Doctoral Dissertation

Study on the Chemical Constituents from *Tridax procumbens* L., *Piper cubeba* Bojer, and *Piper nigrum* L. in Indonesia and their Biological Activity

(Summary)

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Summary

The search for bioactive compounds with therapeutic properties to treat human diseases or to meet other needs for example weed control in agriculture is currently an important issue in many countries and laboratories. Plant tissues are natural matrices which have received the most attention to be exploited for their bioactive compounds because of the availability in nature. Compared with other ecosystems in the world, tropical ecosystem provides more plant species diversity that can be used as an alternative source to develop new drugs, nutraceuticals, functional foods, food additives or other products. Indonesia is a tropical country possessed 10 % of the world's flowering plant species (25,000 species) and to date, the information about biological activities and bioactive compound related to flowering plant species of Indonesia is still limited.

This study elaborates potent bioactive compounds and biological activities from three tropical plant species grown in Indonesia, namely *Tridax procumbens* L, *Piper cubeba* Bojer, and *Piper nigrum* L. Different fractions of *T. procumbens* and essential oils from *P. cubeba* and *P. nigrum* were evaluated for their allelopathic, antioxidant, antihyperuricemic, and antibacterial capacities. Several modern analytical instruments such as thin layer chromatography, column chromatography, high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-electrospray ionization-mass spectrophotometry (LC-ESI-MS) are employed to identify bioactive compounds from those three tropical plant species.

Chapter 1 primarily presents an overview of the dissertation. Background of the study, statement of research problems, objectives, scientific contribution of the study, and structure of the dissertation are explained in this chapter.

Chapter 2 describes the allelopathic activity and allelochemicals identification of *T. procumbens*. The methanol extract of *T. procumbens* was fractioned and assayed on germination and growth of radish (*Rhapanus sativus*). The F_1, fraction separated from ethyl acetate extract showed the strongest inhibition (IC_{50} = 0.22 mg/mL). This fraction induced physiological changes in radish seedlings by reducing the amount of chlorophylls (41.06%) and carotenoids

(35.44%) but increased the accumulation of malondialdehyde (MDA) (21.28%), a respond of cell-membrane on oxidative stresses. Twenty-six compounds were identified from the F_1 fraction by gas chromatography-mass spectrometry (GC-MS) to reveal the presence of 9-principal constituents (guaicol, benzenacettic acid, phenol benzenacetic acid methyl ester, and benzoic acid). Phenolic compounds were the major constituent followed by fatty acids and their derivatives. Dimethyl sulfone, a naturally-derived sulfur compound, was also detected in F_1 fraction. It is found that *T. procumbens* exhibited strong allelopathic activity against radish plant.

Chapter 3 explains the contribution of phenolic acids and dimethyl sulfone on the allelopathic capacity of *T. procumbens*. HPLC and GC-MS were used to identify and quantify phenolic acids and dimethyl sulfone in F_1 fraction of this plant. Contribution on the phytotoxic capacity of *T. procumbens* was presented as total activity of each compound. The results showed that vanillin was the dominant compound (364.689 µg/mg fraction) of F_1 fraction followed by benzoic, ellagic, and ferulic acids (69.888, 17.589, and 3.590 µg/mg fraction, respectively). Among phenolic acids and dimethyl sulfone assayed on germination and growth of radish, benzoic acid presented the highest inhibition (IC_{50} of germination = 5.148 mM). In total activity analysis, this compound also gave the major contribution in germination (0.09), root elongation (0.48), and shoot growth (0.26) inhibitions of radish seedlings. The contribution on radish growth inhibition of phenolic acids and dimethyl sulfone follows the order: benzoic acid > vanillin > dimethyl sulfone > ferulic acid. Thus, benzoic acid is suggested responsible for the phytotoxic effect of *T. procumbens.*

Chapter 4 aims to examine anti-hyperuricemic, antioxidant, and antibacterial activities of *T. procumbens*. Several fractions from ethyl acetate extract separated by column chromatography (CC) were assayed on xanthine oxidase (XO) inhibitory, antioxidant, and antibacterial activities. Among the tested fractions, the $F_{45–47}$ was the most active on XO inhibition (IC_{50} = 133.17 µg/mL), while the $F_{48–50}$ was the strongest on antiradical activity (DPPH and ABTS IC_{50} values = 0.51 and 1.04 mg/mL, respectively). In the antibacterial assay, the $F_{4–5}$ fraction presented the most effective inhibition against the growth of *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Proteus mirabilis*. The liquid chromatography-

electrospray ionization-mass spectrophotometry (LC-ESI-MS) and gas chromatography-mass spectrophotometry (GS-MS) analyses revealed that fatty acids, glycerides, and flavonoids were the main compound of the $F_{45–47}$ fraction. Whilst, glycerides, triose sugar alcohols, and fatty acids were the dominant component of the $F_{48–50}$ fraction and sterols were the major compounds of the $_{F4–5}$ fraction. It was found that *T. procumbens* had potent inhibitory effects to treat hyperuricemia, oxidative stress, and bacterial infection disease. The presence of fatty acids, flavonoids, and sterols in this plant may be correlated with biological activities of *T. procumbens*.

Chapter 5 aims to evaluate antioxidant, antihyperuricemic, and herbicidal effects of essential oils (EOs) from *Piper cubeba* Bojer and *Piper nigrum* L, two pepper species widely distributed in tropics. Identification of their chemical constituents was also provided. Hydro-distillation technique was employed to extract essential oil from dried berries of *P. cubeba* and *P. nigrum* and yielded of 1.23 and 1.11% dry weight of EOs, respectively. In the antioxidant assay, *P. cubeba* EO presented more effective inhibition against DPPH and ABTS free radicals than *P. nigrum* (28.69 and 24.13% greater respectively). *P. cubeba* EO also showed stronger inhibitory effects on xanthine oxidase (IC_{50} = 54.87 μg/mL) than *P. nigrum* EO (IC_{50} = 77.11 μg/mL). In the herbicidal assay, *P. cubeba* EO presented greater inhibition against *Bidens pilosa* and *Echinochloa crus-galli* germination and growth than *P. nigrum* EO. Photosynthetic pigments of *B. pilosa* and *E. crus-galli* were decreased 15.98–73.00%, while electrolyte leakages, lipid peroxidations, prolines, phenolics, and flavonoids contents were increased 10.82–80.82% caused by *P. cubeba* EO treatment at 1.93 mg/mL dose. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analyses revealed that *P. cubeba* and *P. nigrum* EOs mainly consisted of complex mixtures of monoterpenes and sesquiterpenes. Terpinen-4-ol (42.41%), α-copaene (20.04%), and γ-elemene (17.68%) were the dominant components of *P. cubeba* EO, while β-caryophyllene (51.12%) and β-thujene (20.58%) were the major components of *P. nigrum* EO.

Finally, Chapter 6 discusses the major findings and provides concluding remarks of this study. This study is successful to evaluate biological activities of *T.*

procumbens and essential oils of *P. cubeba* and *Piper nigrum* and to identify their chemical constituents. It is found that *T. procumbens* possessed allelopathic, antihyperuricemic, antioxidant, and antibacterial activities. The presence of phenolic acids and flavonoids may be responsible for allelopathic and antihyperuricemic of this plant. While fatty acids and sterols may contribute to antioxidant and antibacterial activities. In the case of *P. cubeba* and *P. nigrum* EOs, both of them exhibited antioxidant, antihyperuricemic, and herbicidal activities. The presence of monoterpenes and sesquiterpenes may be responsible for the biological activities of these EOs. Findings of this study suggest that *T. procumbens,* and *P. cubeba* and *P. nigrum* EOs are promising a source for drug development to treat oxidative stress, hyperuricemia, and bacterial infection diseases as well as weed control in agriculture.