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Spray drying microencapsulation effect on Antibacterial activities of *Citrus limon* Burm powder against pathogenic bacteria

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Abstract

Citrus is an important fruit crop in the world and grown almost every state of India and in North East India, Assam lemon is considered as an indigenous principal lemon cultivar of Assam. This research study aimed to analyzed the antibacterial activity of microencapsulated spray dried *Citrus limon* burm powder by using disk diffusion method against gram-positive *Staphylococcus aureus*-ATCC 25923 as well as gram-negative *Escherichia coli*-ATCC 10536 taking gentamicin as positive control and dimethyl sulfoxide (DMSO) as negative control. The inhibition zone was measured using HiAntibiotic Zone Scale-C and results were categorized as resistant, sensitive and in intermediate zone respectively. The end of this exploration study is to assay antibacterial activity of spray dried *Citrus limon* juice powder as heat can degrade many metabolic compounds and emphasized the antibacterial wealth of spray dried *Citrus limon* juice powder in exploration of natural sources in storage and transportation for the development of pharmaceutical lead in near future.

Key words : indigenous, antibacterial, microencapsulated, resistant, dimethyl sulfoxide.

Citrus fruit typically contains organic acids, lipids, sugar, polysaccharides, minerals, vitamins, carotenoids, flavonoids and volatile compounds. In practically all of India's states, citrus is a significant fruit crop, with Arunachal Pradesh, Assam, and Karnataka being the top producers. Mandarin, sweet orange, and lemon are among the citrus fruits that India is thought to produce the most of. Citrus is grown on 57.2 thousand hectares in the North-Eastern part of India, which is thought to have one of the highest genetic diversity reservoirs[3,4]. One of the significant types of lemon is the Assam lemon, which is well-known for having several distinctive qualities and being widely farmed in India's North-East. The Assam lemon is regarded as the indigenous primary lemon cultivar of Assam. It is distinguished by its superior quality, flavour, and perfume, and it also bears fruits with two distinct peak seasons and seedless fruits with 9-12 segments[12]. Citrus fruits have been found to offer a variety of therapeutic benefits, including those that are cardiovascular, anticancer, antioxidant, antibacterial, anthelminthic, anti-inflammatory, analgesic, antidiabetic, reproductive, gastrointestinal, respiratory and immunological disorders[7]. Fruits and its essential oil contain secondary metabolites such as phenolic acids, coumarins, carboxylic acids, aminoacids, and monoterpenoids, particularly D-limonene[16].

Today, a variety of human diseases are treated with antibiotics derived from a wide range of microorganisms; thus, steps must be taken to limit the use of antibiotics, create novel synthetic and natural medications, and long-term health maintenance. Natural products have always been valuable sources of plants and compounds present in medicinal plant could be used therapeutically as reported by World Health Organization (WHO)[17]. Recent research focuses on encapsulating these bioactive compounds to shield them from physical barriers and increase their stability against nutritional degradation. Spray drying is a quick drying technique with atomization, drying gas circulation with droplets contact and to powder recovery, that turns a liquid slurry to dry powder used to make instant powder[18]. In essence, spray drying process begins with atomization, feed to create droplet prior to its interaction with a hot gas. By using a hot drying gas medium, process of spray drying begins when spray dryer converts liquids into dried particles.Spray drying has been used to create blended or single mixture of powder goods, including milk, fruit juices, herbal extracts, enzymes, essential oils, fragrances, and medications[6]. The feature that makes spray drying an appealing method for drug formulations is control over the end products' properties[11]. Encapsulating agents plays a major role in retention of bioactive compound during processing and provide longer shelf life of the products[8]. Maltodextrin (MD), with its higher stability and low viscosity act as suitable common carrier agent that facilitate the spray drying process[13]. Additionally, spray-dried juice powder made from lemons has good reconstituting properties as well as good storage and longer shelf life with convenient handling and transportation.

The object of this research is to analyse antibacterial activity of spray dried encapsulated lemon juice powder with respect to its raw juice on storage of 90 days against pathogenic bacteria gram positive *Escherichia coli* and gram negative *Staphylococcus*

aureus, gentamicin as positive control.

Materials :

Thefresh fruit samples of lemonwere purchased from Mangaldai, Assam, India. The fruits were stored in a refrigerator at 4°C until further use.Chemicals includes maltodextrin (MD), culture media includes tryptic soy agar (TSA), trypic soy broth (TSB), luria bertani broth (LBB), luria bertani agar, muller hinton agar (MHA), bacterial srains includes *Escherichia coli* (*E. coli*) reference number ATCC®10536™, *Staphyloccocus aureus (S. aureus)* reference number ATCC®25923™ were from ATCC, gentamicin 10mcg/disc. The glassware's used were obtained from Borosil Glass Works Ltd.

Extraction of Citrus limon juice :

The fruits were cleaned and washed properly first with running tap water to again rinsed with distilled water. The peel removed with a sharp stainless steel knife and the fruit was again washed with distilled water. The juice was extracted using a screw press extractor and made it a concentrated extract. This extract was filtered using a muslin cloth to remove any insoluble materials and transferred to a screw capped glass bottles.

Spray dried juice solution preparation :

Initially MDwas added to the single batch of formulated lemon juice at different percentages such as10, 15and 20% level at 150°C inlet temperature using a laboratory scale spray drier. The level chosen based on preliminary runs(not reported here) and from literature review where 10% MD stuck in the drying chamber and powder gets sticky[9]. On an average, 15% MD gave a better powder quality on its reconstitution. In 100 ml of filtered juice 15% of MD was added and maintained brix at 10°B by diluting it with distilled water and stirred thoroughly in a magnetic stirrer to get the uniformity in the solution[19]. Constant feed flow rate for formulations with 10ml/min with the size of nozzle 0.5mm respectively. Obtained powder were sealed in airtight glass container and stored at refrigerated temperature and analysed further its antibacterial properties.

Bacterial strains and culture conditions :

Characterized *E. coli* ATCC 10536 and *S. aureus* ATCC 25293 were activated by inoculating a loop full of the strain in luria bertani broth and tryptic soy broth and incubated on a rotary shaker for 24hrs at 37°C. strains were sub cultured and marinated slants at 4°C for further use. Stock cultures were maintained on respective broth medium with 10% glycerol at -80°C. turbidity of bacterial suspension was maintained using 0.5 McFarland standard, density 1.5×10^8 cfu/ml[20].

Antibacterial testing :

Antibacterial activity was performed by agar disc diffusion method[20]. Bacterial inoculum was spread over muller hinton agar plates using L-shaped slass rods for obtaining uniform microbial growth. 20µl of extract at a concentration of 10 mg/ml of spray powder (SP) and raw juice (RJ) was loaded intosterile blank disc with 10µl initially and allowed to dry and next 10 µl to ensure precise load of extracts. Dimethyl sulfoxide (DMSO) loaded

discs were used as negative control and gentamicin 10mcg/disc taken as positive control for all *E. coli* and *S. aureus* strains. All discs were dried fully and incubated for 16 hours at 37°C. after complete incubation periods diameter of inhibition zone (IZ) around the discs were measured to evaluate the antibacterial activity and expressed as mean zone of inhibition diameter produced by the extract spray dried powder and raw juice using Hi-Antibiotic Zone Scale-C and results were categorized as resistant, sensitive and in intermediate zone respectively[2,10]. Experiments were conducted in triplicates to obtain mean values for standard deviation calculation. For measuring relative efficacy, activity index was calculated using following formula:

$$\text{Activity index} = \frac{\text{zone of inhibition of the fruit extract}}{\text{zone of inhibition of gentamicin}}$$ (Liya *et al*., 2018)

In present study the antibacterial activity of *Citrus limon* Burm raw juice and spray dried juice powder aqueous extract were evaluated against *E. coli* ATCC 10536 and *S.aureus* ATCC 25923. Aqueous extract of lemon powder shows positive results and calculated in ± mean value of standard deviation. Table-1 shows the effect of raw juice and spray dried powder aqueous extract, wherespray dried lemon powder shows highest antibacterial activity on *S. aureus* even in 90 days of storage and strong antibacterial activity. The result of present study were in mean of inhibition zone of all at 10mg/ml concentration, showed antibacterial effect and this result were in agreement with Reddy *et al.,*[15]. Results indicated thataqueous extract of lemon juice powder exhibit better and similar inhibitory effect on *S. aureus* and *E. coli*. To extend the shelf life of perishable food spray dried micro particles could be used in antibacterial food system

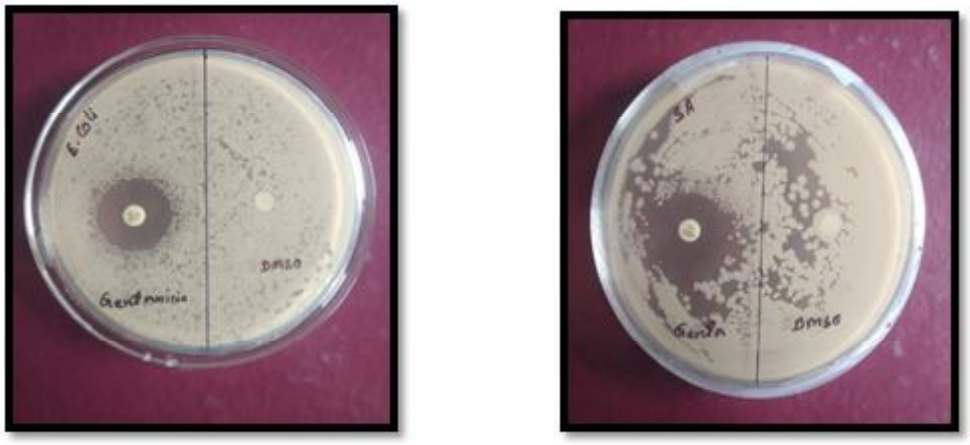

Figure 1. Inhibition zone of GEN 10mcg/disc as positive control and DMSO as negative control on *E. coli* ATCC 10536 and S. aureus ATCC 25923

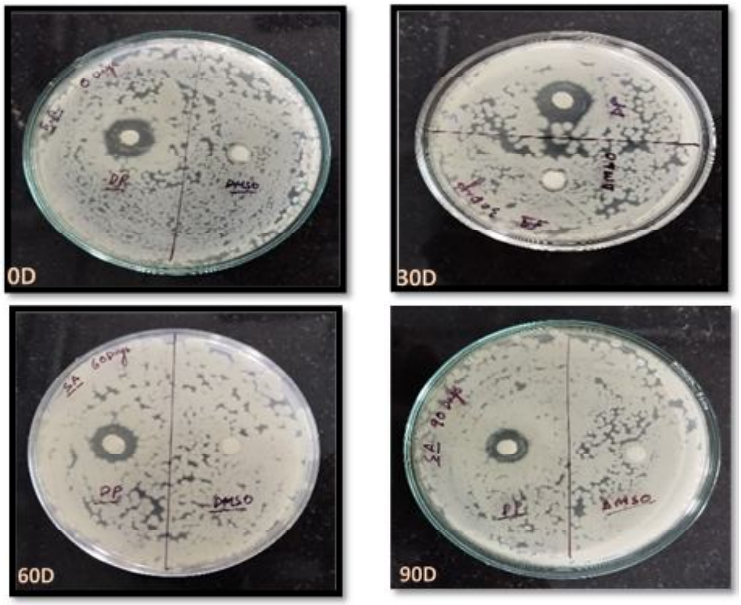

Figure 3. Antimicrobial activity of lemon aqueous extract on 90 days of storage on *S. aureus* ATCC 25923 , DMSO: negative control

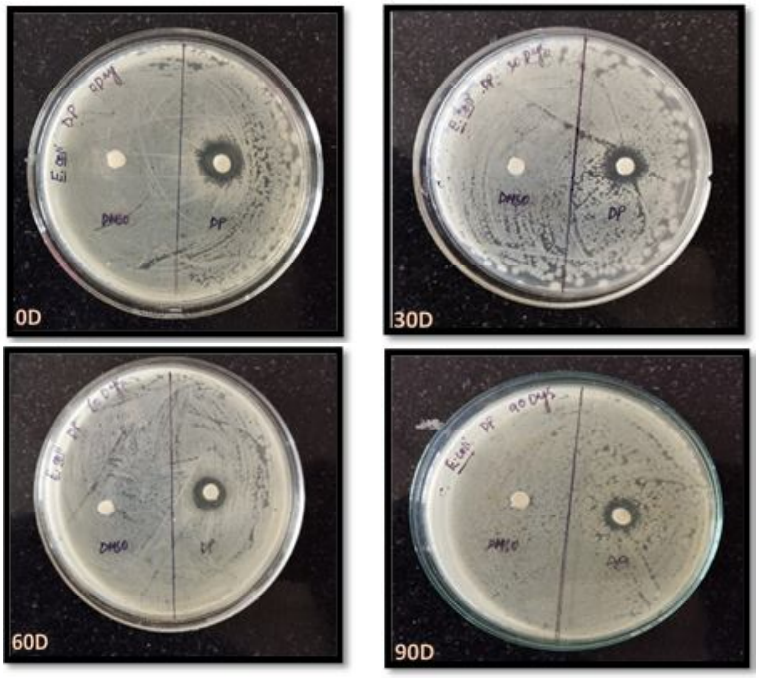

Figure 4. Antimicrobial activity of lemon aqueous extract on 90 days of storage on *E. coli* ATCC 10536, DMSO: negative control

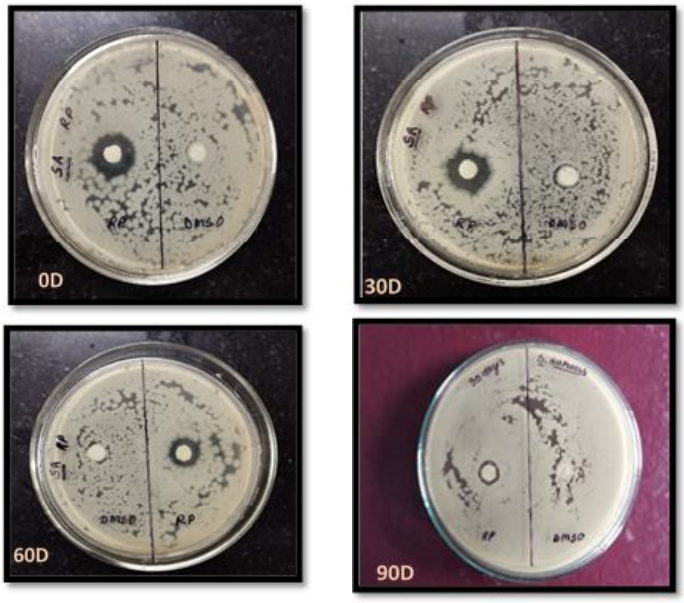

Figure 5. Antimicrobial activity of lemon on 90 days of storage on *S. aureus* ATCC 25923, DMSO: negative control

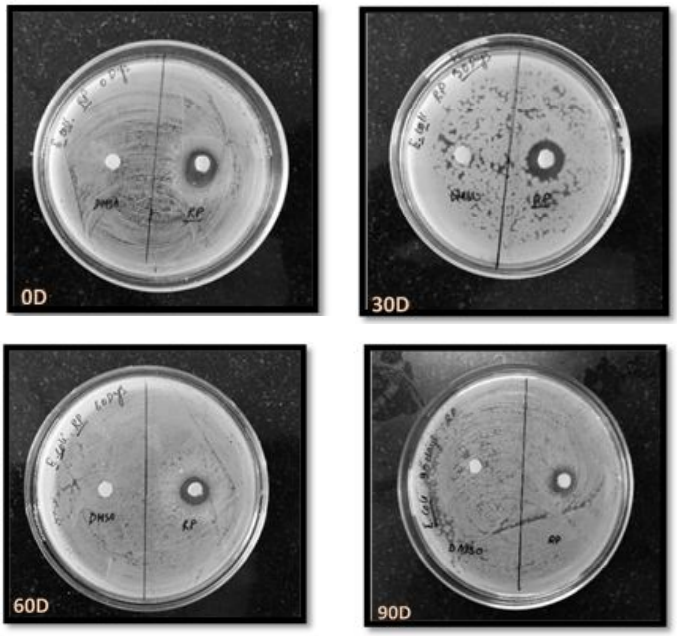

Figure 6. Antimicrobial activity of lemon on 90 days of storage on *E. coli* ATCC 10536, DMSO: negative control

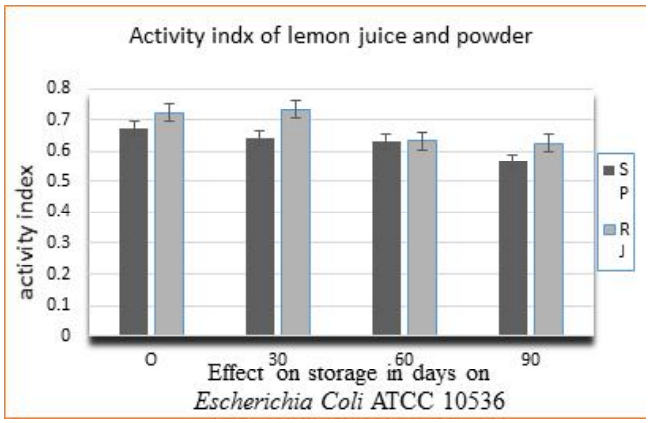

Figure 7. Activity index of lemon RJ and SP aqueous extracts on *Escherichia coli* ATCC 10536 (RJ: raw juice, SP: spray dried powder)

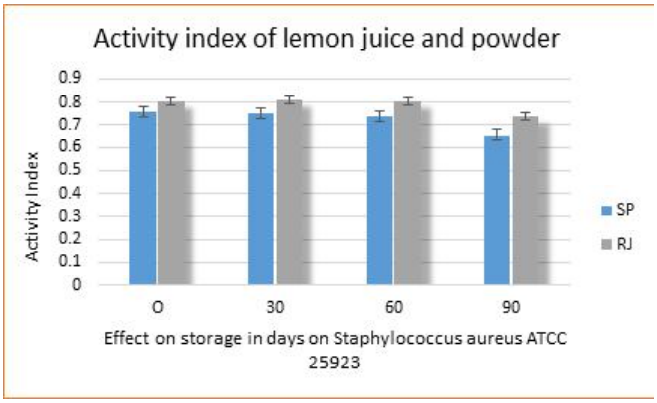

Fig. 5. Activity index of lemon RJ and SP aqueous extracts on Staphylococcus aureus ATCC 25923 (RJ: raw juice, SP: spray dried powder)

Table-1. Antimicrobial test results of raw lemon and spray dried lemon powder aqueous extract

Strain	Days	Zone of inhibition (mm)				Activity Index		Category	
		SP	RJ	Positive Control	Negative Control	SP	RJ	SP	RJ
Escherichia coli ATCC 10536	O	13 ± 0.12	14 ± 0.34	19 ±0.34	-	0.68±0.01	0.73±0.11	I	I
	30	12 ± 0.22	14 ± 0.33			0.63 ± 0.01	0.73 ± 0.23	I	I
	60	12 ± 0.11	12 ± 0.21			0.63 ± 0.06	0.63 ± 0.09	I	I
	90	11 ± 0.21	12 ± 0.42			0.57 ± 0.09	0.63 ± 0.11	I	I
Staphylococcus aureusATCC 25923	O	15 ± 0.33	16 ± 0.29	20±0.41	-	0.75 ± 0.01	0.81 ± 0.12	I	S
	30	15 ± 0.27	16 ± 0.41			0.75 ± 0.09	0.81 ± 0.04	I	S
	60	15 ± 0.28	16 ± 0.37			0.75 ± 0.04	0.81 ± 0.05	I	S
	90	13 ± 0.18	15 ± 0.76			0.65 ± 0.01	0.75 ± 0.05	I	I

(SP: Spray powder, RJ: Raw juice, I: Intermediate, S: Sensitive)

Citric acid in lemon provides a higher degree of acidity that bacterial growth can be inhibited by acidic pH that cause decrease of internal pH of bacterial cells. Presence of flavonoids also able to act in bacterial cell proteins denaturationand exhibit ability to from complex with bacterial cell walls. On spray drying antibacterial activity of juice was improved as encapsulation provides a coating to volatile compounds and stabilised its bioactivity. Results shows, microencapsulation and spray drying shows zone of inhibition in intermediate and sensitive zone at 12 to 16 mm in raw juice and spray dried juice powder which had a strong antibacterial property more in *S. aureus*res pectively. Therefore, this study indicates that spray dried lemon powder holds favourable characteristics to act as novel alternative for development of commercial antibiotics that could use further.

In the present study it was observed that *Citrus limon* juice extract and spray dried juice powder showed intermediate to sensitive antibacterial activity against*S.aureus* where gentamicin as positive control shows 20mm zone of inhibition. This results shows that *Citrus limon* spray dried powder retained its antibacterial compounds which shows its bioactivity even at a higher temperature. These results could be useful in formulation of antibacterial drugs that could be useful to discover natural bioactive products.

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