

PREPARATION OF THIN LAYER CHROMATOGRAPHY PROFILE OF EXTRACTS FROM *EUPHORBIA HIRTA*, LINN.

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Abstract - A thin layer chromatography profile of *Euphorbia hirta*, Linn., commonly known as "gatas-gatas" or "taua-taua", was prepared from extracts of fresh and dried plant materials and the profiles were compared.

TLC profiles were prepared using the following solvents: dichloromethane; hexane: ethyl acetate (8:2); hexane ethyl acetate (7:3); dichloromethane: methanol (10:1); ethyl acetate: methanol: acetic acid (18:1:1) and butanol: water: acetic acid (30:15:15). Comparison of the fresh and dried extracts showed that the fresh extracts had two distinct spots which were not found in the dried extract, while the dried extract gave an additional spot which was not present in the fresh extract. The TLC profiles therefore indicate that some chemical changes may have occurred in the leaf phytochemicals during the drying process.

INTRODUCTION

Herbal medicines are an important traditional remedy for many illnesses. Sophisticated drugs have been formulated and widely distributed all over the world but because they are expensive, herbal plants are continually used specially in rural areas. The Philippines is so rich with these medicinal plants that rural folks continue to use these herbal plants.

The Department of Health, with the help of Department of Science and Technology, has selected ten priority herbal plants to be studied for pharmaceutical use. More than 30 other plants are listed by the Department of Health as herbal plant and the list is likely to increase because there are many medicinal plants not yet known to the scientific world. However, these herbs still need to be studied for efficacy and safety.

One herb that is traditionally used for several illnesses is *Euphorbia pilulifera* / *Euphorbia hirta*, Linn. (Figure 1). Locally known as gatas-gatas or taua-taua, this plant is abundant in the Philippines in waste places and open grasslands. The herb is normally harvested taking the whole plant at its flowering stage. The decoction is prepared by boiling the whole plant for about 15 minutes



Figure 1. *Euphorbia hirta*, Linn..

and the decoction is given to the patient as a tea. The latex of the fresh plant is used for sore eyes and warts.

Early studies of the plant reported that the extract of gatas-gatas contains several components such as gallic acid, the flavonoid quercetin, a considerable amount of amorphous glycosidic material, a levorotatory sugar and a small amount of alkaloidal substance. Other components, such as a monohydric alcohol and a mixture of common fatty acids, chiefly palmitic and linolenic acids, were also identified

in the plant (Quisumbing, 1978). The flavonoid quercetin has been suggested to account for its antidiarrhial activity (Galvez, 1993), but other physiological activities of the plant have not been ascribed to any of the other components mentioned. Several studies have been done on the pharmacological action of *E. hirta*. A number of components have been isolated and identified, although their activities have not yet been established.

Thin layer chromatography (TLC), a simple, quick and a relatively low cost method, is employed to do qualitative analysis of herbal plants. The TLC profile of the plant extract may be used to identify the plant, a provisional method of studying the components pending the identification of the components (Dayrit, 2001).

This study was carried out to establish a TLC profile of the principal components of the *E. hirta* extract. The relative quantities of the components may be calculated using appropriate quantification software. The objectives of this study were:

1. To establish a TLC profile of the extract of fresh gatas-gatas plant and that of dried plant, and
2. To compare the TLC profiles and relative quantities of components obtained from fresh plant with that of the dried plant.

METHODOLOGY

Samples

Fresh samples were gathered and washed thoroughly with water and allowed to drain. The excess water was removed by a tissue paper and the leaves were dried in the air. When the leaves were sufficiently dry these were cut into small pieces (approx < 5 mm²) and were weighed in 1- and 3-gram quantities in an analytical balance. Samples were prepared in duplicate.

Preparation of Extracts

The weighed fresh and dried samples were each extracted with 10 mL methanol in a flask. The mouth of the flask was loosely covered with a funnel and warmed in a water bath at 40

°C for 2 h. The mixture was filtered through a fine mesh filter. The remaining solids were extracted with another two 10 mL portions. The filtrates in the three extractions were pooled together (BFAD, 2001).

The extracts were warmed in a 30°C water bath under vacuum and concentrated to 5 mL. These were then passed through a 5 cm column of silica gel and washed down with 20 mL methanol. The methanol was gently evaporated and then made up to a final volume of 5 mL using a graduated cylinder (BFAD, 2001).

Developing Solvents

Six developing solvents with different polarities were prepared: S1, dichloromethane; S2, hexane: ethyl acetate (8: 2); S3, hexane: ethyl acetate (7: 3); S4, dichloromethane: methanol (10: 1); S5, ethyl acetate: methanol: acetic acid (18: 1: 1); and S6, butanol: water: acetic acid (30: 15: 15).

Development of TLC Plate

Merck pre-coated TLC plates were used. The TLC plate was cut in the desired size and then activated in an oven for an hour at 105 °C. The plates were then allowed to cool to room temperature in a desiccator.

Dried and fresh extracts were spotted on TLC plates 1 cm apart together with an external reference compound (BFAD, 2001). The reference compounds used were cholesterol for the non-polar solvents (S1 to S4) and methyl orange for the polar solvents (S5 and S6).

Five µL were spotted on the TLC plates using a calibrated micropipette. Each extract and reference was spotted thrice. Developed plates were dried in the air and then visualized.

Visualization of Spots and documentation

TLC plates were first visualized in a UV chamber and the UV active components are noted. The plates were then sprayed with 1% vanillin in ethanol, allowed to dry, and then sprayed with 10% sulfuric acid in ethanol. The plates were then heated over a hot plate (about 110 °C) until spots give visible color (BFAD, 2001). Plates were scanned in a scanner for documentation. The images were saved using 256-color bitmap file (Dayrit, 2001).

RESULTS AND DISCUSSION**Profile of Extract from Dried Plant**

Six developing solvents gave the total profile of the *E. hirta* extracts. S1

(dichloromethane), the most non-polar solvent, showed 8 distinct spots (S1-1 to S1-8) with the corresponding R_f values; 0.86, 0.76, 0.68, 0.42, 0.35, 0.29, 0.18, 0.12 (Figure 2).

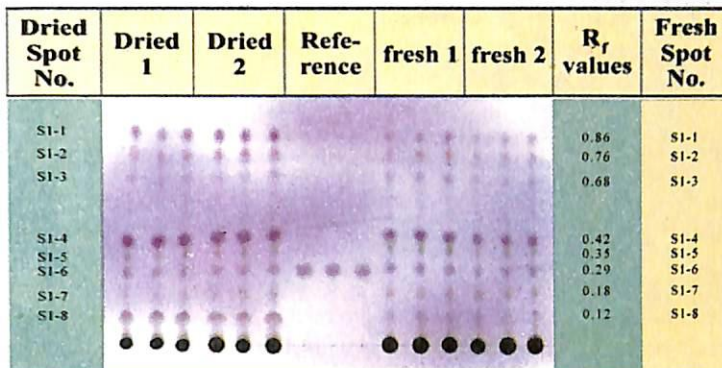


Figure 2. Solvent 1: Dichloromethane Reference compound: Cholesterol

S2 (Hexane: ethyl acetate, 8:2) showed similar profile but shows clearer separation of some overlapping spots in S1 and the

appearance of 2 new, very light spots, S2-8 at R_f 0.18 and S2-9 at R_f 0.11, the ninth component and tenth component (Figure 3).

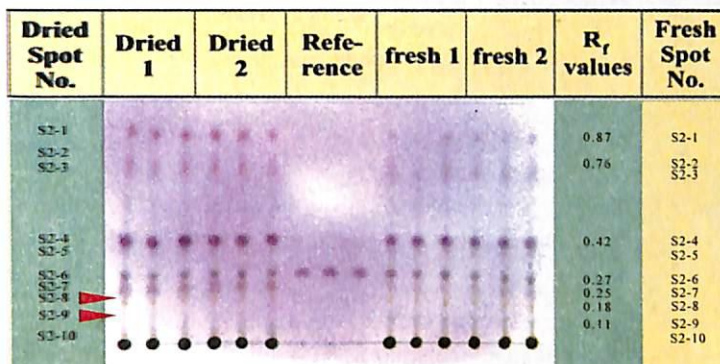


Figure 3. Solvent 2: Hexane: ethyl acetate (8:2) Reference compound: Cholesterol

An appearance of another spot, the eleventh spot (S3-8 at R_f 0.39) can be observed

in S3 (hexane: ethyl acetate 7:3) (Figure 4).

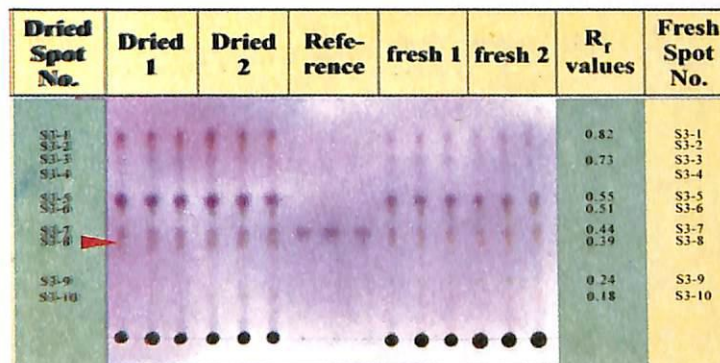


Figure 4. Solvent 3: Hexane: ethyl acetate (7:3) Reference compound: Cholesterol

A very light twelfth component, S4-5 at R_f 0.62 and another light spot, S4-7 at R_f 0.28, the thirteenth can be observed in S4 (dichloromethane: methanol, 10:1) (Figure 5).

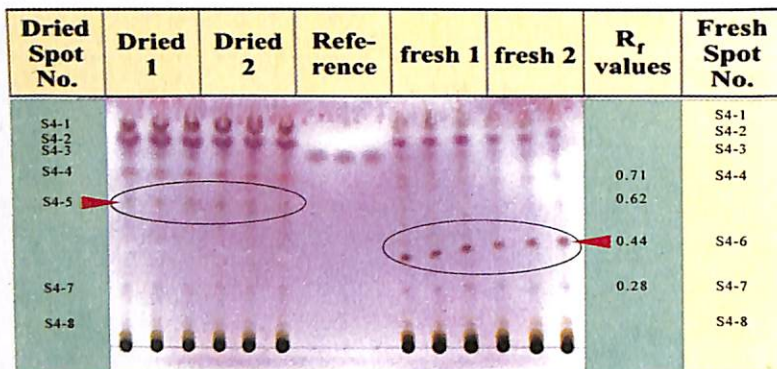


Figure 5. Solvent 4: Dichloromethane: methanol (10:1) Reference compound: Cholesterol

Two UV active spots (S5-6 and S5-7; R_f 0.39 and R_f 0.24) can be observed in the plate developed in S5, ethyl acetate: methanol: acetic acid (Figure 6).

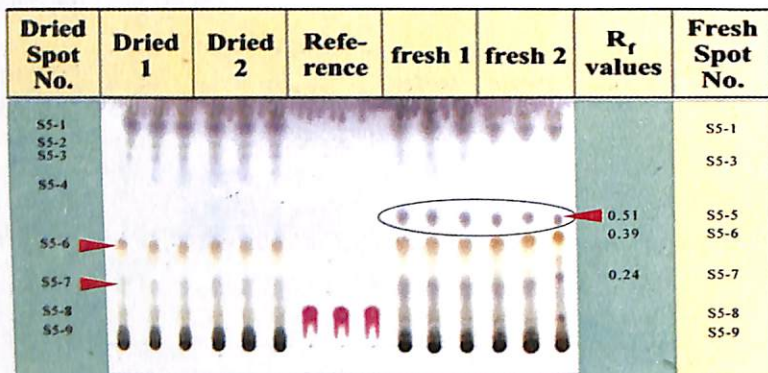


Figure 6. Solvent 5: Ethyl acetate: methanol: acetic acid (18:1:1) Reference compound: methyl orange

In the most polar solvent, S6 (butanol: water: acetic acid, 30:15:15) four UV active spots can be observed. A gray spot S6-1 at R_f 0.71, a brown spot S6-2 at R_f 0.62, a very dark gray spot S6-3 at R_f 0.48 and a very light spot right below S6-4 at R_f 0.44 (Figure 7).

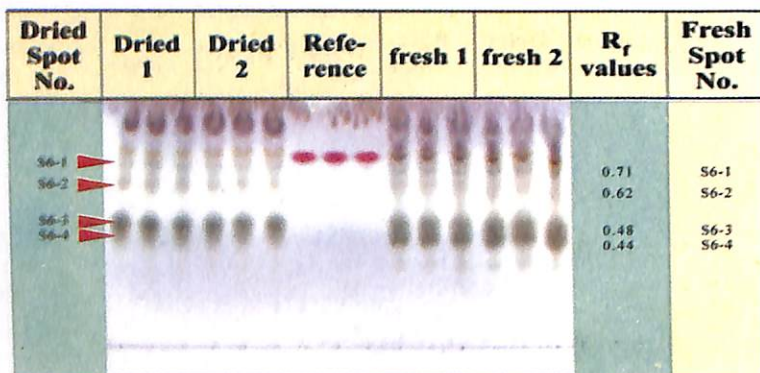


Figure 7. Solvent 6: Butanol: water: acetic acid (30:15:15) Reference compound: methyl orange

Nineteen distinct spots were observed on this plate; S1-1 to S1-8, S2-9, S2-10, S3-8, S4-5, S4-7, S5-6, S5-7 and S6-1 to S6-4.

Profile of Extract from Fresh Plant

The profile of the extract from the fresh sample is more or less similar to that of the dried. S1 showed eight spots with the same R_f values. S2 showed similar spots and 2 more spots, S2-8 at R_f 0.18 and S2-9 at R_f 0.11, components nine and ten. S3 showed similar spots but with one more spot, S3-10 R_f 0.18 as the eleventh component.

A spot, S4-6 at R_f of 0.44, which is the twelfth component, can be observed at S4, which is not found in the profile of the dry sample. A very light spot, S4-7 at R_f 0.28 counts as number thirteen component. In S5, a UV active spot S5-5 at R_f 0.51 can be observed, which is very light or nearly unobservable in the dried, the fourteenth component. S5-6 and S5-7 at R_f 0.39 and 0.24 serves as the fifteenth and sixteenth components.

S6 showed 4 spots (S6-1 to S6-4 at R_f values 0.71, 0.62, 0.48 and 0.44) completes 21 components.

Twenty distinct spots were observed in the profile of the fresh extract.

Comparison of the profiles of fresh and dried extracts

In general, the TLC profiles of the dried and the fresh extracts are nearly similar. Some differences can be observed in S2, S3 and S6. Spot S2-7 at R_f 0.25 appears lighter in the fresh and darker in the fresh. Spot S3-6 at R_f 0.39 appears to be darker and of different color in the dried than in the fresh. S3-7 at R_f 0.24 appears darker in the fresh but hardly noticeable in the dried. S6-4 at R_f 0.44 appears darker in the fresh than in the dried extracts.

Remarkable differences can be observed in S4 and S5. Spot S4-5 at R_f 0.62 is observed in the dried but not in the fresh. Spot S4-7 at R_f 0.44 is observed in the fresh but not in the dried. S5 shows spot S5-2 at R_f 0.51 which is distinct in the fresh but not found in the dried. With these, S5-3 and S5-4 at R_f 0.39 and 0.24 appears lighter in the dried than in the fresh.

These differences in the TLC profile of the dried and fresh extracts of gatas-gatas suggest that there might be chemical change taking place in the components when the plants undergo the drying process.

CONCLUSION

Six developing solvents gave the total profile of the dried and fresh *E. hirta* extracts following specific conditions to obtain a good profile. These solvents are; (S1, dichloromethane; S2, hexane: ethyl acetate, 8: 2, S3, hexane: ethyl acetate, 7:3; S4, dichloromethane: methanol, 10: 1; S5, ethyl acetate: methanol: acetic acid, 18: 1: 1; and S6, butanol: water: acetic acid 30: 15: 15. Using 1% vanillin in ethanol and 10% sulfuric acid in ethanol as spray reagent, the profile of the plant is visualized.

The dried extract showed 19 distinct spots while the fresh extract showed 20 distinct spots as accounted from the profile of the different solvents used. One component was present in the dried but not in the fresh. Two components were observed in the fresh sample, which was not present in the dried sample.

The difference in the TLC profiles of the fresh and dried materials suggests that some materials may undergo chemical change during the drying process. The TLC profile method may be useful for monitoring the identity and quality of fresh and dried gatas-gatas.

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