

## ANTICANCER POTENTIAL OF AGARWOOD DISTILLATE

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### Abstract

Agarwood or Gaharu by definition is the resin impregnated heartwood that produces unique aromatic scent when burnt. Apart from religious rituals and perfumes, agarwood has also been used as traditional medicines leading to the interest of this study which is to screen for anti-cancer property to properly support the unproven traditional medicine claims. In this study, agarwood water distillate (hydrosol) obtained from distillation of agarwood (resin) were screened against MCF-7 cell, cell line commonly used as *in vitro* model for breast cancer and VERO cell in order to investigate the potential anti-cancer property and observe the effects on normal cells. Distillate samples were collected, diluted and directly subjected to three anti-cancer screening assays (cell attachment assay, cell viability assay and sulforhodamine B assay). Based on the data analysis, agarwood distillate possesses anti-cancer activity and exerts no significant effects on normal cells which warrants further investigation and development of alternative remedy against cancer as well as adding more value to the agarwood industry.

**Keywords:** Agarwood, Gaharu, Distillate, Hydrosol, Cancer, Anti-cancer, Cell culture,

### 1. Introduction

The fragrant wood and resin of *Aquilaria* species is known as agarwood, eaglewood or gaharu depending on the country. It is used to fulfil demand in religious, medical and aromatic preparation (Uddin et al., 2008; Yagura et al., 2005). Traditionally, agarwood has been used as sedative, analgesic and digestive medicine (Yagura et al., 2005). Agarwood has also been used as a complex ointment for smallpox, various abdominal complaints and rheumatism (Rana et al., 2010).

More recently, scientific works on *Aquilaria spp.* have shown promising results in the pharmacological field. Feng, Yang and Liu (2009), stated that extract obtained from the leaves of *Aquilaria sinensis* has anti-inflammatory, antitumor, analgesic, therapeutic, and prophylactic activity on constipation, intestinal obstruction, and obesity, therapeutic effect on hemorrhage and cerebral ischemia, and

conducted by Gunasekara et. al (1981), reported that alcoholic extract of *Aquilaria malaccensis* exhibited mild cardiotoxic activity and significant activity against Eagle's carcinoma of the nasopharynx. A work conducted by Huda et. al (2009) discovered that extract from *Aquilaria malaccensis* exhibit potent antioxidant activity. In another study, Takemoto et. al (2007) proved that essential oil vapour from agarwood possessed sedative effects through evaluation of sedation and excitation activities by observing spontaneous motor activity of mice.

Agarwood distillate (also known as hydrosol) is obtained from the essential oil steam distillation process. Other plant hydrosols have been reported to exhibit therapeutic properties. For instance, rose distillate exhibited antibacterial property and cedarwood hydrosol possessed antiviral and antiseptic properties. A study conducted by Pierre Tannous et al., (2004) estimated that a maximum of 29 % of

material suggesting that plant distillate may demonstrate therapeutic properties possessed by the original plant. Despite these findings, plant distillates are often regarded as waste by-products in the distillation process. This is also true in agarwood industry where the sought resin is distilled to produce expensive aromatic

oil, leaving the distillate as waste. While therapeutic properties of agarwood plant and its various parts have been reported, scarce information can be found on its distillate. This present study is therefore crucial in discovering the therapeutic potential of agarwood distillate particularly its anticancer properties.

## **2. Materials and Methods**

### *Sample preparation*

Agarwood hydrosol were collected and diluted with DMSO according to the concentration desired. Both MCF-7 breast cancer cells (ATCC® HTB-22™) and Vero (green monkey kidney cells, ATCC® CCL-81™) representing normal cells were maintained at 37 °C/ 5 % CO<sub>2</sub> in Dulbecco's Modified Eagle medium (DMEM) with 10 % serum.

### *Cell Attachment Assay*

This simple procedure provides rough information about the adhesion system and overall effect of anti-attachment. Cell attachment or adhesion is mediated by specific cell surface receptors for molecules in the extracellular matrix (Freshney, 2005). In this assay, cells were propagated with media containing agarwood distillate sample and anti-attachment effects from sample would reduce the number of viable cells.

### *Cell Viability Assay*

This method relies on the breakdown in membrane integrity that is determined by the uptake of trypan blue into the non-viable cells (Freshney, 2005). Cells were initially propagated with normal media prior to exposure to media adjusted with agarwood distillate sample.

### *Sulforhodamine B (SRB) Assay*

SRB is a bright-pink aminoxanthene dye with two sulfonic groups that bind to basic amino acid residues under mild acidic condition. This method relies on the measurement of cellular protein content to determine total cell density (Vichai &

Kirtikara, 2006). Cytotoxicity effects in this method are represented by the IC<sub>50</sub> values which indicate the required sample concentration to reduce or inhibit 50 % of the controlled cell growth.

## **3. Results and Discussion**

Agarwood distillate also reduced viable cell number in both cell attachment and viability assay while showing no significant effects on VERO cell line suggesting that the distillate possesses anti-cancer effects and poses no significant threat on normal cell line *in vitro*.

### **Conclusions**

Based on simple screening procedures, it can be concluded that agarwood distillate possess anti-cancer properties. The IC<sub>50</sub> value of agarwood distillate was 50 % (v/v). Further investigation is therefore warranted to develop the otherwise underutilized agarwood distillate as an alternative anti-cancer remedy in line with the waste to health concept.

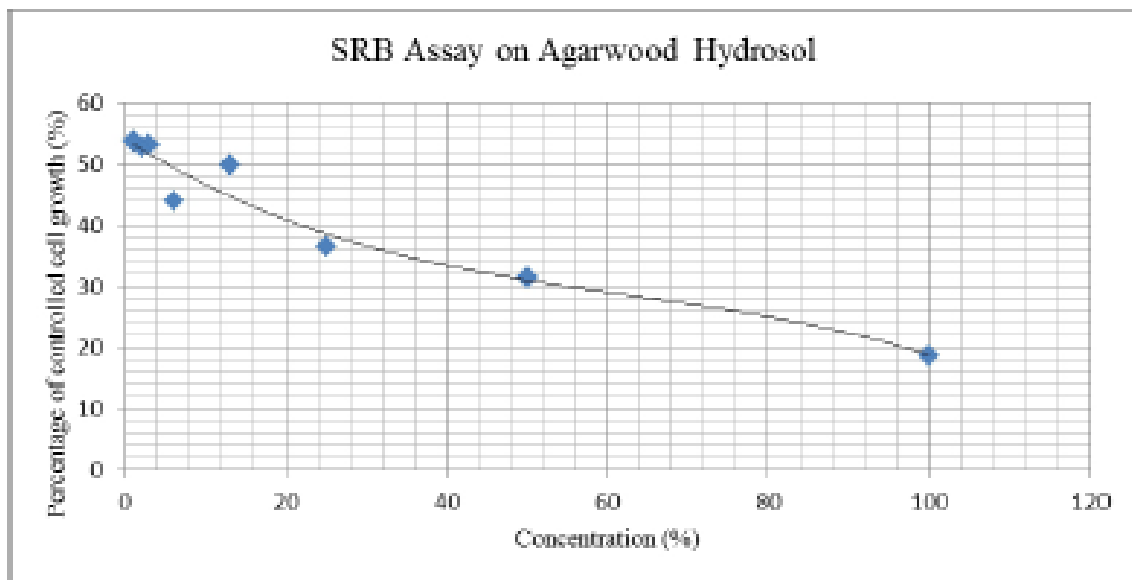


Figure 1: Shows the dose-response fitted plot for agarwood distillate against MCF-7 breast cancer cell line following SRB assay. Agarwood distillate at 50 % (v/v) was able to inhibit 50 % of the controlled cell growth (IC<sub>50</sub>: 50 % v/v).

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