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Investigation of Antioxidants and Anti-Diabetic Activities of Poly Chemical Extraction of S*emecarpus Anacardium* (Leaf) and *Cymbopogon Citratus*

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ABSTRACT:

Diabetes mellitus is a pervasive global health issue that is steadily escalating. It is projected that the global diabetic population would increase to 366 million by 2030. Discovering new antidiabetic medications from medicinal plants that have low or no adverse effects is a tough endeavor, as the currently available drugs for diabetes, such as insulin or oral hypoglycemic agents, often have one or more negative effects. The current study was conducted to examine the antidiabetic and antioxidant properties of Semecarpus anacardium (Linn.) The present investigation revealed that the leaf of the S. anacardium and Cymbopogon citratus plant exhibited the most elevated concentrations of phenolic and flavonoid components. These molecules had strong antidiabetic and antioxidant effects, comparable to the commercial antidiabetic drug metformin and the antioxidant ascorbic acid. The extract derived from Semecarpus anacardium and Cymbopogon citratus shows promise as a natural agent with antidiabetic and antioxidant properties.

Keywords: Diabetic, herbal treatment. Alloxan, antioxidant

INTRODUCTION

Diabetes mellitus (DM), more generally referred to as diabetes, is a condition that is both damaging and long-lasting. It is marked by chronically increased blood glucose levels, which are caused by either insufficient insulin manufacturing or the body's inability to effectively use the insulin that it produces. The fact that diabetes affects people of all ages, genders, and geographical places makes it one of the most ubiquitous causes of death and sickness on a global scale. The development of type 2 diabetes, which accounts for more than 90 percent of all cases, is influenced by a combination of inherited and environmental factors (environmental factors). [1] Each of the two types of diabetes, type 1 and type 2, has its own set of diagnostic criteria. Type 1 diabetes is the more common form of diabetes. The death of pancreatic beta cells by the immune system is the primary cause of type 1 diabetes. The two Obesity and a lack of physical exercise are directly connected with type 2 diabetes, which is determined by genetic factors and is closely associated with obesity. [2] When a woman is pregnant and has gestational diabetes, the likelihood of experiencing complications during pregnancy and childbirth is increased, and this applies to both the mother and the baby. It is three. According to the findings of the study conducted by the Noncommunicable Disease Risk Factor Collaboration in 2023, the probability of both women and men meeting global targets for preventing the rise in the number of cases of diabetes by the year 2026 is extremely low. Less than one percent of women and an even smaller percentage of men have a chance of accomplishing this goal. The two Ischemic heart disease and stroke were placed first and second, respectively, as the primary contributors to the worldwide illness burden in 2023, according to the worldwide Disease Burden 2023 study.[3] This was noted in the report. It is four both of these diseases are thought to have diabetes as a major contributing factor. The results of a recent poll conducted in 2021 showed that 44.7% of people with

diabetes, which is equivalent to 239.7 million people, were unaware of their medical condition. Africa, the Western Pacific, and Southeast Asia had the highest incidence of undiagnosed diabetes, with proportions of 53.6%, 52.8%, and 51.3% correspondingly.[4] These regions also had the highest prevalence of diabetes. On the other hand, the prevalence of diabetes mellitus (DM) that has not been diagnosed is substantially lower in North America and the Caribbean, precisely at a rate of 24.2%. 17 years old Furthermore, the discrepancy between areas with high earnings and those with low to moderate incomes, as well as the frequency of diabetes that has not been identified, continues to be significant. There is a period of latency that occurs during the pre-diagnostic phase of type 2 diabetes, during which the disease is not discovered. The risk factors for both microvascular and macrovascular complications of diabetes are significantly elevated throughout this time period. This is the case for both types of problems. When it comes to people who have trouble controlling their blood sugar levels, this increase is more obvious, and it coincides with the progressive development of difficulties associated with diabetes. The information that was acquired from a number of different investigations demonstrates without a reasonable doubt that diabetes mellitus (DM) experience a significant progression in severity prior to its clinical diagnosis. During the preclinical phase of diabetes, which is characterised by having no symptoms, this pattern signals the development of broad structural abnormalities that are linked to diabetes. [5]This is the number. The progression of type 2 diabetes, which is caused by impaired fasting glucose (IFG), is often marked by a gradual trajectory, in which the clinical symptoms of the disease are sometimes overlooked for extended periods of time. It is vital to note that delays in detecting and diagnosing type 2 diabetes mellitus (T2DM) are significant factors that lead to poor disease treatment and an increased chance of complications. 'Ballataka' or 'Bhilwa' are common names for the plant that belongs to the family Anacardiaceae and is known as Semecarpus anacardium Linn. Since ancient times, it has been utilised in traditional medical practices for the purpose of treating a wide variety of ailments. There is a wide variety of biologically active components that may be found in the nuts of this plant. These substances include biflavonoids, phenolic compounds, bhilawanols, minerals, vitamins, and amino acids. There are many different medicinal properties that these chemicals possess. Antiatherogenic, antiinflammatory, antioxidant, antibacterial, antireproductive, central nervous system stimulant, hypoglycemia, anticarcinogenic, and hair growth promotion are some of the features that the fruit and nut extract possesses. Lemongrass is a herb that is generally known all over the world and belongs to the Gramineae family. Cymbopogon citratus is the scientific name for this plant. Citral is a cyclic monoterpene that is responsible for the characteristic odour of lemon, which is similar to that of a lemon. The term "lemon" originates from this odour by virtue of the existence of citral. Tannins, flavonoids, alkaloids, and a wide range of essential oils are among the phytoconstituents that can be found in lemongrass. It has also been suggested that the secondary active metabolites of a number of the plant's constituents are responsible for the multifaceted pharmacological effects of this plant. "boat-beard" is the literal translation of the Greek word "kymbe –pogon," which is where the term "Cymbopogon" originates from. Cymbopogon citratus is a perennial aromatic grass that is native to South India and Sri Lanka. It is currently being farmed extensively across tropical climates in both the Americas and Asia.[6-9] The essential oil is extracted from leaves that have been gathered at a very recent time and have been partially dehydrated. These leaves are used for treatments. Numerous studies have been conducted to study the pharmacological properties of Cymbopogon citratus. In spite of this, research suggest that new species might have the potential to offer therapeutic therapeutic advantages. There are numerous biocompounds that can be identified in lemon grass tea, including those that are present in its decoction, infusion, and essential oil extracts. Clear proof of lemongrass tea's antioxidant, anti-inflammatory,

antibacterial, anti-obesity, antinociceptive, anxiolytic, and antihypertensive qualities provided strong support for the pharmacological claims that were made about the beverage.[10,11]

METHODS:

Plant Material

The Nainital Forest in Uttarakhand was the location where the fresh leaves of both Semecarpus anacardium (Linn.) and Cymbopogon citratus were collected.

Preparation of Plant extract

Preparation of plant extracts: [12] it was used to create the extract and solvent fractions, with some minor alterations being made. After being dried in the shade, the leaves of the plant were ground into a coarse powder and then added to the powder. Over the course of three days, a total of five hundred grammes of pulverised leaves were submerged in two and a half litres of methanol. After that, the mixture was filtered, and the solvent was extracted by evaporating it in a rotary evaporator at a temperature of fifty degrees Celsius while the pressure was lowered. The methanol extract that was obtained from Semecarpus anacardium (Linn.) and Cymbopogon citratus was 32 grammes in weight after going through this method. It was determined that the extract was appropriate, and it was preserved for potential use in the future. Following the initial dissolution of 20.14 grammes of this extract in 100 cubic centimeters of methanol, the extract was subsequently split into three sections of n-hexane, each of which was 350 cubic centimeters in volume. In order to extract the n-hexane solvent fraction, the top phase, which was composed of n-hexane, was collected and concentrated. Afterwards, the leftover methanol phase was collected, condensed, and then dissolved once more in 100 cubic centimetres of a mixture of water and methanol with a volume-to-volume ratio of 55:45. After that, it was separated into three equal parts of ethyl acetate of 350 millilitres each. Following the collection and concentration of the ethyl acetate solvent fraction, the top phase, which was composed of ethyl acetate, was obtained. Following the collection and concentration of the aqueous-methanol solvent fraction, the lower phase, which was composed of a mixture of water and methanol, was obtained. In preparation for its potential use in the future, each extract and fraction was correctly marked and stored.

Phytochemical analysis: The extracts and fractions were subjected to phytochemical analysis using the procedures:[13,14]

Alkaloids: 1 cm^3 of a 1% hydrochloric acid solution was introduced into a test tube containing 3 cm^3 of the extracts. The concoction was subjected to heat for a duration of 20 minutes, subsequently cooled, and subsequently passed through a filter. The filtrate was utilised in the following manner:

A. Two droplets of Mayer's reagent were introduced to 1 cubic centimetre of the extracts. The presence of alkaloids in the extracts was confirmed by the formation of a creamy precipitate.

B. Two drops of Wagner's reagent were added to 1cm^3 of the extracts. The observation of a reddish brown precipitate confirmed the existence of alkaloids.

Tannins: One cubic centimetre of a freshly produced solution containing 10% weight/volume of potassium hydroxide (KOH) was combined with one cubic centimetre of the extracts. The observation of a discoloured white solid suggests the existence of tannins.

Phenolics: In a test tube, 1cm^3 of the extracts was mixed with two drops of a 5% $FeCl_3$ solution. The appearance of a greenish solid confirmed the existence of phenolics.

Glycosides: A portion of 10 cm^3 of a solution containing 50% sulfuric acid ($H_2 SO_4$) was combined with 1 cm^3 of the extracts. The resulting combination was then subjected to heating in boiling water for a duration of 15 minutes. Subsequently, a volume of 10 cm^3 of Fehling's solution was introduced into the mixture, which was then subjected to boiling. The observation of a brick red precipitate suggests the existence of glycosides.

Saponins:
Foaming test: A 2 cm^3 volume of the extract was forcefully agitated in a test tube for a duration of 2 minutes. Frothing is a clear indication of the presence of saponins.

Emulsion test: A total of five droplets of olive oil were introduced into a test tube containing 3 cm^3 of the extract, and then violently agitated. The presence of saponins was suggested by the formation of a stable emulsion.

Flavonoids: A 1cm^3 portion of a 10% NaOH solution was introduced into 3cm^3 of the extracts. The presence of flavonoids was indicated by a yellow coloration.

Steroids: Five drops of concentrated sulfuric acid ($H_2 SO_4$) were applied to 1cm^3 of the extracts. The presence of steroids is indicated by a red coloration.

Phlobatannins: 1 cubic centimetre of the extracts was added to a solution containing 1% volume/volume hydrochloric acid. Phlobatannins were verified to exist through the observation of a red precipitate.

Triterpenes: 1cm^3 of the extracts was treated with five drops of acetic anhydride. Next, a little amount of very concentrated sulfuric acid ($H_2 SO_4$) was added to the mixture. The combination was then subjected to steam for a duration of 1 hour. After that, it was neutralised using sodium hydroxide (NaOH) and chloroform was subsequently added. The presence of triterpenes was indicated by a colour that was a combination of blue and green.

Carotenoids: Ten millilitres of chloroform were used to extract one gramme of each sample, which was shaken vigorously in a test tube. After filtering the resultant slurry, 85% sulfuric acid was added. The presence of carotenoids was indicated by the interface's blue colour.

Determination of *in vitro* antioxidant activity of the extract: [15]
The methanol crude extract/solvent fractions of Cymbopogon citratus and Semecarpus anacardium (Linn.) were evaluated for their in vitro antioxidant activities using the techniques outlined in Saeed et al. (15).

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl): Using the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) technique, the extracts' capacity to scavenge free radicals was assessed in vitro. In a nutshell, 4 mg of DPPH was dissolved in 100 ml of methanol to create DPPH stock solution (0.1 mM), which was then kept at 20°C until needed. Using the spectrophotometer, the DPPH solution was diluted with methanol to reach an absorbance of approximately 1.2±0.09 at 517 nm, which was the working solution. This solution was combined with 100µl of each of the different concentrations (0-100 µg/cm3) in a 3 cm3 aliquot. After giving the reaction mixture a good shake, it was allowed to sit at room temperature for 30 minutes in the dark. At 517 nm, the absorbance was measured. As previously mentioned, the control was made using methanol in place of the sample. The following formula was used to calculate the percentage of scavenging activity.

DPPH scavenging activity (%) = [(control absorbance−sample absorbance)/ (control absorbance)] ×100

Induction of diabetes

Rats were given an intraperitoneal injection (i.p.) of 120 mg/kg of alloxan monohydrade dissolved in 100 µl of saline, following a 16-hour fast. Following a 72-hour period, a glucometer was used to assess the plasma glucose levels using a sample of rat tail vein blood. More than 11.5–13.5 mmol/l of blood sugar in rats is regarded as moderate diabetes. As a typical control, age-matched, healthy rats were employed.

Experimental design [16]

Thirty long Evans rats were used in the experiment, and they were split into the following six groups to receive extracts, medicines, or a vehicle orally.

Control group (referred to as NC) consisted of 5 subjects treated with a vehicle solution containing 0.5% methyl cellulose.

I. Diabetic Control (Group DC, Vehicle 0.5% Methylcellulose, oral administration, sample size = 5)

II. Diabetic Standard, also known as Group DS, was administered with Metformin HCl at a dosage of 150 mg/kg. The experiment included a sample size of 5. Group SA 100, consisting of 5 subjects, received an oral administration of 100 mg/kg of Diabetic Extract. Group SA 200, consisting of 5 individuals, was administered an oral dose of 200 mg/kg of the diabetic extract.

III. Diabetic rats were administered an extract of 400 mg/kg (referred to as Group SA 400) with a sample size of 5.

Antidiabetic studies of SA extract on alloxan-induced diabetic rats The animals of Group

Group V and Group VI were administered S. anacardium bark extract at doses of 100, 200, and 400 mg/kg body weight once daily for 15 days by intragastric tube. Group III was administered metformin at a dosage of 150 mg/kg body weight, whereas Group II served as the control group for diabetes and received a vehicle solution containing 0.5% MC. The blood samples were examined for blood glucose levels using a Glucometer on the 0th, 5th, 10th, and 15th days of treatment.

Measuring body weight and organ weight (liver, pancreases, kidney, heart, and lung)

The body weights of rats in each group were measured on days 0, 3, 6, 9, 12, and 15 during the treatment period. After the studies concluded, the rats were anaesthetized using diethyl ether. Their chests were then opened, and blood samples were directly extracted from the aorta of the heart using syringes containing heparin. These blood samples were preserved in test tubes containing the anticoagulant EDTA. Subsequently, the liver, kidney, pancreas, heart, and lung were extracted and thoroughly cleansed of the adjacent tissues. The organ weights (OW) were promptly quantified and the organ weights to body weight ratio (O/B) was computed. Some of the samples were preserved in 10% formalin and maintained in a refrigerator at −20°C for histopathological analysis, while others were stored for biochemical estimations.

Statistical Analysis

The mean standard error of the mean is a statistical measure used to quantify the variability of values. Statistical significance was determined using a one-way analysis of variance and Dunnett's't' test. Values were considered significant when the P-value was less than 0.05.

RESULTS & DISCUSSION

After a 15-day treatment period, the survival rates of the groups of rats treated with SA & CC extract were 40%, 60%, and 80% correspondingly. Each group consisted of 5 rats, with Group DC having a survival rate of 40%, Group SA & CC 100 having a survival rate of 60%, and Group SA & CC 200 having a survival rate of 80%. No rats in Group DS, Group SA & CC 400, and Group NC died. Group SA & CC400 exhibited a considerably greater 15-day survival rate compared to Group DC, as indicated in Table 1.

Table: 1 Survival rate after treating with SA & CC Extract

Treatment & Dose	Total Animals	Survivors	Death	Survival rate%
Normal control	5	5	0	100
Diabetic control	5	2	3	40
Diabetic standard	5	5	0	100
SA+CC (100 mg/kg)	5	3	2	60
SA+CC (200 mg/kg)	5	4	1	80
SA+CC (400 mg/kg)	5	5	0	100

Effect of SA extract on blood glucose levels in alloxan induced diabetic rats

Group DC rats had markedly elevated blood glucose levels in comparison to Group NC rats. The blood glucose levels in rats from Group SA & CC 100, Group SA & CC 200, and Group SA & CC 400 were reduced after 5, 10, and 15 days of treatment, respectively. Group SA 200 and Group SA 400 rats had a noteworthy reduction in glucose levels from days 10 to 15, and their effectiveness was similar to that of Group DS. However, Group SA 100 did not have a substantial impact on blood glucose levels compared to Group DS. Following a 15-day treatment with extract, glucose levels were considerably reduced in rats from Group SA& CC 100, SA & CC 200, and SA & CC 400. The observed effect was depending on the dosage, with the most notable impact reported in Group SA & CC 200 and Group SA & CC 400 ($p < 0.05$) (Figure 1).

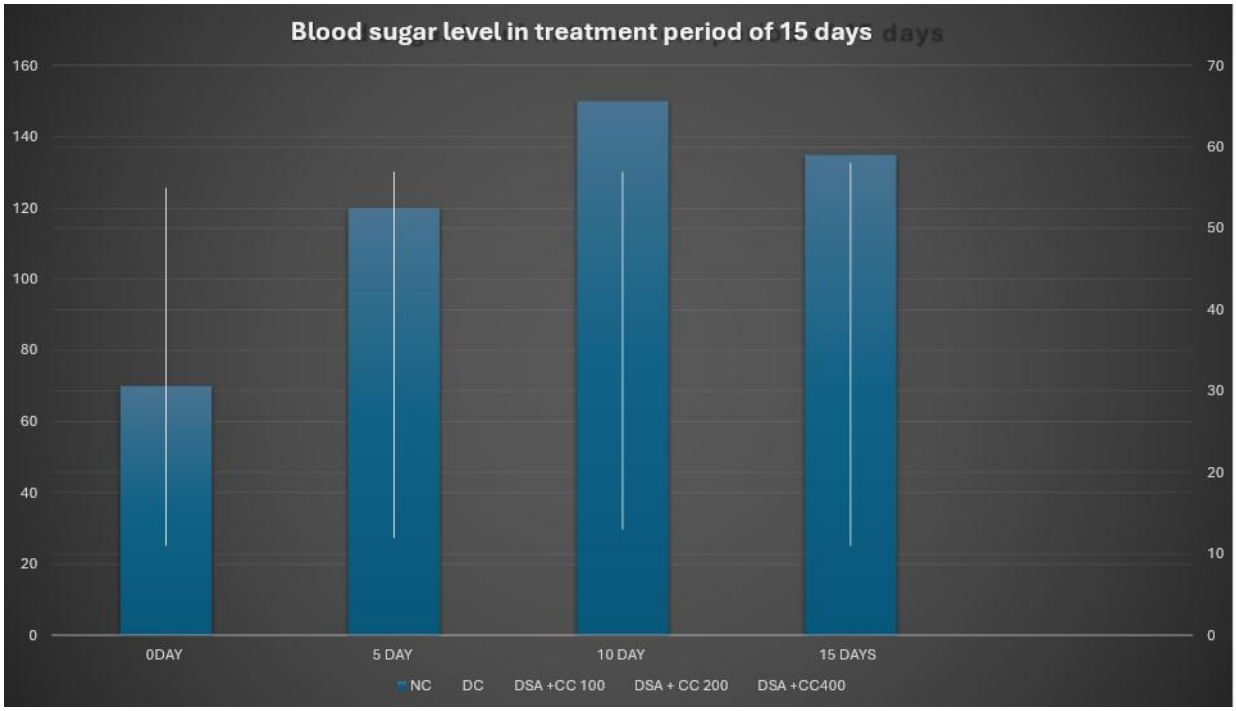

Fig: 1 Blood sugar level in treatment period of 15 days

Effect of SA & CC in body weight

Table 2 displays the notable variations in body weight among the groups of rats throughout the treatment period.

Table: 2 Combine effect of SA & CC in body weight

Group	0 days	3 days	6 days	9 days	12 days	15 days
NC	172.4± 6.4	174.4±4.21	178.6±2.4	180.2±4.1	183.4±3.56	186.1±5.78
DC	165.2±3.2	167.5±4.2	168.4±4.2	17.2±3.2	174.2±3.6	175.0±4.4
SA + CC100	154.4±4.2	157.3±4.2	157.8±4.1	158.4±5.4	163.2±4.2	157.3±3.2
SA + CC 200	153.2±5.6	152.4±5.1	151.5±8.5	156.3±4.5	170.5±5.6	166.4±6.0
SA + CC400	148.5±4.5	146.3±4.6	148.2 ±4.2	161.4±3.3	147.6±4.2	178.2±4.5

Effect of SA and CC extract on organ weight to body weight ratio

The findings indicated that there were no significant alterations in the weight of the heart, kidneys, lungs, and pancreas over a 15-day therapy period. While the liver weight noticeably dropped in Group DC, it returned to normal levels after therapy in Groups SA+CC 200 and SA+CC 400. The experimental groups did not show any notable variations in the ratio of organ weight to body weight, as indicated in Table 3.

Table: 3 Effect of SA +CC Extract on the heart weight, kidney weight, lung weight and pancreas weight

Group	Heart weight	Liver weight	Kidney weight	Lung weight	Pancreas wt.
NC	0.62± 0.05	4.87±0.01	1.1±0.06	0.38±0.02	1.16±0.07
DC	0.51±0.04	3.45±0.4	1.05±0.0	0.36±0.1	1.15±0.5
SA + CC100	0.54±0.02	4.31±0.4	0.95±0.1	0.41±0.04	1.35±0.13
SA + CC 200	0.52±0.02	3.45±0.1	0.98±0.02	0.54±0.05	1.45±0.46
SA + CC400	0.53±0.04	4.20±0.02	0.94±0.04	0.47±0.05	1.39±0.78

DPPH Free radical scavenging

The antioxidant activity of the SA + CC extract was assessed using the DPPH free radical scavenging assay. The IC50 value determines the antioxidant potency of an extract. Figure 2 displays the outcome of the DPPH scavenging activity of the SA extract. The extract demonstrated DPPH radical scavenging action, with IC50 values of 75.28 µg/ml, while ascorbic acid had an IC50 value of 18.45 µg/ml.

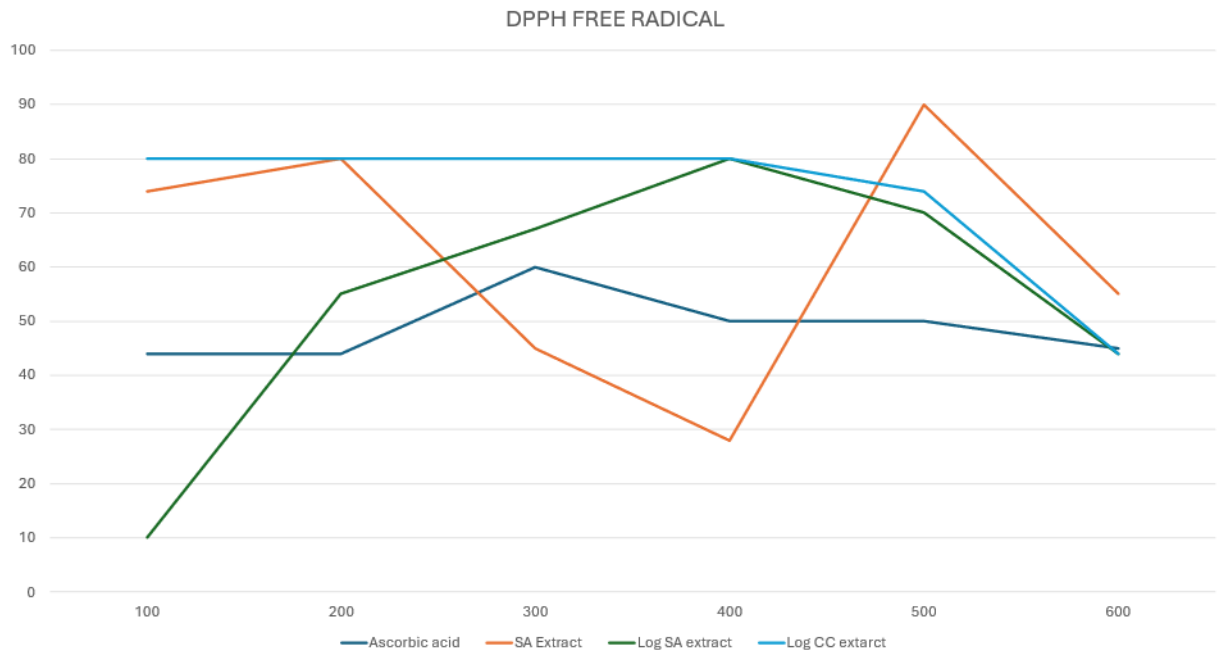

Figure 2 shows the percentage of DPPH free radical scavenging activity at different concentrations (µg/mL) of SA + CC Leaf extract and ascorbic acid.

Phytochemical Screening

Chemicals	SA Extract	CC Extract
Tannins	+	+
Steroids	+	+
Saponins	+	-
Carbohydrates	+	+
Fat/ Lipid	+	+
Alkaloids	+	+

CONCLUSION

The current study demonstrated that the leaf of the *S. anacardium* and *Cymbopogon Citratus* plant contained the highest levels of phenolic and flavonoid compounds. These compounds exhibited potent antidiabetic and antioxidant actions, which were similar to those of the commercial antidiabetic medicine metformin and the antioxidant ascorbic acid. The Semecarpus anacardium and Cymbopogon citratus extract appears to possess potential as a natural antidiabetic and antioxidant agent.

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