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Research Article

ANTIBACTERIAL ACTIVITY GS-MJ NOVEL JUICE AGAINST *Staphylococcus aureus*

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Abstract

In the present investigation, *Gymnema sylvestre* was incorporated with mango juice (GS-MJ) and its antibacterial activity was analysed. Quality assessment was done by a standard methodology. It was well documented that a complete aseptic protocol was followed for the sample preparation. Hazard analysis was done carefully and a systematic procedure was followed for its rejection as well as inclusion. Finally, safety sample was taken for sample preparation and further studies. The final product GS-MJ was efficient in inhibiting growth of *Staphylococcus aureus*. The antibacterial activity of GS-MJ could have therapeutic role in the prevention of respiratory and other kind of infectious diseases which are frequently caused by *Staphylococcus aureus*.

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1. Introduction

Gymnema sylvestre is an herb native to the tropical forests of southern and central India where it has been used as a natural medicine for treatment of diabetes for nearly two millennia. Leaves of *Gymnema sylvestre* are active against glycosuria and many other urinary disorders. The saponin gymnemic acid, constituent of the leaves, was shown to suppress sweet taste sensation and to inhibit glucose absorption in the small intestine. Extracts of *Gymnema* is not only claimed to curb sweet tooth's but also for treatment of various problems as hyperglycemia, obesity, high cholesterol levels, anemia, digestion and anti microbial activity *Gymnema sylvestre* is a woody, climbing plant, native to India. The leaves of this plant have been used in India for 2,000 years to treat madhu meha, or "honey urine," an early term for glucosuria detected by pouring the patients urine onto the ground and observing whether or not insects were attracted to it. Chewing the leaves also destroys the ability to discriminate "sweet" taste, giving it its common name, gurmar or "sugar destroyer. Plant constituents include two resins (one soluble in alcohol), 6 % gymnemic acids, saponins, stigmasterol, quercitol, and the amino acid derivatives betaine, choline and trimethylamine. *Gymnema sylvestre* is a woody, climbing plant native to India. The leaves of this plant have been used in India for over 2000 years to treat diabetes. Chewing the leaves destroys the ability to discriminate the sweet taste, giving it its common name, gurmar or sugar destroyer. Plant constituents include two resins (one soluble in alcohol), gymnemic acids, saponins, stigmasterol, quercitol, and the amino acid derivatives betaine, choline and trimethylamine (Abbasi *et al*., 2010). *Gymnema sylvestre* is a stomachic, diuretic, refrigerant, astringent and tonic. It has been found to increase urine output and reduce hyperglycemia in both animal and human studies. The antidiabetic activity of *Gymnema sylvestre*

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appears due to a combination of mechanisms. Two animal studies on beryllium nitrate- and streptozotocin - diabetic rats found *Gymnema sylvestre* extracts doubled the number of insulin secreting beta cells in the pancreas and returned blood sugars to almost normal (Abbasi *et al*., 2010).

Gymnema sylvestre increases the activity of enzymes responsible for glucose uptake and utilization and inhibits peripheral utilization of glucose by somatotrophin and corticotrophin. *Gymnema sylvestre* treatment for 6 - 8 months was shown to decrease in glycosylated hemoglobin (HbA1c) in diabetic rats. Numerous animal studies have confirmed the hypoglycemic effect of *Gymnema sylvestre.* It was suggested that some of the extracts containing gymnemic acids from *Gymnema sylvestre* leaves suppress the elevation of blood glucose level by inhibiting glucose uptake in the intestine (Ahmed *et al*., 2010). In a recent study showed that *Gymnema sylvestre* inhibits the glycation of lens protein and ameliorates diabetic cataract. In the global market the attractiveness of mango is owing to its stunning colour, striking fragrance, pleasing flavour, good taste and healthy nutritional properties. When it was considered the losses of mango fruit after harvesting especially considering the developing countries, then the post harvest losses of mango are extremely conspicuous. The losses are basically due to the mango fruit harvesting at inappropriate maturity, offensive field handling, chilling injury, fruit softening, mechanical injure, decay of mango fruit, lenticels discoloration, squishy tissue, sap burn and pest or disease damage. The basic nutritive and quality losses are occur due to stiff fruit packing, by using inappropriate transportation and meagre field management (Aleisa *et al*., 2014).

Mango fruit are commonly processed into juice or puree form and added to many different types of food systems, including fruit juice blends. Fruit juice blends containing mango are becoming more popular with the rise of tropical fruit juices. Processed mango products undergo heat treatment or pasteurization to destroy all pathogenic and spoilage organisms. Pasteurization is effective in assuring sanitation. However, application of heat treatments can adversely affect quality characteristics with regard to aesthetic and nutritional quality (Adedeji *et al*., 1992). As the chemical preservation is the most economical method among the other preservation techniques, so commonly the mango pulp was preserved with the help of these chemicals. Prevention of the food spoilage due to microbial attack is done by using the chemical preservatives. These chemicals showed their batter effect when use in different combination and concentration for the control of microbial growth. No preservative on its own is absolutely efficient against the entire microorganisms. For the longer time storage or preservation of fruit pulp especially mango, the frequently used preservatives are potassium metabisulphite (PMS), citric acid and sodium benzoate (SB) because of their superior antimicrobial action (Ahmed *et al*., 1986).

Sodium benzoate concentration has a direct impact on the microorganism inhibition, as greater amount of sodium benzoate manifest the better antimicrobial effect, when applied on different species of *Aspergillus* sp. In the pulp preservation sodium benzoate and potassium metabisulphite have an inhibitory effect to all bacteria and other microorganisms, while addition of chemical preservatives adversely affects the sensory attributes and physico-chemical characteristics of mango pulp. The previous studies proposed that as the storage proceeded of the preserved mango pulp, chemical preservatives significantly affect the sensory profile and physico-chemical characteristics will cause a raise in brix value, acidity contents, reducing sugars constituents while sucrose contents were decreased (Bajwa *et al*., 2003).

The highest level for the utilization of these chemical preservatives as mentioned in the codex standards applied in 2001 and 2006 respectively are 1000 mg/kg sodium benzoate as benzoic acid and 500 mg/kg potassium metabisulphite as remaining SO_2 in the fruit processing and their storage, including nectars, purees and pulp. Unsystematic use of these chemicals is a huge concern to the healthiness and physical conditions of the human beings, and has been the basis for the development of resistant bacteria and microorganisms, and as a result leading to the incidence of food borne

diseases. In the present investigation, *Gymnema sylvestre* was incorporated with mango juice and its antibacterial activity was analysed.

2. Materials and Methods

Preparation of Mango Juice using raw material

Preparation of mango juice using raw material was done by using the methodology followed by Devaraja Agro aseptic Industries, Krishnagiri, Tamil Nadu, India.

Gymnema sylvestre extract

Dried *Gymnema sylvestre* leaves were ground in a miller as the temperature of the container was maintained at less than 50 °C. The powder was extracted with 95 % ethanol (1:10 w/v) for two days with constant stir. Suspensions were filtered through Whatman No.1 filter paper to retain the clear solution. The residue was extracted again. The pooled *Gymnema sylvestre* extract was vacuum evaporated below 50 °C. The dried extracts were stored at 4 °C.

Preparation of GS-MJ novel juice material

The prepared ethanolic extract of *Gymnema sylvestre* extract was incorporated with mango juice prepared as described above. The required concentration for withstanding mango juice's yellow color was found to be 10 µg/ml using Titrmetric method.

Antimicrobial activity

Test Organisms

The Gram positive microorganism (*Staphylococcus aureus*) was used for the present research. Bacterial strains were maintained on freshly prepared Nutrient agar slant and stored at 4 °C. The bacterial strains were procured from Bethasda Hospital, Ambur, Tamilnadu, India.

Storage of bacterial cultures

A single colony was inoculated into 0.85 ml of Nutrient broth and incubated at 37 ^{o}C for 12 hrs. A volume of 0.15 ml of sterile glycerol was added, mixed thoroughly and stored frozen at – 20 ^{o}C.

Antibacterial activity of GS-MJ

Antibacterial activity of GS-MJ was done by Agar well diffusion method. A single colony of bacterial cells was suspended in 1 ml of Nutrient broth and it was added to 25 ml of Nutrient agar media at 45 ^{o}C, mixed thoroughly and poured into plates. After solidification, wells were dug in it and GS-MJ were added and incubated for 24 hrs at 37 ^{o}C. After incubation, inhibition zone was observed and measured as mm in dm.

3. Results and Discussion

Quality assessment was done by a methodology followed by Devaraja Agro aseptic Industries, Krishnagiri, as described above. It is well documented that a complete aseptic protocol was followed for the sample preparation. Hazard analysis was done carefully and a systematic procedure was followed for its rejection as well as inclusion. Finally, safety material was taken for sample preparation and further studies. It is well known that microorganisms have certain general physiological requirements for survival and growth, regardless of the antibiotic sensitivity profile of the organism. These include an appropriate temperature range, nutrients, oxygen moisture and pH. In the human body, microorganisms will encounter nutrients, temperature, moisture and oxygen required for growth.

Virtually all body fluids provide sufficient organic nutrients for optimal bacterial growth. Extremes of acidity or alkalinity can effectively limit growth and survival of microorganisms, and this approach is used widely in the food, pharmaceutical, and cosmetics industries. Studies noted a pH of 4.4 - 9 is the limiting growth range for many organisms, including *Staphylococcus*, *Pseudomonas*, *Streptococcus* and others commonly associated. In the present study, it was noted that GS-MJ was significantly more effective against Gram positive organisms include *Staphylococcus aureus* when compared to positive and negative control. Antimicrobial activity of GS-MJ was done by Agar well diffusion method. We propose that the constituent of GS-MJ protect the Growth of *Staphylococcus aureus in vitro* by preventing its utilization of the Nutrient medium (Ahamed *et al*., 2011).

The GS-MJ is efficient in inhibiting growth of *Staphylococcus aureus*. In addition it's also ameliorates to prevent multiplication in-vitro, the antibacterial activity of GS-MJ could have therapeutic role in the prevention of respiratory and other similar kind of infectious diseases. In the present investigation, antibacterial effect of GS-MJ against *Staphylococcus aureus* was monitored. The drug of interest GS-MJ showed maximum inhibition against *Staphylococcus aureus* (20 mm) and followed by moderate effect shown by *Gymnema sylvestre* (GS) against *Staphylococcus aureus* (15 mm) and mango juice was found to be 8 mm. There was a least effect of control on *Staphylococcus aureus* (No zone of inhibition). Further studies are required to monitor the exact mechanism of action of this antibacterial action as well as *Staphylococcus aureus* inhibiting activity of the novel juice GS-MJ.

Table – 1: Antibacterial activity against *Staphylococcus aureus*

Name of the drug	Zone of Inhibition (mm in dm)
GS-MJ	20
GS	15
MJ	8
Negative control	No zone of inhibition

4. Conclusion

In conclusion, quality assessment was done by a standard methodology. It was well documented that a complete aseptic protocol was followed for the sample preparation. Hazard analysis was done carefully and a systematic procedure was followed for its rejection as well as inclusion. Finally, safety material was taken for sample preparation and further studies. The final product GS-MJ is efficient in inhibiting growth of *Staphylococcus aureus*. In addition it's also ameliorates to prevent multiplication *in vitro*, the antibacterial activity of GS-MJ could have therapeutic role in the prevention of respiratory and other similar kind of infectious diseases. In the present investigation antibacterial effects of GS-MJ against *Staphylococcus aureus* was monitored. The drug of interest GS-MJ showed maximum inhibition against *Staphylococcus aureus* (20 mm) and followed by moderate effect shown by *Gymnema sylvestre* (GS) against *Staphylococcus aureus* (15 mm) and mango juice (MJ) was found to be 8 mm. There was a least effect of purified water (PW) on *Staphylococcus aureus* (No zone of inhibition). Further studies are required to monitor the exact mechanism of action of this antibacterial action as well as *Staphylococcus aureus* inhibiting activity of the novel material GS-MJ.

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