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Antioxidant and Antimicrobial Potential of Ripen and Unripe Juice of *Citrus limon*

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ABSTRACT : *The present study was designed to investigate in vitro antioxidant activity of of both ripen and unripe juice in methanol and antimicrobial potential against two bacterial strians i.e. E. coli and Pseudomonas aeruginosa. A number of antibiotic resistant bacterial strains have been increasing in the past years. So natural products are alternative to such diseases. Among which Citrus has gained a separate dimension. The DPPH scavenging activity of the juices was determined and found to be highest at 100µL/mL as 92.71±0.521 and 94.58±1.100 for ripen and unripe fruit respectively. Antimicrobial potential was determined in terms of MIC and was calculated for the juices and found to be 0.625(v/v) for E.coli for both ripen and unripe juice. However, for Pseudomonas aeruginosa the MIC for ripe was recorded at 0.3125(v/v) and for unripe MIC was found to be at the concentration of 0.625(v/v) which is same as for E.coli. The results obtained reveal that Citrus juice is rich in antioxidant and antimicrobial properties. This provide the base for its use in normal diet and herbal medicine at the global level.*

KEYWORDS : *Antibiotics, anthocyanin, ascorbic acid, carotenoids and phytochemical*

I. INTRODUCTION

Antioxidants are the substances, compounds or nutrients in our foods which can prevent or slow oxidative damage to our bodies. These agents are able to remove the deleterious effects of free radicals within our body. Nowadays, considerable interest is focused on the development and evaluation of natural antioxidants and radical scavengers from plant materials which are rich in polyphenolic compounds. Ascorbic acid is the most important antioxidant in citrus fruit juices and it protects the organism from oxidative stress [1], [2] & [3]. Flavonoids which occur in Citrus fruit are important antioxidant. Pharmacological industries are producing a number of antibiotics; resistance to these drugs by microorganisms is also increasing. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [4]. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [5]. For a long period of time, plants have been a valuable source of natural products for maintaining human health. The use of plant extracts and photochemical, both with known antimicrobial properties can be of great significance in therapeutic treatments [6]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant [7]. These products are known by their active substances e.g. the phenolic compounds which are part of the essential oils, as well as tannin [8].

The antimicrobial properties of lemon was investigated and found that lemon possesses significant antimicrobial activity against *Staphylococcus aureus*, *Klebsiella*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* [9]. The emergence of antibiotic-resistant microorganisms had swiftly reversed the advances of previous fifty years of research on antibiotics [10]. It has also been used as an anti-diabetic [11], antifungal, hypotensive agent, antioxidant, carminative, insect repellent, antibacterial, antiviral [12], uricosuric, anti-yeast, antihepatotoxic and antimutagenic agent. Therefore, it has been a challenge for the researchers to overcome these problems. Over the past two-three decades, researchers have turned their eyes towards the traditional folk medicines or natural products to uncover the scientific basis of remedial effects as antibacterial agents. Beside plants, fruits also have been studied by the researchers for the presence bioactive compounds close related with herbs, commonly referred as phytochemicals such as tannins, carotenoids, polyphenols and anthocyanins. The current research focuses on the determination of antimicrobial activity of Citrus fruits basically lemon which can lead a great change in pharmaceutical industry. The main objective of this antimicrobial study is to identify and to demonstrate antimicrobial activity of lemon juices against two specific bacteria.

II. MATERIALS AND METHODS

2.1 Preparation of juice extract

Juice extract of concentrations crude juice, 0.5, 0.25, 0.125, 0.062.5 and 0.031.25 (v/v) was prepared in water. Prepared extracts of different concentrations were used for the antimicrobial screening as per the protocol given by agar well diffusion method.

2.2 Antioxidant activity by DPPH free radical scavenging assay

The scavenging activity was determined by using DPPH synthetic free radical [13]. Different concentration of juices were prepared (from 12.5µL to 100µL) in methanol. After incubation at room temperature for 30 min, optical density was measured at 517nm and the scavenging activity was calculated by the formula-

Scavenging activity (%) = [(A-B)/A] X 100

Where A = absorbance of DPPH and B = absorbance of fruit juice and DPPH combination.

2.3 Antimicrobial activity and MIC determination

After sterilization, the nutrient agar media was poured on to the plates (15 cm diameter) aseptically and after polymerization 1cm well was prepared. To the center well of each plate, 400µL of streptomycin sulphate (1mg/mL) was transferred and all other wells were adminstrated with juice extracts serially from the original juice upto lowest concentration. Plates were incubated at 37°C for 24 hrs. The diameter of the zone of inhibition was determined and recorded.

III. RESULTS AND DISCUSSION

Table1. Antioxidant activity by DPPH free radical scavenging property (R-ripen, UR-unripe)

Conc. (µL/mL)	UR	R
25	55.81±0.578	50.823±0.388
50	78.07±0.226	77.670±0.606
75	91.53±1.290	89.33±0.388
100	94.58±1.100	92.71±0.521

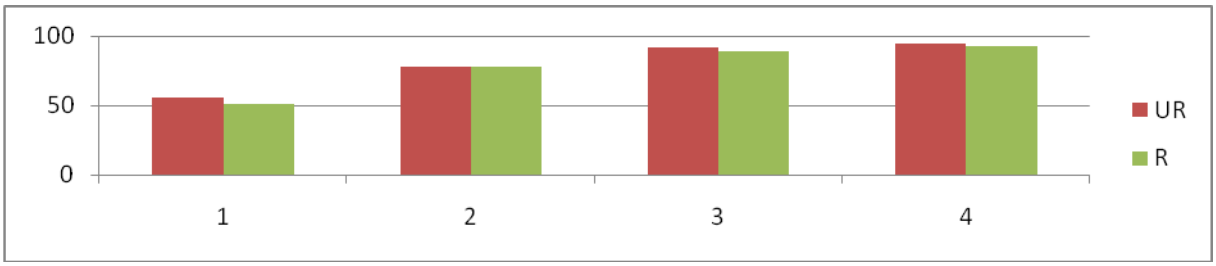

Figure1. Antioxidant activity by DPPH free radical scavenging property (R-ripen, UR-unripe)

Table2. Antimicrobial activity (NZ – No Zone, R-ripen, UR-unripe).

Microbes	Streptomycin (cm)		Zone of inhibition in different juice concentration (cm)											
			Original (pure)		0.5(v/v)		0.25(v/v)		0.125(v/v)		0.625(v/v)		0.3125(v/v)	
	UR	R	UR	R	UR	R	UR	R	UR	R	UR	R	UR	R
E. coli	0.48	0.48	1.0	1.0	0.8	0.8	0.6	0.7	0.3	0.4	0.1	0.2	NZ	NZ
Pseudomonas aeruginosa	0.48	0.45	0.8	1.0	0.7	0.9	0.5	0.6	0.3	0.4	0.2	0.3	NZ	0.1

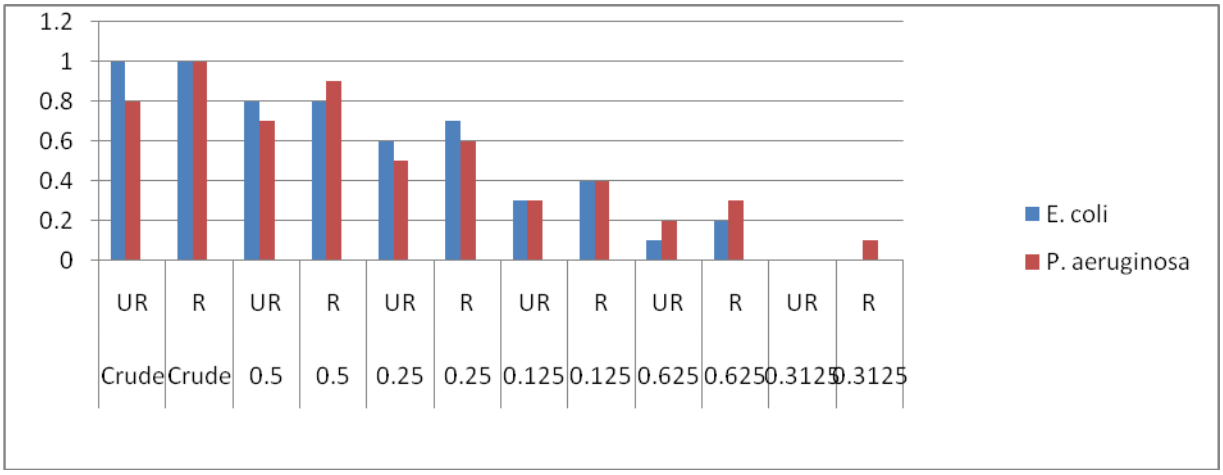

Figure2. Antimicrobial activity (R-ripen, UR-unripe).

IV. CONCLUSION

Antioxidant and antimicrobial properties of plant have been showing a very high impact in the industrial field. Everyone is concerned with the high and growing number of diseases associated with microorganisms especially bacteria and fungi. Till date the mechanism of action and the interaction between these compounds have not been completely understood; in fact, the resulting antimicrobial effect is not always the sum of the single effect, as antagonistic and synergistic interactions. The free radicals are harmful and toxic to the body. They lead to oxidative stress. From the present study it can be concluded that antioxidant and antimicrobial potential is show by the lemon variety used. The variety used in the present study is commonly available in market and is consumed by almost all class of people. If the local variety can be used in normal diet and is commercialized at the industrial level, will lead to the growth of health and economy as well.

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