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Structural Modification in Oleanolic Acid for Antiviral Activity

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Abstract

Oleanolic acid (OA) is a triterpene of the pentacyclic oleanane-type that is derived from several edible and medicinal plants. It exhibits a wide spectrum of pharmacological effects and possesses significant therapeutic potential. The usage of Oleanolic acid doesn't have wide application, to increase its therapeutic effects several studies on structural changes of OA have been conducted. The objective is to study and explain current developments in medicinal chemistry of OA derivatives, with a focus on the structural modification of OA in recent years (2010–21).

Most causes of worldwide deaths are Herpes, Hepatitis, Human immune deficiency virus (HIV) and influenza. There is a critical need for the discovery of new and robust antiviral medications, as evidenced by the dearth of efficient treatments or vaccines for the addressing the many viral diseases through prevention and therapy as well as the sharp rise in new viruses that are resistant to drug. Natural products drug discovery is one of the most valuable sources. There are several different techniques and chromatography platforms that may be utilized in order to extract and isolate oleanolic acid, which is a pentacyclic triterpenoid found in a broad variety of plant species, including fruits and vegetables. This study found that modification of Oleanolic acid structure at various sites for antiviral activity.

Keywords: Oleanolic Acid, Conjugation, Triterpenoid, Antiviral Activity, Derivatives.

I. INTRODUCTION

Many organic compounds have been extracted from their natural habitats. Researchers all around the globe are interested in a class of secondary metabolites found in plants such as phenolics, because of their propensity to remove free radicals that cause oxidative stress and illnesses. Plants produce and secrete a wide variety of metabolites to regulate processes including development, senescence, stress tolerance, and defence (Chianese et al., 2018).

In broader sense, plant's secondary metabolites are required to control interactions between plants and their environments. This is in contrast to plant fundamental macromolecules, such as nucleic acids, carbohydrates, lipids and proteins, which supply structure, energy and media for diverse activities. (Zang and Xu, Sun 2012). Plant secondary metabolites such as alkaloids, waxes, terpeniods, phenolics and flavonoids, protect the plant from harmful ultraviolet radiation, attract pollinators and fruit dispersers, provide mechanical support to the plant and enhance its growth (Atta, Mustafa, Sharif, Jamil, A and Arif, 2017).

The pentacyclic triterpenoids of oleanolic acid's isomer ursolic acid are found in wide variety of land-adapted plant species. These terpenoids exist as free acids or in the case of triterpenoid saponins, as aglycone. An aglycone is a specific kind of saponin; saponins are a broad category of amphipathic

glycosides that have been artificially grouped together to symbolise substances that produce a soap-like lather when dissolved in water.

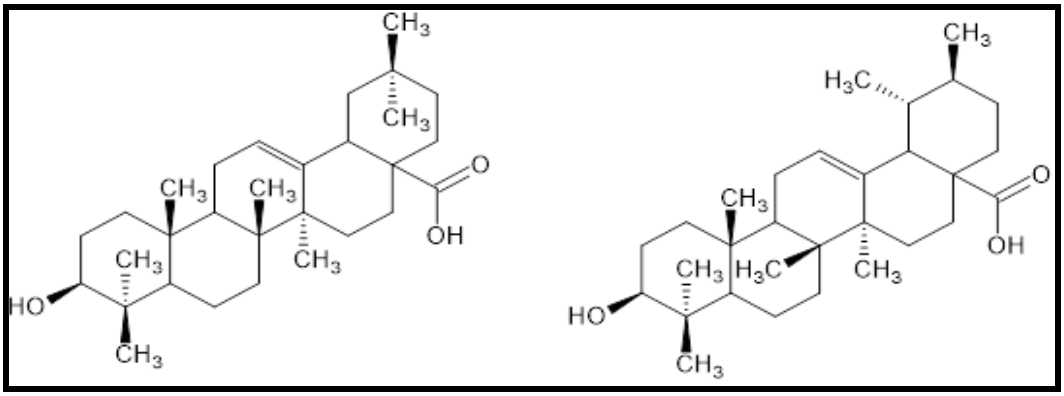

Figure 1: ***Oleanolic acid & Ursolic acid Structures***

Oleanolic acid & Ursolic acid have many of same pharmacological qualities and chemical behaviours since they are structural isomers. All 30 of squalene's carbon atoms are maintained throughout the natural cyclization that produces both OA and UA. Oleanolic acid is produced by modifying β-amyrin of Carbon position-28 of triterpene saponins, whereas ursolic acid is produced by oxidising the α-amyrin. The structure of Oleanolic acid & Ursolic acid is represented in Fig.1.

Oleanolic acid, also known as OA, been extracted from almost 120 different plant species, whereas ursolic acid, also known as UA, has been found in over 1600 different plant species. The most important OA and UA sources are outlined in Table-1, and is abundant in fruit skin and fruit pulp, medicinal plant leaves and few vegetables.

Table 1: ***A list having OA and UA Source***

Plant	**Plant part**	**Abundance in g/100 g of dry matter**	
		Oleanolic acid	**Ursolic acid**
Apple	Peels	0.4	1.05
Cornus	Flower	1.65	-
Bearberry	Leaf	0.27	1.24
Guagyusa and Mate	Leaf	-	1.8
Rosemary	Leaf	1.23	2.95
Marigold	Flower	2	-
Olive	Leaf	3.1	0.18
Hawthorn	Flower and Leaf	0.1	0.52
	Stem	0.11	0.32
Catharanthus Roseus	Leaf	0.22	0.24
	Flower	0.04	0.47
Franberry	Fruit	0.13	0.94
Grapes	Leaf	0.37	1.17
Ligustrum lucidum Ait	Fruit	0.31	0.92
Crataegi pinnatifidae Fructus	Fruit	0.63	0.4 – 0.6
Ziziphora Clinopodiodes	Whole plant	0.07	0.12

These chemical structure of OA and UA have somewhat distinct biological effects due to the substituted methyl group. In contrast, UA which has two methyl functional groups at positions C-19 and C-20, OA only has a single methyl group at position C-20. It has been shown that the molecule's stability, affinity, and/or reactivity towards other molecular reactants may be affected by position of methyl group.

Viral diseases continue to be a large concern for humanity. According to various studies, there is an increase in viral infections that cause mortality and ill health across the world. According to National Health Laboratory Service (NHLS) report, around 6,000 and 11,000 South Africans die from influenza each year. Some viral diseases, like hepatitis C and human immunodeficiency virus (HIV) virus are challenging to treat by usual techniques of treatment.

The alphaherpesvirinae subfamily includes the enveloped DNA virus known as Herpes Simplex Virus Type 1 (Midak-Siewirska et al., 2010). In neonates and immunocompromised adults, HSV-1 can cause serious illnesses such keratitis and herpes simplex encephalitis. Skin lesions and oral and facial lesions (with Herpes labialis as the clinical manifestation) are also brought on by HSV-1 infection (Herpes gladiatorum). As a result, there is an increasing body of data suggesting that HSV-1 infection is associated with neurological diseases such as Parkinson's disease and Alzheimer's disease.

Oleanolic acid has a variety of medicinal effects, including liver protection, anti-tumor, and anti-inflammation properties (Goossens and Pollier, 2012; Wang et al., 2006). Several research have also identified Oleanolic acid's anti-herpesvirus properties. By encouraging production of the pro-inflammatory cytokines IL-12 and IL-6, one of the studies found that OA might suppress HSV-2 (EC50 7.8 g/mL) and HSV-1 (EC50 6.8 g/mL).

Researchers' interest in finding the bioactive chemicals contained in plants and their products has been piqued by the undeniable medicinal properties of plants. Several phytochemicals from plants have been found in numerous studies to have biological effects against chronic diseases. Oleanolic acid (OA) is a natural substance extracted from a different type of plants. Plants of Oleaceae family contains pentacyclic triterpenoid, including the olive tree. OA is commonly found in the epicuticular waxes of many plants, where it acts as a blockade against water loss and pathogens.

II. OA BIOSYNTHESIS:

Oleanolic acid a kind of triterpenoid, belongs to one of a broad family of structurally varied natural compounds called triterpenoids. Synthesis of 2,3-oxidosqualene, a precursor molecule of primary sterol metabolism, occurs in the cytoplasm of plant cells from isopentenyl pyrophosphate (IPP) produced through mevalonate pathway. Secondary triterpenoid metabolism, which ultimately results in oleanolic acid, branched out from primary sterol metabolism at the cyclization of 2,3-oxidosqualene. Cycloartenol is a tetracyclic plant sterol precursor that is produced during the biosynthesis of phytosterols from 2,3-oxidosqualene through cycloartenol synthase (Fig.2). CAS is the enzyme that is responsible for the production of all plant oxidosqualene cyclases that are involved in the secondary metabolism. It‘s been discovered a long time before plants originated.. The BAS (OSC β-amyrin synthase) converts 2,3-oxidosqualene into the pentacyclic oleanane-type triterpenoid backbone β-amyrin, which is then used in the biosynthesis of Oleanolic acid.

The cloning of the BAS enzyme was initially achieved by the replication of genetic material derived from the panax ginseng; a plant known for its therapeutic properties. Following that, the process of cloning was replicated from several other botanical species, such as the olive plant. At the last stage of the biosynthesis of OA, β-amyrin is oxidised in 3 steps of oxidation at Carbon position-28 by single cytochrome P-450 enzyme to yield OA through erythrodiol. This process is carried out by a single cytochrome P-450 enzyme.

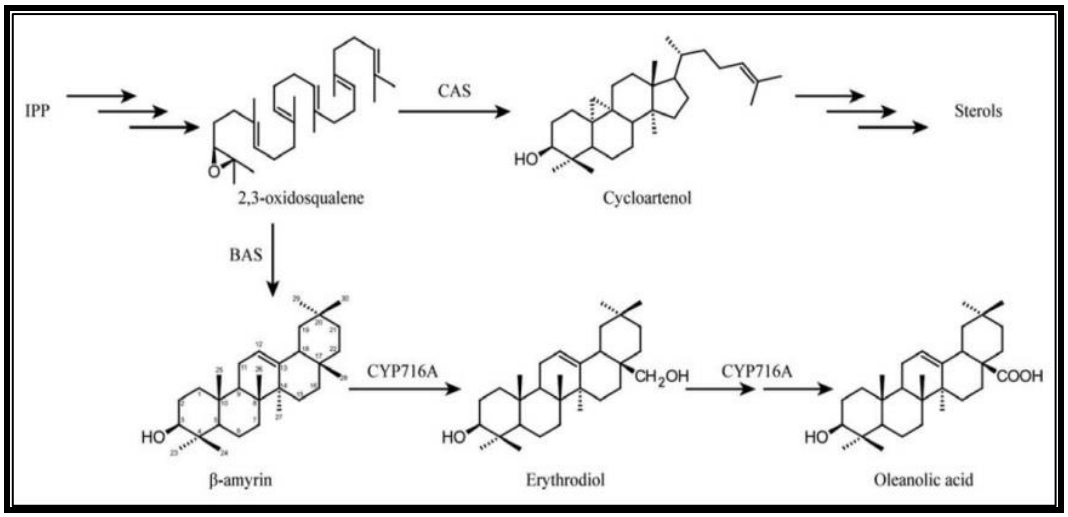

Figure 2: ***Biosynthetic pathway of OA***

III. CHEMISTRY OF OA AND ITS DERIVATIVES:

OA is a pentacyclic triterpenoid that binds free acid to one or more sugar chains and is present in herbs and medicinal plants. Its molecular formula $C_3OH_8O_3$ and molecular weight 456.70 g/mol. Oleanolic acid derivatives was prepared by reaction of 1,3-cyclopropanyl phosphate ester with OA. Structural modification at the various sites in OA (Fig.3) shows different therapeutic activities, one of the derivative is 2- cyano- 3,12 -diox- ooleana-1,9 (11)-dien -28-oic acid (CDDO) which is $2x10^5$ times more efficient anticancer agent. Carbenoxone is also the derivative of OA, it is a potent antiulcer molecule. OA derivative shows less toxicity and more effective in therapy.

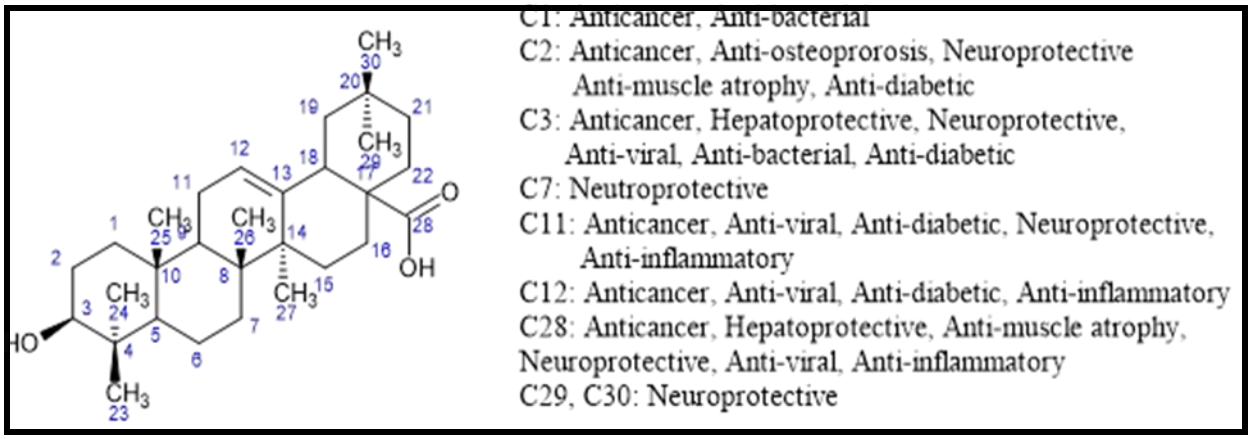

Figure 3: ***Various sites for structural modification in Oleanolic Acid.***

IV. EXTRACTION, ISOLATION AND CHARACTERIZATION OF OLEANOLIC ACID

OA extraction is carried out by soxhlet, UV and microwave aided process. OA is extracted from variety of plants. Analytical platforms utilised in the characterisation and identification of OA include HP-TLC, TLC, HPLC, NMR, MEEC and CD-MEKC. OA is often isolated by defatting the plant's crushed powder using petroleum ether or n-hexane. Following the synthesis of the crude extracts, Oleanolic acid is commonly isolated utilising methods such as column chromatography, precipitation and crystallisation and vacuum liquid chromatography. Similar with other plant metabolites, a variety of factors can affect how effectively OA is extracted and isolated. For the maximum yield during extraction depends on nature of solvents and concentration of solvent, temperature and techniques used. These variables often modified in order to maximise the separation and extraction of Oleanolic acid from its various plants.

4.1. Anti-viral and anti-bacterial activity

Antibacterial activity of OA and its derivatives shows a modification in genes for haemolysis, beta lactamase and metabolism. By its effects on lipid membranes, oleanolic acid increased the action of

several antibiotic medicines, including ampicillin. Oleanolic acid derivatives shown antiviral action against Human Immunodeficiency Virus by inhibiting HIV-1 Protease and prevented influenza viruses from infecting host cells.

4.2. Anti-viral derivatives:

Li et al. conducted a study aimed at mitigating the transmission of the influenza A/WSN/33 (H1N1) virus in MDCK cells. To achieve this objective, the researchers synthesized a range of C-28 modified OA derivatives (Fig.4) by conjugating them with polyphenols. Compound 5a had a greater inhibitory efficacy against A/WSN/33 (H1N1) with an IC-50 value of 7.65 micron meter when compare the IC-50 value of 16.5 micron meter showed by Oseltamivir, which was employed as a positive control in this study. In addition, cytotoxicity testing revealed that a high concentration (100 μM) of 5a was associated with a lower level of toxicity. Molecular docking investigations indicated that polyphenol-pentacyclic triterpene may prevent spread of the virus by interacting with the sialic acid receptor binding domain of the heme-A protein. This might prevent the virus from infecting new cells (Li et al., 2019).

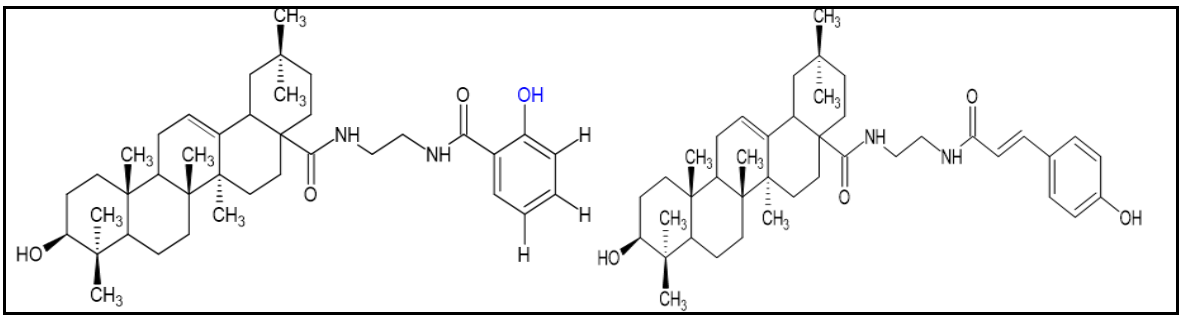

Figure 4: ***Modification of OA and its derivatives by conjugation with polyphenols.***

Yu and his colleagues conceptualized, developed, and synthesised derivatives of OA by conjugation with amino acid (Fig.5), which they then tested in vitro for their ability to inhibit viral replication. At a concentration of 100 μM, the majority of OA derivatives exhibited excellent anti-influenza effectiveness against the H1N1 virus, they also maintained cell viability and had a virus inhibition. Oleanolic acid derivative preventing viruses from attaching themselves to host cell by blocking the binding site in sialic acid receptor for HA and thus interaction disrupted. Compound 9a at 100 microM had 75 percent antiviral activity but these compounds showed higher cytotoxicity.

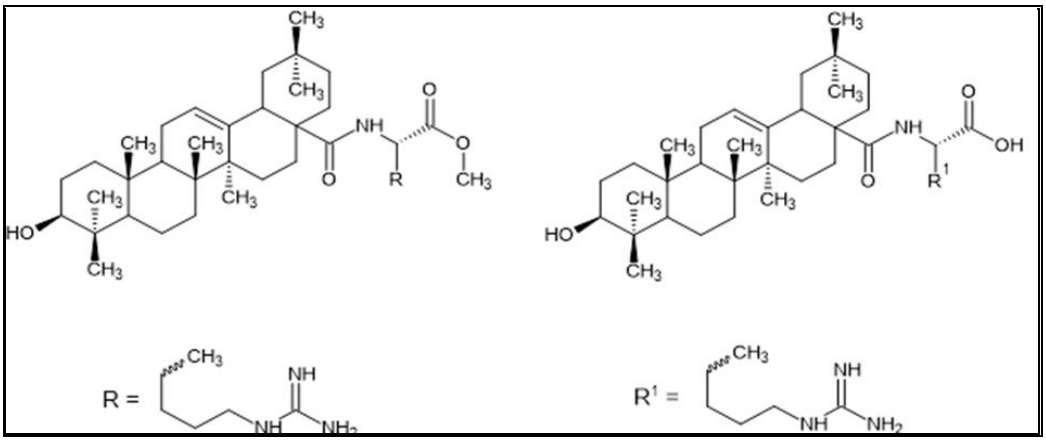

Figure 5: ***Antiviral activity of OA by conjugation with amino acids.***

Antiviral activity was examined for 11 Oleanolic acid analogues, including four derivatives that had not been published before Song and his colleagues carried out the research (Fig.6). Among these, 10a demonstrated promising inhibitory action against influenza A virus multiplication in cells. The antiviral activity is due to introduction of disubstituted amide at 17-COOH group. The EC50 value for this activity was 14.0 microM.

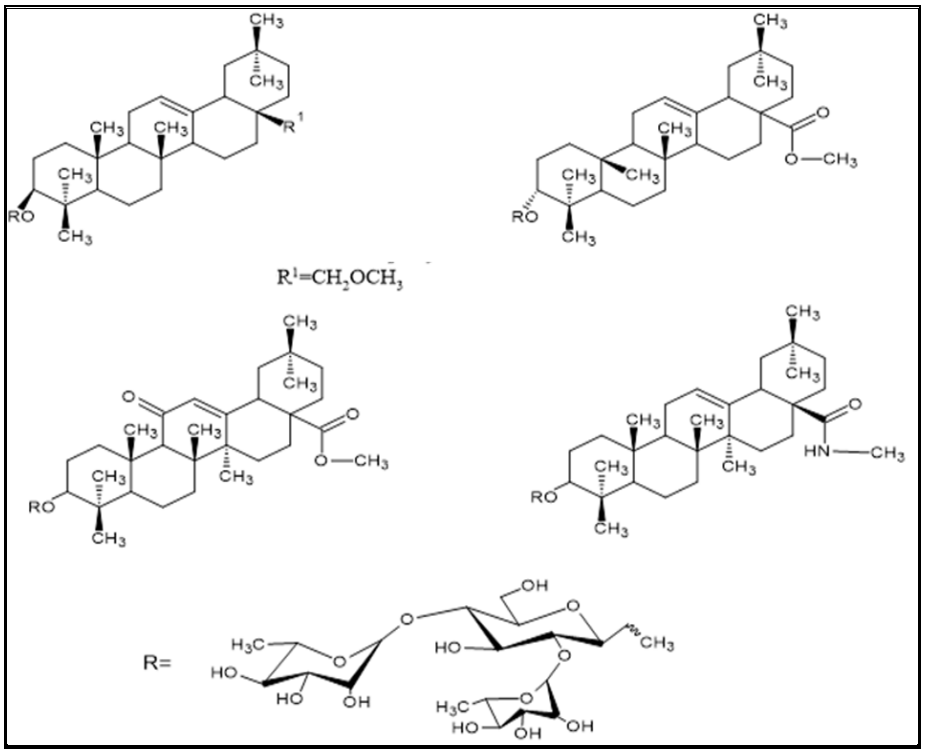

Figure 6: ***Antiviral activity of OA and its derivatives by conjugation of disubstituted amide.***

Researchers Medina-O'Donnell and colleagues synthesised derivatives of OA (Fig.7) that were combined with an acyl group and one or two amino acid groups. To determine the possession of antiviral capabilities as HIV-1-protease inhibitors were tested for derivative presence in compounds for OA. Accordingly results of the in-vitro study, OA derivatives were esterified at C-3 position with carboxylic acid free group exhibited a remarkable capacity to block HIV-1 protease activity.

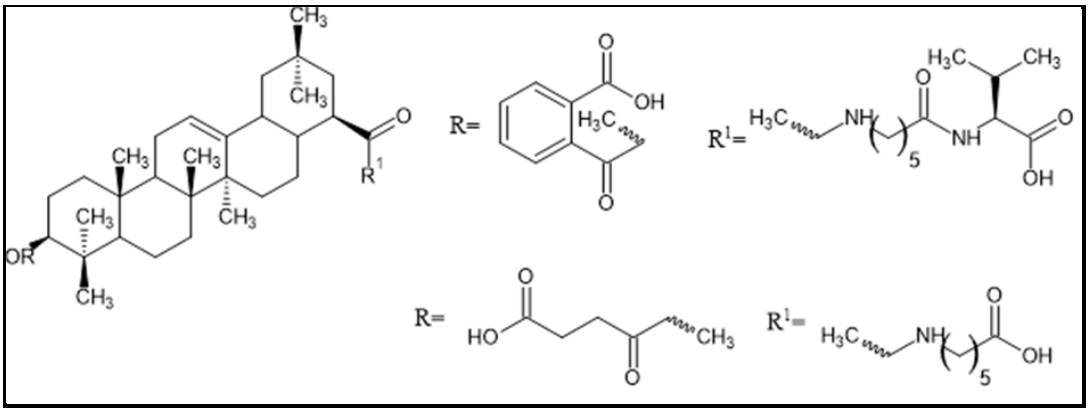

Figure 7: ***Anti-viral activity of OA and its derivatives by conjugation with carboxy acyl group amino acid.***

The compounds (Fig.8) were synthesised by Xiao et al. The compounds were synthesised by introduction of carboxyacyl group at Carbon position-3 of OA which enhance HIV-1 Protease inhibition and conjugation of Ψ-amino acid at C-28. If substitution at C-28 by shorter chain Ψ-amino acid increased the antiviral potency whereas longer chain Ψ-amino acid decreased the effect.

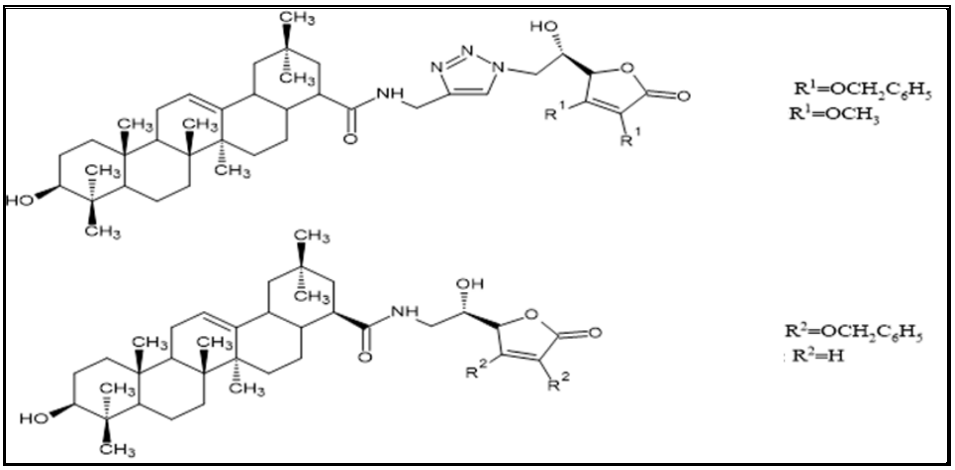

Figure 8: ***Anti-influenza virus activity by OA conjugation with L-ascorbic acid.***

As nonglycosylated neo mucin mimics, conjugation with HSA (Fig.9) were synthesised by Yang et al. for the purpose of preventing influenza viruses from capturing and entering cells. The experiment indicated that was capable of capturing the virus by the process of selective adsorption to the HA on the surface of virus. The compound (OA conjugated HSA) had a capacity to inhibit the entry of all 3 viral strains (H9N2, H3N2 and H1N1) in microM range. H7N9 pseudovirus entry is inhibited by the rate of nearly 90% at a concentration of 20 μM, a potent capacity for viral capture and entry inhibition.

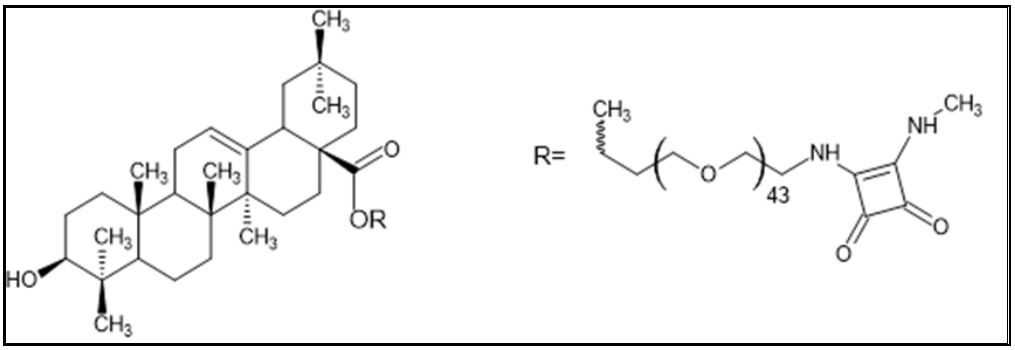

Figure 9: ***Anti-viral activity of OA conjugation with HSA.***

V. CONCLUSION:

Numerous studies of in vitro and in vivo shown a wide range of favourable biological effects associated with OA derivatives. Some OA compounds made it to clinical trials but were never utilised in practise. They were shown to be effective preventative and curative medicines for a wide range of ailments, including cancer, diabetes, and viral infections. The pharmacology of OA derivatives has been the primary subject of previous reviews. Different biological activities, chemical structures and the structural activity connection of oleanolic acid derivatives were discussed and summarised in this study.

The development of antiviral medications shifted its focus to strategies that blocked virus entrance. HA was a tempting target because of how important it is to viral infection. Many people worked hard to find OA derivatives that showed promise as viral entry inhibitors by inhibiting the HA protein and preventing virus penetration into host cells. Several OA analogues with improved affinity and selectivity towards molecular therapeutic targets were discovered after being developed and synthesized, demonstrating the importance of structural and functional diversity in this field. Many sources have hinted that additional research into OA derivatives might lead to its use as an alternative and supplemental treatment for a wide range of ailments. In conclusion, this review's focus helped illuminate the structural activity connection of derivatives of OA and provided visions into rational creation of drugs with exceptional biological activities and targeted molecular mechanisms.

Pure and Crude chemicals obtained from plants have been shown to have anti-HSV-1 properties, making natural products an important source for antiviral drugs. Natural extracts including steroids, tannins,

flavonoids, saponins, phenols, alkaloids, and glycosides for example, are reported to intervention with HSV-1 proliferation.

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Conflicts of Interest

The authors declare no conflict of interest.

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