Structure modification of mefenamic acid and evaluate their bioactivities

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Introduction

Thousands of years medicine and natural products have been closely linked through the use of traditional medicines and natural poisons (Phillipson *et al*., 2001). Lately, pharmaceutical industries mainly pay their attention on natural products and their bioactivities to develop novel drugs which shows promising activities on diseases in humans as well as in plants. Since biological assays are used to detect the biological activity on synthetic compounds and natural products. There are several kinds of bioassays such as, antioxidant, antifungal, phytotoxicity, cytotoxicity and enzyme inhibitory activities (e.g. lipase, α-amylase, α-Glucosidase etc). Though there are several types of bioassays, most pharmaceutical manufacturing industries limited their research activities only towards one or few types of bioassays. There can be hidden bioactivities which are important in medicinally, agriculturally and environmentally. Therefore it is important to study about the other bioactivities too by doing the structure modifications of existing drugs and evaluate their bioactivities using various types of bioassays. By doing the modification of the exiting drug that can be enhanced the efficiency of the drug usage.

Mefenamic acid (MFA) which is used as a pain killer from many years ago. It is a Non-Steroidal Anti-Inflammatory Drug (NSAID) which exhibits poor water solubility properties (Dixit et al., 2012). MFA is a prescription medicine used for the relief of mild to moderate pain, including painful menstrual periods (Asl et al., 2008). There can be some hidden important bioactivities which were not studied yet in addition as a pain-killer. The aim of this study was to improve the usages of the drug for industrially, agriculturally and environmentally. Therefore the drug was synthetically modified by acetylation/acylation reaction methods.

Methodology

Pure MFA was separated from the tablet by using column chromatography technique. Separated pure MFA was subjected to acetylation and acylation reactions according to Arnold *et al*., 2007. Acylation was carried out with acid chlorides such as acetyl chloride (AC), benzoyl chloride (BC) and crotonoyl chloride (CC), while acetylation with acetic anhydride. Efficiency of the drug and its synthetic derivatives were tested by subjecting to bioassays such as α-amylase inhibition assay (Giancarlo et al., 2006), antioxidant assay (DPPH radical scavenging assay) (Mensor *et al*., 2001), phytotoxicty (bio-assay with lettuce seed germination) (Drewes *et al*., 1995) and cytotoxicity (brine shrimp lethality assay) assay using *Artemia salina* (Subbaraju *et al*., 2005).

The Inhibition percentage was plotted against the sample concentration and the fitted line plot was established. Then IC_{50}value (Inhibitory Concentration) was calculated in order to the 50% inhibition.

This would represent the concentration of sample (µg/ml) necessary to decrease the 50% activity of the particular sample.

Results and Discussion

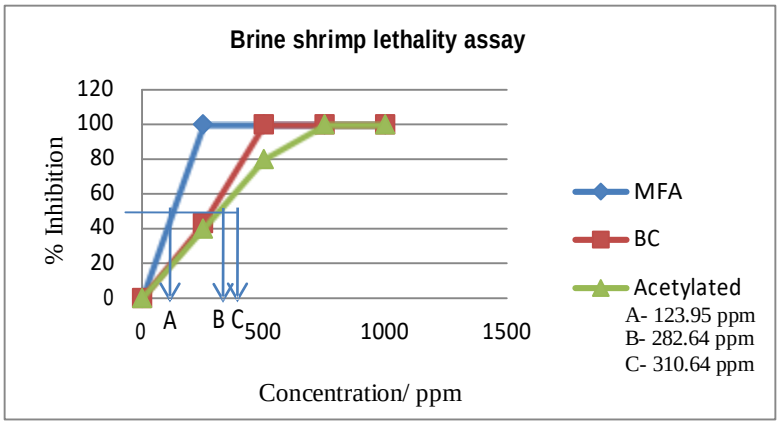

Figure 01: Inhibition of brine shrimp lethality assay

According to the figure 1 brine shrimp lethality assay shows positive inhibition activity in lower concentrations of all three samples (MFA, BC, Acetylated product). After performing the statistical analysis using one-way ANOVA and Tukeys test (P- value= 0.077 > α value) MFA sample shows significant toxicity effect than synthetic derivatives. Pure MFA was shown the lowest IC50 value than its derivatives (123.95 ppm).

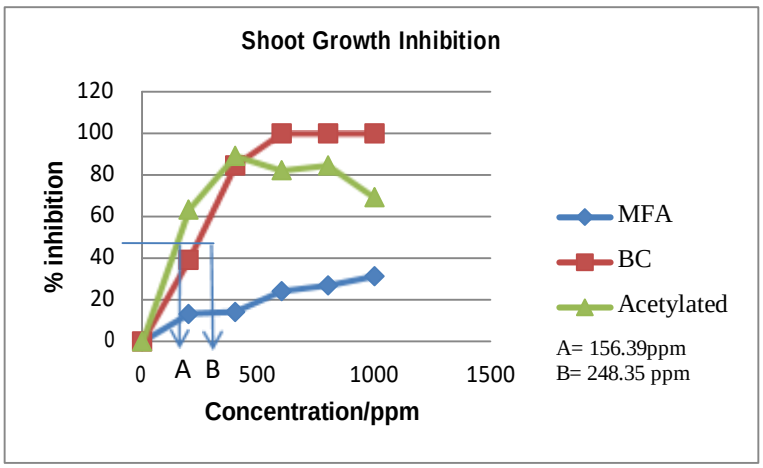

Figure 02: Shoot growth inhibition for phytotoxicity assay

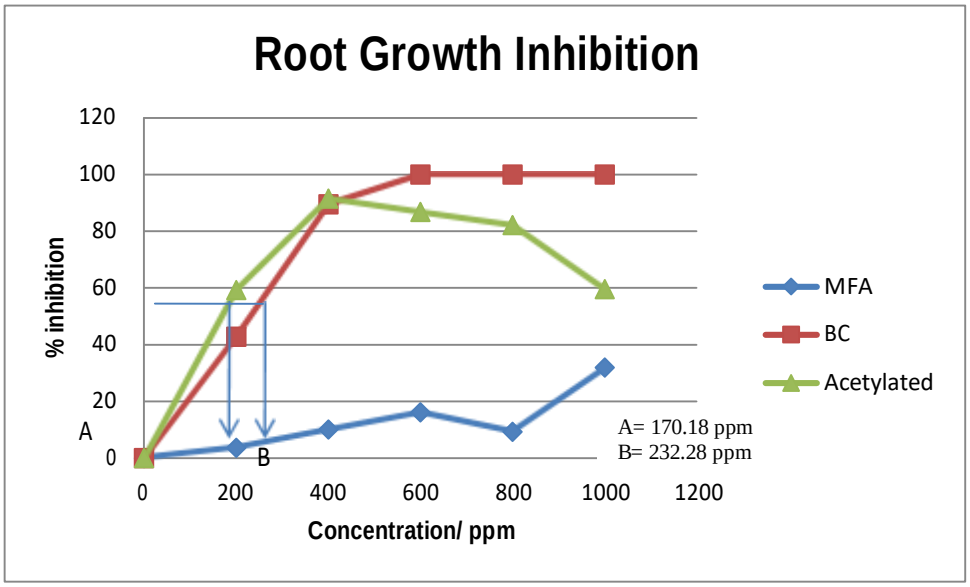

Figure 03: Root growth inhibition assay for phytotoxicity

IC50 values for phytotoxic assay for all 3 samples were observed in the figure 2 and 3. According to those two figures synthetic derivatives have shown better IC50 values than the pure MFA. All three samples were found to be non active for DPPH antioxidant activity (>1000 ppm) and α-amylase inhibition assay (765.53 ppm) when compared to the positive controls. Positive control for DPPH assay was ascorbic acid and acarbose was the positive control for amylase enzyme assay and its IC50 value was 4.16 ppm.

Conclusions

Pure MFA was shown the lowest IC50 value (123.95 ppm) in brine shrimp lethality assay. And also it was shown IC50 values for anti-oxidant activity, phytotoxicity activity for lettuce seeds and amylase enzyme inhibition activity in upper range. Synthetic derivatives of pure MFA (BC/Acetylated product) were shown lowest IC50 values for brine shrimp lethality assay and phytotoxicity assay. IC50 values in upper range of those derivatives were shown for the anti-oxidant activity. Pure MFA as well as its synthetic derivatives also were shown the lowest IC50 values for brine shrimp lethality assay. From one-way ANOVA and Tukeys test (P- value= 0.077 > α value) MFA sample shows significant toxicity effect than synthetic derivatives of the product for brine shrimp lethality assay. These interesting results lend further support to their traditional use. Synthetic derivatives (BC/Acetylated product) were shown the lowest IC50 values rather than Purified MFA in phytotoxicity assay. Because of that they are good for the use as inhibitors against dicotyldonae plants. They can be use in agricultural purposes such as weed-killers by conducting further analysis. Anti-oxidant activity of all three samples were shown significantly higher IC50 values (>1000 ppm) than the positive control. Although the IC50 values were much higher, pure MFA as well as its synthetic derivatives were shown anti-oxidant activity too. Since the purified MFA was showed the amylase enzyme inhibition activity, it can be said that MFA has good enzyme inhibition activity also. Then it can be check for the amylase enzyme inhibition activity of the synthetic derivatives too. It can be great benefit for the industrially as well as for pharmaceutical industries. So, by modifying the existing drugs they can be use for various purposes instead of the pharmaceutical usages. And also, by synthesizing various types of derivatives for the existing drugs and evaluate their activities using various types of bioassays, there can be enhance the utility of the drugs than the traditional usages.

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