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Research Article

EVALUATION OF ANTIBACTERIAL, ANTIMICROBIAL, AND HYPOGLYCEMIC EFFECTS OF THE LEAVES OF *EMBELIA RIBES*

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ABSTRACT

Objective: The purpose of this work is to evaluate the antimicrobial, antibacterial, and hypoglycemic effects of methanolic and ethanolic extracts of *Embelia ribes* leaves using *in vitro* studies.

Methods: Antibacterial activities of the methanolic and ethanolic extract of *E. ribes* leaves against *Escherichia coli*, *Staphylococcus aureus*, *Enterococci*, and *Klebsiella pneumoniae* at different concentrations ranging from 10, 25, 50, and 75 µg/mL and their antibacterial activities were compared to those of the reference controls such as ciprofloxacin and clindamycin. Furthermore, the effect of leaf extracts on α-amylase and α-glucosidase enzymes was assayed.

Results: The methanolic and ethanolic extract of *E. ribes* leaves effectively inhibited the activity of α-amylase and α-glucosidase in a dose-dependent manner. The effect of the methanolic extract was more prominent than that of ethanolic extract. At the same time, both the extracts showed markable inhibition of bacterial growth at a concentration of 75 µg/mL compared to the other three doses (10, 25, and 50 µg/ml) and also commercially available antibiotic drugs ciprofloxacin and clindamycin that were used as positive control drugs. The antibacterial activity of methanolic extract is significantly higher than that of ethanolic extract.

Conclusion: The preliminary results of this study have put forward *E. ribes* into promising herb with respect to its therapeutic potential although further studies are needed to evaluate its mechanism of action.

Keywords: α-amylase, α-glucosidase, *Embelia ribes.*

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INTRODUCTION

In recent years, it has been observed that there has been a shift in universal trend from synthetic to herbal medicines which involve the use of plants for medicinal and therapeutic purpose and also to improve overall human health [1]. It has been well documented that both herbal plants and their derivatives play critical roles in modern drug development, in addition, with the rise of medicinal plants as the natural resources in the development of new drugs [2]. Hence, there is a need in advance research for the development and characterization of new natural drugs with the aid of better screening methods from plants and other natural sources.

Antibiotics are the main basis for the therapy of microbial (bacterial and fungal) infections [3]. However, most of the existing antimicrobial compounds prescribed today are in danger for losing their efficacy because of the increased microbial resistances [4]. In recent times, its impact is considerable with treatment failures associated with multidrug-resistant bacteria, and it has become a global concern to public health [5,6]. Hence, new leads in screening of drugs are being made from natural sources. There are several studies that have reported the antimicrobial and antibacterial activity of different herbal extracts [7,8]. Hence, plants continue to provide a good source for new drugs [9].

Diabetes is a multifactorial disease with diabetic patients more prone to fatal complications such as chronic diabetes microvascular complications, episodes of hypoglycemia, and poor glycemic control. Hence, the use of antidiabetic medications and glucose-lowering drugs may have an impact on metabolism and the risk associated with their use is afflicting many people, from various walks of life in different countries. India presently has the largest number of diabetic patients in the world and has been infamously dubbed as the diabetic capital of the world [10].

Herbal formulations are preferred nowadays due to lesser side effects and low cost, although many drugs are available, they have many side effects [11]. In recent times, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents for the treatment of diabetes [12].

The chief enzymes responsible for metabolizing polysaccharides into monosaccharides are pancreatic α-amylase and α-glucosidase in intestine. The action of these enzymes causes postprandial blood glucose level elevation due to absorption of formed glucose from polysaccharides in the small intestine. Drugs which have an inhibitory action on both of these enzymes possess an ability to control postprandial blood glucose level specifically in type 2 diabetic patients [13]. Acarbose and miglitol are the most commonly prescribed drugs which competitively inhibit above enzymes. However, both of these drugs do have common side effects such as flatulence and abdominal bloating [14,15]. Hence, new drugs or formulations which are devoid of the above side effects will improve the compliance in type 2 diabetic patients. Plants have been an exemplary source of drugs, and it is reported that about 800 plants may possess antidiabetic potential [16]. Medicinal plants exhibit their hypoglycemic activity through their ability to restore the function of pancreatic tissues by causing a rise in insulin output, inhibiting the intestinal absorption of glucose, or facilitating metabolites in insulin-dependent processes [17,18].

Embelia ribes is a large woody shrub with slender branches and gland-dotted leaves. It is distributed in moist deciduous forests of the Western Ghats of South India, Jammu and Kashmir, Himachal Pradesh, Uttar

Pradesh, Assam and Maharashtra, Sri Lanka, Malaya, Singapore, and South China.

The plant has been reported to contain minute quantities of a volatile oil, quinone derivative embelin (3-Undecyl 2, 5-dihydroxy, 1, 4-benzoquinone), quercitol, and fatty ingredients; an alkaloid, christembine, a resinoid, and tannins [19,20]. Other components that have been reported include christembine, quercitol, vilangin, and resinoid. The dried fruits are being used for the preparation of medicine. It is widely used as antihelminthic, anticarminative, antibacterial, anti-inflammatory, antidiuretic, and anti-astringent [21]. Embelin has been also reported as a potent inhibitor of NF-κB activation, which makes it a potentially effective suppressor of tumor cell survival, proliferation, invasion, angiogenesis, and inflammation and has great potential as a therapeutic agent for osteoporosis and cancer-linked bone loss [22,23].

The fruits of *E. ribes* are known to have various pharmacological and therapeutic effects such as antioxidant, hepatoprotective, antihyperglycemic, cardioprotective, neuroprotective, and antitumor effects [24]. With this background, not much work has been carried out on the antimicrobial and antihyperglycemic effect of *E. ribes* leaves. Hence, the present study was designed to evaluate the *in vitro* antimicrobial and hypoglycemic effects of the methanolic and ethanolic leaf extracts of *E. ribes*.

METHODS

Plant material

Leaves of *E. ribes* were procured from the Institute of Trans-Disciplinary Health Sciences and Technology, Bangalore, India. The samples were authenticated by Dr. N.M.Ganesh Babu, Head, Centre for Herbal Gardens, from the same institute.

Extraction procedure

Methanol and aqueous extracts were selected because they have been reported to be among the best solvents for the extraction of antioxidant compounds. 50 g of the dry leaves of *E. ribes* was weighed accurately and soaked in 50 ml of the two solvents (methanol and ethanol) separately and kept in a dark place for 3 d in a shaker. Carbon dioxide was released frequently. After 3 days, samples were filtered and the filtrates were kept in a water bath at about 40°C to concentrate them. The concentrated filtrates obtained were used for further studies at different concentrations.

Alpha-amylase assay

Alpha-amylase activity was carried out as reported by Roux *et al*. [25]. The assay mixture consisting of 120 µl phosphate buffer (40 mM) pH 6.9, along with test sample of various concentrations (dissolved in 1% methanol) and positive control (acarbose) was preincubated with 60 µl of enzyme at 37°C for 10 min. The substrate reagent 250 µl (CNPG3) was added and incubated at 37°C for 8 min. Control tubes were run devoid of test samples. The absorbance was measured at 405 nm using Tecan Microplate Reader.

Alpha-glucosidase assay

Alpha-glucosidase activity was carried out as reported by Matsuo *et al*. [26]. The assay mixture consisting of 250 µl phosphate buffer (40 mM) pH 6.9, along with test sample of various concentrations (dissolved in 1% methanol) and positive control (acarbose) was preincubated with 50 µl of enzyme at 37°C for 30 min. 500 µl of sucrose solution was added and incubated at 37°C for 20 min, heated on a boiling water bath for 2 min to arrest the reaction and then cooled. The glucose concentration was measured by glucose oxidase method.

Glucose oxidase method

100 µl of the sample was mixed with 500 µl of glucose reagent (Glucose reagent kit) and incubated at room temperature for 10 min. The absorbance was measured at 510 nm using semi autoanalyzer (Erba Chem).

Antibacterial studies

The bacterial strains *Staphylococcus aureus* (ATCC 25923), *Enterococcus* species, *Escherichia coli* (ATCC 8739), and *Klebsiella pneumonia* (ATCC BAA 1705) were obtained from the microbiology clinical laboratory which was used for the evaluation of antimicrobial activity. Fresh bacterial cultures were prepared by subculturing stock bacterial cultures into freshly prepared nutrient agar and incubating at 37°C for 24 h. These 24 h old bacterial cultures were transferred into freshly prepared nutrient broth and standardized to 0.5 McFarland turbidity standards using the spectrophotometer to obtain the desired cell density of 1.5×108 (cells/ml). The 0.5 McFarland turbidity standard was prepared by adding 0.05 ml of 1.175 % of barium chloride dihydrate ($BaCl_2 \bullet {}^2H_2O$), with 9.95 ml of 1% sulfuric acid.

Screening for the antimicrobial potential of the plant

The bacteria cultures were grown in nutrient agar medium at 37°C. After 6 h of growth, each microorganism, at a concentration of 106 cells/ml, was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, filter paper discs (6 mm in diameter) saturated either with *E. ribes* extract (25 µl) were placed on surface of each inoculated plate. To evaluate the efficiency of the methodology, each extract was inserted simultaneously in a whole made (50 µl) in new plates. The plates were incubated at 37°C for 24 h. After this period, it was possible to observe inhibition zone. Overall, cultured bacteria with halos equal to or >7 mm were considered susceptible to either the tested extract or phytochemical. Dimethyl sulfoxide (2%) was used to dissolve the extracts in the culture media when necessary. The controls were the solvents used for each extract, and the phytochemicals showed no inhibitions in preliminary studies.

RESULTS AND DISCUSSION

Hypoglycemic activity of the leaf extract of *E. ribes*

Alpha-amylase and alpha-glucosidase are responsible for the hydrolysis of poly- and oligosaccharides into monomers or cleavage of bonds between sugars and non-carbohydrate aglycone. These enzymes are involved in a number of important biological processes such as digestion of carbohydrate into glucose [27]. There is now a great deal of interest in amylase and glucosidase inhibitors because these are important biochemical tools for studying the mechanism of enzymes. The search for amylase and glucosidase inhibitors has yielded a number of chemically distinct inhibitors from plants [28].

Alpha-glucosidase and alpha-amylase concentration determined in ethanolic and methanolic extracts of *E. ribes* leaves at various concentrations and acarbose was used as a standard (Figs. 1 and 2). α-glucosidase activity was significantly inhibited by methanolic extracts whereas compared with ethanolic extracts in a dose-dependent manner (Fig. 1). Whereas α-amylase activity was significantly inhibited by methanolic extracts whereas compared with ethanolic extracts in a dose-dependent manner (Fig. 2). α-amylase inhibitors obtained from natural herbal-based food sources suggest an attractive therapeutic move toward the treatment of postprandial hyperglycemia, and this is done by decreasing glucose release from starch and may likely find its use in the treatment of diabetes mellitus and obesity [29]. The outcome of the present investigation is in agreement with other reports [30-32]. The postprandial glucose suppression through α-glucosidase inhibitory action by methanol extracts of *E. ribes* showed potent inhibitory activity, while less inhibitions were observed in ethanolic extract of *E. ribes*. The retardation of membrane-bound α-glucosidase inhibitory reaction or inhibition of passive glucose transport would successfully flatten the postprandial blood glucose excursions or reduce hyperglycemia. On the basis of these results, methanolic extracts of *E. ribes* showed strongest α-glucosidase inhibitory activity than ethanolic extract of *E. ribes* leaves.

Inhibition of α-amylase and α-glucosidase might lead to delay in carbohydrate digestion and glucose absorption resulting in the diminishing of postprandial hyperglycemic excursions. These inhibitors most often do not whole alter the amount of carbohydrate absorbed. Hence, they influence only the carbohydrate digestion without resulting

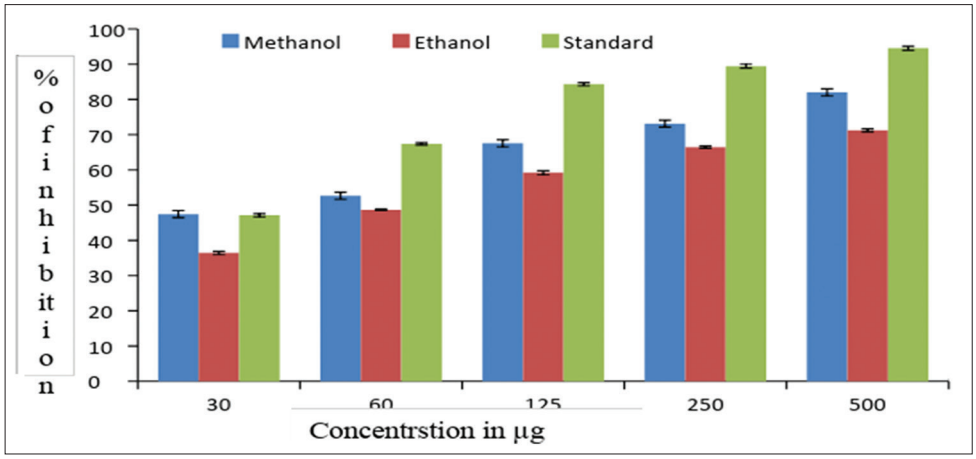

Fig. 1: Effect of *Embelia ribes* on the activity of alpha-amylase. Alpha-amylase concentration determined in ethanolic and methanolic extracts of *E. ribes* leaves at various concentrations and positive control (acarbose) was used as a standard. Each bar represents mean ± standard error of the mean of five sets. Each first bar reveals α-amylase activity was significantly inhibited by methanolic extract when compared with ethanolic extracts in a dose-dependent manner

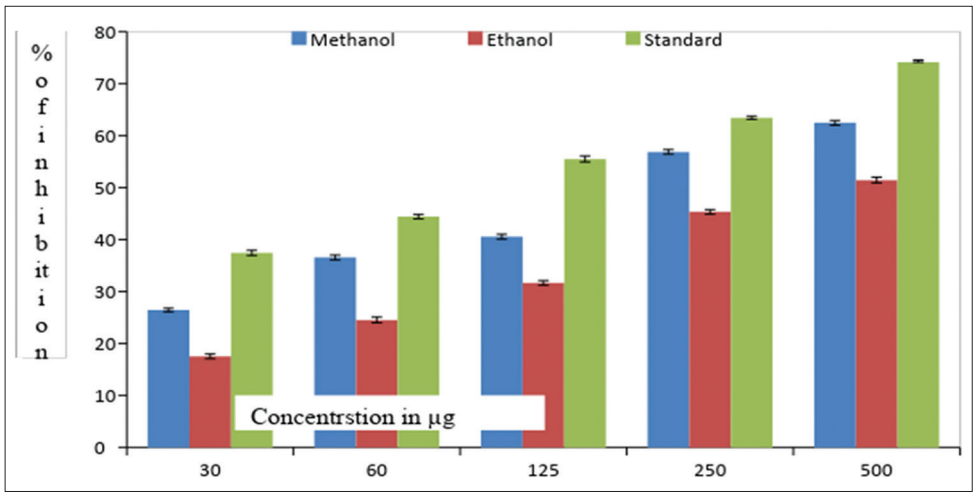

Fig. 2: Effect of *Embelia ribes* on the activity of alpha-glucosidase. Alpha-glucosidase concentration determined in ethanolic and methanolic extracts of *E. ribes* leaves at various concentrations and positive control (acarbose) was used as a standard. Each bar represents mean ± standard error of the mean of five sets. Each first bar reveals α-glucosidase activity was significantly inhibited by methanolic extracts whereas compared with ethanolic extracts in a dose-dependent manner

in any nutritional caloric loss. Persistent hyperglycemia after intake of food could provoke the non-enzymatic glycosylation of a variety of proteins, ensuing in the progress of chronic complications. Thus, control of glucose levels after intake of meal is crucial in the early management of diabetes mellitus and in ameliorating the chronic vascular complications.

Most of the antidiabetic drugs exhibit their effect by raising the insulin secretion and/or by recuperating insulin action [29]. Sulfonylurea-induced insulin secretion by clamping open ATP-sensitive K^{++} channels, thus preventing membrane depolarization and subsequent Ca^{+2+} influx, two of the key initial steps in insulin secretion [33]. In the present study, *E. ribes* had significant effects on insulin secretion and stimulated more than 2-fold insulin release. The extract is, therefore, likely to act at an early stage of the insulin secretion pathway before Ca^{+2+} influx. These characteristics are indicative of sulfonylureas, and this study shows that the antihyperglycemic actions of *E. ribes* are associated with the stimulation of insulin secretion.

Antibacterial and antimicrobial activities of the leaf extract of *E. ribes*

The antibacterial activities of the extracts against both Gram-positive organisms *E. coli*, *S. aureus*, *Enterococci*, and *Klebsiella pneumoniae* at different concentrations ranging from 10, 25, 50, and 75 µg/ml and their antibacterial activities were compared to those of the reference controls (ciprofloxacin and clindamycin). The antibacterial activity of the extracts was found to increase with increasing concentration against all bacterial strains tested, as evidenced by the higher zones of inhibition at higher concentrations (Figs. 3 and 4). Moreover, the extracts showed a remarkable inhibition of bacterial growth at a concentration of 75 µg/ml compared to the other doses (10, 25, and 50 µg/ml), and the activities are comparable to (ciprofloxacin and clindamycin) a commercially available antibiotic drug that was used as the reference control drug.

The extract was significantly active, exhibiting antimicrobial activity against tested organisms, namely *E. coli, S. aureus, Enterococci,* and *K. pneumoniae*. The antibacterial activity of the extracts was found to increase with increasing concentration against all bacterial strains tested, as evidenced by the higher zones of inhibition at higher concentrations. Both the extracts showed are markable inhibition of bacterial growth at a concentration of 75 µg/ml compared to the other three doses (10, 25, and 50 µg/ml) and commercially available antibiotic drug (ciprofloxacin and clindamycin) that was used as a reference positive control drug. The antibacterial activity of methanolic

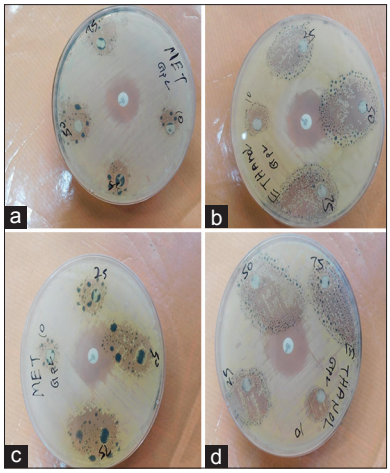

Fig. 3: Antibacterial activity of *Embelia ribes* on Gram-positive bacteria. (a and b) *Staphylococcus aureus and* (c and d) *Enterococcus species*. It represents that the antibacterial activity of the extracts was found to increase with increasing concentration against all bacterial strains tested, as evidenced by the higher zones of inhibition at higher concentrations. Both the extracts showed are markable inhibition of bacterial growth at a concentration of 75 µg/ml compared to the other three doses (10, 25, and 50 µg/ml) and commercially available antibiotic drug (ciprofloxacin and clindamycin) that was used as a reference positive control drug. This figure reveals the antibacterial activity of methanolic extract is significantly higher than that of ethanolic extract

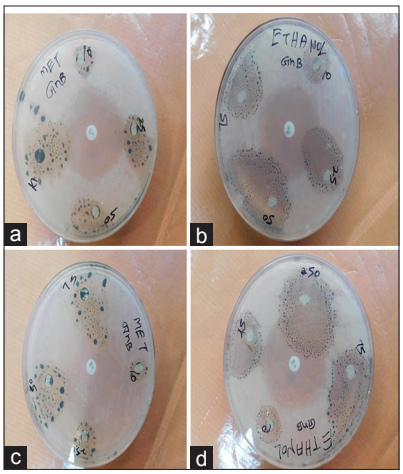

Fig. 4: Antibacterial activity of *Embelia ribes* on Gram-negative bacteria. (a and b) *Escherichia coli* and (c and d) *Klebsiella Pneumonia.* It represents the antibacterial activity of the extracts was found to increase with increasing concentration against all bacterial strains tested, as evidenced by the higher zones of inhibition at higher concentrations. Both the extracts showed are markable inhibition of bacterial growth at a concentration of 75 µg/ml compared to the other three doses (10, 25, and 50 µg/ml) and commercially available antibiotic drug (ciprofloxacin and clindamycin) that was used as a reference positive control drug. This figure reveals the antibacterial activity of methanolic extract is significantly higher than that of ethanolic extract

extract is significantly higher than that of ethanolic extract. These results support the view of developing new antibacterial drugs from the leaf extract of *E. ribes* [34].

CONCLUSION

In the present study, *in vitro* hypoglycemic activity of methanolic and ethanolic extracts of *E. ribes* leaves on alpha-amylase and alpha-glucosidase activity was studied, and it was observed that both the extracts have an antidiabetic activity but when compared to ethanolic extract, the methanolic extracts of *E. ribes* leaves had a higher inhibitory activity on alpha-amylase and alpha-glucosidase enzymes. Similar results were obtained when the antimicrobial and antibacterial activity of the leaf extracts were studied. From the results of the present study, it can be inferred that the methanolic extract of the leaves of *E. ribes* holds more therapeutic potential when compare with the ethanolic extract and further studies are needed to find out the active component responsible for the antibacterial and hypoglycemic efficacy of the *E. ribes* leaves.

CONFLICTS OF INTEREST

There are no conflicts of interest among authors.

AUTHOR'S CONTRIBUTION

All the authors have contributed equally to carry out the work.

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