KINETIC CHARACTERIZATION OF ATPase OF *PLASMODIUM FALCIPARUM* (*Pf*ATP4) & ITS INHIBITION BY *DUTTAPHRYNUS MELANOSTICTUS* (SCNEIDER) SKIN EXTRACT: AN IN-VITRO STUDY

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Abstract: Bufadienolides, which are naturally present in the toxic skin secretions of toads, have recently been discovered to have a variety of antiparasitic effects. These substances are known to inhibit the activity of ATPase enzyme, thereby preventing the ATP usage in the parasites leading to their rapid death. In present study, the crude hydroalcoholic skin extract of Common Asian Toad, *Duttaphrynus melanostictus,* containing bufadienolides was evaluated for its ability to inhibit the ATPase of *Plasmodium falciparum* (PfATP4). PfATP4 was isolated from the trophozoites of *Plasmodium falciparum* 3D7 cells and its kinetic characterization was performed at varying concentrations of ATP, sodium, potassium, hydrogen, and calcium. The results obtained confirmed that PfATP4 followed Michaelis-Menten kinetics when treated with ATP, sodium, and hydrogen while no significant change in the activity was observed after the treatment with potassium and calcium. The inhibition constant of the extract was determined in vitro which was found to be 0.06 µg/ml. The Michaelis-Menten, Lineweaver-Burk and EisenthelCornish-Bowden plots showed that the Km value of the enzyme significantly increased while the Vmax remained unaffected after extract treatment. Therefore, from this preliminary study, it could be concluded that the bufadienolides present in the skin extract possess a potential of being a strong competitive inhibitor of ATPase in *Plasmodium falciparum* and hence could be further explored as a novel antimalarial drug.
Keywords: Bufadienolides; Malaria; ATPase; *Plasmodium falciparum*; Common Asian Toad.

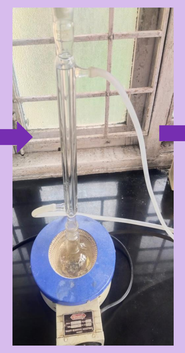
INTRODUCTION

- *Plasmodium falciparum* P-type ATPase *(Pf*ATP4) plays a crucial role in maintenance of Na-homeostasis in malaria parasites during their intraerythrocytic development cycle.
- Drugs that target *Pf*ATP4 therefore, present the prospect of taking us a step closer to malaria elimination in a world that is currently threatened by artemisinin resistance.
- In present study, the skin extract of Common Asian Toad rich in bufadienolides was evaluated for its potential to inhibit *Pf*ATP4 by in-vitro methods.

METHODOLOGY

- Preparation of Toad Skin Extract (TSE) rich in bufadienolides
- Kinetic characterization of *Pf*ATP4 from the trophozoites of *Plasmodium falciparum* 3D7 cells
- Determination of inhibition constant (Ki) of TSE on *Pf*ATP4
- In-vitro inhibition kinetic study of *Pf*ATP4 treated with TSE at various ATP concentrations

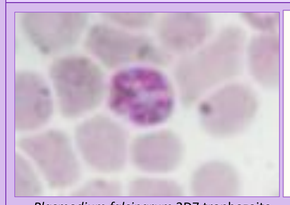
PREPARATION OF TOAD SKIN EXTRACT (TSE)



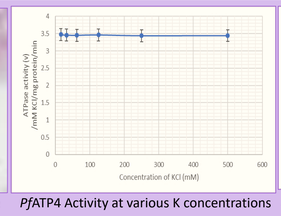
Percentage Yield of TSE = 9.22%

HPTLC Plate showing blue/purple bands indicating presence of bufadienolides in TSE

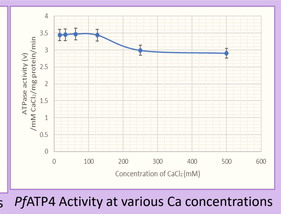
KINETIC CHARACTERIZATION OF *Pf*ATP4



Plasmodium falciparum 3D7 trophozoite

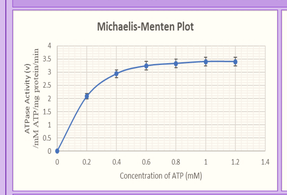


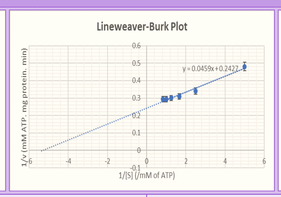
*Pf*ATP4 Activity at various K concentrations

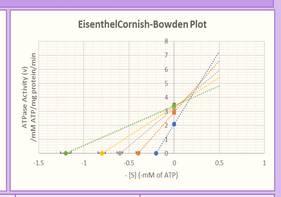


*Pf*ATP4 Activity at various Ca concentrations

KINETIC CHARACTERIZATION OF *PF*ATP4 ACTIVITY AT VARIOUS CONCENTRATIONS OF ATP

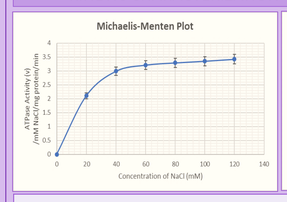


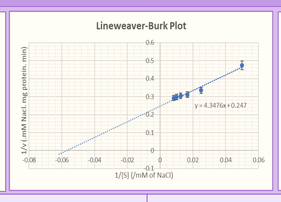


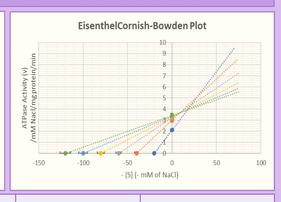


Kinetics	Vmax	Km	Kcat
Michaelis-Menten	3.40	0.16	1.00
Lineweaver-Burk	4.12	0.19	1.21
EisenthelCornish-Bowden	4.12	0.18	1.21

KINETIC CHARACTERIZATION OF *PF*ATP4 ACTIVITY AT VARIOUS CONCENTRATIONS OF SODIUM

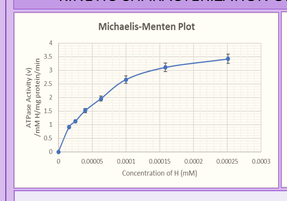


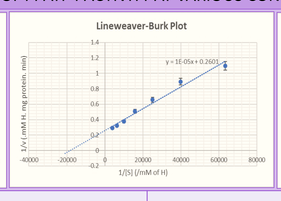




Kinetics	Vmax	Km	Kcat
Michaelis-Menten	3.42	2.48	1.00
Lineweaver-Burk	4.05	2.80	1.19
Eisenthel Cornish-Bowden	4.16	2.83	1.22

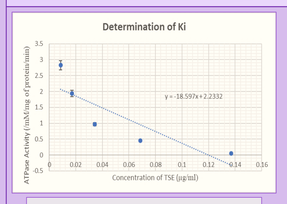
KINETIC CHARACTERIZATION OF *PF*ATP4 ACTIVITY AT VARIOUS CONCENTRATIONS OF HYDROGEN





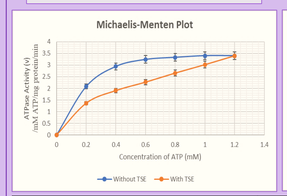
Kinetics	Vmax	Km	Kcat
Michaelis-Menten	3.41	2.01	1.00
Lineweaver-Burk	3.84	2.17	1.13
Eisenthel Cornish-Bowden	4.20	2.40	1.23

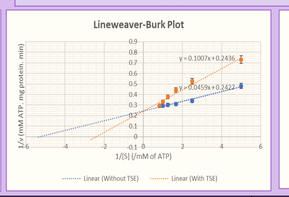
IN-VITRO INHIBITION STUDY

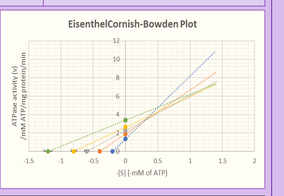


Ki = 0.06 µg/ml

Extract Conc. (µg/ml)	ATPase Activity
0.008	2.83
0.017	1.94
0.034	0.97
0.069	0.45
0.137	0.05







Kinetics		Km	Vmax
Michaelis-Menten	Without Extract	0.16	3.40
	With Extract	0.30	3.40
Lineweaver-Burk	Without Extract	0.19	4.12
	With Extract	0.41	4.11
EisenthelCornish-Bowden	Without Extract	0.18	4.12
	With Extract	0.44	4.12

CONCLUSION

From this study, it can be concluded that the skin extract of Common Asian Toad affects the binding of ATP to *Plasmodium falciparum* Na-H ATPase (*Pf*ATP4), as a competitive inhibitor. Therefore, bufadienolides present in the extract could be prospective novel antimalarial compounds.

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