International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Anti-Psoriatic Activity of *Musa* Mysore Aab (Poovan Banana) Peel Extract Using Human Keratinocyte Cell Line

Nithya Durga .E[1], Mahesh Kumar[2]

[1]M. Tech Scholar, Food and Nutritional Biotechnology, SRM University, Tamilnadu, India

[2]Assistant Professor, (SG), Department of Food and Process Engineering, SRM University

Abstract: ***Banana is one of the most widely distributed and consumed fruit in tropical and subtropical countries. Considering to nutritional aspects, it is one of the worlds leading food crops with a high source of minerals, vitamins, carbohydrates, flavonoids, phenolic compounds etc. The current study was performed to evaluate the antioxidant activity and Anti-psoriatic activity in Musa Mysore AAB peels. The MTT assay was carried out for the determination of antipsoriatic activity and DPPH and HRSA assay was used in identifying antioxidant activity. The presence of antioxidant was confirmed by phytochemical analysis and partial purification of the compound was done by TLC.***

Keywords: Antioxidant, antipsoriatic activity, *Musa Mysore* AAB.

1. Introduction

1.1 Antioxidants

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature. Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods require special equipment and technical skills for the analysis. The different types of methods published in the literature for the determinations of antioxidant activity of foods involve electron spin resonance (ESR) and chemiluminescence methods.

These analytical methods measure the radical scavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical (O2), the hydroxyl radical (OH), or the peroxyl radical (ROO).

The various methods used to measure antioxidant activity of food products can give varying results depending on the specific free radical being used as a reactant. There are other methods which determine the resistance of lipidor lipid emulsions to oxidation in the presence of the antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assays have been used extensively since the 1950's to estimate the peroxidation of lipids in membrane and biological systems. These methods can be time consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix etc. and may not be practical when large numbers of samples are involved. Antioxidant activity methods using free radical traps are relatively straightforward to perform.

The ABTS [2,2'- azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their (Prior et al.) have used the Oxygen Radical Absorbance Capacity (ORAC) procedure to determine antioxidant capacities of fruits and vegetables. In the ORAC method, a sample is added to the peroxyl radical generator, 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) and inhibition of the free radical action is measured using the fluorescent compound, B-phycoerythrin or R-phycoerythrin.Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages and are usually quantified

employing Folin's reagent. Vinson et al.(Hoyer., *et.al* 2005).Have measured phenolics in fruits and vegetables colorimetrically using the Folin-Ciocalteu reagent and determined the fruit and vegetable's antioxidant capacity by inhibition of low density lipoprotein oxidation mediated by cupric ions. A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years .

The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps understand the functional properties of foods. Antioxidant activity has been expressed in various ways including the percentage of the reagent used, the oxidation inhibition rate and so on. An easier way to present antioxidant activity of foods would be to reference a common reference standard. One common reference standard, (S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid, also known as Trolox, serves this purpose (Hebbel., *et.al* 1990).The search for natural products as potential anticancer agents dates back at least to the Ebers papyrus in 1550 B.C. However, the scientific period of this search is much more recent, beginning with the investigation by Hartwell and his co-workers on the application of podophyllotoxin and its derivatives as anticancer agents (Kingston *et al*. 1990). Plants offer scientists searching for novel bioactive compounds the added advantage of ethnobotanical observations, since many species are used in traditional medicine, principally in developing countries.

1.2 Psoriasis

Psoriasis is a common chronic inflammatory dermatosis. Person of all ages may develop the disease. Psoriasis is sometime associated with arthritis, myopathy, enteropathy, spondylitic heart disease or the AIDS.Psoriatic arthritis may be mild or may produce severe deformities resembling the joint changes seen in rheumatoid arthritis. Clinically, psoriasis most frequently affects the skin of the elbow, knees, scalp, lumbosacral areas, intergluteal cleft and glans peniS. The most typical lesion is a well demarcated, pink to salmon colored plaque covered by loosely adherent scales that are characteristically silver white in color. Psoriasis can be one cause of total bodyerythema and scaling known as erythroderma. Nail changes occur in 30% of cases ofpsoriasis and consist of yellow browndiscoloration (often linked to an oil slick),with pitting, dimpling, separation of the nailplate from the underlying bed (onycholysis),thickening and crumbling.

Psoriasis is eitherbenign or localized (hands and feet) orgeneralized or life threatening, withassociated fever, leukocytosis, arthralgias,diffuse cutaneous and mucosal pustules,secondary infection and electrolytedisturbances. Psoriasis may begin at any age,but in most cases, it begins between the agesof 10 and 20. Psoriasis affecting the body folds is common in individuals. Both sexes are equally affected.

Psoriasis is anautosomal, dominantly inherited dermatosis. The disease may flare up as a result infection of the upper respiratory tract (Streptococcalsore throat), bladder, teeth or kidneys. It may also be triggered by physical trauma (Kobnerphenomenon). Currently available allopathic drugs have been associated with a number of side effects. Some drugs such as lithium, β blockers and chloroquine are also provocative factors.

Psoriasis is caused by acceleration in the life cycle of skin cells resulting in development of thick, scaly white skin patches or plaques. Banana peels contain natural anti-inflammatory antiseptic, cooling properties that can help alleviate or reduce the severity of the symptoms.

Mix banana peels and coal tar to make a paste that can be applied to affected areas. To make the paste, use several banana peels and dice into small pieces and place into a blender for a few minutes. Add the coal tar to the mix. According to the National Psoriasis Foundation, coal tar is derived from natural coal and is used to treat skin conditions. Coal tar is available as over-the-counter topical lotions and shampoos. Place the paste on the skin and rub lightly into affected area. Banana peels contain fatty acids. A study published in 2004 in the "Journal of Dermatological Treatment" concluded that the use of the topical medication Exorex, which contained 1 percent coal tar and an analogue of banana peels made up of esterified essential fatty acids, demonstrated positive results in the treatment of mild to moderate psoriasis.

1.3 *MUSA MYSORE* AAB (POOVAN BANANA)

Important commercial variety of Tamil Nadu produced year round in large quantities. Plant tall and vigorous. Bunch - large, 25 kgs, 12 hands, upto 18 fingers, 200 or more fruits. Fruits - medium size, cylindrical, pronounced nipple, skin - thin, bright yellow, peels off easilyPulp - soft, juicy, yellow, acid-sweet taste, good flavour.Crop duration 11-14 months.It is highly susceptible to Banana Mosaic Virus(BBMV) and Banana Streak Virus (BSV) which causes reduction in yield.

Figure 1.1: Poovan Banana

1.4 Nutritional Value of Banana Peel

Just as bananas are packed with nutrients, their peels also pack a punch nutritionally. Banana peel actually contains more fiber (soluble and insoluble) and potassium than the flesh of banana. It also contains tryptophan, an essential amino acid that increases the levels of the hormone serotonin, which is a mood enhancer. Banana peel is a good source of vitamins like vitamin A, and vitamin B6. In addition to potassium, it also contains minerals like calcium, manganese,magnesium, sodium and sulfur. Banana peel also contains antioxidants including lutein and phytochemicals like polyphenols and carotenoids. Interestingly, antioxidant activity of the banana peel extract was found to be stronger than that of the banana pulp extract.

Banana peel protects your heart
The high levels of soluble and insoluble fiber in banana peel are very useful inreducing the levels of bad cholesterol or LDL cholesterol. Also, banana peel is an excellent source of potassium that helps to lower high blood pressure. By keeping the cholesterol and blood pressure levels under check, banana peel offers protective effects to your heart.

Banana peel reduces skin aging
The antioxidants in banana peel fights off the free radicals and prevent the oxidation stress on the cells of your body. This helps in slowing the aging process and also in reducing the signs of aging. Moreover, rubbing banana peel on the skin is an excellent remedy to tone and tighten your skin, thus removing wrinkles and fine lines.

Banana peel relieves eczema and psoriasis:
Banana peel is also used as a natural remedy to get relief from skin conditions like psoriasis and eczema. The peel has excellent exfoliating properties, which helps in removing loose and flaky skin. The antioxidants and other nutrients in banana peel provide the necessary nourishment to your skin. Banana peel has anti-inflammatory properties that help in reducing the inflammation associated with eczema and psoriasis.

Banana skin helps soothe irritation
Banana peel is a wonderful remedy for soothing skin irritation caused by poison ivy, bug bites and other kinds of allergic skin reactions. The vitamins, antioxidants and other nutrients in the peel increases blood circulation, which promotes quicker healing. Banana peel has anti-inflammatory properties that soothes irritation and prevents inflammation, while its antibacterial properties help in preventing bacterial infection.

Banana skin and weight loss
The high amounts of fiber in banana peel give you the sensation of fullness, which prevents you from overeating and snacking in between meals. Banana peels are low in calories and at the same time rich in nutrients. This makes it a healthy and nutritious food for people trying to lose weight.

Banana peel and cancer cure
Banana peel is high in antioxidants that prevent oxidative stress caused to the cells by free radicals. It alsocontains compounds that can prevent cell mutations that may lead to the development of cancer.

1.5 Objectives

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

2. Review of Literature

The present study was designed to investigate the anti-oxidant activity of the methanolic extract of *Portulacaoleracea*. The methanolic extract was evaluated by TLC and HPTLC fingerprint method. Anti-oxidant activity of methanolic extract was determined by DPPH free radical scavenging activity, reducing power by FeCl3, nitric oxide free radical scavenging activity, super oxide scavenging activity by alkaline DMSO method (Sanja*et al.*2009).

Antioxidant activity has been assessed by in vitro method for phytochemical fraction of plant, viz. methanolic and butanol extracts of Cordiamacleodii bark. This investigation was under taken to evaluate methanolic and butanol extract of Cordiamacleodii bark for possible antioxidants potential. The extracts were evaluated for their phenolic content & antioxidant activity. Phenolic content was measured using Folin-ciocalte reagent & was calculated as Gallic acid equivalents. Antiradical activity of both extracts was measured by 1, 1, diphenyl-2, picrylhydrazyl (DPPH) assay & was compared to ascorbic acid and Ferric reducing power (FRAP) of the extract was also evaluated by Oyaizu et al. In the present study three in vitro models were used for evaluate of antioxidant activity. The first two methods were for direct measurement of radical scavenging activity & remaining one method evaluated the reducing power. The present study revealed the Cordiamacleodii bark has significant radical scavenging activity (Pankaj*et al.*,2012).

Free radicals induce numerous diseases by lipid peroxidation, and DNA damage. It has been reported that numerous plant extracts have antioxidant activities to scavenge free radicals. In the present study, the antioxidant properties of crude (aqueous and methanolic) extract of Hibiscus rosasinensis (Malvaceae) were studied in six in vitro models viz. radical scavenging activity by DPPH reduction Assay, Scavenging of SO, H2O2 and NO, reducing power, FRAP assay. The extract was found to contain large amounts of phenolic compounds and flavonoids. Methanolic extract of Hibiscus rosa-sinensis possessed significant antioxidant activity as compare to aqueous extract. These results suggest that hibiscus has potential to develop a new functional dietary agent to treat chronic metabolic diseases, such as diabetes and hyperlipidemia(Garg*et al.*,2012).

Primary attention is given to the antioxidant (and prooxidant) activity of polyphenols arising from their

interactions with iron both in vitro and invivo. In addition, an overview of oxidative stress and the Fenton reaction is provided, as well as a discussion of the chemistry of iron binding by catecholate, gallate, and semiquinone ligands along with their stability constants, UV–vis spectra, stoichiometries in solution as a function ofpH, rates of iron oxidation by O2 upon polyphenol binding, and the published crystal structures for iron–polyphenol complexes. Radical scavenging mechanisms of polyphenols unrelated to iron binding, their interactions with copper, and the prooxidant activity of iron–polyphenol
complexes (Nathan *et al.,* 2013).

Premnaserratifolia Lin., is widely used in Ayurvedic system medicine for the treatment of cardiovascular disorders, arthritis, inflammation etc. The stem-bark and stem-wood were extracted with 95% ethanol and double distilled water and these extracts were screened for their in-vitro antioxidant potential. Inhibition of oxygen-derived free radicals, viz., assays for free radical scavenging by DPPH, reducing power ability and nitric oxide scavenging were performed. All the antioxidant activities were compared with standard antioxidant such as ascorbic acid. Both the extracts of this plant showed effective free radical scavenging activity, reducing power and nitric oxide scavenging activity. All these antioxidant properties were concentration dependent. The highest antioxidant activity was observed with ethanol extracts. Preliminary phytochemical screening revealed the presence of flavonoids, steroids, alkaloids, glycosides and phenolic compounds in the extracts and the results obtained from the current study indicate that *Premnaserratifolia* Lin., is a potential source of natural antioxidants and the extracts have constituents which were capable of showing anti-oxidant activity and the said in-vitro anti-oxidant activity may also be due to the presence of
anti-oxidant principles present in the extracts like flavonoids and phenolic compounds. These findings confirm the great interest of the *Premnaserratifolia*whose phytochemistry and phytopharmacology should be investigated further in order to detect possible hytotherapeutic uses in the prevention of ageing related diseases, cardiovascular disorders and Alzheimer disease (Rajendran*et al., 20*11).

The potential of antioxidant activities of the plant extract Gynuraprocumbens, Achyranthesaspera and Polygenumtomentosum were studied by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) .Antioxidant activity was qualitatively and quantitatively determined. In this analysis Ascorbic acid (Vitamin C) was used as the standard .The antioxidant activities were observed all three plant extracts and the EC50 values of G procumbens A.aspera andP.tomemtosum were 13.7 µg /ml,14.37 µg /ml and 14.35 µg /ml.Among these plants, G.procumbens is more potent antioxidant activity then others. Antitumor activities were found with A.aspera(s2) extracts in the dose of 100ppm in carrot disks and G.procumbens(s1) and P.tomentosum (s3) in the dose of 1000 ppm (Maw.,2011).

Many herbal remedies individually or in combination have been recommended in various medicalexpositions for the cure of different diseases. Chronic plaque psoriasis is an immune-mediated,inflammatory skin disease. Current treatments are unable to counter the inflammatory conditions of psoriasis. The present study was carried out to evaluate whether the presence of aqueous extract of bark of *Pongamiapinnata* in a commercial preparation SUEX GEL that is used in the treatment of psoriasis, has any additional benefits over a similar preparation supplied by the manufacturer that does not have the extract using the rat ultraviolet ray photo dermatitis model. The irradiated rat skin treated with SUEX GEL containing aqueous extract of the bark of *P. pinnata* showed a significant reduction in the total epidermal thickness, retention of the stratum granulosum and the absence of movement of neutrophils, and further substantiated that the SUEX GEL having aqueous extract of the bark of *P. pinnata* has been very useful in the changes that occur in the skin due to irradiation. The presence of the aqueous extract of the bark of *P. pinnata* produces an improvement in the efficacy of the ayurvedic ointment (SUEX GEL) used in the treatment of psoriasis.(Divakara..,2013)

Psoriasis is a chronic inflammatory skin disorder characterized by rapid proliferation of keratinocytes and incomplete keratinization. Discovery of safer and more effective anti-psoriatic drugs remains an area of active research at the present time. Using a HaCaT keratinocyte cell line as an *in vitro* model, we had previously found that ethanolic extracts from three Thai medicinal herbs, namely *Alpiniagalanga*, *Curcuma longa* and *Annonasquamosa*, possessed anti-psoriatic activity. In the current study, we aimed atinvestigating if these Thai medicinal herb extracts played a molecular role in suppressing psoriasis via regulation of NF-κBsignaling biomarkers. Using semi-quantitative RT-PCRand report gene assays, we analyzed the effects of these potential herbal extracts on 10different genes of the NF-κBsignaling network in HaCaT cells. In accordance with ourhypothesis, we found that the extract derived from *Alpiniagalanga* significantly increased the expression of TNFAIP3 and significantly reduced the expression of CSF-1 and NF-κB2. *Curcuma longa* extract significantly decreased the expression of CSF-1, IL-8,NF-κB2, NF-κB1 and RelA, while *Annonasquamosa* extract significantly lowered the expression of CD40 and NF-κB1. Therefore, this *in vitro* study suggested that these herbal extracts capable of functioning against psoriasis, might exert their activity by controllingthe expression of NF-κBsignaling biomarkers.(Chanachai Saelee..,2011)

The plant *Thespesiapopulnea* (Malvaceae) traditionally claimed to be useful in the treatment of cutaneous affections such as scabies, psoriasis, ringworm, guinea worm, eczema and herpetic diseases. Oil prepared by boiling the ground bark in coconut oil is applied externally in psoriasis and scabies. However, there are no established scientific reports for its anti-psoriatic activity. Hence, the plant *Thespesiapopulnea* has been choosen to establishscientific data for its traditional claim as anti- psoriatic. This is firstever study on *Thespesiapopulnea*bark extract.Phytochemical investigation revealed the presence of carbohydrates, glycosides, tannins, flavonoids, triterpenoids,phytosterols, proteins and lipids/fixed oils in the bark of *Thespesiapopulnea*. Further, Thin layer chromatographystudies supported their presence. Chemical tests performed, TLC studies carried out and UV & IR

spectral dataindicates that the isolated compounds TpF-1, TpF-2 might be flavonoids and TpS-2 might be a sterols.

Three compounds TpF-1, TpF-2 & TpS-2 were isolated from the bark powder and an attempt was made to characterizet hem by physical, chemical and spectral data. Screening for anti-psoriatic activity was carried out by topical application of different extracts & isolated compounds (TpF-1, TpF-2 & TpS-2) of *Thespesiapopulnea* bark in theform of a cream using the Perry's scientific mouse tail model. Successive pet-ether extract showed maximum antipsoriatic activity (increased orthokeratoticregion by25%) amongst the extracts tested whereas the compound TpF2exhibited 38%increasein the same. From the above data,it is can be said that, the plant*Thespesiapopulnea*ispromisingforfurtherinvestigationst oproveits anti-psoriaticactivity. (Sidharth..,2009) .

Aqueous extracts of seeds of *Cassia tora* and *Momordicacharantia* and flowers of *Calendula officinalis* exhibited better antibacterial activity as compared to their petroleum ether, methanolic and ethanolic extracts. Among the organisms tested *S. aureus* was more susceptible to the aqueous extracts of all the three herbs. Further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of these herbal extracts in treatingvarious infections and skin diseases like psoriasis.(Roopashre..,2008).

3. Materials and Method

3.1 Collection of Poovan Peel

The peel of *MusaMysore* AAB was collected from the local market of CMBT, Chennai, Tamil Nadu in the month of August.

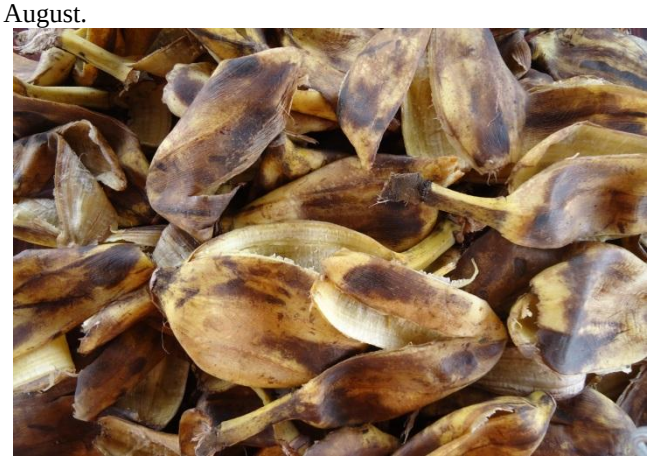

Figure 3.1: Poovan Peels

3.2 Preparation of Peel Material

The peels were washed with the tap water, rinsed with distilled water and shade dried (15days) until it is completely dried .

Figure 3.2: (a) Banana peels kept for shade drying Day 1(b) Dried Banana peels at Day 15

Then it was cut into small pieces and they were ground into coarse powder and stored at room temperature.

Figure.3.3: Dried Banana peel powder

3.3 Extraction with Different Solvents

The powdered samples were subjected to extraction using three different solvents Hexane, ethyl acetate and methanol. 10g of powdered sample was extracted with 100 ml of Hexane, ethyl acetate and methanol in conical flaskunder shaking condition. The extract was decanted into pre weighed glass vials. The process was repeated three times using the same material but in fresh solvents.

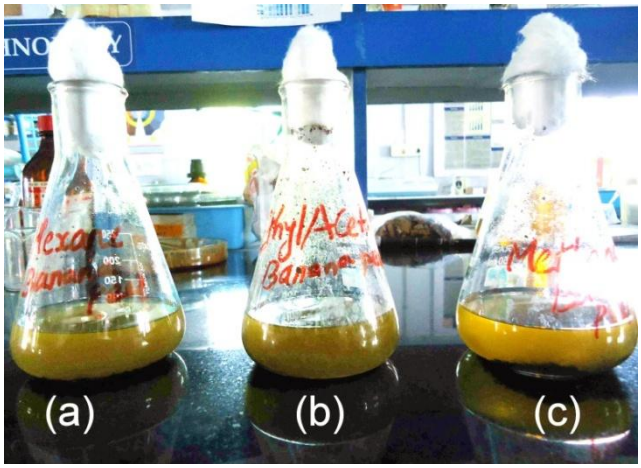

Figure 3.4: Extraction of samples using different solvents (a) Hexane (b) Ethyl Acetate (c) methanol

The remaining solvent was concentrated to dryness under reduced pressure and controlled temperature according to the solvents using condenser (Kshirsagar and Shupadhyay.,2009).

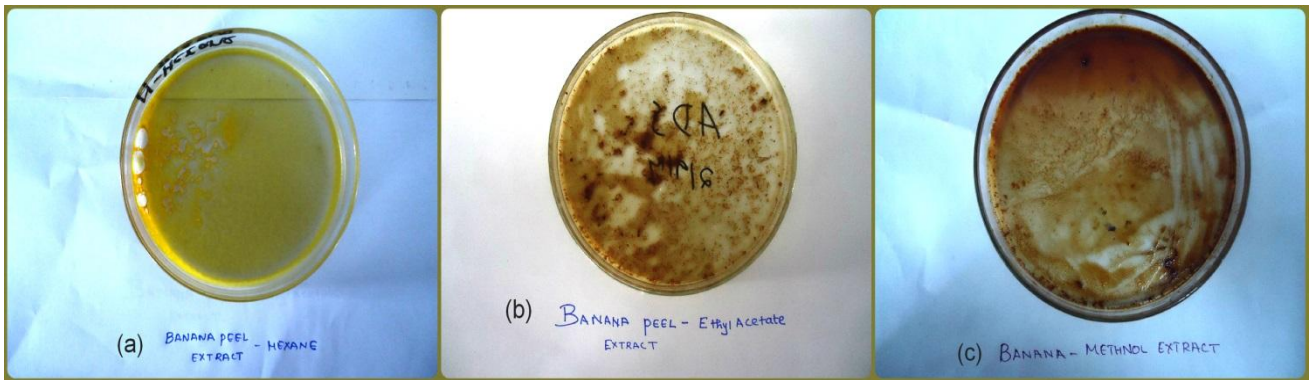

Figure 3.5: Dried Poovan Peel Extract of different solvents (a) Hexane Extract (b) Ethyl Acetate Extract (c) Methanol Extract

3.4 Methods

3.4.1 Determination of Antioxidant Activity

3.4.1.1 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:

$$\text{DPPH scavenging effect (\%)} = A_o - A_1 / A_o \text{ X } 100$$

Where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample.

3.4.1.2 Hydroxyl Radical Scavenging Activity (KLEIN *et al* .,91)
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 80ºC-90ºC/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.

3.5 Phytochemical Screening

3.5.1 Qualitative Analysis

3.5.1.1 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.

Figure 3.6: Detection of Alkalids

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

Figure 3.7: Detection of Carbohydrates

3.5.1.3 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's test(Evans ,1997)

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

Figure 3.8: Detection of Glycosides

3.5.1.4 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.

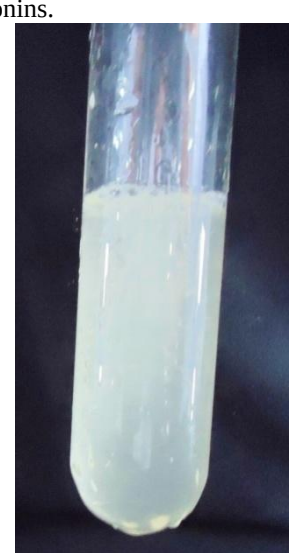

Figure 3.9: Detection of Saponins

3.5.1.5 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.

Figure 3.10: Detection Of Proteins

3.5.1.6 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 compound.

Figure 3.11: Detection Of Phenolic Compound

3.5.1.7 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.

Figure 3.12: Detection of Steroids

3.5.1.8 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.

Figure 3.13: Detection of Flavonoids

3.5.1.9 Detection of Tannins

Neutral ferric chloride test

About 5g of each portion of peel 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.

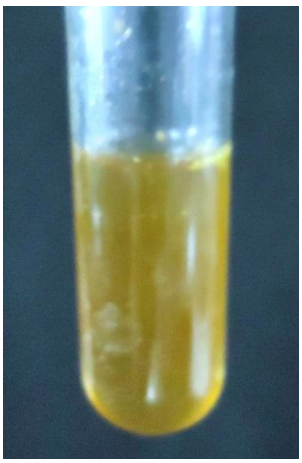

Figure 3.14: Detection Of Tannins

3.5.2 Quantitative Phytochemical Estimation

3.5.2.1 Determination of Total Phenol Content

Folin-ciocalteu's reagent method

The amount of total phenol content, in various solvent extracts of poovan peels 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).

3.5.2.2 Determination Of Total Flavonoid Content

Aluminium chloride calorimetric method

The amount of flavonoid content in various solvent extract of Poovan peels was determined by Aluminium chloride calorimetric method (chang*et 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).

3.6 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.

3.7 Determination of Anti - Psoriatic 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
HaCaTcells 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 MTTreduction assay, as described previously with slight modification. Briefly, HaCaTcells 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 *MUSA MYSORE AAB*(POOVAN BANANA) peel 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.

Results and Discussion

4.1 Extraction of Poovan Peels

The selected sample materials were extracted with different solvents Ethyl Acetate, Methanol and Hexane.

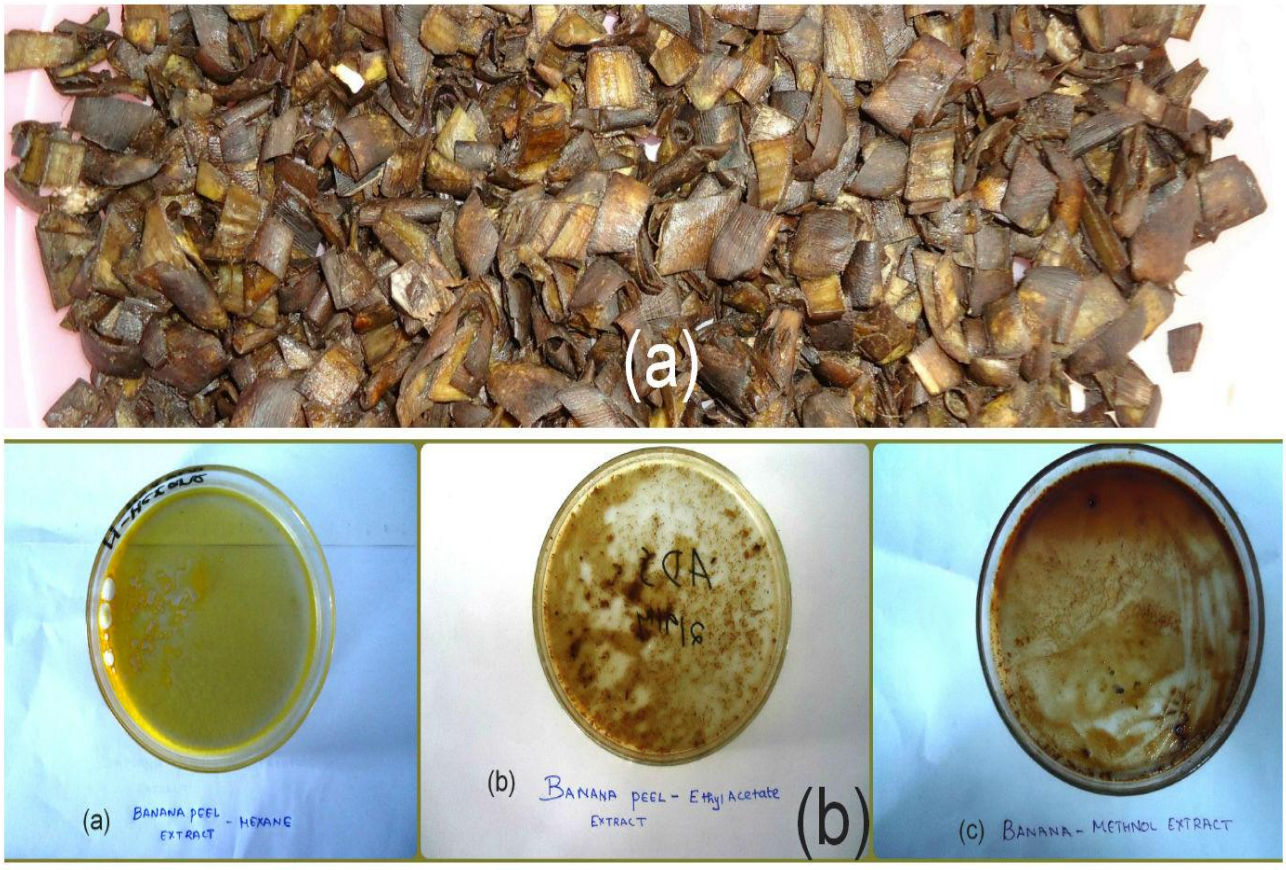

Figure 4.1: (a) Poovan peels (b) Extract of different solvents

4.2 Evaluation of Antioxident Potential

4.2.1 Radical Scavenging Activity (RSA) DPPH Assay
From the dose dependent response curve of DPPH radical scavenging activity of different peel extract of *Musa Mysore AAB*was observed that the Ethyl Acetate extract had higher radical scavenging activity at a concentraton of 200µg/ml,the scavenging activity of Ethyl Acetate extract reached 50%, which was comparable to that of standard chemical.

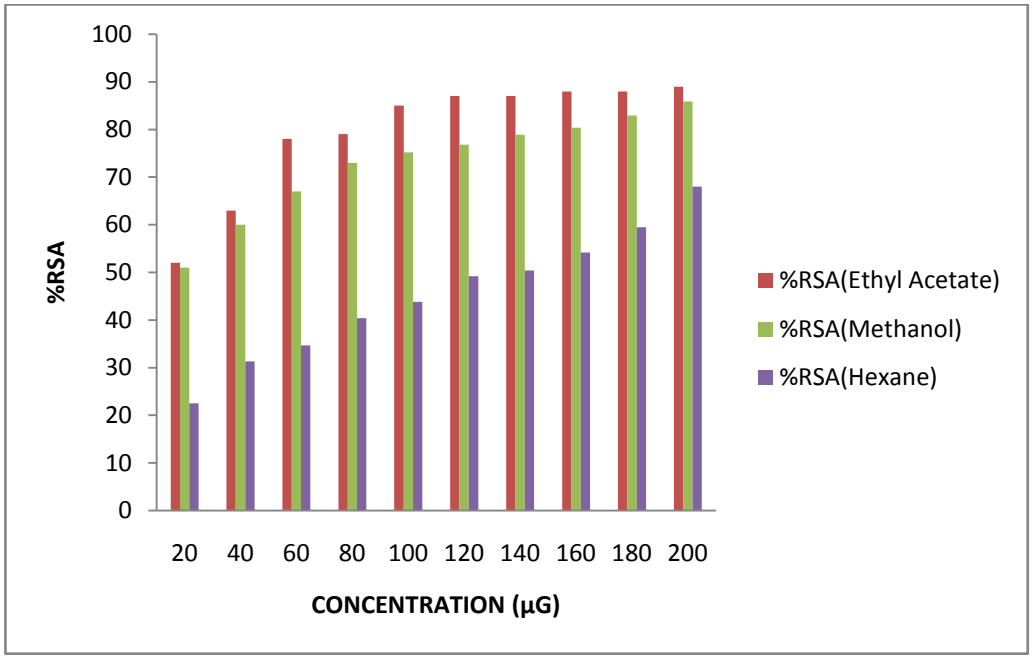

Figure 4.2.1: %RSA of Ethyl Acetate extract, Methanol and Hexane of*Musa Mysore* AAB

4.2.2 HYDROXYL RSA ASSAY

The scavenging capacity of the Ethyl Acetate extract of *Musa Mysore* AABwas studied to be effective at 71.4% (100µg/ml) as shown in fig4.2.2.

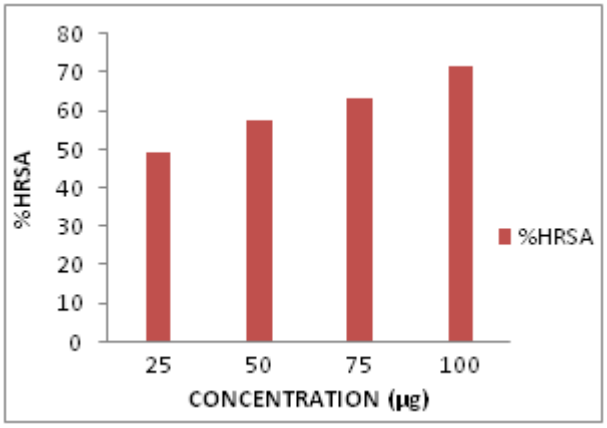

Figure 4.2.2: %HRSA of Ethyl Acetate extract of *Musa Mysore* AAB

4.3 Qualitative phytochemical screening of *Musa Mysore* AAB

The preliminary phytochemical screening of*Musa Mysore* AAB revealed the presence of phenols, proteins, Flavonoids, Gylcosides, Saponins, Carbohydrates, 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.

4.4Quantitative phytochemical analysis

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

Bioactive Compounds	*Amount (**µg/ml**)*
Total phenols	148.36
Total flavonoids	139.49

4.5 Thin Layer Chromatography

The chromatogram developed with 4% Ethyl Acetate in hexane revealed the presence of three major compound at Rf value of 0.619, 0.47, 0.41as visualized under iodine vapour and UV illumination.

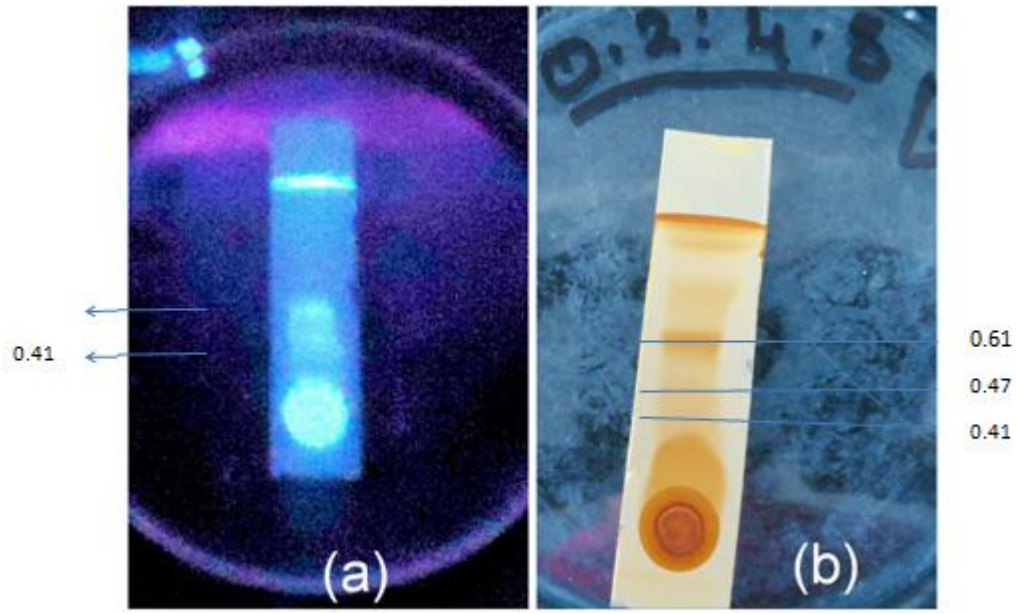

Figure 4.5: TLC under (a) Short UV (b) Iodine chamber

4.6 Evaluation of Antipsoriatic Activity of *Musa Mysore* AAB

The results of MTT assay suggest that the extract was capable of reducing cell viability of selected psoriatic cell line fig 4.6.Also, the IC_{50} of the selected extract was found to be 100µg where the cell viability was recorded as 52.24%.

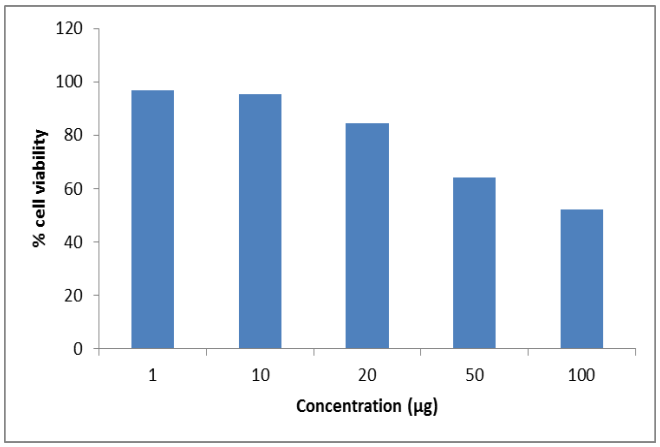

Figure 4.6: MTT assay of Ethylacetate extract of *Musa Mysore* AAB

Summary and Conclusion

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 electron donation ability of natural products can be measured by 2,2′-diphenyl-1- picrylhydrazyl 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. (**NaimaSaeedet al.,2012)**

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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 biomolecules of the living cells. These radical combines with nucleotides in DNA and cause strand breakage leading to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl 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 represent for the potential of *Musa Mysore* AAB. 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 *Musa Mysore* AABdepricts that the extract possed significant inhibits activity on the proliferative of HaCaT cell lines. This denotes that Musa Mysore AAB could be considered as an effective sourse of anti-psoriatic bioactive compounds.

In conclusion EthylAcetate extract of *Musa Mysore* AABshowed phytochemicals such as phenolics, Flavanoids. The dried extract of *Musa Mysore* AABshowed considerable inhibiting activity on free radical..

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