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ANTIBACTERIAL ACTIVITY OF CITRUS SINENSIS PEEL EXTRACTS AGAINST STREPTOCOCCUS AGALACTIAE ISOLATED FROM PLEURAL FLUID

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ABSTRACT

The recent past has shown the increasing evidences indicating the role of traditional plants in the prevention of many diseases. The effectiveness of these medicinal plants mostly depends on the presence of several bioactive constituents that produces a raid action against many bacterial pathogens in a conventional application form. Also, due to the lesser side effects of these plants along with their therapeutic benefits, their usage has been increased thus serving as a crucial source of many ancient medicines. The present study was intended to evaluate the efficacy of the *Citrus sinensis* peel extracts against *Streptococcus agalactiae* isolated from pleural fluid. The analysis of the result for the antibacterial activity was found to be more effective with the methanolic extract with an inhibition zone of 14.0±0.1 mm than the ethyl acetate peel extract which showed a relatively less inhibition zone of 11.0±0.1mm respectively. As the methanolic extract of the plant showed good zone of inhibition against the test organism (*Streptococcus spp.*) therefore, it may be recommended that isolation of the bio-active components is needed for further research using animal model.

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INTRODUCTION

Bacterial infections are one of the prominent causes of health problems, physical disabilities and mortalities around the world [1].The prevalence of resistant bacteria constitutes a major challenge in the treatments of numerous well-known infections and therefore, necessitates the need to find new substances with antimicrobial properties to be used against these microorganisms. Discovery of novel compounds with anti-microbial capabilities against the microorganisms is necessary since resistant bacteria constitute a major problem in the treatment of many well-known illnesses [2].

Also, due to simple accessibility, cost saving and less side effect, it has been estimated that 80% of the world still relies on herbal remedies to cure a variety of disorders. Therefore, herbal remedies have built the foundations of medical pharmacology for centuries and are the corner stone of ancient medicinal systems. It is becoming more popular as a result of improved patient acceptability, particularly in developing nations like India which has great agro-climatic cultural and ethnic biodiversity.

One of the essential constituents of these natural resources that people can investigate are the secondary metabolites from a variety of plant sources that can be employed as pharmaceuticals, agrochemicals, tastes, scents, colors, biopesticides, and food additives. It has been demonstrated that antimicrobials and antibiotics derived from plant sources work more efficiently with fewer side effects and additional beneficial effects [3].

Several clinically important bacterial species are considered for study as they are responsible for causing a number of diseases in various hosts. Among these, the species *Streptococcus agalactiae*, a Gram positive, catalase negative and facultatively anaerobic bacteria causes remarkable perinatal infections in human. It may cause pneumonia, meningitis, bacteremia and other skin or soft tissue infections mostly in immune-compromised people and act as an opportunistic pathogen of the elderly people [4]. Pleural effusion is the abnormal accumulation of fluid in between the parietal and visceral pleura, called the pleural cavity. It can occur by itself or can be the result of surrounding parenchymal disease like infection, malignancy or inflammatory conditions. Pleuraleffusion prevents the lungs from fully inflating, making it hard to breathe. The main types of Pleural Effusion include Tuberculous Pleural Effusion (TPE), Malignant Pleural Effusion (MPE), and Para-pneumonic Pleural Effusion (PPE).The analysis of Pleural Effusion involves a group of tests that look for the cause of pleural effusion. Pleural effusion can have one more symptoms like Chest pain, dry and non productive cough, trouble breathing, fatigue and fever [5]. In the early stages of treating pneumonia, excessive non-steroidal anti-inflammatory medicines are used, which can easily result in pleural effusion [6].

Citrus sinensis is one of the widely grown fruit crop, with total global production reported to be around 120 million tons. About 140 genera and 1300 species make up the Rutaceae family's genus Citrus, *Citrus aurantium* (sour orange), *Citrus medica* (citron), *Citrus sinesis* (orange), *Citrus paradis* (Grapefruit), *Citrus limon* (lemon), *Citrus reticulate* (tangerine), *Citrus grandise* (shadd ock), *Citrus medica* (citron) and *Citrus auranti folia* (lime) are some of significant fruits of the genus. They are widely cultivated in tropical and subtropical climates for its tasty juice and medicinal value [2]. It is one of the most significant fruits grown in many nations with tropical or subtropical climates. The main countries that produce citrus include Brazil, Japan, China, Mexico, Pakistan, and those in the Mediterranean region [4]. Citrus fruits are an important foodstuff for human health because of its excessive contents of health-elevating nutritional as well as phytochemical substances, such as multivitamins, pectins, carotenoids, fatty acids, and especially polyphenols [7].Citrus juice contains various substances including carbohydrates, fiber, vitaminC, potassium, folate, calcium, thiamine, niacin, vitaminB6, vitaminA, phosphorus, magnesium, copper, riboflavin, pantothenic acid and various phytochemicals. It has several chemicals that can give the body additional defenses against chronic diseases and nutritional deficiencies. Citrus fruits offer a wide range of pharmacological properties, including those that are antibacterial, anti-helmintic, insect repellent, antioxidant, anticancer, cardiovascular, anti-inflammatory, analgesics, anti diabetic, reproductive, gastrointestinal, immunological and respiratory properties [8].

MATERIALS AND METHOD:

Collection and Preparation of Citrus peel Sample

The fresh and healthy Citrus fruits were collected from Guwahati city of Assam through random sampling. The collected fruit samples were washed thoroughly using distilled water to remove the surface dirt and transferred to the department of Medical Laboratory Technology, GEA National College. The outer layer of the fruits (peel) were removed carefully, cut into thin slices with a sharpened knife and allowed to dry in shade condition at room temperature for 20 days. The dried peels were then powdered using pestle and mortar and the powder obtained was stored in an air tight container for further use.

Solvent Extraction

Solvent extraction and processing was performed in the Central Instrumentation facility of Assam downtown University, Guwahati, Assam.50g of the stored air tight powder was weighed by electronic balance; it was packed in a filter paper and placed in the separate thimble(s) of two soxhlet extractors. Methanol and Ethyl acetate were used as solvents for the extraction. 500 ml of both the solvents were filled in the round bottom flask of the extractors and extraction was carried out for 15 hours. During the process, the non volatile compounds dissolve in the polar solvents and get concentrated in the round bottom flasks. The filtrates were finally collected and concentrated by using rotator vacuum evaporator.50µg/ml concentrations of the citrus peel extracts were prepared separately in different test tubes preparing a 10mg/ml stock prior to use.

Test organism isolation and preparation

The bacterial isolate (*Streptococcus agalactiae*) was obtained from the pre-collected clinical sample (pleural fluid) from Ayursundra Super speciality Hospital, Guwahati, Assam. The collected was later transferred into Medical Laboratory Technology (MLT) department by streaking on a blood agar slant and preserved at 4˚C for further use.

Initiation of the frozen test organism

The test organism maintained and stored at 4˚C were initiated by thawing the sample using gentle agitation in a water bath that was set for 25˚C for 2 minutes following the standard ATCC guidelines. After thawing, the outer surface of the test tube was decontaminated with 70% ethanol and the entire content of the test tube was transferred to a fresh tube containing 5ml of 0.1% peptone water. Finally, the tubes were incubated under suitable temperature.

Antibacterial susceptibility test

The antibacterial activity of *Citrus sinensis* peel extracts was performed by the agar well diffusion method using CLSI guidelines. The aliquots of the test organism incubated with 5ml of 0.1% peptone water (w/v) were evenly spread onto sterilized blood agar media using sterile cotton swab. The plates were then allowed to dry foe few minutes in laminar air flow hood. Wells of 5mm diameter, 2 cm apart from the edge of the plate were made on the culture medium using a cork borer. For the antibacterial activity, 50µl of all the extracts wells were used.

Statistical analysis

The zones of inhibition of the test organism against the peel extracts were expressed as mean ± standard deviation (SD) of three replicates.

RESULT

Anti –microbial test

The antibacterial activity of both the extracts has been shown in table 1. The antimicrobial activity in terms of the diameter of the inhibition zone of the peel extracts was compared. The findings have shown that methanol extract exhibited maximum inhibition against the tested organism with an inhibition zone of 14.0±0.1 mm in contrast to the ethyl acetate extract with an inhibition zone of 11.0±0.1 mm.

Table 1 - Zone of inhibition against *Streptococcus spp*.

Sl. No	Strains	Zone of Inhibition (in mm) Values represent mean±SD	
		Methanol extract	Ethyl acetate extract
1	*Streptococcus agalactiae*	14.0±0.1	11.0±0.1

DISCUSSION

The entire study was carried out by using methanol extract and ethyl acetate extract of peels of *Citrus sinensis* . Both the extracts were administered against the pathogenic strain of *Streptococcus agalactiae*. The mean ± standard deviation of the inhibition zone diameter of the methanolic extract for the isolate of human origin observed for revealed a significant action of the *Citrus sinesis* peels which could be ascribable to the presence of any of the secondary metabolites Saponin, phytosterol, Phenol, Steroid, Terpenoid, Flavanoid. Presence of any of the secondary metabolites, singly or in combination with others could be responsible for the anti bacterial activity of the plant. From the study, isolated strain *Streptococcus agalactiae* was found to be more susceptible to methanol extract of *Citrus sinesis* peels.

CONCLUSION

The results obtained from the antibacterial activity assay showed characteristic zone of inhibition around the test organism isolated from the pleural fluid of patients suffering from the pleural effusion. Effective bacterial growth inhibition was recorded against the isolated *Streptococcus spp.* and study indicated that these solvents have the capacity of extracting the antibacterial agents and therefore it points out that diverse extracts may have different antibacterial agent, which may have different modes of action. As the methanolic extract of the plant showed good zone of inhibition against the test organism (*Streptococcus spp.*) therefore, it may be recommended that isolation of the bio-active components is needed for further research using animal model.

CONFLICT OF INTEREST

None

ABREVIATION

CLSI - Clinical and Laboratory Standards Institute
SD - Standard Deviation
ATCC - American Type Culture Collection
TPE - Tuberculous Pleural Effusion
MPE - Malignant Pleural Effusion
PPE - Para Pneumonic Pleural Effusion

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