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An In-vitro Study on Methanolic Leaf Extract of Couroupita Guianensis of Antiobesity Activity

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ABSTRACT

From ancient times, medicinal plants are used for the traditional purpose of providing relief for several diseases. They follow the fundamental aspects such as safety, quality, and efficacy to ensure the role of plant-based medicines in healthcare. *Couroupita guianens* is also a type of medicinal plant, which belongs to the *Lecythidaceae* family. It is also called a Cannonball tree. The whole plant of *Couroupita guianens is* has several biological activities such as the anti-ulcer, antimicrobial, antioxidant, hypolipidemic, anti-inflammatory, antiseptic, anti-fertility, antipyretic, anti-stress, analgesic, anti-depressant, wound-healing, anti-biofilm, insecticidal, anti-diarrheal, anxiolytic, hepatoprotective, anti-helminthic, anti-fungal activities. The present study evaluate the Phytochemical screening to determine the presence of chemical constituents used for the antioxidant activity by using DPPH and α-glucosidase inhibition activity for anti-obesity activity and estimates of total phenolic content present in the methanolic extract of *Couroupita guianensis*.

Keywords: α-glucosidase, antioxidant activity, Total phenolic content, *Couroupita guianensis.*

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CONTENTS

1. Introduction

Medicinal plants have more remedies for curing human diseases because they contain more components of therapeutic value. Medicinal plants are the “backbone” of traditional medicines, which means more than 3.3 billion people in less developed countries utilize medicinal plants on a regular basis [Davidson-Hunt, 2000]. Ancient people value the plants as natural healers and they give a prominent position in their livelihood. The medicinal plants are reported to be rich in bioactive secondary metabolites, thereby acting as a renowned supply of drugs being utilized in folklore to modern medicine for therapeutic purposes. The prevalence of obesity is increasing all over

the world. Presently, 300 million peoples are obese due to the usage of medicines, while more than one billion adults are overweight [5]. Approximately, 3.3 million people may increase in the year 2030. This disease has many factors which contribute to its etiology disorders, and psychiatric issues, lack of physical fitness [6, 7].

Previous studies also indicate that people increase their intake of high-energy fast foods when stressed, thereby leading to obesity [8]. In addition to this, labor-saving devices such as elevators, cars, remote controls, personal computers, and sedentary recreational activities such as watching television, browsing the Internet, and playing video games are highly contributed to obesity in the world [9, 10]. This is due to the adverse effects of causing obesity related to these drugs. More current approaches have focused on natural sources that have been reported to manage obesity and hyperlipidemia as well as reduce weight gain with fewer side effects [12].

Nowadays, the potential use of natural agents for the management of obesity is not fully explored and could be an outstanding substitute approach for developing safe and effective anti-obesity drugs. For example, some edible medicinal plants have been used as dietary supplements for body weight management and control in many countries [13, 14].

Couroupita guianensis is a large evergreen tree that grows upto a height of 25 meters. Leaves are alternate, oblong-ovate, up to 20 cm long, entire to slightly serrate, and hairy on the veins beneath. The inflorescence is racemose, arising from the trunk and other large branches. *Couroupita guianensis* leaves are simple, alternate, oblong, or oblong–ovate up to 10 cm long with an entire to sligl serrate margin and a short petiole and hairy beneath the veins. They are arranged in whorls at the end of the shoot.

Botanical classification

Kingdom : Plantae
Subkingdom :Tracheobionta
Division :Magnoliophyta
Class : Magnoliopsida
Order :Lecythidales
Family :Lecythidaceae
Genus :Couroupita
Species : guianensis

Synonyms:

Telugu: Mallikarjuna,Naagamalli, Naagalingam
Tamil : Naagalingam
Hindi : Tope Gola, Nagalinga
English: Cannon ball tree
Odiya :Nagakesara
Malayalam :Naagadanthee
Indonesia : Sala
Thai: Sala Lankaa
Marathi : Kailasapati.

1.1 Chemical constituents

The chemical constituents present in the leaves of Couroupita guianensis are Triterpene ester, β-Amiri palmitate, hydroxycinnamic acids, caffeic acid, Rosamarinic acids, Kaempferol-3-O- neohesperidoside, 20,40-dihydroxy-60-methoxy-30,50-dimethylchalcone,7-hydroxy-5-methoxy-6,8-dimethyl flavanone, 4-hydroxybenzoic acid.

1.2 The pharmacology of *Couroupita guianensis*

Anti-oxidant activity

Couroupita guianensis leaf extract exhibited scavenging activities with respect to DPPH[2,2-diphenyl-1-picrylhydrazyl], hydrogen peroxide, nitric oxide, and hydroxyl radicals. Importantly, Ethylacetateleaf extract has shown the highest scavenging activity against DPPH, hydrogen peroxide, and hydroxyl radicals. In another study, Kekuda mentioned that marked scavenging activity against 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid was exhibited by the methanol and ethanol extract of the *Couroupita guianensis* leaves and it also exhibit antioxidant activities [13].

Anti-inflammatory activity

Different extracts from C.G leaves were explored in an invivo study for inflammatory pain [formalin-induced licking] and acute inflammation [carrageenan-induced peritonitis]. This study showed that hexane, ethanol, and ethyl acetate inhibited leukocyte migration at higher doses [30 and 100mg/kg]. The extract was found to reduce cytokines such as tumor necrosis factor-α and inhibit interleukin-1b and nitric oxide production.

Anti-diabetic activity

Methanolic extract of CG leaves on previously the carbohydrate hydrolyzing enzymes are noticed that the extract could control the enzymes dose-dependently highlight the inhibition of α-amylase and α-glucosidase with sucrose as substrate and α-glucosidase with maltose as substrate. These compounds were reported it is having inhibitory activity against carbohydrate hydrolyzing enzymes [24].

Gold Nanoparticles

Gold nanoparticles are obtained from leaves of the *Couroupita guianensis* they have increased lipid peroxidation, and antioxidant enzymes such as superoxide dismutase, glutathione reductase stress, and catalase enzyme activities in diabetic rats and thus relieve hyperglycemia condition by regulating oxidative.

Anti-Obesity activity

The Methanolic extract of the plant [*Couroupita guianensis*] it reduces the total serum cholesterol, low-density lipoproteins, triglycerides, and very low-density lipoproteins and increased the high-density lipoproteins in obese rats when compared with the standard, Atorvastatin. Further, the extract has shownasignificant inhibition of atherosclerotic plaque formation

Hepatoprotective activity

The hepatoprotective activity of *Couroupita guianensis* Ethanolic leaf extract acts against $CCL_4$and induces liver

damage occurs in 4 rats. Actions of liver marker enzymes, serum glutamate oxaloacetate transaminase, serum glutamic pyruvic transaminase, aspirate aminotransferase, alkaline phosphate, total bilirubin, and total protein, visible a considerable hepatoprotective effect in evaluation with the drug of silymarin.

Anti-ulcer activity

The Ethanolic extract of leaves in experimental rats showed that at doses of 150 and 300 mg/kg body weight there was a significant decrease in ulcer index, gastric volume, and free acidity compared to control. The extract showed significant inhibition of the gastric lesions induced by pylorus ligation-induced ulcer and ethanol-induced gastric ulcer. The *Couroupita guianensis.* Ethanolic leaf extract has the anti-ulcer property that is attributed to the anti-secretory activity [16].

2. Material and methods

2.1 Chemicals

α−naphthol, Conc.H_2SO_4, Fehling's A and B, Benedict's reagent, Selwinoff'sreagent, Ferric chloride solution, Potassium dichromate, Iodine, 10%NaoH, Dil. HCl, Mayer's reagent, Dragendroff's reagent, Nitric acid, Biuret reagent. Bovine albumin serum, Phosphate buffer[6.8 P], α−glucosidase, PNPG[4−Nitrophenyl α−D−Glucopyranoside], Phosphate buffer [6.8 P_H], Acarbose.0.1M Silver nitrate, Distilled water.

2.2 Collection and Authentication

Couroupita guianensis leaf was collected from nearby places of Tirupati, Chittoor district, Andhra Pradesh. The plant material was identified and authenticated by K. Madhava Chetty Ph.D, Asst. Prof. of Department of Botany, Sri Venkateswara University, Tirupati, India. The authentication no: 0794. The plant material was dried in the shade, coarsely powdered through a mixer grinder, and used for the extraction.

Fig: 1 Leaves of Couroupita guianensis

Fig: 2 Whole Plant of Couroupita guianensis

2.3 Preparation of extract

The plant material was collected, washed, and dried under shade. After complete drying, the plant material was powdered in a mixer to obtain a coarse powder and then passed through a sieve no.60 and stored in anair-tight container. The dried powdered is used for extraction, and a weighed quantity of an air-dried powdered drug of *Couroupita guianensis is* taken and kept for maceration respectively by methanol extraction. The extract was evaporated to dryness in a rotary flash evaporator at a temperature of 45°c not exceeding 60°c. A phytochemical test was carried out following standard procedure.

2.4 Phytochemical analysis:

Chemical tests were carried out on the plant extract using standard procedures to identify the constituent molecules as described by sofowora and co-workers. Hydro-alcoholic extract of *Couroupita guianensis* leaves are prepared [MECGL]. Then phytochemical analysis was carried out on plant extract.

Detection of Carbohydrates

1. Molisch's test: To 2ml of the methanolic extract, add 1ml of α-naphthol solution and it is concentrated with sulphuric acid through the sides of the test tube. Appearances of purple or reddish violet color at the junction between the two liquids confirm the presence of carbohydrates.
2. Fehling's test: To 1ml of the methanolic extract, an equal quantity of Fehling's solution A and B were added. Upon heating, a brick red precipitate confirms the presence of carbohydrates.
3. Benedict's test: To 5 ml of the benedict's reagent, add 1ml of the methanolic extract solution and boil it for 2 min, and then cool it. The formation of red precipitate shows the presence of carbohydrates.

DETECTION OF TANNINS AND PHENOLS:

To 1 ml of the methanolic extract, add ferric chloride solution. The formation of a dark blue or greenish black color product shows the presence of tannins. To the extract, add potassium dichromate solution. The formation of a precipitate shows the presence of tannins and phenolics.

DETECTION OF ALKALOIDS:

1. Dragendroff's test: To 1 ml of methanolic extract, 1ml Dragendroff's reagent is added. An orange-red precipitate indicates that alkaloids are present in the extract.
2. Wagner's test: To 1 ml of methanolic extract, 2 ml of Wagner's reagent was added. The appearance of a reddish-brown precipitate specifies the presence of alkaloids.
3. Hagger's test: To 1 ml of Methanolic extract, add 3 ml of Hager's reagent. The appearance of a yellow precipitate indicates the presence of alkaloids.
4. Mayer's test: To 1 ml of the ethanolic extract, add 2 ml of Mayer's reagent. A dull white or creamy precipitate specifies the presence of alkaloids.

Detection of proteins and amino acids:

1. Biuret test: To 1 ml of the extract, add 1 ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulfate solution. The formation of violet color indicates the presence of proteins.
2. Xanthoproteic test: To 1 ml of the extract adds 1 ml of concentrated nitric acid. A white precipitate is formed, and it is boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. The orangecolor indicates the presence of proteins.
3. Lead acetate test: To 1 ml of the extract, 1 ml of lead acetate solution is added. The formation of dull white precipitate indicates the presence of proteins.
4. Ninhydrin test: Add 2 drops of freshly prepared 0.2% of ninhydrin reagent to the extract solution and heat. The development of blue color indicates the presence of proteins.

Detection of Flavonoids:

1. Shinoda's test: To 1 ml of the extract, add magnesium turnings and 1-2 drops of concentrated hydrochloric acid. The formation of red colorindicates the presence of flavonoids.

Detection of triterpenoids:

Dissolve two or three granulesin lead metal in 2 ml thionyl chloride solution. Then add 1 ml of the extract into the test tube. The formation of pink color indicates the presence of triterpenoids.

Detection of saponins:

About 1 ml of extract is diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. A 1 cm layer of foam indicates the presence of saponins.

Detection of steroids:

1. Liebermann Burchard test: Dissolve the extract in 2 ml of chloroform in a dry test tube. Add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue, and finally bluish green indicating the presence of steroids.
2. Salkowski test: Dissolve the extract in chloroform and an equal volume of concentrated sulphuric acid. The formation of bluish red to cherry red color in the chloroform layer and green fluorescence in the acid layer represents the steroid components in the tested extract.
3. Liebermann's reaction: Mix 3 ml of an extract with 3 ml acetic anhydride, heat and cool, and a few drops of concentrated sulphuric acid, blue color appears.

DETECTION OF FIXED OILS:

1. Spot test: Press a small quantity of extract between two filter papers. Oil strains on paper indicate the presence of fixed oils.
2. Saponification test: To 1 ml of the extract, add a few drops of 0.5N alcoholic potassium hydroxide along with a drop of phenolphthalein. Heat the mixture in a water bath for 1-2 hours. The formation of soap or partial neutralization indicates the presence of fixed oils.

Detection of glycosides:

1. Legal test: Dissolve the extract in pyridine and sodium nitroprusside solution to make it alkaline. The formation of pink red to red color shows the presence of glycosides.
2. Bal-jet's test: To 1 ml of the test extract, add 1 ml sodium picrate solution and the transformation of yellow to orange color reveals the presence of glycosides.
3. Bontrager's test: To 1 ml of extract solution, add a few ml of dilute sulphuric acid. Boil, filter, and extract the filtrate with chloroform. The chloroform layer is treated with 1 ml of ammonia. The formation of red color shows the presence of anthraquinone glycosides.
4. Keller Killian test: Dissolve the extract on acetic acid containing traces of ferric chloride and transfer to a test tube containing sulphuric acid. At the junction, the formation of a reddish-brown color, which gradually becomes blue, confirms the presence of glycosides.

2.5 Total phenol content

The total phenolic content of *Couroupita guianensis* leaf extract was analyzed using the folin-ciocalteu colorimetric method described by Biju john et.al.[2014]. About 0.02ml of 2 mg/ml extract solution was mixed with 0.2 ml folin-ciocalteu reagent and 2 ml of distilled water. After 3 minutes, 1 ml of sodium carbonate was added. The mixture was re-incubated for 20 minutes at room temperature. Then, the absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was calculated from the gallic acid standard curve. A stock gallic acid standard. A solution of 1 mg/ml was prepared by dissolving gallic acid in distilled water. The result was expressed as 1 mg gallic acid equivalent per gram of extract.

3. In-Vitro Methods

3.1 Silver nanoparticles of *Couroupita guianensis*:

1. Weigh 5gms of *Couroupita guianensis* leaf powder and were added 150ml of distilled water to the powder and take to a beaker.
2. Boil the powder for 1 hour 75^0c. Then cool the solvent and macerate the solvent overnight. Then powder was filtered with Whatman filter paper. From that filtrate take 2ml and add 3ml of distilled water and add 10ml of silver nitrate 0.02 mM solution.
3. Observe the solution can change its color in to dark brown. Then check out the absorbance with UV-VIS and FTIR analysis.

3.2 DPPH free radical scavenging assay:

The free radical scavenging activity of the methanolic solvent extract was measured by using DPPH with the modified method of Mc Cune and Johns [26]. The reaction mixture [3.0 ml] consisting of 1.0 ml DPPH (0.3 Mm), 1.0 ml extract (different concentrations), and 1.0 ml methanol, was incubated for 10 minutes in a dark place, after which the absorbance was measured at 517nm. Ascorbic acid was used as a positive control. The percentage of inhibition was calculated using the following formula:

$\%Inhibition = [1-(A/B)] \times 100$

Where, B is the absorbance of the blank (DPPH plus methanol) and A is the absorbance of the sample (DPPH, methanol, plus sample).

3.3. α-glucosidase inhibition assay:

Plant extracts were evaluated for α-glucosidase inhibitory activity according to the method given by Unuofin JO et al,(2018)with slight modifications. Plant extracts (50µL) at varying concentrations (50 to500µg/mL) were incubated with 10µL of the α-glucosidase (maltase) ex. Yeast (Sigma Research Laboratories Pvt. Ltd.) enzyme solution (1 U/mL) for 20 min at 37 °C with an additional 125µL of 0.1 M phosphate buffer (pH 6.8). After 20 min, the reaction was started with the addition of 20µL of 1 M pNPG (substrate), and the mixture was incubated for 30 min. The reaction was terminated with the addition of 0.1 N of Na_2CO_3 (50µL) and final absorbance was measured at 405 nm using a spectrophotometer. Acarbose was used as a positive control at varying concentrations (100 to 1000µg/mL). Enzyme activity was calculated as:

$(OD_{BLANK}-OD_{SAMPLE})/OD_{BLANK}$ x 100IC50 (concentration required to inhibit 50% of the enzyme activity) was calculated using a regression equation obtained through plotting concentration in the range from 100 to 1000µg/mL (x-axis) and %inhibition (y-axis) for different extracts and fractions.

3. Results and Discussion

Table: 1 Phytochemicalscreening

S.NO	CHEMICAL TEST	OBSERAVATION	INFERENCE
TEST FOR CARBOHYDRATES			
1	Molish's test	+	Carbohydrates are present
2	Fehling's test	-	Carbohydrates are absent
3	Benedicts test	-	Carbohydrates are absent
4	Selwinoff's test	+	Carbohydrates are present
TEST FOR PHENOLS			
1	Ferric chloride's test	+	Phenols are present
2	Potassium dichromate test	+	Phenols are present
3	Iodine test	-	Phenols are absent
TEST FOR ALKALOIDS			
1	Mayer's test	-	Alkaloids are absent
2	Iodine test	+	Alkaloid are present
3	Dragendroff's test	+	Alkaloid are present
TEST FOR PROTEINS AND AMINO ACIDS			
1	Xanthoproteic test	-	Proteins and amino acids are absent
2	Biuret test	-	Proteins and amino acids are absent
TEST FOR TANNINS			
1	10%NaoH	-	Tannins are absent
TEST FOR FLAVANOIDS			
1	Alkaline test	+	Flavanoids are present
TEST FOR SAPONINS			
1	Foam test	+	Saponins are present

4.2 Total Phenolic content

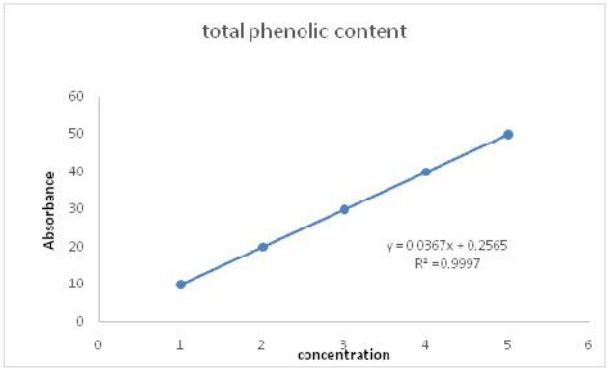

Fig: 3 Calibration curve of total phenolic content showing linearity over the concentration range of 10-50 µg/ml.

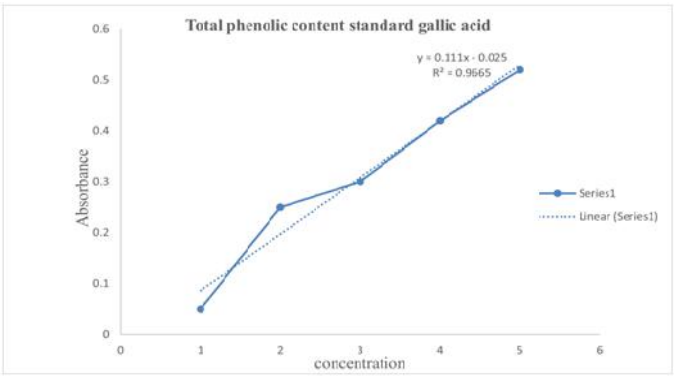

Fig:4 Calibration curve of total phenolic content showing linearity over concentration range of 10-50 µg/ml

4.3 Silver nanoparticles of *Couroupita guianensis*

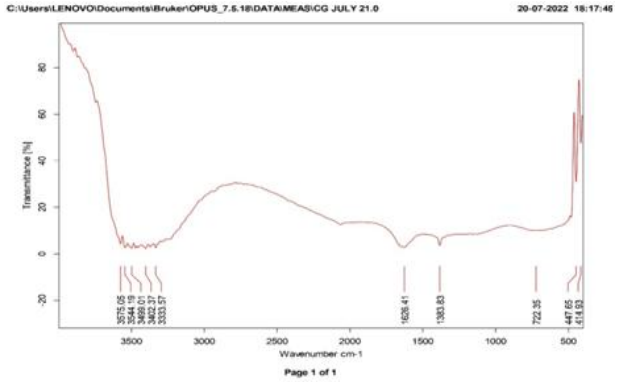

Fig: 5 The Silver Nano Particles FTIR Result of the Methanolic Extract of Couroupita guianensis

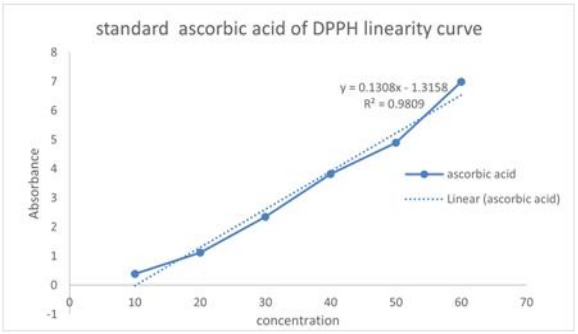

Fig:6 Calibration curve of total standard ascorbic acid showing linearity overconcentration range of 10-50 µg/ml

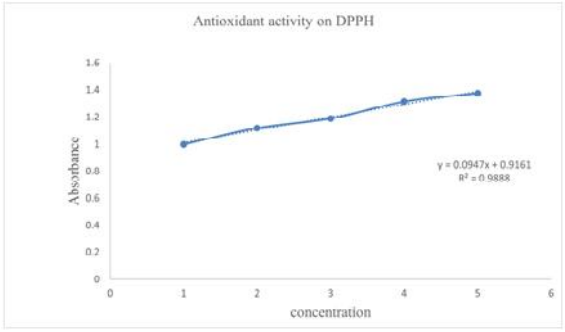

Fig: 7 Calibration curve of Antioxidant activity on DPPH content showing linearity over concentration range of 10-50 µg/ml

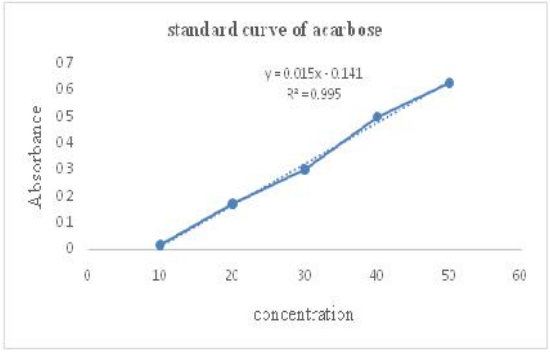

Fig: 8 Calibration curve of acarbose and alpha-glucosidase showing linearity over concentration range from 10-40µg/ml

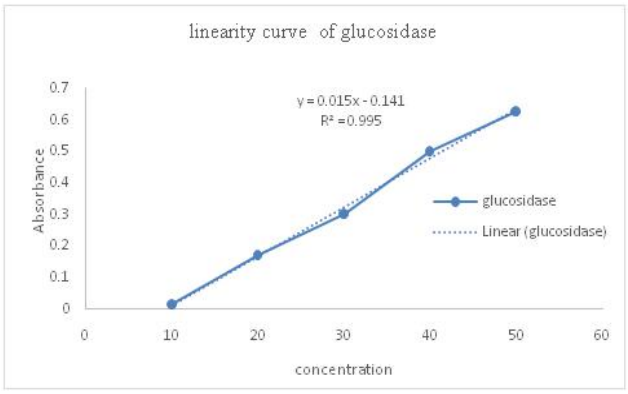

Fig: 9 Calibration curve of acarbose and alpha-glucosidase showing linearity over concentration range from 10-40µg/ml

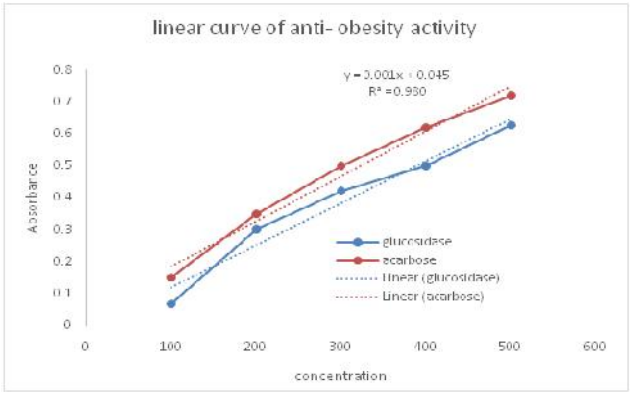

Fig:10 Calibration curve of anti-obesity activityshowing linearity over concentration range from 10- 40µg/ml.

Discussion

The previous studies conformed that the plant Couroupita guianensis having the rich sources of the chemical constituents are the phenols, flavonoids, carbohydrates, proteins, tannins are present and the absence of the steroids and alkaloids. These are the chemical constituents identified by the performing the identification tests. The pharmacological activities are present due to having the chemical constituents are the phenols and flavonoids. In the traditional plants the phenolic content increases with the increase of absorbance of the sample and is equivalent to the standard absorbance of gallic acid absorbance.

4. Conclusion

The present study reveals the methanolic extract of Couroupita guianensis leaves shows the potent activity in decrease the lipid profile and also decreases the blood glucose levels in the body. The phenols are mainly responsible for the Anti-Obesity activity of the plant Couroupita guianensis.

5. Acknowledgments

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