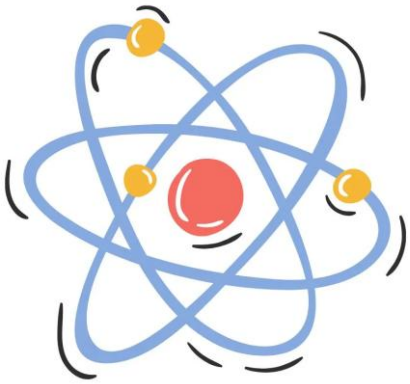

ISSN: 1672 - 6553

JOURNAL OF DYNAMICS AND CONTROL

VOLUME 9 ISSUE 5: 75 - 81

PHYTOCHEMICAL PROFILING OF *TINOSPORA RUMPHII* STEMS CULTIVATED IN VARYING SOIL TYPES

Pedro M. Gutierrez, Jr.

Department of Biology, College of Computing, Artificial Intelligence and Sciences, Cebu Normal University, Cebu City, Philippines

JOURNAL OF DYNAMICS AND CONTROL
DOI: https://doi.org/10.71058/jodac.v9i5007

PHYTOCHEMICAL PROFILING OF *Tinospora rumphii* STEMS CULTIVATED IN VARYING SOIL TYPES

Pedro M. Gutierrez, Jr.

Department of Biology, College of Computing, Artificial Intelligence and Sciences
Cebu Normal University, Cebu City, Philippines
Email: gutierrezp@cnu.edu.ph

Abstract: *This study aims to determine the effects of soil type on the phytochemical profiles (type and abundance) of Tinosphora rumphii stem ethanolic extract collected from Adtuyon Clay loam and hydrosol types of soil. The plant stem extract was concentrated using rotary evaporator until it is semi-solid in form. Phytochemical screening was done to evaluate the plant's phytochemical constituents. Results showed the presence of alkaloids, flavonoids, steroids, and tannins in the T. rumphii extract obtained from two sites with Adtuyon Clay loam and hydrosol types of soil. On the other hand, saponins are present only in the plant sample obtained in Adtuyon type of soil. Results also revealed variations on the abundance of some phytochemicals present in T. rumphii stem collected from the two types of soil in which alkaloids and tannins are less in abundant in Adtuyon clay loam soil while high in abundance in hydrosol type. Hydrosol type of soil is effective in storing alkaloids and tannins in T. rumphii stem could be due to the presence of iron and manganese in Hydrosol type of soil that are necessary for accumulation of such phytochemicals in the plant tissues. On the other hand, Adtuyon clay loam soil is valuable in storing saponins.*

Keywords: *Tinospora rumphii*, phytochemicals, adtuyon clay, hydrosol type, soil type.

Introduction

Tinospora rumphii belongs to the family Menispermaceae, is a climbing vine plant are commonly grown and matured in the wild places and can also be cultivated through artificial propagation like stem cuttings. It is commonly known as *Makabuhay* in the Philippines which literally means "bring backs life" (Salazar *et al*., 1987) . It is one of the folkloric medicinal plants that is widely distributed in Asia and Africa, has been used as herbal remedy for a long time (Ruan *et al*., 2012). It is commonly prescribed as an aqueous extract in the treatment of stomach trouble, indigestion, diarrhea, toothache, and topical ulcers (Tan and Bajo, 2014). Zulkhairi *et al*. (2008) stated that the aqueous crude stem extract of *T. rumphii* was not toxic on normal cell lines and moderately blocked the proliferation of selected human cancer cells.

It has been reported that phytochemicals which are considered as secondary metabolites components are directly responsible for activity such as antioxidant, antimicrobial, antifungal, anticancer, anti-inflammatory among others (Karate, 1997). Phytochemicals constitute one of the most numerous and widely distributed groups of substances in the plant kingdom (Chose and McArthur, 2002). The medicinal value of plants is related to their phytochemical component content and secondary metabolites, including: phenolic compounds, flavonoids, alkaloids, tannins, and other stress gene response products (Mohammedi and Atik, 2011). Phytochemicals can be extracted from either the whole plant or from specific parts of the plant known to contain a concentration of the desired active chemical. Concentrations can occur when bioactive chemicals accumulate in the various parts of the plant, such as leaves, stems, bark, flowers, fruits, seeds, and roots (Shaalan *et al*., 2005).

DOI: https://doi.org/10.71058/jodac.v9i5007

The distinction between primary and secondary metabolites is not always easily made. At the biosynthetic level, primary and secondary metabolites share many of the same intermediates and are derived from the same core metabolic pathways (Figure 1). In the strictest sense, however, secondary metabolites are not part of the essential molecular structure or function of the cell. Secondary metabolites generally, but not always, occur in relatively low quantities and their production may be widespread or restricted to particular families, genera, or even species. it has become increasingly evident that many natural products do have significant ecological functions, such as protection against microbial or insect attack (Hopkins and Huner, 2009).

Phytochemical composition of medicinal plants is known to be affected by a number of environmental factors including altitude and change of season (Nchabeleng *et al*., 2012; Jayanthy *et al*., 2013; Odjegba and Alokolaro, 2013). Effect of seasonal changes on the formation of plant secondary metabolites is influenced by responses to a variety of season specific pathogens (Figueiredo *et al*., 2008). This effect affords plants, unlike other organisms, to survive different seasonal conditions without hibernation. Seasonal changes expose plants to different temperature levels (including extreme levels) that have an effect in their phytochemical compositions, with volatile compounds being the most affected (Usano- Alemany *et al*., 2014). Determination of the seasonal effect on plant phytochemical compositions provides knowledge on the time/season of harvest of individual plant species that afford optimum concentration of active ingredients (Kale, 2010).

Monteiro *et al*. (2006) also stated that environmental factors, such as temperature, rainfall and ultraviolet radiation incidence can affect the concentrations of phenolic (such as flavonoids and tannins) compounds in plants. Among phenolic compounds, the tannins can be influenced by development of the plant and by environmental changes (Hatano *et al*., 1986; Salminen *et al*., 2001). Thus, phenolic compounds and other secondary metabolites represent a chemical interface between plants and environment (Gobbo-Neto & Lopes, 2007). Changes in phenols amounts influence directly the quality of the plant for medicinal application (Santos *et al*., 2006). On the other hand, there is no existing study or information available related to the effect of soil type on the phytochemical profile variations in Makabuhay, *Tinospora rumphii* plant stem especially in the two-study area (Carcar, Cebu, Philippines and Oroquieta City, Misamis Occidental, Philippines). Hence, this study was conducted to evaluate the effects of soil type on the phytochemical variations (type and abundance) of *Tinospora rumphii* stem extract.

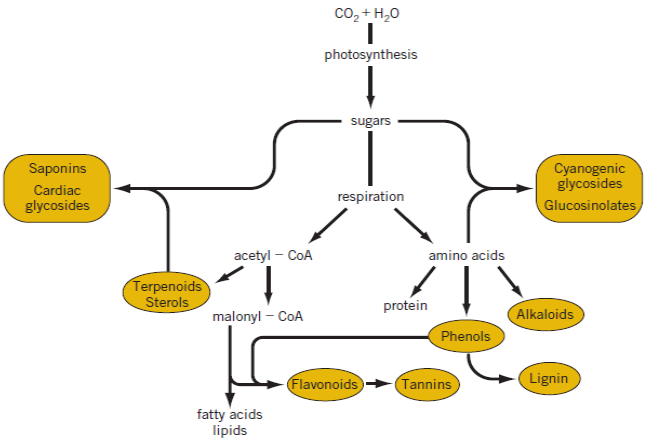

Figure 1. A schematic to illustrate biosynthetic relationships between principal primary and secondary metabolites (circled) (Source: Hopkins *et al*., 2009).

Materials and Methods

A. Collection and Preparation of Plant Samples

Tinospora rumphii mature stem was collected from the botanical garden of Misamis Occidental National High School, Oroquieta City, Philippines, with Adtuyon Clay loam type of soil and Carcar,

DOI: https://doi.org/10.71058/jodac.v9i5007

Cebu, Philippines, with hydrosol type of soil. These were washed with tap water and then rinsed with distilled water. The plant samples were air dried for 96 hours at room temperature. The dried stem of the said plant was chopped using a kitchen knife and pulverized using an electric blender.

B. Extraction of Plants

Pulverized plant samples were placed in a glass container. The stem samples extracted by soaking with concentrated (100%) laboratory grade ethanol and was left to stand for 48 hours. The soluble ethanolic extract was filtered using Whatman filter paper.

C. Rotary Evaporation and Phytochemical Analysis

The filtered ethanolic plant extract was then concentrated using rotary evaporator until it is semi-solid in form. The phytochemical screening was done following the standard procedure as described by Harborne (1998) evaluating the qualitative determination of major phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, steroids, anthraquinone, and cyanogenic glycosides.

Alkaloid

The detection of Alkaloids was carried out through extracting an equivalent of 10 grams of each plant extract that was recently evaporated on an evaporating dish to a syrupy consistency over a steam bath with 5 mL of 3 M HCl; and the filtrate was treated with Mayer's Wagner's reagent.

Flavonoids

The determination of flavonoids was done by taking another extract equivalent to 10 grams of the plant samples that was evaporated to incipient dryness over steam bath. Plant samples were then be added to a room temperature. The residue was defatted by treating it with 95% n-Hexane until the extract is almost colorless. Afterwards, the hexane extract was discarded. Its residue was taken up with 80% alcohol and finally added with hydrochloric acid. The appearance of color red within 10 minutes demonstrated positive test for flavonoids.

Tannins

The presence of tannins was determined through adding 10 grams of evaporated plant extract with 10 ml boiled distilled water, followed by the addition of 5 drops of 10% $FeCl_3$(ferric chloride) to the filtrate. Development of white precipitate was taken positive for the presence of tannins.

Saponins

Saponin content was confirmed by mixing the crude methanolic extracts with 10 mL distilled water. The extract was then shaken vigorously to record froth formation.

Steroids

The detection of steroids was done by extracting 20 grams of sample in 10 mL methanol. Five (5) mL of this methanolic extract was treated with 2 mL glacial acetic acid containing 1 drop of 5% $FeCl_3$solution. This solution was carefully transferred to the surface of 1 mL concentrated of H_2SO_4. The formation of reddish brown ring at the junction of two liquids was the indication of the presence of steroids.

Anthraquinone

Anthraquinone was detected by taking an amount of 10 grams of the plant samples and it was evaporated until it is almost dry over a steam bath. The residue was took up with 10 mL distilled water and filtered. The filtrate was extracted twice with 5 mL portions of benzene. Benzene extract was divided into portion, the other one served as control while the other was treated with 5 mL ammonia solution. The appearance of color pink in the lower alkaline layer indicated the presence of Anthraquinone.

Cyanogenic glycocides

Cyanogenic glycosides was tested through placing 2-5 grams of crushed plant material. It was moistened with enough distilled water and was then added with a few drops of chloroform to enhance enzyme activity. One (1) mL of 1 % emulsion solution was added to ensure hydrolysis of glycoside.

DOI: https://doi.org/10.71058/jodac.v9i5007

The test tubes was tightly covered with cork from which it was suspended with a piece of picrate paper that must not touch the inner side of the test tube. The tubes was warmed up at 35-40°C or kept at room temperature for three hours. Appearance of various shades of red within 15 minutes measures the relative concentration of cyanogenic glycoside.

Results and Discussion

Phytochemical screening of *Tinospora rumphii* stem extract obtained from two different types soil shows the presence of various phytochemicals which are shown in Table 1. The results provide evidence of the presence of alkaloids, flavonoids, steroids, and tannins in the *T. rumphii* extract obtained from two sites with Adtuyon Clay loam and hydrosol types of soil. On the other hand, saponins are present only in the plant sample obtained in Adtuyon type of soil. Results also revealed variations on the abundance of some phytochemicals present in *T. rumphii* stem collected from the two types of soil in which alkaloids and tannins are less in abundan (+) in Adtuyon clay loam soil while high in abundance (+++) in hydrosol type of soil.

Table 1: Phytochemicals present in the Ethanolic Extract of *Tinospora rumphii* obtained from Two Soil types.

Soil Type	**Phytochemicals**						
	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Anthra-quinone	Cyanogenic glycosides
Adtuyon Clay Loam	+	++	+++	+++	+	-	-
Hydrosol Type	+++	-	+++	+++	+++	-	-

Legend: (-) Absent; (+) Low in abundance; (++) Moderate in abundance; (+++) High in abundance

Based on the result, hydrosol type of soil is effective in storing alkaloids and tannins in *T. rumphii* stem, on the other hand, Adtuyon clay loam soil is valuable in storing saponins in the said plant extract. Hydrosol soil is rich in iron and manganese and influenced by seasonal or permanently wet conditions for at least 2-3 months in most years. Hydrosols commonly form in alluvial soil landscapes and so could be expected to be quite fertile. Plants with sufficient manganese has the phenolic compounds such as tannins accumulate in the plant tissues (Lin *et al*., 2005). Clay loam soil is composed mostly of sand, silt and smaller amount of clay. Stevens *et al.* (2013) stated that glycosides such as saponins are abundant in *Populus tremuloides* plant grown in sand and silt rich soil.

In general, the results of this study revealed that the type and abundance of phytochemicals present in the ethanolic extract of *T. rumphii* stem varied based on the type of soil. The chemical variability in the plant extract may reflect environmental influence on phytochemical contents.

Major outcomes of the present investigation revealed that the samples tested contained low to high concentrations of health-enhancing phytochemical constituents, including flavonoids, saponins, flavonoids, steroids and tannins. Flavonoids are strong antioxidants and are effective antibacterial substances *in vitro* against a large number of microorganisms by inhibition of the membrane-bound enzymes (Cowan, 1999). They also showed substantial anticarcinogenic and antimutagenic activities due to their antioxidant and anti-inflammatory properties and also they are an important class of natural products, are the main bioactive constituents of a lot of medicinal or dietary plants, they have been reported to show extensive benefits to human health, including antioxidant, anti-inflammatory, and anti-cancer activities (Li-Weber, 2009). Terpenoids are potent antioxidants, with anti-inflammatory, antibacterial, antiviral and anticancer properties (Lay *et al*., 2014). Alkaloids are toxic against cells of foreign organisms. Potential use in the elimination and reduction of human cancer cell lines. Saponins

DOI: https://doi.org/10.71058/jodac.v9i5007

are cancer protective agents acting as antioxidants, & antimutagens (Nobori *et al*., 1994) and also involved in plant disease resistance because of their anti-microbial activity (Anyasor *et al*., 2010). This plant also contain steroids, which are known to mediate cardiotonic activities and possess insecticidal and antimicrobial properties, while tannins, which are also found in these plants, are known to possess general antioxidant activities (Rievere *et al*., 2009). In addition, tannins are able to inactivate and kill microorganisms. They used in the treatment of varicose ulcers, hemorrhoids, minor burns, frostbite as well as inflammation of gums, in recent years, these compounds have demonstrated their antiviral diseases (Cowan, 1999).The saponins of extracts described here, are typically used in treatment of hypercholesterolemia, hyperglycemia, as antioxidants, anticancer antifungal, antibacterial, anti-inflammatory and in weight loss (Manjunatha, 2006).

Conclusion

The study revealed the presence of different phytochemicals such as alkaloids, flavonoids, steroids, and tannins in the *T. rumphii* extract obtained from two sites with Adtuyon Clay loam and Hydrosol types of soil. On the other hand, saponins are present only in the plant sample obtained in Adtuyon type of soil. Results also revealed variations on the abundance of some phytochemicals present in *T. rumphii* stem collected from the two types of soil. Based on the result, Hydrosol type of soil is effective in storing alkaloids and tannins in *T. rumphii* stem, on the other hand, Adtuyon clay loam soil is valuable in storing saponins in the said plant extract. Moreover, the ethanolic stem extract of *T. rumphii* showed availability of biologically active components which potentially of high significant pharmacologically importance. The ability of *T. rumphii* to store high amount of alkaloids and tannins could be due to the presence of iron and manganese in Hydrosol type of soil that are necessary for accumulation of such phytochemicals in the plant tissues.

References

Anyasor, G., KO Ogunwenmo, OA Oyelana *et al.* (2010). Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl (Costaceae). *Afr J Biotechnol*. 9(31): 4880-4884.

Chose, D. and C. McArthur. (2002). Rethinking the Role of Many Plants Phenolics Protection from Photodamage, Oikos, 999, 161-172

Cowan, M. (1999). Plant products as an antimicrobial agents. *Clin. Microbiol. Rev*. 12:564- 582.

Figueiredo, A., Barroso, J., Pedro, L., Scheffer, J. (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Fragr. J. 23:213-226.

Fernandez, T.Jr. (1996). Panyawan (*Tinospora rumphii*) as dewormer for goats, *Regional R and D [Research and Development] Symposia*

Guangqiu, Q., Chongling, Y., Haoliang, L. (2007). Influence of heavy metals on the carbohydrate and phenolics in mangrove, *Aegiceras corniculatum* L., seedlings. *Bull Environ Contam Toxicol 78*: 440-444.

Gutierrez, P. Jr., Antepuesto, A., Eugenio, B., Santos, M. (2014). Larvicidal Activity of Selected Plant Extracts against the Dengue vector, *Aedes aegypti* Mosquito. *International Research Journal of Biological Sciences.* Vol. 3(4), 23-32

Hatano, T., Kira, R., Yoshizaki, M., Okuda, T. (1986). Seasonal changes in the tannins of *Liquidanbar formosana* reflecting their biogenesis. *Phytochemistry 25*: 2787- 2789.

DOI: https://doi.org/10.71058/jodac.v9i5007

Hopkins, W., Huner, N. (2009). *Introduction to Plant Physiology*, 4th Edition. John Wiley and Sons, Inc., Hoboken, USA.

Jayanthy, A., Prakash, K., Remashree, A. (2013). Seasonal and Geographical Variations in Cellular Characters and Chemical contents in *Desmodium gangeticum* (L) DC. - An Ayurvedic. Medicinal plant. *Int. J. Herb. Med.* 1:34-37.

Kakate, K. C. (1997). *Practical pharmacognosy* .4th ed. Delhi: Vallabh Prakashan. p. 218.

Kouki, M., Manetas, Y. (2002). Resource availability affects differentially the levels of gallotannins and condensed tannins in *Ceratonia siliqua*. *Bioch Syst Ecol 30*: 631- 639.

Lay, M., Karsani, S., Mohajer, S., Nurestri, S., Malek, A. (2014). Phytochemical constituents, nutritional values, phenolics, flavonols, flavonoids, antioxidant and cytotoxicity studies on *Phaleria macrocarpa* (Scheff.) Boerl fruits. BMC *Comp Altern Med.* 14(152).

Li-Weber, M. (2009). New therapeutic aspects of Flavones: the anticancer properties of Scutellaria and its main active constituents wogonin, Baicalein and Baicalin. Cancer Treat Rev. 35: 57-68.

Lin, C., Chen, L., Lui, Z. (2005). Rapid effect of copper on lignin biosynthesis in soybean roats. *Plant Sci 168*: 855-861.

Manjunatha, B. (2006). Antibacterial activity of Pterocarpus santalinus. *Indian J Pharm Sci*. 68(1):115–6.

Mohammedi, Z., Atik, F. (2011). Impact of solvent extraction type on total polyphenols content and biological activity from *Tamarix aphylla* L. Karst. *Int J Pharm Bio Sci*.2(1):609–15.

Nchabeleng, L., Mudau, F., Mariga, I. (2012). Effects of chemical composition of wild bush tea (*Athrixia phylicoides* DC.) growing at locations differing in altitude, climate and edaphic factors. *J. Med. Plant Res*. 6:1662-1666.

Nobori, *et. al.* (1994). Deletion of the cyclin-dependent kinase-4 inhibitor gene inmultiple human cancers. *Nature*, 368(6473)**:**753-756

Odjegba, V., Alokaloro, A. (2013). Simulated Drought and Salinity Modulates the Production of Phytochemicals in *Acalypha wilkesiana*. J. Plant Stud. 2:105-112.

Rievere, C., Nguyen, V., Pieters, L., Dejaegher, B., Heyden, Y., and Minh, C. (2009). Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*. Phytochemistry. 70:86–94

Ruan, C., Lam, C, Chi, T., Lee, S., and Su, M. (2012). "Borapetoside C from *Tinospora crispa* improves insulin sensitivity in diabetic mice," *Phytomedicine*, vol. 19, no. 8-9, pp. 719–724,

Salazar, N., Sabordo, N., Romero, R., Montalban, C., Acacio, M., Tarrayo, M., Bustos, D. and Moragas, TR. (1987). "*Tinospora rumphii* Boerl. (Makabuhay) in the treatment of scabies", *Phil J. Microbial Infect Dis*, vol. 16, no. 1, pp. 25-29

Salminen, J., Ossipov, V., Haukioja, E., and Pihlaja, K. (2001). Seasonal variation in the content of hydrolysable tannins in leaves of *Betula pubescens*. *Phytochemistry 57*: 15-22.

Santos, S., Costa, W., Batista, F., Santos, L., Ferri, P., Ferreira, H., Seraphin, J. (2006). Seasonal variation tannins in barks of barbatimao. *Rev Bras Farmacogn 16*: 552- 556.

DOI: https://doi.org/10.71058/jodac.v9i5007

Treutter, D. (2010). Managing phenol contents in crop plants by phytochemical farming and breeding-visions and constraints. *Int J Mol Sci 11*: 807-857.

Zulkhairi, A., Abdah, M., Kamal, N., Nursakinah, I., Moklas, M., Hasnah, B., Fazali, F., Khairunnur, F., Kamilah, K., Zamree, M., and Shahidan, M. (2008). Biological properties of *Tinospora crispa* (Akar Patawali) and its antiproliferative activities on selected human cancer cell lines. *Malaysian Journal of Nutrition*, 14(2), 173-187.