extract treatment, indicating that the oxidative stress associated with diabetes was potentially mitigated by the plant extract. The relationship between the antioxidant system and diabetic nephropathy is well established, but our results showed contradictory findings regarding GST levels in the liver. In contrast, GST activity in the kidney decreased under diabetic conditions but increased upon administration of the drug and plant extract, suggesting a restoration of GST levels; this is consistent with Kyznetsova et al. (2015), who also reported low GST levels in the kidneys of diabetic rats and an improvement following treatment with plant extracts and standard drugs. The decrease in kidney GST activity aligns with Anwar and Meki (2003). Lower levels of antioxidant enzymes in diabetic patients compared to healthy individuals have been noted, indicating possible tissue damage or diabetic nephropathy (Annadurai et al., 2014). This decrease may be due to enzyme inhibition or depletion caused by increased free radical production. In our study, rats treated with 200 mg of FRDF showed higher GST activity compared to the untreated diabetic groups.

Catalase is an important enzyme that breaks down harmful hydrogen peroxide into oxygen. In our study, Catalase activity was higher in the liver and kidney of the diabetic control group, with lower rises observed in the glibenclamide and plant extract (100 and 200 mg) treated rat groups. This aligns with findings from Qujeq and Rezvani (2007), who also observed increased Catalase activity in diabetic conditions. The increase in Catalase activity may be due to higher production of hydrogen peroxide in response to oxidative stress or hyperglycemia (Asmat et al., 2016). Upon administration of glibenclamide and plant extract, Catalase activity showed a significant difference from the diabetic control group, indicating the efficacy of these treatments in reducing H_2O_2 levels.

MDA, a final product of lipid peroxidation, is associated with free radical damage. Higher MDA levels indicate greater damage from oxidative stress (Roosdiana et al., 2020). In our study, MDA levels increased in untreated diabetic rat groups in both the liver and kidney, with liver levels rising to twice those of the control group (Annadurai et al., 2014). STZ may also directly affect organs such as the liver and kidney (Ghasemi et al., 2014). Similar findings were observed by Samadi-Noshahr et al. (2021), where MDA levels rose in the liver of diabetic rats. Higher MDA levels in the kidneys of diabetic rats compared to normal controls suggest ROS accumulation and lipid peroxidation in the tissue. However, administration of plant extracts reduced MDA levels, demonstrating the plant's antioxidative properties, consistent with Qi et al. (2020). STZ-induced rats show numerous pathological alterations, including distortion of liver architecture, irregular hepatocyte arrangement, and enlarged sinusoids, similar to findings by Sharmen et al. (2022). However, normal liver architecture was restored with treatment. Safitri et al. (2021) observed similar improvements in diabetic rats treated with 500 mg/kg bw of *Ruellia tuberosa* L. root extracts. Histological studies of the kidneys in STZ-diabetic rats revealed shrinkage of the glomerulus, distorted visceral layers, and slightly thickened parietal layers and basement membranes, consistent with findings by Hazam and Rai (2022). The group treated with 200 mg FRDF showed signs of healing and noticeable renal improvement. Restoration of normal kidney architecture was seen in diabetic mice treated with *Moringa oleifera* Lam. leaf and seed extract in a study by Aljazzaf et al. (2023).