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Biological Activity of Betulinic Acid: A Review

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ABSTRACT

Betulinic acid is a known natural product which has gained a lot of attention in the recent years since it exhibits a variety of biological and medicinal properties. This review provides the most important biological properties of betulinic acid.

Keywords: Betulinic Acid; Betulin; Triterpene; Natural Product; Medicinal Properties

1. Introduction

Betulinic acid, (3*β*-hydroxy-lup-20(29)-en-28-oic acid) (**Figure 1**) is a naturally occurring pentacyclic lupane-type triterpenoid which exhibits a variety of biological and medicinal properties such as inhibition of human immunodeficiency virus (HIV) [1,2], anti-bacterial [2,3], anti-malarial [4], anti-inflammatory [5-8] anthelmintic [9], antinociceptive [10], anti-HSV-1 [11,12], and anticancer activities [13-16]. Betulinic acid is widely distributed throughout the plant kingdom [17]. The birch tree (*Betula* spp., Betulaceae) is one of the most widely reported sources of betulinic acid and betulin which can be obtained in considerable quantities [18-20]. Betulinic acid could be also isolated from various sources include *Ziziphus* spp. (Rhamnaceae) [16,21,22], *Syzygium* spp. (Myrtaceae) [1,23], *Diospyros* spp. (Ebenaceae) [24-26] and *Paeonia* spp. (Paeoniaceae) [27]. The reduced congener of betulinic acid, betulin (3*β*-lup-20(29)-ene-3, 28-diol) (**Figure 1**), was one of the first natural products isolated in 1788 from the bark of the white birch, *Betula alba* [28]. This review focuses on the pharmacological properties of betulinic acid.

2. Anticancer Activity

Kumar *et al*. [29] isolated betulinic acid from the methanolic extract of *Dillenia indica* L. fruits. The methanolic extract showed significant anti-leukemic activity in human leukemic cell lines U937, HL60 and K562. The isolated betulinic acid showed IC_{50} of values at 13.73, 12.84, 15.27 mg/ml in U937, HL60 and K562 cell lines, respectively.

Betulinic acid was isolated from the MeOH extract of the aerial part of Vietnamese *Orthosiphon stamineus* and tested for its cytotoxicity towards highly liver metastatic murine colon 26-L5 carcinoma cells [30]. It was found that betulinic acid shows the cytotoxicity with an ED_{50} value of 75.4 μg/ml.

Betulinic acid was obtained from the ethanol extract of *Engelhardtia serrata* Bl. by bioassay-guided isolation and was tested for its cytotoxic and apoptosis-inducing activities against the K562 cell line [31]. Betulinic acid showed an inhibitory activity on the growth of K562 tumor cell line with IC_{50} value of 6.25 μg/ml and also induced 35% apoptosis at 25 μg/ml.

Betulinic acid was isolated from the $CHCl_3$ extract of the bark of *Bischofia javanica* and was evaluated for its inhibitory effects on DNA Topoisomerases (Topos) II activity [32]. Betulinic acid was found to be catalytic inhibitor of Topo II activity with IC_{50} value of 56.12 μM which was comparable to that of 52.38 μM for a classic Topo II inhibitor, etoposide. It was suggested that betulinic acid is potent DNA Topo II inhibitor. The ED_{50} values of betulinic acid and etoposide were found to be 7.19 and 2.59 μM against A549 cancer cell line.

Betulinic acid was isolated from the methanol extract of the dried leaves of *Nerium oleanderand* and was tested for its *in vitro* anticancer activity on the basis of the cell growth inhibitory activities toward three kinds of human cell lines, *i.e*. WI-38 fibroblast cells, VA-13 malignant tumor cells, and HepG2 human liver tumor cells [33]. Betulinic acid showed significant cell growth inhibitory to WI-38 cells, moderate cell growth inhibitory activity to VA-13 cells and moderate cell growth inhibitory activity to HepG2 with IC_{50} values of 1.3, 11.6 and 21 μM, respectively.

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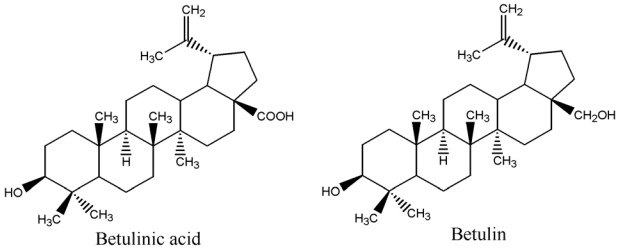

Figure 1. Structures of betulinic acid and betulin.

3. Anti-HIV Activity

Betulinic acid was isolated from the MeOH extract of the leaves of *Syzrgium claviflorum* by Fujioka *et al*. [2] and was tested on HIV-1 replication in H9 lymphocyte cells. It was found that betulinic acid shows an inhibitory activity against HIV-1 replication with an EC_{50} value of 1.4 μM and inhibited uninfected H9 cell growth with an IC_{50} value of 13 μM. This finding prompted them to prepare derivatives of betulinic acid and to evaluate their anti-HIV activity.

Theo *et al*. [34] isolated betulinic acid from the stem bark of *Peltophorum africanum*, a traditional South African medicinal plant. They evaluated it for the inhibitory activities against HIV-1_{NL4-3} (X4-HIV-1) and HIV-1_{JRCSF} (R5-HIV-1). Betulinic acid inhibited against HIV-1_{NL4-3} and HIV-1_{JRCSF} with IC_{50} values of 0.04 and 0.002 μg/ml, respectively. They suggested that betulinic acid could be used as potential therapeutics for HIV-1.

Betulinic acid was isolated from the leaves and twigs of *Cratoxylum arborescens* and was tested in the HIV-1 RT assay and syncytium assay [35]. Betulinic acid showed anti-HIV-1 activity in the syncytium assay with IC_{50} value of 9.8 μg/ml and in the RT assay with IC_{50} of 10.8 μg/ml. The results showed that betulinic acid is the most active of isolated compounds in the syncytium assay.

4. Anti-Bacterial Activity

Woldemichael *et al*. [36] isolated betulinic acid from the CH_2Cl_2-MeOH extract of the Argentinean legume *Caesalpinia paraguariensis* Burk and tested it against *Bacillus subtilis*, methicillin-sensitive and -resistant *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Betulinic acid was found to be inactive against the tested organisms with MICs (minimum inhibitory concentrations) greater than 128 μg/ml.

Betulinic acid was isolated from the crude chloroform bark extract of *Syncarpia glomulifera* (Myrtaceae) by Setzer *et al*. [37]. It was found that the crude extract of *S. glomulifera* shows anti-bacterial and cytotoxic activity. They reported that the relatively large abundance (10% of the crude extract) and high degree of activity of betulinic acid are responsible for the bioactivity of the crude bark extract.

The stem bark of the Brazilian medicinal plant *Zizyphus joazeiro* was phytochemically investigated by Schühly *et al*. [4]. Betulinic acid was isolated from the dichloromethane extract which showed a considerable activity against Gram-positive bacteria.

The anti-bacterial activity of isolated betulinic acid from leaves of *Vitex negundo* L. was tested against *Bacillus subtilis* and *Escherichia coli*, by the paper disc method [3]. Betulinic acid did not show any inhibition zone (total area-disc area) against *Escherichia coli* at concentrations of 1000 μg/disc, 500 μg/disc, 250 μg/disc and 125 μg/disc. However, betulinic acid showed 18.8 mm^2 of inhibition zone against *Bacillus subtilis* at concentration of 1000 μg/disc while at a concentration of 500 μg/disc and below, the betulinic acid did not show any inhibition zone. Kanamycin was used as a standard compound which showed an inhibition zone of 207.2 mm^2 at a concentration of 0.05 μg/ml.

The extract of *Forsythia suspensa* VAHL was studied by Shin *et al*. [38]. It showed a strong inhibition of the urease activity of *Helicobacter pylori*. The active compounds were isolated from the extract of *Forsythia suspense* VAHL. Two active compounds, betulinic acid and oleanolic acid, were found in the extract. At the same concentration level, betulinic acid inhibited urease activity of *H. pylori* stronger than oleanolic acid.

5. Antimalarial Activity

The *in vitro* and *in vivo* antimalarial activity of betulinic acid was investigated [39]. Betulinic acid was isolated from an ethanol extract of the root bark of the Tanzanian tree *Uapaca nitida* Müll-Arg. (Euphorbiaceæ). The *in vitro* antiplasmodial IC_{50} values of betulinic acid against chloroquine resistant (K1) and sensitive (T9-96) *Plasmodium falciparum* were found to be 19.6 μg/ml and 25.9 μg/ml, respectively. Betulinic acid was also tested for in vivo activity in a murine malaria model (*P. berghei*). It was found that the top dosage employed of 250 mg/kg/day is ineffective at reducing parasitaemia.

Bringmann *et al*. [5] was isolated betulinic acid from the *Triphyophyllum peltatum* and Ancistrocladus heyneanus. *In vitro* antimalarial activity of betulinic acid was tested against asexual erythrocytic stages of the human malaria parasite *Plasmodium falciparum*. Betulinic acid showed the antimalarial activity between of moderate to good.

6. Other Biological Activities

Zhang *et al*. [40] isolated betulinic acid from the ethanol extract of *Tovomita krukovii*. They found that betulinic acid shows inhibitory effects against Candida albicans secreted aspartic proteases (SAP) with IC_{50} values of 6.5 μg/ml.

Enwerem *et al*. [10] examined the anthelmintic activity of the methanol, hexane and ethyl acetate extracts of the stem bark of *Berlina grandiflora*. The ethyl acetate extract was found to be the most active. The isolated betulinic acid from the ethyl acetate fraction at 100 and 500 ppm showed stronger anthelmintic activities than piperazine.

Krogh *et al*. [41] was isolated betulinic acid from a medicinal plant *Ipomoea pes-caprae* (L.) R. Br. The results demonstrated that betulinic acid shows pronounced antinociceptive properties in the writhing test and formalin test in mic.

Betulinic acid was isolated from *Diospyros leucomelas* and was tested for anti-inflammatory activity in the carrageenan and serotonin paw edema and TPA and EPP ear edema [26]. The results showed that betulinic acid is the most affected.

The antifeedant activity of isolated betulinic acid from the leaves of *Vitex negundo* L. was studied against the third instar larvae of castor semilooper (*Achoea janata*) [42]. The percentage feeding reduction, at a dosage of 10 μg/cm^2, for betulinic acid was 71.18%, 84.75% and 73.34% after 24, 48 and 72 hr time period, respectively.

Betulinic acid was isolated from the methyl ethyl ketone extract of *Tetracera boiviniana* and was monitored for DNA polymerase β inhibition [43]. Betulinic acid showed an inhibition of DNA polymerase β with IC_{50} value of 14 μM in the presence of bovine serum albumin (BSA) and 6.5 μM in the absence of BSA.

Domínguez-Carmona *et al*. [44] isolated betulinic acid from the crude extract of the leaves of *Pentalinon andrieuxii* (Apocynaceae). They tested betulinic acid for its antiprotozoal activity against *Leishmania amazonensis* (LA), *Leishmania braziliensis* (M2903), Trypanosoma cruzi tulahuen (TULA) and *Plasmodium falciparum* (F32). Betulinic acid showed a moderate trypanocidal activity against T. cruzi with IC_{50} value of 50.0 μM and a good antiplasmodial activity with IC_{50} value of 22.5 μM against *P. falciparum*. No any leishmanicidal activity was detected for betulinic acid against *L. amazonensis* and *L. braziliensis*.

Betulinic acid was isolated from the MeOH extract of the roots of *Saussurea lappa* C. B. Clarke (Compositae) and was evaluated *in vitro* for protein tyrosine phosphatase 1B (PTP1B) inhibitory activity [45]. Betulinic acid inhibited PTP1B activity with IC_{50} value of 0.7 μg/ml, which was comparable to those of ursolic acid and RK-682 used as positive controls.

Substantial amounts of betulinic acid was obtained from the stem barks of five *Uapaca* species (Euphorbiaceae) include *Uapaca acuminata*, *Uapaca guineensis*, *Uapaca heudolotti*, *Uapaca paludosa* and *Uapaca vandhoutei* [46]. Isolated betulinic acid was screened *in vitro* for inhibitory activity against *Trypanosoma brucei* glycolytic enzyme GAPDH. Betulinic acid inhibited *T. brucei* GAPDH with an IC_{50} value of 240 μM and was a competitive reversible inhibitor of this enzyme with respect to its cofactor NAD^+.

Betulinic acid was obtained from the dried and powdered roots of *Clusia nemorosa* L. (Clusiaceae) and was tested for its antiobese activity in the adult male Swiss mice on a high-fat diet (HFD) [47]. It was found that mice treated with betulinic acid (50 mg/L, in drinking water) and fed a HFD showed significantly decreased body weights, abdominal fat accumulation, blood glucose, plasma triglycerides, and total cholesterol relative to their respective controls fed no betulinic acid during 15 weeks. It was suggested that betulinic acid has an antiobese potential through modulation of fat and carbohydrate metabolism.

Betulinic acid was isolated and identified from the methanolic extract of the stem bark of *Clusia ellipticifolia* and was studied for its antinociceptive activity [48]. The pharmacological study using the abdominal contortions model induced by acetic acid showed significant antinociceptive activities to the isolated compounds and the highest effect was attributed to the betulinic acid.

Betulinic acid was isolated from the powdered rose hip with and without fruits (Rosae pseudofructus cum/sine fructibus, *Rosa canina* L., Rosaceae) and was tested *in vitro* for inhibition of cyclooxygenase (COX-1, COX-2) and 5-LOX-mediated leukotriene B_4 (LTB_4) formation [49]. Betulinic acid was found to act as moderate inhibitors of COX-1, COX-2 and LT formation *in vitro* with IC_{50} values of >125, >125 and 102.2 μM, respectively.

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