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RESEARCH ARTICLE

Screening of Peel Extracts as Antioxidants, Anticancer Agents and Antimicrobials

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ABSTRACT

In India several plants and plant derived products have been inferred for various studies whose phytochemicals reveals various apoptotic, antiproliferative, antioxidant and antimicrobial activities which gains clinical significance. The present study aimed at exploiting the various bioactive metabolites in pomegranate and sweet lime peel extracts for use as antimicrobial compounds and as anti cancerous agents. The dried mill powdered peels were extracted with various solvents - methanol, ethyl acetate, chloroform and water and their phytochemistry revealed the potency of methanolic extract being a rich source in extracting the phytochemicals for their antimicrobial activity against the pathogenic E. coli, Pseudomonas sp., Klebsiella sp., Vancomycin and Methicillin Resistant Staphylococcus aureus. Also MTT assays conferred that 50µg/ml concentration of sweet lime and pome peel methanolic extract showed efficacy against Hep - 2 and MCF - 7 cell lines respectively. Further, antioxidant activity via DPPH assay concluded that the methanolic extract of sweet lime and pome was potent than the other extracts. Hence, these results provide rationale to develop sweet lime peel extract enriched with secondary metabolites into a value added nutraceutical product for cancer prevention.

***KEYWORDS**: Sweet lime, Hep -2, MCF -7, MRSA, Antioxidants*

INTRODUCTION

In India several plants have been inferred for various studies for their medicinal value. Recent studies is much concentrated on the plant derived products like fruit pulp juices, seed, flower etc. whose phytochemistry reveals various apoptotic, anti proliferative, antioxidant and antimicrobial activities which gains clinical significance in the field of drug discovery and therapeutics [1].

Among various fruits of commercial value, pomegranate (*Punica granatum*) and sweet lime (*Citrus limetta*) are known worldwide for their delicious taste and health promoting properties. Recent studies indicate that the peel yields 1000 folds more phenolic compounds than pulp. The pomegranate (*Punica granatum* L.) is one of the oldest widely grown edible fruit in tropics. Reviews suggest that flavonoids and phenolics were significantly greater in peel than the pulp and hence their fruit husk extracts shows antiproliferative activity against a panel of human oral, colon and prostate cancer cell lines [1]. Similarly, Citrus fruits were historically used for their high content of vitamin C. Various studies elucidate their total radical- trapping anti oxidative potential (TRAP) that the TRAP was significantly higher in peels than in peeled fruits [2]. These citrus fruits contain high concentrations of phenols, hydroxycinnamates, flavonoids, glycosides, herperidin and its flavone analogue, diosmin, etc., that all have exhibited anti carcinogenic activities in various *in vivo* studies [3].

The present study aims at exploiting the various bioactive metabolites in pomegranate and sweet lime peel extracts for use as antimicrobial compounds. Also further studies to use them as anti cancerous agents can be elucidated via *in vitro* animal tissue culture techniques. Inferences on the qualitative analysis of phytochemicals and their major importance in clinical areas- more specifically against the emerging drug-resistant bacteria and also in breast and liver cancer cell lines were also determined.

MATERIALS AND METHODS

Collection of fruit peels

The pomegranate and sweet lime peels were collected from in and around Chennai fruit stalls. The peels were shade dried and mill-powdered.

Extraction of Metabolites

20 grams of the powdered peel material was weighed and extracted using different solvents such as methanol, ethyl acetate, chloroform and water in a soxhlet apparatus, filtered using the distillation flask and dried in a rotary vacuum evaporator. The crude extract was collected, partially dried and stored in a cool place until use.

Antimicrobial Activity

The antimicrobial activities of the crude extracts obtained with different solvents were screened against *Pseudomonas sp., Escherichia coli, Klebsiella sp., Methicillin resistant Staphylococcus aureus and Vancomycin resistant Staphylococcus aureus* using agar well diffusion assay methods [4]. Muller Hinton agar plates were inoculated with 0.5 McFarland standard of the test organism and 5mm diameter wells were punched and filled with 30µl of the extract. After 24 hrs of incubation at 37°C, diameters of the zones of inhibition were measured.

Antioxidant Activity (DPPH Method)

The free radical scavenging activity of the peel extract and the standard reference compound was analyzed by the DPPH assay. In this assay, 100 µl of 0.01% methanolic 2, 2-diphenylpicrylhydrazyl (DPPH) was added onto 50µl of the extract in a microtitre plate and incubated for 30minutes in dark. Positive samples with observed discoloration from purple to yellow were used for quantitative analysis. In quantitative assay (5) 0.2ml of extract at a concentration of 25, 50, 75 and 100 µg/mL was combined with 2.7ml of methanol and 1ml of 0.1 % methanolic DPPH, incubated for 30 mts in dark and the absorption maxima were measured at 517nm. The control contained all reagents except the extract fraction while methanol was used as blank. 0.16% of Butylated Hydroxy Toluene (BHT) was used as the standard.

$$\%\,\text{Antioxidant activity} = \frac{\text{O.D. of control O.D. of sample}}{\text{O.D. of control}} \times 100$$

Phytochemical Analysis

The extracts were subjected to various phytochemical tests to determine the active constituents present in the crude extracts obtained with different solvents. Qualitative presence of alkaloids, carbohydrates, glycosides, flavanoids, tannins, phenolics and saponins were analysed. The slightly modified method of Okerulu and Ani [6] was used.

Anticancer Activity of the peel

The cell lines of Hep - 2 (hepato cellular liver carcinoma), MCF - 7, (breast cancer cell line) and Vero cell lines (Control) were obtained from National Center for Cell Sciences, Pune. The cells were cultured in a growth medium (MEM, pH 7.4), supplemented with 10% fetal bovine serum (FBS) and antibiotics, penicillin (100 units/mL), and streptomycin (100 µg/mL). Since methanolic extract of peels showed the strongest antioxidant and antimicrobial activity, it was chosen for anticancer assay according to the method of Zhao *et al*., 2007. In brief, the cells were seeded into 4 wells of a 24 -well plate at 1 x 10^5 cells per well with 100 µL MEM growth medium and then incubated for 24 hours at 37◦C under 5% CO2 in a humidified atmosphere. MTT Assay was performed to assess cell viability and cytotoxicity. Different concentrations of the methanolic extract (1:2 to 1:16 dilutions - 10 µg to 150 µg) were prepared from the diluted stock (1mg /ml), and the assay was performed in MCF-7 and Hep 2 cells in tetrads. Cell Control containing drug free medium and neat drug controls were included. After 3 days of incubation at 37◦C under 5% CO2, the absorbance was determined by ELISA reader (Bio-Rad, Hercules, California, USA) at 492 nm. The concentration of drugs showing complete cytotoxic effect was recorded. The Minimum concentration nontoxic in VERO but completely inhibiting the cancer cells (MCF-7 or Hep-2) is recorded as the "effective drug concentration" The inhibition of cell growth was calculated as a percent anticancer activity using the following formula: percent anticancer activity ($A_c - A_s/A_c$) x 100%, where A_c and A_s referred to the absorbances of control and the sample, respectively.

RESULTS AND DISCUSSION

The results reveal a higher degree of antimicrobial activity with the methanolic extract comparative to other solvents extracts of pomegranate and sweet lime (Tab. 1& Fig.1). Also, the methanolic extract of pomegranate peel possessed more anti microbial efficacy even against the drug resistant (MRSA AND VRSA) *S. aureus* strains rather

than sweet lime (Tab. 1& Fig.1).

Table. 1 Anti-bacterial activities of peel extract of Pomegranate and Sweet lime:

Microorganism tested	Zone of Inhibition (in diameter, mm)							
	Pomegranate peel extract				Sweet lime peel extract			
	Methanol	Ethanol	Chloroform	Water	Methanol	Ethanol	Chloroform	Water
Pseudomonas sp.	**18**	**7**	**R**	**7**	**6**	**3**	**R**	**R**
E. coli	**20**	**10**	**R**	**7**	**3**	**R**	**R**	**R**
Klebsiella sp	**11**	**R**	**R**	**9**	**4**	**R**	**R**	**R**
MRSA	**11**	**10**	**R**	**8**	**7**	**R**	**R**	**R**
VRSA	**21**	**11**	**R**	**8**	–	–	–	–

R – Resistant, – - Nil.

Table. 2 Anti cancer activites of methanol extracts of fruit peels

Methanolic extract of peel	Cell line	% Anticancer activity			
		Concentration (μg/mL)			
		10	**50**	**100**	**150**
Pomegranate	MCF – 7	42	50	68	90
	HEP2	26	28	45	55
Sweet lime	MCF – 7	48	54	66	92
	HEP2	44	53	65	78

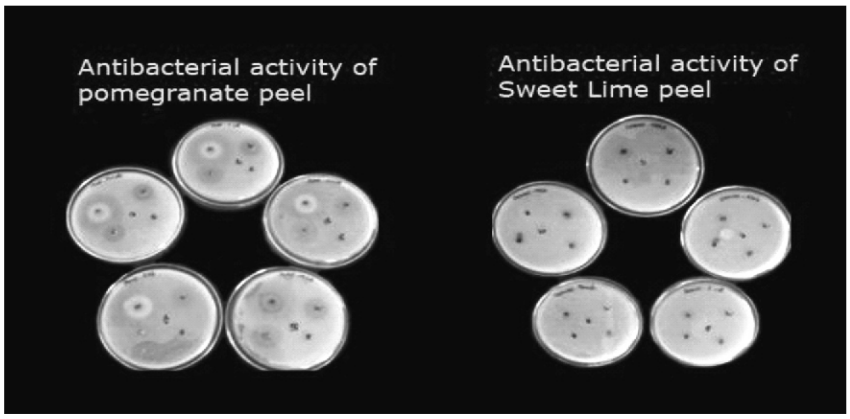

Figure.1 : Anti microbial activity of peel extracts

Further the anti oxidant activity of methanol extract of fruit peels showed a significant free radical scavenging activity generated by DPPH compared to other solvent extracts(Fig. 2). Scavenging activity of pome peels was higher than the sweet lime. However, sweet lime peels gave considerable 50% of DDPH radical inhibition revealing the presence of anti oxidants. Similar evidences can be obtained from the literature, showing the presence of polyphenol and polymethoxyphenols in citrus fruits at higher levels, owing to their antioxidant activity [1]. The phytochemistry also reveals the presence of glycosides, saponins, tannins, phenolics and flavonoids in the extracts qualitatively. However alkaloids were absent in both the peel extracts.

All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested as well as antioxidant activities. The study concludes that, the methanolic extract of fruit peel was more effective comparative to other solvents such as ethyl acetate, chloroform and water. Inferences via

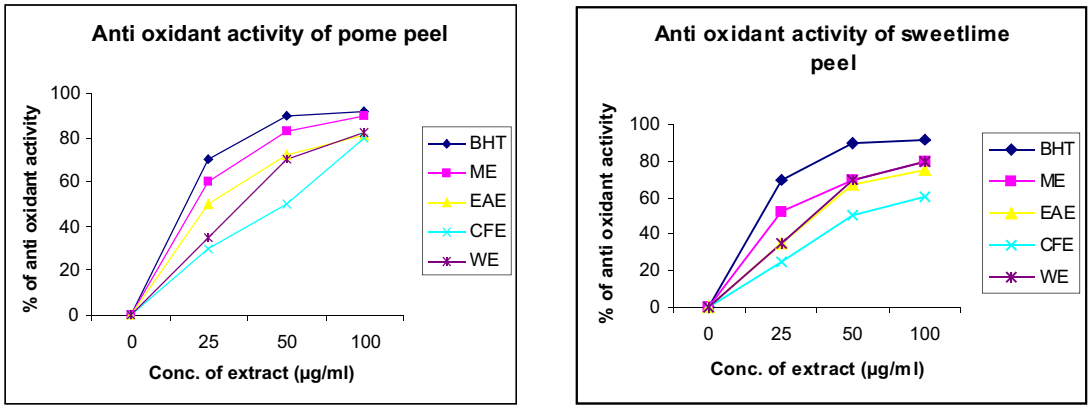

Figure.2: Anti oxidant activity of peel extracts

antibacterial and antioxidant activity of various solvents show that methanol acts as a better solvent in the extraction of various phytochemicals. The presence of phytochemicals, in particular, the phenol and flavone had already been reported in other fruit peel emphasizing to be in higher amount rather than fruit pulps. This makes suggestive of exploiting the individual type of flavones and phenolics to be isolated from the peel extract for antimicrobial and anticancer assays for further studies.

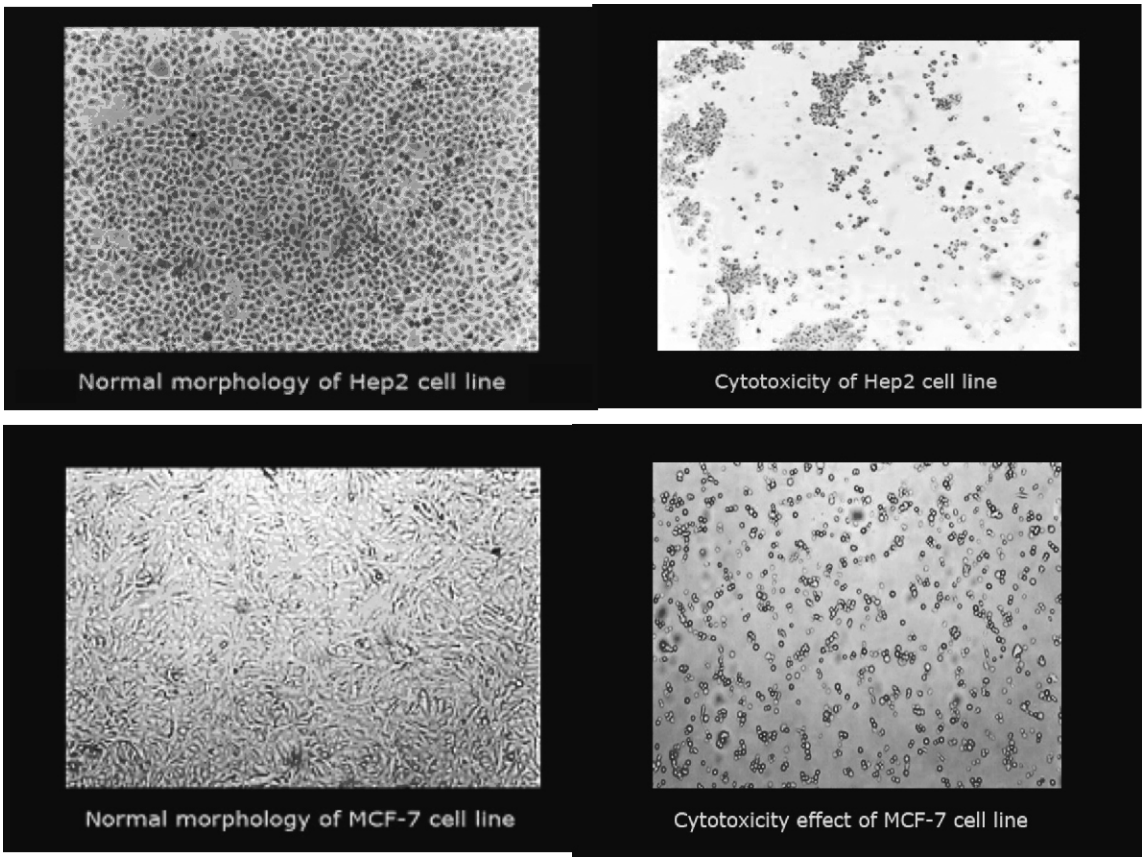

Figure.3 :Anti cancer activity of peel extracts on Hep -2 & MCF – 7 cell lines

The anticancer activity of methanolic extracts against the cell lines further revealed growth inhibition at a concentration of 50µg/ml (Table.2 & Fig. 3). Since more than 50% of anti cancer activity is considered to be significant, the activity was observed to be from the concentration of 50µg/ml for
both extracts except the pome extract against Hep 2 cell lines. Cell counting and MTT assay confers sweet lime peel extracts to be more effective being against both the cell lines compared to pome peel extract with an effective drug concentration of 50µg/ml. Hence, several anti cancerous compounds can be elucidated from sweet lime peels in future studies.

Foods rich in antioxidant phytochemicals are important for the prevention of diseases related to oxidant stress such as heart disease and cancer [1]. Supportive evidences from phytochemical analysis indicating the presence of flavones (flavanoids) and phenolic contents, contribute as natural antimicrobials as well as antioxidants. This in turn reveals the anticancerous activity of fruit peel extract against Liver and Breast cancer which can insight, in future, in drug development strategies including apoptosis. Altogether, these results provide rationale to develop sweet lime peel extract enriched with secondary metabolites into value added nutraceutical product for cancer prevention.

REFERENCES

[1] Seerama, N.P., Adamsa, L.S., Henninga, S.M., Niua, Y., Zhang, Y., Nair, M.G., & Hebera, D. (2005) *In vitro* antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *Journal of Nutritional Biochemistry*.,16**(6):**360-367.

[2] Gorinstein, S., Belloso, O.M., Milan, A.L., Fortuny, R.S., Park, Y.S., Abraham Caspi, A., Libman, I., & Trakhtenberg, S. (2002) Comparative content of some phytochemicals in Spanish apples, peaches and pears. *Journal of the Science of Food and Agriculture*., 82**(10):**1166 1170.

[3] Tanaka, T., Makita, H., & Ohnishi, M. (1997) Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis in rats by flavonoids references diosmin and hesperidin, each alone and in combination. *Cancer Res*., **57:** 246252.

[4] Perez, C., Pauli, M., & Bazevque, P.(1990) An antibiotic assay by the agar well diffusion method. *Acta Biologiae et Medicine Experimentalis* ., **15:**113-115.

[5] Blois, M.S. (1958) Antioxidant determinations by the use of a stable free radical., *Nature*., **181:**11991200.

[6] Okerulu, I.O., & Ani, C.J.(2001) The phytochemical analysis and antibacterial screening of extracts of *Tetracarpidium* conophorum. *Journal of Chemical society of Nigeria*., **26 (1):** 53 -55.