Tannin Identification of 4 Species Pterydophyta from Baluran National Park

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Abstract. Tannins are one polyphenol antioxidants which have higher molecular weight and having two ring aromatic group containing -OH. Antioxidant polyphenols in plants are potentially beneficial to human health as antibacterial, astringent, antidiarrheal, and antioxidant. The aim of this study was to identify tannins from 4 Pterydophyta species from Baluran National Park. This type of research is qualitative. The research sample consisted of rhizom and frond extracts of *Pseudocyclosorus ochthodes*, *Dyopteris hirtipes*, *Phymatodes sp.*, and *Pteris vittata*. The extraction process was carried out using maceration method using 96% methanol and phytochemical screening was tested followed by TLC test. The results showed all samples were positive tannins and their Rf range were 0.62-0.86.

1. Introduction
Tannins are one of the antioxidant polyphenols which have a higher molecular weight and have 2 aromatic rings containing -OH group. Tannin could classified to in 2 groups main : tannins that can hydrolyzed ( tannin hydrolyzed ) and tannin condensed [1].On reaction with acid or enzymes, tannins hydrolyzed broken to be compounds which are more simple, temporary tannin condensed generate the products which cannot dissolve in the water [2].

Hydrolyzed tannins consist of gallic acid and condensation products in dimer, hexahydroxydiphenic acid, esterified to polyols, especially glucose [3]. These metabolites can be oxidatively condensed into other galloyl or hexahydroxydiphenic molecules and form high molecular weight polymers. As the name suggests, this tannin is easily hydrolyzed by acids, alkalis and hot water and by enzymatic action, which produces polyhydric alcohols and phenylcarboxylic acids [4]. According to the nature of the latter, hydrolyzed tannins can be further divided into gallotannin, which comes from gallic acid, or ellagitannins, which are derived from hexahydroxydiphenic acid and which take their name from ellagic lactone acid. The most well-known hydrolyzed tannin is tannic acid (Figure 1), which is a gallotannin consisting of pentagalloyl glucose molecules which can then be esterified with five other gallic acid units [5].
Figure 1. Structure of tannin acids (Bravo, 2009)

Tanin condensed or proanthocyanidins is polymer with high weight molecule. The monomer unit is flavan-3-01 (catechin, epicatechin, etc.), with flavan-3 molecule, 4-diol orleucoanthocyanidin as the precursor. Condensation oxidative happen between carbon C-4 from heterocyclic and adjacent carbon C-6 or C-8 units [2].

Tannins as antioxidant antioxidants in plants are potentially beneficial to human health as antibacterial, astringent, anti-diarrhea, and antioxidants. So far the way natural polyphenols act as medicine and drugs is concerned, there is clear evidence that natural polyphenols have the potential to act in three predetermined general areas (eg, complex transition metal ions, as antioxidants in the state of cellular proxydants, and associate with proteins and peptides) [1]. Based on many effect positive from womb polyphenols, purpose main research this is analyze contain of polyphenols on rhizome and frond of 5 species plant Pterydophytes in National Parks Baluran with method screening phytochemicals and Thin Layer Chromatography (TLC).

2. Method
2.1 Location and Time Research
Research this held on Months from April to June 2018. Sample were from the National Park Baluran Situbondo, Java East. The examination contain research done in Materia Medica Stone and Herbarium Malangensis, University State of Malang.

2.2 Preparation Sample
Samples used in research this were rhizom and frond of Pterydophytes that have been identified in the Herbarium Malangensis University Malang. They are Pseudocyclosorus ochthodes, Dyopteris hirtipes, Phymatodes sp., and Pteris vittata. Sample dried aerate and smoothed with way blended or ground then extracted with solvent methanol 96%.

2.3 Extraction compound bioactive
Extraction sample use solvent 96% methanol for 24 hours. Extract filtered and evaporated use vacuum rotary evaporator to obtained pure extract. Extract obtained viscous saved in refrigerator with temperature ± 4°C.

2.4 Identification Tanin with method screening phytochemicals
2 ml of extract sample was added 20 ml of warm distilled water, then the filtrate was filtered and put into a test tube. Identification of gallotannins with added sodium acetate and 3 drops of 1% FeCl₃ and identification of catechol tannins by adding a small amount of 3% formaldehyde solution: concentrated HCl (4: 2) and heated at 90°C. Positive results of gallotannins are blackish brown, blackish blue, or blackish green. The positive results of catechol tannin are red deposits

2.5 Identification Tanin with method thin layer chromatography
2 grams of extract sample added 10 ml of ethanol P.A. The results are then filtered, take the filtrate and then put into a test tube. Then it was sprayed on a 20 x 20 60F254 silica gel plate along the plate at a distance of 1 cm from the bottom line and 1 cm from the edge. Then eluted using n-Butanol: Acetic Acid: Aquades with a ratio of 4: 1: 5. In the calculation of the Rf value is done by Camag TLC Scanner 3.
3. Results

The research sample of rhizom and frond extract *Pseudocyclosorus ochthodes, Dyopteris hirtipes, Phymatodes sp.*, and *Pteris vittata* showed positive results containing gallotannins and catechol tannins on phytochemical screening tests. These results can be seen in table 1.

| Sample Name                  | Gallotannin | Cathecol tannin |
|------------------------------|-------------|----------------|---|
| *Pseudocyclosorus ochthodes* frond | +           | +             |  |
| *Pseudocyclosorus ochthodes* rhizom   | +           | +             |  |
| *Dyopteris hirtipes* frond       | +           | +             |  |
| *Dyopteris hirtipes* Rhizome    | +           | +             |  |
| *Phymatodes* frond             | +           | +             |  |
| *Phymatodes* Rhizome           | +           | +             |  |
| *Pteris vittata* frond         | +           | +             |  |
Specific reagent such as FeCl₃ that mostly polar use so can interact with samples based on the principles of 'like dissolve like'. The formation of complex compounds between tannins and FeCl₃ because of the presence of Fe³⁺ ions as central atoms and tannins have O atoms have free electron pairs that can coordinate to the central atom as ligand. The Fe³⁺ ion in the reaction binds three tannins which have 2 donor atoms namely O atom at position 4 and 5' dihydroxy, so there are six free electron pairs which can be coordinated to the central atom. Atom O in position 4 and 5' dihydroxy has lowest energy in formation complex compounds, making it possible become a ligand [6]. The reaction between FeCl₃ and tannins can be seen in Figure 2.

![Figure 2. The reaction between FeCl₃ and tannins](image)

After a phytochemical screening test, the TLC test was continued. The results showed that all positive samples of catechol tannin and tannin tannin compounds and had a range of Rf 0.62-0.86. Table 3.2 shows the results of the TLC test.

| Sample Name                      | Result | Rf     | Peak Display                      |
|----------------------------------|--------|--------|-----------------------------------|
| Pseudocyclosorus ochthodes frond | +      | 0.62-0.71 | ![TLC Peak Display](image)       |
| Pseudocyclosorus ochthodes rhizome | +      | 0.68-0.80 | ![TLC Peak Display](image)       |
Dyopteris hirtipes frond + 0.65-0.77

Dyopteris hirtipes Rhizome + 0.62-0.71

Phymatodes frond + 0.62-0.81

Phymatodes Rhizome + 0.64-0.85

Pteris vittata frond + 0.62-0.75
4. Discussion

Based on the table 2, rhizome and fronds extracts from the *Pseudocyclosorrus ochthodes* (Kunze) Holtum, *Dryopteris hirtipes* (Bl.) Kuntze, *Phymatodes scolopendria* (Burm.) Ching, *Pteris vittata* L. showed positive tannin content indicated by Rf values including Rf range tannins based on acid eluents Acetate : Aquadest (4: 1: 5) which is 0.70 - 0.80. The lowest Rf range value is shown by *Pseudocyclosorus ochthodes* fronds (0.62-0.71) and *Dryopteris hirtipes* rhizome (0.62-0.71) while the highest Rf range is shown by *Pteris vittata* rhizome (0.63-0.86).

The presence of tannins in all samples is influenced by environmental factors that support the tannin biosynthesis pathway. The tannin biosynthesis pathway is included in the shikimic acid pathway. Pathway of shikimic acid, secondary metabolites synthesized through the acidic pathways include cinnamic acid, phenol, benzoic acid, lignin, tannins, benzoic amino acids and quinones. Shikimic acid pathway is alternative pathway to the aromatic compounds, particularly L-phenylalanine, L-tyrosine, and L-tryptophan. This pathway takes place in only in microorganisms and plants, not take place in the animal [7].

The tannin structure can be very complicated although most plant scientists may not be interested in the details of esoteric variations in tannin structure, knowledge of tannin biosynthesis can provide insight into differences in tannin content between their species and tissues, and ultimately their biological and evolutionary consequences. For example, typical plant cells do not produce GT and ET simultaneously, and efficient ET production must have direct negative effects on PA and flavonoid biosynthesis. For this reason both ET and PA do not accumulate to high concentrations in the same tissue and all of this can be inferred from the branches shown in the tannin biosynthesis pathway [8].

Factors affecting secondary metabolite production are (1) formulation / composition of culture media, (2) physical factors (temperature, light, humidity etc.), (3) genetic factors (cell genotypes), and (4) environmental stress factors (heavy metals, elicitor, UV light) [9]. Further research is needed to determine the factors that influence tannin production from this study.

5. Conclusion

The results of this study indicate that rhizom and frond of *Pseudocyclosorrus ochthodes* (Kunze) Holtum, *Dryopteris hirtipes* (Bl.) Kuntze, *Phymatodes scolopendria* (Burm.) Ching, *Pteris vittata* L. which was tested using phytochemical screening method and TLC showed positive results on tannin content which is influenced by environmental factors that support tannin biosynthesis.

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