(c) ***Herpes simplex:*** Herpes simplex infection of mucosal cells results in cold sores and occasionally encephalitis and genital herpes. This virus comes in contact with the broken skin or the lining of the mouth, vagina or anus. Then goes to the nuclei of the cells and tries to reproduce itself. Sometimes the virus's replication process destroys the cells it has invaded causing blisters or ulcers to form on the skin.

(d) ***Warts:*** Warts are actually benign tumors of the epidermis caused by a virus. The virus is called as papilloma viruses that cause skin cells to proliferate and produce a benign growth. That is known as a wart or papilloma. This disease transmitted through direct contact.

Non-infective category diseases: The other types of skin disorders are like eczema and dermatitis, psoriasis, acne, skin cancer, etc. These types of diseases are easily curable by using various modern herbal formulations.

Dermatitis and eczema: Dermatitis is used to describe a specific type of rash with many causes. It is refers to any inflammation (swelling, itching, and redness) possibly associated with the skin. There are many types of dermatitis, including:

(a) ***Atopic dermatitis (eczema):*** This is a very common hereditary dermatitis, which causes an itchy rash primarily on the face, trunk, arms, and legs. It generally develops in infancy, but can also appear in early childhood. It may be associated with allergic diseases such as asthma and seasonal, hay fever, environmental and food allergies.

(b) ***Contact dermatitis:*** This is developed when the skin comes into contact with an irritating substance. The best-known cause of contact dermatitis is poison ivy, but there are many others, including chemicals found in laundry detergent (irritant contact dermatitis), cosmetics, perfumes and metals like nickel plating on jewelry, belt buckles, and the back of a snap.

(c) ***Seborrheic dermatitis:*** This oily rash, which appears on the scalp, face, chest, and back, is related to an overproduction of sebum from the sebaceous glands. This condition is common in infants and adolescents.

(d) ***Allergic contact dermatitis:*** It is a delayed hypersensitivity reaction involving allergens and antibodies.

(e) ***Stasis dermatitis:*** It occurs on the ankles and lower legs of people with venous insufficiency.

(f) ***Dyshidrotic dermatitis:*** It is also known as pompholyx. It is generally located on the hands or feet. It is characterized by redness, scaling and deep blisters.

(g) ***Nummular dermatitis:*** Coin shaped patches that occur anywhere on the body in relation to dry skin.

(h) ***Lichen simplex chronicus:*** It is a rash caused by long-term scratching of an area producing thickened skin.

Psoriasis: Psoriasis is a skin disease that causes itchy or sore patches of thick, red skin with silvery scales. It is a chronic, non-contagious autoimmune disease. It affects the skin and joint and is generally located on elbows, knees, scalp, back, face, palms and feet. The scaly patches caused by psoriasis, called psoriatic plaques, are areas of inflammation and excessive skin production. Skin rapidly accumulates at these sites and takes on a silvery-white appearance. Fingernails and toenails are frequently affected (psoriatic nail dystrophy) and can be seen as an isolated finding. Psoriasis arthritis is also a kind of disease due to inflammation of the joints caused by psoriasis. Generally, ten to fifteen percent of people with psoriasis are suffering by this psoriatic arthritis pain.

Acne: Acne is the most common cutaneous disorder. Generally, it is cause by *Acne vulgaris*. Nearly 80% of people aged between 11 and 30 years are affected. It is a disease of

was stopped by addition of glacial acetic acid. The reaction mixture was stirred and shaken with 4 ml of n-butanol and was allowed to stand for few minutes. After centrifugation, the color intensity of the chromogen in butanol layer was measured by colorimeter at 520 nm against control. The specific activity of the enzyme is expressed as enzyme required for 50% inhibition of nitroblue tetrazolium reduction/min/mg Hb for erythrocyte lysate and enzyme required for 50% inhibition of nitroblue tetrazolium reduction/min/mg protein for tissues.

Further the same method was modified by Guzman et al. (2001). The superoxide radical generated by hypoxanthine and xanthine oxidase system was determined. Basic principle involved the reaction was performed in the absence and presence of plant extracts or allopurinol or superoxide dismutase (reference compounds), containing 1 mM EDTA, 100 µM hypoxanthine, 100 µM NBT with final volume adjusted to 1.2 ml by 50 mM phosphate buffer (pH 7.4). The $O_2^{\bullet -}$ generation was initiated by the addition of 0.066 U xanthine oxidase and detected by the NBT reduction/min spectrophotometrically at 560 nm.

A reaction mixture with a final volume of 1 ml/tube was prepared with 50 mM $KH_2PO_4^-$ KOH pH 7.4 containing 1 mM EDTA, 100 µl hypoxanthine, 100 µM nitroblue tetrazolium and 0.666 U per tube of xanthine oxidase in 100 µl of phosphate buffer. The reaction mixtures were incubated at 25°C for 5 minutes and the absorbance was measured at 560 nm against standard ascorbic acid. The results are expressed as the percentage inhibition of nitroblue tetrazolium reduction rate with respect to the reaction mixture without test compound.

Catalase Enzyme Activity

The catalase activity was determined in erythrocyte lysate and tissue homogenate by the method described by Sinha (1972). The assay was based on the principle of dichromate in acetic acid was reduced to chromic acetate, when heated in the presence of hydrogen peroxide with the formation of perchromic acid as an unstable intermediate. The catalase preparation was allowed to split hydrogen peroxide for various period of time. The reaction was stopped at different time intervals by the addition of dichromate acetic acid mixture in hot condition. The remaining hydrogen peroxide forms hydrogen peroxide-chromic acetate which was determined colorimetrically at 590 nm. The used reagents are phosphate buffer 0.01 M, pH 7.0, hydrogen peroxide 0.2 M, 5% potassium dichromate, dichromate-acetic acid reagent (potassium dichromate and glacial acetic acid were mixed in the ratio of 1:3. From this mixture 1.0 ml was diluted with 4.0 ml acetic acid) and standard hydrogen peroxide 0.2 mM. The procedure as follows, tissue homogenate was prepared in phosphate buffer. To 0.9 ml of phosphate buffer, 0.1 ml of erythocyte lysate or tissue homogenate and 0.4 ml hydrogen peroxide were added. The reaction was arrested after 15, 30, 45 and 60 sec by adding 2.0 ml dichromate-acetic acid mixture. The tubes were kept in a boiling water bath for 10 minutes, cooled and color developed was measured at 590 nm. The specific enzyme activity is expressed as micro mole of hydrogen peroxide utilized/min/mg of Hb for erythrocyte lysate and micro moles of hydrogen peroxide utilized/min/mg of protein for tissues.

Further the method was modified by the method described by Aebi (1984). As per the method, packed erythrocytes were hemolyzed by adding 100 volumes of distilled water, then 20 µl of this hemolyzed sample was added to a cuvette and the reaction was started by the addition of 100 µl of freshly prepared 300 mM hydrogen peroxide in phosphate buffer (50 mM, pH 7.0) to give a final volume of 1 ml. The rate of hydrogen peroxide decomposition was measured spectrophotometrically at 240 nm during 120s. The catalase activity was expressed as µM H_2O_2/ml erythrocytes/min.

and was prepared freshly and warmed at 37°C. Aliquots of 40 µL sample filtrate was mixed with 0.2 ml of distilled water and 1.8 ml of FRAP reagent. Then the absorbance was measured at 593 nm using spectrophotometer after incubation at 37°C for 10 minutes. Gallic acid, ascorbic acid, BHA and trolox were used as the standard. The final result was expressed as the concentration of antioxidants having ferric reducing ability equivalent to that of mg of standard used per gram of sample on dry weight basis.

Trolox Equivalent Antioxidant Capacity (TEAC)

The method was based on the ability of antioxidant molecules to quench the $ABTS^{\bullet+}$ [2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)], a blue-green chromophore with characteristic absorption at 734 nm, compared with that of Trolox (water-soluble vitamin E analog). The addition of antioxidants to the preformed radical cation reduced it to ABTS, determining a decolorization. The method was developed by several researchers namely, Rice-Evans et al., 1994; Miller and Rice-Evans, 1996 ; Poungbangpho et al., 2000. The required chemicals were $ABTS^{\bullet+}$, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid) and myoglobin. All other chemicals were of analytical grade. Metmyoglobin was purified after adding the stock myoglobin solution (100 µM) in 5 mM isotonic phosphate buffer saline (PBS buffer), pH 7.4 to an equal volume of freshly prepared 740 µM potassium ferricyanide. The solution was dialysed against PBS buffer, pH 7.4 twice at 4°C for 24 hours, and the metmyoglobin was collected and calculated for the concentration as described by Miller and Rice-Evans (1996). All compounds were dissolved in PBS buffer, except Trolox or samples dissolved in 95% ethanol. The standard antioxidative curve of Trolox (concentration 0–2.5 mM) was obtained at the concentration of 108 µM H_2O_2 and 100 µM metmyoglobin at 20 minutes. The percentage inhibition of Trolox was calculated of the blank at an absorbance 734 nm by UV-vis spectrophotometer (Shimadzu, UV-1201v) and then was plotted as a function Trolox concentration. The total antioxidant capacity of several plant extracts were examined and compared with a standard antioxidant, Trolox. The Trolox equivalent antioxidant capacity was defined as the antioxidant capacity of 1 mg crude extracted to imol of Trolox and represented as TEAC value.

This method was described by Schlesier et al., 2002. The method is based on the reduction of $ABTS^{\bullet+}$ [2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)] radical nation by antioxidants. 25 mM of Trolox was prepared in ethanol and use as standard that was prepared by mixing $ABTS^{\bullet+}$ (7 mM) with 2.45 mM of potassium persulphate in water. The mixture was kept for 12–24 hours at room temperature in the dark until reaction to be completed. To prepare $ABTS^{\bullet+}$ working solution, $ABTS^{\bullet+}$ stock solution was diluted with water to an absorbance of 0.700 ± 0.02 at 734 nm. A stock solution of Trolox (1 mM) was prepared with water. Then for photometric assay, 1 ml of $ABTS^{\bullet+}$ working solution and 10 µl of 1 mM Trolox stock or 10 µl of a range of dilutions of plant extracts were mixed for a few seconds and measured immediately after 1 minute at 734 nm. The antioxidant activity of the test substances was calculated by determined the decreased in absorbance using the following formula:

$$\%\text{Antioxidant activity} = \{(E[ABTS^{\bullet+}]/E[\text{standard}]/E[ABTS^{\bullet+}]\} \times 100$$

Trolox equivalents were estimated by linear extrapolation of the antioxidant activity from the Trolox standards.

Hydrogen Peroxide Method

This is one of the most simple method for determination of antioxidant activity of the compounds. Jayaprakasha, et al. 2004, were described the method. Hydrogen peroxide solution (20 mM) was prepared in phosphate buffer saline (PBS) at pH 7.4. Various concentration of extracts or standard were prepared in 1 ml of methanol and further 2 ml

they are much cheaper and extend the shelf life of the product more than natural alternatives. But synthetic preservatives can cause allergies in susceptible people, including dermatitis and other side effects. Not only that, natural preservatives can also be harmful if they are uses in excess but natural preservative are not as toxic as synthetic preservative. Natural preservative are much safer because they are widely existed in nature and are known to the immune system of the body. Most preservatives derived from plants are safe for humans but the main drawback against natural preservative is that they are no powerful enough. Ideally no preservatives should be used but many products would not stay fresh even for short time without them. Hence preservatives should add to the cosmetic formulations in balance amount. Some of the synthetic preservatives which are commonly use by the cosmetic industries are Imidiazolidinyl urea (Germall 115) and diazolidinyl urea, DMDM hydantoin, methyl paraben, propyl paraben, 2-bromo-2-nitro-propane-1 3-diol, benzalkonium chloride, chloromethylisothiazolinone and isothiazolinone, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), methylisothiazolinone, etc. All these chemicals are having side effects commonly cause contact dermatitis, allergies and skin rashes. Whereas, commonly used natural preservatives are: Tea tree essential oil, Thyme essential oil, grapefruit seed extract, d-alpha tocopherol acetate (vitamin E), citric acid, neem oil, lemon, honey, bee propolis, rosemary extract, etc.

Tea tree oil: It is also known as Melaleuca oil. It is with pale yellow color fixed oil. The oil is procure from leaves of tea tree, scientifically known as *Melaleuca alternifolia*, belongs to family Myrtaceae.

Thyme oil: The oil is obtained from thymus vulgaris, belongs to the family Labiatae. It is mainly uses as antiseptic agent.

Grapefruit seed extract: It is obtained from *Vitis vinifera* belongs to the family of Vitaceae. It is rich in vitamin E, flavonoids, etc. It is a natural antibiotic, antiseptic, disinfectant and preservative. It is used to promote the healing of almost any atypical skin condition.

Alpha tocopherol acetate: It is also known as vitamin E. It is an antioxidant and nutrient. Vitamin E is abundant in whole wheat, rice germ, and vegetable oils. It is destroyed by the refining and bleaching of flour. Vitamin E is used because it prevents oils from going rancid. Recent studies indicate that large amounts of vitamin E may help reduce the risk of heart disease and cancer.

Citric acid: It is an important metabolite in all living organisms and is especially abundant naturally in citrus fruits and berries. It is an acid, flavoring and chelating agent in ice cream, sherbet, fruit drink, candy, carbonated beverages. It is used as an powerful antioxidant.

Neem oil: Neem oil is one of the most powerful oils obtained from seeds of *Azadirachta indica*, belongs to the family Meliaceae. The oil having great demand on the market today. It is antifungal, antibacterial as well as anti-protozoan and a spermicide.

Lemon: It is obtained from *Citrus lemon* belongs to family of Rutaceae. The acid produced mostly by the citrus and identified as $C_6H_8O_7$ promotes preservation. The lemon is rich in vitamin C and much like salt removes moisture to prevent spoilage and rotting.

Honey: It is known for being highly stable against microbial growth because of it is low moisture content and water activity, low pH and antimicrobial constituents. It is the saccharine liquid prepared from the nectar of the flowers by the hive bee *Apis mellifera* belongs to family Apidae.

Bee propolis: It is a mixture of beeswax and resins collected by the honey bee from plants, particularly flowers and leaf buds, it is used to line and seal the comb. The propolis is effective in protecting the hive offering both antibacterial and antifungal properties.