Thin Layer Chromatographic Identification of the Whole Plant of Sangketan (Achyranthes Aspera)

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Abstract. Medicine plant is a great source of information for variety of phytochemicals which can be developed as drug with the right selectivity. One of the potential plants developed as anti-cancer drugs is Sangketan (Achyranthes aspera). In the in vivo study showed that Achyranthes aspera causes apoptosis and healing to breast cancer cells induced by benzopyrene. This study deals with early phytochemicals screening using thin layer chromatography of Achyranthes aspera. Sangketan powder is extracted by reflux method on water bath with temperature of 50°C using ethanol, methanol and petroleum ether solvent. The extract from filtering is concentrated on water bath using porcelain dish. After obtaining the extract concentrated, it is dissolved with a suitable solvent, then elluted on Silica Gel 60 F254 stationary phase with various mobile phase comparison (chloroform : methanol). In qualitative analysis, phytochemical compounds such as steroids, triterpenoid, sugar, alkaloids, phenolic compounds, flavonoids were extracted in Sangketan using standard methods. The screening uses thin layer chromatography with chloroform : methanol of (8: 2 v/v), showing some gray bands after spraying vanillin - sulphate acid reagent. This result reveals that Achyranthes aspera is an important source of various active therapeutic and pharmacological properties.

1. Introduction

Achyranthes aspera Linn, Family: Amaranthaceae or its common name Apamarga is stiff, erect herb found commonly as weed throughout India up to an altitude of 900 m. Stem erect, base woody, angular or ribbed, simple or branched. Leaves are opposite, petiolate, ovate-elliptic obovate-rounded, apex usually rounded, finely or softly pubescent on both sides. Flowers are in an auxiliary or terminal spikes, which are more than 50 cm long, greenish white, bracteates and bracteolate. Stamens 5 in number, staminodes are truncate, fimbriate, ovary oblong, subcompressed and ovule solitary. Fruit easily disarticulate oblong or ovoid and utricle. Seeds are inverse, testa coriaceous, embryo annular and surrounded by floury albumin. The plant has been mentioned in manuscripts of Ayurveda and Chinese medicines. The plant has been reported to passes number of medicinal properties. The herb is widely used to treat various kinds of ailments. The whole plant used as diuretic in renal dropsies and general anasarca(1)

Ethnomedicinal study deals with the study of traditional medicine since ancient times mankind has been using herbal plants, organic materials from the sea, rivers etc .for its betterment. Recently much attention has directed towards extracts and biologically active isolated from popular plant species .In the
present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their bioactive constituents. The curative properties of medicinal plants are mainly due to presence of various complex chemical substances of different compositions which occur as secondary metabolites (2). Study conducted toward Achyranthes aspera leaves ethanol extract given to female mice mated with a dose of 300 mg/kg bw orally cause a decline in the number of pregnant mice, the site of implantation, the number of corpus luteum and the number of fetus conceived during a period of pregnancy. Alkaloid as antimitotic and antitelomerase have the ability to bind to tubulin, which is a protein that compiles microtubules by inhibiting or blocking the polymerization of protein into microtubules causing the destruction of microtubules resulting cells will stop dividing so that will be followed by the occurrence of cell death (apoptosis) (3).

2. Materials and Methods
2.1. Collection plant material
The specimen was collected from University Botanical Garden, Department of Pharmacy, University of Gadjah Mada, Yogyakarta. The whole plant (Achyranthes aspera) were washed thoroughly 2-3 times with running tap water and then with sterile distilled water, air dried at room temperature. After complete drying were powdered well using a mixer. Powdered samples were extracted by reflux method on water bath with temperature of 50°C using ethanol, methanol and petroleum ether solvent. The extract from filtering is concentrated on water bath using porcelain dish. The crude extract were collected in amber coloured sample bottles and stored. All chemicals and reagents used in this study belongs to the solvents of analytical grade.

2.2. Chromatographic studies
Ethanol, methanol and petroleum ether extract were evaluated by TLC for the presence of alkaloids, phenolic compounds & steroids etc. using specific solvent systems and detecting reagents, to substantiate the presence of these constituents, detected in Qualitative chemical tests, and to know how many compounds are present (4). Phytochemical identification from extract by thin layer chromatography was performed as per the method. Briefly, the extract were drawn into capillary tubes and applied as spots on a stationary phase (Silica Gel 60 F254) about 1 cm from the base. The plates was then dipped into various mobile phase comparison (chloroform : methanol) and placed in a well covered tank. Chromatographic tank was saturated with mobile phase at room temperature for 5 min prior to development. After that the plates were removed, dried and processed for the identification of separated compounds (as colored spots) and the Rf values were calculated using the formula.

\[ Rf \text{ value } = \frac{\text{Distance moved by the compound}}{\text{Distance moved by the solvent front}} \]

3. Results and Discussion
To determine the presence of phytochemicals tested by thin layer chromatography (TLC), using stationary phase Silica Gel 60 F254 while mobile phase using various comparison chloroform and methanol. From the experiment shows the presence of alkaloids, saponins, tannins and cardiac glycosides, flavonoids, carbohydrates and steroids are present in ethanolic extract (5). Whereas methanolic extracts have been screened for qualitative determination of different secondary metabolites like starch, alkaloids, flavonoids, tannins, reducing sugars, amino acids and lignins (6). Amino acid, flavonoids, carbohydrates and phenols were found to be present in petroleum ether extract (7). Thinner layer chromatographic studies revealed that a comparison using more polar mobile phase is a mixture of chloroform : methanol with a ratio of 3 : 7, and more non polar mobile phase of chloroform and methanol with a ratio 8 : 2. By using non polar mobile phase phytochemicals retained on various retardation factor (Rf) after spraying vanillin - sulphate acid reagent, good separation showed marker compound in form of gray spot under UV light 254 nm at Rf value 0.47. While using more polar mobile phase, the phytochemicals retained in bad separation showed under UV light 254 nm. It means that the use of more non polar mobile phase can show better phytochemicals contained in Achyranthes aspera linn.
Figure 1. Phytochemicals qualitative measurement results with thin layer chromatography, gray bands showed the presence of phytochemicals. Eluent chloroform : methanol in ratio 8 : 2, after spraying vanillin - sulphate acid reagent.

Figure 2. TLC test of phytochemicals Achyranthes aspera, the mobile phase chloroform : methanol in ratio 8 : 2, showed under UV light 254 nm.
Figure 3. TLC identification of phytochemicals from whole plant extract of Achyranthes aspera. Eluent chloroform : methanol in ratio 3 : 7, showed at visible light.

Figure 4. TLC identification of phytochemicals from whole plant extract of Achyranthes aspera. Eluent chloroform : methanol in ratio 3 : 7, showed under UV light 254 nm.

4. Conclusion
Fast screening of the triterpenoids of methanol Sangketan extract using applicability of thin layer chromatography (TLC) showed that better separation with mobile phase chloroform : methanol (9 : 1 v/v). The bands were identified after spraying of vaniline - sulphuric acid reagent, the gray band with Rf value 0.53 was attributed as oleanolic acid for A. aspera.(8). Results of the analysis for the marker compounds of Sangketan in which TLC profile was obtained based on the Rf value. The gray band under UV light 254 nm at Rf value 0.47 was assossiated to triterpenoids for A. aspera. In this study, observation of these bands can be associated to oleanolic acid compound.

5. References
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