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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF ANTIMICROBIAL PROPERTIES OF *RUTA GRAVEOLENS* AND *PLUMBAGO ZEYLANICA* AGAINST *LEUCONOSTOC MESENTEROIDES*

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ABSTRACT

The carbohydrate polymer dextran, majorly formed by *Leuconostoc mesenteroides* (gm + ve Lactic acid bacterium) is widely recognized as causing agent of large sucrose losses in sugar industry during different stages of processing. Recently the pathogenic potential of L mesenteroides has been established. Antibacterial activities of *Ruta graveolens* and *Plumbago zeylanica* extracts against several bacterial strains have been reported. The aim of this study was to evaluate the possible antimicrobial potential of *Ruta graveolens* stem and *Plumbago zeylanica* leaf extracts (chloroform, methanol, ethanol, aqueous) against major sugar loss causing *Leuconostoc mesenteroides* (MTCC *107). For this purpose, the bacterium *L. mesenteroides* was tested. Antimicrobial activity was done using agar well diffusion method. Tested extracts showed antibacterial activity against the selected bacterium *L. mesenteroides*. The test microorganism was more susceptible to methanolic extracts of both *Ruta graveolens* and *Plumbago zeylanica* with mean diameters of inhibition being 18.22mm, 22.35mm and MIC being 12.5mg/ml, 6.25mg/ml respectively, followed by ethanol, chloroform and aqueous extracts. Relatively less antimicrobial inhibitory activity was shown by aqueous extracts of both plants. The *P. zeylanica* leaves extracts shown comparatively better antimicrobial property than R. graveolens stem extracts as evidenced by mean zone of inhibition and MIC. These promising findings suggest the presence of antibacterial activity of the tested plants, exhibited by their bioactive compounds, and serving them as an alternative antimicrobial agents against sugar loss causing microorganism *L. mesenteroides*. Further, results are beneficial for sugar industry.

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INTRODUCTION

Dextran is the name given to a group of high molecular weight polymers composed of D-glucose units connected by α -1,6 linkages and with various of side branches linked with α-1,2, α -1,3, or α -1,4 to the main chain (Khalikova, 2005). The enzyme dextran sucrase converts a molecule of sucrose to fructose that can be used by the microorganisms; dextran is the by-product of this enzymatic reaction (Day, 1993). The viscous polymer dextran, majorly formed by *Leuconostoc mesenteroides* (gm + ve Lactic acid bacterium) are widely recognized as causing agent of large sucrose losses in sugar industry during different stages of processing(Tallgren,1999). *Leuconostoc mesenteroides* infections incurs sugar beet and sugarcane deterioration also (De Bruijn, 2000, Eggleston, 2005), particularly when humid and warm environmental conditions prevail. The deterioration products including mannitol and D-lactic acid, which in moderate and severe cases can disrupt normal processing operations in sugar industries. Commercially available Dextranases (microbial enzymes which breaks down dextrans) are sometimes applied to hydrolyze dextran polysaccharide in sugar manufacture, when *Leuconostoc mesenteroides* deterioration of sugar beet has occurred. Unfortunately, dextranases only have a small market and low volume sales compared to many other industrial enzymes due to unoptimised reaction conditions, and they are highly specific towards the low molecular weight dextrans than the larger ones (Eggleston, 2005).

Hence, a better approach to tackle the sucrose losses and overall quality in sugar industry is to look for inhibition of *L. mesenteroides* than handling of Dextran. Though, obvious next choice is to look for antibiotics, there has been increased resistance to existing antibiotics due to injudicious use. Biologically active compounds from natural resources have always been of great importance to deal with microbial infections. A number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants (Brantner, A and E. 1994, Perumal-Samy, 1997). There is an ever increasing demand for plant based natural products, which

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are non narcotic and easily available at affordable prices with no side effects. To work in this direction, we have chosen two plants for our studies, *Ruta graveolens* is a member of Rutaceae family. It is a hardy, evergreen shrub of up to one meter tall, with a characteristic grayish green colour and a sharp unpleasant odour. The leaves are small, oblong, deeply divided, pinnate, glandular dotted. The stems are much ramified. Its flowers are small, yellow and in clusters during spring and summer. Rue (*R. graveolens)* is a native of Southern Europe. In England Rue is one of the oldest garden plants, cultivated for its medicinal use.

The leaves are the main part used medicinally. Rue is traditionally used for a very wide range of ailments including menstrual disorders, spasm, loss of appetite, dyspeptic complaints, circulatory disorders, fever, high blood pressure, heart palpitations, inflamed mucosa, toothache, hysteria, arthritis, sprains, injuries and skin diseases. Rue has been shown to induce apoptosis (cell death) and could be beneficial in cancer therapy (Preethi, 2006). The aqueous extracts of rue been shown to be effective against *Trichomonas vaginalis* in vitro (Al Heali, 2006). Rue has been shown to have antimicrobial and cytotoxic activities in vitro (Ivanova, 2005).

The *Plumbago zeylanica* is commonly known as chitrak is perennial, one of the common plants used in Indian traditional system of medicine. *Plumbago zeylanica*, belongs to the family Plumbaginaceae, which are distributed in several parts of India which is a subscadent, pretty perennial shrub with semi woody stems and numerous branches. Its leaves are simply alternate, ovate, narrowed into petiole, oblong-lanceolate and acute. The plant can be common, wild or in cultivation due to its more therapeutic uses (Chetty, 2006). Its roots are used in traditional system of medicine to cure various ailments like body pain, headache, fever and inflammation (Mittal, 2010) and also as laxative, expectorant, astringent, abortifacient, and in dysentery (Bhattacharjee, .1998).Tincture of root bark is used as antiperiodic. The leaves are used as aphrodisiac and in scabies and also has antimicrobial effect (Dhale, 2011). *P. zeylanica* roots were reported to possess antioxidant, hypolipidemic, anti atherosclerotic, central nervous system stimulant and anti-fertility properties (Kirtikar, 1975, Mallikadevi, 2010). In an urgent need to address the problem of sugar losses by *L. mesenteroides* during processing in sugar industry and to combat its pathogenic abilities, this study is planned to evaluate the antimicrobial potential of plant extracts of *Ruta graveolens* (stem) and *Plumbago zeylanica* (leaves).

MATERIALS AND METHODS

Collection and preparation of plant materials

Stems of the *Ruta graveolens* were collected from Tumkur district, Karnataka, south India and air dried for 7-10 days in a shade and ground into fine powder using a mechanical grinder. The extraction of the powdered plant material (25 g) were done by soaking in 400 ml of chloroform, methanol, ethanol, and water and kept for 72 hours in a shaker. The extract were filtrated on filter paper, concentrated to dryness and stored at 4^0C for further studies.

The extract was dissolved in Dimethyl sulfoxide (DMSO) under aseptic condition to prepare the desired dilutions (Pinkee Pandey, 2011). Leaves of the medicinal plant *Plumbago zeylanica* were collected from Tumkur district, Karnataka, south India. The leaves were shade dried at room temperature for 10 days. Extraction of the plant material which was dried and powdered (30g) was done by Sox-let extraction with 300 ml of each of chloroform, methanol, ethanol, water respectively for 48 hours at temperature below the boiling point of the solvents. The extracts were filtered through whatman No.1 filter paper and concentrated in vacuum at 40^0C. Each extract was transferred to small beaker and kept at 4^0C before use (Dhale, 2011).

Microorganism used and culture medium

The microbial strain namely *L. mesenteroides* (MTCC *107) was used for the antibacterial activity collected from the Institute of Microbial Technology, Chandigarh, India. The De Man Rogosa Sharpe (MRS) medium (De Man, 1960) was used for the activation of the microorganism. The microbial strain was transferred on nutrient agar slants and transferred in to nutrient broth and stored at 4^0C until required for the study.

Inoculum

The microorganism was inoculated into MRS medium and incubated at 23 ± 2°C for 4 hours. The turbidity of the resulting suspension was diluted with MRS to obtain a transmittance of 25.0 % at 580 nm. That percentage was found spectrophotometrically comparable to 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/ml. The Bausch and Lomb® spectrophotometer, Model Spectronic 20 was used to adjust the transmittance of the working suspensions.

The Antimicrobial activity by agar well diffusion assay

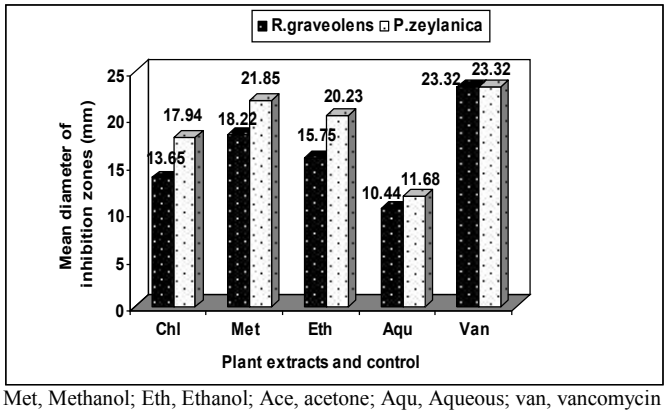
The chloroform, methanol, ethanol, and aqueous extracts of *R. graveolens* stem and *P. zeylanica* leaf were used for the screening. Antimicrobial activity of various extracts was determined by the agar well diffusion method (Okeke, 2001). The 100µl of inoculum of test organism, *L. mesenteroides* was spread onto the MRS agar media plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells or cups of 8mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium. The extracts were reconstituted in 20% Dimethylsulphoxide (DMSO) for the bioassay analysis (Rajasekaran 2008). A 100µl volume of each extract was propelled directly into the wells (in triplicates) of the inoculated MRS media agar plates for test organism. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24 hours (Khokra, 2008, Rios, 1980). Sterile DMSO and respective blanks served as the negative control and vancomycin served as the positive control. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition diameter was greater than 8mm (Hammer, 1999). The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated.

Determination of minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration of a compound or extract or drug that completely inhibits the growth of the microorganism in 24 hours (Thongson, 2004). The MIC for the extracts was determined by following the modified agar well diffusion method (Okeke, 2001). A twofold serial dilution of each extract was prepared by first reconstituting the powder in 20% DMSO in case of *R. graveolens* and chloroform, methanol, ethanol and aqueous blanks incase of *P. zeylanica* followed by dilution in sterile distilled water to achieve a decreasing concentration range of 100mg/ml to 0.39mg/ml. A 100µl volume of each dilution was introduced into wells (triplicate) in the MRS medium agar plates already seeded with 100µl of standardized inoculum (10^6 cells/ml) of the test microbial strain. The test plates were incubated at 25°C for 24 hours and observed for the inhibition zones. The lowest concentration of each extract of *R. graveolens* stem and *P. zeylanica* leaves and showing a clear zone of inhibition, considered as the MIC, was recorded for the test organism, *L. mesenteroides*.

RESULTS AND DISCUSSION

The comparative evaluation of results of antimicrobial activity of chloroform, methanol, ethanol and aqueous extracts by agar well diffusion method revealed that all the four extracts of *R. graveolens* stem showed antimicrobial activity against sugar loss causing *L. mesenteroides,* and all extracts (chloroform, methanol, ethanol and aqueous extracts) of *P. zeylanica* also shown significant antimicrobial activity against the same. highest mean diameter of inhibition zone was produced by the methanolic extracts (18.22mm of *R. graveolens* stem and 22.35mm of *P. zeylanica* leaf) followed by ethanol (15.75mm of *R. graveolens* stem and 20.23mm of *P. zeylanica* leaf), chloroform (13.65mm of *R. graveolens stem* and 17.94mm of *P. zeylanica* leaf). Relatively less antimicrobial inhibitory activity was shown by aqueous extracts (10.65mm of *R. graveolens* stem and 11.32mm of *P. zeylanica* leaf) of both plants.



Met, Methanol; Eth, Ethanol; Ace, acetone; Aqu, Aqueous; van, vancomycin

Fig. 1. Comparative antibacterial activity of four extracts of *Ruta graveolens and Plumbago ze*ylanica against sugar loss causing *L. Mesenteroides* along with the positive control (vancomycin)

The *P. zeylanica* leaf extracts showed comparatively better antimicrobial property than *R. graveolens* stem extracts as evidenced by mean zone of inhibition and MIC (12.5mg/ml of *R. graveolens* stem extracts and 6.25mg/ml of *P. zeylanica* leaf extracts). The positive bacterial control vancomysin showed an antimicrobial inhibitory zone of 23.32mm, at a concentration of 3mg/ml, while the negative control i.e. DMSO and respective blanks produced no observable zone against the tested microorganism *L. mesenteroides.*

Table 1. Minimum Inhibitory Concentration (MIC) of *R. graveolens and P zeylanica against L. mesenteroides*

Plant extracts	MIC (mg/ml)	
	Ruta	*Plumbago*
Methanol	12.5	6.25
Ethanol	12.5	6.25
Chloroform	12.5	6.25
Aqueous	12.5	6.25

We chose *L.mesenteroides* as test microorganism for our study because it has been implicated in infection of sugar cane and sugar loss in industry (Eggleston, 2005). A strain of *Leuconostoc mesenteroides*, for example, is revealed to have been the pathogen responsible for an outbreak of illness in a Spanish hospital as late as 2006 when six people became infected. The outbreak seemed to be linked to patients already having poor immune systems (German Bou, 2008). There is a significant amount of salt at the end of fermentation by *L. mesenteroides*, which can disrupt the environment in many ways may range from decreased vitality in plants and soils to soils decreasing in porosity.

Results of our study reveal that all the tested extracts of *Ruta graveolens* and *plumbago zeylanica* exhibited growth inhibitory activity against sugar loss causing bacterial strain. The zone of inhibition of the positive control for bacteria i.e. vancomysin when compared to the methanolic extract of both *R. graveolens and p. zeylani*ca was more or less the same. It is also observed that the methanolic extract of *both plants* was more potent against *L. mesenteroides* compared to other tested extracts. The methanol, ethanol and chloroform extracts of both plants showed greater antimicrobial activity than the corresponding aqueous extracts. This finding is interesting, because in the traditional method of treating a microbial infection, decoction of the plant parts or boiling the plant in water was employed, whereas according to the present study, preparing an extract with an organic solvent (methanol, ethanol and chloroform) shows a better antimicrobial activity.

It is also important to note that susceptibility of the tested pathogen varied with solvent extract and aqueous extract. This indicates the involvement of more than one active principle of biological significance. Moreover, the potential for developing antimicrobial drugs from plants appears rewarding, as it will lead to the development of a phytomedicine that will act more effectively against microorganisms. Therefore, such screening experiments form a primary platform for further phytochemical and pharmacological studies that may open the possibilities of finding new clinically effective antimicrobial compounds. Thus, results from the present studies have established that *Ruta graveolens and Plumbago zeylanica* are potential candidate plants, which could be used against *L. mesenteroides* to prevent sugar loss in industry and also against its potential pathogenic effects.

Conclusion

The *Ruta graveolens* stem and *Plumbago zeylanica* leaf solvent extracts were highly effective against the tested sugar loss causing bacteria *L.mesenteroides*. The antimicrobial activities can be enhanced if the specific phytoactive components are purified and adequate dosage determined for proper administration. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern antibacterial agents especially plants like *Ruta graveolens* and *Plumbago zeylanica* should be emphasized for commercial utilisation to effectively control the sugar loss, infection and ill effects of fermentation caused by *L.mesenteroides.*

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