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ANTI-OXIDANT STUDIES ON *KSHEERAPAKA* OF FRESH AND DRIED ROOTS OF *BALA (SIDA CORDIFOLIA*. L) IN SHSY-5Y CELL MODEL OF NEURODEGENERATIVE DISEASES

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Abstract

Background: Neurodegenerative diseases are characterized by progressive neuronal loss, often exacerbated by oxidative stress. Traditional Ayurvedic formulations like *ksheerapaka* (medicated milk decoctions) have been explored for their therapeutic potential.

Objective: This study evaluates the antioxidant potential of *ksheerapaka* prepared from fresh and dried roots of Bala (*Sida cordifolia L.*) using in vitro activities.

Methods: Antioxidant activity was assessed through DPPH scavenging, nitric oxide scavenging, and superoxide anion scavenging activities. The capability of *ksheerapaka* to reduce Reactive Oxygen Species (ROS) was evaluated using the DCFDA assay in SH-SY5Y neuroblastoma cell lines. Statistical analysis was conducted to compare the activity of *ksheerapaka* prepared from fresh and dried roots of *Bala* (*Sida cordifolia*.L)

Results: Both preparations demonstrated concentration-dependent antioxidant activity, with the *ksheerapaka* prepared from dried root of *Bala* (*Sida cordifolia*.L) showing significantly higher efficacy ($p < 0.001$) across all assays. The DCFDA assay for invitro ROS measurement further revealed a considerable reduction in ROS levels by the dried root preparation.

Conclusion: The potent antioxidant activity of *ksheerapaka*, prepared from dried roots of *Bala* (*Sida cordifolia*.L), highlights its potential as a neuroprotective agent in mitigating oxidative stress implicated in neurodegenerative diseases. These findings warrant further in vivo and clinical studies to explore its therapeutic activity.

Keywords: Anti-oxidant activity, *Sida cordifolia*. L, *Ksheerapaka, Balamoola*, Neurodegenerative diseases.

I. Introduction

Neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, are debilitating conditions associated with progressive neuronal loss and functional decline. A hallmark of these disorders is oxidative stress, arising from an imbalance between the production of Reactive Oxygen Species (ROS) and the body's antioxidant defense mechanisms. Prolonged oxidative stress exacerbates neuronal damage, contributing to the progression of these diseases.

In Ayurveda, *Sida cordifolia L.*, commonly known as *Bala*, is revered for its *Balya, Vatahara, and Rasayana* (rejuvenative) properties. Traditional formulations like *ksheerapaka*, are believed to enhance the therapeutic potency of Bala. Recent studies have suggested that drying herbs may further concentrate their active constituents, potentially augmenting their efficacy.

This study aims to evaluate the antioxidant potential of *ksheerapaka* prepared from fresh and dried roots of *Sida cordifolia L.* using in vitro assays, including DPPH scavenging, nitric oxide scavenging, and superoxide anion scavenging, alongside the DCFDA assay in SH-SY5Y neuroblastoma cell lines, to assess the efficacy of these preparations in combating oxidative

stress. By comparing the activity of fresh and dried roots, this research aims to provide insights into optimizing the preparation of *ksheerapaka* of *Bala* for neuroprotective activity.

II. MATERIALS AND METHODS

A. Collection of the drug

The study drug *Sida cordifolia*. L was collected from Vencode, Kanyakumari district in October. The whole plant and its roots were collected in the respective flowering season from locations free from contamination and infestation. The plants were collected in dry weather and kept in a clean sack in the shade. The weeds and unwanted plant parts were carefully removed. The roots were sorted and washed carefully in clean water using a high-powered spray nozzle to clean off the mud. The water was drained, and one part of the roots were kept for drying in the shade during the morning hours and the other part was kept as fresh. The roots attained uniform drying in about 9 to 10 days.

B. Identification

The faculty identified the collected plant specimens in the Department of Dravyaguna vigyana, Govt. Ayurveda College, Tripunithura. The plant was authenticated at the Department of Botany St Albert's College, Ernakulam (Voucher No:585). A herbarium specimen was prepared for th plant samples. The whole plant along with its flower was pressed to prepare the herbarium.

C. Preparation of *Ksheerapaka* (milk decoction)

The *ksheerapaka* was prepared following the guidelines outlined in Sarangadhara Samhitha.

Ksheerapaka of fresh roots were prepared by crushing the roots using motor and pistil and the *ksheerapaka* of dried roots were prepared by taking the fresh roots of *Sida cordifolia* L. It was then dried, and chopped into small pieces, made into a coarse powder and stored in airtight containers.

To prepare the *ksheerapaka*, the drugs, milk, and water were combined in a 1:8:32 ratio, respectively, in a steel vessel. In vitro studies require only a small amount of *ksheerapaka*. The process began with the addition of 31.25g of the crushed drug, followed by 250 ml of

milk, and then one litre of water. The mixture was boiled and reduced to the quantity of milk. A 250ml of *ksheerapaka* was prepared daily and filtered through 10 layers of muslin cloth to remove sediments.

D. Determination of in vitro antioxidant activities of *ksheerapaka* of fresh and dried roots of *Bala* (*Sida cordifolia*. L).

i.Anti-oxidant activity and Acetylcholinesterase (ACHE) Inhibitory Activity were assessed using in vitro biochemical studies.

ii.Cellular responses for Reactive Oxygen Species (ROS) quantification were evaluated using in vitro studies on SH-SY5Y Human neuroblastoma cell lines.

i. In vitro biochemical studies.

a. Anti-oxidant Assays

Antioxidant studies are conducted using test tube methods, to evaluate the ability of compounds to neutralize oxidative stress. Here DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Assay, Nitric Oxide Scavenging Activity, and Super Oxide Free Radical Scavenging Activity were performed.

1) DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Assay

Aim

To study the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging activity of *ksheerapaka* prepared from the fresh and dried roots of *Bala-Sida cordifolia*. Linn.

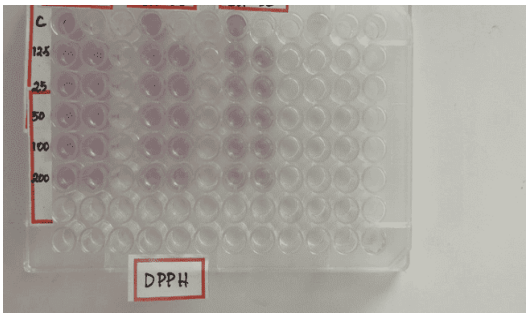
Reagent preparation

0.1 mM DPPH solution was created by dissolving 4 mg of DPPH in 100 ml of methanol.

Procedure

Different concentrations of the sample, ranging from 12.5 µl/mL to 200 µl/mL, were prepared from the stock solution, and adjusted to a final volume of 20 µl with DMSO. Then, 1.48 ml of DPPH (0.1 mM) solution was added. A control was set up without the test compound, using an equivalent amount of distilled water. The reaction mixture was allowed to sit in the dark at room temperature for 20 minutes. After this period, the absorbance of

the mixture was measured at 517 nm using a SHIMADZU (UV-1900i) UV-VIS spectrophotometer. For the control, 3 ml of DPPH solution was used. Ascorbic acid (10 mg/ml in DMSO) served as the reference. The scavenging activity was then calculated using a specific formula.[1]



Pic 1: 96 well plate containing *ksheerapaka* of dried roots of *Sida cordifolia.* L and DPPH radical scavenging assay reagents

Calculation:

Radical scavenging activity = (OD of Control- OD of Test) x100

OD of Control

2) Nitric Oxide Scavenging Activity

Aim:

To study the nitric oxide scavenging activity of *ksheerapaka* prepared from the fresh and dried roots of *Bala (Sida cordifolia*. Linn.)

Procedure

A volume of 1.5 mL of sodium nitroprusside (5 mmol/L concentration in phosphate-buffered saline at pH 7.4) was combined with varying concentrations of the test sample, ranging from 125 µL/mL to 2000 µL/mL, derived from a stock concentration of 10 mg/mL, and subjected to a 60-minute incubation at 25°C. A control lacking the test compound but containing an equivalent quantity of distilled water was concurrently prepared. Following the incubation period, 1.5 mL of the resultant solution was extracted and subsequently diluted with 1.5 mL of Griess reagent (comprising 1% sulphanilamide, 2% phosphoric acid, and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore, formed

by the diazotization of nitrate with sulphanilamide followed by coupling with N-1-naphthyl ethylenediamine dihydrochloride, was measured at 546 nm using a spectrophotometer. a UV-visible-light spectrophotometer (SHIMADZU- UV-1900i), and the scavenging activity percentage was evaluated to the standard gallic acid.[2]

Calculation

$$\text{Nitric oxide Scavenging (\%)} = \frac{(\text{OD of control} - \text{OD of test}) \times 100}{\text{OD of control}}$$

3) Super Oxide Free Radical Scavenging Activity

Aim:

To study the superoxide free radical scavenging activity of *ksheerapaka* prepared from the fresh and dried roots of *Bala (Sida cordifolia*. Linn).

Procedure

Various concentrations of the sample, ranging from 125 to 2000µL/mL derived from a 10mg/mL stock solution, were combined with 0.05mL of Riboflavin solution (0.12mM), 0.2mL of EDTA solution (0.1M), and 0.1mL of Nitro-blue tetrazolium solution (NBT) (1.5mM) were taken in a test tube. Subsequently, the reaction mixture was diluted to 2.64mL with phosphate buffer (0.067M). A control sample, containing an equivalent amount of distilled water but lacking the test compound, was also prepared. The absorbance of the solution was measured at 560 nm after 5 minutes of incubation under fluorescent light, and again after 30 minutes of incubation at 560 nm, using a UV-visible spectrophotometer (SHIMADZU-UV-1900i).[3]

Pic 2: Fluorescence illumination in superoxide scavenging assay

Calculation

Super Oxide Free Radical Scavenging (%) = (OD of control - OD of test) x 100
OD of control

4. Acetylcholinesterase (AChE) Inhibitory Activity

Aim:

To study the Acetylcholinesterase (AChE) Inhibitory activity of *ksheerapaka* prepared from the fresh and dried roots of *Bala(Sida cordifolia*. Linn). using a microplate assay.

Procedure

The enzymatic activity of acetylcholinesterase (AChE) was quantified utilizing a modified 96-well microplate assay based on Ellman's method. In this assay, the substrate acetylthiocholine is enzymatically hydrolyzed by AChE, yielding thiocholine, which subsequently reacts with Ellman's reagent (DTNB) to generate 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate, detectable at 412nm.A 50 mM Tris-HCl buffer at pH 8.0 was consistently employed throughout the experiment unless otherwise specified. The AChE utilized in this study was procured from Electrophorus electricus (electric eel) (Type VI-S lyophilized powder, with an enzymatic activity of 222 U/mg solid or 268 U/mg protein). The enzyme stock solution, with an activity of 222 U/mL, was maintained at -80˚C. Subsequent dilutions of the enzyme were prepared in 0.1% BSA solution in the buffer. DTNB was dissolved in a buffer solution containing 0.1 M NaCl and 0.02 M $MgCl_2$, while acetylthiocholine iodide was dissolved in deionized water.To initiate the assay, 100µL of 3mM DTNB, 20µL of 0.26 U/mL AChE, and 40µL of buffer (50 mM Tris, pH 8.0) were dispensed into a 96-well plate. Furthermore, 20µL of each extract at varying concentrations (25, 50, 100 µL/mL) dissolved in buffer was introduced into the plate. Following a 15-minute incubation period at 25 ˚C, the absorbance was assessed at 412nm. Subsequently, the enzymatic reaction was initiated by the addition of 20µL of Acetylthiocholine iodide. Absorbance readings were then recorded at two distinct time points: 5 minutes and 20 minutes after mixing.[4]

a. Calculation

% inhibition = (E-ΔOD) *100
E

Where E is the absorbance of the control and ΔOD represents the absorbance of the sample +inhibitor.

In addition to antioxidant assays, quantitative ROS (reactive oxygen species) measurements were also done to explore the antioxidant activity. This study was done in the *ksheerapaka* of dried roots of *Bala(Sida cordifolia*. L)

Culturing of the SH-SY5Y cell line was needed for further studies. The in vitro cytotoxic effect of *Ksheerapaka* was assessed and further pre-conditioning and post-conditioning were done to find the concentration at which cells experience maximum viability. Further, that concentration was used for quantitative ROS (reactive oxygen species) measurement.

ii. In vitro ROS measurement using DCFDA in SH-SY5Y (Human Neuroblastoma cells) cell line.

Aim:

To measure the invitro Reactive Oxygen Species using DCFDA in SH-SY5Y (Human Neuroblastoma cells) cell line.

Procedure:

The SH-SY5Y (Human Neuroblastoma cells) cell line was cultured using standard procedures. Beta Amyloid (10μM) was added to induce toxicity. After 1-hour samples, *Ksheerapaka* of *Sida cordifolia.* L *(*25μL/ml each) was added and then incubated at 37ºC in a humidified 5% CO_2 incubator for 24 hours. Non-treated control cells and β-amyloid treated cells were also maintained.

The cells were washed with PBS, added with 50 μl of DCFDA, and incubated for 30 minutes. After incubation, the excess dye was rinsed off with PBS, and fluorescence was captured using a fluorescent microscope (Olympus CKX41 with Optika Pro5 CCD camera). Fluorescence intensity was measured with a fluorimeter (Qubit 3.0, Life Technologies, USA) at an excitation wavelength of 470 nm and an emission wavelength of 635 nm, and the results were recorded in arbitrary units.

III. RESULTS

In vitro biochemical assays conducted in test tubes for determining the antioxidant activity of *ksheerapaka* of fresh and dried roots of *Sida cordifolia*. L showed the following observations.

i. Antioxidant assays

1) DPPH(2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical that appears pink in color but turns yellow when neutralized. In this assay, antioxidants (denoted as H-A) react with DPPH, reducing it to DPPH-H, as shown in the reaction:

$$DPPH+[H\text{-}A] \rightarrow DPPH\text{-}H+(A)$$

As the antioxidant interacts with DPPH, the absorbance decreases, reflecting a change in color from pink to yellow. The extent of this color change indicates how effectively the antioxidant compound or extract can donate hydrogen and neutralize free radicals. The ability of the *ksheerapaka* to scavenge DPPH radical was determined in terms of percentage inhibition. The percentage inhibition of different concentrations of *ksheerapaka* of fresh and dried roots of *Sida cordifolia.* have been given in the table below.

Table 1: Percentage inhibition of different concentrations of *ksheerapaka* of fresh and dried roots of *Sida cordifolia.* L in DPPH radical scavenging assay

Concentration of *ksheerapaka* (µL)	**Percentage inhibition (%) of *ksheerapaka* of dried roots of *Sida cordifolia*. L**	**Percentage inhibition (%) of *ksheerapaka* of fresh roots of *Sida cordifolia*. L**
Control	0	0
12.5	16.675 ±0.65997	6.55 ±0.53
25	25.781 ±1.23068	18.39±0.12
50	33.507 ±0.73827	26.76±0.20
100	45.573 ±0.86806	33.33±0.42
200	64.931 ±0.82503	42.65±0.35

2) Nitric oxide scavenging activity

The process is based on the ability of sodium nitroprusside in an aqueous solution at physiological pH to spontaneously generate nitric oxide (NO). This NO then reacts with oxygen to form nitrite ions, which can be quantified using Griess reagent. Elevated levels of NO may result in tissue damage. Nitric oxide scavenging activity was measured spectrophotometrically and the efficacy of *ksheerapaka* in neutralizing nitrite ions is quantified in terms of percentage inhibition. The percentage inhibition of different concentrations of *ksheerapaka* of fresh and dried roots of *Sida cordifolia.* L has been given in the table below.

Table 2: Percentage inhibition of different concentrations of *ksheerapaka* of fresh and dried roots of *Sida cordifolia.*L in nitric oxide anion scavenging activity.

Concentration of *ksheerapaka* (µL)	**Percentage inhibition (%) of *ksheerapaka* of dried roots of *Sida cordifolia*. L**	**Percentage inhibition (%) of *ksheerapaka* of fresh roots of *Sida cordifolia*. L**
Control	0	0
125	6.8598 ±1.99	7.773±0.80
250	31.199 ±2.23	15.30±0.73
500	48.77 ±3.31	32.63±0.90
1000	65.335 ±2.08	41.81±0.30
2000	74.919±2.91	53.25±0.14

3) Superoxide-free radical scavenging activity

Superoxide is a reactive molecule that can generate other harmful substances like singlet oxygen and hydroxyl radicals. In this test, superoxide is produced using a system that involves riboflavin and NADH (Nicotinamide adenine dinucleotide, a coenzyme). The superoxide then reacts with the chemical NBT (nitroblue tetrazolium), which turns blue when reduced, forming a product called formazan. The intensity of the blue colour indicates the amount of superoxide present. The percentage inhibition of different concentrations of *ksheerapaka* has been given in the table below.

Table 3: Percentage inhibition of different concentrations of *ksheerapaka* of fresh and dried roots of *Sida cordifolia.* L in Superoxide-free radical scavenging activity.

Concentration of *ksheerapaka* (µL)	**Percentage inhibition (%) of *ksheerapaka* of dried roots of *Sida cordifolia*. L**	**Percentage inhibition (%) of *ksheerapaka* of fresh roots of *Sida cordifolia*. L**
Control	0	0
125	14.266 ±1.37	8.023±0.35
250	33.915 ±1.19	14.07±0.46
500	50.91 ±1.11	30.18±0.12
1000	70.394 ±0.84	38.55±0.95
2000	77.186 ±1.28	54.82±0.40

4. Acetylcholinesterase (AChE) Inhibitory Activity

Acetylcholinesterase (AChE) enzyme helps in the degradation of acetylcholine, a neurotransmitter essential for memory and learning. In the context of neurodegenerative diseases, acetylcholine levels are notably diminished, which contributes to cognitive decline. Inhibiting AChE slows the breakdown of acetylcholine, resulting in elevated levels of this neurotransmitter in the brain. The efficacy of *ksheerapaka* in inhibiting AChE was evaluated using a microplate assay, and the results are presented in terms of percentage inhibition in the table below**.**

Table 4: Percentage inhibition of different concentrations of *ksheerapaka* of fresh and dried roots of *Sida cordifolia*. L in Acetylcholinesterase (AChE) inhibitory activity

Concentration of *ksheerapaka(*µL*)*	**Percentage inhibition (%) of *ksheerapaka* of dried roots of *Sida cordifolia*. L**	**Percentage inhibition (%) of *ksheerapaka* of fresh roots of *Sida cordifolia*. L**
Control	0	0
25	11.685 ±0. 59	4.433±0.32
50	20.956 ±0. 68	12.97±0.60
100	31.619 ±0. 81	22.92±0.99

ii. In vitro ROS studies on SH-SY5Y neuroblastoma cell lines.

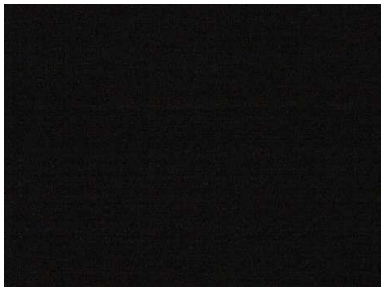
Invitro ROS measurement using DCFDA

The purpose of this assessment was to quantitatively evaluate intracellular reactive oxygen species (ROS) levels and to assess the effectiveness of *ksheerapaka* of dried roots of *Sida cordifolia*. L , in reducing oxidative stress, a significant factor in neurodegenerative processes.

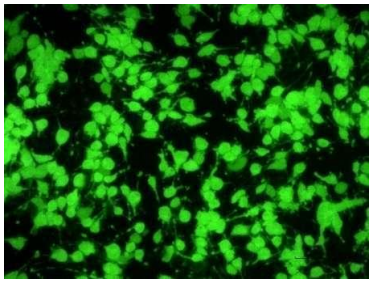
For the quantification of intracellular ROS levels, DCFDA (2',7'-dichlorofluorescein diacetate), a fluorogenic dye that undergoes conversion to a green fluorescent compound upon oxidation was used. The experimental procedure involved incubating cells with DCFDA, removal of excess dye through washing, and subsequent imaging and quantification of fluorescence. The obtained data were then compared among untreated (control) cells, cells treated with 25µL of *ksheerapaka* of dried roots of *Sida cordifolia*. L, and cells administered with beta-amyloid (10µM) with excitation at 470 nm and emission at 635 nm.[5]

Table 5: Invitro ROS measurement of *ksheerapaka* of dried roots of *Sida cordifolia*. L

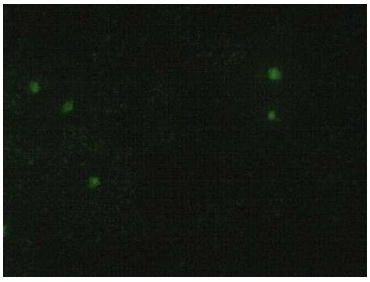
The sample treated in SH-SY5Y cells	**Fluorescence Intensity (AU)**
Control(untreated)	657.79
Beta-amyloid	21340.55
Beta amyloid+ *Sida cordifolia. L ksheerapaka*	3269.00



Fluorescence image of SH-SY5Y control cells.



Fluorescence image of beta-amyloid induced SH-SY5Y cells with DCFDA dye



Fluorescence image of beta-amyloid induced and post-treated with *ksheerapaka* of *Sida cordifolia*. L, SH-SY5Ycells with DCFDA dye

Picture 3: Microphotographs representing the invitro ROS measurements of SH-SY5Y cells on post-treatment with *ksheerapaka* of dried root of *Sida cordifolia.* L.

IV. ANALYSIS

The antioxidant activity of *ksheerapaka* (milk decoction) of fresh and dried roots of *Bala* (*Sida cordifolia*. L), was compared at different concentrations. The data were expressed as the mean of three replicates with standard error. Independent t-test was used to analyze the significance between various concentrations of *ksheerapaka* of fresh and dried roots of *Bala* (*Sida cordifolia*. L). Statistical significance was determined using p-values, where $p < 0.05$ indicates significance and $p < 0.001$ indicates high significance.

Table 6: Statistical results from the independent t-tests comparing the DPPH scavenging activity of *Ksheerapaka* from fresh and dried roots of *Bala*(*Sida cordifolia*. L) at different concentrations are as follows

Concentration (µL) of *ksheerapaka*	**p-value**	**Interpretation**
12.5	0.000046	Highly significant difference
25	0.008628	Significant difference
50	0.002349	Highly significant difference
100	0.000263	Highly significant difference
200	0.000066	Highly significant difference

Table 7: Statistical results from the independent t-tests comparing the nitric oxide anion scavenging activity of *Ksheerapaka* from fresh and dried roots of *Bala* (*Sida cordifolia*. L) at different concentrations are as follows

Concentration (µL) of *ksheerapaka*	p-value	Significance
125	0.7462	Not significant
250	0.0066	Significant
500	0.0261	Significant
1000	0.0009	Highly significant
2000	0.0779	Not significant

Table 8: Statistical results from the independent t-tests comparing the Superoxide-Free Radical Scavenging Activity of *Ksheerapaka* from fresh and dried roots of *Bala*(*Sida cordifolia*. L) at different concentrations are as follows

Concentration (µL) of *ksheerapaka*	p-value	Significance
125	0.0379	Significant
250	0.0026	Significant
500	0.0029	Significant
1000	0.000009	Highly significant
2000	0.000011	Highly significant

Table 9: Statistical results from the independent t-tests comparing the Acetylcholinesterase (AChE) inhibitory activity of *ksheerapaka* from fresh and dried roots of *Bala* (*Sida cordifolia*. L) at different concentrations are as follows

Concentration (µL) of *ksheerapaka*	p-value	Significance
25	0.0047	Significant
50	0.0226	Significant
100	0.0022	Significant

V. DISCUSSION

At all concentrations, the differences between the antioxidant activities of *ksheerapaka* from fresh and dried root are statistically significant ($p < 0.05$). The antioxidant activity of *Ksheerapaka* of dried root of *Bala* (*Sida cordifolia*. L) is consistently higher than that of fresh roots. Mean Percentage Inhibition at every concentration is higher for the dried root preparation than for the fresh root preparation.

On Statistical analysis, the DPPH scavenging activity of *Ksheerapaka* from fresh and dried roots of *Bala* (*Sida cordifolia*. L) at all concentrations showed significant or highly significant differences. the nitric oxide anion scavenging activity showed significant or highly significant differences at intermediate concentrations (250 µL, 500 µL, and 1000 µL), reinforcing that the dried root preparation is consistently more effective. The *ksheerapaka* of dried roots of *Bala* (*Sida cordifolia* L**.**) shows significantly higher superoxide-free radical scavenging activity than fresh roots at all tested concentrations, with highly significant results at higher doses. At all tested concentrations (25 µL, 50 µL, and 100 µL), the difference in AChE inhibitory activity between *ksheerapaka* of dried and fresh roots of *Bala* (*Sida cordifolia*. L)is statistically significant. The *ksheerapaka* prepared from dried root of *Bala* (*Sida cordifolia*. L) consistently exhibits higher AChE inhibitory activity than the *ksheerapaka* prepared from fresh root of *Bala* (*Sida cordifolia*. L), suggesting enhanced efficacy.

In all the three antioxidant assays, the increase in concentration of *ksheerapaka* improved the antioxidant activity. Thus, indicating that the free radicals involved in the different antioxidant assays were scavenged by both the *ksheerapaka* in a concentration-dependent manner. Thus, suggesting that both the *ksheerapaka* can be useful in neurodegenerative diseases. The *ksheerapaka* of dried roots of *Sida cordifolia*. L showed better percentage inhibition than the *ksheerapaka* of fresh roots of *Sida cordifolia*. L.

In the Acetylcholinesterase (AChE) inhibitory activity, all three concentrations were found to exhibit highly significant acetylcholinesterase (AChE) inhibitory activity ($p< 0.001$). The inhibitory activity was found to increase with the increase in concentration of the *ksheerapaka* and the maximum inhibitory activity was exhibited at 100µL for both

samples. However, the percentage inhibition of *ksheerapaka* of dried roots of *Sida cordifolia*. L was greater than that of *ksheerapaka* of fresh roots of *Sida cordifolia*. L.

Upon measuring the invitro Reactive Oxygen Species using DCFDA in the SH-SY5Y (human neuroblastoma) cell lines, the untreated control cells showed a fluorescence intensity of 657.79AU, when treated with beta-amyloid (10 μM) the fluorescence intensity was observed as 21340.55AU. The test cells were treated with beta-amyloid (10 μM), incubated for 1 hour, and later treated with 25 μL of *ksheerapaka* of dried roots of *Sida cordifolia.* L in separate well plates and incubated for 24 hours and the fluorescence intensity was observed as 3269.00AU for *ksheerapaka* of dried roots of *Sida cordifolia*. L

VI. CONCLUSION

The antioxidant activity of the drug was assessed using DPPH scavenging assay, nitric oxide scavenging assay, and superoxide anion scavenging activity, with both *ksheerapaka* preparations showing highly significant antioxidant activity ($p < 0.001$) in a concentration-dependent manner. Between the two, the *ksheerapaka* of dried roots of *Bala (Sida cordifolia.* L)exhibited superior activity, likely due to enhanced phytochemical concentrations. These assays suggest that *ksheerapaka* effectively scavenges free radicals like hydroxyl, superoxide, and nitric oxide, which are key contributors to neurodegeneration. Furthermore, the in vitro Reactive Oxygen Species (ROS) assay using DCFDA in SH-SY5Y neuroblastoma cell lines demonstrated that the *ksheerapaka* of dried roots of *Bala* (*Sida cordifolia* L). significantly reduced ROS levels, underscoring its potential as a neuroprotective agent. These findings pave the way for further in vivo and clinical studies to explore its efficacy in managing oxidative stress-related neurodegenerative conditions.

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