Anti-Psoriatic Activity of Orange Peel Extract using Monocytic Cell Line

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***Abstract*: - An orange, specifically, the bitter orange (Citrus aurantium) is the most commonly grown tree fruit in the world. Orange trees are widely cultivated in tropical and subtropical climates for the bitter fruit, which is peeled or cut (to avoid the bitter rind) and eaten whole, or processed to extract orange juice, & also for the fragrant peel. Citrus fruits are at the top not only in total production, but also in Economic value. The anti oxidant activity of the orange peel is evaluated by DPPH and HRSA assay and the Anti psoriatic activity is determined by MTT assay. The presence of Phytochemicals like Proteins, Carbohydrates, Glycosides, Alkaloids, Phenols, Flavonoids,Tannins and Saponins are screened. TLC is carried out in partial purification of Bioactive compounds.**

I. INTRODUCTION

1.1 Antioxidant

Oxygen is essential to life, but as our bodies use oxygen, we generate by-products known as reactive oxygen species (ROS) or, more commonly, free radicals. These compounds are a normal part of the body's stress response, but they can damage healthy cells and are especially likely to attack the fats that provide structure to the membranes surrounding body cells. Free radicals are also produced from exposure to cigarette smoke, excess exposure to the sun, drinking alcohol, from exposure to large amounts of heavy metals and during any inflammatory response. Antioxidants neutralise the effects of free radicals, but activity may be limited to specific antioxidants.

Sales of antioxidant supplements and cosmetics have increased dramatically with the hope they may have anti-ageing effects, although their true role is much more than skin deep.

Antioxidants function in many body systems and it's important to sort out the genuine research from the marketing hype.

The body produces a range of its own protective antioxidants. Some foods are also rich in antioxidants and these may boost the body's own supply. There is some evidence that antioxidants in plant foods may become especially important as we age and produce more free radicals.

Plants produce hundreds of antioxidants for their own protection. Some that may also be useful to us are present in vegetables, fruits, herbs and spices, nuts and whole grains. Tea, coffee, extra virgin olive oil, red wine and dark bitter chocolate are also rich in antioxidants.

Antioxidants produce the bright colours in fruits and vegetables and the flavour of extra virgin olive oil, tea or coffee.

Bitter compounds in foods such as rocket are also potent antioxidants.

Claims that a particular food is the 'richest source of antioxidants' can be confusing as the claim may depend on the type of measurement used or refer to a particular antioxidant, such as lycopene in tomatoes, and does not necessarily consider how well particular antioxidants can be absorbed.

The variety and combination of antioxidants in their natural food sources may also be important as isolated antioxidants may not function in the same beneficial way.

Antioxidant action is also part of the role of vitamins C,E, folate and beta carotene and also the minerals selenium, manganese, copper and zinc.

Much of the marketing of antioxidants concentrates on these nutrients. However, studies of antioxidant minerals and vitamins taken as supplements have been disappointing and it appears that the complex array of antioxidants present naturally in plants as well as those the body produces in reaction to stress may be more important.

Research into antioxidants is in its infancy. Epidemiological studies (studies of large populations that try to link disease in that population with a cause) show that a diet rich in foods with high levels of antioxidants is associated with longevity and good health. Evidence from laboratory studies indicates that particular antioxidants may have specific roles in disease prevention. However, most clinical trials using antioxidant vitamins have not shown expected results.

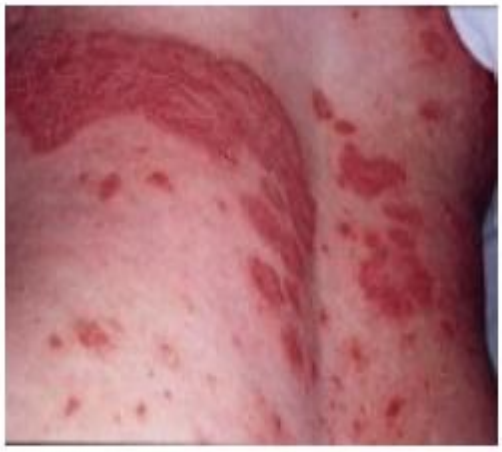
Anti aging

For many people, the greatest interest is in antioxidants' anti-ageing potential. Since the body's production of its own antioxidants. However, there is

no evidence that extra antioxidants stop hair greying, prev ent wrinkles or provide a 'fountain of youth'.

1.2 Psoriasis

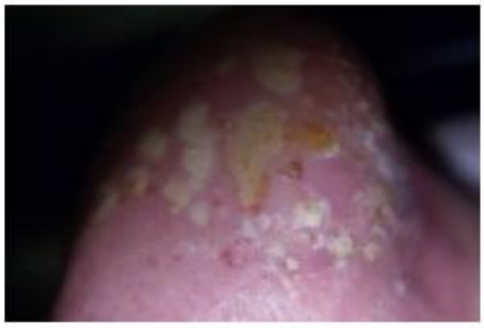
Psoriasis is a chronic inflammatory skin disease. Patients with psoriasis who are obeseare redisposed to diabetes and heart disease. Psoriasis can be initiated by certain environmental triggers.
A predisposition for psoriasis is inherited in genes. Psoriasis is not contagious. Psoriasis gets better and worse spontaneously and can have periodic remissions (cl ear skin). Psoriasis is controllable with medication. Psoria sis is currently not curable. There are many promising t herapies, including newer biologic drugs. Future research for psoriasis is promising. Psoriasis is a common and chronic incurable but treatable skin disorder. Plaque psoriasis is the most common form and appears as elevated plaques of red skin covered with silvery scale that may itch or burn. The involved areas are usually found on the arms, legs, trunk, or scalp but may be found on any part of the skin. The most typical areas are the knees, elbows, and lower back.



Plaque psoriasis on the back.

Psoriasis is not contagious but can be inherited. Research indicates that the disease may result from a disorder in the immune system.Factors such as smoking, sunburn, alcoholism, and IV infection may prolong the severity and extent of the condition.A signifcant percentage of people with plaque psoriasis also have psoriatic arthritis. Individuals with psoriatic arthritis have inflammation in their joints and may have other arthritic symptoms.

Sometimes plaque psoriasis can evolve into more inflammatory disease, such as pustular psoriasis or erythro dermic psoriasis. In pustular psoriasis, the red areas on the skin contain small blisters filled with pus. In erythrodermic psoriasis, extensive areas of red and scaling skin are present.



Pustular psoriasis. Image

Psoriasis affects children and adults. Men and woman are affected equally. Females develop plaque psoriasis earlier in life than males. The first peak occurrence of plaque psoriasis is in people 16-22 years of age. The second peak is in people 57-60 years of age.

Psoriasis can affect all races. Studies have shown that more people in western European and Scandinavian populations have psoriasis than those in other population groups.

Psoriasis Causes

Research indicates that the disease results from a disorder in the immune system. The immune system programs the white blood cells that protect the body from infection. In psoriasis, T lymphocytes (a type of white blood cell) abnormally trigger inflammation in the skin.

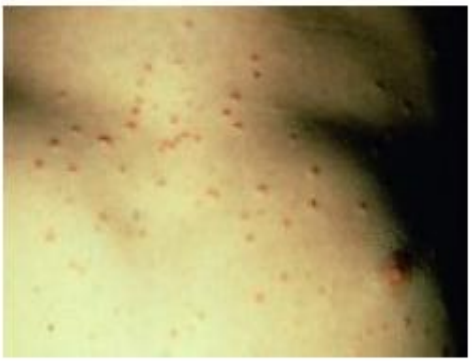
These T cells also cause skin cells to grow faster than normal and to pile up in raised plaques on the outer surface of the skin.

Those with a family history of psoriasis have an increased chance of having the disease.

Some people carry genes that make them more likely to develop psoriasis. When both parents have psoriasis, the child has a 50% chance of developing psoriasis. About one-third of those with psoriasis can recall at least one family member with the disease.

Certain factors may trigger psoriasis.

- Injury to the skin: Injury to the skin has been associated with plaque psoriasis. For example, a skin infection, skin infl ammation, or even excessive scratching can trigger psoriasis.
- Sunlight: Most people generally consider sunlight to be beneficial for their psoriasis. However, a small minority find that strong sunlight aggravates their condition. A bad Sunburn may worsen psoriasis.
- Streptococcal infections: Streptococcal sore throats may trigger a guttate psoriasis, a type of psoriasis that looks like small red drops on the skin.



Guttate psoriasis.

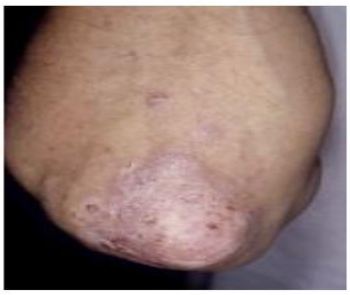
HIV: Psoriasis may worsen after an individual has been infected with HIV.

However, psoriasis often becomes less active in advanced HIV infection.

- Drugs: A number of medications have been shown to aggravate psoriasis. Some examples are as follows:
    1. Lithium: Drug that may be used to treat bipolar disorder
    2. Beta-blockers: Drugs that may be used to treat high blood pressure
    3. Antimalarials: Drugs used to treat malaria, arthritis, and lupus.
    4. NSAIDs: Drugs, such as ibuprofen (Motrin and Advil) or naproxen(Aleve), used to reduce inflammation.
- Emotional stress: Many people note an increase in their psoriasis when emotionally stressed.
- Smoking: Cigarette smokers have an increased risk of chronic plaque psoriasis.
- Alcohol: Alcohol is considered a risk factor for psoriasis. Even moderate intake of beer may trigger or worsen psoriasis.
- Hormone changes: The severity of psoriasis may fluctuate with hormonal changes.
- Disease frequency peaks during puberty and menopause. During pregnancy, psoriatic symptoms are more likely to improve. In contrast, flares occur in the postpartum period.

Psoriasis Symptoms and Signs

Plaque psoriasis, the most common form, usually produces plaques of red, raised, scaly skin affecting the scalp, elbows, and knees. The plaques may itch or burn.

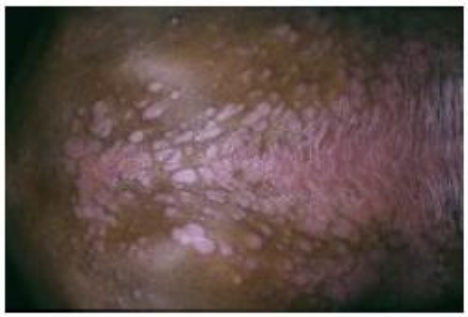


Plaque psoriasis on the elbow.

The flare-ups can last for weeks or months. Psoriasis can spontaneously resolve only to return later.

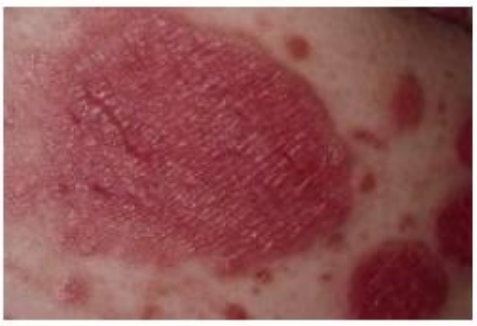
General characteristics

- Plaques: They vary in size (1 centimeter to sever al centimeters) and may be limited or quite exten sive. The shape of the plaque is usually round with irregular borders. Smaller pla que areas may merge, producing wide areas of in volvement.



Plaque psoriasis.

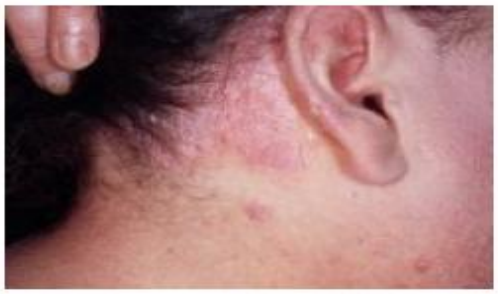
The skin in these areas, especially when over joints or on the palms or feet, can split and bleed.



Plaque psoriasis with fissures, which are splits in the skin.

Plaques sometimes have an area around them that looks like a halo or ring (Ring of Woronoff) that is especially noticeable after effective treatment as the lesions are resolving.

- Red color: The color of the affected skin reflects the inflammation present and is caused by increased blood flow.
- Scale: The scales are, thin, and silvery white. The thickness of the scales may vary. When the scale is removed, the skin underneath looks smooth, red, and glossy. This shiny skin usually has small areas of pinpoint bleeding(Auspitz sign).
- Symmetry: Psoriatic plaques tend to appear symmetrically on both sides of the body. For example, psoriasis is usually present on both knees or both elbows.



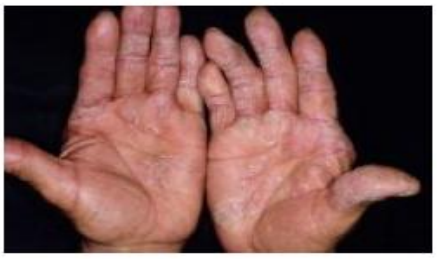
Psoriasis of the scalp.

- Nails: Nail changes are common in psoriasis. The nails may have small indentations or pits. The nails can be discolored and separate from the nail bed at the fingertip. (See Nail Psoriasis.) This may be identical in appearance to fungal nail infections and may actually encourage the acquisition of, or coexist with, a fungal infection.

Nail psoriasis

Psoriasis in children: Plaque psoriasis may look slightly different in children compared to adults. In children, the plaques are not as thick, and the affected skin is less scaly. Psoriasis may often appear in the diaper region in infancy and in flexural areas in children. The disease m ore commonly affects the face in children as compared to adults.

Other areas: Although the most common body areas affected are the arms, leg, back, and scalp, psoriasis can be found on any body part. Inverse psoriasis can be found on the genitals or buttocks, under the breasts, or under the arms and may not show the scale typically seen in other body areas. These areas can feel especially itchy or burning.



Psoriasis on the palms.

1.3 Health Benefits of Eating Orange Peels

1. Prevent Cancer

Flavonoids in orange peel assist vitamin C in working for efficiently. They also slow down growth of cancer cells. One study shows how orange peel can reduce the number of squamous cell carcinoma, which is a fatal type of skin cancer. People who consume orange peel regularly have less risk of being diagnosed with skin cancer and lung cancer than those who only eat the orange inside.

2.Lower Cholesterol Level

Orange peels contain properties (hesperidin) that help lower cholesterol levels.

3. Reduce Weight

Orange peel has been recommended by many health experts as a cure to weight loss. Since it helps increase yo ur metabolism rate, you will be able to burn fat from your body faster.

4. Better Digestion

Thanks to its anti-inflammatory properties orange peel can help people with digestion Problems as well as gastrointestinal problems like heartburn, diarrhoea and mild cases of acidity. Additionally, since it contains pectin, orange peel can be very beneficial in preventing constipation and help lower blood sugar levels.

5. Fight Infection, Colds and Flu

Since it's full of vitamins C and A, orange peel acts as a natural antioxidant that helps strengthen your immune system and fight off germs and viruses.

6. Cure Hangovers

Amazingly, if you don't feel like falling apart the day after a heavy of drinking, orange peel can be the only remedy you'll ever need.

Orange peel is actually one of the few things of nature that can cure a hangover. All you have to do is boil the peel for around 15-20 minutes and drink it like a tea.

7. Relieve Bronchitis

Bronchitis is extremely painful and hard to deal with. By drinking orange peel often, you can help heal the infection eventually and it also has an instant soothing effect.

8. Treats Bad Breath

Orange peels are a great cure for bad breath. By chewing small pieces, you can get rid of any foul odor in your mouth. It also fights against cavities and keeps your breath fresher for longer.

9. Anti-cholesterol

Almost all the anti-cholesterol compounds in oranges are found in the peels. These compounds help combat the LDL or 'bad' cholesterol in our bodies that form clots and plaque culminating in clogged heart arteries. Therefore, including orange peels to your diet can lower the total cholesterol levels in the body.

10. Averts Cancer

Apart from repelling oxygen free radicals that steal oxygen from healthy cells, the chemical compounds in orange peels also help inhibit the growth and division of cancerous cells.

11. Heartburn

Suffer from chronic heartburn? There's a natural relief to get rid of severe heartburns-orange peels! Research shows that an active chemical in orange peels helps relieve heartburn. When used over a period of 20 days, you can get an extended relief from heartburns.

12. Digestive problems

Orange peels contain a lot of dietary fiber. 100 grams of orange peels contain at least 10.6 grams of dietary fiber. Dietary fiber is useful for preventing irritable bowel syndrome including constipation and bloating. You can prepare a relaxing tea with orange peel extract to soothe your digestive system. Long ago, people treasured orange peels for their natural healing properties. The ingredients extracted from orange peels were used as medicine to cure various digestive disorders including indigestion. The rich dietary fiber helps regulate bowel movement, thereby soothing the digestive system.

1.4 Objectives

- To collect sample material and to obtain dried peel powder.
- To obtain the orange peel extract from different solvents.
- To Evaluate the Anti-oxidant activity and Anti-psoriatic activity.
- To elucidate the photochemical of the compound.
- To check In-Vivo anti psoriatic activity using Monocytic cell line.

III. MATERIALS AD METHOD

3.1 SPECIES DISCRIPTION

Orange peel

The Orange peel were collected from the shops of local market , CMBT , Chennai.

Fig.No.3.1 Orange peels

Family :Citrus aurantium

Peels:

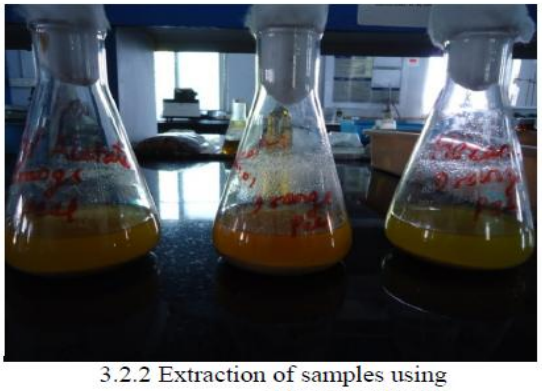
The peels were washed with the tap water, rinsed with distilled water and shade dried until it is completely dried. Then it was cut into small pieces and they were ground into coarse powder and stored at ro om temperature.

Fig.No.3.2 Dried peels

3.2 EXTRACTION

The samples were grounded into powder are extracted with different solvents namely Hexane, Ethyl Acetate and Methanol. About 10 gms of samples were extracted with the solvents under shaking condition for 24 hrs .The samples are extracted in a fresh solvents for 3 times.

3.2.1Dried Banana peel powder



3.2.2 Extraction of samples using different solvents
(a) Hexane (b) Ethyl Acetate (c) methanol

3.3 METHODS

3.3.1 DETERMINATION OF ANTIOXIDANT ACTIVITY

3.3.1.1 DPPH radical scavenging activity(Dharini,2011)

10mg of extract was dissolved in 1ml of DMSO. At various concentration of extract were added (20, 40 , 60 , …. 200 µg) with 2.96 ml of DPPH solution under dark condition. It was incubated for 20mins. The absorbance were noted at 517nm. DPPH radical's concentration was calculated using the following equation:

DPPH scavenging effect (%) = Ao – A1 / Ao X 100

Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample.

3.3.1.2 Hydroxyl radical scavenging activity (kleinet al .,1991)

The sample was taken in different concentration (250, 500, 750, 1000 µg). The 1ml of Iron EDTA solution , 0.5ml of EDTA solution, 1ml of DMSO and 0.5ml of Ascorbic acid was added to the sample. Then the sample was kept in the boiling water bath at 80ºC90ºC/15mins. 1ml of Ice cold TCA was added to the solution along with that 3ml of Nash Reagent was added and placed at the room temperature for 15 mins. The absorbance was noted at 412nm.

3.4 PHYTOCHEMICAL SCREENING

3.4.1 QUALITATIVE ANALYSIS

3.4.1.1 DETECTION OF ALKALOIDS (Evans 1997)

Solvent free extract 50mg was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows

Mayer's test

To a few ml of filtrate, a drop or two of mayer's reagents was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

Mayer's Reagents

Mercuric chloride (1.358g)was dissolved in 60ml of water and potassium chloride (5g) was dissolved in 10 ml of water. The solutions were mixed and made up to 100ml with water.

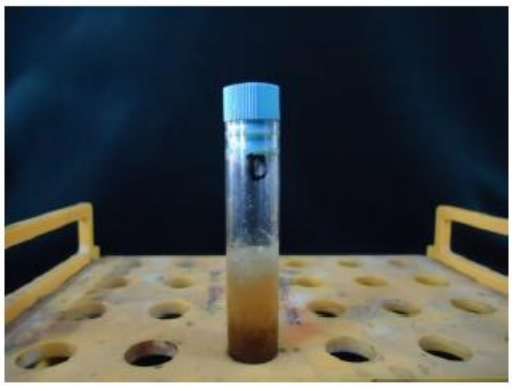


Fig.No.3.3 Detection of Alkaloids

3.4.1.2 DETECTION OF CARBOHYDRATES (Ramakrishnan et al ., 1994)

The extract (100mg) was dissolved in 50ml of water and filtered. The filtrate was subjected to the fehling's test.

Fehling's test

1ml of filtrate was boiled on water bath with 1ml each of fehling's solution I and II. A red precipitate indicated the presence of sugar.

Fehling's solution I

Copper sulphate (34.66g) was dissolved in distilled water and made up to 500ml with distilled water.

Fehling's solution II

Potassium sodium tartarate (173g) and sodium hydroxide (50g) was dissolved in water and made up to 500ml.

Fig.No.3.4 Detection of Carbohydrates

3.4.1.3 DETECTION OF GLYCOSIDES

50mg of extract was hydrolysed with concentrated hydrochloric acid for 2hours on a water bath, filtered and the hydrolysate was subjected to the Borntrager's test.

Borntrager'stest(Evans ,1997)

To 2ml of filtrate hydrolysate, 3ml of chloroform was add ed and shaked.Chloroform layer was seperated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

Fig.No.3.5 Detection of Glycosides

3.4.1.5 DETECTION OF SAPONINS

Foam Test (Kokate,1999)

The extracted (50mg) was diluted with distilled water and made up to 20ml the suspension was shaken in a graduated cylinder for 15mins. A 2cm layer of foam indicated the presence of saponins.

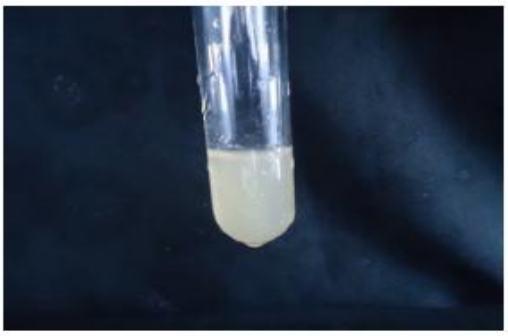


Fig.No.3.6 Detection of Saponins

3.4.1.6 DETECTION OF PROTEINS

(Ruthmann , 1970)

The extract 100mg was dissolved in 10ml of distilled water and filtered through wattman no.1 filter paper and the filtrate was subjected to test of protein and amino acid.

Biuret Test (Gahan, 1984)

An aliquot of 2ml of filtrate was treated with one drop of 2%copper sulphate solution. To this, one ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of protein.

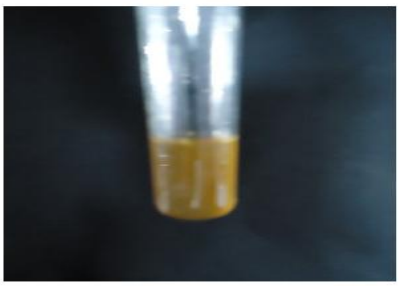


Fig.No.3.7 Detection of Protiens

3.4.1.7 DETECTION OF PHENOLIC COMPOUND

Ferric chloride test (Mace,1963)

The extract 50mg was dissolved in 5ml of distilled water. To this, few drops of netural 5% ferric chloride solution were added. A dark-green color indicated the presence of phenolic.

Fig.No.3.8 Detection of Phenolics

3.4.1.8 DETECTION OF STEROIDS

Salkowski Test

0.5 of the extract will be dissolved in 2 ml of chloroform. Sulfuric acid is then carefully added to form a lower layer. A reddish brown color at the interface will indicate the presence of a steroidal ring.

Fig.No.3.9 Detection of Steroids

3.4.1.9 DETECTION OF FLAVONOIDS

Ferric chloride test

About 0.5 of each portion was boiled with distilled water and then filtered. To 2ml of the filtrate, few drops of 10% ferric chloride solution was then added. A green-blue are violet colouration indicated the presence of flavonoids.

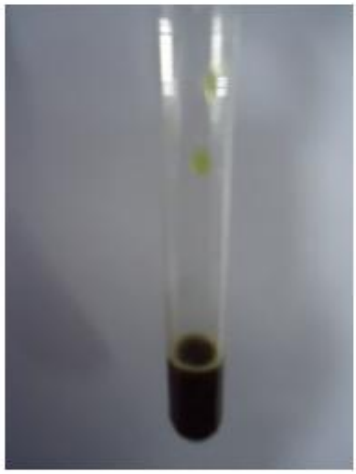


Fig.No.3.10 Detection ofFlavonoids

3.4.1.10 DETECTION OF TANNINS

Neutral ferric chloride test

About 5g of each portion of leaf extract will be stirred with 10ml distilled water, filtered and ferric chloride reagent will then be added to the filtrate. A blue-black, green or blue-green precipitate is taken as evident for presence of tannins.

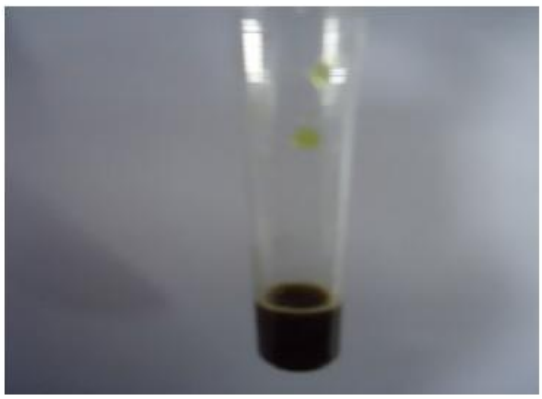


Fig.No.3.11 Detection of Tanins

3.4.2 QUANTITATIVE PHYTOCHEMICAL ESTIMATION

3.4.2.1 Determination of total phenol content

Folin-ciocalteu's reagent method

he amount of total phenol content, in various solvent extracts of leaves was determined by Folin-ciocalteu's reagent method (Mc Donald et al.,2001). 0.5ml of extract and 0.1ml (0.5N) folin-ciocalteu's reagent was mixed and mixture was incubated at room temperature for 15mins then 2.5ml saturated sodium carbonate solution was added and further incubated for 30mins at room temperature and the absorbance was measured at 760nm. Gallic acid was used as a positive control. Total phenolic value was expressed in terms of gallic acid equivalent (mg/g of extracted compounds).

3.4.2.2 Determination of total flavonoid content

Aluminium chloride calorimetric method

The amount of flavonoid content in various solvent extrct of leaves was determined by Aluminium chloride calorimetric method (changet al., 2002). The reaction mixture 3ml consist of 1ml of sample (1mg/ml) and 0.5ml of (1.2%) aluminium chloride and 0.5ml (120mM) potassium acetate was incubated at room temperature for 30mins.the absorbance of all samples was measured at 415nm. Quercetin was used as positive control. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

3.5 PARTIAL PURIFICATION OF BIOACTIVE COMPOUND

Thin layer chromatography

In TLC, 10µg of the plant extract was separated on TLC plate using as eluents solvent system of different polarities, namely ethyl acetate/hexane and ethyl acetate/chloroform. (Eloff., 2004) The loaded TLC plate is carefully placed in the TLC chamber with the sample line toward the bottom. The plate whose top is leaned against the jar wall should sit on the bottom

of the chamber and be in contact with the developing solvent (solvent surface must be below the extract line). The TLC chamber is covered. The TLC plate is allowed to remain undisturbed. When the solvent front has reached three quarters of the length of the plate, the plate is removed from the developing chamber and the position of the solvent front is immediately marked.

3.6 DETERMINATION OF ANTIPROLIFER-ATIVE ACTIVITY

MTT assay

Chemicals and reagents:

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) invitrogen, USA. Acridine orange were obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

Cell culture:

THP -1 cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/mL), gentamycin (100ug/mL) andamphotericin B (1mg/mL) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37.C in a humidified atmosphere of 5% CO2. Cells were allowed to grow to confluence over 24 h before use.

Cell growth inhibition studies by MTT assay:

Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modif ication. Briefly, THP-1 cells were seeded at a density of 5×103 cells/well in 96-well plates for 24 h, in 200ul of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (1–100µg/mL) of Ethyl acetate extract of Citrus aurantium was added and incubated for 48 h. After treatment cells were incubated with MTT (10µl, 5mg/mL) at 37 .C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments.

IV. RESULTS

4.1 EXTRACTION OF ORANGE PEEL

The selected Orange peel was extracted using Methanol, Ethyl acetate and Chloroform. (Fig 4.1)

(a)

Fig.4.1 (a) Dried peels

(b)

(b) Extract

4.2 EVALUATION OF ANTIOXIDENT POTENTIAL

4.2.1 RADICAL SCAVENGING ACTIVITY (RSA) DPPH ASSAY

From the given graph of Anti oxidant potential of Orange peel extract of the different solvents showed Ethyl Acetate at higher activity among three different solvents at concentration of 100µg of 87% RSA .

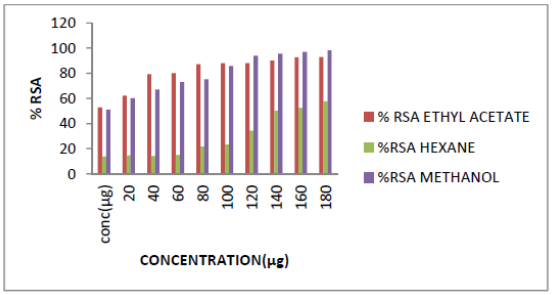


Fig.4.2.1 %RSA of Ethyl Acetate extract, Methanol and Hexane of Citrus aurantium

4.2.2 HYDROXYL RSA ASSAY

The Hydroxy Scavenging activity of the Ethyl Acetate extract of Orange peel showed higher activity at the concentration of 100µg of 73.3% HRSA.

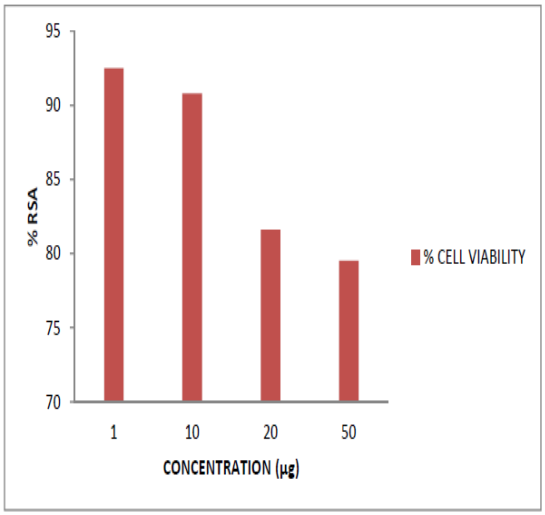


Fig.4.2.1 %HRSA of Ethyl Acetate extract of *Citrus aurantium*

4.3 Qualitative phytochemical screening of Cirus aurantium

The preliminary phytochemical screening of Citrus aurantium revealed the presence of phenols, proteins, Flavonoids, Gylcosides, Saponins, C arbohydrates, Tanins, Setroids in high amounts followed by Alkaloids in trace.

Table 1: Qualitative phytochemical screening

S No.	Compound	Result
1	Alkaloids	++
2	Flavonoids	+++
3	Glycosides	++
4	Saponins	++
5	Carbohydrates	+
6	Tannins	++
7	Phenols	+++
8	Proteins	++
9	Sreoids	+

+: Present in trace amounts. ++: Present in moderate amounts.
+++: Present in higher amounts. - : Not detectable using the assay followed.

4.4 Quantitative phytochemical analysis

The result of total phenol content, Flavonoid content from the given table Phenolic content was found to be mo re in Ethyl Acetate extract followed by flavonoids content .

BIOACTIVE COMPOUNDS	AMOUNT *µg/ml*
Total phenols	156.2
Total flavonoids	142.3

4.5 THIN LAYER CHROMATOGRAPHY

The chromatogram developed with 10% Ethyl Acetate in hexane revealed the presence of three major compounds at Rf value of 0.75, 0.42, 0.33 were noticed under short UV and in the Iodine chamber.

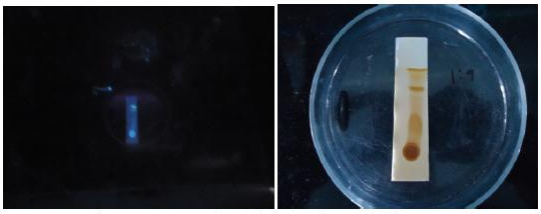


Fig.No. 4.5 TLC in Iodine chamber and Short UV

4.6 EVALUATION OF ANTI PSORIATIC ACTIVITY OF CITRUS AURANTIUM

The results of MTT assay suggest that the extract was capable of reducing cell viability of selected Psoriatic cell line fig 3.6.Also, the IC50 of the selected extract was found to be 100µg where the cell viability was recorded as 82.24%.

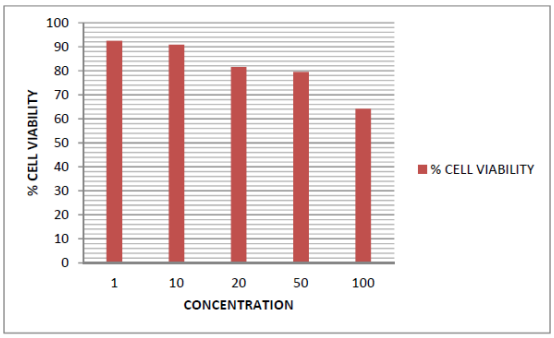


Fig 4.6 MTT assay of Ethylacetate extract of *Citrus aurantium*

V. DISCUSSION AND CONCLUSION

The electron donation ability of natural products can be measured by 2,2 '-diphenyl-1picrylhydrazyl radical (DPPH) purple-coloured solution bleaching. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test. In the present

Study among all the fractions tested, ethyl acetate showed significantly higher inhibition percentage and positively correlated with total phenolic content and total flavonoid content. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage. (Naima Saeedet al.,2012)

Several techniques have been used to determine the antioxidant activity in vitro in order to allow rapid screening of substances since substances that have low antioxidant activity in vitro, will probably show little activity in vivo. Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. (Saeed et al.,2012)

The •OH radical is an extremely reactive in biological systems and has been implicated as highly damaging species in free radical pathology, capable of damaging bio molecules of the living cells. These radical combines with nucleotides in DNA and cause strand breakage leadin g to carcinogenesis, mutagenesis and cytotoxicity. Hydrox yl radical (•OH) scavenging capacity of an extract is directly related to its antioxidant activity. (Khan et al., 2012) Ethyl Acetate was the most effective for hydroxyl radical scavenging activity.

Since the result of the study revealed the presence of phenols, flavonoids in major amounts, it can be derived that these phytochemicals might be respresent for the potential of Citrus Aurantium. Further mechanistic studies are required to isolate , purify and analyse the specific bioactive compound respectively for the antioxidant activity.

The MTT assay performed to study the Antipsoriatic activity of Citrus Aurantium depricts that the extract possed significant inhibits activity on the proliferative of THP1 cell lines. This denotes that Citrus Aurantium could be considered as an effective sourse of Anti psoriatic bioactive compounds.

In conclusion EthylAcetate extract of Citrus Aurantium showed phytochemicals such as phenolics, Flavanoids. The dried extract of Citrus Aurantium showed considerable inhibiting activity on free radical.

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