The Bioscan 19(2): S.I (1), 238-249, 2024 www.thebioscan.com
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

Comparative in vitro study of the antidiabetic, anti-inflammatory, and antioxidant potential of the Piper cubeba, Piper betle, and Piper nigrum

Shreya Naga Devika C.[1], Keerthana M.[1], Myrene Roselyn Dsouza[2], Sharangouda J. Patil[3] and Premalatha S.J.[1]***

[1]PG Department of Studies in Biochemistry, Maharani's Science College for Women, Mysuru, Karnataka, India

[2]Department of Biochemistry, Mount Carmel College Autonomous, Bengaluru, Karnataka, India

[3]Department of Zoology, NMKRV College for Women, Bengaluru - 560011, Karnataka, India

DOI: https://doi.org/10.63001/tbs.2024.v19.i02.S.I(1).pp238-249

KEYWORDS

Antidiabetic, Anti-inflammatory, Antioxidant, Phytochemicals, Piperaceae

Received on:

04-08-2024

Accepted on:

23-11-2024

ABSTRACT

The study was performed in vitro to evaluate and compare the antidiabetic, anti-inflammatory, and antioxidant effects of aqueous, ethanol and methanol extracts of Piper nigrum, Piper cubeba, and Piper betle. Piper betle leaves and seeds of P. cubeba and P. nigrum were collected from different regions and analyzed for their phytochemical components. The investigation included the assessment of the total phenolic, alkaloid, flavonoid, tannin, and terpenoid contents. The antidiabetic effects of the extracts of the three species were determined through several assays, such as α-amylase inhibition, glucose adsorption capacity, and glucose uptake by yeast. The methanolic extracts exhibited significant inhibition of α-amylases, with P. cubeba showing 71% inhibition, P. betle 88%, and P. nigrum 70% at 100 mg/ml concentration. In the glucose adsorption capacity assay, the methanolic extracts of P. cubeba, P. nigrum, and P. betle demonstrated glucose adsorption rates of 39%, 38%, and 47%, respectively, from a 100 mM glucose solution. Furthermore, in the glucose uptake by yeast assay, the methanolic extracts showed a markedly higher percentage of glucose uptake compared to the other extracts, exhibiting activity similar to that of the standard acarbose. The anti-inflammatory properties were evaluated using a protein denaturation assay, which indicated that P. cubeba and P. nigrum had the highest inhibitory activities in methanol extracts, while P. betle showed significant activity in ethanol extracts. The antioxidant properties of the aqueous, methanol, and ethanol extracts were assessed, focusing on their ability to scavenge DPPH free radicals and their remarkable reductive potential towards ferric chloride ions and phosphomolybdate in the tests carried out. Importantly, the methanolic extract of P. betle leaves exhibited superior antioxidant capacity compared to the aqueous and ethanol extracts of P. cubeba and P. nigrum.

INTRODUCTION

Medicinal plants play a crucial role in the daily life of individual. These plants not only act as supplement or alternative to modern medicine, but also have significant value in daily life, closely linked to various economic aspects related to geriatric disease and mortality [1]. Traditional Ayurvedic practices use plant extracts to treat various health problems. Plants produce phytochemicals, compounds that provide protection against environmental threats. Research has shown that these phytochemicals also have beneficial effects on human health. About 4500 phytochemicals have been identified and categorized based on their protective properties and chemical characteristics. These compounds are found in various parts of plants including roots, stems, leaves, flowers, fruits and seeds [2]. There is currently increasing interest in natural plant-derived medications due to their wide-ranging therapeutic benefits. The therapeutic uses of the Piperaceae family have historically provided a variety of medicinal and culinary spices to numerous cultures. The secondary metabolites that confer these beneficial properties also serve as chemical defenses for many species within the genus Piper. This family includes various chemical compounds such as alkaloids, phenols, tannins, terpenoids, flavonoids, glycosides, carbohydrates, and proteins which exhibit antioxidant, antibacterial and antifungal activities against human pathogens, as well as therapeutic and preventive potential against several chronic diseases. In addition, Piper species have antidiabetic, anti-inflammatory, antipyretic, antiproliferative, neuropharmacological, antihyperglycemic and analgesic effects [3].

P. cubeba, a member of the Piperaceae family, is primarily indigenous to Java and Borneo. It is commonly referred to as Java pepper or tailed pepper. This species is part of a larger group of approximately 700 species that are widespread in tropical and subtropical regions worldwide. Cultivation of *P. cubeba* is mainly grown in South Africa, Indonesia, India, and parts of southern and western Europe. The plant is extensively utilized as a spice in traditional practices and is grown primarily for its berries, which are rich in essential oils [4].

P. betle belongs to the Piperaceae family and its leaves are commonly called "*paan*." There are approximately 2,000 species of *P. betle* worldwide. This plant is primarily cultivated in regions such as Africa, West and South Asia, the Himalayas, India, China, Nepal and Sri Lanka [5]. Cultivation focuses primarily on the heart-shaped leaves. The *P. betle* thrives in a tropical climate with by high humidity and does best in well-drained, fertile soil [6]. Betel leaves are widely used for various reasons, particularly due to the chewing habits prevalent in many countries. These leaves are believed to provide several benefits including preventing bad breath, the strengthening of gums, preserving teeth, and stimulating the digestive system. In Indonesia they are traditionally used for douching, while in India they are used as a mouthwash and remedy for dental problem, headaches, arthritis and joint pain. In addition, boiled betel leaves are used to treat respiratory catarrhs and are known for their tonic or astringent properties as well as their biological activities [7].

P. nigrum is a member of the Piperaceae family and is widely considered as the king of spices. It is commonly referred to as black pepper or Kali mirch. This plant is primarily cultivated for its fruits, which are typically dried and used as a spice. Black pepper is indigenous to India and is predominantly grown in tropical and subtropical areas. With over 600 varieties of pepper, India is often referred to as the home of black pepper. In the past, black pepper was termed black gold due to its significant commercial and economic value [8].

MATERIALS AND METHODS

Materials

Gallic acid, Picric acid, Gelatin, Ferric chloride, Ascorbic acid, 1,1-Diphenyl-2- picrylhydrazyl (DPPH), Lead acetate, Biuret reagent, Benedict's reagent, Sodium carbonate, Sulphuric acid, Glacial acetic acid, Copper sulfate, Mayer's reagent, dinitrosalicylic acid, Potassium hydroxide, Sodium chloride, Ammonia, Ammonium hydroxide, Copper sulfate, Potassium hydroxide (KOH) pellet,, Folin-Ciocalteu phenol reagent, Quercetin, Ammonium chloride, Sodium hydroxide, Sodium nitrite, Anthrone, Potassium ferricyanide, Trichloroacetic acid, Sodium phosphate dibasic. Sodium phosphate monobasic, Ammonium molybdate, concentrated hydrochloric acid (HCl), Ammonia, Sodium hydroxide, Ethanol and Methanol.

Plant Material and Preparation of Extracts

P. cubeba was purchased from an online retailer and then ground into powder using a blender. *P. nigrum* was sourced from a nearby store and also processed into a powder with the aid of a blender. *P. betle* was purchased from a local market, allowed to dry at room temperature for 3 to 5 days, and then ground into a powder using a blender. 10 g of *P. cubeba*, *P. nigrum* and *P. betle* were weighed and transferred to a clean and dry beaker. They were then subjected to ethanol, methanol and aqueous extraction for 48 h. The extract was filtered through Whatman filter paper and the residue obtained was used for further analysis [9].

Phytochemical screening

Phytochemicals are naturally occurring chemical compounds in plants that can have both beneficial and detrimental effects on health. Notable examples of phytochemicals include alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides, terpenes, terpenoids, and coumarin. The process of phytochemical screening is carried out to verify the presence of these compounds in plant extracts [10, 11].

Determination of Total Phenol Content

The content of total phenols in plant extracts was assessed using the modified Folin-Ciocalteu method, with gallic acid serving as the reference standard. The reaction mixture included 1 ml of the extract, 5 ml of distilled water, and 0.5 ml of Folin-Ciocalteu phenol reagent, which was then mixed thoroughly. After a 5-min incubation, 2 ml of a 20% sodium carbonate solution was added, and the mixture was left in the dark for 60 min. The absorbance values of both test and standard solutions were measured at 690 nm, and compared to the reagent blank. The amount of total phenols was then expressed as milligrams of GAE per gram of extract **[12, 13]**.

Determination of Total Flavonoid Content

The total flavonoid content in the plant extract was determined using the aluminium chloride method. 0.5 ml of the plant extract was added to 4 ml of distilled water. Then, 0.3 ml of 5% $NaNO_2$ was added, followed by incubation in the dark for 6 min. After that, 0.3 ml of 10% $AlCl_3$ was added, and the mixture was incubated again in the dark for 6 min. The mixture was then diluted with 2 ml of 1 M NaOH to a final volume of 10 ml. A set of standard solutions of quercetin was prepared with concentrations of 20, 40, 60, 80, and 100 µg/ml. The absorbance of both the standard and extract solutions was measured against a reagent blank at 510 nm. Total flavonoid content was determined using the calibration curve and expressed in milligrams of quercetin equivalent (QE) per gram of extract **[12, 13]**.

Determination of Total Tannin Content

The Folin-Ciocalteu method was used to determine the total tannin content of plant extracts. 0.1 ml of plant extract was dissolved in 7.5 ml of distilled water, followed by the addition of 0.5 ml of FC reagent. Then, 1 ml of 35% Na_2CO_3 was added and the mixture was diluted to 10 ml with distilled water. The solution was shaken well and allowed to sit at room temperature for 30 minutes. A set of reference standard solutions of gallic acid with concentrations of (20, 40, 60, 80, and 100 µg/ml) was prepared. The absorbance of both test and standard solutions was measured with a spectrophotometer at 680 nm against the blank. The tannin content was reported in milligrams of GAE per gram of extract **[12, 13]**.

Determination of Total Alkaloid Content

The alkaloid content of the extract was expressed in milligrams of QE per gram of extract. To test the alkaloid content, the plant extract was first denatured in DMSO, then 1 ml of 2N HCl was added and filtered. The solution was transferred to a separatory funnel, to which 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken vigorously with 1, 2, 3, and 4 ml of chloroform, then collected in a 10 ml volumetric flask and diluted with chloroform to obtain the desired volume. Finally, the absorbance of the mixture was measured at a wavelength of 470 nm **[13]**.

Determination of Total Terpenoid Content

100 mg of dried plant extract (initial weight-Wi) was steeped in 9 ml of ethanol for 25 h and then filtered using Whatman filter paper. Using a separatory funnel, the filtrate was extracted with 10 ml of petroleum ether, and the ether extract was separated and completely dried in preweighed glass vials (final weight-Wf). The ether was evaporated, and the yield of total terpenoids was calculated using the formula. The total terpenoid content was determined by subtracting the final weight (Wf) from the initial weight (Wi) and multiplying the result by 100 **[14]**.

Antidiabetic assays

α-Amylase Inhibition Assay

Plant extracts of various concentrations (10, 20, 40, 60, 80, and 100 mg/ml) were mixed with 0.5 ml alpha-amylase solution (0.5 mg/ml) and 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) to perform the experiment. The mixture was incubated at room temperature for 10 min, followed by the addition of 0.5 ml starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was then incubated for another 10 min, and the reaction was terminated using 1 ml of dinitro salicylic acid reagent. The test tubes were placed in a boiling water bath for 5 min and cooled to room temperature before diluting with 10 ml of deionized water. The absorbance was determined at 540 nm, and the absorbance of the blank and control samples was measured using buffer instead of extract and amylase solution [15]. Acarbose was used as the standard drug and the inhibition of alpha-amylase was calculated using the following equation:

$$\%\ inhibition\ of\ alpha\ amylase = \frac{Ac - As}{Ac}\ x\ 100$$

Where Ac corresponds to the absorbance of the solution without extract and with alpha-amylase solution, and As corresponds to the solution with extract and alpha-amylase solution.

Determination of Glucose Adsorption Capacity

Samples containing plant extracts at a concentration of 1% were added to 25 ml of glucose solution at increasing concentrations of 10, 50, and 100 mM, respectively. The mixtures were stirred and incubated at 37°C for 6 h, followed by centrifugation at 4,000 × g for 20 min. The glucose content in the supernatant was then determined [16, 17]. The bound glucose was calculated using the following formula:

$$Glucose\ bound = \frac{G1 - G6}{Weight\ of\ the\ extract * Volume\ of\ the\ solution}$$

Where G1 represents the initial glucose concentration, and G6 represents the glucose concentration after 6 h.

Glucose Uptake by Yeast Cells Assay

A glucose uptake assay was performed using yeast cells. The commercial baker's yeast was suspended in distilled water and repeatedly centrifuged (3,000×g, 5 min) until a clear supernatant was obtained. A 10% (v/v) suspension of the yeast in distilled water was then prepared. Different concentrations of solvent extracts of *P. cubeba*, *P. betle* and *P. nigrum* (10 to 100 mg/ml) were added to 1 ml of glucose solution (10, 50 and 100 mM) and incubated together for 10 min at 37 °C. The reaction was initiated by adding 160 µl of the yeast suspension, followed by vortexing

and further incubation at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and the amount of glucose in the supernatant was determined. Acarbose was used as the standard drug [18].

The percentage increase in glucose uptake by the yeast cells was calculated using the following formula:

$$Increase\ in\ glucose\ uptake = \frac{As - Ab}{Ac - Ab} x\ 100$$

Where, As sample is the absorbance of the test sample, and Ac is the absorbance of the control reaction (containing all reagents except the test sample).

Anti-inflammatory Assay

Protein Denaturation Assay

The experiment was performed by combining 200 µl of bovine serum albumin, 2.8 ml of phosphate-buffered saline (PBS) with pH of 6.4, and 2 ml of samples or control. The mixtures were then incubated at 37°C for 15 min and heated in a water bath at 70 °C for 5 min. After cooling for 5 min, the absorbance of each sample was measured at 660 nm with a spectrophotometer [18].

The percentage inhibition of protein denaturation was calculated using the formula:

$$\%\ inhibition\ of\ protein\ denaturation = 1 - \frac{As - Ab}{Auc - Ab}\ x\ 100$$

Where, As is the absorbance of the reaction mixtures, Auc is the absorbance of the mixture without the samples or positive control, and Ab is the absorbance of deionized water.

Antioxidant assays

DPPH (1,1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging Assay.

The antioxidant capacity of the extract was determined by measuring its ability to scavenge the stable DPPH free radical. Different concentrations of the extract (50.100, 150, 200, and 250 µg/ml) were added to 3 ml of 0.004% DPPH solution along with 1 ml of solvent as a control. The mixtures were then incubated in the dark for 30 min. Ascorbic acid was used as a reference standard to determine antioxidant activity **[19 -22]**. Absorbance was measured at 517 nm after 30 min and the % inhibition was calculated using the formula:

$$Total\ antioxidant\ capacity\ (\%) = \frac{Ac - As}{Ac}\ x\ 100$$

Where Ac is the absorbance control reaction; As is the absorbance of DPPH free radical + sample extract

Reducing Power Assay

The ability of the sample extracts to act as antioxidants was evaluated using the reducing power assay. Different concentrations of the standard (ascorbic acid) and sample extracts (50, 100, 150, 200, 250 µg/ml) were mixed with 2.5 ml of phosphate buffer (200 mM; pH 6.6) and 2.5 ml potassium ferricyanide, and then incubated at 50 °C for 20 min. Subsequently, 2.5 ml of 10% TCA was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The top layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and freshly prepared 0.5 ml of 0.1% ferric chloride solution. A blank reagent was prepared in the same way, but without adding extract. The absorbance was measured at 700 nm. The results showed that the extracts with higher absorbance values exhibited greater reducing power **[19-22]**.

$$Total\ antioxidant\ capacity\ (\%) = \frac{Ac - As}{Ac}\ x\ 100$$

Where Ac is the absorbance control reaction; As is the absorbance of sample extract.

Phosphomolybdate Assay

The phosphomolybdate method was employed to assess the antioxidant activity of sample extracts. One hundred microliters of the extract solution (containing 1 mg/1 ml) were added to 1 ml of the reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The tubes were then placed in a boiling water bath at 95 °C for 90 min. The samples were then allowed to cool to room temperature and the absorbance of the aqueous solution of each sample was measured at 695 nm. The phosphomolybdate reduction potential (PRP) of the studied extracts was expressed in %, and ascorbic acid was used as a standard [22]. The antioxidant capacity was calculated using the following formula:

$$Total\ antioxidant\ capacity\ (\%) = \frac{Ac - As}{Ac}\ x\ 100$$

Where Ac is the absorbance control reaction; As is the absorbance of sample extract.

Results and Discussion

Phytochemical Screening

Numerous historical texts provide comprehensive details about the medicinal uses of ethnobotanical plants, particularly in alleviating numerous common health issues. These plants are abundant in bioactive compounds, which have been instrumental in traditional medicinal practices, specifically in managing inflammation and disorders linked to it. Various scholars have attributed antioxidant, anti-inflammatory and antidiabetic properties to plant extracts employed by various ethnic communities worldwide to combat a wide range of diseases, some of whose origins are intertwined with oxidative stress. The global importance of utilizing these compounds as phytochemical treatments for inflammation has grown significantly. Initial phytochemical analysis of the hydromethanolic methanolic, ethanolic and aqueous extracts of revealed the presence of tannins, flavonoids, terpenoids, saponins, carbohydrates, glycosides, coumarins, alkaloids, phenolics, anthroquinones, anthocyanin, leucoanthocyanin and protein (Table 1).

Table 1: Phytochemical investigation of *P. cubeba*, *P. betle* and *P. nigrum*

	Extracts of *P. cubeba*			Extracts of *P. betle*			Extracts of *P. nigrum*		
Phytochemicals	Met	Eth	Aque	Met	Eth	Aque	Met	Eth	Aque
Tannins	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	+	-	+	-	-	-
Carbohydrate	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	-	+	+	-
Alkaloids	+	+	+	+	+	+	+	+	+
Phenolics	+	+	+	+	+	+	+	+	+
Anthroquinones	+	+	+	+	+	+	+	+	+
Anthocyanin	-	-	-	-	-	-	-	-	-
Leucoanthocyanin	-	-	-	-	-	-	-	-	-
Protein	+	+	+	+	+	+	+	+	-

Met-methanol; Eth-ethanol; Aque-aqueous

Total phenolic, total flavonoid, total tannin and total alkaloid contents

Phenolics have been shown to moderate carbohydrate and lipid profiles, alleviate hyperglycemia, dyslipidemia and insulin resistance, stimulate insulin release, improve fat tissue breakdown and decrease oxidative stress [23 - 25]. Besides, they exhibit biochemical activities including anti-allergic, anti-atherosclerotic, anti-inflammatory, anti-microbial, antioxidants, anti-coagulant, heart disease preventing and vasodilation [27 - 29]. Total phenolic content was estimated using standard gallic acid. The results were determined using a calibration curve ($y=0.0159x+0.373$ $R^2= 1.750$) for gallic acid in the concentration range of 20-100 µg/ml, and the results were expressed as gallic acid equivalents (GAE) per unit of dry weight (Table 2). Among the extracts, methanol extracts of *P. cubeba, P. betel,* and *P.*

nigrum exhibited the highest phenolic content, while the ethanol extract had the lowest phenol content.

Flavonoids, the most common secondary metabolite provides health benefits through their antioxidant activity. Total flavonoid content was determined using a quercetin standard. The results were obtained from the calibration curve (y = 0.0083x + 0.4493, R^2 = 0.0019) for quercetin concentrations in the range of 20 to 100 µg/ml. The highest flavonoid content was observed in *P. betel* and *P. nigrum* when extracted with ethanol, while P. cubeba showed the highest content when extracted with methanol (Table 2).

Table 2: Total Phenolic, Total Flavonoid, Total Tannin, Total Alkaloid Contents of *P. cubeba*, *P. betle* and *P. nigrum*

Samples	Total Phenolic			Total Flavonoid			Total Tannin			Total Alkaloid		
	mg GA/g DE			mg Q/g DE			mg GA/g of DE			mg Q/g DE		
	Met	Eth	Aq	Met	Eth	Aq	Met	Eth	Aq	Met	Eth	Aq
Piper cubeba	6.76	1.02	1.38	3.88	2.53	-	0.16	0.16	0.11	0.85	-	3.32
Piper betle	9.50	1.24	2.17	7.02	14.91	5.56	10.71	1.6	1.48	3.99	3.26	-
Piper nigrum	7.38	1.26	1.35	3.43	2.029	0.38	-	-	0.078	1.52	2.42	0.28

Tannins also called tannic acid, are polyphenol, which dissolves in water, and found in numerous plant-base diets. They are implicated in reduced feed intake, growth rate, feed conversion efficiency and the net metabolizable energy, as well as impaired protein digestibility in experimental animals. The overall tannin concentration was assessed utilizing gallic acid [30]. The amount of tannins present in the extracts were obtained from a calibration curve (y=0.0159x+0.373 R2= 1.750) for gallic acid (20-100µg/ml) and were expressed as gallic acid equivalent (GAE) per unit of dry extract weight. The methanol exhibited the highest tannin content, while the aqueous solution demonstrated the lowest.

Along with phenolics, alkaloids constitute 20% of plant secondary metabolites and are relevant to human medicine and natural defense. They defend plants against the herbivores and also control plant growth. Therapeutically, they are anaesthetic, cardio protective, and anti-inflammatory drugs [31]. The total alkaloid content was assessed using a standard quercetin compound. The outcome was obtained from the calibration curve (y = 0.0083x + 0.4493, R^2 = 0.0019) for quercetin within the concentration range of 20-100 µg/ml.

α-Amylase Inhibition Assay

The inhibition of α-amylase leads to a postponement in carbohydrate digestion, which subsequently reduces postprandial hyperglycemia [32]. Enzyme inhibitors are viewed as potential therapeutic targets for managing diabetes mellitus. While several synthetic antidiabetic medications, including Acarbose, Miglitol, and Voglibose, are available, they often come with gastrointestinal side effects such as abdominal pain, bloating, and diarrhea [33]. Numerous herbal products, along with various metals and minerals, have been identified for their potential benefits in diabetes management. Plant-derived products are generally regarded as less toxic and significantly less likely to cause side effects compared to their synthetic counterparts [34]. The inhibition of carbohydrate-hydrolyzing enzymes by medicinal plants may be attributed to several factors, including the encapsulation of starch by dietary fibers present in the samples, which reduces starch availability to the enzymes, or the direct binding of the enzyme to fibers, which diminishes enzyme activity [35].

In the α-amylase inhibitory test, various concentrations of *P. cubeba*, *P. betle*, and *P. nigrum* were evaluated for their inhibitory effects on α-amylase, with acarbose serving as the standard reference (Fig 2). The methanolic extracts of *P. cubeba*, *P. betel*, and *P. nigrum* demonstrated superior antidiabetic activity compared to the other extracts, namely the ethanol and aqueous extracts.

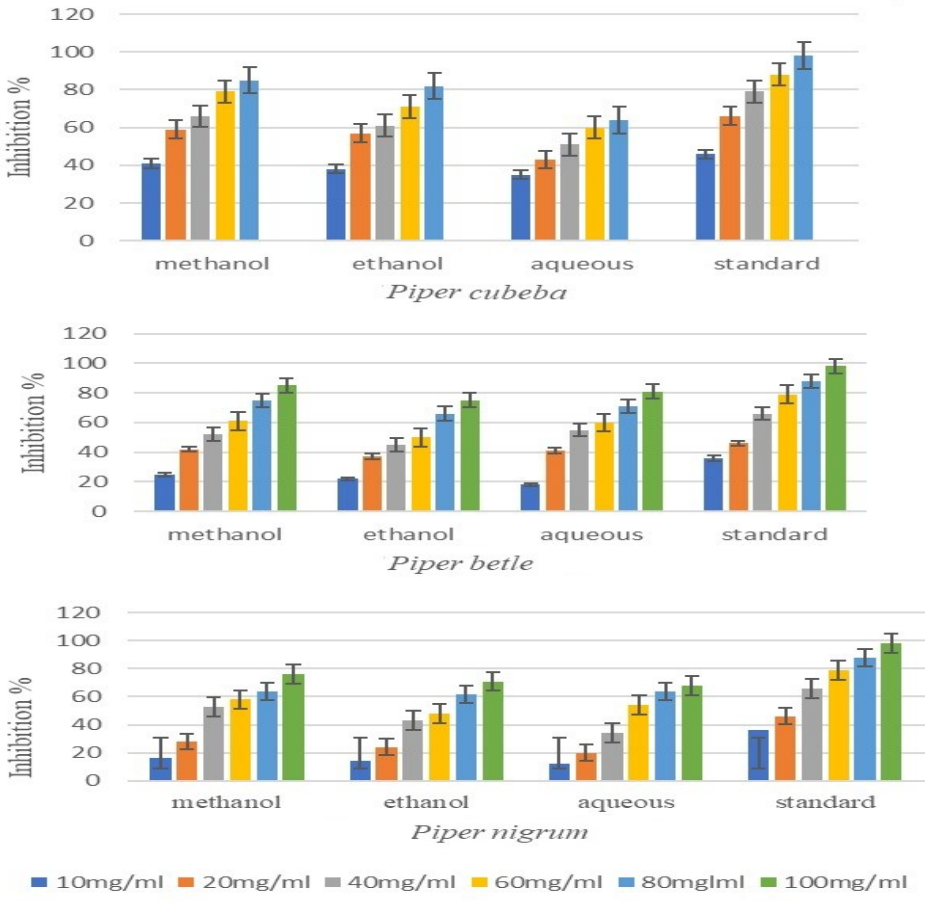

Fig 1: α-amylase inhibitory capacity of *P. cubeba*, *P. betle*, and *P. nigrum*.

Glucose Adsorption Capacity Assay

The study revealed that the adsorption capacities of all extracts were positively correlated with the molar concentration of glucose, indicating that greater quantities of glucose were bound as the concentration increased [35]. The samples demonstrated efficacy in adsorbing glucose across a range of concentrations, specifically at 10, 50, and 100 mM. Notably, the extract of *P. betel* was able to adsorb 47% of glucose from a 100 mM glucose solution, which was the highest amount observed among the extracts analyzed. In comparison, the extracts of *P. cubeba* and *P. nigrum* showed adsorption rates of 39% and 38% of glucose, respectively. This could be attributed to the insoluble and soluble constituents and fibers present [27, 28]. Absorption of glucose would thus reduce the amount of glucose available for transport across the intestinal lumen, consequently reducing postprandial hyperglycemia event [36].

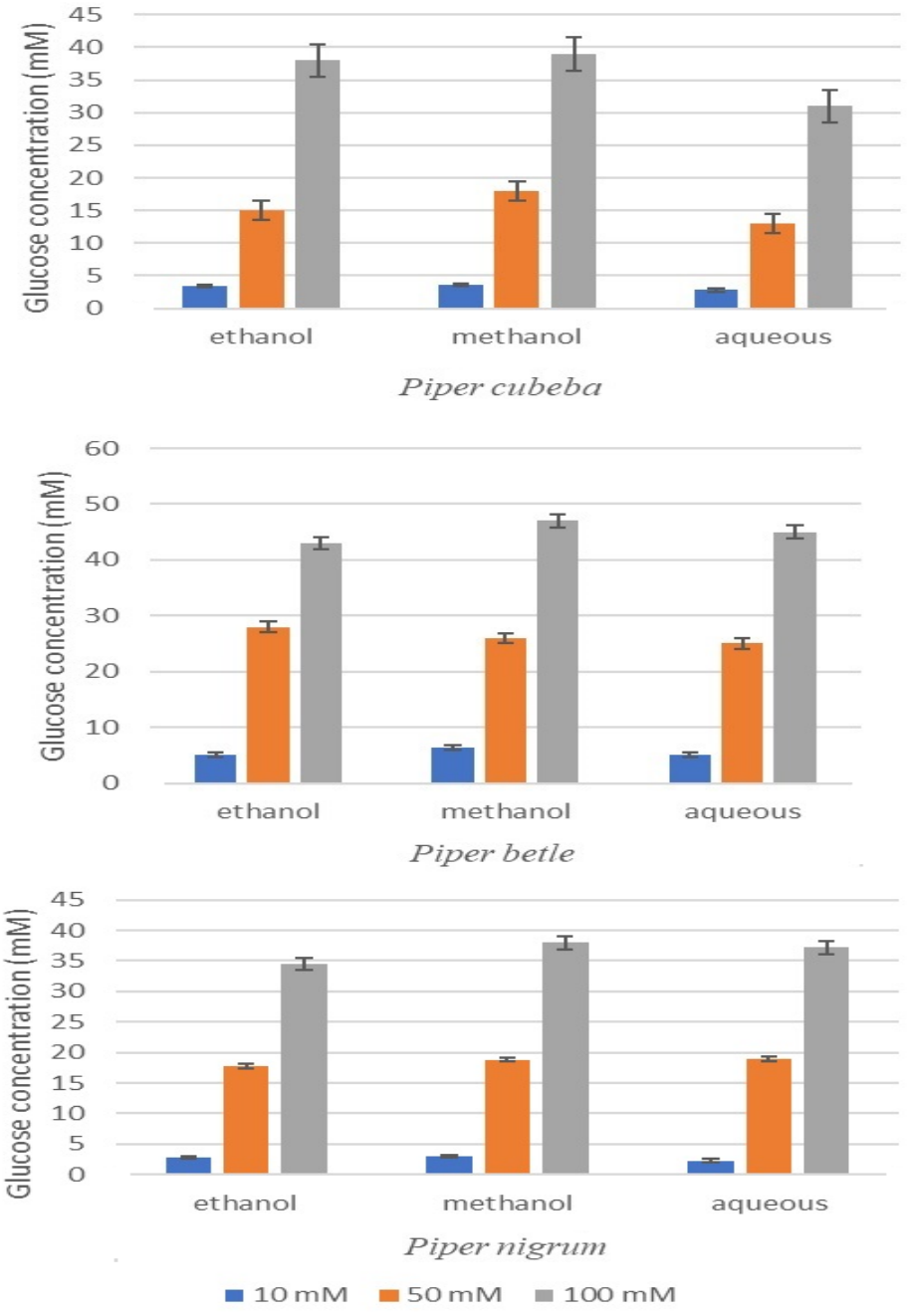

Fig 2: Glucose absorption capacity of *P. cubeba*, *P. betle*, and *P. nigrum*.

Glucose Uptake by Yeast Cell Assay

Facilitated carriers are specific carriers that transport solutes down the concentration gradient, and it is stressed that this effective transport is only achieved provided there is exportation of glucose out of the cellular compartment [36]. Thus, glucose transport occurs only where intracellular glucose is effectively reduced utilised to drive other metabolic functions. The rate of glucose uptake by yeast cells increases in tandem with the rise in glucose molar concentration [18, 37]. To find the glucose absorption at a concentration of 10 mM, many extracts (ethanol, methanol, aqueous) of *P. cubeba, P. betle*, and *P. nigrum* were evaluated. When the glucose content was 10 mM, the uptake by methanolic extracts was found to be 230%, 210%, and 261% *P. cubeba, P. betle*, and *P. nigrum* respectively (Fig 3). A similar trend was seen in the control drug acarbose.

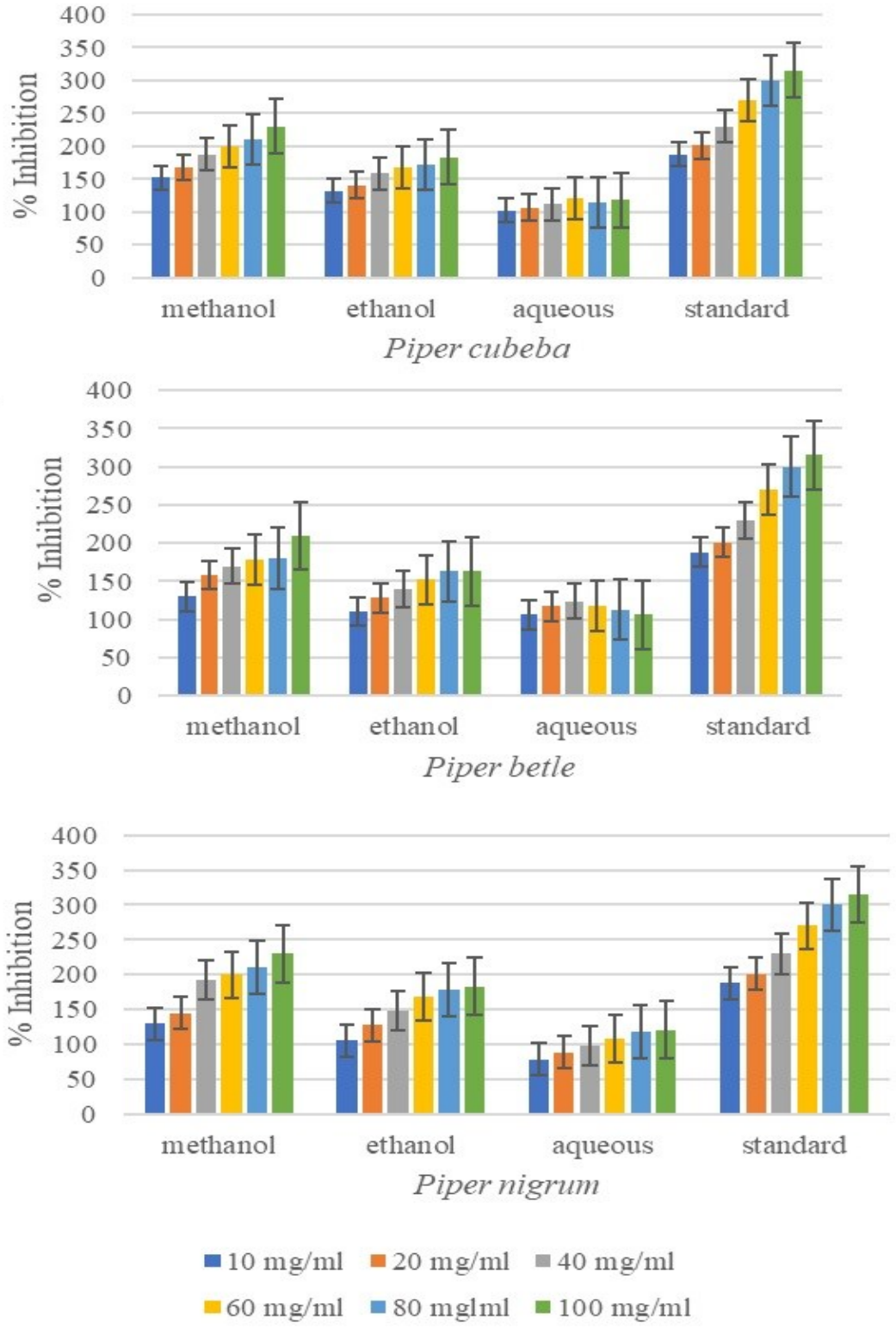

Fig 3: Glucose uptake capacity of *P. cubeba*, *P. betle* and *P. nigrum*.

Protein Denaturation Assay

Protein denaturation is the unfolding of secondary and tertiary structure of proteins by applying stress externally, using compounds like, strong acid or base, concentrated inorganic salt, organic solvent or heat [27]. One cause of inflammation is the denaturation of proteins leading to the loss their biological function. Protein denaturation assay was done to determine the inhibitory potential of methanol, ethanol and aqueous extracts of *P. cubeba*, *P. betle*, and *P. nigrum* on protein denaturation. The result indicates that methanolic extracts have maximum anti-inflammatory potential in *P. cubeba* and *P. nigrum* while *P. betle* has shown maximum anti-inflammatory capacity in ethanolic extract.

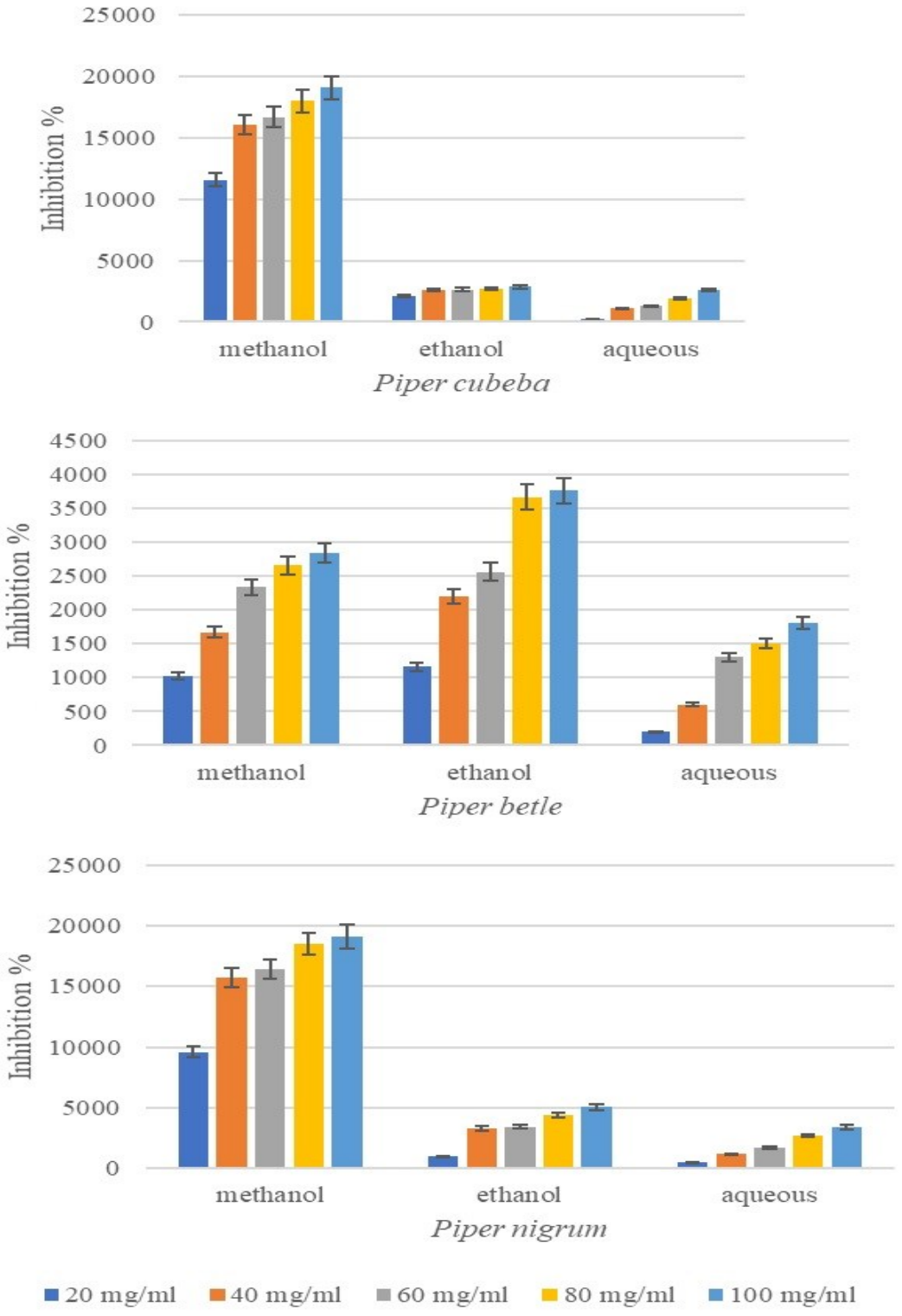

Fig 4: Protein Denaturation Potential of *P. cubeba*, *P. betle* and *P. nigrum*.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity indicates the ability of the test compound to acts as a scavenger for free radicals. The method is based on the capability of 1,1 diphenyl 2-picrylhydrazyl (DPPH), a stable free radical to decolorize the test compound due to the presence of antioxidants [38]. DPPH, a protonated radical, has the absorption maximum at 517 nm, which decreases with the scavenging activity of the proton radical. This characteristic property has been widely used to evaluate the free radical scavenging effect in the natural antioxidant [39]. However, the antioxidant activity of ascorbic acid, a known antioxidant used as the positive control, was comparatively more effective than that *P. cubeba, P. nigrum* and *P. betle* (Fig 5). Overall, methanol extract *P. betle* leaves shows highest antioxidant activity at 82% compared to other aqueous and ethanolic extract of *P. cubeba* and *P. nigrum,* which had low antioxidant activity.

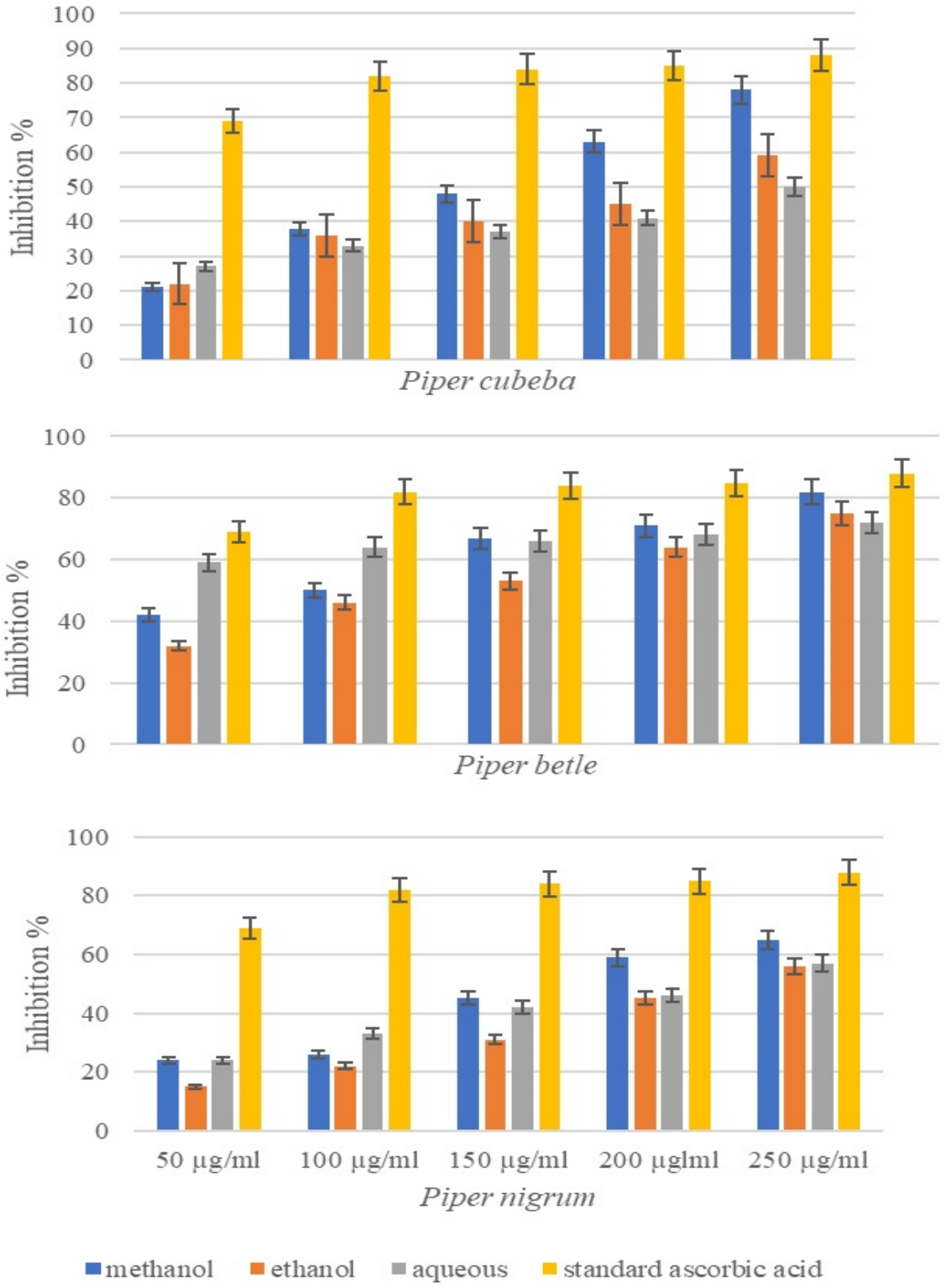

Fig 5: DPPH Radical Scavenging Activity of *P. cubeba*, *P. betle* and *P. nigrum* Extracts.

Reducing power assay

The reducing power assay is as method based on the principle that substances with reducing potential react with potassium ferriocyanide to form potassium ferrocyanide, and then both reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm [40]. In this method, ascorbic acid used as a standard was found to have a stronger reducing power than the extract. The reducing power of ascorbic acid and the extract increases with the concentration of the sample and standard. The concentrations used ranged between 50-250 µg/ml. Ascorbic acid exhibited the highest antioxidant activity when compared to the extracts of *P. cubeba, P. nigrum* and *P. betle*. The plant extracts could reduce most Fe^{3+} ions, which had lower reductive activity than the standard of ascorbic acid. The reducing capability of methanolic extract of *P. betle* shows highest reduction activity at concentration of 250 µg/ml, followed by ethanolic and aqueous extract of different samples (Fig 6).

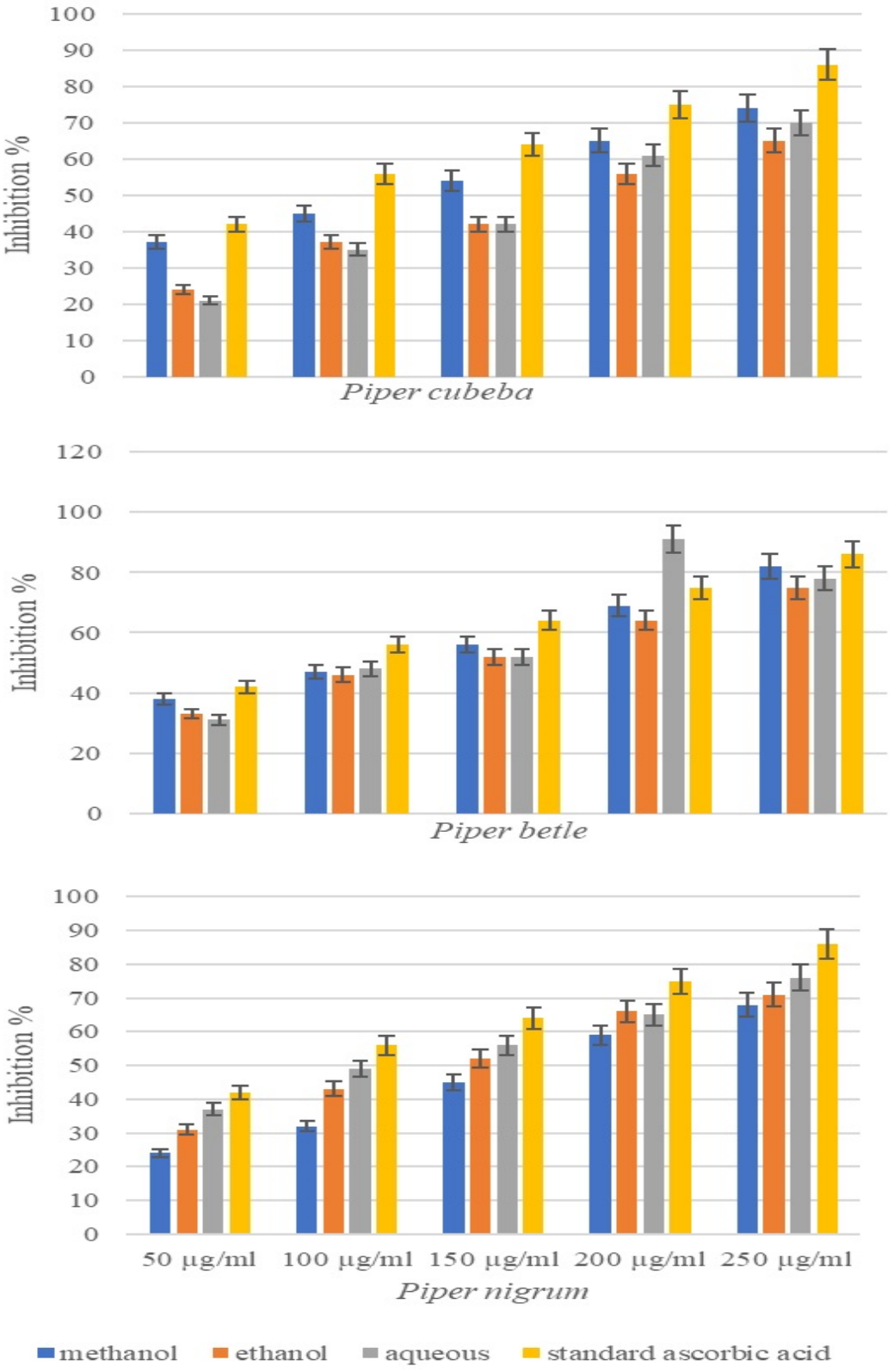

Fig 6: Reducing power activity of *P. cubeba*, *P. betle* and *P. nigrum* extracts.

Phosphomolybdate Assay

This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant compound, resulting in the formation of a green phosphate/MoV complex [41]. Antioxidant activity was calculated based on % inhibition. The standard ascorbic acid was found to be 96%. The antioxidant activity of the methanolic and ethanolic extracts is as depicted (Fig 7). The maximum antioxidant activity was observed in the methanolic extract of *P. betle* at 93% compared to aqueous and ethanolic extracts of different samples. Aqueous extract of *Piper nigrum* has low antioxidant properties when compared to other sample extracts.

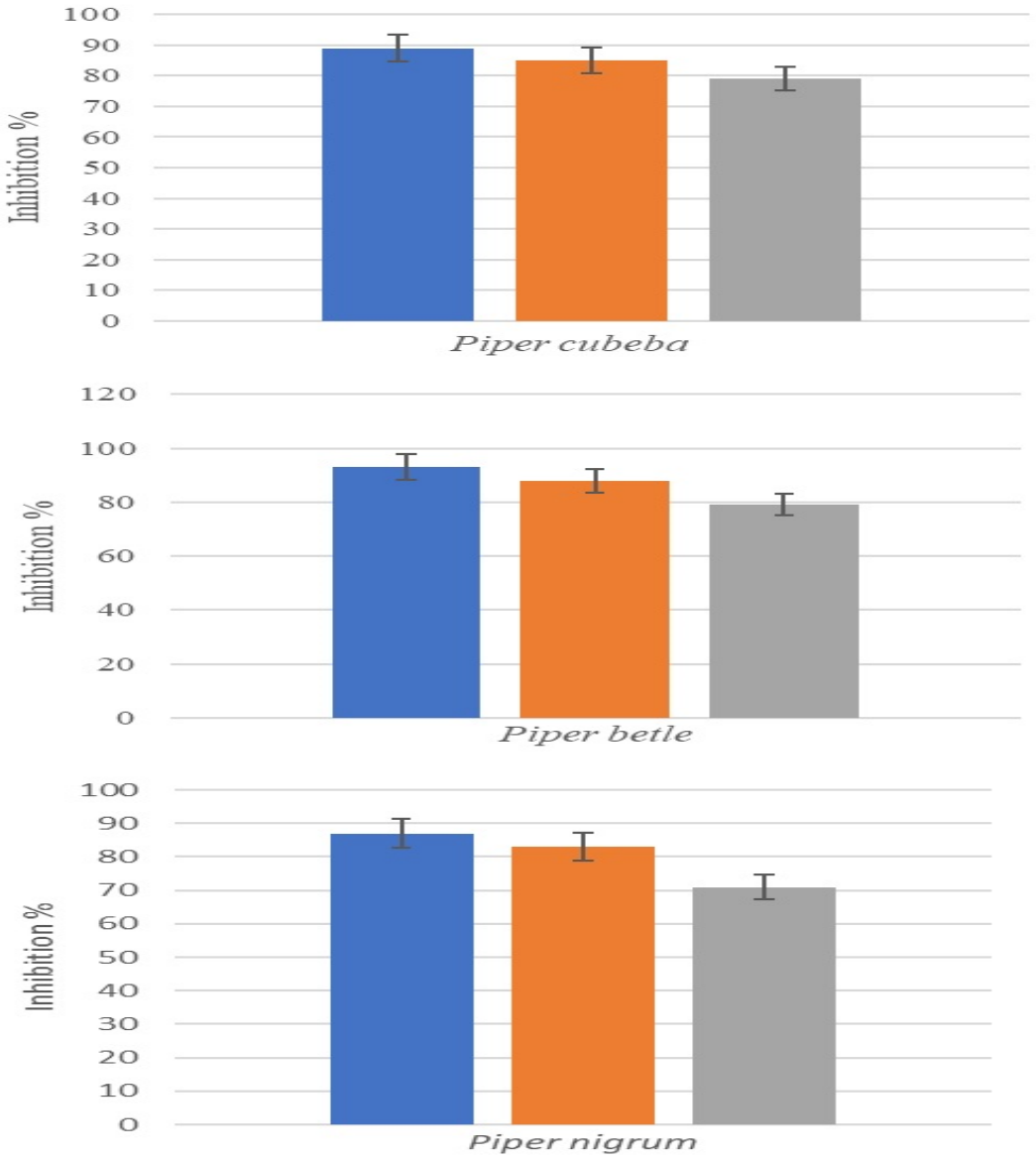

Fig 7: % Inhibition of the *P. cubeba*, *P. betle* and *P. nigrum* by the Phosphomolybdate Assay

CONCLUSION

The present research successfully extricated data related to phytochemical content and bioactivity in *P. cubeba, P. betle,* and *P. nigrum* extract. The phytochemical test showed the presence of tannins, flavonoids, terpenoids and phenolics identified from the methanolic, ethanolic and aqueous extracts exhibited bioactivities that justify their folkloric use. Of the three plants, phenolics and flavonoids were highest, as they are the most important compounds for antioxidant, anti-inflammatory, and antidiabetic properties. Interestingly, the methanolic extracts of these plants also showed greater α-amylase inhibition, glucose adsorption, and protein denaturation than the aqueous extracts of the plants studied here that might have potential applications in the diabetic and inflammatory conditions. *P. betle* possessed the greatest glucose absorption capacity, and *P. cubeba* and *P. nigrum* significantly inhibited glucose uptake in the yeast cell model. Furthermore, the maximum percentage inhibition of DPPH radicals was observed in the methanol extract of *P. betle*.

The results thus show that these ethnobotanical plants have a high level of therapeutic activity in reducing oxidative stress and inflammation as well as regulating hyperglycemia. The results extend the existing literature on the potential of phytochemicals as a source of bioactive ingredients for development of advanced drugs against chronic diseases associated with metabolic disorder and oxidative stress. Future studies are needed to identify and determine the *in vivo* activity of these potential phytoconstituents.

REFERENCES

- Thakur SH, Kaurav HE, Chaudhary GI. Karanj (*Pongamia pinnata*)–an ayurvedic and modern overview. Asian J Pharm Clin Res. 2021;14(6):86-92.
- Koche DE, Shirsat RU, Kawale MA. An overview of major classes of phytochemicals: their types and role in disease prevention. Hislopia J. 2016;9(1/2):1-1.
- Salehi B, Zakaria ZA, Gyawali R, Ibrahim SA, Rajkovic J, Shinwari ZK, Khan T, Sharifi-Rad J, Ozleyen A, Turkdonmez E, Valussi M. Piper species: A comprehensive review on their phytochemistry, biological activities and applications. Molecules. 2019 Apr 7;24(7):1364.
- Kumar P. A review on medicinal plant *Piper cubeba* L. and its pharmaceutical properties. Int J Sci Food Agric. 2021;5:174-9.
- Dwivedi V, Tripathi S. Review study on potential activity of *Piper betle*. Journal of Pharmacognosy and Phytochemistry. 2014;3(4):93-8.
- Rekha VP, Kollipara M, Gupta BR, Bharath Y, Pulicherla KK. A review on *Piper betle* L.: nature's promising

medicinal reservoir. American Journal of Ethnomedicine. 2014;1(5):276-89.
- Depi S. Review of traditional use, phytochemical and pharmacological activity of *Piper betle* L. Galore International Journal of Health Sciences and Research. 2020;5(3):59-66.
- Saleem A, Naureen I, Naeem M, Tasleem G, Ahmed H, Farooq U. Therapeutic role of *Piper nigrum* L (Black Pepper) and pharmacological activities. Sch. Int. J. Biochem. 2022;5(1):15-21.
- Muchandi AA, Jadhav AS, Patil SB, Patil SA, Jadhav NB. Antioxidant and in vitro antidiabetic activity of methanol extract of *Piper cubeba* L. International Research Journal of Pharmacy and Medical Sciences. 2018;1(3):1-4.
- Vazhacharickal PJ, Sajeshkumar NK, Mathew JJ, Babu A. Phytochemical and anti-diabetic activities of different plant part extracts among black pepper (Piper nigrum) varieties in comparison with *Piper longum* and *Piper betel*: An overview. Scientific Study, 2017, 79.
- Haleshappa, R., Keshamma, E., Girija, C.R., Thanmayi, M., Nagesh, C.G., Lubna Fahmeen, G.H., Lavanya, M. and Patil, S.J. (2020). Phytochemical study and antioxidant properties of ethanolic extracts of *Euphorbia milii*. Asian Journal of Biological Sciences, 13(1): 77-82.
- Kolgi, R.R., Haleshappa, R., Sajeeda, N., Keshamma, E., Karigar, C.S. and Patil, S.J. (2021). Antioxidant studies, in vitro cytotoxic and cell viability assay of flavonoids and alkaloids of Leucas aspera (Wild.) Linn leaves. Asian Journal of Biological and Life Sciences, 10(1): 165-171.
- Haleshappa, R., Patil, S.J., Usha, T. and Murthy, S.K.M. (2020). Phytochemicals, antioxidant profile and GCMS analysis of ethanol extract of *Simarouba glauca* seeds. Asian Journal of Biological and Life Sciences, 9(3): 379 385.
- Londonkar, R.L., Patil, S.J., P atil, S.B. (2009). Phytochemical and contraceptive property of *Sida acuta* Burm Fi. Ind. in albino rats. International Journal of Pharmatech Research, 1(4): 1260 1266.
- Bagewadi, S. and Giri, S. Phytochemical profile, antimicrobial, antidiabetic and anti-inflammatory studies of *Gynandropsis pentaphylla* leaves extracts. J Adv Zool., 44(S 6): 01:08.
- Patil, S.J., Renuka Jyothi S., Sadashiv S.O., Vishwanatha T., Adam, J.K. and Naidu, S.B.K. (2023). Antioxidant potential of *Zephyranthes citrina* seed extract in *Saccharomyces cerevisiae*'s oxidative stress response system seeds. Current Concepts in Biotechnology and Pharmacy, 17(Suppl. Iss- 3A): 1129 – 1140
- Roy, U.B., Seethalaxmi, R., Renuka Jyothi, S., Dsouza, M.R., Mahishi, P., Premalatha, S.J. and Patil, S.J. (2023). Herbal medical product for metabolic diseases: A new pharmacological approach. Pakistan Heart Journal, 56(2): 1451-1450.
- Dakshayini, P.N., Roy, U.B., Kolgi, R.R., Singh, S.R., Sharma, S., Shrivastava, R. and Patil, S.J. (2023). Efficacy of phytoextracts on female reproduction and impact on diabetes mellitus. J Adv Zool., 44(4): 1154–1160.
- Bairagi J.H., Haritha G., Yadav K., Garg S., Rani V., Pulipati S., Kolgi R.R., Pundir R. and Patil S.J. (2023). To study of *Artemisia nilagirica* leaves for their antithyroid, oxidative and antihyperglycemic properties. J. Advanced Zool., 44(S4): 40–51.
- Akbar PN, Jahan IA, Hossain MH, Banik R, Nur HP, Hossain MT. Antioxidant capacity of *Piper longum* and *Piper nigrum* fruits grown in Bangladesh. World Journal of Pharmaceutical Sciences. 2014 Sep 1:931-41.
- Chaves N, Santiago A, Alías JC. Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. Antioxidants. 2020 Jan 15;9(1):76.
- Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong public health and research perspectives. 2013 Oct 1;4(5):246-54.
- Naji KM, Al-Shaibani ES, Alhadi FA, Al-Soudi SA, D'souza MR. Hepatoprotective and antioxidant effects of single clove garlic against CCl4-induced hepatic damage in rabbits. BMC Complementary and Alternative Medicine. 2017 Aug; 17:411.
- Ningthoujam S, Dsouza MR, Alisha Pharmacological evaluation of *Parkia speciosa* Hassk. for antioxidant, anti-inflammatory, anti-diabetic and antimicrobial activities *in vitro*, International Journal of Lifesciences. 2018 Jan; A11:49-59.
- Giri, S., Ankali, S., Pavani, M., Binorkar, S.K., Keshamma, E., Kolgi, R.R., Lavanya, L. and Patil, S.J. (2024). Anti-inflammatory, anticancer and phytochemical potential of *Indigofera cordifolia* various extracts. African Journal of Biological Sciences, 6(6): 5419-5429.
- Dsouza MR, Rufina K, Hana D. Extraction of diosgenin from fenugreek and evaluation of its pharmacological role in alleviating metabolic syndrome in vitro. Res J Biotechnol. 2018 Dec; 13(12):10-17.
- Alisha, Dsouza MR, Ningthoujam S. Pharmacognostical overview on *Terminalia arjuna*: A justification of folkloric belief. International Journal of Pharmaceutical Sciences and Research. 2018 Apr; 9(12):1000-13.
- Hana CD, Rufina K, Dsouza MR (2019) Diosgenin from *Dioscorea alata*: Extraction and potential effects on enzymes related to metabolic syndrome. International Journal of Pharmacy and Biological Sciences, 9: 177-185 (Special Issue – NCBTIL).
- Shediwah FMH, Naji KM, Gumaih HS, Alhadia FAA, Al-Hammamia AL, D'Souza MR. (2019) Antihyperlipidemic activity of *Costus speciosus* on atherogenic diet induced hyperlipidemia in rabbits. Journal of Integrative Medicine. 2019 May; 17:181-191.
- Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Critical reviews in food science and nutrition. 1998 Aug; 38(6):421–464.
- Heinrich M, Mah J, Amirkia V. Alkaloids Used as Medicines: Structural Phytochemistry Meets Biodiversity-An Update and Forward Look. Molecules. 2021 Mar; 26(7):1836.
- Bell DS. Type 2 diabetes mellitus: what is the optimal treatment regimen?. The American journal of medicine. 2004 Mar 8;116(5):23-9.
- Apostolidis E, Lee CM. In vitro potential of *Ascophyllum nodosum* phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition. Journal of food science. 2010 Apr;75(3):H97-102.
- Surya Dhandapani SD, Subramanian VR, Senthilkumar Rajagopal SR, Nalini Namasivayam NN. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats.
- Ou S, Kwok KC, Li Y, Fu L. In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. Journal of Agricultural and Food Chemistry. 2001 Feb 19;49(2):1026-9.
- Ahmed F, Urooj A. In vitro studies on the hypoglycemic potential of *Ficus racemosa* stem bark. J Sci Food Agric. 2010; 90:397-401.
- Myrene RD. *In vitro* antidiabetic potential of *Quercus infectoria* galls and in silico docking studies of isolated rosmarinic acid on α-glucosidase activity. International Journal of Biology, Pharmacy and Allied Science. 2022 Sept; 11(9):4080-4096.
- Naji KM, Thamer FH, Numan AA, Dauqan EM, Alshaibi YM, Dsouza MR. Ferric-bipyridine assay: A novel spectrophotometric method for measurement of antioxidant capacity. Heliyon. 2020 Jan; 6(1):e03162.
- Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958 Apr 26;181(4617):1199-200.
- Haleshappa, R., Patil, S.J. and Murthy, S.K.M. (2021). Phytochemical analysis, in vitro evaluation of antioxidant and free radical scavenging activity of *Simarouba glauca*

seeds. Advances in Pharmacology and Pharmacy, 9(1): 01 08.

- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry. 1999 May; *269*(2):337–341.