

EVALUATION OF ANTIMICROBIAL EFFICIENCY AND ALPHA-GLUCOSIDASE INHIBITION OF *RUBUS ELLIPTICUS* SMITH. LEAF EXTRACTS AND ITS PHYTOCHEMICAL ANALYSIS

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ABSTRACT

Objective: The aim of this study was to investigate phytochemicals, antimicrobial and α -glucosidase inhibition effects of *Rubus ellipticus*.

Methods: Various solvent extracts were investigated for its phytochemical analysis. Total phenol content (TPC) and total flavonoid content (TFC) were determined by using standard methods. The extracts of *R. ellipticus* were tested for antimicrobial activity and *in vitro* α -glucosidase inhibition effect.

Results: The phytochemical tests revealed the presence of phytoconstituents significantly in methanol extract with high TPC and TFC. Methanol extract showed significant antibacterial activity against Gram-positive and negative strains, as well as a strong antifungal activity. The maximum inhibition zone is found against *Staphylococcus aureus*, *Micrococcus luteus* and methicillin-resistant *S. aureus* (MRSA) (22, 17 and 18 mm) respectively. Against fungus the inhibition zone ranged between 19 mm and 23 mm. Methanolic extract showed minimum inhibitory concentration value with 32.5 μ g/ml against *S. aureus*, 62.55 μ g/ml against MRSA, *Aspergillus flavus*, *Trichophyton mentagrophytes*, *Trichophyton rubrum* 15 μ g/ml against *Aspergillus niger*. The methanol extracts and standard acarbose showed significant α -glucosidase inhibition effect with 86.14% and 92.46%.

Conclusions: Correlation achieved by antimicrobial activity and α -glucosidase inhibition of *R. ellipticus* and its chemical composition insinuates that the activities may be easily ascribed to phenolic compound and flavonoids present in the high percentage in the leaf. Thus, our findings could provide a basis of future studies on *R. ellipticus* leaves used in food and pharmaceutical applications.

Keywords: *Rubus ellipticus*, Phytochemical, Phenolic and flavonoid content, antimicrobial activity, α -glucosidase inhibition.

INTRODUCTION

The herbal healthcare system is the catchword of 21st century. For all the side-effects of allopathic drugs and successive accumulation in the biological system, drugs obtained from plant sources are the best possible alternatives. Herbs have been healing the people from time immemorial. Microbes cause serious infections in humans as well as animals. Currently, different sources of natural antibiotics have been used for several infectious diseases, generally bacterial and fungal. Although hundreds of plant species have been tested for antimicrobial properties, a huge mass have not been sufficiently evaluated [1]. The development of safe, effective and inexpensive antimicrobial drugs is among top global priorities in drug development, as many bacterial and fungal diseases are not yet curable. Antimicrobial compounds are a group of chemical compounds, which are either destroy or suppress the growth and metabolism of a variety of microorganisms. Some antimicrobial agents from plant source are effective in controlling infectious diseases in plants, animals and humans. The wrong and excessive dose of antibiotics is a serious problem in antimicrobial chemotherapy, which causes resistance and ineffective antimicrobial treatment [2]. Appearance of undesirable side-effects, as well as the development of drug resistance of certain antibiotics, has led to the search of novel antibacterial agents in particular from medicinal plants [3]. Phytochemical analysis of medicinal plants has proved that abundant compounds in plants traditionally used for medicinal purposes have several therapeutic properties. Hence, medicinal plants are widely recognized as sources of active antimicrobial metabolites.

According to the World Health Organization estimation, almost 3 million deaths are occurring annually as a result of diabetes, and there will be 366 million cases of diabetes by the year 2030 [4]. As a major endocrine disorder, diabetes mellitus is affecting approximately 5% of the world's population. Diabetes mellitus is characterized by hyperglycemia arising as a result of a deficiency of insulin secretion, resistance to insulin action or both [5]. Although the synthetic antidiabetic agents such as biguanide,

sulfonylureas, thiazolidinedione, and α -glycosidase inhibitors are currently used to control the hyperlipidemia and hyperglycemia, these drugs fail to appreciably modify the course of diabetic complications and have restricted use because of undesirable side-effects and high percentage of secondary failure [6]. Hence, it is indispensable to search for more effective anti-diabetic agents with fewer side-effects. Ethno medicinal plants with anti-diabetic properties can be a useful source for the development of effective and safer oral hypoglycemic agents. An effective means of lowering the levels of postprandial hyperglycemia have been offered by α -amylase and α -glucosidase inhibitors [7].

The genus *Rubus* includes over 750 species in 12 subgenera, and is found on all continents except Antarctica [8]. *Rubus ellipticus* is a stout weakly climbing evergreen shrubs belongs to *Rosaceae* family, grows abundantly at high altitudes. The plant is known as yellow raspberry native to tropical and subtropical India. *Rubus* species are traditionally used to cure diabetes mellitus, ulcers and inflammatory disorders. In the folk medicine, it used for antimicrobial, gastralgia, antifertility, analgesic, wound healing, dysentery, antiepileptic [9]. The phytochemical research based on ethno-pharmacological information is usually considered as a successful approach in the discovery of new anti-infective agents from higher plants [10]. The young shoot is chewed raw to relieve sudden stomach pain. The root juice drunk against urinary tract infection and its fruits are edible and were listed in the top 10 wild edible, medicinal plants in Tanahun district of Western Nepal [11].

In Nilgiris, India, the raw fruits are used to cure sore throat and mouth ulcers by Kattunayaks. Kurumbas use root to cure stroke and as a pain reliever. The leaf bud juice is used in the treatment of peptic ulcers. In view of the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial, antifungal and anti-diabetic agents, an *in vitro* investigation was carried out to screen the different solvent leaf extracts of *R. ellipticus* for its phytochemicals, antibacterial, antifungal activity and α -glycosidase inhibition.

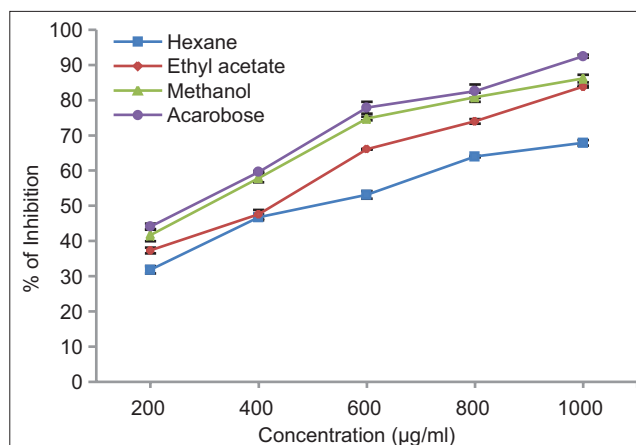


Fig. 1: α -Glucosidase inhibition effect of different concentrations (200-1000 $\mu\text{g/ml}$) of *Rubus ellipticus* hexane, ethyl acetate, methanol extracts and standard acarbose. Each value represents the mean \pm standard error mean of triplicate experiments

METHODS

Chemicals and reagents

All solvents and chemicals used were of analytical grade and were procured from Merck (Mumbai, India).

Plant procurement and extraction

The plant leaves were collected near Doddabedda, Udahamandalam, Nilgiri District of Tamil Nadu, India. It approximately lie between $11^{\circ}28'0''$ N longitudes and $76^{\circ}41'0''$ E latitude. Identification of the plants was done by Dr. G. Jayajothi Taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai. The voucher specimen is preserved in the herbarium of the department (LCH-305). The plant parts were shade dried and powdered. The powder (1 kg) was extracted three times by cold percolation method with 3 L of hexane, ethyl acetate and methanol respectively at room temperature for 72 hrs. The filtrates were concentrated under reduced pressure at 40°C and stored in a refrigerator at $2-8^{\circ}\text{C}$ for use in subsequent experiments. Thereafter extract was dried using a rotary evaporator and dried extract was put to the process of standardization.

Preliminary phytochemical analysis

The preliminary phytochemical studies were carried out with the hexane, ethyl acetate and methanol extracts using standard methods with some modifications [12-14]. The plant extracts were assayed for the presence of alkaloids, tannins, phenolic compounds, quinone, saponins, flavonoids, flavones, glycosides, carbohydrates, terpenes, triterpenes and proteins.

Determination of total phenolic content (TPC) by spectrophotometric method

Total phenolic concentration in different extract was measured by Folin-Ciocalteu assay described by Siddhuraju and Becker [15] with a few modifications. Briefly, with 0.1 ml of extracts (200-1000 $\mu\text{g/ml}$), 1.9 ml distilled water and 1 ml of Folin-Ciocalteu's reagent was added in a tube, and then 1 ml of 100 g/l Na_2CO_3 was added to neutralized the reaction. The reaction mixture was incubated at 25°C for 2 hrs and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate. Quantification was done on the basis of a standard curve prepared out of catechol. TPC was standardized against catechol and expressed as mg catechol equivalents per gram of extract. Data are reported as means \pm standard deviation (SD) for at least three different experiments.

Determination of total flavonoid content (TFC)

The TFC was determined by the method described by Zhishen et al. [16]. A 0.5 ml of different solvent extract of leaf was mixed with 0.15 ml of 5%

NaNO_2 solution and 2 ml of distilled water. After 6 minutes, 0.15 ml of 10% AlCl_3 solution was added and allowed to stand for 5 minutes and then 2 ml of 4% NaOH solution was added to the mixture. Immediately the water was added to bring the final volume to 5 ml and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510 nm versus water blank. Quercetin was used as a reference for the calibration curve. Results of TFC were expressed as mg quercetin equivalents. Data are reported as means \pm SD for at least three different experiments.

Screening solvent extract for antibacterial activity

Media used

Muller-Hinton agar (MHA) and broth (Hi-media, Mumbai, India), sabouraud dextrose agar pH 7.3 ± 0.2 (Hi-media), were used for antibacterial and antifungal activity, respectively.

Test microorganism

The hexane, ethyl acetate, and methanol leaf extracts of *Passiflora mollissima* were screened for antibacterial activity against, a total of thirteen human pathogenic bacterial strains and six fungal strains. Gram-positive bacteria such as *Staphylococcus aureus* (ATCC 96), *Micrococcus luteus* (MTCC 106), *Bacillus subtilis* (MTCC441), *Enterococcus faecalis* (ATCC29212), *Staphylococcus epidermidis* (MTCC3615), methicillin-resistant *S. aureus* (MRSA). Gram-negative bacteria such as *Klebsiella pneumoniae* (MTCC109), *Enterobacter aerogenes* (MTCC111), *Vibrio parahaemolyticus* (MTCC 451), *Yersinia enterocolitica* (MTCC 840), *Salmonella typhimurium* (MTCC 1251), *Shigella flexneri* (MTCC 1457) and *Proteus vulgaris* (MTCC1771). Fungi such as *Aspergillus flavus* (H-08), *Trichophyton mentagrophytes* (TM-11), *Trichophyton rubrum* (TR-2), *Scopulariopsis* sp. (101/01), *Aspergillus niger* (MTCC1344) and *Curvularia lunata* (46/01). All cultures were obtained from American Type Culture Collection (ATCC), MTCC, clinical strain preserved at Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. The fresh bacterial broth cultures were prepared before the screening procedure.

Determination of inhibitory effect of *R. ellipticus*

The antimicrobial activity of *R. ellipticus* leaf extract was determined by disc diffusion method according to Durairamian and Ignacimuthu [17]. 20 ml of MHA sterile medium for bacteria and 20 ml of Sabouraud dextrose agar for fungi were poured into each petridish. Approximately 200 μL of suspension (2×10^8 cfu/mL) of the test microorganisms was smeared onto the agar media after it had solidified and allowed to dry for 10 minutes. 6 mm sterile disc were loaded by different concentrations of crude extracts (5, 2.5 and 1.25 mg/disc). The impregnated discs were kept on the surface of the medium and left for 5 minutes at room temperature for compound diffusion. The plates were kept for incubation overnight at 37°C for 24 hrs for bacteria and at for 72 hrs at 27°C for fungi. After incubation, the diameters (mm) of the inhibition zone were measured by using zone-scale from Himedia. Reference antibiotic streptomycin (10 $\mu\text{g/disc}$) for bacteria and fluconazole (30 $\mu\text{g/disc}$) for fungi were used as positive controls. The same procedure was performed in triplicates.

Determination of the minimum inhibitory concentration (MIC) assay using Broth micro dilution method

MIC was defined as the complete growth inhibition at low concentration of the plant extract. The MIC for the crude extract against bacteria and fungi was performed using the method by Saravana Kumar et al. [18]. The extracts were dissolved in water with 2% dimethyl sulfoxide. MHB was prepared and sterilized. The required concentrations of the extract (1000 $\mu\text{g/mL}$, 500 $\mu\text{g/mL}$, 250 $\mu\text{g/mL}$, 125 $\mu\text{g/mL}$, 62.5 $\mu\text{g/mL}$, 31.25 $\mu\text{g/mL}$, and 15.625 $\mu\text{g/mL}$) were added to the 96 well microtiter plate containing 0.1 ml broth. An amount of 3 μL of log phase culture was introduced into to the respective well, and final inoculum size was 1×10^5 cfu/mL. The plates were incubated at 37°C for 18 hrs negative and positive controls were also included. Fluconazole for fungi and streptomycin for bacteria were served as positive controls. To observe

the viability of the organism, an amount of 5 µl of test broth was introduced on plain MHA plates. All the assays were done in triplicates.

α-Glucosidase inhibition of *R. ellipticus*

The mode of α-glucosidase inhibition by the *R. ellipticus* leaf extract was determined by the lowest IC₅₀ according to the modified method described by Dahlqvist [19]. An *in vitro* α-glucosidase inhibition assay was performed to investigate the inhibition activity of hexane, ethyl acetate and methanol extracts. After 20 hrs fasting, the part between duodenum and the cecum in the small intestine of mouse was cut, rinsed with ice cold saline, and homogenized with 12 ml of maleate buffer (100 mM, pH 6.0). The homogenate was used as the α-glucosidase enzyme solution. The assay mixture consisted of 100 mM maleate buffer (pH 6.0), 2% (w/v) each sugar substrate solution (100µl), and the plant sample extract (200-1000 µg/mL). It was preincubated for 5 minutes at 37°C, and the reaction was initiated by adding the crude α-glucosidase solution (50 µl) to it, followed by incubation for 10 minutes at 37°C. The glucose released in the reaction mixture was determined with the kit described above. The rate of the carbohydrate decomposition was calculated as the percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of prevention was calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{[\text{Amount of glucose produced by the positive control}] - [\text{Amount of glucose produced by the addition of sample}]}{[\text{amount of glucose produced by the positive control}]} \times 100.$$

Statistical analysis

Analysis was performed using Microsoft Excel 2007. The one-way ANOVA test was used to determine statistically significant difference in the MIC of the extracts and the antibiotics. $p < 0.05$ were considered significant.

RESULT AND DISCUSSION

Preliminary phytochemical screening

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Our study shows the presence of bioactive compounds alkaloids, tannins, phenols, saponins, flavonoids, flavones, glycosids, carbohydrates, terpenes, triterpenes and proteins significantly present in methanol and moderately in ethylacetate extracts of *R. ellipticus* leaf and the results were given in Table 1. Quinones was completely absent whereas carbohydrates and proteins were absent in hexane and ethyl acetate extract. Different phytochemicals have been found to possess a wide range of activities, which may help in the protection against chronic diseases. The screening of plant extracts has been of great interest to scientist for the discovery of new drugs effective in the treatment of several diseases [20]. *Rubus* species is well known for its pharmacological properties.

Determination of TPC and TFC

Determination the TPC and TFC of the leaves was studied by spectroscopic method and the results are shown in Table 2. The present

Table 1: Qualitative phytochemical analysis of *Rubus ellipticus* leaf

Phytochemicals	Hexane	Ethylacetate	Methanol
Alkaloids	+	+++	++
Tannin	-	++	+++
Phenols	-	++	++
Quinones	-	-	-
Saponins	-	+	+++
Flavonoids	+	++	+++
Flavones	-	+	++
Glycosides	++	++	++
Carbohydrates	-	-	+
Terpenes	+	++	+++
Triterpenes	+	++	++
Proteins	+	-	+

+++ : High concentration, ++ : Moderate concentration, + : Low concentration, - : Absent

study showed that the leaf extract exhibited a significant amount of phenols and flavonoids. The TPC of hexane, ethyl acetate and methanol extracts of *R. ellipticus* was found to be 102.48±1.09, 147.23±0.52 and 276.51±0.28 mg catechol equivalent/gram extract, respectively. The TPC of hexane, ethyl acetate and methanol extracts was found to be 121.46±0.59, 167.07±1.18 and 283.51±1.34 mg catechin equivalent/gram extract, respectively. The plant methanolic extract exhibited a significant amount of TPC and TFC among three solvent extracts (hexane, ethyl acetate and methanol) whereas flavonoid is more than TPC. The phenolic acids and flavonoids present in the plants are natural antioxidants and also have proved to antimicrobial activity [21]. Flavonoids, the major group of phenolic compounds, are reported for their antimicrobial and antiviral activity. They are important for prevention of diseases associated with oxidative damage of the membrane, proteins and DNA.

Antimicrobial assay of various solvent extracts of *R. ellipticus* by disc diffusion method

Our results clearly exhibit the variable zone of inhibition against all the bacterial strains as well as fungal pathogens tested in a dose-dependent manner (Table 3). Among the three extracts attempted, methanolic extract of *R. ellipticus* showed significant inhibitory zone whereas ethyl acetate was moderate, and hexane extract showed lesser activity against all the tested organisms. Further, all the bacteria were found to be more susceptible to methanolic leaf extract. Methanol and ethyl acetate extract showed the maximum zone of inhibition produced against the bacteria *S. aureus* (16, 20 and 22 mm) and (14, 17 and 19 mm) followed by *M. luteus* (12, 15 and 17 mm) and (12, 15 and 16 mm), *B. subtilis* (10, 11 and 12 mm) and (10, 11 and 12 mm), *E. faecalis* (9, 10 and 11 mm) and *Staphylococcus epidermis* (9, 10 and 11 mm) and (10, 11 and 13 mm) and MRSA (14, 16 and 18) and (12, 14 and 15 mm), *K. pneumoniae* (8, 10 and 11 mm), *E. aerogenes* (10, 11 and 12 mm), *V. parahaemolyticus* (11, 12 and 14 mm) and (11, 12 and 13 mm), *Y. enterocolitica* (12, 15 and 17 mm) and (10, 11 and 13), *S. typhimurium* (11, 13 and 14 mm), *S. flexneri* (13, 14 and 20 mm) and (10, 12 and 16 mm), *P. vulgaris* (12, 13 and 15 mm) and (13, 15 and 16), *A. flavus* (19, 21 and 22 mm) and (12, 13 and 14 mm), *T. mentagrophytes* (19, 20 and 21 mm), *T. rubrum* (17, 18 and 19 mm) and (19, 20 and 21 mm), *A. niger* (21, 22 and 23 mm) and (19, 20 and 21 mm), *Scopulariopsis* sp. (21, 22 and 23 mm) and (19, 20, 21 mm) at concentrations 1.25, 2.50 and 5 mg/mL respectively. On the other hand, none of the extract gave inhibitory effect against the fungus *C. lunata*. Hexane extract never exhibited activity against *E. faecalis*, *A. flavus* and *T. mentagrophytes*. Similarly, ethyl acetate never showed activity against *E. faecalis*, *E. aerogenes*, and *T. mentagrophytes*. The investigated phytochemicals of *R. ellipticus* are known to have activity against several pathogens and, therefore, could suggest their traditional use for the treatment of various illness [22,23].

MIC value of different extracts against bacteria and fungi

The MIC of the extracts was defined as the lowest concentration of plant extract that caused growth inhibition of more than 90% at 48 hrs, when compared to the control. The MIC value of hexane, ethyl acetate and methanol extract against *S. aureus* was 250, 250 and 31.25 µg/mL, followed by *M. luteus* 500, 250 and 500 µg/mL, *S. epidermis* 500 µg/mL, MRSA, 250, 250 and 62.5 µg/mL, *V. parahaemolyticus* 500 µg/mL, *S. typhimurium* 500 µg/mL, *S. flexneri* 500, 1000 and 250 µg/mL, *P. vulgaris* 500 µg/mL respectively. Methanol extract gave significant MIC value than other extracts against *S. aureus* and MRSA whereas ethylacetate exhibited moderate value. Among fungi, methanol extract significantly exhibited MIC value of 15 µg/mL

Table 2: TPC and TFC of different solvent leaf extracts of *Rubus ellipticus*

Phytochemicals	Hexane	Ethyl acetate	Methanol
TPC (CE/g) ^a	102.48±1.09	147.23±0.52	276.51±0.28
TFC (CE/g) ^b	121.46±0.59	167.07±1.18	283.51±1.34

^amg CE/100 g of dry mass, C - catechol, ^bmg CE/100 g of dry mass, C - catechin. TPC: Total phenolic content, TFC: Total flavonoid content

against *A. niger*, 62.5 µg/mL against *A. flavus*, *T. mentagrophytes* and *T. rubrum* whereas a moderate level of activity was shown by ethyl acetate extract with MIC value of 31.25 µg/mL against *T. rubrum* and *A. niger*, 62.5 µg/mL against *Scopulariopsis* sp., 500 µg/mL against *A. flavus*. Hexane showed less MIC value, as shown in Table 4. Thus *in vitro* antimicrobial test results revealed methanolic extract of *R. ellipticus* were found to exhibit significant antibacterial as well as pronounced antifungal property. The plant showed highest TPC and TFC which may have contributed for its antibacterial activity. The presence of antimicrobial substances in higher plants is thus well established. Saklani et al. [24] reported that the ethanolic extract of *R. ellipticus* fruit showed the presence of glycoside, flavonoids, phenols, resin and tannin. Besides the fruit extract exhibited significant antibacterial activity against *Escherichia coli* (MTCC 729 and MTCC 443) and *Streptococcus pyogenes*.

α-Glucosidase inhibition efficacy of *R. ellipticus*

The *in vitro* α-glucosidase inhibitory results exhibited that all the extracts of *R. ellipticus* leaf possess inhibitory activities. Fig. 1 elucidates the results of α-glucosidase inhibition assay of different solvent extracts of *R. ellipticus* and standard acarbose. The concentration for 50% inhibition of hexane, ethyl acetate, methanol extracts and acarbose were found to be 520.53±1.47, 431.21±1.23, 319.55±1.98 and 289.33±2.34 µg/mL respectively Fig. 1. The hexane extract showed less inhibition compared to methanol and ethyl acetate extracts. However, methanol extract exhibited the strongest inhibition of the enzyme than the ethyl acetate. Methanol extract of *R. ellipticus* effectively reduced the glucose level during α-glucosidase inhibition assay. Most of the studies reported that methanol extracts of several medicinal plants are having higher antibacterial activities than that of any other alcoholic solvents [25,26]. The activity was mainly due to the presence of phenolics, flavonoid,

Table 3: Antimicrobial activity of leaf extracts of *Rubus ellipticus* using the disc diffusion method

	Zone of inhibition in mm									Streptomycin
	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	
Gram-positive										
96	14.66±0.57	16.66±0.57	18.66±0.57	14.66±0.57	17.33±0.57	19.33±0.57	16.33±0.57	20.33±0.57	22±1	24.66±2.18
106	11.33±0.57	12.66±1.5	15.66±0.57	12.5±0.5	15.33±0.57	16±1	12.66±0.57	15±1	17.33±0.57	17.66±1.1
441	10.33±0.57	11.66±0.57	12.33±0.57	10±1	11.66±0.33	12±1	10.33±0.33	11.33±0.57	12.33±0.57	13.66±1.15
29212	0	0	0	0	0	0	9.66±0.18	10.33±0.57	10.66±0.33	17.66±1.15
3615	11.66±0.57	11.66±0.57	14.33±0.57	10.33±0.57	11.33±0.28	13±1	9.66±0.57	10.33±0.66	11.33±0.57	13.33±0.57
MRSA	13±1	14.33±0.57	15.33±0.57	12.33±0.57	14.33±0.18	15.6±0.57	14.33±0.57	16.66±0.57	18.33±0.57	26.66±0.57
Gram negative										
109	10.33±0.57	10.66±0.57	11.66±0.57	8.33±0.33	10.33±0.28	11.33±0.57	8.33±0.33	10.33±0.28	11.66±0.66	13.66±0.57
111	10.33±0.58	11.66±0.57	12±1	0	0	0	10.33±0.28	11.66±0.18	12.33±0.57	26.33±2.01
451	12.5±0.57	14.16±0.28	15.36±0.77	11±1	12.33±0.57	13.9±0.85	11.33±0.57	12.66±0.57	14.66±0.66	22.33±0.57
840	11.33±0.57	12.33±0.57	15.33±0.57	10.33±0.66	11.33±0.57	12.33±0.57	12±1	15.33±0.57	17.16±0.76	20±1
1251	11.33±0.57	11.66±0.57	14.33±0.33	11.33±0.57	13.33±0.33	14.33±0.57	11±1	13.33±0.33	14.5±0.5	16.33±0.57
1457	13.16±0.18	14.23±0.68	14.93±0.11	10.33±0.78	12.33±0.66	16.33±0.66	13.26±0.46	14.26±0.46	20±1	20.33±1.5
1771	12.33±0.57	14.16±0.28	17.33±0.57	13.33±0.57	15.66±0.57	16.66±0.57	12.66±0.57	13.33±0.57	15.33±0.577	26.66±1.1
Fungi										Fluconazole
H-08	0	0	0	12.66±0.57	13.16±0.18	14.16±0.28	19.66±0.57	21.33±2.01	22.33±0.57	25.66±1.1
66/02	0	0	0	0	0	0	19.33±0.57	20.33±1.5	21.33±1.5	22.33±0.57
57/01	11±1	12±1	13.05±0.57	19.34±0.66	20.33±1.52	21.33±0.66	17.66±0.66	18.26±0.76	19.33±0.57	20.33±1.5
102	12.66±0.57	13.01±0.57	14.25±0.57	16.16±0.28	17.16±0.76	18.33±0.28	18.26±0.46	19.66±0.66	20.66±0.57	22.33±1.5
1344	11.66±0.68	12.23±0.57	13.33±0.66	19.33±0.58	20.33±0.57	21.66±0.18	21.33±0.57	22.33±0.57	23±1	25.33±0.57
46	0	0	0	0	0	0	0	0	0	20±1

0: No activity, Standard - Streptomycin (10 µg/ml) for bacteria, Fluconazole (30 µg/ml) for fungi. **Gram-positive:** *Staphylococcus aureus* (MTCC 96) *Micrococcus luteus* (MTCC 106), *Bacillus subtilis* (MTCC 441), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus epidermis* (MTCC 3615) and Methicillin resistant *Staphylococcus aureus* (MRSA). **Gram-negative:** *Klebsiella pneumoniae* (MTCC109), *Enterobacter aerogenes* (MTCC111), *Vibrio parahaemolyticus* (MTCC451), *Yersinia enterocolitica* (MTCC 840), *Salmonella typhimurium* (MTCC1251), *Shigella flexneri* (MTCC1457) and *Proteus vulgaris* (MTCC1771). **Fungi:** *Aspergillus flavus* (H-08), *Trichophyton mentagrophytes* (66/01), *Trichophyton rubrum* (57/01), *Aspergillus niger* (MTCC1344), *Scopulariopsis* sp. (102/01) and *Curvularia lunata* (46)

Table 4: MIC of leaf extracts of *Rubus ellipticus*

	MIC (µg/ml)												
	96	106	441	29212	3615	MRSA	109	111	451	840	1251	1457	1771
Hexane	>250	>500	>1000	>1000	>500	>250	>1000	>1000	>500	>500	>500	>500	>500
Ethyl acetate	>250	>250	>500	>1000	>500	>250	>1000	>1000	>500	>1000	>500	>1000	>500
Methanol	>31.25	>500	>1000	>1000	>500	>62.5	>1000	>1000	>500	>500	>500	>250	>500
Streptomycin	>15	>6.25	>125	>15	>6.25	>6.25	>25	>25	>30	>30	>12.5	>62.5	>15
	H-08	66/02	57/01	102	1344	46							
Hexane	>1000	>1000	>500	>500	>500	>1000							
Ethyl acetate	>500	>1000	>31.25	>62.5	>31.25	>1000							
Methanol	>62.5	>62.5	>62.5	>62.5	>15	>1000							
Fluconazole	>15	>15	>30	>30	>15	>30							

0: No activity, Standard - Streptomycin (10 µg/ml) for bacteria, fluconazole (30 µg/ml) for fungi. **Gram-positive:** *Staphylococcus aureus* (MTCC 96) *Micrococcus luteus* (MTCC 106), *Bacillus subtilis* (MTCC 441), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus epidermis* (MTCC 3615) and methicillin resistant *Staphylococcus aureus* (MRSA). **Gram-negative:** *Klebsiella pneumoniae* (MTCC109), *Enterobacter aerogenes* (MTCC111), *Vibrio parahaemolyticus* (MTCC451), *Yersinia enterocolitica* (MTCC 840), *Salmonella typhimurium* (MTCC1251), *Shigella flexneri* (MTCC1457) and *Proteus vulgaris* (MTCC1771). **Fungi:** *Aspergillus flavus* (H-08), *Trichophyton mentagrophytes* (66/01), *Trichophyton rubrum* (57/01), *Aspergillus niger* (MTCC1344), *Scopulariopsis* sp. (102/01) and *Curvularia lunata* (46), MIC: Minimum inhibitory concentration

tannins, saponin and alkaloid compounds. Agents with α -glucosidase inhibitory activity have been useful as oral hypoglycemic agents to control hyperglycemia in patients with diabetes. George *et al.* [27]. reported that the leaf methanol extract of *R. ellipticus* also has anti-inflammatory, central and peripheral analgesic and antipyretic activity.

CONCLUSION

The results of the present study revealed that the leaf methanol extract of *R. ellipticus* possess significant antibacterial, strong antifungal and pronounced α -glucosidase inhibitory activities. Our result of antimicrobial and α -glucosidase inhibitory activity correlated with George *et al.* [27] reports that confirms the traditional use of *R. ellipticus* to treat various disorders by tribes in Nilgiris, India. Since diabetics, inflammation and cancer are interrelated further investigation will lead to the innovation of anti-diabetic and anti-cancer novel compounds from *R. ellipticus*.

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