INVITRO EVALUATION OF ANTICHOLINE ESTERASE ACTIVITY OF TRADITIONAL VEGETABLE'S JUICE

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ABSTRACT:

The purpose of this study was to investigate the in-vitro acetyl cholinesterase inhibitory activity of various commonly consumed traditional vegetables using Ellman's method, and hence the neuroprotective potential of these vegetables. Green cucumber, radish, ridge gourd, snake gourd, and carrot are all examples of vegetables. The primary enzyme associated with Alzheimer's disease pathogenesis is AChE. Alzheimer's disease is a neurodegenerative condition of cortical cholinergic neurons that causes sensory memory loss, odd behaviour, personality changes, and cognitive impairment. As a result, the current study takes a different strategy from a preventive standpoint, employing typical veggies as well as the substance of interest under inquiry. Natural dietary agents are an excellent source of nutrients and contain a variety of phytochemicals that may provide significant health advantages in addition to basic nutrition.The enzyme hydrolyzes the substrate Acetyl thiocholine, producing thiocholine as a byproduct.Which, when combined with Ellman's reagent (DTNB), produces 2-Nitrobenzoate, 5-mercaptothiocholine, and 5-thio 2-Nitro benzoate, which may be detected at 412nm. Among the veggies, green cucumber inhibited AchEase activity at a statistically significant greater level. The current investigation shed light on a variety of supporting evidences concerning protein concentration, polyphenol concentration, and other parameters. Apart from inhibiting AchEase activity, carrot is a rich source of antioxidants, and reducing oxidative stress is one of the key strategies for preventing and slowing the progression of Alzheimer's disease.

KEYWORDS: Acetyl cholinesterase, Alzheimer's disease, Ellman's assay.

1)INTRODUCTION

NEURODEGENERATIVE DISEASES:

The prevalence of neurodegenerative illnesses has increased in the global population, most likely as a result of greater life expectancy due to improved health care. Chronic neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and Amyotrophic Lateral Sclerosis (ALS), as well as those caused by an acute first insult, such as traumatic brain injury and stroke, are examples of neurodegenerative diseases. The primary basic mechanisms involved in chronic neurodegenerative illnesses are complex and caused by genetic, environmental, and

endogenous factors. Pathologically, these illnesses have one thing in common: the selective loss of a specific population of neurons for unclear reasons. Despite the fact that each disease has its unique biological processes andclinical manifestations, some general pathways might be recognised in different pathogenic cascades. neurodegenerative diseases[1-3].

ALZHEIMER'SDISEASE

Alzheimer's disease (AD) is the fourth greatest cause of death and the most common cause of dementia in the senior population among neurodegenerative disorders.4,5.

Alzheimer's disease is characterised by protein buildup in the brain. They are distinguished in two ways: Plaques are beta-amyloid protein deposits that form in the gaps between nerve cells. Tangles are deposits of the protein tau that build up inside nerve cells. In recent years, scientists have uncovered a genetic component in the development of Alzheimer's disease [6].

Nutritional substances are the earliest known kind of medicine, and the importance of human nutrition in maintaining health status dates back to Hippocrates (460-370BC), who declared, "Let food be your medicine, and medicine be your food." Healthy food is simply food that is believed to be healthy, either because it has a healthy image or because it contains elements usually assumed to be beneficial, such as fibre, anti-oxidants, and poly unsaturated fatty acids, and helps to reduce the risk of a specific chronic disease.[7,8].

Natural dietary agents such as fruits, green vegetables, and spices are high in fibre and vitamins. They also include polyphenols, terpenes, alkaloids, and glycosides, which may provide significant health advantages beyond basic nourishment and thus have a high nutritional value. They have attracted a lot of interest from the scientific community as well as the general population due to their proven capacity to suppress several chronic diseases[9,10].

Given the prominent preventive and therapeutic significance of dietary vegetables in human disease since ancient times, we chose a group of vegetables to evaluate their anticholinesterase activity in fresh juices and their role in inhibiting the enzymatic activity of

acetylcholinesterase, which is involved in the major pathological aspect and is a prominent target.Aside from that, the veggies' phenolic and antioxidant properties were being studied[11,12]. Extensive literature searches have revealed no evidence of in vitro acetylcholinesterase inhibitory actions for the vegetables listed below.

1. Green cucumber (Keera dosa)
2. Radish (Mullangi)
3. Snake gourd (Potlakaaya)
4. Ridge gourd (Beerakaaya)
5. Carrot (carrot)

2. MATERIALS AND METHODS

Vegetable		**Species name & family**	**Part used**
Green cucumber(GC)		*Cucumis sativus*, Cucurbitaceae	Fruit
Snake gourd (SG)		*Trichosanthes cucumerina*, Cucurbitaceae	Fruit
Radish (RD)		Raphanus sativus, Brassicaceae	Root
Ridge gourd (RG)		Luffa actangula , Cucurbitaceae	Fruit
Carrot (CT)		Daucus carota , Apiaceae	Root

Chemical constituents: [13,14]

Green cucumber(GC): Cucurbitacin, Oils, Niacin, Riboflavin, Water content, Dietary fiber.

Snake gourd (SG):proteins, fats, carbohydrates, Ca, P, Fe, thiamine, riboflavin, niacin, folate and ascorbic acid .
Radish (RD):4-methylthio-3-trans-butenyl isothiocyanate (Prota4u),Glucosinolates, Carbohydrates, Thiamine, Riboflavin (Prota 4 u).

Ridge gourd (RG):The Ridge gourd contains lactose, arabinose, rhamnose, ribose, xylose, galactose, glucose, mannose, fructose, sucrose and maltose

Carrot (CT): Beta-carotene, starch, extractine gluten, albumin, volatile, malic acid, niacin

2.1.Preparation Of Vegetables Juice:

Fresh vegetables were properly washed in running water before being chopped into little pieces. In a food grade blender, a paste of chopped and weighed veggies was made. To get juice, the paste was put onto a clean and sterile cotton cloth and squeezed. To compute the % yield, the volume of the resulting juice was measured in a measuring cylinder. The juice was centrifuged at 5000 rpm for 30 minutes at room temperature to achieve a clear supernatant.

2.2.Preliminary Phytochemical Analysis [15]:

The collected vegetable juices were subjected to chemical testing using the methods listed below to identify the individual contents using established procedures.

The weight/volume of fresh vegetable juice was determined at random using a specific gravity bottle using the formula w1-w2, where w2 is the weight acquired after evaporating the liquid and w1 is the beginning weight of vegetable juice..

Preparation of 20µg/ml ,40 µg/ml ,60 µg/ml, µg/ml80 and 100mg/ml concentrations

The wt/ml value of vegetable juices was determined, and the value obtained is being used to construct the sample's planned dilutions. utilising the formula

$C_1V_1 = C_2V_2$

2.3.ANALYSIS OF TOTAL POLYPHENOLIC AND PROTEIN CONTENT

Total polyphenols and total protein in the juice were analyzed as follows:

Determination of Total Polyphenol content (F-C method)

The total polyphenol content was determined using the Folin-Coicalteu reagent, as previously reported. In summary, fresh vegetable juice (25L) was reconstituted in 2.5mL distilled de-ionized water, then Folin-Coicalteu reagent (1N, 250L) and sodium carbonate (20% w/v Na2CO3, 250L) were added. For 60

minutes, the mixture was incubated at room temperature. On a microplate reader, absorbance (765nm) was measured spectrophotometrically. The total polyphenolic content was measured in micrograms of Gallic acid Equivalent per millilitre of juice (g GAE mL-1)[16].

2.4.Phytochemical Constituents Present in Traditional vegetable juice's

Sl. No.	Test	Results
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Fats	+
4.	Glycosides	-
5.	Phenolic compounds	+
6.	Proteins	+
7.	Tannins	-
8.	Aminoacids	+
9.	Gum and Mucilages	+

- Indicates Absent + Indicates Presence

2.5.TOTAL PROTEIN ESTIMATION

BRADFORD METHOD [17]

Method:

The 1mg/mL BSA was used to generate standards at 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8mg/mL. A 96 well plate was filled with 240 l of 1X Bradford reagent and 10 l of each standard. On a microplate reader, absorbance (595nm) was measured spectrophotometrically. The standard curve was calculated as Y = mX + C, with absorbance on the Y-axis and concentrations (mg/mL) on the X-axis. This equation was used to compute the protein in our sample by replacing Y with their relative absorbance.

$$x = \frac{y-c}{m}$$

Total protein content was expressed as micrograms of BSA Equivalent per millilitre of juice (µg BSAE mL^1

2.6.In-vitro Acetylcholinesterase activity:

Method for estimating RBC bound acetylcholinesterase activity:

Whole blood or washed human erythrocytes were used to create a very stable solution. Because the acetylcholinesterase is located on the cell membrane, hemolysis was not required. The blood test was performed as follows:

(1) A phosphate buffer (pH 8.0, 0.1 M) suspension of blood cells was produced.

(2) A cuvette was pipetted with 3.0 ml of the suspension.

(3) A total of 25l of DTNB reagent was added. The photometer was loaded with the cuvette.

(4) The photometer's slit was set so that the absorbance of the suspension in the cuvette at 412nm was zero. This cuvette received

(5) 20l of substrate. Changes in absorbance at 412nm were measured every 2 minutes for 20 minutes.

(6) Worksheets:The number of moles of substrate hydrolyzed per minute per RBC = (4.41) (l0-14). A/ RBC

Where 4.41 (l0-14) denotes the dilution factor, extinction coefficient, and unit changes.

A = absorbance change per minute, RBC = red cell count (in millions per mm3).

CALCULATION OF % INHIBITION OF AchE

$$\%\text{AchE inhibition} = \frac{\text{Absorbance(control)} - \text{Absorbance } [\ \frac{\text{Test}}{\text{Juice}}\ ]}{\text{Absorbance(control)}} \times 100$$

Calculation of IC_{50} for enzyme inhibition activity

IC_{50} was calculated by linear interpolation method using the formula

IC_{50}=50-A/B-A×(D-C)+C

Where

A =Percentage of inhibition, that is immediately less than50%

B = Percentage of inhibition, that is immediately greater than or equal to 50%

C = The concentration of inhibitor that gives A% inhibition

D = The concentration of inhibitor that gives B % inhibition

2.7. Statistical analysis:

The experimental data were statistically analysed using ordinary one-way ANOVA followed by Tukey's multiple comparisons test using the trial version of Graph Pad Prism, San Diego version (Prism graph pad version 8.0.2 (263, GraphPad Software, Inc. La Jolla, CA USA).

3.RESULTS

3.1.Preparation of vegetable's juice and Evaluation of parameters

Percentage yield of the vegetables' juice

$$\%\text{Yieldofjuice} = \frac{\text{Quantity of juice obtained (mL)}}{\text{Weight of vegetable taken (gm)}} \text{X100}$$

Vegetables	% Yield (mL $100g^{-1}$)
Green cucumber (GC)	41.2
Ridge gourd (RG)	42.6
Snake gourd (SG)	42.8
Radish (RD)	45.1
Carrot (CT)	36.1

Values are average of triplicate readings.

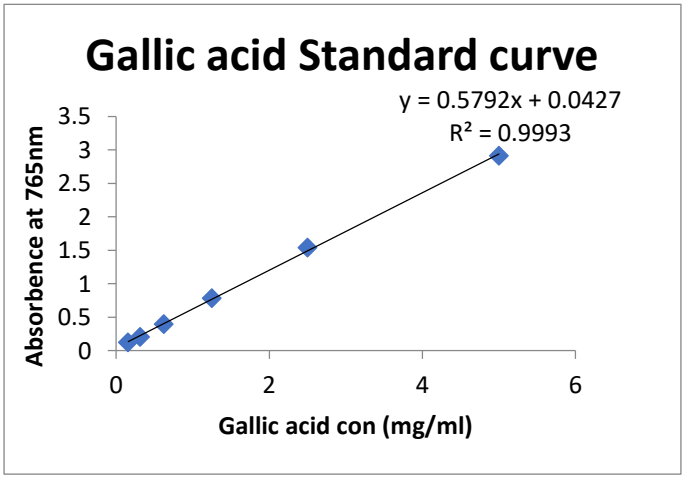
According to the findings, the average yield from various veggies ranged from 33-56%. The larger output of the RD could be ascribed to its porus stem, whereas the lowest yield of CT can be related to its hard modified root. Because IG is a little fruit vegetable and CH is a solid medium sized vegetable, the low yield in their produce is clear.The amount of juice does not indicate the availability of proteins and polyphenols..

3.2.PROTEIN ESTIMATION :

The absorbance readings of the test vegetable juice samples were substituted as y, and the protein content was calculated [BSA E=log(y-0.024)/0.723]. Although all three samples were high in protein, this investigation found substantial variances in total protein level in different vegetable juices. The RG had the highest protein concentration (1,255.60.10g/mL-1 BSA E). However, the protein content of SG (760.80.03g/mL-1 BSA E) and CT (752.30.08g/mL-1 BSA E) were comparable.All of the vegetables contain protein, however the RD has the lowest protein concentration (629.40.04g/mL-1 BSA E).

3.3.POLYPHENOL ESTIMATION:

There is a linear relationship between the phenolic concentration of the sample and its antioxidant capacity. In my investigation, I evaluated the total polyphenolic content of the samples using Gallic acid as the reference standard and quantified it as Gallic acid equivalents. The standard curve was created by plotting the average absorbance and their relative Gallic acid concentrations. In the equation obtained as y = 0. 579x + 0.042, R^2 = 0.999



3.4.TOTAL POLY PHENOL & PROTEIN CONTENT

Total protein and Polyphenol content in vegetables.

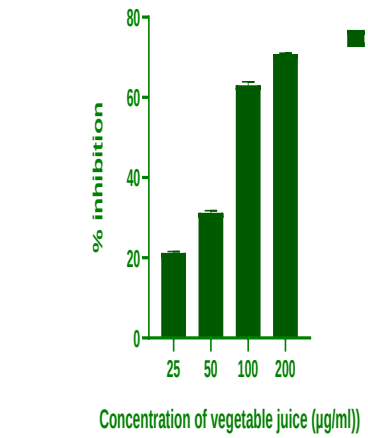
Vegetable	Total polyphenols (µg GAE mL^{-1})	Total Protein (µgBSAEmL^{1})
GC	143.8±0.01	633.1±0.04
RG	313.3±0.02	1255.6±0.10
SG	137.1±0.02	760.8±0.03
RD	187.9±0.01	629.4±0.04
CT	85.6±0.02	752.3±0.08

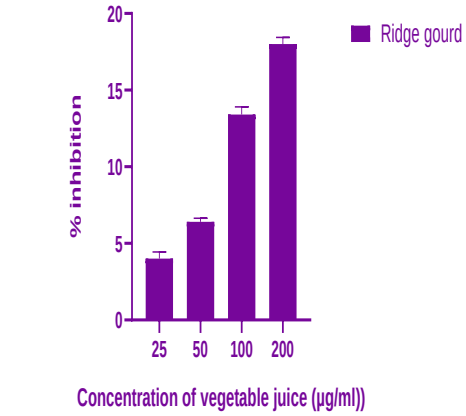
The overall polyphenol content concentration varied considerably. Snake gourd (137.10.02 gGAE/mL-1) and CT (85.60.02 gGAE/mL-1) had the lowest polyphenol concentration.

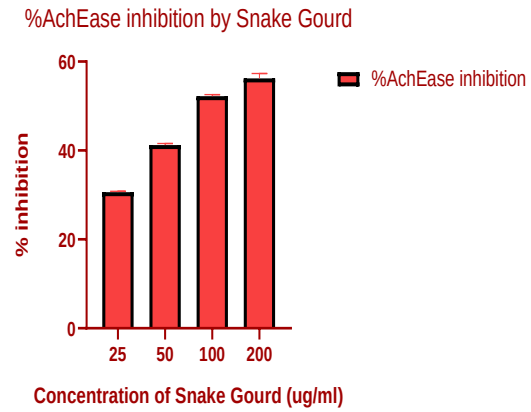
The juice of RG (313.30.02gGAE/mL-1), RD (187.90.010gGAE/mL-1) and GC (143.80.010gGAE/mL-1) had more polyphenols.

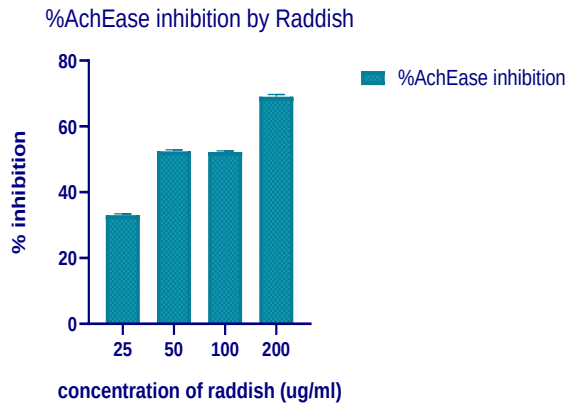
The juices of RG,SG,CT were found to be a rich source of protein, whilst the juices of veggies, RG, and GC were shown to be a rich source of both polyphenols and protein.

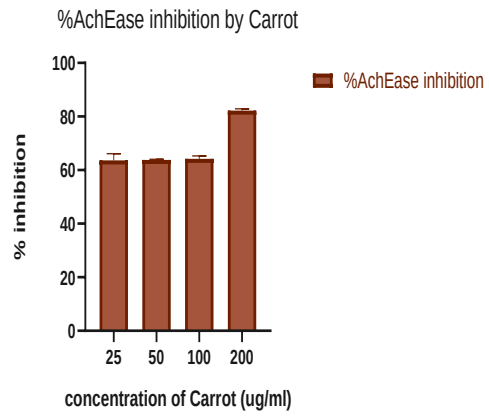
CONCENTRATION OF VEGETABLE"S JUICE (µg/ml)	GREEN CUCUMBER	RIDGE GOURD	SNAKE GOURD	RADISH	CARROT
25	21.200±0.3741657	4.000±0.4472136	30.600±0.244949	33.000±0.4472136	63.600±2.541653
50	31.200±0.5830952	6.400±0.244949	41.200±0.3741657	52.400±0.5099019	63.800±0.200
100	63.000±0.8944272	13.400±0.5099019	52.200±0.3741657	52.200±0.3741657	64.200±1.113553
200	70.800±0.200	18.000±0.4472136	56.200±±1.113553	69.000±0.7071068	82.200±0.663325
IC_{50} Values	68.19(µg/ml)	83.01(µg/ml)	51.11(µg/ml)	48.16(µg/ml)	37.41(µg/ml)

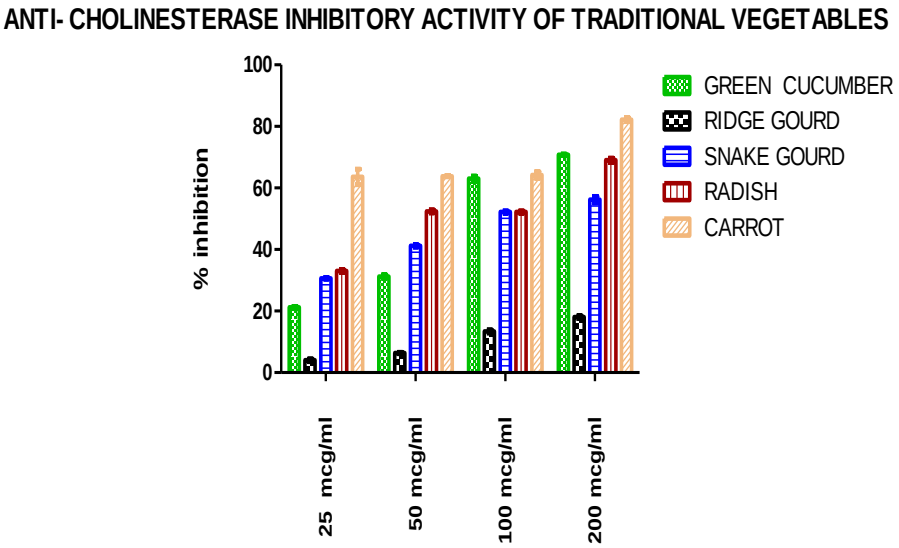

% AchEase INHIBITION BY JUICE OF GREEEN CUCUMBER
Green cucumber
% inhibition
0
20
40
60
80
25
50
100
200
Concentration of vegetable juice (µg/ml))


% AchEase INHIBITION BY JUICE OF RIDGE GOURD
Ridge gourd
% inhibition
0
5
10
15
20
25
50
100
200
Concentration of vegetable juice (µg/ml))


%AchEase inhibition by Snake Gourd
%AchEase inhibition
% inhibition
0
20
40
60
25
50
100
200
Concentration of Snake Gourd (ug/ml)


%AchEase inhibition by Raddish
%AchEase inhibition
% inhibition
0
20
40
60
80
25
50
100
200
concentration of raddish (ug/ml)


%AchEase inhibition by Carrot
%AchEase inhibition
% inhibition
0
20
40
60
80
100
25
50
100
200
concentration of Carrot (ug/ml)



4.CONCLUSION:

The purpose of this study was to investigate the in-vitro acetyl cholinesterase inhibitory activity of vegetable juices using Ellman's method, and therefore the neuroprotective potential of various commonly consumed traditional vegetables such as green cucumber, radish, ridge guard, snake guard, and carrot. The primary enzyme associated with Alzheimer's disease pathogenesis is AChE. As a result, the current study attempts to use traditional vegetables of our interest under inquiry. The current investigation shed light on a variety of supporting evidences concerning protein concentration, polyphenol concentration, and other parameters. Prism Graph Pad version 8.20 estimated the IC50 values for Green cucumber, Ridge gourd, and Snake gourd to be 68.19 g/ml, 83.01 g/ml, 51.11 g/ml, 48.16 g/ml, and 37.41 g/ml. Carrot and radish, respectively. These findings reveal that carrots block the AchEase enzyme more effectively than other vegetables.Aside potentially inhibiting AchEase activity, carrot is a rich source of antioxidants, and reducing oxidative stress is one major strategy for preventing and slowing the progression of Alzheimer's disease.

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