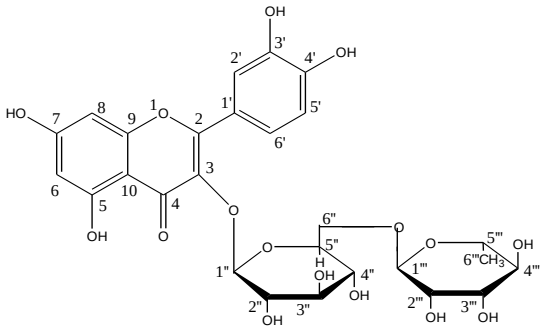
ABSTRACT

The present Ph.D. dissertation is composed of two parts. Part A deals with the isolation and structure elucidation of protease inhibitors from plant sources.

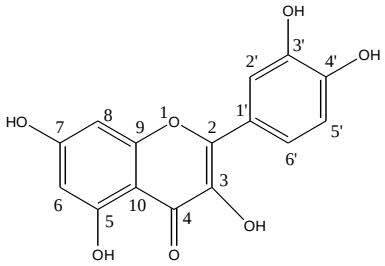
Twenty nine plants of nineteen families were preliminary screened against proteases, among which nine plants namely; *Citrus sinensis* (Family: Rutaceae), *Camellia sinensis* (family: Theaceae), *Cinnamomum zeylanicum* (Family: Lauraceae), *Impatiens bicolor* (Family: Balsaminaceae), *Datura innoxia* (Family: Solanaceae), *Melia azedarach* (Family: Meliaceae), *Artemisia roxburghiana* (Family: Asteraceae), *Colchicium luteum* (Family: Liliaceae) and *Adhatoda vasica* (Family: Acanthaceae), showed significant proteases inhibition activity. The active crude extracts, were further extracted with different solvents, using partition chromatography, and subjected to proteases inhibition assay. Bioassay guided fractionation of the active extracts, through column chromatography, followed by TLC and HPLC yielded; rutin (**1**), hesperetin (**2**), and hesperidin (**3**) from *Citrus sinensis*; catechin (**4**) and caffeine (**5**) from *Camellia sinensis*; caffeic acid (**6**) and cinnamic acid (**7**) from *Cinnamomum zeylanicum*; ferulic acid (**8**) from *Impatiens bicolor*; vanillin (**9**) from *Melia azedarach*; colchicine (**10**) from *Colchicium luteum*; hyoscine (**11**) from *Datura innoxia*; vasicine (**12**) from *Adhota vasica* and artemisinin (**13**) from *Artemisia roxburghiana*. The structures of all purified compounds, were determined through spectroscopic studies (UV, IR, HREIMS, FABMS, ^{1}H NMR, COSY 45^{o}, ^{13}C NMR, BB, DEPT, HMQC and HMBC), except cinnamic acid and ferulic acid, which were confirmed through X-ray analysis. The order of protease inhibition activity of the inhibitors was; rutin (**1**) > caffeic acid (**6**) > catechin (**4**) > hesperetin (**2**) > colchicines (**10**) > ferulic acid (**8**) > hesperidin (**3**) > cinnamic acid (**7**) > caffeine (**5**) > vasicine (**12**) > hyoscine (**11**) > artemisinin (**13**) > vanillin (**9**). Kinetic studies of **1**, **2**, **3**, **4**, **6** and **10** were carried out using GraFit 7.0 software. All tested compounds showed non-competitive inhibition with *Ki* values ranged from17.5±0.6 to 690±1.6 µM.

Part B of the thesis describes microbial transformation of some of the purified compounds.

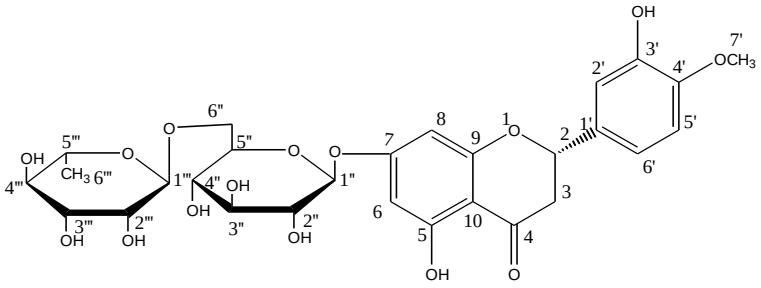
Rutin (**1**) was transformed to quercetin (**14**) in the culture of *S. griseus*, which enhanced the protease inhibition activity of compound **1** as compared to **14**. Colchicine (**10**) was transformed into demethyl colchicine (**15**) in the culture of *B. licheniformis*, while vanillin (**9**) and artemisinin (**13**) were converted into vanillyl alcohol (**16**) and 5-hydroxy artemisinin (**17**) respectively with *A. niger*. Vanillyl alcohol (**16**) and 5-hydroxy artemisinin (**17**) were more active than their parent molecules while protease inhibition activity of demethyl colchicine (**15**) was less than colchicine (**10**).



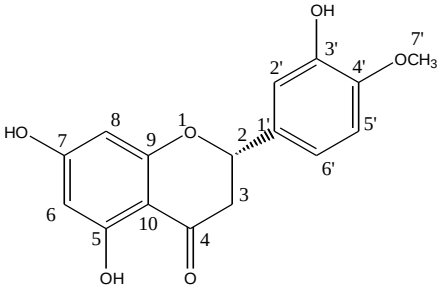
Rutin (**1**)



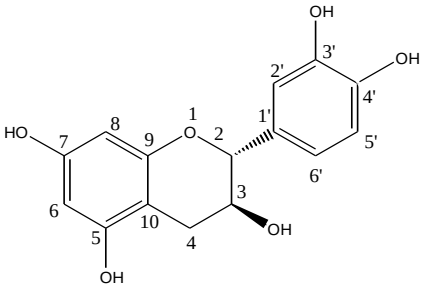
Quercetin (**14**)



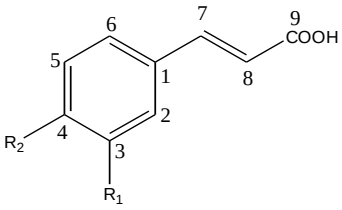
Hesperedin (**3**)



Hesperetin (**2**)



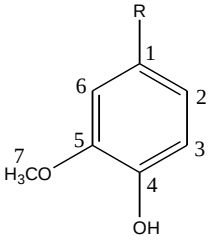
Catechin (**4**)



Caffeic acid (**6**) = R_1, R_2=OH

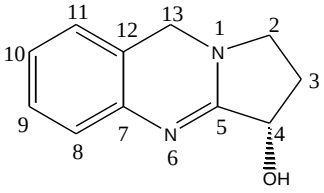
Cinnamic acid (**7**) = R_1, R_2=H

Ferulic acid (**8**) = R_1= OCH_3, R_2= OH

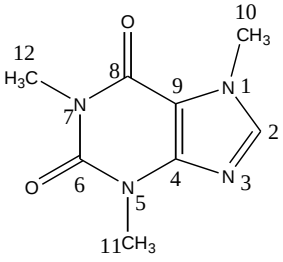


Vanillin (**9**) = R=CHO

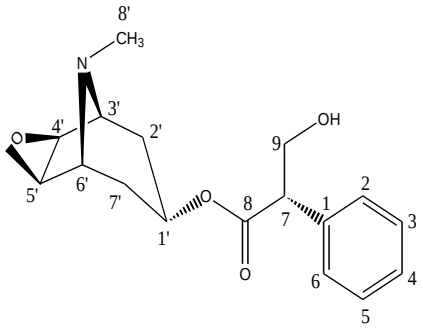
Vanillyl alcohol (**16**) = R = CH_2OH



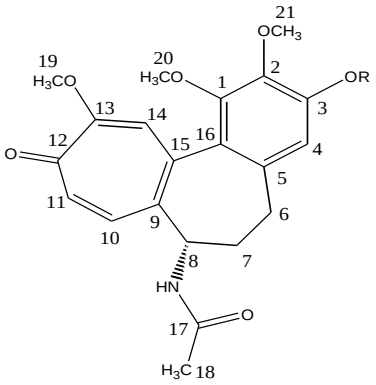
Vasicine (**12**)



Caffeine (**5**)

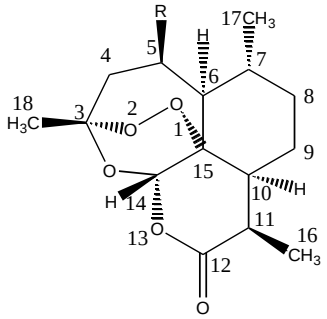


Hyoscine (**11**)



Colchicine (**10**) = R=CH_3

Demethyl colchicine (**15**) = R =H



Artemisinin (**13**) = R= H

5-hydroxy artemisinin (**17**) = R= OH