International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.2, pp 829-833, April-June 2014

# Comparative Study of HPTLC Finggerprint of β-Asarone Content between Leaves and Rhizome of *Acorus calamus* L.

**Abd. Malik[1], Ajhar Kurniawan[2] and Ahmad Najib[1]***

**[1]Phytochemistry Division, Faculty of Pharmacy,
[2]Pharmacognosy Division, Faculty of Pharmacy,
Universitas Muslim Indonesia, Jl. Urip Sumiharjo KM 5
Makassar 90132, Indonesia.**

***Corres. Author: ahmad.najib@umi.ac.id
Phone : +6281524045514**

**Abstract:** ß-asarone as a major compound of Sweet Flag (*Acorus calamus* L.) family acoraceae. ß-asarone accumulate on rhizome and distribute generally on the other part of plant including on leaves. The aim of this study is determine of ß-asarone contents of Sweet Flag (*A. Calamus* L.) between rhizome and leaves by HPTLC method. Rhizome and leaves of Sweet Flag (*A. Calamus* L.) extracted by Stahl steam distillation. Extract rendemen is 2.4% mL/g from rhizome and 4.5% mL/g from leaves. Extract and ß-asarone eluted by n-hexane:aethyl acetat (9:1). TLC plate scaned at maximum wavelength 298 nm. The result of ß-asarone contens between rhizome and leave are 5.1386 µg/mL equivalent 0.23246 µg/g and 4.21866 µg/mL equivalent 0.22499 µg/g respectively.
**Key word:** ß-asarone, *Acorus calamus* L., HPTLC.

## Introduction

*Acorus calamus* L. (AC) is a perennial plant with flavoring scent that grow in aquatic environments. It has a long history of medical, cultural, and ritual use and hence was spread outside its indigenous areas in Asia and is now found across Australia, Europe, and North America[1] In India, Traditional use of AC in Ayurvedic medicine is documented for treatment of insomnia, neurosis, and remittent fever [2, 3].

AC has been widely used alone or combined with other herbs in traditional Chinese medicine over centuries. Recent studies have suggested that ß-asarone is one of the main bioactive constituents of its essential oil. Growing evidence has demonstrated that ß-asarone has the properties of antifungal[4]. ß-asarone is affect to central nervous system (CNS)[5], induces apoptosis at colon cancer cell[6], hallucinogenic[7]. The rhizome and essential oil preparations thereof contain high concentrations of α- and ß-asarone, which are believed to be pharmacologically active components[1,8]. The aromatic constituents namely asarylaldehyde in roots asarone in leaves are responsible for the smell of volatile oil[9].

Currently HPTLC is often used as an alternative to HPLC for the quantification of plant products because of its simplicity, accuracy, cost-effectiveness and rapidity[10]. HPTLC fingerprint has better resolution and estimation of active constituents is done with reasonable accuracy in a shorter time[11].

## Material and Method

Twenty five gram of the air dried leaves and rhizome powder was subjected to hot extraction using Sthal steam distillation for 5 hours. The distillation of sample was repeated three times until came by oily substance. The oily substance from distillation was collected and measured the volume. HPTLC process base on Gunalan, G., *et al* method with any modification; the oil resulting from both of samples were applied to a commercial 10 cm × 10 cm precoated HPTLC silica gel 60-plate (Merck) on various concentration, β-asarone used as a standard. Fifteen milliliters of mobile phase consisting of n-hexane and ethyl acetate in the ratio of 9:1 v/v was added into a single-trough chamber, to saturate it for 15 min. The plate in the chamber was developed upward over a path of upper mark. The fluorescent image was examined under UV 254 nm by using a UV viewer cabinet (CAMAG). They were captured with a WinCATS Planar Chromatography Manager documentation system (CAMAG). The excitation wavelength was 298 nm in reflection mode and the exposure time was 3 secon.[12]

## Result and Discussion

Sthal steam distillation results shown on table 1. Rendemen of 2.4% mL/g from rhizome and 4.5% mL/g from leaves.

**Table 1**. **Distillation Result**

| **Sample** | **Sample Weight (g)** | **Volume of Water (mL)** | **Oily Substance (mL)** |
|---|---|---|---|
| Leaves | 75 | 750 | 1.8 |
| Rhizomes | 75 | 750 | 3.4 |

Most of the phenylpropanoids were isolated by steam distillation. An oily substance namely calamol was extracted which was found to be an allyl trimethoxy benzene derivative. It is isomeric with asarone[13].

Total ß-asarone content from samples shown on table 2. ß-asarone content on rhizome is 5.10795 µg/mL and ß-asarone content on rhizome is 4.21866 µg/mL.

**Table 2**. **Total ß-asarone Content from Samples**

| Sample / Repetitions | | Area | β-asarone Content (µg/µl) | Average Content (µg/µl) | Average Content (µg/mL) |
|---|---|---|---|---|---|
| Leaves | I | 12498.88 | 4105.62 | 4218.66 | 4.21866 |
| | II | 14850.07 | 4937.76 | | |
| | III | 11105.85 | 3612.60 | | |
| Rhizomes | I | 18111.98 | 6092.01 | 5107.95 | 5.10795 |
| | II | 19077.00 | 6433.56 | | |
| | III | 8804.65 | 2798.28 | | |

HPTLC chromatogram shown on figure 1 and β-asarone fingerprint from samples showed 10 tracks on figure 2.

Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to ITM (Indian Traditional Medicine) and TCHM (Chinese traditional herbal medicine). The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs. HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug[12].

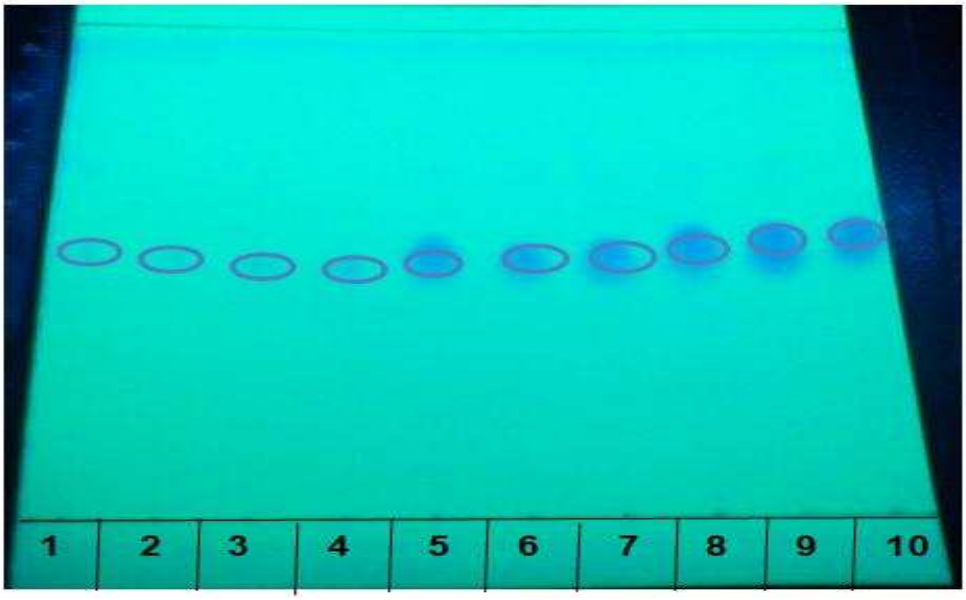



**Figure 1**. **HPTLC chromatogram (1-4 ß-asarone standard, 5-7 leaves and 8-10 rhizome)**

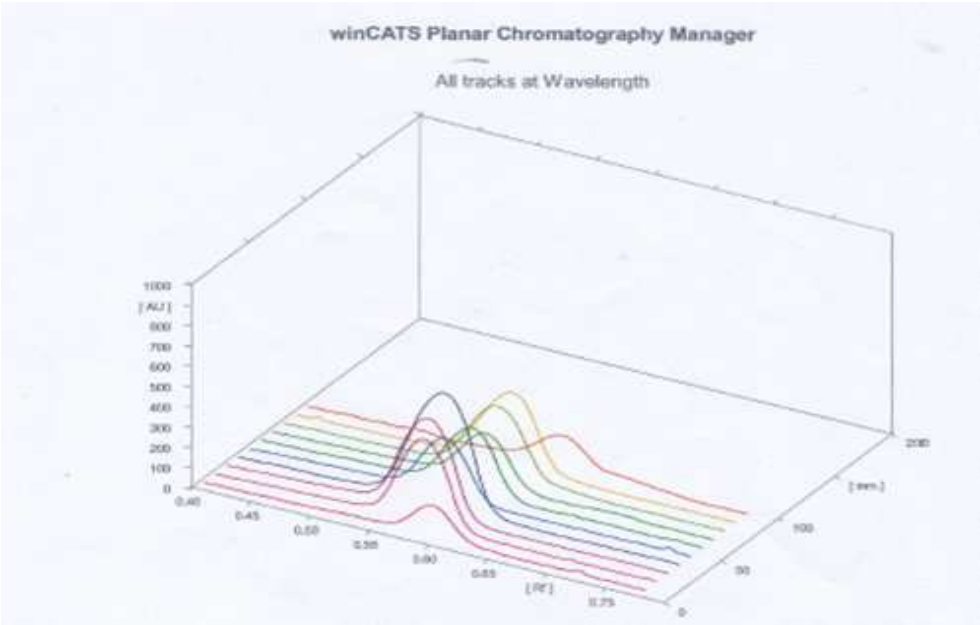
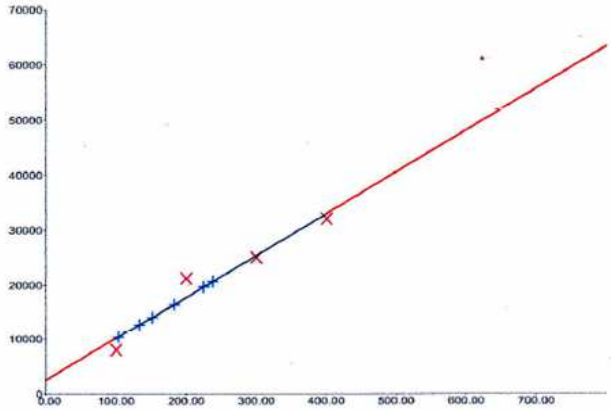



**Figure 2. Fingerprint of ß-asarone (4 tracks in front are standard, 3 tracks in middle are leaves and 3 tracks rhizomes)**

Characteristic TLC/HPTLC fingerprinting of particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species. Thus the present study will provide sufficient information about therapeutic efficacy of the drug and also in the identification, standardization and quality control of medicinal plant[12].

Standard curve on figure 3 was used for determine of β-asarone content on both of sample. On this research we use serial standard from 100 ppm, 200 ppm, 300 ppm and 400 ppm.




| Concentration | Area |
|---|---|
| 100 ppm | 6335.49 |
| 200 ppm | 19612.35 |
| 300 ppm | 23449.10 |
| 400 ppm | 30489.07 |

y = 897.1+76.3 x

r = 0.97089

**Figure 3. Standard curve for samples determination**

The maximum wave length showed on figure 4, this figure base on measurement of β-asarone standard at 298 nm.

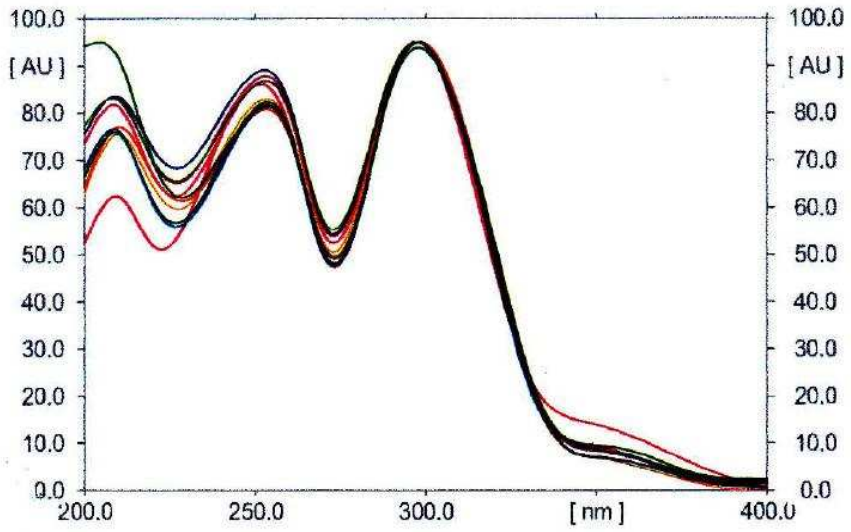



**Figure 4. Spectrum of maximum wave length from samples**

Calculation result base on the regression standard curve obtained that β-asarone content on rhizome and leaves are 5.10795 µg/mLand 4.2186 µg/mL.

## Conclusion

The existence of β-asarone on leaves can be used to determine the concentration. By comparing the concentration on rhizomes can give a data for identification and quality control on AC plant.

## Acknowledgments

The authors are thankful to Director of Study Center of Biopharmaca, Faculty of Pharmacy. Hasanuddin University-Indonesia, for providing technical facilities to conduct this research. The authors also thankful to Head of Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia (Indonesia) for the fully support to design this research.

## References

1. C. Rätsch. The Encyclopedia of Psychoactive Plants. Park Street Press, Rochester, VT, 2005.
2. S.B. Vohora, S.A. Shah, and P.C. Dandiya. Central nervous system studies on an ethanol extract of *Acorus calamus* rhizomes. J Ethnopharmacol. 1990, 28: 53–62.
3. C. Deng, S. Lin, T. Huang, G. Duan, and X. Zhang. Development of gas chromatography/mass spectrometry following headspace solid-phase microextraction for fast determination of asarones in plasma. Rapid Commun. Mass Spectrom. 2006, 20: 2120–2126.
4. Begum, J., et al.In vitro antifungal activity of asaron isolated from rhizome extract of *Acorus calamus* L. Pakistan Journal of Biological Science, 2004, 7(8), 1376-1379.
5. Yang et al. Beta-asarone, a major component of *Acorus tatarinowii* Schott, attenuates focal cerebral ischemia induced by middle cerebral artery occlusion in rats, BMC Complementary and Alternative Medicine 2013, 13:236.
6. Xi Zou et al. Beta-asarone Induces Lo Vo Colon Cancer Cell Apoptosis by Up-regulation of Caspases through a Mitochondrial Pathway in vitro and in vivo, Asian Pacific J Cancer Prev, 2012, 13 (10), 5291-5298.
7. Björnstad K, Helander A, Hultén P, Beck O. Bioanalytical investigation of asarone in connection with *Acorus calamus* oil intoxications. J Anal Toxicol. 2009, 33:604–609.
8. C.E. Dennehy, C. Tsourounis, and A.E. Miller. Evaluation of herbal dietary supplements marketed on the internet for recreational use. Ann. Pharmacother. 2005, 39: 1634–1639 (2005).
9. Venakutonis.P.R. and Dagilyte. Journal of Essential oil Research, 2003, 15(5),313-318.
10. Wasim Aktar MD, Rajlakshmi Poi and Anjan Bhattacharya. Status of sennosides content in various Indian herbal formulations method standardization by HPTLC. Bangladesh J Pharmacol 2008, 3: 64-68.
11. Pawar RK , Sharma Shivani , Singh KC , Sharma Rajee v KV. Physico chemical standardization and development HPTLC method for the determination of Andrographonin in Kalmgh Navyas Loha. An Ayurvedic formulation. Int.J Research in Ayurveda and Pharmacy 2011, 2 (1): 295-301.
12. Gunalan G, Saraswathy A and Vijayalakshmi K. HPTLC fingerprint profile of *Bauhinia variegata* Linn. leaves. Asian Pac J Trop Disease 2012, S21-S25.
13. Arasan Elaya Raja, M Vijayalakshmi and Garikapati Devalara, *Acorus calamus* linn. : Chemistry and Biology Research J. Pharm. and Tech. 2009, 2 (2).

*****