CHAPTER

Biological Activity of Triterpenoids - An Overview
2.0.0 BIOLOGICAL ACTIVITY OF TRITERPENOIDS - AN OVERVIEW

Triterpenoids are the most ubiquitous non-steroidal secondary metabolites in terrestrial and marine flora and fauna. Their presence, even in non photosynthetic bacteria, has created interest both from evolutionary and functional aspects. Although medicinal uses of this class of compounds are rather limited. Considerable recent work in this regard, strongly indicates, their great potential as drugs. Extensive exploratory activities in this area have been underway during recent years. Some exciting and thought provoking results are reproduced with a view to provide deep insight into wide spectrum of pharmacodynamic activities of triterpenoids.

2.1.0 ANTITUMOUR AND ANTILEUKEMIC ACTIVITY

Triterpenoid glycosides, foetoside C (1) and cyclo foetoside B from *Thalictrum foetidum* and thalicoside A from *T. minmus* were studied for their anti-tumour activity in rats with implanted tumours[13]. Each agent given in dose of 30-50 mg kg\(^{-1}\) i-p daily for 10 days, had an appreciable anti-tumour activity. Foetoside C was found to be most effective, especially in the treatment-resistant types of tumours. According to the

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Glycoside foetoside

Giganteaside D

\[ R^1 = \text{gly}^{2\text{ara}} \]

\[ R^2 = \text{Rham} - \text{gly}^4 - \text{gly}^4 \]
authors, the *Thalictrum glycosides* are promising agents in the development of new neoplasm inhibitors. Saikosaponins from *Bupleurum kummingenese* have been found by Japanese workers to inhibit *in vitro* growth of Hela cells and leukemia cell L-1210\[14\]. Two oleanolic acid glycosides giganteaside D (2) and flaccidin B(3) isolated from *Anemone flaccida* showed an inhibitory effect on reverse transcriptase from RNA tumour virus\[15\]. Bioassay directed fractionation of the cytotoxic antileukemic extracts of *Prunella. Vulgaris, Psychotria Serpens* and *Hyptis capitata* led to the isolation of ursolic acid(4) as one of the active principles.\[16\] Ursolic acid showed significant cytotoxicity on the lymphocytic leukemia cells P-388 and L-1210 as well as the human lung carcinoma cells A-549. It also demonstrated marginal cytotoxicity in the KB and human colon (HCT-8) and mammary (MCF-27) tumour cells. Esterfication of the hydroxyl group at C-3 and the carboxyl group at C-17 yielded a compound with decreased cytotoxicity in human tumour cell lines, but with equivalent or slightly increased activity against the growth of L-1210 and P-388 leukemic cells. Reddy *et al.*\[17\] isolated spinosides A and B, 11-deoxocucurbitacin I(5) and its 23,24 dihydro derivative from *Desfontainia spinosa* by following the cellular toxicity of fractionated materials against Eagle KB strain of human carcinoma of nasopharynx.

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\[ R = \text{Glu} - \text{xyl} - \]

\text{Flaccidin B}

\[ R = \text{Glu} - \text{xyl} - \]

\text{URSOLIC ACID}
The ethanolic extract of the plant was previously reported to have significant activity against KB cells in culture and P-388 mouse leukemia\cite{18}. The ED_{50} of the above four compounds indicated that the glycosidation of the C-16 position in the cucurbitacin skeleton reduces the cytotoxicity to some extent and reduction of the double bond in the side chain leads to complete loss of activity. Tubeimoside-1, a cyclic bisdesmoside isolated from the bulb of *Bolbostemma paniculation*, showed moderate antitumour activity in primary *in vivo* pharmacological study\cite{19}. The anti cancer effect of tegafur, a pyrimidine derivative possessing anticancer property, was enhanced when applied in combination with ginseng extract.\cite{20} A higher survivor rate was obtained with the oral administration of an emulsion, containing tegafur and extract powder in mice bearing P-388 leukemia cells, compared with tegafur alone. The combination, composition, inhibited 62.7% of sarcoma cell growth in mice. Also this combination reduced toxicity in animal studies.

The relation between chemical structure and anticancer activity of some pentacyclic and tetracyclic triterpenoids was studied by Ling et al\cite{21}. The anticancer effects were tested against human cancer cell lines ME-180, V-87 MG, SK-HEP-1, CALU-1, CAMA-1, SK-OV-3 and HEC-1-A. Among the pentacyclic triterpenoids epimanidiol(6) (3β,16-dihydroxy-

\[ R_1 = R_2 = H \]

11 - dexo - cucurbitacin 1

Epimanidiol
olean-12-ene) was found to be cytotoxic at 100μg ml⁻¹ against HEC-1-A. CAMA-1, ME-180, V-87 MG, CALAU-1, and SK-OV-3, the required concentration producing 50% inhibition against HEC-1-A was approximately 10μg ml⁻¹. Maniladiol, the 16β epimer exhibited cytotoxicity against ME-180 and CAMA-1 at 100μg ml⁻¹. Whereas sophoradiol(7) (3β, 22β-dihydroxyolean-12-ene) was cytotoxic only against ME-180 at 100 μgml⁻¹. This result suggested that the presence of a 16 hydroxy group is important for the appearance of cytotoxicity of 12-oleanenes. Glycyrrhetic acid (8) (3β-hydroxy-11-oxo-olean-12-en-30-oic acid) and 11-oxo-β-amyrin, both with a free 3β-OH group were active at 100 μg ml⁻¹ against SK-OV-3 and CAMA-1 respectively while sodium glycyrrhetic acid succinate, with the esterified 3β-hydroxy group was found to be inactive.

The effect of triterpene glycoside, synthesised on the basis of betulafolientriol, ginsenoside Rb1 from ginseng, betulafolientriol and its 3-epimer on the growth of the Ehrlich tumour cell cultures was studied.[22] It was shown that, in relation to the quantitative composition and sites of carbohydrate residue linkage to aglycone as well as configuration of the alpha- or beta- hydroxyl group at C-3, the activity of the triterpenoids of the dammaraneric series changed within wide ranges, 3 and 12-0-beta-D-

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spohoradiol

Glycerrhetic acid
glycosides of 3-cpiibetulafolicnlriol proved to be most active. The antitumour agent sculponeatin C is produced in the leaves of *Rabdosia Sculponea*.[23]. The leaves are extracted with diethyl ether and the extract is concentrated to dryness.

To investigate the biological activities of the hopane group of pentacyclic triterpenoids, Nagumo, et al.[24] isolated one hopanoid, bacteriohopane-32-ol from *Rohodosaudomonas palustris* and tested its cytotoxicity against mouse leukemia cells in vitro. The IC-50 of hopanoid for L-1210 and P-388 was 22 and 19 micro M respectively. This activity was slightly reduced by co-incubation with cholesterol. The mechanism of cytotoxic action, disturbance of membrane function and metabolism were observed.

Two new cucurbitane type triterpenes, 15-oxo-cucurbitacin F (9) and 15-oxo-23, 24-dihydrocucurbitacin F were isolated from the leaves and branches of *Cowania mexicana*[25], were found to be inhibitors of Epstein-Barrvirus, early antigen activation induced by 12-o-tetradecanlyphorbol-13-acetate,a well-known tumour promotor.

A new cytotoxic (P-388 ED 50 4 micro gm/ml) aryl napthalene lignan were isolated from the Mexican medicinal plant *Hyptis verticillata* (lamiaceae) and characterized as 5-methoxy dehydropodophyllotoxin

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15-oxo-cucurbitacin

(9)

Arjunilic Acid

(10)
Eight additional lignans were also obtained by bioactivity-directed fractionation using the brine shrimp lethality test. Of these, the dehydro-beta-peltatin methyl ether 2(P-388 ED₅₀ 1-8 micro gm/ml) was reported. The other bioactive compounds were identified as dehydropodophyllotoxin, deoxy-dehydropodophyllotoxin, 4-demethyl-deoxypodophyllotoxin, isodeoxy-podophyllotoxin, deoxy-picropodophyllin and beta-apopicropodophyllin. Each of these compounds was evaluated against a panel of cell lines comprising a number of human cell types (breast, colon fibrosarcoma, lung, prostrate, KB and KBr-1) and murino lymphocytic leukemia (p-388).

Betulinic acid -- a common triterpene was shown to be inhibitor for growth of the leukemia cell lines P-388.[27] Oral adminstration of 18 β-olean-12-ene-3β, 23,28-triol, tri o-hemiphthalate sodium and olean-11 13(18)-dien-3β-ol-30-oic acid-3-0-β-D-glucuro-nopyranosyl-(1→2)-β-D-glucuronopyranoside sodium suppressed carcinogenesis[28] in mouse skin induced by DMBA and TPA. This was the first report of an effective oral administration of triterpenoid compounds suppressing skin tumour promotion in mice. Arjunilic acid(10) oleanene triterpene (2α,3β, 23-trihydroxy-olean-12-enoic-acid) isolated from the rhizome of Cochlosperm tinctorium and its derivatives (triacetate & methylester triacetate) were

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Pfaffic Acid

Saikosaponins
tested.\textsuperscript{[29]} Their inhibitory effects on skin tumour promoter were found to be greater than those of previously studied natural products. Pfaffic acid, a new hexacyclic norrtriterpene isolated (11) from \textit{Pfaffia peniculata} also showed high inhibitory effects on growth of cultured tumour cells such as melanova (B-16), Hela (S-3) and lewis lung carcinoma cells at 4-6 μg ml\textsuperscript{-1}\textsuperscript{[30]}. The antitumour and antibacterial activities of 11-triterpene quinones isolated from \textit{Maytenus horrida} and \textit{Rzedowskia tolantonglum} were studied in cultures of Hela-cells and several bacteria respectively.\textsuperscript{[31]} A known steriod, in addition to triterpenoids, anthraquinones, napthalenes and a new anthraquinone glycosides, xanthopurpurin 3-O-beta-D-glucoside, were isolated from the roots of \textit{Rubia akane}\textsuperscript{[32]} grown in Taiwan. Mollugin - a naphthohydroquinone showed strong inhibition of arachidonic acid (AA)-induced and collagen-induced platelet aggregation. In contrast, 2-methyl-1,3,6-trihydroxyl-9,10-anthraquinone, xanthopurpurin, 3-0-β-D-glucoside and xanthopurpurin showed mainly inhibition of collagen induced platelet aggregation.

The cytotoxic activity of nine triterpenoids and flavonoids isolated from \textit{Artemisia annua} were tested \textit{in vitro} on several human cell lines.\textsuperscript{[33]} These compounds are artemisinin, deoxyartemisinin, artemisinic acid, artoannuin-B, stigmasterol, Friedelin, friedelan-3-beta-ol, artemetin, and

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quercetagetin, 3,4,6,7-tetramethyl ether, friedelane-type triterpenoids isolated from this plant. Artemisinin and quercetagetin 3,4,6,7-tetramethyl ether showed significant cytotoxicity against P-388, A-549, HT-29, MCF-7 and KB tumour cells. Two cytotoxic pentacyclic triterpenoids from *Naruim oleander*[^34] were found to be antineoplastic agents.

To search for possible antitumour-promoters, screening of 24, 29-nor-cucurbitacin glucosides, isolated from the roots of *Cayaponia tayuya* using an *in-vitro* synergistic assay system was carried out by Kanoshima-T; et al.[^35]. Of these glucosides, cayaponosides B,B-3,D, D-3b and C2 exhibited significant inhibitory effects on Epstein-Barrvirus (EBV) activation induced by the tumour promoter, 12-0-tetradecanoylphorbol-13-acetate (TPA). Further more cayaponosides B and C 2 exhibited remarkable anti-tumour promoting effects on mouse skin tumour promotion in an *in-vivo* two stage test.

**2.2.0 MOLLUSCIDAL ACTIVITY**

Millions of people living in Asia, Africa and South America are affected with schistosomiasis. This disease is linked with certain species of aquatic snails because they serve the parasite as intermediate hosts. Mollusicicidal or snail-killing activities of plants are of special importance for the control of schistosomiasis as they are less expensive than synthetic

[^34]: Siddique-BS; Begum-S; Siddique-S; Lichter-W (1995) *Phytochemistry* 39 (1); 171-4
compounds. Two species of Swartzia (leguminoseae) were extensively studied for active components. Four monodesmosidic and one bisdesmosidic saponins isolated from S. madagascariensis[36], saponins from S. simplex[37] were investigated for their molluscicidal activities. They were shown to be glucuronides of oleanolic acid, gypsogenin and gypsogenic acid. The result of biological testing showed that a saponin which was identified as the 3-O-α-L-rhamnopyranosyl-[1→3]-β-D-glucuronopyranoside of oleanolic acid exhibited the highest molluscicidal activity (3 mg l⁻¹) of the isolated compounds against schistosomiasis transmitting snails. Biomphalaria glabrata Saponins with disubstituted glucuronic acid as well as those with gypsogenin and gypsogenic acid as aglycone had a lower activity (>25 ppm). By a study of general structure-activity relationships with other molluscicidal saponins, it was observed that bisdesmosidic saponins had no snail killing activity. Two monodesmosides of oleanic acid isolated from Xeromphis spinosa were subjected to the molluscidal test against Biomphalaria glabrata.[38] In both saponins, the glycone portion was linked to OH-3β of oleanolic acid. Of the two isolated saponins, one is a disaccharide and other was found to be a trisaccharide. The disaccharide and trisaccharide were found to be lethal against the snails at concentrations of 15 and 20 ppm respectively.

2.3.0 ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity of some triterpenoid derivatives of oleanane series were examined on arachidonic acid (AA) induced ear edema in mice.\textsuperscript{39} Of the compounds examined, dihemiphthalate derivatives of 18\,\beta-olean-12-ene-3\beta,3\alpha-diol, 18\beta-olean-9(11), 12-diene-3\beta,3\alpha-diol showed a strong inhibition of ear edema on both topical and oral administration. The most effective time for the typical administration of the compounds against ear edema was found to be 0-30mts before AA application. This is different from dexamethasone which requires a time lag for reaction glycyrrhetinic acid and deoxoglycyrrhetol. The parent compounds of these derivatives showed no detectable inhibition on edema. The same result was also obtained from the similar study on TPA- induced mouse ear edema\textsuperscript{40}, 11-oxo-oleanoic acid and 11-oxohedragenin inhibited corticoid 5\,\beta- reductase\textsuperscript{41} and the inhibitory effect of the former was found to be higher than the latter. To study the corticoid like activities, 11-keto-triterpenoids were prepared and their anti-inflammatory activities were tested in carragenin induced hind paw edema in rats.\textsuperscript{42} Corticoid-5\,\beta-reductase inhibition was also evaluated. All the oxo-triterpenes tested, inhibited corticoid-5\,\beta-reductase and 11,19-diketo-18,19-secoursolic acid

\textsuperscript{39} Inone, H., Mori, T., Shibata, S & Koshihara, Y. (1989) \textit{J. Pharm. Pharmacol} 40, 272
\textsuperscript{40} Inone., H., Mori, T., Shibata, S & Koshihara, Y (1989). \textit{J. pharmaco.} 96 204
\textsuperscript{42} Han, B. H., Han, Y. N. and Kim, T. H. (1986). \textit{Saengya Hakhoechi} 17, 178.
was found to have highest inhibitory potency. 3β-hydroxy-11-oxo-olean-12 en-30-oic acid (glycyrrhetinic acid) inhibited carragenin-induced edema in the rat paw and inhibited leukocyte migration in the pleural space induced by dextran injections[43]. The mechanism of the anti-inflammatory action of papyriogenins A and C, two tri-tetaterpenoids isolated from *Tetrapanax papyiferum* was studied by sugishita *et al*[44]. The cotton pellet granuloma test in normal and adrenalectomized rats, the blockade by antiglucocorticoids of vascular permeability caused by serotonin, and the competition on 5β-reduction of steroidal compounds were followed for the investigation. Papyriogenin A was found to be more potent than papyriogenin C as an inflammation inhibitor of caragenin-induced paw edema in mice. Pre-treatment with progesterone completely blocked the anitinflammatory effects of papyriogenin A or C against serotenin induced paw edema. The effects of papyriogenin A and C orally, on the cotton pellet granuloma test in adrenalectomized rats were similar to those of normal rats. On the other hand the competitive effects of papyriogenin A and C on 5β-reduction of testosterone and cortisol were recognized to be significant. Pyracremic acid, isolated from the bark of *Pyracantha crenulata* and characterized as 3β-3',4'-dihydroxy cinnamoyloxylup 20(29)-en-28-oic acid was tested for its anti-inflammatory activity by the cotton

method and was shown to be a potential inhibitor of the formation of granulation tissue[45].

The fruit juice of *Ecballium elaterium*, in which active principle isolated was characterised as triterpenoid namely *cucurbitacin B*, was known to be used in turkey for the treatment of sinusitis and it was investigated for its anti-inflammatory activity in mice[46]. This is the first report that cucurbitacin B has a significant anti-inflammatory acitivity. Triptotriterpenic acid A, a new oleanane triterpene isolated from the roots of *Tripterygium wilfordii* was found to be an effective antiinflammatory agent[47]. Stearyl glycyrrhetinate and glycyrrhetinyl stearate, two olean-12-en-30-oic-acid derivatives were shown to possess significant anti-inflammatory properties as detected by the rat foot test and the cotton pellet test[48]. An ointment of 3β-hydroxy-11-oxo-olean-12en-30-oic acid triterpenoid, was also found to be effective in the treatment of caragenin-induced edema in rats and UV light-induced crythema in guineapigs, in a dose related manner. In patch tests in human subjects, di-potassium or dis-odium, derivative of 3β-hydroxy-11-oxo-olean-12en-30-oic acid triterpenoid added to the lotion of a cold hair waving preparation reduced

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the skin irritation induced by the lotion. Olean-12-ene-3β, 30-diol showed anti-ulcer, anti-inflammatory and antiallergic activities in rats[49].

2.4.0 ACTION ON METABOLISM

The mechanism of mineralocorticoid action of 11-oxo-olean-12-en-30-oic acid 3β-succinate, triterpenoid was studied by Armani et al[50]. *In vitro* and *in vivo* studies showed that this type of triterpenoid has demonstrable affinity for rat renal mineralocorticoid receptor, intrinsic mineralocorticoid activity in the adrenalectomized rats.

A pharmacological study on the antihepatitis effect of curcurbitacins triterpenoid has been reported[51] in rats, with experimental fatty liver (CCl₄-induced) the serum glutamate pyruvate transaminase and hepatic collagen levels were significantly decreased whereas the serum β-lipoprotein level was increased by administration of cucurbitacin triterpenoid. The hepatic damages, including fibrosis and cirrhosis were also markedly reduced by this triterpene. The serum c AMP and c GMP ratio was increased following intravenous injection of curcurbitacins triterpenoid, suggesting that the changes in cyclic nucleotide balance might be related to the therapeutic mechanism of action of curcurbitacins in hepatits.

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3,11,23,-oxo, 7β,15α-hydroxy lanost-8-en-26-oic acid, a triterpenoid acid, and its derivatives isolated from ganoderma lucidum were shown to be inhibitors of cholesterol biosynthesis[52]. In a similar study[53] 27-nor-24-dihydrolanosterol was found to be markedly active in depressing cholesterol biosynthesis.

Growth inhibiting properties of azadirachtin a bioeffective triterpenoid compound isolated from neem seeds was evaluated[54] on the rice moth, Coryra cephalonica staint, a serious pest of stored products. Two triterpenoids with an unusual 14-carboxyl group, penasterone[55] and acetyl penasterol were isolatd from okinawan marine sponge penaros incrustans were found to be potent inhibitor of histamnine release.

2.5.0 MISCELLANEOUS

Oleanolic acid was effective in the prevention of experimental liver injury induced by injection of CCl₄ in rats.[56] The results suggested that oleanolic acid posses a potent protective action of CCl₄ induced liver injury.

11-oxo-olean-12-en 30oic acid,3β-succinate was shown to provide a protective effect to experimentally induced lower urinary tract infections in the rabbit model.[57]

54 Sharma, G.K. (1992) Phytoparasitica. 20 (1) 47-50
3-oxo,16β-OH; 23-OAc-24,25-epoxy-protast-13(17)-ene triterpenoid isolated from *Alisma orientale* was found to be inhibitor of experimentally induced contractions in isolated rat ileum.[58] The antitussive and expectorant activities of 3β-oh-11-oxo-olean-12 en-30-oic acid choline was evaluated in experimental animals including guinea pigs and mice[59]. The antiviral activity of some dammar resin triterpenoids was investigated by Pochland *et al*.[60]. Nine triterpenoid isolated from dammer resin showed antiviral activity against *Herpes simplex* virus Type I & II *in vitro*. Each compound caused a significant reduction in viral cytopathic effect when vero cells exposed continuously to 1-10 μg ml⁻¹ of compound for 48 hrs. after viral challenge.

Mariestic acid A and other triterpenoid acids, having normal and rearranged lanostane skeletons isolated from *Abies mariesii* and *Abies firma* exhibited antimicrobial activity against Gram-positive bacteria and actinomycetes[61,62]. These results suggested that not only the carboxylic group but also the hydrophobic moiety, played an important role in revealing the inhibitory activity. The inhibitory effects of 10 lanostane triterpenes (including five new) isolated from *Ganoderma lucidum* on angiotensin converting enzymes (ACE) action were

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determined[63]. Gentiatriculin, a new triterpene ester isolated from the herbs of *Gentiana-flavo maculata* protected mice against CCl₄ induced hepatotoxicity[64].

The circulatory effects of oleanolic acid sodium hydrogen succinate (OSS)- an analouge of the antiulcer drug carbenoxolone were investigated by Filzewiski et al[65].

Two new triterpenoids isolated from the leaves and fruits of *Skimmia Japonica* exhibited an increased growth inhibitory activity against the silk worm[66].

Oleanane-type triterpenoids isolated from *Dillenia papnana*[67] showed antibacterial activites against *Bacillus subtilis*, *Escherichia coli* and *micrococcus luteus* Konoshima-T et al[68], found significant inhibitory effects of triterpenoids, 23, 24-dihydrocucurbitacin F, 25-acetyl-23,24-dihydrocucurbitacin F, 2-O-beta-D-glucopyranosyl- 23,24-dihydrocucurbitacin F on Epstein-Barr virus (EBV)

Triterpenoids namely Betulinic acid 1 and plantanic acid-2 isolated from leaves of *Gyzium claviforum* were found to be inhibitors of HIV replication in H-9 lymphocyte cells[69]. Evaluation of anti HIV activity with eight derivatives of Betulinic acid 1 revealed that dihydrobetulinic acid

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67 Nick-A; Wright-AD; Sticher-O; Rali-T (1994), *J Nat. Prod.* 57 (9) 1245-50
68 Konoshima T; Takasaki-M; Tatsumoto.T (1994) *Biol-Pharm-Biol. 17(5) ;* 668-71
was also a potent inhibitor of HIV replication. The C-3 hydroxy group and C-17 carboxylic acid group, as well as the C-19 substituents, contribute to enhanced anti-HIV activity. The inhibitory activity of these compounds against protein kinase C (PKC) was also examined. However there was no correlation between anti HIV activity and the inhibition of PKC among these compounds.

Fujio T; Kashiwada-Y; Kilkuskiec-RE Cosentino-LM; Ballas-LM; Jiang-JD (1994). J-Nat-Products 57(2); 243-7