Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2021, 12, 297-308
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Effect of Antimicrobial Peptides and Chemicals Produced by Animals on Liver Microsomal Enzymes CYP450

Al-Baraa Akram, Glen McCann

Department of pharmaceutical biotechnology, De Montfort University, Leicester, UK
Email: albraaakram94@gmail.com

How to cite this paper: Akram, A.-B. and McCann, G. (2021) Effect of Antimicrobial Peptides and Chemicals Produced by Animals on Liver Microsomal Enzymes CYP450. *Advances in Bioscience and Biotechnology*, **12**, 297-308.
https://doi.org/10.4236/abb.2021.129019

Received: August 11, 2021
Accepted: September 25, 2021
Published: September 28, 2021

Copyright © 2021 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

The problem of multidrug-resistant pathogens as bacteria, fungi and yeast in addition to the restriction of using antibiotics as growth promoting substances in feed has attracted attention for looking for alternative sources instead of conventional antibiotics like natural products which have antimicrobial activity. Much interest and researches have been focused on using natural antimicrobial peptides and chemicals extracted from animal secretions and some insect's venoms as they exhibit antimicrobial activity against pathogens with lower resistance and higher synergistic effects if they were given in combination with blends of them. In this paper, some antibacterial peptides extracted from honeybees venom and expression with yeast *Saccharomyces cerevisiae* will be discussed as well as antimicrobial chemicals extracted from giraffes in addition to their inhibitory effects on liver microsomal enzymes CYP450 will be discussed also with its activity against microorganisms which are *Agrobacterium rhizogenes*, *Aspergillus niger*, *Candida albicans and E. coli* using optical density analysis technique then their minimum inhibitory concentrations (MIC) will be determined as well as ICs 50 to measure the potency to inhibit a biological function using programmes like Gene5, graph pad prism and clone manager as well as testing antimicrobial activity of some chemicals which are provided in animal secretions.

Keywords

Antimicrobials, CYP450, Antimicrobial Peptides

1. Introduction

1.1. Cytochrome P450 Enzymes

Cytochrome P450 groups are haem containing enzymes responsible for phase 1

oxidative metabolic reaction; they are not involved in electron transport (cytochrome b and c). A member of a complex family of microsomal enzymes (mono-oxygenases) is present in the endoplasmic reticulum that detoxifies compounds [1].

1.2. Peptides of Honeybees

There have been former study states that total ribonucleic acid has been transcribed from deoxyribonucleic acid from the venom glands of queen bees and cloned into PSTI website of inclusion body pPR322 [2] as there are extremely specialised cells manufacturing giant amounts of polypeptides and play a serious role in organic chemistry analysis; there are varied resources for purification specific mRNAs that had been used for learning macromolecule synthesis in cell free systems which had been thought of as a beginning material for constructing body DNA acid clones which might be considered as a begin purpose to purify and isolate genes. The synthesis of melittin is that the peptide of bee venom (**Figure 1**), consists of twenty-six amino acids [3] that this lytic peptide is isolated foremost from a primary precursor known as pre-pro-melittin through several pathway steps to rework it into pro-melittin then melittin using different enzymes (**Figure 1**).

The Giraffe produces these chemicals which might be detected in totally different ways; methylene chloride or DMSO of hair samples of male and female Giraffes were analysed by gas chromatography/mass spectroscopy that produces 2 chemicals which are indole and 3-methyl indole (**Figure 2**) which are powerfully accountable to the robust scent of the giraffe. Alternative chemical extracts are p-cresol (**Figure 2**), heptanal, octanal, nonanal, benzaldehyde, octane, hexadecenoic acid and tetra-decanoic acid.

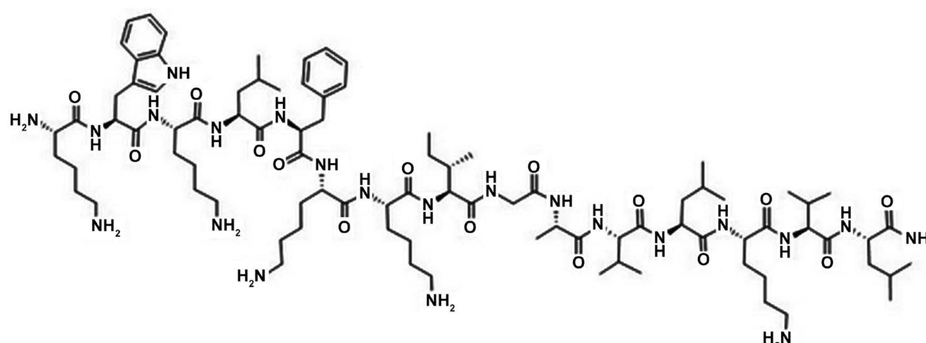

Figure 1. Chemical structure of melittin.

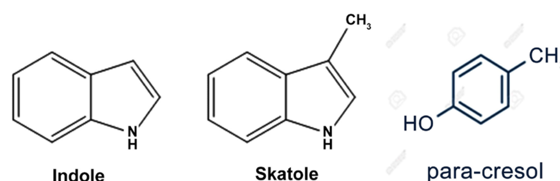

Figure 2. The chemical structure of main chemicals of giraffes' hair indole, skatole and p-cresol.

1.3. Cytochrome P450s Activities

Multiple drug medical care could be a difficult drawback in medication today that crystal rectifier to drug-drug interactions which are magnified hugely thus it's involved in drug analysis and development. Some medications were withdrawn from market as terfenadine and mibefradil thanks to drug-drug interactions [4] [5] thanks to metabolism by CYP450s that are thought of as the main route for drug elimination by the processes of metabolism and excretion.

Inhibition of CYP450s could be a contend at CYP active sites like prosthetic haem iron and substrate-binding region of CYP proteins and therefore the activity of CYPs enzymes may be resorted *in vivo* and *in vitro* when depletion. As irreversible inhibitors, most compounds inhibit CYPs act by irreversible binding or they're born-again by CYPs to an energetic matter foremost that bind irreversibly to the positioning at the centre of the accelerator resulting in protein activity permanent loss.

Irreversible loss of the protein activity by remodelling to an energetic matter is completed by CYP-catalysed reaction thus it may be thought of as mechanism-based inhibition and during this case, the reactive matter forms stably advanced with the haem molecule of CYP that is termed similar irreversible inhibition or modifies covalently CYP proteins irreversibly.

Generally, in each of these cases, the protein activity cannot be recovered by retreating the CYP substance from the reaction. In distinction reversible inhibition that the inactivation of CYPs by reactive matter results in future effects on the pharmacology of the medication consists of the processes of absorption, distribution, metabolism and excretion because the inactivated accelerators are replaced by new synthesized CYP enzyme.

Though the bulk of drug-drug interactions are happening by reversible inhibition, the irreversible inhibitors aren't most popular and are avoided in drug formulation and development. As they forever fail within the method of drug development thanks to toxicity and drug-drug interaction in addition as drug-food interaction considerations, it's necessary to grasp drug-drug interaction and therefore the irreversible inhibitors within the early stage of drug development before additional valuable resources are done to compounds that have less likelihood to pass to the hospitals or clinics.

Though a lot of serious issues are caused by irreversible inhibition of CYPs in drug formulation, differentiation between reversible and irreversible isn't the main issue for CYP screening approaches that the irreversible inhibition of CYP450 in mammals' granule enzymes are known liquid natural process (LC) or LC-MS/MS to analyse metabolites of the marker. This effortful and high-value assay limits the flexibility to handle compounds with high volumes and it's been a big obstacle for screening the irreversible inhibitors throughout drug development however recently, synthesis and drug screening of drug discovery led to high numbers of drug Candidates.

The requirement for higher ways to screen irreversible inhibitors became terribly clear, during this paper, we've investigated the flexibility and result of inhi-

bition between chemicals indole, alkyl group indole, p-cresol and mix of those 3 chemicals that are extracted from Giraffe hair victimisation DMSO on three forms of liver granule enzymes CYP1A1, CYP1A2 and CYP1B1 to tell apart between the result of every chemical one by one and screen the synergistic effect of their combination to dilute the nephrotoxic effect of p-cresol and cut back the antimicrobial resistance at constant time.

2. Materials and Methods

2.1. CYP450 Protocols

The typical parameters used for fluorescent substrates using microsomal P450s is by adjusting conditions' assays per 100 μL volumes, black 96 well plates and 100 mM potassium phosphate buffer with PH 7.4. Regenerating system is by frozen solution A and solution B

Regenerating system solution A stock is held by 65.42 mM magnesium chloride solution containing 26.13 mM $NADP^+$ and 65.77 mM of glucose-6-phosphate. In each well $NADP^+$ 1.3 mM, glucose-6-phosphate 3.3 mM in magnesium chloride buffer 3.3 mM.

Regenerating system solution B stock 5 mM tribasic sodium citrate contains 40 units per ml of glucose-6-phosphate dehydrogenase. In each well 0.04 units of glucose-6-phosphate dehydrogenase in buffer sodium citrate 50 μM.

General conditions:

1) Concentration of final well substrate is 2 to 15 μM;

2) Concentration of final well P450 volumes is 0.5 μL to 4 μL according to P450;

3) Concentration of final well of the solvent is aimed to keep at 0.5% but it can go towards 1%. Usually use acetonitrile/DMSO for substrates/inhibitor testing;

4) Keep the temperature of the reaction at 37˚C, 30˚C is good and acceptable;

5) These reactions are linear from 20 min to 1 hour according to P450 and set-up conditions;

6) Make the required multiplies of P450 mixture by diluting the required volume of a total volume of 50 μL in 100 mM potassium phosphate buffer PH 7.4 and then dispense 50 μL/well after that add master mix to every well.

Master mix standards (Table 1):

25 μL 200 mM potassium phosphate buffer PH 7.4 top up to give the final volume 50 μL of water:

5 μL solution A;

1 μL solution B.

Table 1. Assay parameters per reaction showing P450 families, name of substrate, final well substrate by μM, volumes of P450s μL/well and volume of 100 mM potassium phosphate μL.

P450	Substrate	Final well [Substrate] μM	Volume of P450s μL per well	Volume of 100 mM K phosphate μL
CYP1A1	EROD	5	1	49
CYP1A2	CEC	15	1	49
CYP1B1	EROD	5	1	49

Substrate is normally between 0.1 μL to 0.5 μL/well, normally fix the volume to avoid P450 solvent destruction.

2.2. CYP Inhibition

Studying CYP450 inhibition is very important because it measures the effect of the chemicals on the liver enzymes and screens the metabolism of liver function; it is also useful in studying drug-drug interactions, drug-food interactions, drug-excipient interactions and drug-gene interactions.

2.3. CYP1A2 Assays

Prepare blend of indole, skatole and p-cresol by taking 1/50 dilution of 100 mM Cresol, 1/250 dilution of 500 mM indole and 1/125 dilution if 250 mM of skatole to get mixture consists of:

20 μL of cresol in 980 μL of 10% DMSO; 10 μL in 2490 μL of 10% DMSO; 10 μL of skatole in 1240 μL of DMSO.

Prepare stock solution consists of CYP1A2 mixed with substrate CEC (3-cyano-7-ethocxycoumarin), 0.5 M with PH 7.4 of potassium phosphate buffer. Put the stock with cresol in the first three columns, indole in the next three columns, skatole in the next three columns and blend in the last three columns. Put DMSO (dimethyl sulfoxide) in all wells.

In a clear 96 well plate put cresol, indole, skatole and blend in the first four wells individually and as before Insert 200 μL of 2 mM of stock from the first raw and transfer from it 100 μL in 100 μL 10% DMSO in to the second raw then transfer 100 μL in 100 μL of DMSO in the third raw then transfer 20 μL from the third raw to the forth raw with 180 μL of DMSO, then transfer from it 100 μL in 100 μL in 10% DMSO to the fifth raw then transfer 100 μL in 100 μL of DMSO in the sixth raw then add 20 μL in 180 μL of 10% DMSO in the 7th raw and in the 8th raw transfer 100 μL in 200 μL DMSO.

In the last raw put 200 μL of DMSO as a positive control and add 0.5 M tris base acetonitrile as a killing and stop solution which is considered a negative control and consists of 80% acetonitrile and 20% tris OSM not PHed as the concentration of pure acetonitrile is 10 mM (**Table 2**).

Add substrate solution A which is buffer consists of NADPH glucose-6-phosphate magnesium chloride and enzyme solution B glucose-6-phosphate dehydrogenase tribasic sodium citrate to form master mix in a petri dish which consists of:

20 μL of CEC (cyano-7-ethocxycoumarin);

3250 μL potassium phosphate;

650 μL solution A;

130 μL solution B;

2450 μL distilled water.

Mix them gently and pipette 50 μL to each well in 96 black well plate then measure the rate of inhibition using gen5 software which will produce this (**Figure 3**).

Table 2. Scheme of the contents with concentrations of the 96 well plate showing their concentations.

Wells	Dilutions	1	2	3	4	5	6	7	8	9	10	11	12
			Cresol			Indole			Skatole			Blend	
A	Stock	100 µM	100 µM	100 µM	2000 µM	2000 µM	2000 µM	2000 µM	2000 µM	2000 µM	2000 µM	2000 µM	2000 µM
B	½	50 µM	50 µM	50 µM	1000 µM	1000 µM	1000 µM	1000 µM	1000 µM	1000 µM	1000 µM	1000 µM	1000 µM
C	½	25 µM	25 µM	25 µM	500 µM	500 µM	500 µM	500 µM	500 µM	500 µM	500 µM	500 µM	500 µM
D	1/10	2.5 µM	2.5 µM	2.5 µM	50 µM	50 µM	50 µM	50 µM	50 µM	50 µM	50 µM	50 µM	50 µM
E	1/2	1.25 µM	1.25 µM	1.25 µM	25 µM	25 µM	25 µM	25 µM	25 µM	25 µM	25 µM	25 µM	25 µM
F	1/2	0.625 µM	0.625 µM	0.625 µM	12.5 µM	12.5 µM	12.5 µM	12.5 µM	12.5 µM	12.5 µM	12.5 µM	12.5 µM	12.5 µM
G	1/10	62.5 nM	62.5 nM	62.5 nM	1.25 µM	1.25 µM	1.25 µM	1.25 µM	1.25 µM	1.25 µM	1.25 µM	1.25 µM	1.25 µM
H						200 µL DMSO						10 mM Acetonitrile	

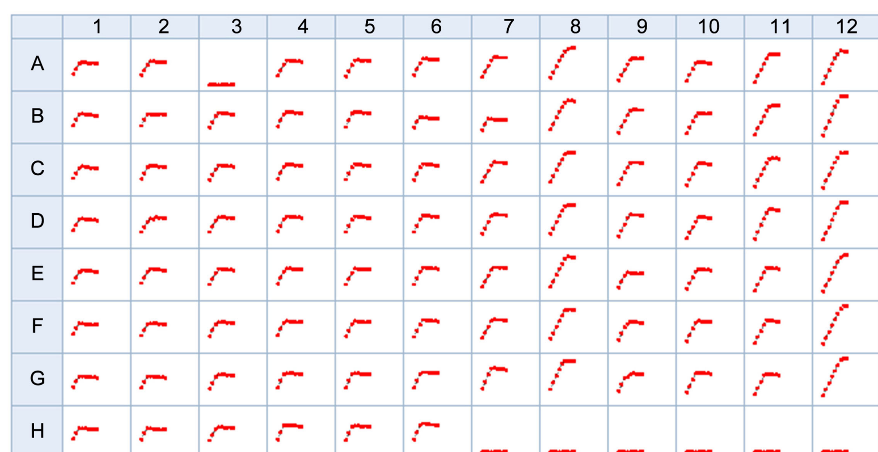

Figure 3. Explanation figure shows the inhibition grades of CYP1A1, distant points refer to low inhibition, closed or near points refer to high inhibition and flat point for negative controls resulted from using stop solution acetonitrile tris.

Transfer your data into excel sheet and divide data related to blend on 3 to obtain the inhibition effect of each chemical individually within the combination. Calculate the mean, standard deviation and standard error of the mean for both positive and negative control values. Calculate the percentage of inhibition by the law:

% of inhibition = 100 − ((amount of inhibition/mean value of the positive control) × 100).

2.4. CYP1A1 Assays

1) In a new clear 96 well plate put cresol, indole, skatole and blend in the first four wells individually and as before Insert 200 µL of 2 mM of stock from the first raw and transfer from it 100 µL in 100 µL 10% DMSO in to the second raw then transfer 100 µL in 100 µL of DMSO in the third raw then transfer 20 µL from the third raw to the forth raw with 180 µL of DMSO, then transfer from it 100 µL in 100 µL in 10% DMSO to the fifth raw then transfer 100 µL in 100 µL of DMSO in the sixth raw then add 20 µL in 180 µL of 10% DMSO in the 7th raw

and in the 8th raw transfer 100 µL in 200 µL DMSO.

2) In the next successive four wells dilute with 60 µL water and 80 µL of 200 mM KPI which is phosphate buffer consists of 0.6 ml of 1M K2HPO4 + 9.4 ml of 1 M KH2PO4 mixed and made up to 50 ml with sterile ultra-pure water. and pipette 20 µL cresol from the first column with them in the 6th column, 20 µL indole from second column in the 7th column, 20 µL skatole from the third column in the 8th column and 20 µL blend from the 4th column in 9th column (Table 3).

Prepare master mix from CYP 1A1 and KPI by the ratio 1:130.

1 µL CYP 1A1 * 130 = 130 µL;

9 µL KPI * 130 = 1170 µL.

In Eppendorf tube prepare CYP mix by mixing 1170 µL of 100 mM KPI and 130 µL of CYP 1A1 and pipette 10 µL in each well of a black 96 well plate.

Note: in each well add KPI first before addition of water.

In the black 96 well plate:

take 40 µL of the 6th clear column and transfer it into black columns 1, 2 and 3, take 40 µL of the 7th clear column and transfer it into black columns 4, 5 and 6, take 40 µL of the 8th clear column and transfer it into black columns 7, 8 and 9, take 40 µL of the 9th clear column and transfer it into black columns 10, 11 and 12.

In the last down 6 wells in the right pipette 75 µL of acetonitrile stop solution to potassium phosphate buffer and CYP1A1 as a negative control. Prepare master mix in a petri dish which consists of:

3250 µL 200 mM potassium phosphate buffer;

70 µL 1mM stock EROD substrate (7-ethoxyresorufin O-deethylation);

650 µL solution A;

130 µL solution B;

2405 µL distilled water.

Mix them gently and pipette 50 µL to each well in 96 black well plate then measure the rate of inhibition using gen5 software. Transfer your data into excel sheet and divide data related to blend on 3 to obtain the inhibition effect of each chemical individually within the combination. Calculate the mean, standard deviation and standard error of the mean for both positive and negative control values. Calculate the percentage of inhibition by the law:

Table 3. scheme of the contents of the clear 96 well plate showing their diluents.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cresol	Indole	Skatole	Blend		KPI+ water+ cresol	KPI+ water+ indole	KPI+ water+ skatole	KPI+ water+ blend			
B	cresol +DMSO	Indole +DMSO	Skatole +DMSO	Blend +DMSO		KPI+ water+ cresol	KPI+ water+ indole	KPI+ water+ skatole	KPI+ water+ blend			
C	cresol +DMSO	Indole +DMSO	Skatole +DMSO	Blend +DMSO		KPI+ water+ cresol	KPI+ water+ indole	KPI+ water+ skatole	KPI+ water+ blend			
D	cresol +DMSO	Indole +DMSO	Skatole +DMSO	Blend +DMSO		KPI+ water+ cresol	KPI+ water+ indole	KPI+ water+ skatole	KPI+ water+ blend			
E	cresol +DMSO	Indole +DMSO	Skatole +DMSO	Blend +DMSO		KPI+ water+ cresol	KPI+ water+ indole	KPI+ water+ skatole	KPI+ water+ blend			
F	cresol +DMSO	Indole +DMSO	Skatole +DMSO	Blend +DMSO		KPI+ water+ cresol	KPI+ water+ indole	KPI+ water+ skatole	KPI+ water+ blend			
G												
H												

% of inhibition = 100 − ((amount of inhibition/mean value of the positive control) × 100).

2.5. CYP1B1 Assays

In a new clear 96 well plate put cresol, indole, skatole and blend in the first four wells individually and as before Insert 200 μL of 2 mM of stock from the first raw and transfer from it 100 μL in 100 μL 10% DMSO in to the second raw then transfer 100 μL in 100 μL of DMSO in the third raw then transfer 20 μL from the third raw to the forth raw with 180 μL of DMSO, then transfer from it 100 μL in 100 μL in 10% DMSO to the fifth raw then transfer 100 μL in 100 μL of DMSO in the sixth raw then add 20 μL in 180 μL of 10% DMSO in the 7th raw and in the 8th raw transfer 100 μL in 200 μL DMSO.

In the next successive four wells dilute with 60 μL water and 80 μL of 200 mM KPI and pipette 20 μL cresol from the first column with them in the 6th column, 20 μL indole from second column in the 7th column, 20 μL skatole from the third column in the 8th column and 20 μL blend from the 4th column in 9th column.

Prepare master mix from CYP 1B1 and KPI by the ratio 1:130.

1 μL CYP 1B1 * 130 = 130 μL;

9 μL KPI * 130 = 1170 μL.

In Eppendorf tube prepare CYP mix by mixing 1170 μL of 100 mM KPI and 130 μL of CYP 1B1 and pipette 10 μL in each well of a black 96 well plate.

In the black 96 well plate:

take 40 μL of the 6th clear column and transfer it into black columns 1, 2 and 3, take 40 μL of the 7th clear column and transfer it into black columns 4, 5 and 6, take 40 μL of the 8th clear column and transfer it into black columns 7, 8 and 9, take 40 μL of the 9th clear column and transfer it into black columns 10, 11 and 12.

In the last raw H keep the first 6 wells as positive control and in the next 6 wells pipette 70 μL stop solution of acetonitrile to kill enzyme as a negative control.

Prepare master mix in a petri dish which consists of:

3250 μL 200 mM potassium phosphate buffer;

70 μL 1mM stock EROD substrate;

650 μL solution A;

130 μL solution B;

2405 μL distilled water.

Mix them gently and pipette 50 μL to each well in 96 black well plate then measure the rate of inhibition using gen5 software and wait for about 20 min. Transfer your data into excel sheet and divide data related to blend on 3 to obtain the inhibition effect of each chemical individually within the combination. Calculate the mean, standard deviation and standard error of the mean for both positive and negative control values. Calculate the percentage of inhibition.

2.6. Statistical Analysis of CYP450s

Using graph pad insert the data from excel sheet to draw the curve illustrating the relative fluorescence units (RFU) versus concentration by µM showing the inhibition of these chemical to microsomal enzymes CYPs.

3. Results

3.1. CYP450 Inhibition

3.1.1. CYP1A2 Assays

The results obtained in the tables below illustrate final well concentrations by µM, inhibition values, and percentage of inhibition according to the equation:

% of inhibition = 100 − ((amount of inhibition/mean value of the positive control) × 100).

The results of CYP1A2 inhibition assays are illustrated below (Table 4):

For positive controls the mean value = 21,402.2222, standard deviation = 1551.13183 and SEM = 517.043942.

For negative controls mean value = 791.666667, standard deviation = 51.0718448 and SEM = 17.0239483.

3.1.2. CYP1A1 Assays

The results of CYP1A1 inhibition assays are illustrated below (Table 5):

For positive controls the mean value = 98.56482, standard deviation = 11.3913778 and SEM = 4.65051051.

For negative controls the mean value = 0.092593, standard deviation = 0.03950135 and SEM = 0.01612636.

Table 4. Inhibition values related to CYP450 1A2 under the effect of cresol, indole, skatole and blend with calculated percentage of inhibition.

		Cresol			Indole			Skatol				Blend		
		1	2	3	1	2	3	1	2	3		1	2	3
	Final well concentration µM										Final well concentration µM			
A	100	21,844	25,254	23,444	19,719	19,799	21,620	9036	9219	9732	33.3333	4539	4681	5318.667
B	50	20,252	19,953	20,599	25,287	26,897	26,651	10,984	10,904	11,598	16.666667	5277.6666	5016.666	5478.666
C	25	19,861	19,937	20,198	19,952	21,426	18,787	13,603	13,364	14,373	8.3333333	5716.333	5739.333	6263.3333
D	2.5	20,639	23,474	19,967	20,366	23,511	20,399	19,380	19,131	20,090	0.8333333	6342	6609	6737.333
E	1.25	20,087	23,262	20,338	20,409	20,129	19,938	18,513	18,686	19,547	0.41666667	6520	6713.666	6952
F	0.625	24,135	20,407	37,439	21,567	21,171	19,660	18,904	18,403	20,142	0.208333	6426.333	12451	6959
G	0.0625	21,016	21,276	20,725	20,598	20,066	19,724	19,954	20,043	20,435	0.02083333	6847	7254.333	7120.333
H	Pos	21,446	24,563	20,709	20,565	23,397	20,041	20,109	20,729	21,061				
	Neg	755	770	850										
	% of inhibition	−2.06417	−17.9971	−9.54003	7.8647	7.49091475	−1.0175475	57.78009	56.92503	54.52809		78.79192	78.12844	75.149

Table 5. Inhibition values related to CYP450 1A1 under the effect of cresol, indole, skatole and blend with calculated percentage of inhibition.

		Cresol			Indole			Skatol				Blend		
		1	2	3	1	2	3	1	2	3		1	2	3
	Final well concentration μM										Final well concentration μM			
A	100	85.77315	82.51389		90.37963	90.80556	102.0139	91.80093	96.07407	90.21296	33.3333	33.08796	33.77777	35.8
B	50	90.50926	94.34259	92.0092	100.8333	106.0509	88.83111	91.7037	100.6898	95.39352	16.666667	31.7	33.9	39.2
C	25	86.39352	95.64352	91.35185	100.5417	100.6435	101.287	93.53704	98.28241	95.91204	8.3333333	31.9	33.2	35.48
D	2.5	89.42593	82.25463	93.05556	99.11111	100.1852	103.9815	98.23611	102.6204	102.3657	0.8333333	30.15586	36.7685	36.077
E	1.25	89.81944	94.65741	93.00926	99.37963	96.59259	102.3102	92.58333	97.03241	102.6852	0.41666667	32.3	33.57	33.375
F	0.625	88.25926	90.2963	94.46759	102.6944	100.4491	98.14815	95.72685	99.21296	101.1713	0.208333	33.9	33.05	33.5
G	0.0625	85.0463	89.22685	93.13889	102.875	107.1343	111.5787	114.4213	111.1898	110.9074	0.02083333	35.9	35.37	34.3
H	Pos	84.38889	87.52315	95.30093	105.032407	105.625	113.5185							
	Neg	0.171296	0.069444	0.083333	0.0648148	0.0787037	0.087963							
	% of inhibition	12.97793	16.28464		8.3043727	7.8722408	−3.4993	6.862377	2.527017	8.47347	66.18131	66.43025	65.7304	63.67872

3.1.3. CYP1B1 Assays

In this case take skatole inhibition values only into considerations because it has high inhibition effect comparable to blend.

The results of CYP1B1 inhibition assays are illustrated below (Table 6):

For positive controls the mean value = 76,565.5, standard deviation = 19,608.78 and SEM = 8005.25148.

For negative controls the mean value = 2456.333, standard deviation = 899.8417 and SEM = 367.358832.

3.2. Statistical Analysis of CYP450s

For CYP1A2 it showed high inhibition of skatole as IC50 equals 25.85 μM where its range is between 17.6 μM to 37.95 μM as shown below (Figure 4).

For CYP1B1 it showed high inhibition of skatole as IC50 equals 39.76 μM where its range is between 13.41 μM to 117.9 μM as shown below (Figure 5).

4. Discussion

For the graph pad related to CYPs enzymes it was shown that skatole is an inhibitor for CYP1A2 and 1B1 and according to Gene5 results, the percentage of inhibition of blend is much higher than the percentage of inhibition related to indole, skatole and cresol individually which supports the synergistic effect of their combination.

Skatole in this case is more potent inhibitor especially to CYP1A2 so skatole may be an inhibitor against anticancer drugs metabolised by CYP1A2.

For chemicals, the blend of indole, skatole and cresol can be used for topical uses to protect the body against dermatological disturbances. This blend can be used topically as patches, ointments or creams especially for some dermatologic disorders.

As well as using it for plants as onions to repel Aspergillus Fumigatus which infects plants especially onions, the physical properties of these chemicals can be beneficial if they were used as spray to repel insects as flies and mosquitoes.

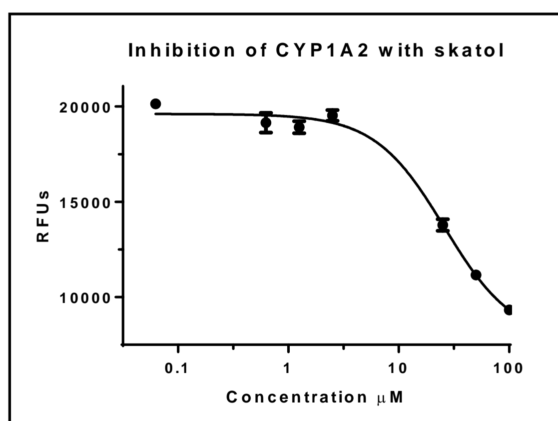

Figure 4. GraphPad shows RFU by Nano meter against log of skatole related to CYP1A2.

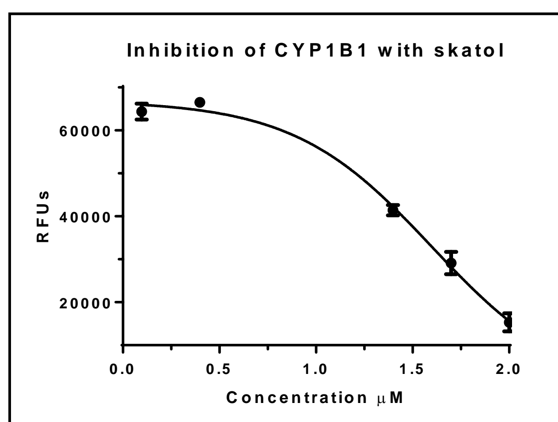

Figure 5. GraphPad shows RFU by Nano meter against log of skatole related to CYP1B1.

Table 6. Inhibition values related to CYP450 1B1 under the effect of skatole and blend with calculated percentage of inhibition.

		09-Jul.		Skatole			Blend	
		μM						
	A	100	11,155	17,329	17,509	33.3333	19,865	20,600	21,622.33
	B	50	23,907	31,915	31,490	16.666667	21,550.33	22,651.667	23,404
	C	25	39,020	42,182	43,045	8.3333333	21,569.67	24,520	24,049.33
	D	2.5	67,675	67,304	64,660	0.8333333	21,917.33	22,125.667	24,120.67
	E	1.25	66,521	60,598	65,942	0.41666667	19,202.33	20,139	22,126.33
	F	0.625	51,702	49,870	54,946	0.208333	15,662.33	16,107.333	17,791.67
	G	0.0625	35,367	28,880	33,246	0.02083333	9345.667	9542	11,672.33
	H 1-6	Pos	98,315	92,030	83,020	76,067	65,857	44,104	
	H 7-12	Neg	1901	3265	3906	1821	1997	1848	
% of inhibition			85.4	77.367	77.1		74.05	73.09	71.76

According to the results related to CYP450, the inhibitory effect of these chemicals especially skatole alone or blend can be useful to determine the drug-drug interactions related to taking these chemicals with other drugs which will affect their concentration as well as drug-disease interactions which were seen with patients suffer from chronic kidney diseases who cannot metabolize cresol and this substance is contraindicated to them.

5. Conclusions

The future work based on these results, His-tag cloning may be further done as some tags participate in the loss of activity of the peptide and optical density may be measured for measuring its inhibitory effects using N and C terminals.

Cloning and expression with yeasts can be done further with other protein peptides as lactoferrin and lactotransferrin which are found in the milk of many species of mammals and have antimicrobial activity. It can be extracted from milk of whales, camels and dolphins depending on their hydrophobicity and β-sheets which facilitates its penetration to the cell wall of microbes.

Further studies are needed to understand the overall properties of this combination and its effect against more microorganisms and its inhibitory effect on liver microsomal enzymes.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ortiz de Montellano, P.R. (1995) Cytochrome P450: Structure, Mechanism, and Biochemistry. Kluwer Academic/Plenum Publishers, New York. https://doi.org/10.1007/978-1-4757-2391-5

[2] Vlasak, R., Unger-Ullmann, C., Kreil, G. and Frischauf, A.M. (1983) Nucleotide Sequence of Cloned cDNA Coding for Honeybee Prepromelittin. *European Journal of Biochemistry*, **135**, 123-126. https://doi.org/10.1111/j.1432-1033.1983.tb07626.x

[3] Habermann, E. (1972) Bee and Wasp Venoms: The Biochemistry and Pharmacology of Their Peptides and Enzymes Are Reviewed. *Science*, **177**, 314-322. https://doi.org/10.1126/science.177.4046.314

[4] von Moltke, L.L., Greenblatt, D.J., Duan, S.X., Harmatz, J.S. and Shader, R.I. (1994) *In Vitro* Prediction of the Terfenadine-Ketoconazole Pharmacokinetic Interaction. *The Journal of Clinical Pharmacology*, **34**, 1222-1227. https://doi.org/10.1002/j.1552-4604.1994.tb04735.x

[5] Griffin, J.P. (1998) The Withdrawal of Mibefradil (Posicor). *Adverse Drug Reactions and Toxicological Reviews*, **17**, 59-60.