International Journal of Natural and Engineering Sciences 3 (2): 23-27, 2009
ISSN: 1307-1149 www.nobel.gen.tr

Screening of Some Plant-Derived Alkaloids and Phenolics for Catechol-*O*-Methyltransferase Inhibition

Dilek YALÇIN Oğuz BAYRAKTAR*
Izmir Institute of Technology, Department of Chemical Engineering, Gulbahce Koyu, 35437, Urla-Izmir, Turkey

*Corresponding Author
e-mail: oguzbayraktar@iyte.edu.tr

Received: December 11, 2008
Accepted: January 15, 2009

Abstract:

The purpose of this study was to screen natural compounds extracted from plant species (*Peganum harmala, Pistacia terebinthus, Sarcopterium spinosum, Pistacia lentiscus, Hypericum empetrifolium*) as medicine alternates in order to investigate the inhibition characteristics on catechol-*O*-methyltransferase (COMT) activity. Enzyme activity and inhibition assays were performed flourometrically where S-adenosylmethionine (SAM) and Aesculetin (ES) were used as methyl donor and acceptor substrates, respectively. The K_m values of SAM and ES were determined as 3.5 ± 0.3 µM and 6.4 ± 0.4 µM in absence of inhibitor, respectively. Inhibition performances of the natural, plant derived polyphenolics and alkaloids were determined and found that alkaloids extracted from *P. harmala* seeds showed the strongest inhibition as well as 3,5-dinitrocatechol (3,5-DNC), positive control. Inhibition mechanism of alkaloids was defined with tight binding uncompetitive Michaelis-Menten approach like 3,5-DNC. However, the inhibition types of polyphenolic extracts were found as mixed type inhibitors according to the Michaelis plots their K_i values were determined.

Key words: Parkinson's Disease, catechol-*O*-methyltransferase, inhibition, alkaloids, phenolics

INTRODUCTION

Plants have been used as medicine against certain diseases for the human being. In the last century, the use of natural sources as medicine has expanded and there is a growing interest in obtaining biologically active compounds from natural sources. These compounds are classified as phytochemicals. Especially, ones including catecholic structures have antioxidant and free radical scavenging properties. Based on this knowledge and recent studies, it is known that these properties can also gain some neuroprotective features to the phytochemicals like catechols [1, 2, 3]. In administration of catechols, it is known that they are methylated by an enzyme known as Catechol-*O*-metyhltranferase (COMT; EC 2.1.1.6) that deactivates the biologically active catechols like catecholamine neurotransmitters (dopamine, epinephrine), levodopa (L-DOPA), carbidopa and flavonoids [4, 5].

It is known that increment in COMT activity leads to Parkinson's Disease [6]. In current therapies for Parkinson's disease, selective COMT inhibitors, mainly synthetic nitrocatechol compounds (entacapone, tolcapone), are used in combination with L-DOPA and dopa decarboxylase inhibitors, although there are several well known adverse side effects of these drugs [4, 5]. Therefore, researchers have focused on the potency of natural products having ability of inhibiting COMT. Among several natural sources, tea catechins have been mostly investigated compounds by several research groups and found that flavonoids in *C. sinensis* have great inhibitory potency against COMT [7, 4, 3]. In this study, the natural compounds extracted from the leaves of *P. terebinthus, S. spinosum, P. lentiscus, H. empetrifolium* and from the seeds of *P. harmala* were used as natural inhibitors of COMT in order to investigate the inactivation performance of methylation of aesculetin to scopoletin.

MATERIALS AND METHODS

Preparation of Raw Plant Materials and Extraction Procedure

The leaves of *P. terebinthus, S. spinosum, P. lentiscus* and *H. empetrifolium* were washed, dried and ground. The leaves of these two plants were extracted with 70 % aqueous ethanol solution at 30 °C for 2 hours. After evaporation of alcohol by using a rotary evaporator, the aqueous crude extracts were dried by using lyophilizator. The seeds of *P. harmala* were ground. The grounded seeds were extracted with methanol by using a Soxhlet apparatus at 70 °C for 8 hours. Then, methanol was evaporated to dryness. Dried particles were dissolved with 5% HCl solution and it was extracted two times with 30 ml petroleum ether, the acidic part was collected and basified to pH 9.0 with NH_4OH which was further extracted with 50 ml chloroform for four times. Finally, chloroform layer was collected and evaporated to dryness.

Determination of Enzyme Activity and Inhibition Study

Enzymatic assays were performed according to the method reported by J. Veser's and M. Kurkela et al's studies applying minor differences [8, 9]. The followed reaction which was catalyzed by COMT was conversion of aesculetin to scopoletin at 37°C. Aesculetin was dissolved in dimethyl sulfoxide (DMSO) and diluted with aqueous buffer solution containing 100 mM phosphate, 5 mM $MgCl_2$, 20 mM L-cysteine (pH 7.4), for a final DMSO concentration of 2% in the 200 µl of reaction mixture. All other reagents were dissolved in the same buffer solution. Fluorometric measurements were performed at 355 nm emission and 460 nm excitation wavelenghts for 2 hours by using Microfluor white, 96 well plate. Enzyme and aesculetin concentrations were kept constant at 11.0 µg/ml and 4 µM, respectively while five different SAM concentrations varied from 10 µM to 300 µM. 3,5-DNC and the crude extracts obtained from plant species were dissolved in DMSO and diluted with the buffer solution for a final DMSO concentration of 4%. Final

concentrations of them were 100 µg/ml, 50 µg/ml ve 20 µg/ml in 250 µl.

Curve Fitting and Data Analysis

In data analysis, GraphPad Prism 5.0 software was used. One-way of ANOVA following with Tukey's test was applied for all assay data. Linear regression and nonlinear regressions were used to obtain the best fitted double-reciprocal plots and michaelis plots.

RESULTS AND DISCUSSIONS

In inhibition study, SAM was prepared in between 10 – 300 µM range, while enzyme and aesculetin concentrations were taken constant at 11.0 µg/ml and 4 µM in 250 µl total reaction volume, respectively. Final inhibitor concentrations were 100 µg/ml, 50 µg/ml ve 20 µg/ml. A representative percentage values of COMT inhibition obtained for the assay performed at 100 µM SAM concentration were given on Figure 1. Inhibition performance of alkaloids were found as comparable to that of obtained for positive control, 3,5-DNC. On Figure 1, it was also revealed that among polyphenolic extracts, *P. terebinthus* and *P. lentiscus* leaf extracts showed higher inhibition than that of *S. spinosum* and *H. empetrifolium*. As a result, alkaloids were found to be more potent COMT inhibitors than the polyphenolics and it was thought that nitro groups content of them plays important role in COMT inhibition.

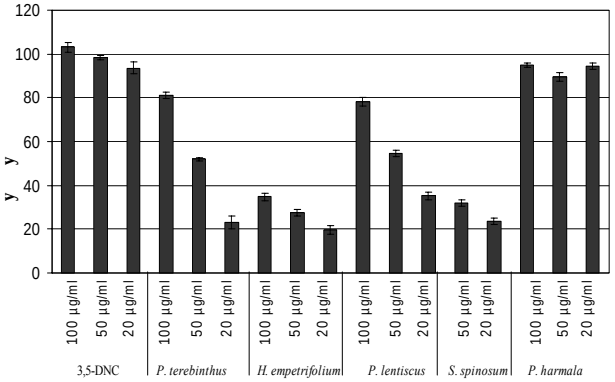


Figure 1. Inhibition percentages of crude extracts with respect to 3,5-DNC. (SAM concentration was 100 µM)

Kinetic Analysis for Inhibition Study

The inhibition mechanisms and also inhibition constants for crude extracts obtained from plants could be determined by kinetically analyzing data. A sudden and sharp decrease of formation rate of scopoletin, V (nmol/min. mg protein) was observed in presence of the positive control and alkaloid extract, expectedly. Because, almost all nitrocatechols are known as tight binding inhibitors of COMT and also they behave uncompetitively with respect to SAM [5]. It was thought that the structural similarities between the alkaloids and 3,5-DNC were very effective in achieving strong inhibitory activity. The kinetic data given on Figure 2 and Figure 3 was obtained by nonlinear fitting of data by using GraphPad software as uncompetitive inhibition. From this plot, the tight binding and uncompetitive nature of 3,5-DNC and alkaloid extracts were observed, however, under these concentration range of inhibitors, IC_{50} values and other kinetic parameters could not be identified but results revealed that the 50 % inhibitory concentrations should be much lower than 20 µg/ml [5].

The kinetic analysis results applied on data obtained for polyphenolic extracts showed different inhibition manner from that of obtained for 3,5-DNC and *P. harmala* alkaloids. In these cases, it was found that there is a inversely linear relationship between the formation rates of scopoletin and inhibitor concentrations. Rate obtained in presence of *P. terebinthus* leaf extract of which the michaelis plot was given on Figure 4A, was fitted nonlinearly by using GraphPad software in which regression equation was for mixed type inhibition model as given on Figure 4B.

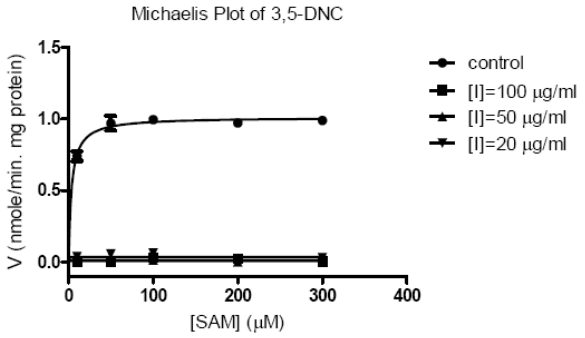


Figure 2. Plot of formation rate against various SAM concentrations in presence of 3 different 3,5-DNC concentrations ($P < 0.01$, means of inhibited ones are significantly different from control)

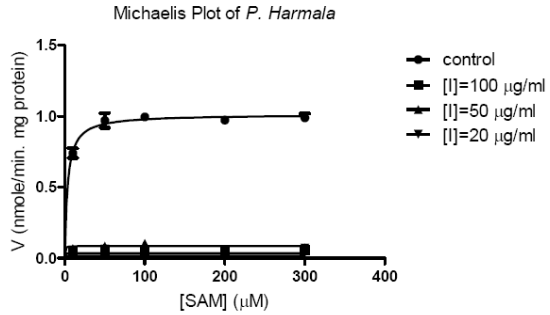


Figure 3. Plot of formation rate against various SAM concentrations in presence of 3 different concentrations of crude alkaloid extracts obtained from *P. harmala* seed ($P < 0.01$, means of inhibited ones are significantly different from control)

The kinetic parameters for this plant extract were calculated from the software and K_i value was found as 13.1 ± 4.7 µg/ml. The apparent K_m was calculated as 3.5 ± 1.0 µM with respect to SAM that indicates inhibition takes place noncompetitively with respect to SAM and competitively with respect to catecholic substrate, Aesculetin. It was found also reasonable that there can be a competition between the catecholic compounds with aesculetin due to phenolic and flavonoid content of *P. terebinthus* leaf extract like apigenine, luteoline, luteoline 7-O-glucoside and quercetin which were the examples of first generation COMT inhibitors [10].

The kinetic analysis of data obtained in presence of *S. spinosum* leaf extract revealed that this plant specie inhibited COMT as mixed type manner shown on Figure 5B. Also, from the nonlinear fitting of data given on Figure 5A, the

inhibition constant, K_i was found the highest among other polyphenolic extract having value of 103.5 ± 11.3 µg/ml. It was found that these β – carotene rich plant specie was not very effective for COMT inhibition as Pistacia species [11].

The observations obtained for the crude extracts of *P. lentiscus* leaves were found very similar to that of other *Pistacia* specie, *P. terebinthus* as expected. On Figure 6A and 6B, the kinetic analysis results were given for the extracts of *P. lentiscus* leaves.

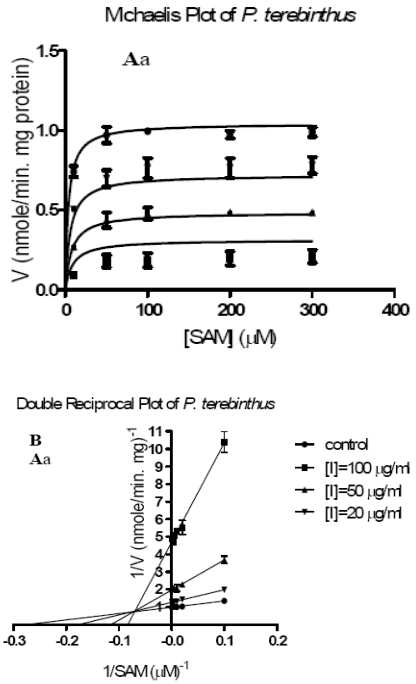
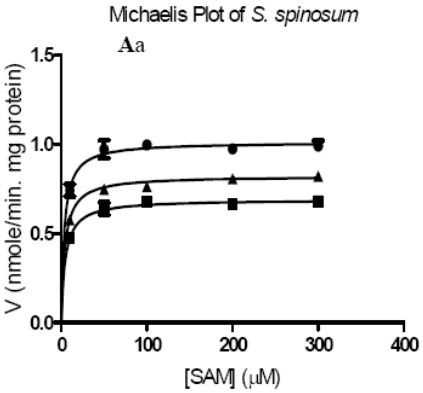


Figure 4. A) Plot of formation rate **B)** Lineweaver-Burke Plot (Double reciprocal plot) of formation rate against various SAM concentrations in presence of 3 different concentrations of crude extracts obtained from *P. terebinthus* leaves (Means obtained for different inhibitor concentrations are significantly different from each other and from control, (P<0.01))



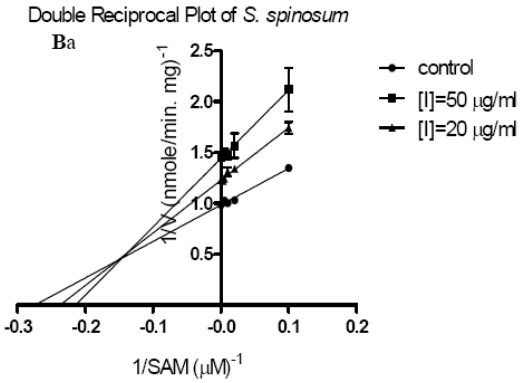


Figure 5. A) Plot of formation rate **B)** Lineweaver-Burke Plot (Double reciprocal plot) of formation rate against various SAM concentrations in presence of 3 different concentrations of crude extracts obtained from *S. spinosum* leaves (Means obtained for different inhibitor concentrations are significantly different from each other and from control, (P<0.01))

The nonlinearly fitted data (Figure 6A) obtained in presence of *P. lentiscus* leaf extract showed that the highest inhibitory effect on COMT activity with 12,7 ± 3,1 µg/ml of K_i value. It was thought that the same extracted compounds or analogs of them which are known as gallic acid derivatives, flavonol glycosides and anthocyanins existed in both Pistacia species were effective on the inhibition of COMT activity [12, 10]. However, their performances were found less than alkaloids of *P. harmala* seed. Additionally, the inhibition mechanism for the *P. lentiscus* leaf extract was determined as again mixed type model according to the Figure 6B.

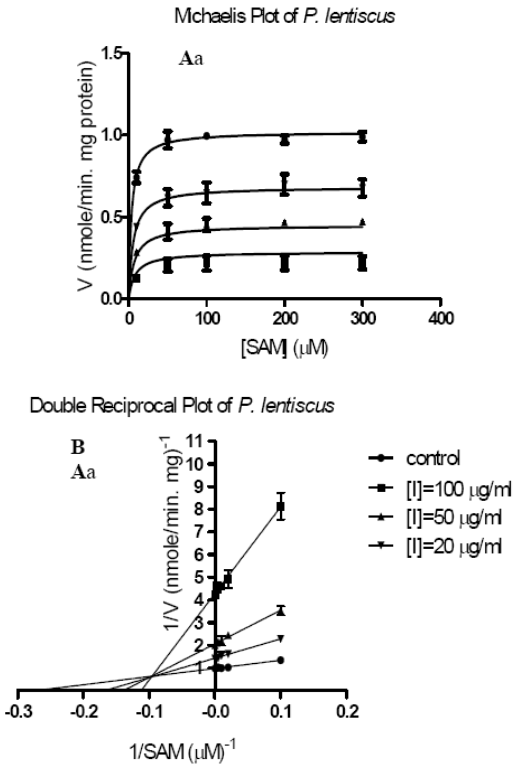


Figure 6. A) Plot of formation rate **B)** Lineweaver-Burke Plot (Double reciprocal plot) of formation rate against various SAM concentrations in presence of 3 different concentrations of crude extracts obtained from *P. lentiscus*

leaves (Means obtained for different inhibitor concentrations are significantly different from each other and from control, (P<0.01))

Similar calculations were also applied for the data obtained in presence of *H. empetrifolium* leaf extract and the same inhibition mechanism was observed with that of all polyphenolic extracts. On Figure 7A and 7B, the kinetic results were given and it was obvious that *H. empetrifolium* leaf extracts inhibited COMT in mixed type manner which means inhibition takes place noncompetitively with respect to SAM and competitively with respect to aesculetin. The K_i for this plant extract was determined as 41,8 ± 2,4 µg/ml which revealed that the inhibition performance of *H. empetrifolium* leaf extracts were higher than *S. spinosum* however, it was less than the other two *Pistacia* species.

CONCLUSION

The alkaloid extracts obtained from *P. harmala* seed were found as the most potent inhibitors and obviously, they showed the strongest inhibitory activity against COMT, but under these experimental conditions, IC_{50} values of them could not be determined but predictively, they should be in nanomolar range. Also, the inhibition type was determined as tight binding uncompetitive manner for the alkaloid extract as it was for 3,5-DNC.

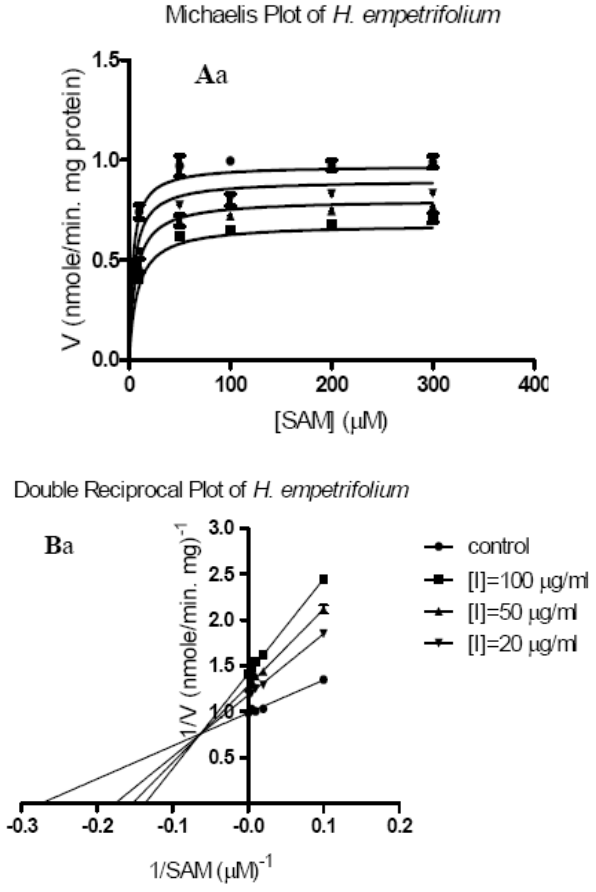


Figure 7. A) Plot of formation rate **B)** Lineweaver-Burke Plot (Double reciprocal plot) of formation rate against various SAM concentrations in presence of 3 different concentrations of crude extracts obtained from *H. empetrifolium* leaves (Means obtained for different inhibitor concentrations are significantly different from each other and from control, (P<0.01))

On the other hand, the polyphenolic extracts of all other plant species, *P. terebinthus, S. spinosum, P. lentiscus, H. empetrifolium* leaves, were found as less effective COMT inhibitors than alkaloids. Among these polyphenolics, the extract of *P. terebinthus* and *P. lentiscus* showed greater inhibitory effect than that of *H. empetrifolium*. The least inhibitory effect was obtained in presence of *S. spinosum* leaf extract. In addition to that, all of the polyphenolic extracts inhibited COMT in mixed type inhibition manner which means inhibition takes place noncompetitively with respect to SAM and competitively with respect to catecholic substrate, aesculetin. K_i values of *P. terebinthus, S. spinosum, P. lentiscus, H. empetrifolium* leaves were determined as 13.1 ± 4.7 µg/ml, 103.5 ± 11.3 µg/ml, 12.7 ± 3.1 µg/ml and 41.8 ± 2.4 µg/ml, respectively.

As a result of this study, it was observed that the second metabolites obtained from plants can be an alternative source of medicine in treatment of Parkinson's disease. Mainly, alkaloids from *P. harmala* and phenolics from *Pistacia* species were found to be worth to perform their further in vivo studies in order to investigate their pharmacotoxicological properties.

ACKNOWLEGDEMENTS

Support from the Turkish Scientific Research Council under Project MAG-107M307 and from the Izmir Institute of Technology under Project 2007IYTE04 is gratefully acknowledged.

REFERENCES

[1] Heath MC. 2002. In this issue: more secondary metabolites – phenolics. Physicol. Mol. Plant Pathol. 61:1-2.

[2] Duursen MBM, Sanderson JT, Jong PC, Kraaij M, Berg M. 2004. Phytochemicals Inhibit Catechol-*O*-Methyltransferase Activity in Cytosolic Fractions from Healthy Human Mammary Tissues: Implications for Catechol Estrogen-Induced DNA Damage. Toxicol. Sci. 81:316-324.

[3] Kadowaki M, Ootani E, Sugihara N, Furuno K. 2005. Inhibitory Effects of Catechin Gallates on *o*-Methyltranslation of Protocatechuic Acid in Rat Liver Cytosolic Preparations and Cultured Hepatocytes. Biol. Pharm. Bull. 28:1509-1513.

[4] Chen D, Wang CY, Lambert JD, Ai N, Welsh WJ, Yang CS. 2005. Inhibition of human liver catechol-*O*-methyltransferase by tea catechins and their metabolites: Structure–activity relationship and molecular-modeling studies. Biochem. Pharmacol. 69:1523-1531.

[5] Bonifacio MJ, Palma PN, Almeida L, Silva PS. 2007. Catechol-*O*-methyltransferase and Its Inhibitors in Parkinson's Disease. CNS Drug Reviews 13:352-379.

[6] Bonifacio MJ, Vieira-Coelho MA, Soares-da-Silva P. 2003. Kinetic inhibitory profile of BIA 3-202, a novel fast tight-binding, reversible and competitive catechol *O*-methyltransferase inhibitor. Eur. J. Pharmacol. 460:163-170.

[7] Weinreb O, Mandel S, Amit T, Youdim MBH. 2004. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. J. Nutr. Biochem. 15:506–516.

[8] Veser J. 1987. Kinetics and Inhibition Studies of Catechol *O*-Methyltransferase from the Yeast *Candida tropicalis*. J. Bacteriol. 169:3696-3700.

[9] Kurkela M, Siiskonen A, Finel M, Tammela P, Taskinen J, Vuorela P. 2004. Microplate screening assay to identify inhibitors of human catechol-*O*-methyltransferase. Anal. Biochem. 331:198-200.

[10] Topçu G, Ay M, Bilici A, Sarıkürkçü C, Öztürk M, Ulubelen A. 2007. A new flavone from antioxidant extracts of Pistacia *terebinthus*. Food Chem. 103:816–822.

[11] Barbosa-Filho JM, Alencar AA, Nunes XP, Tomaz ACA, Sena-Filho JG, Athayde-Filho PF, Silva MS, Vanderlei de Souza MF, Leitão da-Cunha EV. 2008. Sources of alpha-, beta-, gamma-, delta- and epsilon-carotenes: A twentieth century review. Braz J. Pharmacog. 18:135-154.

[12] Benhammou N, Atik Bekkara F, Kadifkova Panovska T. 2008. Antioxidant and antimicrobial activities of the *Pistacia lentiscus* and *Pistacia atlantica* extracts. Afr. J. Pharm. Pharmacol. 2:22-28.