ISSN: 2395-0536

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

ISOLATION AND GEL FORMULATION OF CRUDE FLAVANOIDS FROM CITRUS FRUITS AND IT'S INVITRO ANTICANCER ACTIVITY AGAINST SKIN CANCER CELL LINES

*** P.Venkatesh, T.Durga Snehitha, B.Jhansi, K.Kishore Babu, P.Madhavi**

Department of Pharmaceutical Chemistry, Sir C R Reddy college of Pharmaceutical Sciences, Shanthi Nagar, Eluru-534007, Andhra Pradesh, India

ABSTRACT

Isolation of crude flavonoids from citrus fruits was carried out and isolated crude flavonoids were formulated to topical gel. Formulated gel was evaluated for parameters such as physical appearance, pH, spreadibility, stability, extrudability and viscosity. Results revealed formulated gel was stable and it is suitable for topical application. *In vitro* anti-cancer activity of formulated gel was carried out by MTT assay against three skin cancer cell lines (A375, NF-103, and HSC-1). Formulated gel showed moderate and good activity against cancer cell lines.

Key words: crude flavonoids, citrus fruits, topical gel, skin cancer cell lines.

Author for correspondence:
P.Venkatesh,
Department of Pharmaceutical Chemistry,
Sir C R Reddy college of Pharmaceutical Sciences, Shanthi Nagar, Eluru-534007,
Andhra Pradesh, India
Email id: venkat7pharma@redifmail.com

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (1).

Citrus fruit intake is associated with a reduced risk the risk of specific types of kidney stones. Grapefruit is another fruit juice that can be used to lower blood pressure because it interferes with the metabolism of calcium channel blockers. Lemons have the highest concentration of citrate of any citrus fruit, and daily consumption of lemonade has been shown to decrease the rate of kidney stone formation (2).

Skin cancers are cancers that arise from the skin. They are due to the development of abnormal cells that have the ability to invade or spread to other parts of the body. There are three main types: basal-cell cancer (BCC), squamous-cell cancer (SCC) and melanoma. The first two together along with a number of less common skin cancers are known as nonmelanoma skin cancer (NMSC). Basal-cell cancer grows slowly and can damage the tissue around it but is unlikely to spread to distant areas or result in death. It often appears as a painless raised area of skin that may be shiny with small blood vessel running over it or may present as a raised area with an ulcer. Squamous-cell

ISSN: 2395-0536

cancer is more likely to spread. It usually presents as a hard lump with a scaly top but may also form an ulcer. Melanomas are the most aggressive. Signs include a mole that has changed in size, shape, color, has irregular edges, has more than one color, is itchy or bleeds.

Greater than 90% of cases are caused by exposure to ultraviolet radiation from the Sun. This exposure increases the risk of all three main types of skin cancer. Exposure has increased partly due to a thinner ozone layer. Tanning beds are becoming another common source of ultraviolet radiation. For melanomas and basal-cell cancers exposure during childhood is particularly harmful. For squamous-cell cancers total exposure, irrespective of when it occurs, is more important. Between 20% and 30% of melanomas develop from moles. People with light skin are at higher risk as are those with poor immune function such as from medications or HIV/AIDS. Diagnosis is by biopsy (3-5).

Skin cancer is the most common form of cancer, globally accounting for at least 40% of cases. It is especially common among people with light skin. The most common type is nonmelanoma skin cancer, which occurs in at least 2-3 million people per year. This is a rough estimate, however, as good statistics are not kept. Of nonmelanoma skin cancers, about 80% are basal-cell cancers and 20% squamous-cell cancers. Basal-cell and squamous-cell cancers rarely result in death. In the United States they were the cause of less than 0.1% of all cancer deaths. Globally in 2012 melanoma occurred in 232,000 people, and resulted in 55,000 deaths. Australia and New Zealand have the highest rates of melanoma in the world. The three main types of skin cancer have become more common in the last 20 to 40 years, especially in those areas which are mostly Caucasian (6).

Literature review revealed that citrus fruits have been used for various diseases in folklore medicine and it was supported scientific studies. Hence we aimed to isolate flavonoids from citrus and to carry out *in vitro* anticancer activity.

MATERIALS AND METHODS

Materials

Methanol, petroleum ether, ethyl acetate, Carbapol 940, propylene glycol, methyl paraben, and Triethanolamine other chemicals used were analytical grade.

Isolation of Flavanoids

Citrus fruits collected were washed in running tap water to remove dust. Citrus fruits were shade dried, powdered, weighed and stored separately for extraction. Each of the dried powdered and weighed samples was soxhlet extracted in 80% methanol for 24 hrs and filtered. The filtrate obtained from each sample was subsequently extracted in petroleum ether, diethyl ether and ethyl acetate following the method of Subramanian and Nagarajan (7). Petroleum ether fraction was discarded due to its being rich in fatty substances. Ether fraction was used for free flavonoids whereas ethyl acetate fraction for bound flavonoids. Ethyl acetate fraction was washed with distilled water to neutrality, dried and weighed.

Procedure of gel preparation

Method for Preparation of Gel Containing Extract

1 g of Carbopol 934 was dispersed in 50 ml of distilled water kept the beaker aside to swell the carbopol 934 for half an hour and then stirring should be done to mix the carbopol 934 to form gel. Take 5 ml of distilled water and required quantity of methyl paraben were dissolved by heating on water bath. Solution was cooled and Propylene glycol 400 was added. Further required quantity of isolated crude flavanoids was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. The same method was followed for preparation of control sample without adding isolated flavanoids of the citrus fruits.

Evaluation of Topical gel formulation

Gel formulation was evaluated for physical appearance, pH, spreadibility, stability, extrudability

and viscosity at 30°c for 1st month, 2nd month and 3rd month using standard procedure.

***In vitro* anti-cancer activity of isolated crude flavanoids**

In vitro anti-cancer activity of isolated flavanoids was carried out by MTT assay against three skin cancer cell lines (A375, NF-103, HSC-1).

MTT Assay

The MTT assay, based on the conversion of the yellow tetrazolium salt-MTT, to purple-formazan crystals by metabolically active cells, provides a quantitative determination of viable cells. Cells are plated on to 96 well plates at a cell density of 2×105 mL-1 per well in 100 µL of RPMI 1640 and allowed to grow in CO2 incubator for 24 h (37 ˚C, 5 % CO2). The medium is then removed and replaced by fresh medium containing different concentrations of sample for 48 h. The cells are incubated for 24-48 h (37 ˚C, 5 % CO2). Then, 20 µL MTT ([3- (4, 5-dimethylthiazol-yl)-2, 5- diphenyltetrazolium bromide]) stock solution (5 mg/mL in PBS) is added to each well and incubated for 5 h. The medium is removed and 200 µL DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min and the optical density is measured at 560nm. Untreated cells (basal) are used as a control of viability (100 %) and the results are expressed as % viability (log) relative to the control. The percentage inhibition was determined using the formula (8, 9).

% Inhibition = 100-(optical density of sample/optical density of control) × 100.

RESULTS AND DISCUSSION

Isolation of crude flavonoids

Crude flavonoids were done by method of Subramanian and Nagarajan. The yield of the isolated crude was found to be 8.99%.

Preparation of topical gel of isolated crude flavonoids

As per method described formulae the formulation was prepared and tabulated in Table-1.

Table-1 Control and isolated flavonoids formulation prepared with these ingredients along with quantity

S.No	Ingredients	Control	Formulation
1.	Carbopol 934	1 gm	1 gm
2.	Methyl Paraben (0.5%)	0.4 ml	0.2 ml
3.	Propylene glycol 400 (5%)	5 ml	5 ml
4.	Triethanolamine (q.s)	1.2ml	1.2ml
5.	Distilled water	Upto 100 ml	Upto 100ml
6.	Isolated crude flavanoids	_	50mg

Evaluation of topical gel formulation

Gel formulation was evaluated for physical appearance, pH, spreadibility, stability, extrudability and viscosity at 30°c for 1st month, 2nd month and 3rd month. Results revealed that the evaluated topical gel was stable for three months and it is suitable for topical application.

***In vitro* anti cancer activity of isolated crude flavanoids**

In vitro anti cancer activity of formulated gel was carried out by MTT assay against three skin cancer cell lines (A375, NF-103, HSC-1) and the absorbance are given table-2.

Table-2 Absorbance of topical gel formulation against skin Cancer cell lines (A375, NF-103, HSC-1)

S.No	Concentration(ng/ml)	Absorbance		
		A375	NF-103	HSC-1
1	0.001	0.210	0.124	0.198
2	0.01	0.331	0.241	0.254
3	0.1	0.412	0.342	0.364
4	1	0.501	0.421	0.464
5	10	0.593	0.493	0.521
6	100	0.612	0.521	0.602
7	1000	0.798	0.592	0.698
8	10000	0.819	0.654	0.714
9	100000	0.912	0.698	0.798
10	Control	1.234	1.103	1.099

% inhibition of topical gel formulation was calculated using the formula

% Inhibition = 100-(optical density of sample/optical density of control) × 100

and results are given in table-3.

IC50 values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell.

Table-3 % inhibition of topical gel formulation against skin cancer cell lines (A375, NF-103, HSC-1)

S.No	Concentration(ng/ml)	% Inhibition		
		A375	NF-103	HSC-1
1	0.001	17.01	11.24	18.01
2	0.01	26.82	21.84	23.11
3	0.1	33.49	31.00	33.39
4	1	40.59	38.16	42.22
5	10	48.00	44.69	47.40
6	100	49.59	47.23	54.77
7	1000	64.66	53.67	63.51
8	10000	66.36	59.29	64.96
9	100000	73.90	63.28	72.61

IC50 values of gel formulation against skin cancer cell lines A375 was 1000 ng/ml it indicates that gel formulation showed moderate activity. IC50 values of gel formulation against skin cancer cell lines NF-103 was 1000 ng/ml it indicates that gel formulation showed moderate activity. IC50 values of gel formulation against skin cancer cell line HSC-1 was 100 ng/ml it indicates that gel formulation showed potent activity.

CONCLUSION

Crude flavanoids was isolated from citrus fruits. Isolated crude flavanoids was formulated to topical gel and evaluated. *In vitro* anti-cancer activity of formulated gel was carried out by MTT assay against three skin cancer cell lines (A375, NF-103, HSC-1). Results revealed the formulated gel possess moderate and good activity against the cancer cell lines.

ACKNOWLEDGEMENT
Authors are thankful to management Sir C R Reddy college of Pharmaceutical Sciences for providing necessary facilities to carry out this work

REFERENCES

1. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology* 2008; 7 (12): 1797-1806.
2. González CA, Sala N, Rokkas T (2013). "Gastric cancer: epidemiologic aspects". *Helicobacter* 18 (Supplement 1): 34–38. .
3. *National Cancer Institute*. Retrieved 10 June 2014.
4. "*Skin Cancer Treatment* (PDQ®)". NCI. 2013-10-25. Retrieved 30 June 2014.
5. Cakir, BÖ; Adamson, P; Cingi, C (November 2012). "Epidemiology and economic burden of nonmelanoma skin cancer.". *Facial plastic surgery clinics of North America* 20 (4): 419–22.
6. Leiter, U; Garbe, C (2008). Epidemiology of melanoma and nonmelanoma skin cancer--the role of sunlight. *Advances in experimental medicine and biology* 624: 89–103.
7. Yadav S, Padma K. Isolation of flavanoids. *International Journal of Life Sciences and Pharma Research*. 2012, 2(3), 1-5
8. Ramzi A Mothana, Ulrike Lindequist, Renate Gruenert and Patrick J Bednarski. "Studies of the in vitro anticancer, antimicrobial and antioxidantpotentials of selected Yemeni medicinal plants from the island Soqotra. *BMC Complement Alt Med*. 2009; 9:7.
9. Scudiero DA, Shoemaker RH, Paull KD. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res*. 1988; 48:4827–4833.