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Analysis of antimicrobial activity of *Salaciareticulata* root extract

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Abstract
Salacia reticulata Wight (*Hypocrataceae*) known as Ponkoranti, have been used for thousands of years for the treatment of diabetes. Traditionally, *S. reticulata* is used in the preparation of herbal teas for diabetes. It appears to greatly increase the number of insulin secreting beta cells in the pancreas, while returning blood sugar levels to near normal. It increases the activity of enzymes responsible for glucose uptake and utilization. Different species of *Salacia* have medicinal principles with a high pharmacological significance. Extracts of ***Salacia*** roots, stems, and leaves have been used in Asia for hundreds of years for this folkloric treatment of diabetes and other health problems.

Keywords: *Salacia reticulata*, beta cells, diabetes

Introduction

Medicinal plants are an important element of the indigenous medical systems in India, where about 35 % of the population, even at present, de-pends on traditional systems of medical care (Smith *et al*. 2006) [5]. The genus, *Salacia* belongs to the family *Hippocrateaceae*, class Magnoliopsida and order-Celastrales, having 407 different species. These species are widely distributed in South-West India, Peninsular region of India, Sri Lanka, Vietnam, China, Indonesia, Brazil, South Africa, Malaysia, Thailand and Philippines (Anshul *et al*, 2013) [11]. *Salacia reticulate*, a species widely known for its antidiabetic properties has been investigated in Japan and the United States and patented.*S. reticulata* have led to increase the consumption of the species across the world and it has now become a subject of broad studies for diabetes management. In order to meet the ever-increasing demand, commercial exploitation of the species simultaneously with the scientific investigations is of paramount important. Though many toxicological studies carried out with rodents have proved that little or no adverse effects of *S. reticulata*, clinical trials are crucial to further confirm the safety of the use of Salacia extracts. Numerous biologically active compounds, such as anthocyanidins, triterpenes, phenolic compounds, glycosides and coloring agents have been isolated from plants of the Salacia species, which show various medicinal properties (Zhang *et al*., 2008). Anti-diabetic (especially type 2 diabetes) (Duke and Ayensu, 1985), antiinflamatory (Govindaraj *et al*., 2009), nephroprotective (Singh *et al*., 2010), antioxidant and anti-tumor (Guha *et al*., 1996) are the main pharmacological activities of the genus Salacia. However, further mechanistic studies are needed to illustrate as to how different usage of *S. reticulata* interact with other therapeutic interventions. Interest in the use of Salacia extracts have risen in recent years for a number of reasons, including the rapid increase in the incidence of diabetes and pre-diabetes, the need for safe and effective drugs and medicinal foods that can assist in the control of blood sugar as well as lipid levels, and the fact that Salacia extracts exhibit multiple mechanisms of action with respect to carbohydrate and lipid metabolism (Li *et al*., 2008).

Materials and Methods

Collection of Plant Material Fresh healthy plants of *Salacia reticulata* was identification and collected from Kanyakumari District, Tamil Nadu and brought to the laboratory for further sample processing. Fresh leaves were washed under running tap water, shade dried at room temperature and then homogenized to fine powder and stored in air tight bottles. Preparation of leaf extracts Sample extraction was prepared by dissolving the one gram of dried powder of leaf in 20 ml of ethanol (75%), chloroform and aqueous (Merck, extra pure) then mixed for 1 min using an Ultra Turax mixer (13,000 rpm) and percolate overnight at room temperature. The sample extracts were then filtered through Whattman No. 1 filter paper in a Buchner funnel. The filtered solution was concentrated under vacuum in a rotary evaporator at 40°C to a constant weight and re-dissolved in the respective solvents such as ethanol, chloroform and aqueous. The samples were stored at 18°C for further studies. Test Organisms The test microorganisms like Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Lactobacillus sps, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Proteus mirabilis and Streptococcus mutants were collected from (MTCC) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA) for further assays. Broth culture preparation Pure culture from each strains were inoculated into Nutrient Agar plate and sub cultured at 37ºC for 24h. culture Inoculums were prepared by aseptically inoculate the fresh culture into 2 ml of sterile 0.145 mol/ L saline tube and the cell density was observed with 0.5 McFarland turbidity standard to yield a fine bacterial suspension of 1.5×108 cfu/ml. Standardized inoculum for Anti-microbial assay. Antimicrobial Test The assay media was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121oC for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture *viz*. *Enterococcusfaecalis*, *Staphylococcusaureus*, *Bacillussubtilis*, *Lactobacillus*, *Escherichiacoli*, *Pseudomonasaeruginosa*, *Proteusvulgaris*, *Klebsiellapneumonia*, *Proteusmirabilis* and *Streptococcusmutans*. Finally, the positive control (streptomycin 25mg) and sample loaded disc was then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, zone of clearance was measured around the disc with transparent ruler in millimeters. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence or low activity (Kohner *et al*., 1994, Mathabe *et al*., 2006) [12].

The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam, 2010).

Result and discussion

Table 1

Strain Name	Positive control	Ethanolic extracts of *S. reticulata*	Chloroform extract of *S. reticulata*	Aqueous extract of *S. reticulata*
Zone of inhibition in mm				
S. aureus	24	15	10	-
B. subtilis	27	20	10	-
S. mutans	17	17	13	-
Lactobacillus sps	22	18	-	-
E. faecalis	25	16	11	-
E. coli	25	17	10	-
K. pneumonia	21	16	12	-
P. mirabilis	20	18	-	-
P. aeruginosa	22	19	12	-
P. vulgaris	25	21	13	-

Ethanolic leaf extract of *Salacia reticulata* showed maximum inhibition against *P. vulgaris* (21mm) and minimum zone of inhibition against *S. aureus* (15mm). Inhibitory zone against *B. subtilis*, *S. mutans*, *Lactobacillus* sp., *E. faecalis*, *E.coli*, *K. pneumonia*, *P. mirabilis* and *P. aeruginosa* were found to be 20mm, 17mm, 18mm, 16mm, 17mm, 16mm, 18mm and 19mm respectively. Chloroform leaf extract showed maximum inhibitory zone against *S. mutans* and *P. vulgaris* (13mm). Inhibitory zone was noticed in three bacterial species *S. aureus*, *B. subtilis* and *E.coli*. *E. faecalis*, *K. pneumonia* and *P. aeruginosa* showed 11mm and 12mm inhibitory zones. No zone of inhibition was noticed in Chloroform leaf extract against *Lactobacillus* species and *P. mirabilis*. No zone of inhibition was produced by aqueous extract of *S. reticulata* against all the tested bacterial pathogens. Positive control showed good antibacterial activity against all the test organisms. Positive control showed maximum inhibition against *B. subtilis* and minimum inhibition against *S. mutans*.

Discussion

Medicinal plants have been used as remedies for human diseases for centuries; this is because they contain components with therapeutic properties in their parts. The antibacterial effect of several medicinal plant extracts has been proved (Habbal *et al*., 2011; Yaouba *et al*., 2012) [13, 14]. Although a variety of solvents have been used in the extraction of bioactive components, it is still uncertain as to what kind of solvent is the most effective and suitable for extraction. In the present study, the ethanolic leaf extracts of *Salaciareticulata* inhibited the growth of all the bacterial pathogens tested, but their effectiveness varied. Chloroform extract was inactive against *Lactobacillus* and *Proteusmirabilis*. Aqueous extract was not effective against all the tested pathogens. The variation in the microbial activity of extracts prepared using different solvents might be due to the polarity of the solvents used, polarity of the compounds being extracted by each solvent and, in addition to their extrinsic bioactivity and by their ability to dissolve in the solvent used in the assay (Anjana *et al*., 2009) [15]. The other possibility may be due to the loss of some active compounds during extraction of the sample, lack of solubility of active constituents in the solvent (Kumar *et al*., 2008) [16] or the presence of active compounds in low quantities in the extracts to show their activity with the dose levels employed (Taylor *et al*., 2001) [17]. Extracts which have lesser antibacterial activity may be active against other untested bacterial species (Shale *et al*., 1999) [18]. In the present study ethanolic extracts showed good antibacterial activity. Previously Sastry and Rao (1994) [19] have revealed that extracts prepared using solvents like ethanol, hexane and methanol are capable of inhibiting both Gram-positive and Gram-negative bacteria. The antimicrobial activity of plant extracts might be due to the presence of lipophilic compounds that might bind within the cytoplasmic membrane (Jabeen *et al*., 2008) [20].

Conclusion

Diabetes has now become an epidemic affecting millions of people worldwide, neither insulin nor other modern pharmaceuticals has been shown to modify the course of diabetic complications mainly due to the multifactorial basis that involves both genetic and environmental risk factors. Therefore, effort is being devoted to find new therapeutics aimed at multiple targets, which has become a new paradigm in drug discovery.

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