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RESEARCH ARTICLE

CHARACTERISATION AND *IN SILICO* STUDY OF SKIN EMOLLIENT ACTIVITY IN *OCIMUM TENUIFLORUM*, *AZADIRACHTA INDICA* AND *ALOE VERA* EXTRACTS

***Khoushika Raajshree, R. and Chitra, P.**

Department of Biochemistry, Sri Ramakrishna College of Arts and Science for Women, Coimbatore, Tamilnadu, India

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ABSTRACT

Emollients have been part of human life for centuries. It softens and smoothes the skin. They improve the skin barrier function by decreasing the Transepidermal water loss. Leaves of *Ocimum tenuiflorum* and *Azadirachta indica* as well as peel of *Aloe vera* have been principle ingredients in emollient creams. They were subjected to qualitative tests and quantitative total phenolic content assay. It was found that the ethanolic extracts of the sample had high phenolic content and it had a good correlation with DPPH free radical scavenging assay. GC-MS analysis of the ethanolic extracts of the samples gave several compounds and of which Thymol, Methylparaben, Vitamin E, 1-acetyl pyrrolidine and n-Hexadecanoic acid were selected for the study. It was docked against Human Retinoic Acid Receptor (RXR)-Gamma. *In silico* docking studies showed that n-Hexadecanoic acid as an active compound with high emollient activity with a fitness score of 33.05. This paves way for treating Eczema and photodamaged skin at molecular level.

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INTRODUCTION

Emollients have been part of human life for centuries. Records suggest that the ancient Greeks used wool fat on their skin as early as 700BC (Marks, 2001). Emollients in the modern day are much more user-friendly than raw wool fat. While they are commonly used for cosmetic purposes, they are also vital for the treatment of dry skin conditions and for the promotion of skin health. The word emollient is a Latin derivation and implies a material that softens and smoothes the skin (Loden, 2003a). Emollients should have the effect of reducing the clinical signs of dryness, such as roughness or scaling, and improving sensations, such as itching and tightness. They should also be acceptable cosmetically, that is in a way that permits the person to fulfil their lifestyle at the same time as promoting adherence to treatment (Loden, 2003a). Emollients work to moisturise the skin by increasing the amount of water held in the stratum corneum (Cork, 1997, Marks, 1997, Loden, 2003b). Specifically, depending on the constituents of the emollients, they work either by occlusion, 'trapping' moisture into the skin (which slows the evaporation of water), or in an 'active' way by drawing moisture into the stratum corneum from the dermis (Fendler, 2000, Flynn *et al.*, 2001, Rawlings *et al.*, 2004). Occlusion is most effectively achieved if greasy (heavy sealing) substances, such as petrolatum are used (Fendler, 2000). The occlusive effect traps water in the stratum corneum (preventing transepidermal water loss by evaporation) and thereby mimics the role of natural emollients such as sebum and natural moisturising factor (NMF). Indeed, (Rawlings *et al*., 2004) report that petrolatum jelly moisturisers reduce water loss by 98%, whereas other oils only manage to reduce water loss by 20–30%. The second mode of action involves the active movement of water from the dermis to the epidermis. Emollients that have this effect contain substances known as humectants, e.g., urea and glycerine. These have a low molecular weight and water-attracting properties (Loden, 2003b) and as they penetrate the epidermis they draw water in from the dermis. Some cream and lotion emollients contain a mixture of occlusive and humectant substances—the humectant draws water into the epidermis while the occlusive element ensures that it is trapped there. Research work carried out in the field of eczema provides some useful evidence for the impact of emollients on the barrier function of the skin (Rawlings *et al.,* 1994 and Cork, 1997). The stratum corneum was compared to a brick wall — the corneocytes represent the bricks and the intercellular lipids the mortar (Elias, 1993).

****Corresponding author: Khoushika Raajshree, R.***
Department of Biochemistry, Sri Ramakrishna College of Arts and Science for Women, Coimbatore, Tamilnadu, India.

These lipid bilayers are composed of ceramides, cholesterol and free fatty acids (Downing and Stewart, 2000). As the skin loses moisture and becomes dry, the corneocytes shrink and gaps develop between the cells, thus compromising the barrier function of the skin. When applied to the skin, the emollient will trap water, thus rehydrating the corneocytes. As the emollient penetrates the stratum corneum, it mimics the natural lipids that are so vital to the barrier function. The distinct expression patterns of the retinoid receptors RAR-a, RAR g, and RXR a in normal keratinocytes *in vivo* and in culture (Karlsson, 2002).

Vitamin A and metabolites (retinoid) are crucial for normal epidermal maturation. Physiological effects are mediated by retinoic acid (RA) that activates nuclear retinoic acid receptors (RARs) and complexes with retinoid X receptors (RXRs), resulting in altered gene transcription. Retinoid exert their effect by binding to specific nuclear receptors and thereby regulate cell growth and cellular proliferation, differentiation and apoptosis. This effect is a class effect, but each retinoid has a higher affinity for specific receptors. Retinoid receptors are divided into two groups: RAR-α,β,γ and RXR-α,β,γ. In the skin the predominant retinoid receptor is the RAR-γ/RXR-α heterodimer (Cheng *et al*., 2008).

Ocimum tenuiflorum, also known as *Ocimum sanctum,* holy basil, is an aromatic plant in the family Lamiaceae which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. It is a natural skin emollient which improves firmness & texture thus make skin soft & supple. It also has Anti-bacterial and Anti-fungal properties. *Azadirachta indica*, also known as Neem, Nimtree and Indian Lilac is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*. It is an excellent moisturizer , high in vitamin E , rich in emollient and fatty acids. It has a very good Anti-Bacterial property. *Aloe vera* is a succulent plant species. The species is frequently cited as being used in herbal medicine since the beginning of the first century AD. Extracts from Aloe *vera* are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing, or soothing properties. There is scientific evidence of the effectiveness Aloe *vera* extracts for cosmetics. It has a very good emollient property.

The present study is aimed to Characterize and study the Skin emollient activity in *Ocimum tenuiflorum*, *Azadirachta indica* and Aloe *vera extracts* by *in silico* methods.

The objectives of the current study are as follows:

1. To collect the *Ocimum tenuiflorum*, *Azadirachta indica, Aloe vera* peel samples
2. To Shade dry the samples for a week
3. To sequentially extract the plant secondary metabolites of *Ocimum tenuiflorum*, *Azadirachta indica, Aloe vera* peel using hexane, ethyl acetate and ethanol
4. To perform qualitative analysis of the plant extracts
5. To quantify the phenolic content in the plant extracts
6. To determine the free radical scavenging potential of the plant extracts by DPPH assay
7. To understand the phytoconstituents present in the plant extracts by GC-MS
8. To perform in silico modeling of target protein and molecular docking of the phytoconstituents against the target

Review of Literature

Emollients and moisturizing creams are used to break the dry skin cycle and to maintain the smoothness of the skin. The term 'moisturizer' is often used synonymously with emollient, but moisturizers often contain humectants in order to hydrate the stratum corneum. Dryness is frequently linked to an impaired barrier function observed, for example, in atopic skin, psoriasis, ichthyosis, and contact dermatitis (Loden, 2003). Dry skin is a common skin condition as well as a key aspect of a number of diseases such as atopic dermatitis and psoriasis but also of other diseases and systemic conditions. Dry skin has an impact on the patient in terms of discomfort, pruritus and impaired quality of life. Within the overall treatment regimen for these diseases, the use of emollients to manage dry skin plays a considerable role in managing skin conditions. In atopic dermatitis and psoriasis, emollients help to improve skin condition and to reduce pruritus alongside more potent pharmacological agents. It is important to choose an emollient that not only soothes and rehydrates the skin but also offers numerous other dermatological supporting roles, especially induction of proper epidermal differentiation. This review will explain the role of emollients within the management of diseases with dry skin as a major symptom and the components of an ideal emollient (Proksch, 2008).

The enucleate layer of the epidermis, i.e. the stratum corneum, is responsible for certain critical protective functions, such as epidermal permeability barrier function. Within the epidermal membrane lamella component, ceramides are the dominant lipid class by weight (over 50%) and exhibit the greatest molecular heterogeneity in terms of sphingoid base and fatty acid composition. It is now evermore important to understand how ceramide production and functions are controlled in the epidermis, since decreased epidermal ceramide content has been linked to water loss and barrier dysfunction. During the past several years, critical enzymes in ceramide biosynthesis have been identified, including ceramide synthases (CerS) and ceramide hydroxylase/desaturase. In this review, we describe the molecular heterogeneity of ceramides synthesized in the epidermis and their possible roles in epidermal permeability barrier functions. We also describe recent studies that identified the family of CerS (CerS1-CerS6) in mammals. We further focus on the roles of specific isoforms of these enzymes in synthesizing the epidermal ceramides, especially in relation to chain-length specificity. In addition, we provide experimental information, including our recent findings, as to how applying ceramide or ceramide-containing substances to skin, orally or directly, can benefit skin health (Mizutani *et al.,* 2009).

The epidermal keratinocytes produce and secrete lipids to maintain the water barrier of the epidermis. To clarify the regulation of epidermal lipid synthesis, we investigated the

hormonal effect on the activity of fatty acid synthase (FAS) of the keratinocytes, and the expression of FAS in the human skin. In cultured keratinocytes, the FAS activity, assayed by measuring the oxidation of NADPH, was slightly increased by hydrocortisone or testosterone, but not influenced by thyroid hormone, estrogen, progesterone or insulin (Uchiyama, 2000).

The permeability barrier is mediated by a mixture of ceramides, sterols, and free fatty acids arranged as extracellular lamellar bilayers in the stratum corneum. Whereas prior studies have shown that cholesterol and ceramides are required for normal barrier function, definitive evidence for the importance of nonessential fatty acids is not available. To determine whether epidermal fatty acid synthesis also is required for barrier homeostasis, we applied 5-(tetradecyloxy)-2-furancarboxylic acid (TOFA), an inhibitor of acetyl CoA carboxylase, after disruption of the barrier by acetone or tape stripping. TOFA inhibits epidermal fatty acid by approximately 50% and significantly delays barrier recovery. Moreover, coadministration of palmitate with TOFA normalizes barrier recovery, indicating that the delay is due to a deficiency in bulk fatty acids. Furthermore, TOFA treatment also delays the return of lipids to the stratum corneum and results in abnormalities in the structure of lamellar bodies, the organelle which delivers lipid to the stratum corneum. In addition, the organization of secreted lamellar body material into lamellar bilayers within the stratum corneum interstices is disrupted by TOFA treatment. Finally, these abnormalities in lamellar body and stratum corneum membrane structure are corrected by co application of palmitate with TOFA. These results demonstrate a requirement for bulk fatty acids in barrier homeostasis. Thus, inhibiting the epidermal synthesis of any three key lipids that form the extracellular, lipid-enriched membranes of the stratum corneum results in impairment in barrier homeostasis (Quiong *et al*., 1993).

In addition to substances considered as actives, e.g. fats and humectants, moisturizers contain substances conventionally considered as excipients (e.g. emulsifiers, antioxidants, preservatives). Recent findings indicate that actives and excipients may have more pronounced effects in the skin than previously considered. Some formulations may deteriorate the skin condition, whereas others improve the clinical appearance and skin barrier function. For example, emulsifiers may weaken the barrier. On the other hand, petrolatum has an immediate barrier-repairing effect in delipidized stratum corneum. Moreover, one ceramide-dominant lipid mixture improved atopic dermatitis and decreased transepidermal water loss (TEWL) in an open-label study in children. In double-blind studies moisturizers with urea have been shown to reduce TEWL in atopic and ichthyotic patients. Urea also makes normal and atopic skin less susceptible against irritation to sodium laurilsulfate. Treatments improving the barrier function may reduce the likelihood of further aggravation of the disease (Loden, 2003). In order to have optimum effect it is conceivable that moisturizers should be tailored with respect to the epidermal abnormality. New biochemical approaches and non-invasive instruments will increase our understanding of skin barrier disorders and facilitate optimum treatments. The chemistry and function of dry skin and moisturizers is a challenging subject for the practicing dermatologist, as well as for the chemist developing these agents in the pharmaceutical/cosmetic industry (Loden, 2003). When the cells were incubated with 1-30 micromol L-1 nicotinamide for 6 days, the rate of ceramide biosynthesis was increased dose-dependently by 4.1-5. 5-fold on the sixth day compared with control. Nicotinamide also increased the synthesis of glucosylceramide (7.4-fold) and sphingomyelin (3.1-fold) in the same concentration range effective for ceramide synthesis. Furthermore, the activity of serine palmitoyltransferase (SPT), the rate-limiting enzyme in sphingolipid synthesis, was increased in nicotinamide-treated cells. Nicotinamide increased the levels of human LCB1 and LCB2 mRNA, both of which encode subunits of SPT. This suggested that the increase in SPT activity was due to an increase in SPT mRNA. Nicotinamide increased not only ceramide synthesis but also free fatty acid (2.3-fold) and cholesterol synthesis (1.5-fold). Topical application of nicotinamide increased ceramide and free fatty acid levels in the stratum corneum, and decreased transepidermal water loss in dry skin. Nicotinamide improved the permeability barrier by stimulating de novo synthesis of ceramides, with upregulation of SPT and other intercellular lipids (Tanno *et al*., 2000).

MATERIALS AND METHODS

Collection and preparation of plant materials

Ocimum tenuiflorum leaves, *Azadirachta indica* leaves, Aloe *vera* were collected from healthy environment at Teynampet, Chennai. They were rinsed thoroughly. *Aloe vera* was scrapped off and the peel was taken for further analysis. *Ocimum tenuiflorum* leaves, *Azadirachta indica* leaves, *Aloe vera* peel were then shade dried for a week and ground to a coarse powder using mortar and pestle. The samples were stored in an airtight container at room temperature for further analysis.

Extraction of plant material

50g of air dried, powdered material (leaves and peel) was separately macerated with three solvents namely hexane, ethyl acetate and ethanol in the ratio (1:3). The mixture was then filtered after 48 hours. Filtered and collected the solvent. The solvents were evaporated to obtain the extracts.

Qualitative analysis

Phytochemical tests

The methods described by Trease and Evans, 1989 and Abalaka *et al*., 2011 were used for phytochemical screening of the extracts.'

Test for Carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Appearance of purple or reddish color indicates the presence of carbohydrates.

Test for Tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Appearance of dark blue or greenish black indicates the presence of tannins.

Test for Saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for Flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for Quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Appearance of red color indicates presence of quinones.

Test for Glycosides

To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Appearance of pink color indicates presence of glycosides.

Test for Cardiac glycosides

To 0.5ml of plant extract, 2ml of glacial acetic acid and few drops of 5% Ferric chloride is added. This was under layered with 1ml of Concentrated Sulphuric acid. Formation of brown ring indicates the presence of Cardiac glycosides.

Test for Terpenoids

To 0.5ml of plant extract, 2ml of chloroform and 1ml of Concentrated sulphuric acid was added. Appearance of red brown colour at interface indicates the presence of Terpenoids.

Test for Phenols

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Appearance of blue or green color indicates presence of phenols.

Test for Coumarins

To 1ml of plant extract, 1ml of 10% NaOH was added. Appearance of yellow colour indicates the presence of Coumarin.

Test for Steroids and phytosteroids

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Test for Phlobatannins

To 1ml of plant extract, add few drops of 2% hydrochloric acid. Appearance of red colour indicates the presence of phlobatannins.

Test for Anthraquinones

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

Quantitative analysis

Total Phenolic Content (TPC)

Total phenolic content of 9 extracts were assessed according to the Folin–Ciocalteau method (Slinkard and Singleton, 1977) with some modifications. Briefly, 0.1 ml of extracts (200, 600 and 1000µg/ml), 1.9 ml distilled water and 1 ml of Folin–Ciocalteau's reagent were seeded in a tube, and then 1 ml of 100 g/l Na_2CO_3 was added. The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. The results were compared to a catechol calibration curve and the total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

Amount TPC = Sample OD/Standard OD * Respective Amount of extract

Anti oxidant activity

DPPH free radical scavenging assay

The ability of the 9 extracts to annihilate the DPPH radical (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by Blois, 1958. Stock solution of extracts was prepared to the concentration of 10 mg/ml. Different concentration of the extract (200, 600 and 1000 µg) of extracts were added, at an equal volume, to Methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. Ascorbic acid was used as standard controls. The annihilation activity of free radicals was calculated in % inhibition according to the following formula,

% of Inhibition = (A of control – A of Test)/A of control * 100

Gas chromatography—mass spectrometry

The extracts (1 from each sample) with high DPPH free radical scavenging activity were mixed in the ratio 1:1:1 and subjected to GC-MS analysis using the software XCALIBUR (ver-2.2). The GC-MS analysis of unknown compounds of deals with using a TSQ QUANTUM XLS Gas Chromatography. It ionizes compounds and measures their mass number equipped with DB-5MS (30m X 0.25mm X 0.25um) and mass detector turbo mass gold of the company which was operated at in EI mode.

Helium was carried the gas at the flow rate of 1 ml / min, the injector was operated at 280˚C and the oven temperature was programmed as follows: 70˚C @ 8˚C /min to 150˚C (1 min) @ 8˚C / min to 280˚C (10 min). The identification of components was based on comparison of their mass spectra with NIST-011S library.

Molecular docking

GOLD is a program for calculating the docking modes of small molecules in protein binding sites and is provided as part of the *GOLD Suite*, a package of programs for structure visualization and manipulation (Hermes), for protein-ligand docking (GOLD) and for post-processing (GoldMine) and visualisation of docking results. Hermes acts as a hub for many of CCDC's products, for more information please refer to the Hermes product page. The product of a collaboration between the University of Sheffield, GlaxoSmithKline plc and CCDC, GOLD is very highly regarded within the molecular modelling community for its accuracy and reliability.Gold Score performs a force field based scoring function and is made up of four components, Protein-ligand hydrogen bond energy (external H-bond); Protein-ligand vander Waals energy (external vdw); Ligand internal vander Waals energy (internal vdw); and Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore Fitness = S (hb_ext) + 1.3750*S(vdw_ext) + S(hb_int) + 1.0000*S(int)
S (int) = S (vdw_int) + S(tors)

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intra-molecular hydrogen bond in the ligand and S (vdw_int) is the score from intra-molecular strain in the ligand.

Out of several compounds obtained , the below are the five compounds with emollient activity were selected for molecular docking. They are as follows

1. Methylparaben
2. Vitamin E
3. 1 acetyl pyrrolidine
4. n-Hexadecanoic acid
5. Thymol

The crystal structure of the (target) Human Retinoic Acid Receptor RXR-Gamma was retrieved from Protein Data Bank (PDB ID-2GL8). Molecular docking of the selected compounds against the target was performed using Genetic Optimisation for Ligand Docking (GOLD) and GoldScore was calculated. The fitness score (GoldScore) was taken as the negative of the sum of the component energy terms, so that larger fitness scores are better.

RESULTS AND DISCUSSION

Collection and preparation of plant materials

Healthy fresh leaves of *Ocimum tenuiflorum* leaves, Azadirachta *indica* leaves, *Aloe vera* are collected from Teynampet, Chennai. The leaves are rinsed with distilled water and dried at room temperature under well ventilated shade. The dried leaves are powdered and stored in air-tight container for further analysis.

Extraction of plant material

The extract is prepared by adding 150 ml of hexane, ethyl acetate and ethanol to 50 g of powdered samples. After 24 hours, the solvent is allowed to evaporate at room temperature to obtain the hexane, ethyl acetate and ethanol extracts.

Qualitative analysis

Phytochemicals are the potent bioactive components that provide the therapeutic effect in medicinal plants (Doss *et al*., 2009).

Phytochemical tests

The presence (+) or absence (-) of the phytoconstituents in hexane, ethyl acetate and ethanol extracts of *Ocimum tenuiflorum* are depicted in the Table 1.

Table 1. Results of phytochemical tests for *Ocimum tenuiflorum* - Hexane, Ethyl acetate and Ethanol Extracts

S.No.	Phytochemical Tests	Results		
		Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
1	Carbohydrates	+	+	+
2	Tannins	+	+	+
3	Saponins	-	-	-
4	Flavonoids	Weakly +	+	+
5	Alkaloid	-	-	-
6	Quinones	-	-	-
7	Glycosides	-	-	-
8	Cardiac glycosides	-	-	-
9	Terpenoids	-	-	-
10	Phenols	+	+	+
11	Coumarins	-	-	-
12	Steroids and Phytosteroids	Phytosteroid +	Phytosteroid +	Phytosteroid +
13	Phlobatannins	-	-	-
14	Anthraquinones	-	-	-

Phytochemical tests for *Ocimum tenuiflorum* - Hexane, Ethyl acetate and Ethanol Extracts are performed and found that Carbohydrates, tannins, flavonoids , phenols and phytosteroids are present in all the extracts. Flavonoids are weakly present. The above results are similar to the study of (Somkuwar and Kamble, 2013) in which the presence of the carbohydrates, tannins, flavonoids were reported in the extracts of *Mangifera indica.*

The presence (+) or absence(-) of the phytoconstituents in hexane, ethyl acetate and ethanol extracts of *Azadirachta indica* are depicted in the Table 2.

Phytochemical tests for *Azadirachta indica* - Hexane, Ethyl acetate and Ethanol Extracts are performed and found that Carbohydrates, tannins, flavonoids , phenols and phytosteroids are present. Flavonoids are weakly present. The above results are similar to the study of (Somkuwar and Kamble, 2013) in which the presence of the carbohydrates,

tannins, flavonoids were reported in the extracts of *Mangifera indica*.

Table 2. Results of phytochemical tests for *Azadirachta indica* - Hexane, Ethyl acetate and Ethanol Extracts

S.No	Phytochemical Tests	Results		
		Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
1	Carbohydrates	+	+	+
2	Tannins	+	+	+
3	Saponins	-	-	-
4	Flavonoids	-	Weakly +	+
5	Alkaloid	-	-	-
6	Quinones	-	-	-
7	Glycosides	-	-	-
8	Cardiac glycosides	-	-	-
9	Terpenoids	-	-	-
10	Phenols	+	+	+
11	Coumarins	-	-	-
12	Steroids and Phytosteroids	Phytosteroid +	Phytosteroid +	Phytosteroid +
13	Phlobatannins	-	-	-
14	Anthraquinones	-	-	-

The presence (+) or absence(-) of the phytoconstituents in hexane, ethyl acetate and ethanol extracts of *Aloe vera* are depicted in the Table 3.

Table 3. Results of phytochemical tests for *Aloe vera* peel - Hexane, Ethyl acetate and Ethanol Extracts

S.No	Phytochemical Tests	Results		
		Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
1	Carbohydrates	+	+	-
2	Tannins	-	+	+
3	Saponins	-	-	-
4	Flavonoids	+	+	+
5	Alkaloid	-	-	-
6	Quinones	-	-	-
7	Glycosides	-	-	-
8	Cardiac glycosides	-	-	-
9	Terpenoids	-	-	-
10	Phenols	+	+	+
11	Coumarins	-	-	-
12	Steroids and Phytosteroids	Phytosteroid +	Phytosteroid +	Phytosteroid +
13	Phlobatannins	-	-	-
14	Anthraquinones	+	+	+

Phytochemical tests for *Aloe vera* - Hexane, Ethyl acetate and Ethanol Extracts are performed and found that Carbohydrates, tannins, flavonoids, phenols, anthraquinones and phytosteroids are present. The above results are similar to the study of (Somkuwar and Kamble, 2013) in which the presence of the carbohydrates, tannins, flavonoids are reported in the extracts of *Mangifera indica.*

Quantitative analysis

Total phenolic content

Phenolic compounds are among the most important plant components as they possess a variety of biological activities including antioxidant activity, therefore it is quite important to evaluate the total phenolic content in tested extracts (Elzaawely and Tawata, 2010).

The quantitative analysis of *Ocimum tenuiflorum*- Hexane, Ethyl acetae and Ethanol extracts are presented in the Table 4.

Table 4. Total Phenolic Content of *Ocimum tenuiflorum* – Hexane, Ethyl acetate and Ethanol Extracts

Total Phenolic Content		
Concentration (µg/ml)	Name of the extract	Amount of Phenol*
200		23.101695
600	Hexane	54.07574
1000		69.7226
200	Ethyl acetate	33.93709
600		55.05189
1000		74.0634
200		46.7462
600	Ethanol	58.85554
1000		84.14986

*Amount of Phenol is expressed as mg of catechol per gram of extract

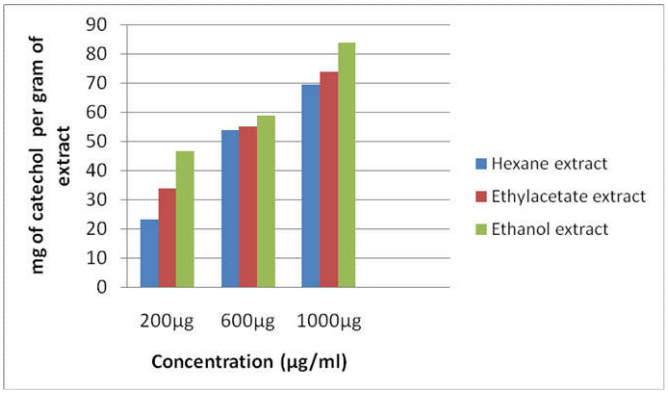

Figure 1. Total Phenolic Content of *Ocimum tenuiflorum* – Hexane, Ethyl acetate and Ethanol Extracts

The total phenolic content of *Ocimum tenuiflorum* - Hexane, Ethyl acetate and Ethanol Extracts are assayed by the quantitative method using standard catechol as the positive control. Ethanolic extract has the highest total phenolic content (84.14 mg catechol equivalents/g of extract). This is in accordance with the study of (Elzaawely and Tawata, 2010) in which the ethanol extract of *Mangifera indica* had high phenolic content. The quantitative analysis of *Azadirachta indica* - Hexane, Ethyl acetae and Ethanol extracts are presented in the Table 5.

Table 5. Total Phenolic Content of *Azadirachta indica* – Hexane, Ethyl acetate and Ethanol Extracts

Total Phenolic Content		
Concentration (µg/ml)	Name of the extract	Amount of Phenol*
200		32.21258
600	Hexane	55.05189
1000		71.99207
200	Ethyl acetate	40.59653
600		58.95652
1000		75.88256
200		51.40998
600	Ethanol	61.59888
1000		88.97695

*Amount of Phenol is expressed as mg of catechol per gram of extract

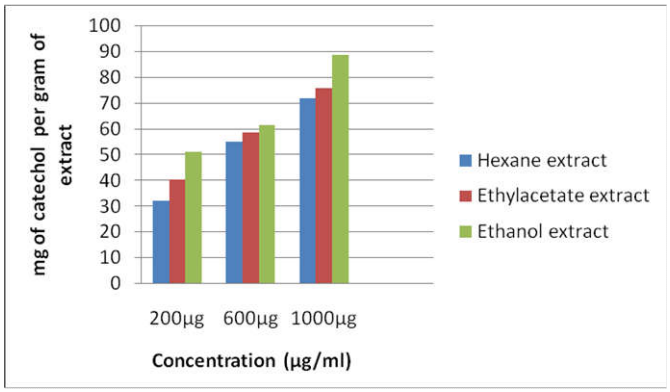

Figure 2. Total Phenolic Content of– *Azadirachta indica* Hexane, Ethyl acetate and Ethanol Extracts

The total phenolic content of *Azadirachta indica* - Hexane, Ethyl acetate and Ethanol Extracts are assayed by the quantitative method using standard catechol as the positive control. Ethanolic extract has the highest total phenolic content (88.97 mg catechol equivalents/g of extract). This is in accordance with the study of (Elzaawely and Tawata, 2010) in which the ethanol extract of *Mangifera indica* had high phenolic content.

The quantitative analysis of *Aloe vera* - Hexane, Ethyl acetae and Ethanol extracts are presented in the Table 6.

Table 6. Total Phenolic Content of *Aloe vera* – Hexane, Ethyl acetate and Ethanol Extracts

Total Phenolic Content		
Concentration (µg/ml)	Name of the extract	Amount of Phenol*
200		32.2342
600	Hexane	54.9845
1000		71.9020
200	Ethyl acetate	40.5748
600		59.0070
1000		75.8285
200		50.9761
600	Ethanol	61.4978
1000		88.9769

*Amount of Phenol is expressed as mg of catechol per gram of extract

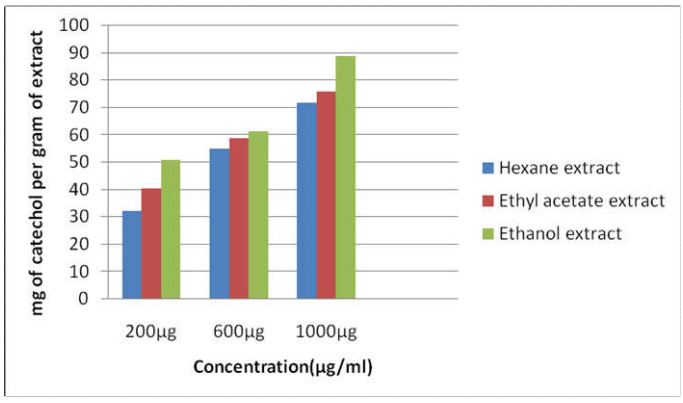

Figure 3. Total Phenolic Content of– *Aloe vera* Hexane, Ethyl acetate and Ethanol Extracts

The total phenolic content of *Aloe vera* - Hexane, Ethyl acetate and Ethanol Extracts are assayed by the quantitative method using standard catechol as the positive control. Peel extract has the highest total phenolic content (88.97 mg catechol equivalents/g of extract). This is in accordance with the study of (Elzaawely and Tawata, 2010) in which the ethanol extract of *Mangifera indica* had high phenolic content.

Anti oxidant activity

DPPH free radical scavenging assay

Scavenging of the stable radical DPPH is considered a valid and easy assay to evaluate scavenging activity of anti oxidants (Nanjo *et al.,* 1996). It is quite important to evaluate the DPPH scavenging activity of plant extracts.

The DPPH scavenging activity of *Ocimum tenuiflorum* - Hexane, Ethyl acetae and Ethanol extracts are presented in the table 7.

Table 7. DPPH Scavenging activity of *Ocimum tenuiflorum* – Hexane, Ethyl acetate and Ethanol Extracts

Concentration (µg/ml)	Name of the extract	Control	DPPH Scavenging Activity	
			% of Inhibition	
			% Inhibition of Sample	% Inhibition of Ascorbic acid*
200	Hexane	0.6508	47.95636	18.56177
600		0.6508	51.58267	52.36632
1000		0.6508	51.62876	80.02459
200		0.6508	57.36017	18.56177
600	Ethyl acetate	0.6508	58.65089	52.36632
1000		0.6508	58.98894	80.02459
200	Ethanol	0.6508	46.55808	18.56177
600		0.6508	56.80701	52.36632
1000		0.6508	63.09158	80.02459

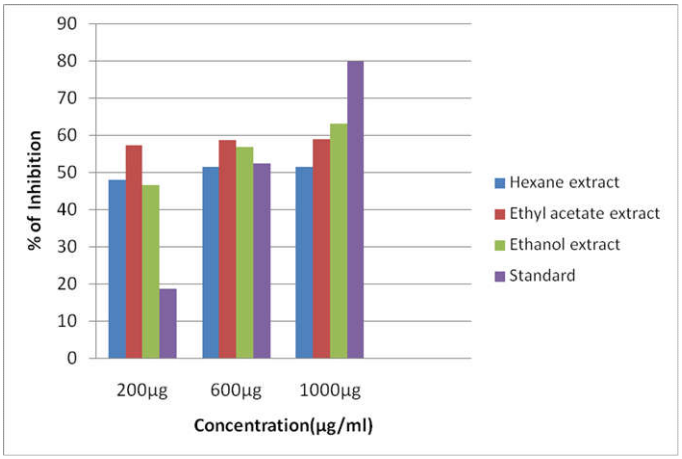

Figure 4. DPPH scavenging activity of *Ocimum tenuiflorum* – Hexane, Ethyl acetate and Ethanol Extracts

The in vitro antioxidant activity determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay of *Ocimum tenuiflorum* - Ethanol extract revealed that it has high antioxidant activity with 63% inhibition at 1000µg/ml concentration of extract. These activities are lesser than ascorbic acid. These results are in accordance to the work of (Kaur *et al.,* 2015) who stated that the ethanol extracts of

Mangifera indica had high DPPH scavenging activity. The more rapidly the absorbance decreases the more potent is the antioxidant activity of the extract.

The DPPH scavenging activity of *Azadirachta indica* - Hexane, Ethyl acetae and Ethanol extracts are presented in the Table 8.

Table 8. DPPH Scavenging activity of *Azadirachta indica* - Hexane Ethyl acetate and Ethanol Extracts

Concentration (µg/ml)	Name of the extract	Control	DPPH Scavenging Activity % of Inhibition	
			% Inhibition of Sample	% Inhibition of Ascorbic acid*
200	Hexane	0.6508	31.86847	18.56177
600		0.6508	49.80025	52.36632
1000		0.6508	52.71973	80.02459
200		0.6508	41.73325	18.56177
600	Ethyl acetate	0.6508	58.49723	52.36632
1000		0.6508	73.40197	80.02459
200	Ethanol	0.6508	75.29195	18.56177
600		0.6508	81.97603	52.36632
1000		0.6508	86.27843	80.02459

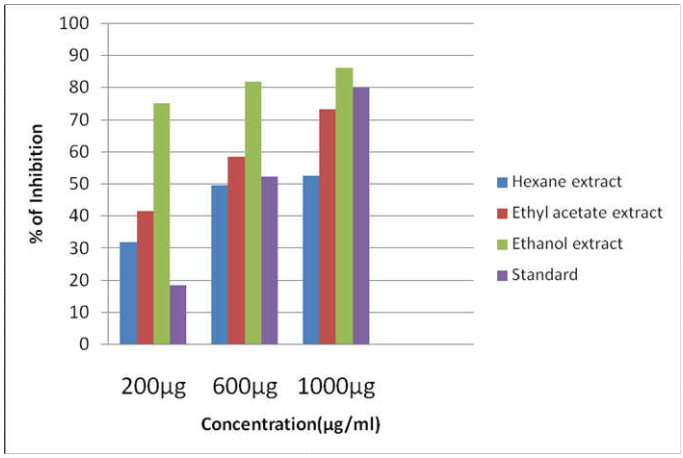

Figure 5. DPPH scavenging activity of *Azadirachta indica* – Hexane, Ethyl acetate and Ethanol Extracts

The *invitro* antioxidant activity determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay of *Azadirachta indica* - Ethanol extract revealed that it has high antioxidant activity with 86% inhibition at 1000µg/ml concentration of plant extract. These activities are lesser than ascorbic acid. These results are in accordance to the work of (Kaur *et al.,* 2015) who stated that the ethanol extracts of *Mangifera indica* had high DPPH scavenging activity. The more rapidly the absorbance decreases the more potent is the antioxidant activity of the extract.

The DPPH scavenging activity of *Aloe vera* - Hexane, Ethyl acetae and Ethanol extracts are presented in the Table 9.

The in vitro antioxidant activity determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay of *Aloe vera* peel - Ethanol extract revealed that it has high antioxidant activity with 97% inhibition at 1000µg/ml concentration of plant extract, These activities are lesser than ascorbic acid. These results are in accordance to the work of (Kaur *et al.,* 2015) who stated that the ethanol extracts of *Mangifera indica* had high DPPH scavenging activity. The more rapidly the absorbance decreases the more potent is the antioxidant activity of the extract.

Table 9. DPPH Scavenging activity of *Aloe vera* – Hexane, Ethyl acetate and Ethanol Extracts

Concentration (µg/ml)	Name of the extract	Control	DPPH Scavenging Activity % of Inhibition	
			% Inhibition of Sample	% Inhibition of Ascorbic acid*
200	Hexane	0.6508	81.66872	18.56177
600		0.6508	83.5126	52.36632
1000		0.6508	96.46589	80.02459
200		0.6508	80.57775	18.56177
600	Ethyl acetate	0.6508	82.71358	52.36632
1000		0.6508	90.61156	80.02459
200	Ethanol	0.6508	97.00369	18.56177
600		0.6508	97.21881	52.36632
1000		0.6508	97.941	80.02459

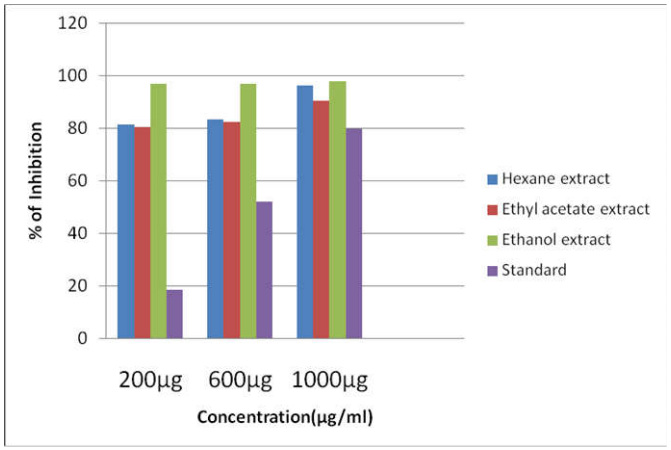

Figure 6. DPPH scavenging activity of *Aloe vera* –Hexane, Ethyl acetate and Ethanol Extracts

Gas chromatography—mass spectrometry

The study of the organic compounds from plants and their activity has increased by the combination of a best separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative analysis for volatile and semi-volatile bioactive compounds (Grover *et al.,* 2013). The present study carried out in the mixture revealed the presence of many active constituents. The results are presented below.

Molecular docking

Five compounds with emollient activity are selected. They are Methylparaben, Vitamin E, 1 acetyl pyrrolidine, n-Hexadecanoic acid and Thymol. Each one is docked against the target protein Human Retinoic Acid Receptor RXR-Gamma using GOLD and fitness score is calculated. During the docking process n-Hexadecanoic acid has high fitness score of 33.05 and is considered as an active compound.

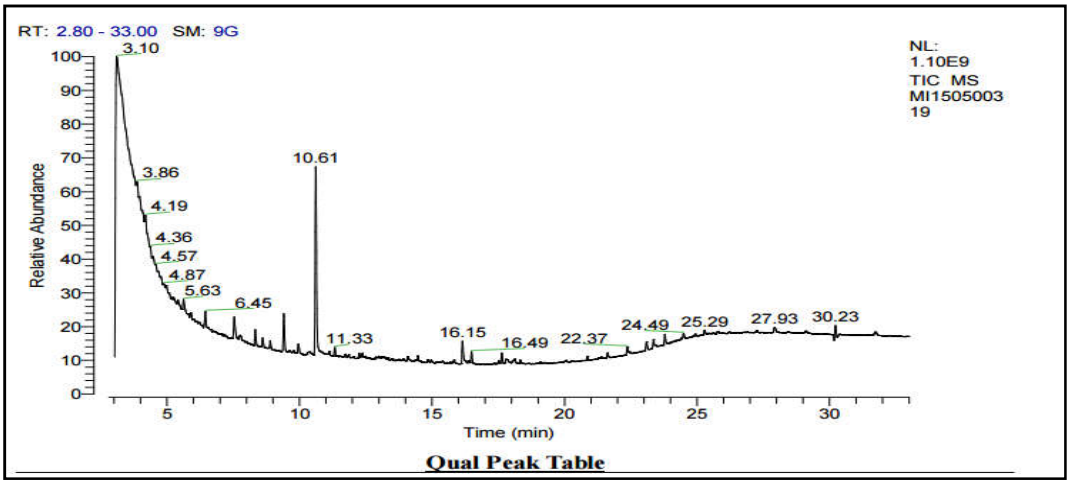

Figure 7. GC-MS chromatogram of Ethanolic extracts of *Ocimum tenuiflorum* (leaves), *Azadirachta indica* (leaves) *and Aloe vera* peel

Table 10. The Constituents of Ethanolic Extracts *Ocimum tenuiflorum* (leaves), *Azadirachta indica* (leaves) *and Aloe vera* peel

S.No	Compound name	Molecular formula	Retention time	Area %
1.	1,2,3-Propanetriol,1-acetate	$C_5H_{10}O_4$	5.63	3.05
2.	Decamethyl cyclopentasiloxane	$C_{10}H_{30}O_5Si_5$	6.45	3.68
3.	5-Hydroxymethylfurfal	$C_6H_6O_3$	7.54	6.27
4.	1-acetyl-L-pyrrolidine	$C_6H_{12}NO$	8.33	3.36
5.	Thymol	$C_{10}H_{14}O$	8.61	1.64
6.	Dodecamethyl cycloHexasiloxane	$C_{12}H_{36}O_6Si_6$	8.90	1.49
7.	Eugneol	$C_{10}H_{12}O_2$	9.41	7.21
8.	Vanillin	$C_8H_8O_3$	9.96	2.47
9.	Methylparaben	$C_8H_8O_3$	10.61	40.74
10.	2,4-bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$	11.33	1.52
11.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	16.15	5.80
12.	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	16.49	2.53
13.	Phytol	$C_{20}H_{40}O$	17.64	1.89
14.	Tetratetracontane	$C_{40}H_{90}$	23.09	1.71
15.	Squalene	$C_{30}H_{50}$	23.36	1.36
16.	Octacosane	$C_{28}H_{58}$	23.79	2.15
17.	7-Hexyl Eicosane	$C_{26}H_{54}$	24.49	2.65
18.	9-octyl heptadecane	$C_{25}H_{52}$	25.29	4.57
19.	Vitamin E	$C_{29}H_{50}O_2$	25.78	3.14
20.	Hexamethyl cyclotrisilane	$C_6H_{18}O_3Si_3$	30.23	2.76

Table 11. The docking results

Compound Name	Atom from Ligand	Atom from Protein	H-Bond Distance	Score
1 acetyl pyrrolidine	O11	ALA106,N	3.015	26.29
Methylparaben	O4	SER91,OG	2.886	29.08
n-Hexadecanoic acid	O18	LEU58,N	2.67	33.05
Thymol	O11	LEU58,N	2.831	23.64
Vitamin e	No H Bonds			18.1

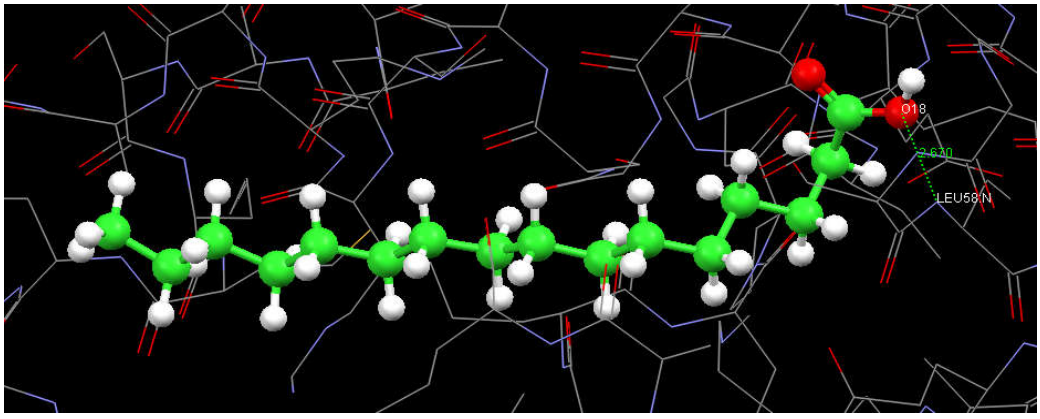

Figure 8. n-Hexadecanoic acid in complex with Human Retinoic Acid Receptor RXR-Gamma

Conclusion

The active compound n-Hexadecanoic acid has high fitness score of 33.05 when docked against the target protein Human Retinoic Acid Receptor RXR-Gamma using GOLD software. Thus the *insilico* method adopted in the present study helps in finding out the best ligand for Human Retinoic Acid Receptor RXR-Gamma. This method not only reduces the time and cost in designing an emollient before it enters the clinical trials but also throws light into the natural bio world for being used to treat Atopic Dermatitis (Eczema) and Photodamaged skin.

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