

## Extraction, Isolation and Purification of Betulinic Acid from *Melaleuca* sp.

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### Introduction

Betulinic acid  $C_{30}H_{48}O_3$  is an unsaturated monobasic triterpene hydroxy-acid. It could be derived from betulin or betulinaldehyde via oxidation reaction, probably by air oxidation in nature. Betulinic acid was isolated throughout plant kingdom in traces and is soluble in many organic solvents such as pyridine, ethanol, acetone, methanol and ethyl acetate. Betulinic acid was reported to selectively kill human melanoma cell<sup>1</sup> while leaving healthy cells alive and retard the progression of HIV infection.<sup>2</sup> In addition, betulinic acid has antibacterial properties in which inhibits the growth of both *Staphylococcus aureus* and *E. coli* sp. and shown some antimalarial activity.<sup>4</sup>

*Melaleuca* sp belongs to the Myrtaceace family, and commonly called Gelam (local name). The tree appears to prefer lowlying swampy areas. The leaf of *Melaleuca* sp may be subjected to steam distillation to extract the *cajiputi* oil which has germicidal and other medicinal properties<sup>3</sup>.

### Materials and Methods

Analytical Thin Layer Chromatography (TLC) was performed on commercially available Merck DC-plastic sheets pre-coated with Kieselgel JF 254 (thickness 0.2mm). The identification of spot was carried out under UV light or exposed to iodine vapours. Column chromatography was employed using Merck silica gel 60 (230-400 mesh ASTM) Art. No: 9385. Infra-red (IR) spectra was measured on Beckman IR spectrophotometer. Melting point were determined using a Kohler micro melting point apparatus equipped with a microscope XSP-12 model 500. Samples were collected from various places in Johor and Selangor. The plant material (i.e. leaves, seed, branches, bark and flow-

ers) was air dried at room temperature. The plants materials were soaked in methanol, chloroform and hexane; for more than 48h. It was then filtered and the solvent was removed by rotary evaporator. After TLC analysis using various solvents combination; the crude extract was subjected for column chromatography followed by crystallization from various solvents.

### Results and Discussion

Our investigation from the methanol extract of *M. cajiputi* collected from various places in Johor and Selangor, established the isolation of bioactive triterpenes, named as betulinic acid and ursolic acid (Ahmad *et al.*, 1997). However, the methanol extract from dried flower of the said plant produced only Betulinic acid alone (Ahmad *et al.*, 1999). The structure of the isolated product was confirmed by spectroscopic data together by direct comparison (m.p and mix.m.p) with the authentic materials. Chemical investigation from other part of the plant gave various terpenes compounds. Example, the methanol extract from the leaf of the plant, as expected finished with mixture of monoterpenes together with betulinic acid, and other triterpenes. Interestingly some acetophenone derivatives was also isolated for the first time from the 1st stage of the leaf. Furthermore, the bioactivities of the extract obtained from the leaf depended on the maturity of the leaf itself. The methanol extract from the 1st stage leaf shown more fungicidal activities in contrast the 3rd stage leaf shown higher cytotoxicity effect.

### Conclusions

Chemical investigation of seed and flower of *M. cajiputi* afforded the isolation of known triterpene compounds. Chemical examination from other part of the plant also produces terpene

compounds. This plant could be preserved for the source of bioactive natural compounds in future life.

### Benefits from the study

Some natural anti-cancer compound named as betulinic acid and ursolic acid, and probably some other analogous material together with some antibacterial compounds could be isolated from our local and native plant.

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