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EVALUATION OF THE CHEMICAL CONSTITUENTS AND THE ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *CITRUS KARNA* FRUIT PEEL

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Key words:

Citrus karna, Essential oil, GC-MS, Zone of inhibition, MIC

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ABSTRACT: The present study was concerned with analysis of the chemical constituents of essential oil of *Citrus karna* fruit peels by GC-MS technique and also its antibacterial activity against some pathogenic bacteria. The essential oil was extracted from fruit peels by steam distillation using Clevenger's type apparatus. GC-MS analysis of essential oil showed presence of D-limonene and β-pinene as the major constituents. The antibacterial activity was detected by agar well diffusion method against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphyloceceus aureus*. The zones of inhibitions obtained were recorded and analyzed against standard control of Ampicillin. The essential oil showed higher antibacterial activity of 18.0 mm against *P.aeruginosa* and least antibacterial activity of 13mm against *B. subtilis*. The MIC of the essential oil on the susceptible bacterial isolates was between 111-333µg/ml. Present study concludes that essential oil from fruit peels of *Citrus karna* have a broad spectrum antibacterial activity against human pathogens.

INTRODUCTION: The genus Citrus, belonging to the Rutaceae or Rue family, comprises of about 140 genera and 1,300 species. *Citrus sinensis* (Orange), *Citrus paradise* (Grapefruit), *Citrus limon* (Lemon), *Citrus reticulate* (tangerine), *Citrus grandis* (shaddock), *Citrus aurantium* (sour orange), *Citrus medica* (Citron), and *Citrus aurantifolia* (lime) are some important fruits of genus Citrus [1]. Citrus fruits and their by-products are of high economic and medicinal value because of their multiple uses, such as in the food industry, cosmetics and folk medicine [2]. In addition to large scale consumption as fresh fruits, the Citrus fruits are mainly processed to produce juice. Specifically, the Citrus peels, commonly treated as agro-industrial waste, are a potential source of valuable secondary plant metabolites and essential oils [3].

Citrus peel essential oils are reported to be one of the rich sources of bioactive compounds namely coumarins, flavonoids, carotenes, terpenes and linalool etc [4]. Recently, Citrus peel essential oils have also been searched for their natural antioxidant and antimicrobial properties [5, 6]. It is widely accepted that biological activities of plant materials are strongly linked with their specific chemical composition, mainly the secondary metabolites such as plant phenolics and flavonoids [7]. Increase in the emergence of new bacterial strains that are multi-resistant coupled with the non-availability and the high cost of new generation antibiotics have resulted in increase morbidity and mortality [8, 9]. Keeping in view the significance of Citrus essential oils, the present

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investigation aimed at study of chemical composition of essential oil of *Citrus karna* fruit peels and evaluation of its antibacterial activity against some pathogenic bacteria was taken.

MATERIALS AND METHODS:

Plant Material:

The fruits of *Citrus karna* were collected from the Horticulture fields of SHIATS, Allahabad in the month of January, 2013. The plant material was identified and authenticated in the post graduate department of Horticulture, SHIATS, Allahabad.

Extraction of essential oil:

The sample of fresh and ambient dried Citrus peels was subjected to hydro distillation for 3h using a Clevenger type apparatus. Distillates of essential oil were dried over anhydrous sodium sulphate, filtered and stored at -4^{0}C until analyzed**[10].**

The amount of the oil obtained from citrus peels was calculated as –

$$\text{Oil (\%v/w)} = \frac{\text{observed volume of oil (ml)}}{\text{weight of sample (g)}} \times 100 = 1.86\%$$

GC-MS analysis of essential oil:

The essential oils were analyzed by GC/MS according to [11]. GC/MS analysis was performed on a Thermo quest Finnegan Trace. GC/MS was equipped with column (60mx0.25mm) with film thickness 0.25µm. The injections temperature was 200^{0}C and oven temperature was raised from 60^{0}C. (5min hold) to 260^{0}C (10 min hold) at a rate of 4^{0}C/min. transfer line temperature was 260^{0}C. 1µml of sample was injected and helium was used as the carrier gas at a rate of 1.0 ml/min. the mass spectrometer was scanned over the 40 to 60 m/z with an ionizing voltage of 70eV and identification was based on standard mass to detected possibilities of essential oils components.

Antibacterial activity assay:

Test organisms:

The organisms used comprises of two gram-positive bacteria (*S. aureus* and *B. subtilis*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*). The test organisms were obtained from the Research Laboratory of Microbiology and Fermentation Technology SHIATS, Allahabad, India.

Agar well diffusion method:

Agar well diffusion method elucidated by [12, 13] with modifications was followed. The antibacterial activity of essential oil of *Citrus karna* fruit peels against four pathogenic bacteria was evaluated by using agar well diffusion method. The Nutrient agar plates were prepared by pouring 15 ml of molten media into sterile petri-plates. About (10^8-10^9) colony- forming unit per ml were used. Wells or cups of 5mm size were made with sterile borer into agar plates containing the bacterial inoculum. 10µL of microbial broth was spread on the surface of nutrient agar plates; 20µL volume of the essential oil of density (2.0mg/ml) measured by weight: volume ratio was poured into a well of inoculated plates. Ampicillin (2mg/ml) was used as a positive control which was introduced into the well instead of essential oil.

Solvent DMSO was used as a negative control which was introduced into well instead of essential oil. The plates thus prepared were kept at room temperature for ten minutes allowing the diffusion of the extract into the agar [14]. After incubation for 24 hrs at 37^{0}C, the plates were observed. The antibacterial activity was present on the plates; it was indicated by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters. Antibacterial activity was recorded when radius of zone of inhibition was greater than 4 mm [15]. The antibacterial activity results was considered as inactive if < 4.5 mm; 4.5-6 mm as partially active ; while 6.5-9 mm as active and greater than 9mm as very active [16].

Minimum Inhibitory Concentration assay:

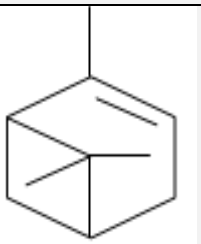
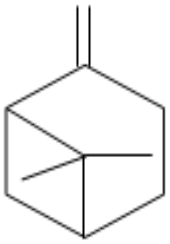
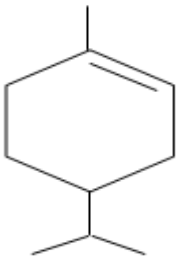
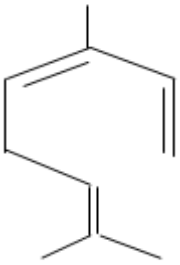
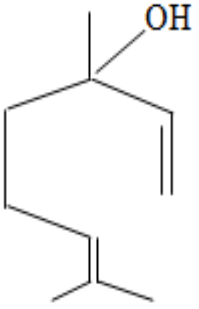
Minimum Inhibitory Concentration of essential oil from fruit peels was also determined [17, 18]. Dilution of the essential oil of *Citrus karna* fruit peels was prepared in sterile nutrient broth to achieve a decreasing concentration ranging from 1000µg/ml to 12µg/ml in sterile tubes labeled 1 to 5. Each dilution was seeded with 10µl of the standardized bacterial inoculums (108-109) CFU/ml. The inoculated culture tubes were incubated at 37°C for 24hrs. A set of tubes containing only seed broth (*i.e.* without essential oil) was kept as control. The lowest dilution of the essential oil that retained its inhibitory effect resulting in no growth (absence of

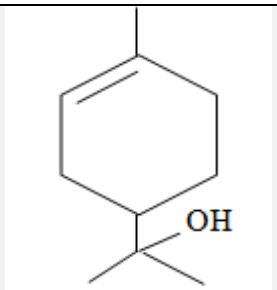
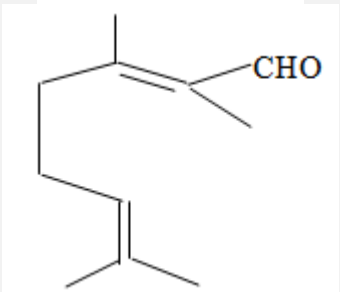
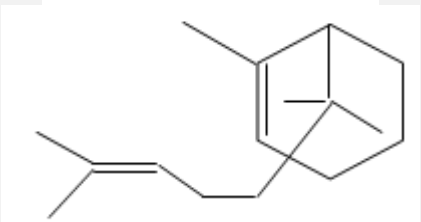
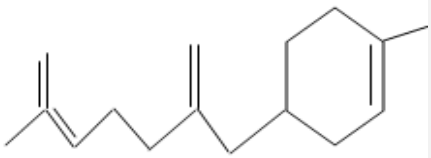
turbidity) of a microorganism was recorded as the MIC value of the extract. After incubation, 10µl of content of each test tube was transferred with a loop on to nutrient agar media. Agar plates were incubated for 24 hours at 37°C. The lower concentration that did not permit any visible growth when compared with the control was considered as the MIC.

The components present in the essential oil were analyzed by GC/MS using the retention time of the components. The GC/MS analysis of the essential oil shows the following compounds (**Table 1**) along with their structure and percentage. It shows that there are nine components present in the essential oil, while the main components being the limonene (93.50%), β-pinene (2.979%) and α-pinene (0.792%).

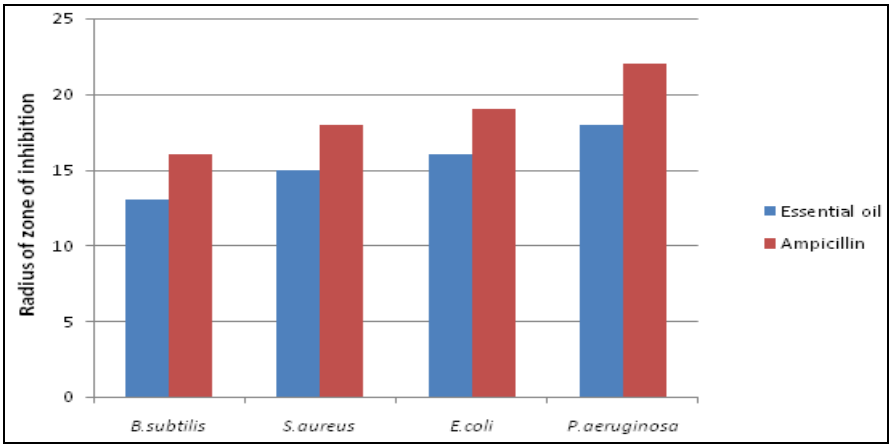
RESULTS AND DISCUSSION:

TABLE 1: GC/MS ANALYSIS OF ESSENTIAL OIL

S.No.	Compounds	Percentage	Retention Time	Structure
1	α-pinene	0.792	12.865	
2	β-pinene	2.979	14.996	
3	Limonene	93.50	16.831	
4	Cis-ocimene	0.235	17.347	
5	Linalool	0.206	19.554	

6	α-terpineol	0.142	23.461	
7	(E)-Citral (geranial)	0.150	25.970	
8	α-bergamotene	0.448	31.567	
9	β-bisabolene	0.365	33.879	

Antibacterial activity: The antibacterial activity reveals that essential oil from *Citrus karna* fruit peels is highly active against both Gram positive and Gram negative bacteria. The essential oil shows highest zone of inhibition (18mm) *P.aeruginosa* against followed by (17mm) zone of inhibition against *E.coli*, (15mm**)** against *S.aureus* and lowest (13mm) zone of inhibition against *B.subtili*. **Graph 1** summarizes the microbial growth of methanolic extract and ampicillin used as a positive control



GRAPH 1: ANTIBACTERIAL ACTIVITY (IN MM) OF ESSENTIAL OIL AND AMPICILLIN (STANDARD)

Ampicillin used as a positive control showed wide zones of inhibition against all the test organisms while dimethyl sulphoxide (DMSO) negative control shows no zones of inhibition. It was completely resistant to all the test organisms. The MIC which is the lowest concentration of a plant extract that still retained an inhibitory effect against the growth of a microorganism was assessed by using broth dilution method. The MIC values of essential oil of *Citrus karna* fruit peels for different pathogenic bacteria were ranged from 111 to 333µg/ml (**Table 2**).

TABLE 2: MIC OF *CITRUS KARNA* ESSENTIAL OIL (µg/ml) AGAINST SELECTED BACTERIA

Conc. (µg/ml)	*Citrus karna* Essential oil (µg/ml) against bacteria			
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
1000	-	-	-	-
333	*	-	*	*
111	+	*	+	+
37	+	+	+	+
12	+	+	+	+
4	+	+	+	+
MIC	**333**	**111**	**333**	**333**

(+) Growth, (*) MIC, (-) No Growth

CONCLUSION: From the present investigation, it may be concluded that essential oil from *Citrus karna* fruit peels has potent antibacterial activity against both Gram positive and Gram negative bacteria due to the presence of different constituents mostly terpenoids. However, further studies are needed to isolate the active molecule responsible for antibacterial activity. The study thus may lead to the formulation of a potent antimicrobial agent from natural sources.

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