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Potential of Secondary Metabolites Isolated From Clausena dentata in Endosulfan Degradation – An Innovative Approach

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Abstract: *In recent times the usage of plant secondary metabolites for degradation of pesticide is cheaper and safer to environment. The present paper mainly focused on isolation of secondary metabolites from Clausena dentata and its degradation potential of endosulfan pesticide. The significant degradation was found in saponins for both 1000 and 2000 µg/ml concentration of endosulfan with 1 and 2 ml of secondary metabolites with corresponding P value =< 0.0001. Amongst all secondary metabolites the saponins and terpenoids are degrade endosulfan efficiently.*

Keywords: *Secondary metabolites, Clausena dentata, endosulfan.*

I. Introduction

Plants produce diverse biochemical compounds which directly involve in metabolic roles known as primary metabolites, which do not involve in growth and development are known as secondary metabolites. There are number of definitions on secondary metabolites (Roze et al., 2011) but, they are classified into three main groups terpenes (Volatile compounds, Glycosides, carotenoids and sterols), Phenolics (coumarins, stilbenes, flavanoids, tannins, and lignin) and Nitrogen based compounds (alkaloids and glucosinolates) (Agostini-Costa et al., 2012). However, organic chemists have been working on these compounds since 1850's on separation techniques, spectroscopic approaches to understand their structure and function in a variety of ways (Croteau et al., 2000). Development of modern technologies like AAS, HPLC, GC and GC-MS added advantage in knowing these compound, which made a shift from non academic use of these compounds as dyes, polymers, fibers, glues, oils, waxes, flavouring agents, perfumes and drugs to academic functions as specific compounds for drug, antibiotics, insecticides and herbicides. Adding to this evolution the present paper attempts to identify degradation and detoxification of xenobiotic compounds like pesticides.

Clausena dentata is a flowering plant in the citrus family, Rutaceae. It was first defined by the Dutch botanist Nicolaas Laurens Burman in 1768 (Clausena, 1768). Genus Clausena is an evergreen trees, occur in tropical belt Asia (Burkill, 1966), easily grown by farmers since they are pest and disease free and can withstand heavy lopping (Swarbrick, 1997). Secondary metabolites of this plant was identified for number of biological activities like anti cancer, antimicrobial, antioxidant, antidiabetic, pesticidal etc. (Arbab et al., 2012).

Endosulfan is an organochlorine insecticide which was effectively used to increase agricultural productivity. The pesticidal activity of endosulfan has become a highly controversial agrichemical due to its potential in bioaccumulation, and as an endocrine disruptor which threatens human health and environment (Sreekumar and Prathapan, 2013). Inspite of its agreement on ban under the Stockholm Convention in April 2011 this is one of the important pesticides for many farmers.

II. Materials And Methods

The healthy leaves of Clausena dentata were collected from Kolli Hills, Namakkal, Tamil Nadu was washed thoroughly, shade dried at room temperature and powdered. Extraction was done using soxhlet apparatus using ethyl acetate. Crude extract from the solvent was separated using Rotary evaporator.

Determination Of Secondary Metabolites

The secondary metabolite Alkaloids of C. dentata were determined by Harborne., et al., 1973. The flavanoids were quantified according to Kumaran et al., 2006. Saponins determined by Obdoni., et al., 2001. The tannins were estimated by using the tannic acid as a standard with reference to (Makkar et al., 1993). By using the gallic acid as a standard the phenols present in the C. dentata were determined based on Mc Donald et al., 2001. Terpenoids were determined by Ferguson, 1956.

Endosulfan Standard Preparation

Stock solution of endosulfan (100mg/litre) was prepared in ethanol and working standard was prepared by appropriate dilution of the stock. 1ml of alcoholic potassium hydroxide (2%) was added to an aliquot of working standard of endosulfan along with 10ml of 0.1N hydrogen peroxide. 0.1ml of diphenyl amine (0.1%)

was added to give a light violet colour after liberating sulphur dioxide by oxidizing endosulfan to sulphate. The solution was kept aside for 5 minutes before taking absorbance and absorbance was measured at 605 nm against reagent blank. (Venugopal et al.2011).

Pesticide Degradation

Endosulfan degradation was estimated through addition different concentration of endosulfan (1000µg/ml, 2000 µg/ml) and different concentration of secondary metabolites such as alkaloids, flavanoids, terpenoids, saponins (1ml and 2ml) were added and incubate the tubes for 7 days in triplicates and OD value was taken at 590 nm. By using the endosulfan reference standard graph concentration of endosulfan in the treated and control samples were estimated.

III. Results

From the dried leaves of C. dentata the quantity of secondary metabolites (Alkaloids, Flavanoids, Saponins, Terpenoids, Phenolics and Tannins) estimated revealed the presence of high levels of Phenolics (25%) and low levels of Flavanoids (2%). Next to Phenolics the terpenoids (14%) present second highest compound followed by alkaloids (12%). Saponins (5%) and tannins (5%) are second least compounds present in C.dentata.

Endosulfan reference standard curve showed R^2=1 and Y=769.2+40, with these standard reference curve degradation of 1000µg/ml endosulfan by 1ml and 2ml of secondary metabolites alkaloids, flavanoids terpenoids and saponins showed significant degradation (Figure 1). Degradation amongst the different secondary metabolites showed highly significant with p values is < 0.0001 for both 1ml and 2ml. Endosulfan degradation by saponin is the highest and significantly different from all other secondary metabolites (P=<0.001) except terpenoids (P=<0.05). Flavanoids showed least degradation among different secondary metabolites (P=<0.05).

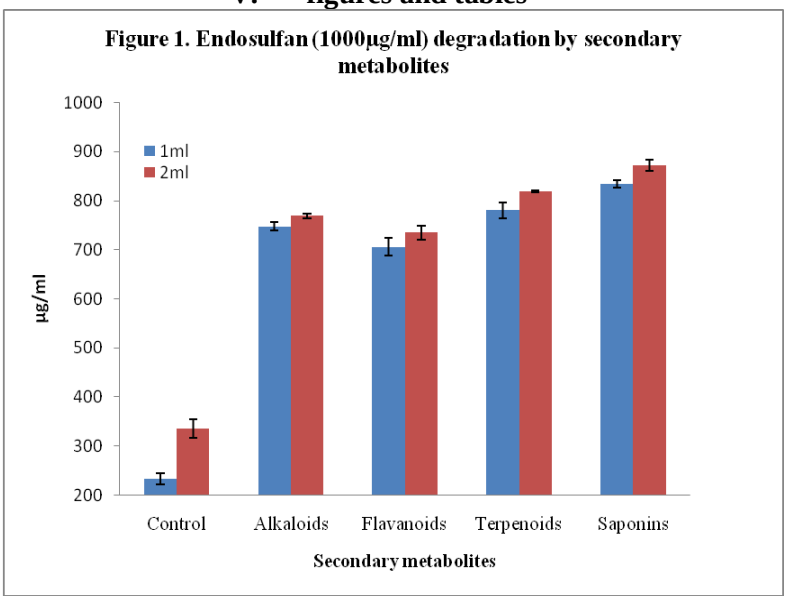
Degradation of 2000µg/ml endosulfan by 1ml and 2ml of secondary metabolites alkaloids, flavanoids terpenoids and saponins showed significant degradation (Figure 2). Degradation amongst the different secondary metabolites showed highly significant with 1ml and 2ml with p values is < 0.0002 and <0.0001 respectively. Endosulfan degradation by saponin is the highest and highly significantly different from all other secondary metabolites (P=<0.001) except terpenoids (P=<0.05) in both 1ml and 2ml. Alkaloids showed least among different secondary metabolites and distinctly not significant from all other metabolites (P=>0.05). The terpenoids and saponins were not significant with P value >0.05.

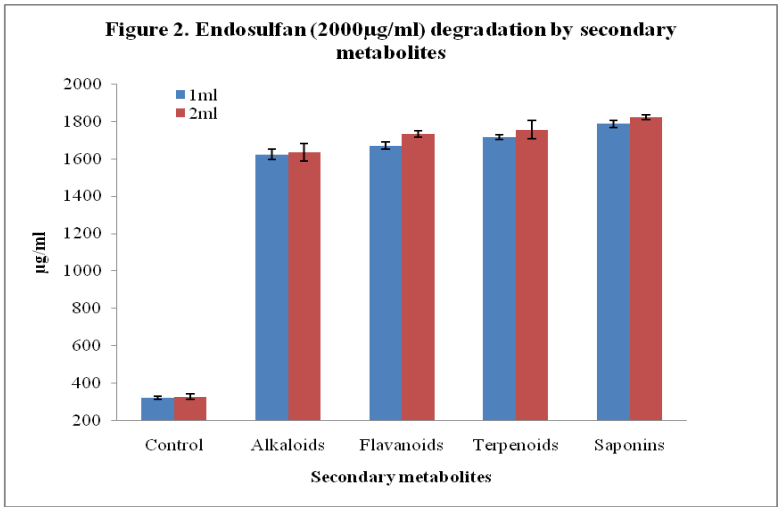
IV. Discussion

Development of science and technology lead to industrialization and decreased land to man ratio. Where a crises emerged throughout the globe, to feed the growing population with existed production potential of agricultural systems. As a result high yielding varieties and substitution of fertilizer and pesticides bloomed into alternative solution. But in the recent past indiscriminate use of fertilizer and pesticide lead to accumulation of pesticide in the soils and entering the food chain effecting the human population with dreadful ill effects like cancer, nervous disorder, genetic disorders, etc. Hence the present paper is relevant which attempts to degrade and dispose safer through secondary metabolites from Clausena dentata. Which was earlier reported to degrade endosulfan (Archaya, et al 2014; Subashini, et al., 2007; Malarvannan et al., 2008), but their mechanism was not explored completely, which said that microbes associate with the plant is responsible for degradation. However, C. dentata have been traditionally farmers using this plant biomass in their field which is surrounded by tapioca cultivation were pesticides are evident, but there was no incidence of pesticide pollution in the region.

Though secondary metabolites of C. dentata were isolated for a variety of uses (Govindarajan, 2009) its pesticide degradation potential has not been studied so far. However, it has been identified that the plant posses pesticide degradation potential (Archaya, 2014). Very few studies are available on secondary metabolites in pesticide degradation where terpenoids was identified by Li-Gen et al., (2008), alkaloids to degrade DDT by Khamid et al., (2012), lignin to degrade PCBs (polychlorinated biphenyls) and PAHs (polycyclic aromatic hydrocarbons) by Sung-Cheol Koh et al., (2000). The present plant showed high percentage of Phenolics (25%), followed by Terpenoids (14%) and Alkaloids (12%). Isolated secondary metabolites (Alkaloids, Flavanoids, Saponins, Terpenoids) showed significant endosulfan degradation with P value 0.0001 in both 1ml and 2ml. Among the different secondary metabolites saponins and terpenoids showed highest degradation in both 1ml and 2ml concentration with significant increase in degradation amongst other metabolites with P<0.001. Flavanoids and Alkaloids showed the least degradation in 1ml and 2ml concentration respectively and remained at non significant relation.

V. figures and tables





VI. Conclusion

Saponins are the second lowest amount of secondary metabolites present in C.dentata. Even though all of the secondary metabolites of C.dentata has a potential and significant degradation of endosulfan pesticide. From the estimated secondary metabolites the saponins are the major endosulfan degrading compound at 1000µg/ml and 2000µg/ml by 1ml and 2ml concentrations.

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