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Pharmaceutical Sciences **Research Article……!!!**

‘‘FORMULATION AND EVALUATION OF PARTIALLY PURIFIED OLEANOLIC ACID FROM DIFFERENT FRUITS AND LEAVES”

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ABSTRACT

Oleanolic acid (3βhydroxy-olea-12-en-28-oic acid) is a naturally occurring pentacyclic triterpenoid present in various parts of the plant such as fruits, leaves, stem bark, etc. Oleanolic acid is a secondary plant metabolite. The oleanolic acid was found to have wide range of activities such as anti-diabetic, anti-inflammatory, anti-tumor, hepato-toxicity, and anti-aging etc. The main focus of this research is to extract cuticular wax from various fruit peels and leaves followed by isolation of oleanolic acid from using different solvents further subjected for partial purification process for the same. The % yield of partially purified oleanolic acid was calculated and the TLC studies were carried out using a reference standard. The partially purified Oleanolic acid was further subjected for different formulations. The cream, gel and peel of mask are formulated and evaluated for its organoleptic and physical properties. This investigation shows that there is enormous scope in future for the formulation of Oleanolic acid as an anti-aging agent.

INTRODUCTION:

Aging is the process of becoming older. The term refers especially to human beings many animals and fungi. In broader sense aging can refer to single cell with an organism which have ceased dividing called as cellular senescence or to the population of a species called as population.

It has been known that oxygen is toxic to organism specifically aerobic organism when they are exposed to it in higher concentration than that of normal air. Intracellular Reduction of oxygen to highly reactive species or free radical leads to toxicity. These are several numbers of ways in which free radicals are formed, but the most common source is mitochondria (which uses 90% of oxygen used by human body), where oxygen is reduced to produce water which produces a number of intermediates like super oxides (O_2^-) hydrogen peroxides (H_2O_2) and hydroxyl radical (OH^-). $H_2O_2$is also found to be toxic to the human cells and cause free radical formation when it reacts with reduced transition metals to form hydroxyl radical[1].

Radicals from all these sources damage biological macromolecules. Free radicals react with polyunsaturated fatty acids to form lipid peroxides which decompose to yield a cascade of reactions including formation of known mutagen malondialdehyde. Lipid peroxide is known to produce an impairment of membrane fluidity and elasticity which leads to rupture of cells. The breakdown product of lipid peroxide produce lipo fuschin that accumulates with age and show animal senescence.

Anti-aging products are the preparations which are designed to reduce the aging factors in an appearance. They prevent fine lines, wrinkles, sagging, dark spots, and other visible signs of aging. Phytoconstituents are the chemical compound by plants through primary or secondary metabolism. These are found to protect against insect attacks and plant diseases. They also exhibit number of protective functions for human consumers.

Secondary plant metabolites are the organic compounds produced by plants which are not directly involved in the growth and development of plants. Absence of secondary plant metabolites does not lead to the death of plants unlike primary metabolites. Phtyoconstituents which are actively used for anti-aging purposes are Polyphenolic compound, Phenolic acids, Flavonoids and Penta cyclic triterpenoids.

Triterpenes are a class of chemical compounds composed of three terpene units with the molecular formula $C_{30}H_{48}$; they may also be thought of as consisting of six isoprene units. Animals, plants and fungi all produce triterpenes, including squalene, which is the precursor to all steroids. Triterpenes are

basically hydrocarbons which donot contain any heteroatoms. Triterpenoids possesses a rich chemistry and produces many pharmacological effects. It contains several penta-cyclic compounds[2].

Penta-cyclic Triterpenoids are widely distributed in the vegetable kingdom including oleanane ursane and lupane groups are present in many medicinal plants for example *Olea europae*. Oleanolic acid, Ursolic acid maslinic acid are the few examples of Penta cyclic triterpenoids.

These show multiple biological activities with apparent effects on glucose absorption, glucose uptake, insulin secretion, diabetic vascular dysfunction. They are also used in the treatment of many cardiovascular complication, tumor and cell proliferation and different kind of inflammations and Aging.

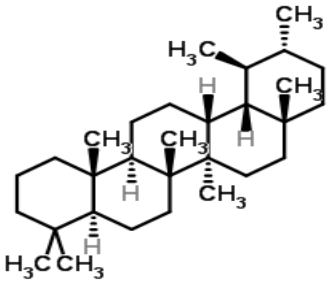

Fig 1. Oleanane

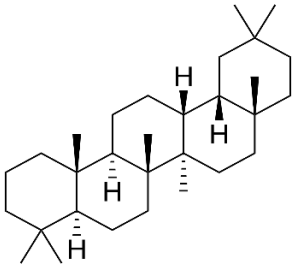

Fig 2. Ursane

Oleanolic acid is naturally present in various parts of plants such as fruits, leaves, stem bark etc. Oleanolic acid is a Penta cyclic triterpenoid which can be isolated from leaves fruits and other sources.

It exists as free acids or as an aglycone of triterpenoid saponins with it is often found with its isomer ursolic acid. The molecular formula and molecular weight of oleanolic acid $C_{30}H_{48}O_3$and 456.70 gram per mole respectively. Oleanolic acid has a wide range of activity such as anti-tumor, anti-inflammatory, anti-diabetic, anti-oxidant, anti-microbial. The main focus of this research is to check its anti-aging action. Oleanolic acid works on the mechanism of anti-oxidation for the purpose of antiaging[3].

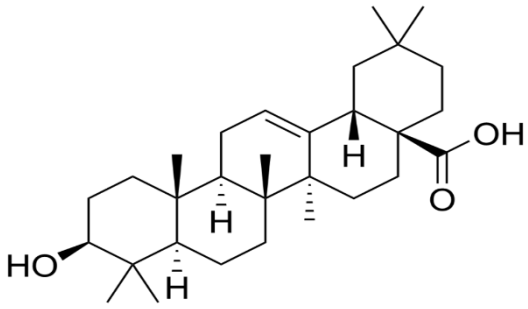

Fig 3. Oleanolic acid

Objective:

- Purpose of this study is isolation of bioactive compound from various fruits and its formulation. In this paper we are reporting isolation of known compound oleanolic acid (3βhydroxy-olea-12-ene-28-oic acid) from various fruits.
- A revolution in chemical technology has occurred in the last 50 years. New technologies have enabled the isolation, identification, and subsequent synthesis of biological compound. Although some chemical compounds found in fruits ad leaves cannot be synthesized today because of technical or economic constraints an increasing number of chemical compounds are being produced in laboratory. Despite these capabilities, renewed interest has developed. In using naturally produced chemicals from fruits as a source of medicinal and therapeutic agents.
- Since there are only few research articles that have worked on oleanolic acid having anti-aging activity and no such formulation is available, it generated a curiosity in us to work on this property of OA.
- This research mainly focuses on the antioxidant property of OA which is found in abundance in fruit peel specially apple, guava, papaya, and leaves specially mango, clustered fig, coconut, banyan ad jamun trees.
- The isolated partially purified OA from the fruit peel was further used for the formulation and evaluation of cream.

Material and methodology[4-6]: The leaves of plant *Mangifera indica* (F: Anacardiaceae), *Ficus racemosa* (F: Moraceae), *Ficus benghalensis* (F: Moraceae), *Coccus nucifera* (F: Arecaceae), *Syzygium cumini* (F: Myrtaceae) were collected in the month of October. The collected plant material was sundried for two days. The extraction of cuticular wax was done on dried sample. The solvent used for isolation; purification was of analytical grade.

The fruits procured for isolation of cuticular wax were *Malus domestica*(apple), *Psidium guajava* (guava), *Carica papaya* (papaya) from the market. The peel of the samples was shade dried for 10-12 days and later on dried in oven for approximately 4-6 hours at 45^0 C before use to remove the moisture content. The solvents used for isolation, partial purification and formulation were of analytical grade.

Extraction of cuticular material: The collected samples of leaves were sundried for 2 days in the month of October and powdered. The powdered sample was then treated to obtain the cuticular wax. Cuticular wax was obtained by soaking 10 grams of powder of dried leaves in chloroform for 30 seconds at room temperature. The extract was decanted and again soaked in hot chloroform for 120

seconds. The extract obtained from both was mixed which contain cuticular wax. The extract was evaporated on electrical water bath and the solid obtained was weighed and percent yield was calculated.

The cuticular wax from these fruit peels were obtained by first immersing in 25ml chloroform and shaken for approximately 30 seconds at room temperature and decanted. The same sample after decantation was again shaken with hot chloroform (60^0C) for about 2 minutes and again decanted. The decanted extract at both the stages were mixed, filter and evaporated at 50^0 C and % yield of cuticular wax was calculated and further subjected for partial purification of oleanolic acid. This procedure was repeated for each fruit peel.

Partial purification of oleanolic acid: The cuticular material obtained from the extraction was subjected for partial purification. The solvent used for partial purification was petroleum ether. Cuticular wax was washed with petroleum ether (40-60) to remove the lipid. The residue obtained was added to a mixture of petroleum ether (40-60) and acetone (70:30v/v) and kept in contact for 2-5 mins, Oleanolic acid dissolved in acetone. The resultant solution was then filtered and filtrate was kept at 4°C for 30 mins to precipitate out. The precipitate was then separated by filtration and dried at room temperature. The solid material was dissolved in methanol and water was added drop wise until precipitation was completed and the sample obtained from the partial purification was further taken for TLC[4].

Thin-layer chromatography: The TLC for oleanolic acid extract was done on trial-and-error basis by preparing three mobile phase and silica gel as a stationary phase. The spot was treated with iodine chamber and anisaldehyde as a post derivatizing agent[5].

Preparation of mobile phase: As discussed above total 3 mobile phase were prepared. The mobile phase 1 was prepared by mixing benzene and ethyl acetate in the ratio 8:2 respectively. The mobile phase 2 was prepared by using Petroleum Ether: Ethyl acetate: Acetone in the ratio of 8.2: 1.8: 0.1. Mobile phase 3 was prepared by mixing toluene and methanol in the ratio 9:1 respectively. Fresh mobile phase was prepared each time for each plate.

Preparation of post derivatizing agent: Post derivatizing agent used in this research was iodine chamber and anisaldehyde-sulphuric acid reagent. Iodine chamber was prepared by mixing silica gel and iodine. Anisaldehyde-sulphuric acid reagent was prepared by mixing 0.5 ml of anisaldehyde, 10ml of glacial acetic acid followed by 85 ml methanol and 5 ml concentrated sulphuric acid in the given

order. The TLC plate was dipped in anisaldehyde reagent and then evaluated in UV 365 nm. The reagent was not reusable when red violet colour was obtained.

Precoated silica gel TLC plate was used with dimensions of 10×10 cm a line was drawn at 2cm from the bottom on the TLC plate. The sample was dissolved in pure ethanol and was spotted on the line using capillary and then allowed to dry. On the same TLC plate a standard oleanolic acid was also spotted. The dry plate was placed in 3 mobile phase and the beaker was covered. Sample through capillary action rose and plate was removed when it was 2 cm from the top and this was marked as solvent front. The plate was allowed to dry, and the spot was developed. The plate was kept in iodine chamber and anisaldehyde reagent. The Rf value for each plate was calculated using scale [6].

Formulation table and procedure [7]:

Formulation 1: Gel

Sr.no.	Ingredients	Quantity (100 grams)
1.	Oleanolic acid	0.003 g
2	Carbopol 940	2 g
3.	Triethanolamine	1.65 ml
4.	Purified water freshly boiled and cooled	100

Procedure: Dissolve the Oleanolic acid in methanol. Carbopol 940 was soaked in warm water for around 25-30 mins. Add oleanolic acid mixture to the soaked carbopol 940. Triethanolamine was added drop wise to adjust the pH. Cool it to room temperature.

Formulation 2: Peel off mask [9]

Sr.no.	Ingredients	Quantity (100gm)
1.	Oleanolic acid	0.003 g
2.	Polyvinyl alcohol	10g
3.	Carbopol 940	0.5g
4.	Propylene glycol	10g
5.	Methyl paraben	0.2g
6.	Sodium lauryl sulphate	2g
7.	Ethanol 96%	20g
8.	Perfume	Qs
9.	Distilled water	Qs to 100g

Procedure: Polyvinyl alcohol was dissolved in distilled water by heating over water bath at 353 K .(mixture 1). Carbopol 940 was soaked in 20 parts of hot water and add it to the mixture. Dissolve the drug in ethanol. Add and mix propylene glycol, methyl paraben, sodium lauryl sulphate, ethanol mixture and drug.

Formulation 3: Cream [10,11]

Sr.no.	Ingredients	Quantity (100g)
	Alcoholic extract of oleanolic acid (0.18mg/ 10ml)	1.8ml
	Glycerin	5%
	Aq. Cream base	q.s 100g
	Methyl paraben	0.018%
	Perfume	0.02%
	Aq.cream base formula	Quantity 1000g
1.	Emulsifying ointment	300g
2.	Chlorcresol	1g
3.	water	699g
	Emulsifying ointment formula	Quantity 1000g
1.	Emulsifying wax	300g
2.	Soft paraffin	500g
3.	liquid paraffin	200g

Procedure:

1. Preparation of emulsifying ointment: Heat (65-70^{0}C) all the ingredients in porcelain dish. Melt it and stir it then cool gradually to room temperature.

2. Preparation of aqueous cream base: Melt emulsifying ointment. Dissolve chlorocresol in water, warm to room temperature (60-70^0 c) mix aqueous phase to oily phase. Stir gently.

3.Preparation of anti-aging cream: Mix all ingredients and triturate it with aqueous cream base in geometrical proportion in mortar. Add perfume

Evaluation parameters*: a*ll formulations were evaluated for physical and Organoleptic properties [10,11]

1. ***Organoleptic properties****:* The colour of the formulation was observed visually. The odour of the formulation was evaluated by smelling. The consistency of the formulation was evaluated visually.

2. ***Homogenity***: The homogeneity was evaluated visually for the presence of particles.

3. ***pH***: The pH was checked by using pH paper.

4. ***Spreadability***: 2g of product was taken on a transparent tile and another tile was placed on it and a load of 2g was placed over it. Then the diameter was measured, and the area was calculated.

5. ***Peel time***: The small amount of sample was applied on the skin and allowed to dry and the peel time was measured.

6. ***Irritation***: Small amount of sample was applied on 10 volunteers and irritability of the product was checked for any allergic reaction.

7. ***Viscosity***: Viscosity was measured using the Brookfield Viscometer.

8. ***WASHABILITY***: A small amount of sample was applied on the skin and kept for few minutes and then washed away.

9. ***Stability at room temperature and cold temperatures***: The product was kept at room temperature and cool temperature and then the stability was visually checked.

Result:

The results of the cuticular yield are given in the [Table 1]. The results indicated that the cuticular yield obtained of Apple peel and *Mangifera indica* leaves was comparatively higher than other fruit peel powder and different leaves respectively was selected for further partial purification of oleanolic acid and formulations. The TLC studies carried out on the sample obtained was also found to be successful and the appropriate mobile phase was found to be Toluene: Methanol in the ratio 9: 1(mobile phase -3) [Table 3]. We subjected partially purified Oleanolic acid for 3 formulations that is cream, gel and peel off mask which passed all the evaluation tests carried out on them. [Table 4].

Discussion:

This study is report of isolation and formulation of partially purified OA from Apple peel and *Mangifera indica* leaves. Due to adverse effects of synthetic drugs, many scientists are working for alternative medicines. Plant or fruit-based drugs are less toxic and have no adverse effects. The OA have various properties such as anticancer, antioxidant, anti-inflammatory, hepatoprotective and anti-aging. It's hoped this compound will lead to formulation of new and more potent anti-aging drugs that will be useful in aging. In TLC technique sample is applied as a rectangular band because it provides more resolution and better separation of spot. The result obtained from TLC analysis, extraction and formulation indicates the presence of oleanolic acid in the isolated sample. This investigation shows that there is enormous scope in future for the formulation of Oleanolic acid as an anti-aging agent.

Table No. 1 % Yield of Cuticular Wax from leaves

Sr.no.	Sample name	Weight of sample taken	Extract solid (wt)	% Yield of cuticular wax
1.	*Mangifera indica* leaves	10 grams	0.25 gram	2.5 % w/w
2.	*Ficus racemose* leaves	10 grams	0.24 gram	2.4 % w/w
3.	*Ficus benghalensis* leaves	10 grams	0.01 gram	0.1 % w/w
4.	*Cocus nucifera* leaves	10 grams	0.15 gram	1.5 % w/w
5.	*Syzygium cumini* leaves	10 grams	0.16 gram	1.6 %w/w
6.	Guava peel	10 grams	0.08 gram	0.8% w/w
7.	Apple peel	10 grams	0.11 gram	1.1% w/w
8.	Papaya peel	10 grams	0.05 gram	0.5% w/w

Table No. 2 % Yield of Partially purified Oleanolic acid

Sr.no.	Sample name	Weight of sample taken	% Yield of cuticular wax	% Yield of partially purified oleanolic acid
1.	*Mangifera indica* leaves	10 grams	2.5 % w/w	1 % w/w
2.	*Ficus racemose* leaves	10 grams	2.4 % w/w	0.9 % w/w
3.	*Ficus benghalensis* leaves	10 grams	0.1 % w/w	0.01 % w/w
4.	*Cocus nucifera* leaves	10 grams	1.5 % w/w	0.4 % w/w
5.	*Syzygium cumini* leaves	10 grams	1.6 % w/w	0.5 %w/w
6.	Guava peel	10 grams	1.1% w/w	0.08%w/w
7.	Apple peel	10 grams	2.9% w/w	0.11%w/w
8.	Papaya peel	10 grams	1.3% w/w	0.05%w/w

Table No. 3 Rf value of partially purified oleanolic acid from Mangifera indica and apple peel in various mobile phases used in TLC after derivatization in iodine chamber and anisaldehyde reagent.

	Mobile phase 1		Mobile phase 2		Mobile phase 3	
	Benzene: ethyl acetate (8:2)		pet ether: ethyl acetate: acetone (8.2 :1.8 :0.1)		Toluene: methanol (9:1)	
	Apple peel	Mangifera indica	Apple peel	Mangifera indica	Apple peel	Mangifera indica
Solvent front	6.7	6.7 cm	7.4	7.4 cm	5.5	5.5 cm
Spot front	3.8	3.6 cm	3.4	3.2 cm	1.8	1.9 cm
Retention factor value	0.5671	0.53	0.459	0.43	0.3272	0.34

Table No. 4 Evaluation of formulations

Sr.No	Evaluation parameters	Gel	Peel off mask	Cream
1.	Organoleptic			
a)	Colour	Colorless	Colorless	White
b)	Odour	Pleasant	Pleasant	Pleasant
c)	Consistency	Semi-solid	Semi-solid	Semi-solid
2.	Homogeneity	No gritty particles	No gritty particles	No gritty particles
3.	pH	7	6	6
4.	Spreadability	38 cm^2	81.6 cm^2	
5.	Irritation	No irritation	No irritation	No irritation
6.	Peel Time	-	15 mins 26 sec	71.8cm^2
7.	Viscosity	Pseudo plastic flow	Plastic Flow	Plastic Flow
8.	Washability	Easily washable	Easily washable	Easily washable
9.	Physical stability at:			
	Room Temp (3 months)	Stable	Stable	Stable
	Cold Temp (3 months)	Stable	Stable	Stable

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