Enzyme inhibitory properties, free radical scavenging activity and phytochemical profile of *Vitex simplicifolia* leaf

Odeghe, Othuke Bensandy[1]*, Opajobi, Adefunke Olukemi[1], Ajala, Michael Olawale[2] and Orororo, Clement Osuvwe[1]

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, P.O. Box 01, Abraka, Delta State, Nigeria.
[2]Department of Chemical Pathology and Metabolic Medicine, Eko University of Medical and Health Sciences, P.M.B. 2009, Ijanikin, Lagos State, Nigeria.

Corresponding Author's phone number and e-mail Address: +2348066768804, odegheothukebensandy@delsu.edu.ng or bensandym@yahoo.com

Abstract

The phytochemical constituents, antioxidant capabilities and enzyme inhibitory potentials of aqueous extract of *Vitex simplicifolia* leaf were investigated. The plant was assessed for its phytochemical availability using GC-FID; antioxidant activity was assessed while lactate dehydrogenase (LDH) inhibitory potential was analyzed by chemical method. The result revealed the presence of phytochemicals in which Anthocyanin (Flavonoid) showed the highest significant ($p \leq 0.05$) increase in concentration when compared to other secondary metabolites. The DPPH and superoxide anion radical inhibitory potential of the plant extract revealed its maximal inhibition of 72.46±1.55 % ; 92.41±0.08 % at a concentration of 10000µg/ml compared to ascorbic acid (87.55±0.19 %) and quercetin (97.33±0.15 %) at 10000µg/ml.. The IC_{50} of DPPH and superoxide anion radical inhibition of the extract was found to be 48.15±0.17µg/ml; 37.15+1.56µg/ml as compared to ascorbic acid (9.02±0.05µg/ml) and quercetin (5.23±0.17µg/ml) respectively. The extract was also found to rapidly scavenge nitric oxide radical at different dose dependent manner. Km and Vmax of LDH were 20.5 µg/ml and 7.9 µmol/min.mg-1 protein respectively and displayed non competitive inhibition for aqueous extract double reciprocal plot. The inhibition Constant (Ki) was 4.5 µg/ml which implies the extract possessing strong inhibitory activity on lactate dehydrogenase. The secondary metabolites found in V. simplicifolia leaf extract could serve as therapeutic plant that may scavenge free radicals and also inhibit LDH by altering carbohydrate metabolism resulting to the reduction rate of proliferation of malaria parasite and tumour cells.

Keywords: Enzyme inhibition, antioxidant ability and phytochemistry

DOI: 10.48047/ecb/2023.12.8.707

Introduction

Over the past decade, increasing indication suggest the capability of the plant active components (phenolics, carotenoids, thiol and anthocyanins) through their various mechanism of action to prevent oxidative damage caused by free radicals. (Odeghe et al., 2020b). Researchers have been showing greater interest in Lactate dehydrogenase because it is an essential therapeutic tool for malaria and cancer (Odeghe *et al*., 2016a). It has been shown that in *in vivo* malaria research, reactive oxygen species are produced in abundant amounts (Odeghe *et al*., 2020a) and also enzymes like LDH plays a role in tumor initiation and metabolism (Odeghe *et al*., 2016a). *Plasmodium falciparum* express pfLDH isoform as an essential enzyme for malaria parasite energy production. Studies have shown that Cancer cells rely on anaerobic respiration through Warburg effect to increase the rate of glucose conversion to lactate thereby facilitating glycolysis in invasive tumor cells through a therapeutic glycolytic enzyme known as Human lactate dehydrogenase (hLDH5) present in the liver and muscles which is responsible for pyruvate conversion to lactate hence oxidizing NADH to NAD^+ (Odeghe *et al*., 2016a). However, one of the measures of solving this challenge is to investigate any enzyme that functions majorly in both malaria parasite and cancer cells. Many investigations have revealed useful relationship between energy produced from glucose and cancer

continuance. In addition, the results obtained suggest that when a specific part of glucose metabolism is inhibited it may be a good and tolerable method of preventing malaria parasite and cancer cell growth (Granchi *et al.*, 2010).

Vitex simplicifolia leaf is generally used locally in South East of Nigeria to treat different medical diseases and it was assessed for its phytochemical constituents, free radical scavenging capability and its inhibitory effects on LDH activity in a set of analysed systems. And to reassure that this plant extracts are potential anticancer and antimalaria therapies.

The use of *Vitex simplicifolia* in the management and treatment of malaria and cancer has not been given scientific base. This study investigates the phytochemical profile, free radical scavenging capability and the lactate dehydrogenase inhibitory potentials of the plant.

Materials and Methods

Plant Identification and Authentication:-

Vitex simplicifolia leaf was collected in April, 2023 at Abraka area, Delta State, Nigeria. The plant was holistically identified and authenticated at International Centre for Ethnomedicine and Drug Development (INTERCEDD) situated at Nsukka, Enugu State, Nigeria and the plant was assigned a voucher number of INTERCEDD/ 868 and kept in the herbarium center for further purposes.

Preparation of extract:

The plant material was washed and unneeded substances were discarded. *Vitex simplicifolia* leaf was kept to air-dry. The Aqueous extract was prepared by dissolving 120g of the pulverized leaf with 1.2 litre of distilled water and kept for one day. The extracts obtained were filtered by the use of a membrane filter and kept at 4^{o}C for other purposes.

Ethical Issues

This is not applicable

Qualitative Phytochemical Analysis

The leaf sample was investigated for the presence of phytochemicals according to the methods of Nwiloh et al., (2016).

Quanlitative Phytochemical Analysis

The presence of the quantity of bioactive components in the pulverized sample was determined using Odeghe et al, (2016b) methods.

Quantitative phytochemical analysis using Gas Chromatography Flame Ionisation Detector (GCFID)

The GCFID of *Vitex simplicifolia* leaf was analysed based on the method Nwiloh et al., (2016).

DPPH radical-scavenging assay

The DPPH radical scavenging analysis method of Odeghe *et al*. (2020b) was used for the determination.

Superoxide radical (O2.-)-scavenging assay:-

Superoxide anion radical investigation was done according to the method of Odeghe *et al*., (2016b).

Nitric oxide radical (NO.) Scavenging assay:-

The nitric oxide analysis was carried out based on the method of Odeghe *et al*., (2020b)

Enzyme Assay:-

Source of Lactate Dehydrogenase

The purified rabbit muscle lactic dehydrogenase (L2500-25KU) possessing enzymatic activity of 942 units/mg protein was obtained from Sigma Company.

Lactate dehydrogenase Assay

The method of Odeghe et al., (2016a) was used to investigate protein concentration.

Effect of Substrate Concentration

The substrate concentration effect was evaluated based on the method of Odeghe et al., (2016a)

Effect of aqueous and ethanol extracts of *Vitex simplicifolia* on LDH activity

The method of Odeghe et al., 2016a was used to determine the LDH activity

Statistical Analysis

The statistical analysis for the data investigation was done using One-way Analysis of Variance (ANOVA) while Windows 7 Microsoft Excel 2010 package was used to obtain the graphs.

Results and Discussions

Qualitative Screening on Phytochemical Profile

The Qualitative screening of Vitex simplicifolia leaf can be found in Table 1 below.

Table 1: **Qualitative Screening of Vitex simplicifolia**

Phytochemical constituent	**Inference**
Alkaloids	++
Flavonoid	+++
Glycoside	++
Tannin	+++
Saponin	+++
Protein	++
Carbohydrate	++
Steroids	+++

KEY: ++ Present in high amount, +++ Present in very high amount

The qualitative analysis investigated on *Vitex simplicifolia* leaf showed the presence of significant phytochemical constituents such as Alkaloids, Flavonoids, Glycoside, Tannin, Saponin, Protein, Carbohydrate and Steroids which is similar to the research of Odeghe et al., 2020a.

Quantitative investigatiom using GC-FID

The GC-FID Qualitative analysis of Vitex simplicifolia leaf displayed the presence of phytochemical constituents such as Spartein, Phytate, Anthocyanin, Tannin, Naringerine, Ribalinidine, Catechin, Epicatechin, and Kaempferol as summarized in Table 2 below.

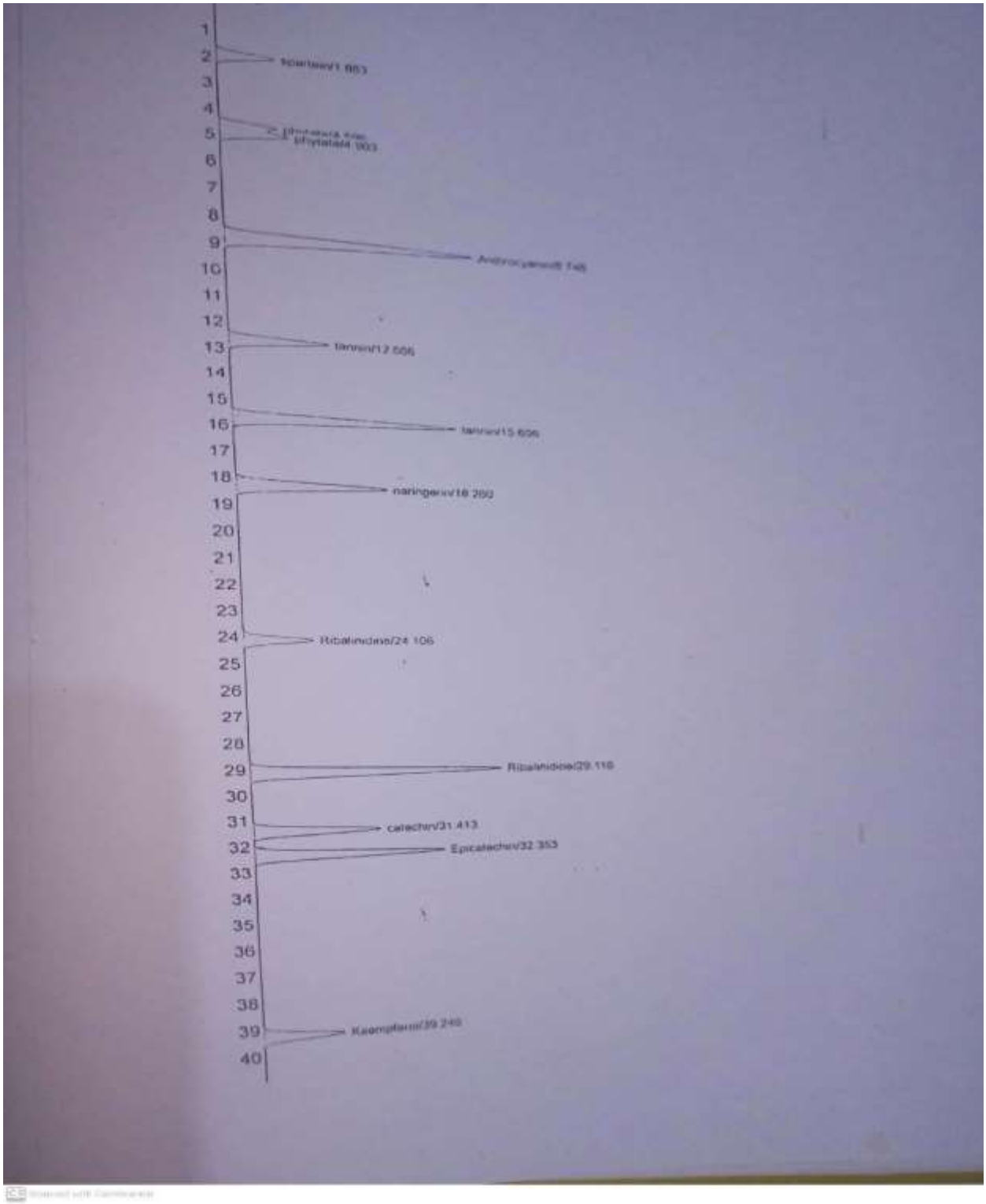

Figure 1: Chromatogram displaying phytochemical constituents of *Vitex simplicifolia leaf* extract

S/No.	Phytochemical	Retention Time	Area	Height	Conc. (µg/ml)	% Composition
1.	Spartein	1. 88	3733.45	212.02	0.0024	0.000024
2.	Phytate	4.59	3462.53	210.93	0.15	0.0015
3.	Anthocyanin	8.75	17782.14	968.05	344.39	3.44
4.	Tannin	12. 87	6589.14	373.98	29. 84	0.29
5.	Naringerine	18.26	10783. 81	576.22	19.17	0.19
6.	Ribalinidine	24.01	4627,23	262.97	2.01	0.02
7.	Catechin	31.41	8523.09	483.91	62.04	0.62
8.	Epicatechin	32.36	13097.72	739.54	26.50	0.27
9.	Kaempferol	39.25	5361.22	304.46	25.34	0.25
	Total				**509. 44**	

The phytochemical screening of Vitex simplicifolia leaf using GC-FID displayed that Anthocyanin which is a flavonoid had the highest concentration of (344.39 µg/ml) while lowest concentration of (0.0024 µg/ml) was found in Spartein (Alkaloid) when compared to other secondary components and this similar to the research of Odeghe et al., 2020b medicinal plants possessing those phytochemical moderate amount may be used use for therapeutic purpose. Flavonoids like Anthocyanins are known to possess anti-inflammatory and anti-tumor properties, as well as boost immunity and increase the production of detoxifying enzymes in the body (Nwiloh et al, 2016). The identification of Alkaloid particularly Spartein is similar to the research of Odeghe et al., 2016b that this plant may be used in the treatment of malaria.

TABLE 3: DPPH radical scavenging activity of *Vitex simplicifolia leaf* compared to Ascorbic acid.

Concentration of Extract(µg/ml)	% Inhibition	Concentration of Ascorbic acid (µg/ml)	% Inhibition
10000	72.46±1.55	10000	87.55±0.19
5000	70.15±1.12	5000	84.02±1.06
2500	70.25±0.24	2500	80.25±0.17
1250	54.15±2.03 [a]	1250	70.07±1.55 [a]
625	41.23±1.07	625	67.06±0.15
312.50	39.23±1.03 [a]	312.50	62.16±2.03 [a]
156.25	30.05±0.15	156.25	60.97±0.08
78.13	29.23±1.44	78.13	58.22±0.01
39.06	27.16±0.09	39.06	41.68±2.22
19.53	25.41±1.23 [a]	19.53	34.06±0.98 [a]
IC_{50}	**48.15±0.17**	**IC_{50}**	**9.02±0.05**

Data represented as MEAN ± SEM of n=3. Values indicated by superscript letters represent significant differences (P<0.05).

Effect of aqueous extract of *Vitex simplicifolia leaf* on DPPH radical

Table 3 shows the DPPH free radical scavenging potential of aqueous extract of *Vitex simplicifolia* leaf. At a concentration of 10000 µg/ml the extract displayed significant (P<0.05) difference at the highest scavenging potential of 72.46±1.55 % while at the same concentration the Ascorbic acid standard was 87.55±0.19 %. The IC_{50} of the aqueous leaf extract was 48.15±0.17 µg/ml when compared to the standard 9.02±0.05 µg/ml This finding is similar to the research of Rahman et al., 2015 that the acceptable mechanism for antioxidant screening potential of plant

extracts is by DDPH approach due to its ability to transfer electron. Free radical scavenging activity is an important model to prevent radicals in diseases such as malaria and cancer (Odeghe et al., 2020a).

TABLE 4: Superoxide radical (O^{2-}) scavenging activity of *Vitex simplicifolia leaf* compared to Quercetin

Concentration of Extract(µg/ml)	**% Inhibition**	**Concentration of Quercetin (µg/ml)**	**% Inhibition**
10000	92.41±0.08	10000	97.33±0.15
5000	80.21±0.22	5000	95.44±0.13
2500	76.12±0.54 [a]	2500	94.01±0.74 [a]
1250	69.16±0.35	1250	82.38±0.06
625	52.11±0.23	625	71.50±0.02
312.5	41.03±0.15 [a]	312.5	63.12±0.67 [a]
156.25	31.25±0.74	156.25	53.44±0.02
78.125	31.03±0.83	78.125	42.13±0.66
39.0625	19.22±0.45	39.0625	40.59±0.52
19.53125	16.09±0.85 [a]	19.53125	31.41±0. 80 [a]
IC_{50}	**37.15+1.56**	**IC_{50}**	**5.23±0.17**

Data represented as MEAN ± SEM of n=3. Values indicated by superscript letters represent significant differences (P<0.05).

Effect of *aqueous extract of Vitex simplicifolia leaf* on Superoxide radical (O^{2-})

The **Superoxide radical (O^{2-}) scavenging potentials as found in Table 4 showed that the aqueous extract of Vitex Simplicifolia leaf at the** 10000 µg/ml concentration displayed **the highest** significant (p<0.05) **reduction of nitro blue tetrazolium (NBT) at** 92.41±0.08% when compared to the quercetin 97.33±0.15% . The IC_{50} of the aqueous leaf extract was 37.15+1.56µg/ml when

compared to the standard 5.23+0.17 µg/ml. This is similar to the report of Odeghe et al., 2016b that plant extract could prevent OH radical synthesis.

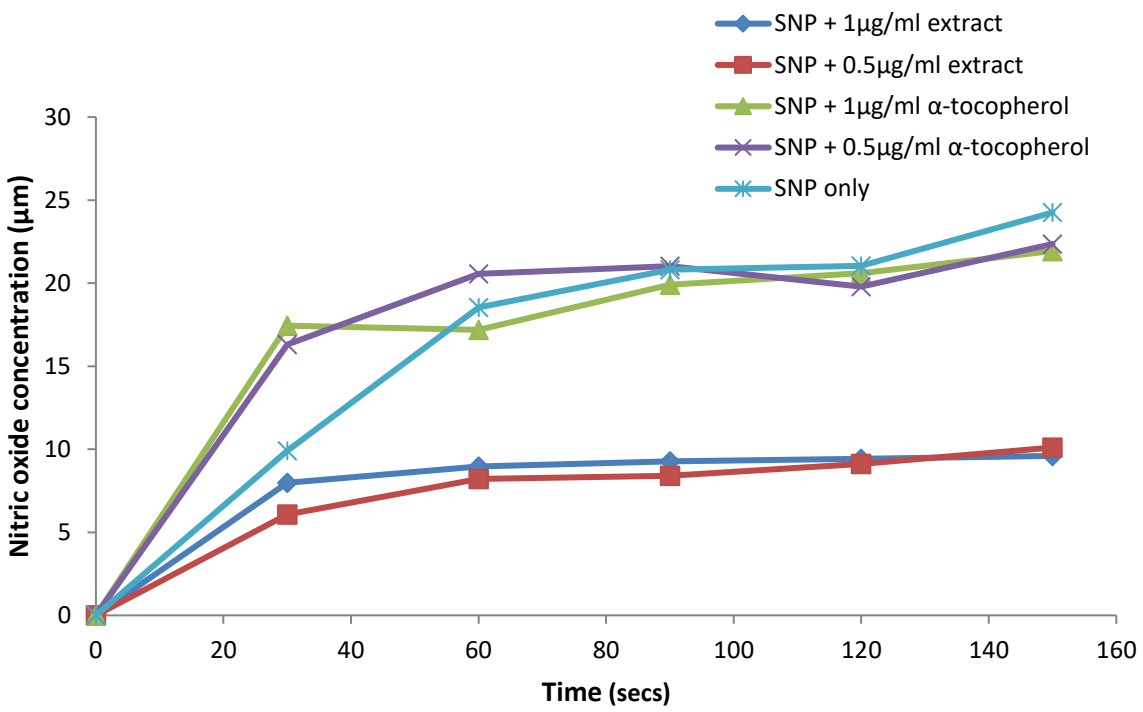

Figure 2: Effect of extract on nitric oxide (NO^-) radical production

From Figure 2, the aqueous extract of *Vitex simplicifolia* leaf revealed high quality Nitric oxide (NO^-) scavenging activity that can alter the physiology of different cellular parts. The presence of the extract could also reduce the accumulation of nitrite that exist as an established NO- byproduct formed from sodium nitroprusside (SNP) and thereby serves as oxidative damage prevention mechanism (Sourav et al., 2011). The *Vitex simplicifolia* leaf aqueous extract

concentration of 1 g/ml displayed the highest percentage inhibition of nitric oxide when compared to α–tocopherol which is the standard. The indispensable function of nitric oxide (NO^-) in oxidative damage is evidently substantial irrespective of its supportive result (Rahman et al., 2015). This is due to the reaction of superoxide and nitric oxide to release peroxynitrite anion that can degrade to form NO_2 and ^-OH due to its prevailing oxidant properties (Odeghe et al., 2016b).

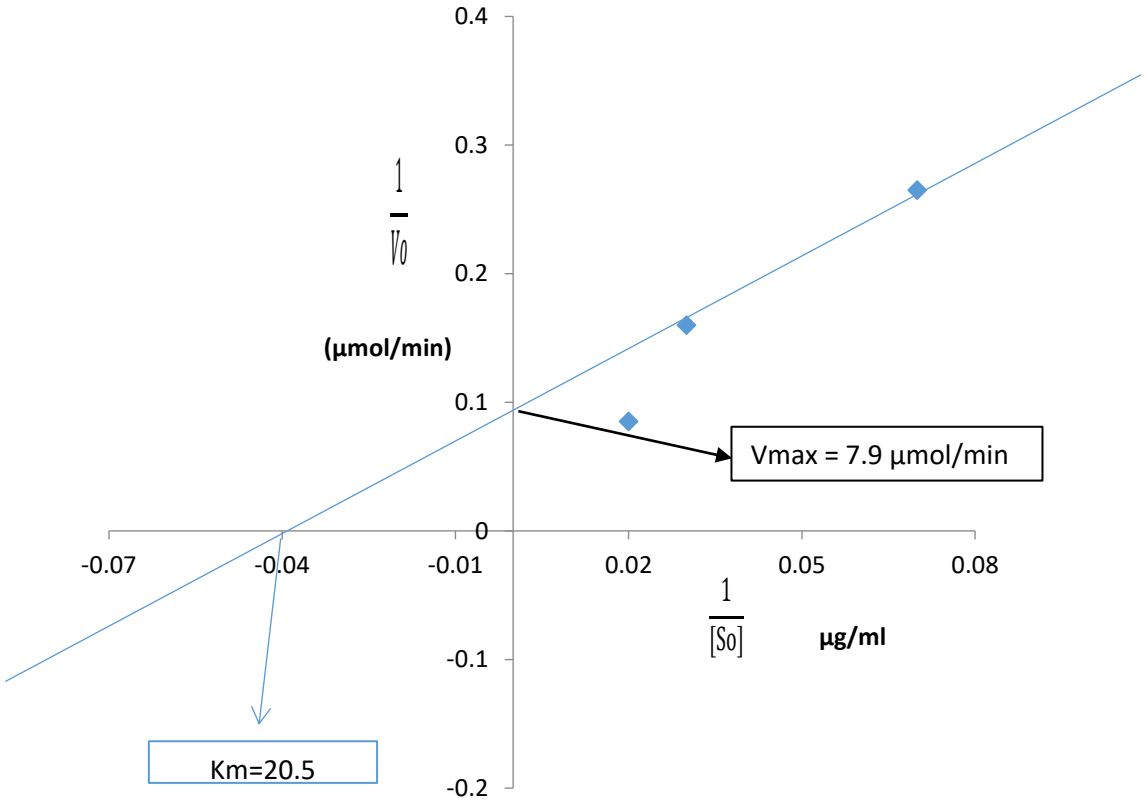

Figure 3: Plot of LDH activity versus Substrate Concentration of *V. simplicifolia* leaf

The result in Figure 3 revealed Km value of 20.5 µg/ml and Vmax of 7.9 mol/min. This finding also showed alteration in the Vmax variables, unchanged Km and a decrease in enzyme concentration which may be as a result of the inability of the inhibitor (extract) or enzyme unable to alter their binding activity and this correspond to the study of Odeghe et al., 2016a.

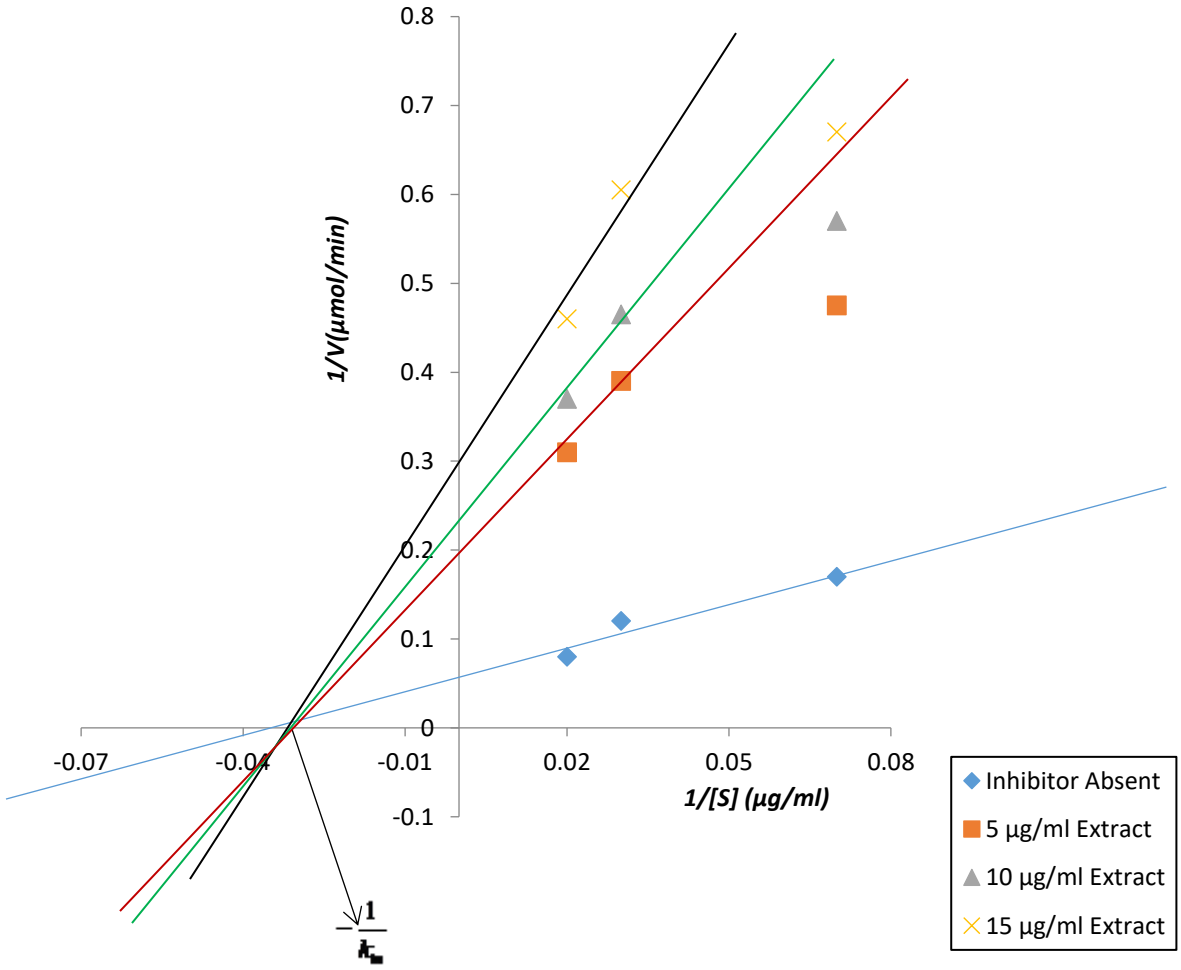

Figure 4: Lineweaver-Burk's plot for *Vitex simplicifolia* leaf aqueous extract on LDH Activity displaying non-competitive inhibition.

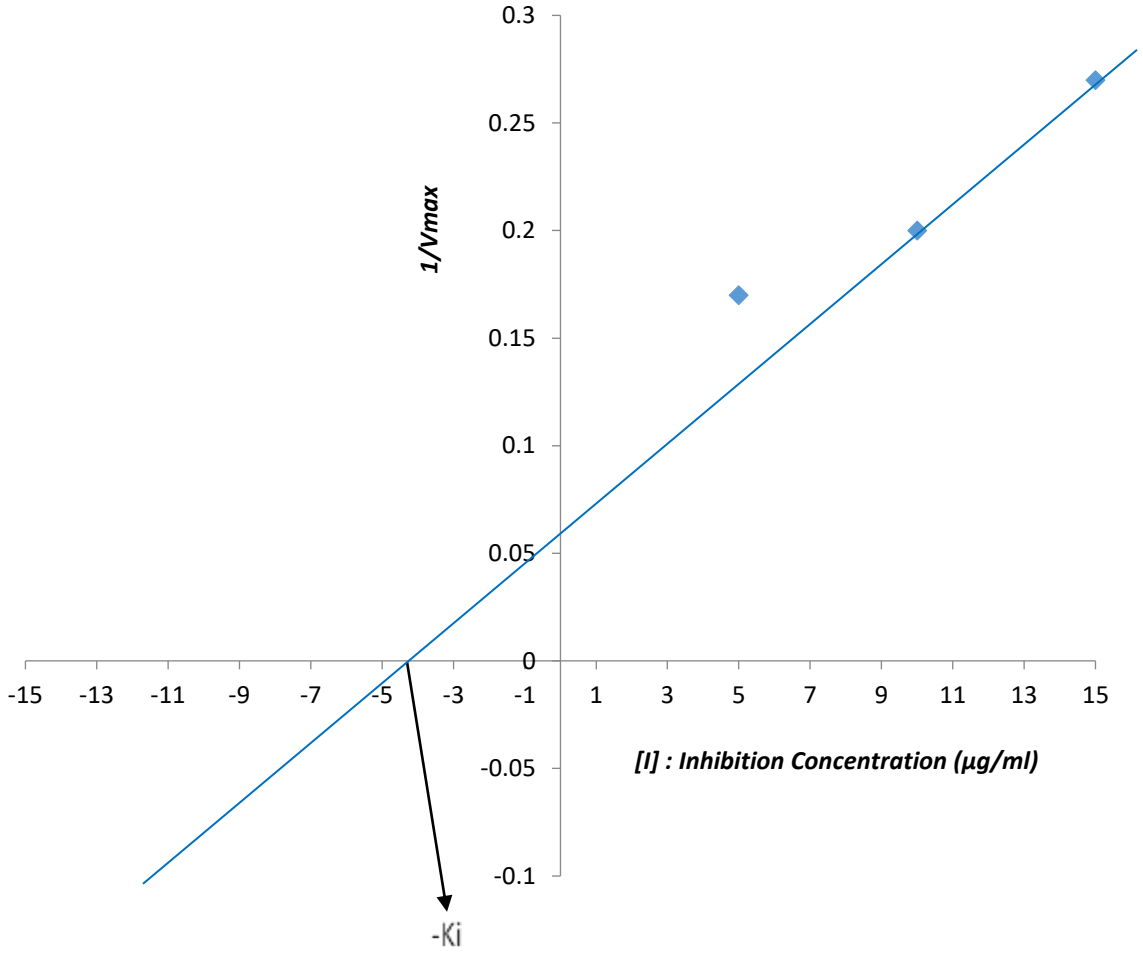

Figure 5: Plot for *Vitex simplicifolia* aqueous extract Inhibition Constant (Ki) showing 4.5 µg/ml.

The result of the activity of LDH from *V. simplicifolia* leaf aqueous extract displayed the binding of the inhibitor on the enzyme at a distinct site of the substrate which depicts non-competitive inhibition as shown in figure 4.

This may be due to the alteration of the catalytic site of the enzyme thereby affecting the conformational state by permitting the binding of the extract on the enzyme at a site distinct from that of the substrate. The reduced Ki value of 4.5 µg/ml (Figure 5) obtained from *V. simplicifolia* leaf extract depicts the high enzyme affinity for the extracts. From this result, the plant extract showed high inhibitory properties in a dose-dependent approach which could be a therapeutic mechanism of developing a novel drug (plant extract) for the treatment of malaria and cancer.

Conclusion:- The secondary metabolites found in V. simplicifolia leaf extract could serve as therapeutic plant that may scavenge free radicals and also inhibit LDH by altering carbohydrate metabolism resulting to the reduction rate of proliferation of malaria parasite and tumour cells.

Reference

Granchi, C., Bertini, S., Macchia, M. and Minutolo, F. (2010) Inhibitors of LDH Isoforms and their Therapeutic Potentials. *Current Medicinal Chemistry*; 17:672-697.

Nwiloh, B.I., Uwakwe, A.A., Akaninwor, J.O. (2012). Phytochemical screening and GC-FID analysis of ethanolic extract of root bark of Salacianitida L. Benth. Journal of Medicinal Plant Studies. 4(6): 283-287.

Odeghe, O.B., Monanu. M.O., Anacletus, F.C. (2016a). In vitro antioxidant abilities and inhibition of rabbit muscle Lactate dehydrogenase by aqueous extract of Hannoa klaineana stem bark. International Journal of Advanced Research;4(2):509-515.

Odeghe, O.B**.,** Monanu, M.O. and Anacletus, F.C. (2016b). Phytochemistry of Anchomanes difformis stem, Dioscorea bulbifera stem bark, Fadogia cienkowskii leaf, Hannoa klaineana stem and Vitex simplicifolia leaf. Advances in multidisciplinary and scientific research; **2**(1):1-8.

Odeghe Othuke Bensandy, Adikwu E., John Ebubechi Frances (2020a). Antiplasmodial activity of *Anchomanes difformis* aqueous leaf extract on *Plasmodium berghei* infected mice. *Trends in Pharmaceutical Sciences (TIPS)* ; **6**(4): 271-278.

Odeghe, O.B**.,** Adikwu E, Ojiego, C.C. (2020b). Phytochemical and antioxidant assessments of *Dioscorea bulbifera* stem tuber. Biomedical and Biotechnology Research Journal (BBRJ);**4**:305-11.

Rahman, M.M., Islam, M.B., Biswas, M. *et al.* (2015). In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Res Notes* 8, 621 https://doi.org/10.1186/s13104-015-1618-6

Sourav, M., Bibhabasu, H., Rhitajit. S., Santanu, B. and Nripendranath, M. (2011). Assessment of the Antioxidant and Reactive Oxygen Species Scavenging Activity of Methanolic Extract of *Caesalpinia crista* Leaf. Evid Based Complement Alternat Med. 2011: 173768.