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Evaluation of *moringa oleifera* and *brassica oleracea* for its inhibitory properties on dietary enzymes and cytotoxicity in ku812 cell, *in vitro*

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Abstract
Brassica oleracea and *Moringa oleifera* are two tropical plants of medicinal properties. The current study aims at investigating inhibitory action of plant extract of *Brassica oleracea* and *Moringa oleifera* on dietary enzymes (lipase and α amylase) as well as assessing the toxicity profile of the extracts. In this study phytochemical constituents, total polyphenol and total flavonoid content, free radical scavenging, inhibitory activity, toxicity profile of the plant in animal cell line model was determined. However, Phenolic extracts from the leaves inhibited α-amylase and lipase in a concentration dependant manner. So, this study offers the effect of using *Brassica oleracea* and *Moringa oleifera* leaves have potential functional properties on dietary enzymes and estimated the cytotoxicity in KU812 cell.

Keywords: *Brassica oleracea*, *Moringa oleifera*, dietary enzymes, lipase, α amylase, KU812 cell

Introduction

The M*oringa oleifera* is also known as moringa, is native to north India but is now found throughout the tropic. Moringa is also known as horse radish tree, drumstick tree and mother's friend and is a versatile plant with high nutritive, agricultural, medicinal, domestic, industrial and environmental benefits [1]. It is effective in combating malnutrition, especially among infants and nursing mothers [2]. All of the part of the tree can be used in variety of way. Moringa is full of nutrients and vitamins and is good in your food as well as in the food of your animals. M*oringa* help to clean dirty water and is a useful source of medicine. It produces lots of leafy material that is useful when using alley cropping system.

Kale (*Brassica oleracea*) is cabbage-like plants native to the eastern Mediterranean or to Asia Minor. Their wild forms have become widely distributed from their place of origin and are found on the coasts of northern Europe and Britain. Apparently, all the principal forms of kale we know today have been known for at least 2,000 years. Kale (also known as non-heading cabbage and broccoli) are hardy cool-season greens of the cabbage family and rich in vitamin A and C. Although kale tolerates summer heat, it grows best in the spring and fall. The highly curled, bluish-green leaves (some varieties have plain leaves) do not form a solid head. Flower-like cultivars of kale are quite colourful. They are planted as an accent or as pot plants. The leaves develop their highest color under cool fall weather. In addition to serving as ornamentals, kale plants are used for greens as a garnish or in salads and may be cooked in place of cabbage [3].

In human, we have different enzymes in different systems. Those that are concerned with digestion are known as digestive enzymes that are secreted by special glands or mucosal cells along the digestive pathway. Their chief function is to metabolize the ingested food so that its components can be absorbed and utilized by our body. This whole process of digestion, absorption and utilization is known as "assimilation". Enzymes are biological catalysts which are an indispensable component of biological reactions. The use of chemical catalysts has been followed for a very long time. Chemical catalysis though widely used was very cumbersome. The disadvantages that this method poses include need for high temperature and pressure for catalysis and the moderate specificity. These limitations were overcome by the use of enzymes. Enzymes work at milder conditions when compared to that required by chemical catalysts for operation. Also enzymes are highly specific and catalyse reactions faster than chemical catalysts.

Lipases catalyse the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. Lipases occur widely in nature, but only microbial lipases are commercially significant. The many applications of lipases include speciality organic syntheses, hydrolysis of fats and oils, modification of fats, flavour enhancement in food processing, resolution of racemic mixtures, and chemical analyses. In contrast to esterase, lipases are activated only when adsorbed to an oil–water interface and do not hydrolyse dissolved substrates in the bulk fluid.

Amylase is critical in the digestion of starch into sugars to make them available energy sources for the body. Amylase is found in two primary places within the human body, and the two types are classified according to where they are found. Salivary Amylase is a component of saliva, and breaks starch into glucose and dextrin. It hydrolyses the bonds between long-chain polysaccharides found in food, breaking compounds such as glycogen and starch into their useful monomers, glucose and maltose. Pancreatic Amylase is added to the small intestine to further digest starches; amylase is denatured in the acidic stomach. Amylase is also present in blood where it digests dead white blood cells. The current study aims at investigating inhibitory action of plant extract of *Brassica oleracea* and *Moringa oleifera* on dietary enzymes (lipase and α amylase) as well as assessing the toxicity profile of the extracts.

The specific objectives of the study are to:

a. Determine the phytochemical constituents of the plant extract of *Brassica oleracea* and *Moringa oleifera*.
b. Determine the total polyphenol and total flavonoid content of the plant extract.
c. Determine the free radical scavenging activity of the plant extract
d. Determine the inhibitory activity of the plant extract on the dietary enzymes.
e. Determine the toxicity profile of the plant in animal cell line model.

Methodology

A. Selection of plants

Brassica oleracea and *Moringa oleifera* were selected. The readymade powdered form of plants was available from Sabala agro product Pvt. Ltd. from Bangalore. They harvested at their peak. Dehydrated and powdered into nutrient rich magic.

B. Extraction of plants

15g dried plant powder was extracted in 70% ethanol. The dried powder of samples was dissolved in ethanol in conical flask and the total volume was made up to 150ml with distilled water. Adjust the pH of the extract 2 by pH meter using concentrated HCl. The mouth of the conical flask was covered with cotton and aluminium foil. Then the extracts were then kept into the shaker for 5 hours at 29.1ºC.Obtained extract was filtered first through muslin filter and normal filter paper, finally filtered through what man No.1 filter paper to remove the debris. Rotary flash evaporator (heidolph) was used to remove the ethanol. After flash, the concentrated samples were transferred into 3 steel bowls for lyophilisation. The samples were kept for lyophilisation overnight. The powdered forms of samples were obtained and dissolved the sample with distilled water. The dissolved samples were stored at -20ºC until analysis.

C. Phyto- chemical evaluation

The tests for Alkaloids, carbohydrates, phenolic compounds, saponin, terpanoids, flavonoids, steroids, anthocyanin, coumanins, free radical scavenging and oxalates were evaluated and analysed.

D. Enzyme inhibitory assay

The inhibitory activity of selected enzymes (lipase and amylase) was estimated by line Weaver- Burk Plot of kinetic analysis by p-NPB at different concentration of M*oringa oleifera* and *Brassica oleracea.* From this, the inhibitory activity of the selected plants were calculated using Vmax $(mm)^{-1}$ and Km (mm).

E. Cell culture

The effect of the ethanolic extract of *Moringa oleifera* and *Brassica oleracea* plant on cell viability was assessed in a cell culture system using cells from the human basophilic leukemic cell line KU812.The cell line were grown in RPMI 1640 (Rose well Park Memorial Institute medium).Supplemented with10% fetal bovine serum. The pH 7.4 was maintained and cells were incubated at 37ºC with 5% CO2 in a suitable incubator. A 5% in air atmosphere is recommended.

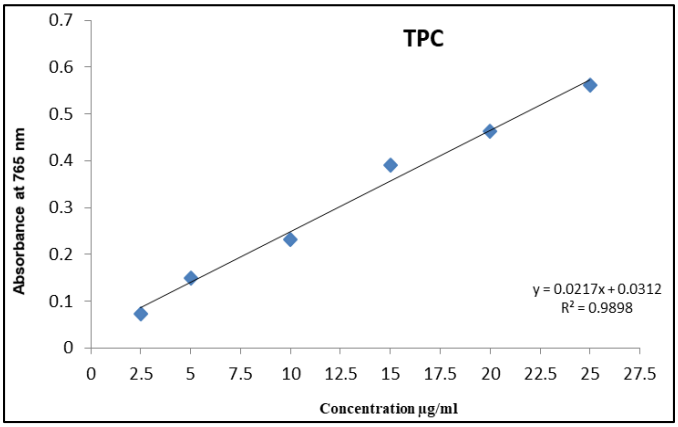
Result and discussion

a. Qualitative Phytochemical Assessment of M*oringa oleifera* and *Brassica oleracea*

The summary of identified phyto constituents in the ethanolic extract of M*oringa oleifera and Brassica oleracea* are *Alkaloids, Carbohydrates, Amino acid, Tannin, Saponin, Terpanoids, Flavonoids, Steroids, Anthocyanin, Coumarins, Oxalates and Glycosides.*

b. Determination of total phenolic content

Total phenolic content of *Moringa oleifera and Brassica oleracea* was calculated as per the standard method mentioned in Kiranmai *et al* (1998). A standard curve was made based on a series of different Gallic acid concentrations was 2.5,5,10,15,20,25 and its absorbance was 0.73, 0.150, 0.231, 0.39, 0.462, and 0.561 respectively. The sample concentration was 250µg/ml and 500µg/ml. The absorbance at 765nm of *Moringa oleifera* was 0.355 and 0.650 respectively. *Brassica oleracea* had an absorbance of 0.316 and 0.515 respectively.
Calibration curve of concentration against the absorbance was plotted. Showing in the Graph 5.1.The total polyphenol content of extract was determined by using linear regression equation derived from the graph.



Graph 1: standard calibration curve of Gallic acid for the determination of total phenolic content.

$X = \frac{Y-0.0312}{0.0217}$ Y=absorbance of sample

Total phenolic content in both *Moringa oleifera and Brassica oleracea* described in table 5.1

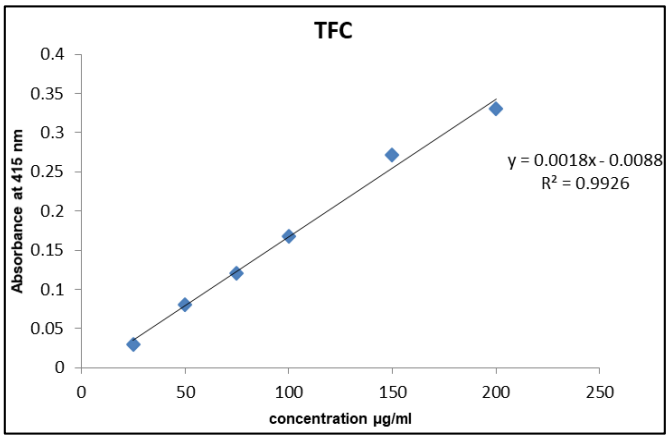
Table 1: GAE=Gallic acid equivalent

Sl No;	Extract	Total phenolic content
1	M*oringa oleifera*	59.6µg GAE/g extract
2	*Brassica oleracea*	52.4µg GAE/g extract

From the table it's clear that the total phenolic content was higher in *Moringa oleifera* (in both concentrations) when compare to *Brassica oleracea* (52.4µg GAE/g extract)

c. Determination of Total flavonoid content

To perform the calculation of total phenolic content in the studied plants of *Moringa oleifera* and *Brassica oleracea* using Kiranmai *et al*., method, a standard curve is needed which is obtained from a series of different Quercetin concentrations. The concentration of Quercetin was 25,50,75,100,150, and 200 mg/ml respectively. 0.03, 0.08, 0.121, 0.168, 0.272 and 0.33 was the absorbance of Quercetin in sequence. The absorbance of *Moringa oleifera* and *Brassica oleracea* were 0.05, 0.15 and 0.02, 0.041 respectively at the sample concentration of 250µg/ml and 500µg/ml.



Graph 2: standard calibration curve of Quercetin for the determination of total flavonoid content

X=Y+0.008/0.001 Y=absorbance of sample

Calibration curve of concentration against the absorbance was plotted. Showing in the Graph 5.2.The total flavonoid content of extract was determined by using linear regression equation derived from the graph. Total flavonoid content in both *Moringa oleifera and Brassica oleracea* described in table 5.2

Table 2: QE=Quercetin Equivalent

Sl No;	Extract	Total Flavonoid content
1	*Moringa oleifera*	232µg QE/g extract
2	*Brassica oleracea*	116µg QE/g extract

From the table 5.2, *Moringa oleifera* have higher concentration of flavonoid content, 232µg QE/g extract when compared with the *Brassica oleracea* (116µg QE/g extract).

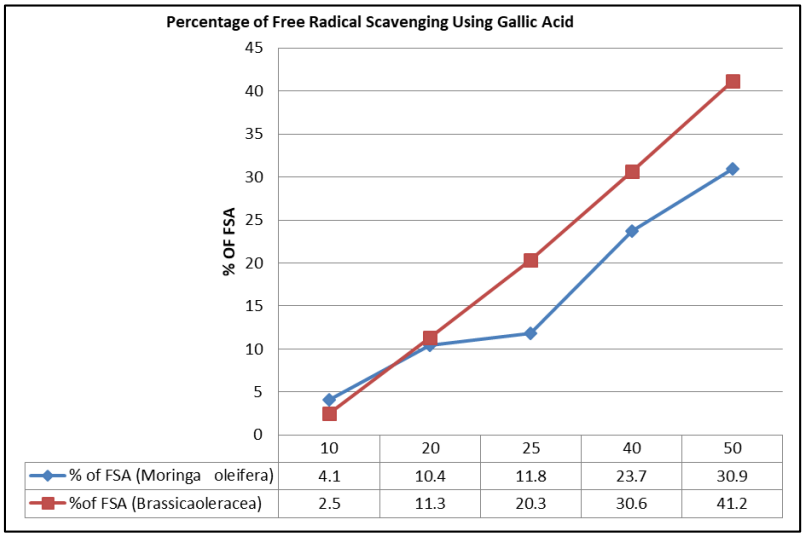
d. DPPH radical scavenging activity

The DPPH radical scavenging activity of the sample were calculated by using linear regression equation derived from the graph of percentage% scavenging activity vs. sample concentration and the IC_{50} of the sample were calculated. Free radical scavenging activity of Gallic acid was 3.3, 10.9, 22.4, 34.8, 47.7, and 63.2 respectively at the absorbance of 0.759.

For the determination of the % of scavenging activity of the samples, different concentrations of samples were taken.

Table 3: Free radical scavenging activity of the plants

Concentration of samples(µg/ml)	% of FSA (*Moringa oleifera)*	% of FSA *(Brassicaoleracea)*
10	4.1	2.5
20	10.4	11.3
25	11.8	20.3
40	23.7	30.6
50	30.9	41.2



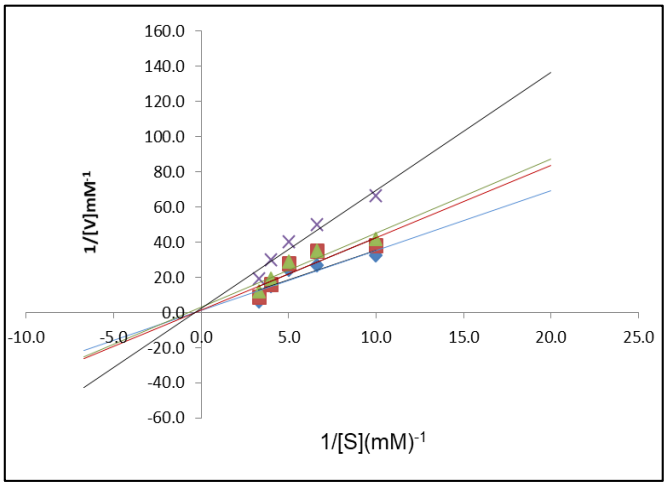
Graph 3: free radical scavenging activity of Moringa oleifera and Brassica oleracea sample concentration vs. percentage of inhibition

All extracts were able to reduce the stable free radical DPPH· to the yellow-coloured DPPH.

From the equations, derived from the graph (Both standard and sample) IC_{50} of the control and samples were calculated and it was expressed in terms of standard deviation. IC_{50} Of the control was 45µg/ml ±0.44 and for *Moringa oleifera*, $IC_{50}$was 79µg/ml ±0.32 and for *Brassicaoleracea*IC_{50} was59µg/ml ±0.82.The best free radical scavenging activity was exerted by *Moringa oleifera*. But both plant extracts showing higher radical scavenging activity than the Gallic acid (45µg/ml ±0.44).

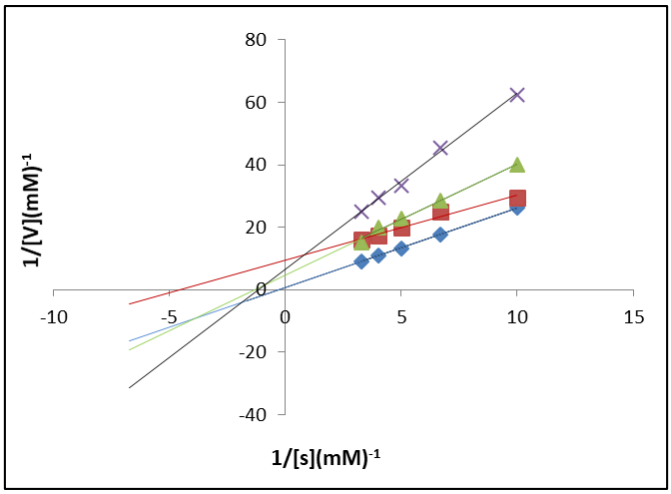
Lipase inhibitory activity

Lipase Line weaver-Burk plot from the relevant Michaelis-Menten equation, was determined for enzyme kinetic study using NPB as the variable substrate by measuring the hydrolysis of p-NPB to p-nitro phenol and with *Brassica oleracea*, *Moringa oleifera* in different concentration.



Graph 4: The Line weaver- Burk plot of kinetic analysis for inhibition of pancreatic lipase by p-NPB at different concentration (blue line) and Brassica oleracea in different concentration (green line-6µlred line-8µl, black line-10µl)

From the above graph the Km value of substrate and plant extract was calculated. The summary of kinetic parameters of the inhibition was shown in table13, Km and Vmax values were increased with increasing with concentration of sample in 8 and 10 µl.



Graph 5: The Line weaver- Burk plot of kinetic analysis for inhibition of pancreatic lipase by p-NPB at different concentration (blue line) and Moringa oleifera in different concentration (red line-6µl, green line-10µl, black line-8µl)

From the above graph the Km value of substrate and plant extract was calculated. The summary of kinetic parameters of the inhibition was shown in table5.4; vmax and Km were increased with increasing with concentration of sample.

Table 4

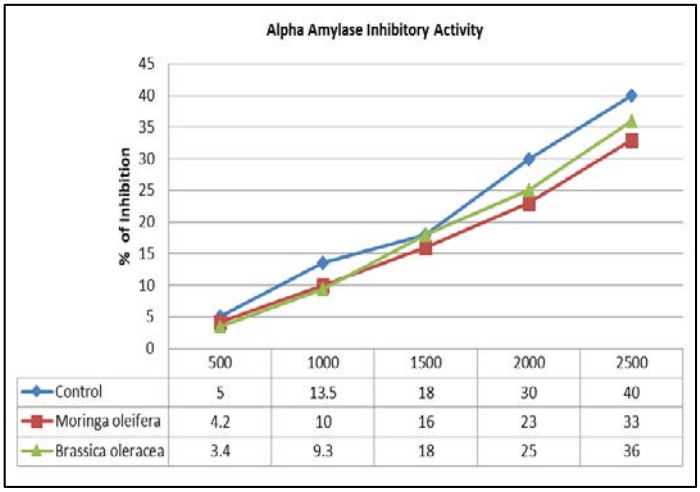
	Brassica oleracea			*Moringa oleifera*		
	6(µl)	8(µl)	10(µl)	6(µl)	8(µl)	10(µl)
Vmax $(Mm)^{-1}$	1	0.34	0.5	0.11	0.16	0.2
Km $(Mm)^{-1}$	1	1.25	3.3	0.22	0.67	0.83

f. Amylase inhibitory activity

In order to investigate the inhibitory effect of *Moringa oleifera* and *Brassica oleracea* extracts, an *in vitro* α-amylase inhibition test was performed. The following table 5.5 showing the percentage of α amylase inhibitory activity of *Moringa oleifera* extracts and control

Table 5

CON:µg/ml	% of Inhibition		
	Control	*Moringa oleifera*	*Brassica oleracea*
500	5	4.2	3.4
1000	13.5	10	9.3
1500	18	16	18
2000	30	23	25
2500	40	33	36

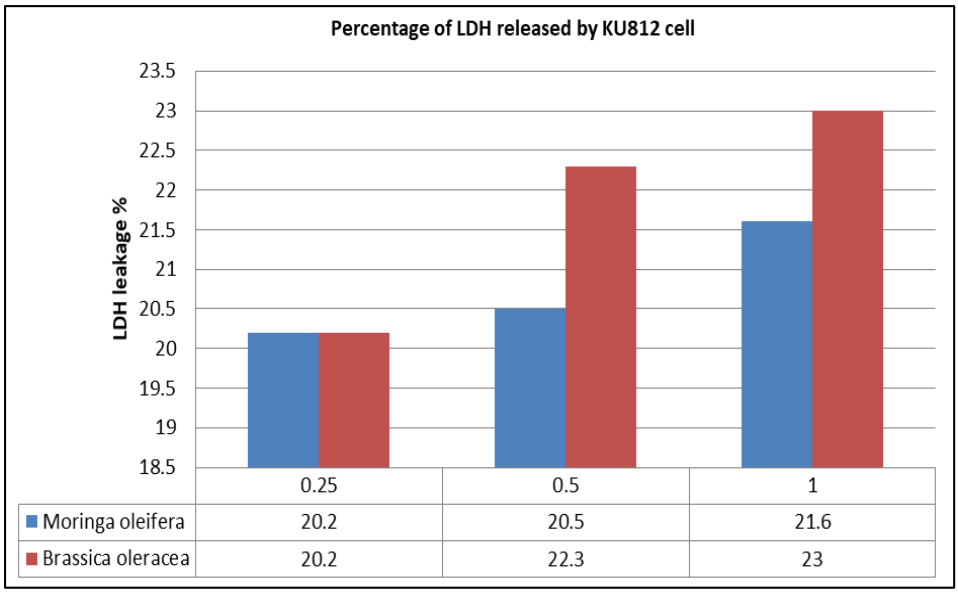


Graph 6: showing the % of α amylase inhibitory activity of starch as a control, Moringa oleifera and Brassica oleracea

From the above data we can concluded that both plants showing enzyme inhibitory activity. When the concentration of plant extract was increased, the percentage of inhibition also increased. In lower concentration (500µg/ml) M*oringa oleifera* & *Brassica oleracea* was showing inhibition like 4.2 and 3.4 % respectively. In high concentration (2500µg/ml) *Moringa oleifera*, *Brassica oleracea* showed inhibition at 33 and 36% respectively

Cytotoxicity assay

Cytotoxicity of plants was determined by the release of LDH in to the incubation medium. The LDH released curve for KU812 cell line treated with different concentration of the plants extract were down for the determination of the cytotoxic effect of the plants. This was compared with control (KU812 in medium without plant extract). The percentage of LDH released of control was 26%.



Graph 7: showing the percentage of LDH released by KU812 cell line treating with different concentration of Moringa oleifera and Brassica oleracea

Conclusion

The result of the present study showed that the ethanol-extract of *Moringa oleifera* and *Brassica oleracea* contains a total of twelve phytochemicals. Furthermore, total phenolics and flavonoid content along with the total antioxidant capacity for the extracts were determined. The results showed that these plants can be one of the potential sources of safer natural antioxidants. An array of antioxidant compounds are present in the extracts can effectively scavenge reactive oxygen species including superoxide Anions and hydroxyl radicals as well as other free radicals *in vitro.* And contains a good amount of phenolic antioxidants to counteract the damaging effects of free radicals and may protect against mutagenesis. Thus, replacement of synthetic antioxidants with secondary metabolites exhibiting safe and effective antioxidant activities (because of their manifestations on human health) from abundantly available plant sources such as *M. oleifera* and *B. oleracea* may be advantageous.

This study primarily investigated the potential inhibitory activity of the M. *oleifera and B. oleracea*, focusing on the inhibitory effects on lipase and a-amylase, there by the plants can reduces the some metabolic diseases such as diabetes mellitus, obesity etc.

Based on the *in vitro* cytotoxic study, the plant revealed that no cytotoxic effect when tested in KU812 cell line, used in the present study. So the oral consumption of this plant can ameliorate several disease conditions.

In conclusion, the results from this study present scientific support on the use of *M. oleifera* and *B. oleracea* for the treatment of diabetes, obesity by significantly inhibiting the activity of lipase and a-amylase.

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