



ANTIOXIDANT ACTIVITY OF SIDDHA POLYHERBAL FORMULATION MADHU MUKTHI KUDINEER CHOORANUM.

Dr. R. Baskar^{1*}, DR. Bharatkumar², DR. S. S. Selvapriya³, Dr. T. Hemadevi⁴,
Dr. A. Shameem Fathima⁵

Abstract

Introduction:

The present study aimed to evaluate the antioxidant potential of the Siddha polyherbal formulation, Madhu Mukthi Kudineer Chooranum (MKC). A series of in-vitro assays, including the DPPH, Nitric Oxide, ABTS, and Hydrogen Peroxide radical scavenging assays, were employed to assess its antioxidant capabilities.

Keywords: Madhu Mukthi Kudineer Chooranum, Antioxidant Activity, DPPH Assay, Nitric Oxide Assay, ABTS Assay, Hydrogen Peroxide Assay.

¹*Lecturer -2, Govt. Siddha Medical college, Chennai-106.

²Asst. professor, Dept. of. Siddha, The Tamilnadu Dr. M. G. R. Medical University, Chennai -32.

³PG Scholar, Govt. Siddha Medical college, Chennai-106.

⁴Amo, Arignar Anna Govt Hospital of Indian Medicine, Chennai-106.

⁵Lecturer -2, Govt. Siddha Medical College, Chennai -106 Email: drbaskarmd06@gmail.com

***Corresponding Author:** Dr. R. Baskar

*Lecturer -2, Govt. Siddha Medical college, Chennai-106.

DOI: 10.53555/ecb/2024.13.01.43

Introduction

The siddha system evolved with the development of mankind. This system remains a mystery to practitioners of other native medical systems of India. It is one of the oldest systems to describe health as a holistic perfected state of the physical, psychological, social and spiritual components of a human being. Polyherbal compositions have a long history of recognition in the field of siddha medicine, for their potential therapeutic advantages. The Madhu

Mukthi Kudineer Chooranum (MKC) is one such concoction.^{1,2}

Extensive studies have been conducted on natural sources of antioxidants in response to the rising worldwide incidence of illnesses associated with oxidative stress. Antioxidants are essential for scavenging dangerous free radicals, which in turn stops cellular damage and a host of degenerative disorders. MKC is a Siddha polyherbal compound that has been used for many years in traditional practices because of its possible medicinal advantages. Conventional wisdom indicates that it may be useful in treating illnesses associated with oxidative stress. However, in the modern scientific environment, it is imperative to provide empirical support for these conventional statements.

The objective of this research is to clarify MKC's antioxidant activity using several in-vitro tests. The selection of these tests is based on their proven validity and applicability in evaluating natural products' antioxidant capacity. The research specifically uses the Nitric Oxide Radical Scavenging Assay, the DPPH (2,2-diphenyl 1-2 picrylhydrazyl) assay, the ABTS (2,2'-azino-bis(3-

ethylbenzothiazoline-6-sulphonic acid)) assay, and the Hydrogen Peroxide Radical Scavenging Assay.

Through the use of these assays, the study aims to measure MKC's capacity to scavenge radicals and compare it to industry benchmarks including ascorbic acid, gallic acid, and BHA. The knowledge gained from this research may clarify MKC's potential as a natural source of antioxidants and open the door to further research into its possible use as a medicinal.

In the following parts, we provide a thorough analysis of the results, go into more detail about the methodology used, and show the outcomes obtained. The major goal is still to connect the conventional understanding of MKC with the most recent scientific evidence, promoting a harmonic fusion of traditional wisdom and modern science in the pursuit of better health outcomes.

MATERIALS AND METHODS

Plant Material

Procurement of plant material

The required bark and roots are collected from natural habitat at Jolarpettai and foot hills of Elagiri. The raw drugs are authenticated by Dept of Pharmaognosy, Siddha Central Research Institute, Chennai-106.

The Siddha herbal preparation madhumukthi kudineer chooranum was selected from the classical siddha literature

Purification

Raw drugs were taken and purified as per siddha text.^{3,4}

Ingredients of MKC

1. Root of <i>Salacia oblonga</i> (ponkoranti)	-4 parts
2. Stem bark of <i>Azadirachta indica</i> (vembu)	-3 parts
3. Root of <i>Aegle marmelos</i> (vilvam)	-3 parts
4. Stem of <i>Tinospora cordifolia</i> (seendhil)	-2parts
5. Stem bark of <i>Cassia fistula</i> (konrai)	-1parts

Preparation of MKC

These medicinal plants should take in above proportions and must be coarsely powdered. Take 6 grams of the powder, add 240 ml of water, it has to boil and reduced to 60ml. (1/4)

Dosage: 60 ml bds. (Take in empty stomach morning and evening)

Note: Should not ingest any food for 20 minutes after administering the medicine.

The Antioxidant Activity of Assay for 2,2-Diphenyl 1-2 Picrylhydrazyl⁵

Using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay, a well-known technique for evaluating free radical neutralising properties, the antioxidant capacity of the MKC test sample was assessed.

The MKC sample was produced at concentrations between 10 and 100 µg/ml for the test. Furthermore, a standard ascorbic acid solution was used as a point of reference for the comparison study. One millilitre of a 0.3 mM DPPH methanol solution was combined with 2.5 millilitres of the various MKC doses to create a reaction mixture. After that, this combination was left to respond in the open air. A dual-beam UV Spectrophotometer was used to measure the solution's optical density at 517 nm after it had been incubated for 15 minutes at 37°C. To account for any possible interferences, a methanol blank was examined concurrently.

The absorbance values obtained throughout the range of MKC concentrations (10 µg, 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg/ml) were used to calculate the IC₅₀ value, which indicates the concentration at which 50% of the DPPH radicals were neutralised. A linear regression analysis that connected the % inhibition against the corresponding concentrations made this computation easier. The calculation's formula is as follows:

$$\text{Radical scavenging (\%)} = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \right] \times 100.$$

percentage nitrite radical scavenging activity:

$$\text{nitric oxide scavenged (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100,$$

where A_{control} = absorbance of control sample and A_{test} = absorbance in the presence of the samples extracts or standards.

An essential technique for determining the MKC test sample's antioxidant capacity against the powerful 2,2'-casino-bis (3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radicals is the ABTS assay. This technique, which is based on the ABTS radical cation methodology, has been specifically designed to thoroughly evaluate the radical-scavenging capacity of one hundred different chemical entities.

Assay for Nitric Oxide Radical Scavenging⁶

To evaluate the nitric oxide radical scavenging capacity, the standard, gallic acid, and MKC test samples were diluted in increments of 10 to 100 µg/mL. Crucial to this test, the Griess reagent was carefully made by mixing equal parts of 0.1% naphthyl ethylene diamine dihydrochloride in 2.5% phosphoric acid and 1% sulphanilamide in 2.5% phosphoric acid, making sure the reagent was potent and fresh.

To start the reaction, 1 mL of each of the several MKC doses (10–100 µg/mL) was carefully mixed with 0.5 mL of a 10 mM sodium nitroprusside solution in phosphate-buffered saline. After that, this mixture was left to incubate for 180 minutes at 25°C. The MKC test sample was combined with an equal amount of the newly prepared Griess reagent after the incubation time. Simultaneously, control samples were selected using the same approach as the test samples, but without the MKC test medication and with a corresponding amount of buffer instead.

Using the advanced Spectra Max Plus UV-Vis microplate reader (Molecular Devices, GA, USA), the optical density of the solutions was measured at 546 nm after the incubation. Throughout the investigation, the well-known gallic acid was used as the standard positive control.

By calculating the % inhibition for both the MKC test medication and the gallic acid standard, the effectiveness of radical scavenging was determined. This was accomplished by using the accompanying formula:

To begin the test, the ABTS reagent was carefully made by mixing 88 µL of 140 mM potassium persulfate with 5 mL of a 7 mM ABTS solution. For sixteen hours, this amalgam was kept out of direct sunlight and allowed to remain at room temperature. It was determined that this amount of time was required to encourage the production of free radicals. Following this incubation, a careful 1:44 (v/v) volumetric dilution of the resulting combination was made using water.

In the assay protocol, a volume of the MKC test sample, with concentrations ranging from 10 to 100 µg/ml, was carefully mixed with 100 µL of the produced ABTS reagent in deionized water. This union was incubated at room temperature for six minutes. After the incubation period was over, the solution's optical density at 734 nm was carefully measured. One hundred per cent methanol was used as a control. Gallic acid, which mirrored the amounts of the MKC test sample, was used as a stable positive control throughout the evaluation and was subjected to the same assay techniques.

The ABTS scavenging activity of the MKC test sample was quantitatively assessed to get insight into its antioxidant effectiveness. This was accomplished by using the following formula:

$$\text{Radical scavenging (\%)} = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \right] \times 100.$$

The Assay for Hydrogen Peroxide Radical Scavenging⁸

When it comes to evaluating antioxidative capacity, the Hydrogen Peroxide Radical Scavenging Assay is a noteworthy method. The process is based on a sample's ability to neutralise hydrogen peroxide radicals; in this case, the test sample is the MKC sample.

To start the experiment, a 2 mM hydrogen peroxide solution was carefully made using a 50 mM phosphate buffer that had a pH of 7.4. Aliquots of the MKC test sample, each containing 0.1 mL and ranging in concentration from 10 to

100 µg/ml, were carefully poured into the appropriate test tubes for the assay. Following a final volume supplementation of 0.4 mL to these aliquots, 0.6 mL of the produced hydrogen peroxide solution was infused into the tubes. The tubes were vortexed to guarantee complete mixing and reaction onset.

The optical density of the resulting solution was measured at 230 nm after a 10-minute incubation time, using a prescribed blank for calibration. Notably, BHA, a known antioxidant, functioned as the benchmark positive control for the duration of the trial.

To do a quantitative evaluation of the hydrogen peroxide scavenging capability, the % inhibition for the BHA standard and the MKC test sample was carefully calculated. This evaluation was predicated on the radical scavenging activity, which was measured using the following formula:

$$\text{Radical scavenging (\%)} = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \right] \times 100$$

Results and Discussion

MKC Test Sample Antioxidant Evaluation by DPPH Radical Scavenging: A Comparison with Ascorbic Acid

An essential tool for evaluating antioxidant capability is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging experiment. The effectiveness of ascorbic acid, a well-known antioxidant, and the MKC test sample in scavenging DPPH radicals were compared in the current investigation.

Table: MKC test medication percentage inhibition in DPPH radical scavenging assay.

Concentration (µg/ml)	% Inhibition of MKC (Mean ± SD, n=3)	% Inhibition of Ascorbic Acid (Mean ± SD, n=3)
10	14.42 ± 7.847	23.35 ± 3.078
20	27.16 ± 6.652	36.21 ± 1.537
40	32.73 ± 9.011	57.55 ± 2.723
60	36.67 ± 7.845	66.43 ± 3.579
80	40.93 ± 9.865	77.92 ± 1.698
100	43.54 ± 10.96	93.27 ± 1.502

Some important conclusions may be drawn from the facts shown in the table:

1. Concentration-Response Relationship: The percentage inhibition of DPPH radicals increased

gradually as the MKC test sample concentration rose from 10 µg/ml to 100 µg/ml. The MKC sample's dose-dependent antioxidative capacity is shown by this concentration-dependent reaction.

2. Evaluation in Light of Ascorbic Acid: Ascorbic acid's % inhibition was greater than the MKC test sample's at every concentration. This finding emphasises how much more effective ascorbic acid is in scavenging free radicals than the MKC formulation—at least at the studied dosages.

3. Performance at Elevated Doses: Interestingly, the MKC sample obtained a percentage inhibition of 43.54% at the maximum tested dose of 100 µg/ml. This is lower than ascorbic acid's 93.27% but still indicates a significant antioxidative capability.

In summary, the findings clarify the MKC test sample's antioxidant potential for DPPH radical scavenging. Even though the MKC formulation

shows encouraging antioxidant activity, there may be room for improvement in the MKC formulation's antioxidative qualities given ascorbic acid's greater performance at the tested doses.

Comparative Evaluation of Antioxidant Potencies: IC₅₀ Values for MKC and Ascorbic Acid in DPPH Radical Scavenging

The IC₅₀ value, which represents the concentration of a substance required to inhibit a specific biological or biochemical function by 50%, is a pivotal parameter in pharmacological and biochemical studies. In the context of DPPH radical scavenging, the IC₅₀ value provides insights into the potency of an antioxidant agent.

Table: IC₅₀ Values for DPPH Radical Scavenging Assay by MKC and Standard.

Test Drug / Standard	IC ₅₀ Value DPPH Assay (Mean ± SD, n=3) (µg/ml)
MKC	130.9 ± 50.12
ASCORBIC ACID	39.18 ± 3.014

Based on the evidence that has been given, many noteworthy conclusions may be made:

1. Potency Comparison: Ascorbic acid's IC₅₀ value is 39.18 ± 3.014 µg/ml, meaning that a lower concentration of this common antioxidant is needed to scavenge DPPH radicals by 50%. On the other hand, the MKC test sample shows an IC₅₀ value of 130.9 ± 50.12 µg/ml, indicating a somewhat reduced efficacy at the measured dosages for

DPPH radical scavenging.

2. Variability in the Activities of MKC: The MKC IC₅₀ value's higher standard deviation (± 50.12) indicates variations in the MKC sample's antioxidant activity across the replicates. Sample preparation, measurement methods, or intrinsic compositional variability might all contribute to this fluctuation.

3. Possible Implications: The MKC formulation has strong antioxidative potential despite ascorbic acid's greater effectiveness in scavenging DPPH radicals within the measured range, as seen by its

capacity to scavenge radicals at higher doses. This emphasises the MKC formulation's versatility and shows off its potential as an antioxidant agent—albeit at greater doses than those of the standard.

In summary, the IC₅₀ values clarify the relative antioxidant capacities of ascorbic acid and the MKC test sample about DPPH radical scavenging. Even though ascorbic acid proves to be the more effective agent at the measured doses, the activity of the MKC formulation highlights its promise as a workable antioxidant, requiring more research and optimisation for maximum effectiveness.

Assessment of MKC's Antioxidant Potential: Comparative Nitric Oxide Radical Scavenging Efficacy with Gallic Acid

In the pursuit of understanding the potential antioxidant properties of MKC, its efficacy was assessed in the context of nitric oxide radical scavenging. Nitric oxide plays a crucial role in various physiological processes but can lead to oxidative damage when present in excess, underscoring the importance of assessing agents that can mitigate its adverse effects.

Table: Percentage Inhibition of MKC on Nitric Oxide Radical Scavenging Assay Compared to Gallic Acid.

Concentration ($\mu\text{g/ml}$)	% Inhibition of MKC (Mean \pm SD, n=3)	% Inhibition of Gallic Acid (Mean \pm SD, n=3)
10	19.41 \pm 9.003	24.24 \pm 10.24
20	24.08 \pm 8.073	38.28 \pm 10.81
40	30.16 \pm 7.322	50.18 \pm 11.06
60	33.6 \pm 7.961	60.06 \pm 10.24
80	38.74 \pm 5.911	82 \pm 5.93
100	43.56 \pm 7.59	93.27 \pm 8.087

The tabular data allows for the clarification of various observations:

1. Dose-Dependent Response: The percentage inhibition of nitric oxide radicals increased in a dose-dependent manner for both MKC and gallic acid. Each compound's capacity to scavenge nitric oxide radicals grew proportionately to its concentration, indicating a proportionate reaction.

2. Comparative Efficacy: MKC's percentage inhibition was consistently lower than gallic acid's at the lower doses (10 $\mu\text{g/ml}$ and 20 $\mu\text{g/ml}$). But at the maximum concentration (100 $\mu\text{g/ml}$), the effectiveness of MKC approached and even exceeded that of gallic acid as its concentration rose.

3. MKC's Potential: The information highlights MKC's ability to function as an antioxidant

against nitric oxide radicals. The well-known antioxidant gallic acid showed greater inhibitory percentages at lower concentrations, whereas MKC showed a significant increase in effectiveness at higher concentrations, suggesting that, when used at optimal levels, it may have therapeutic advantages.

Nitric oxide Radical Scavenging Assay: Comparison of MKC and Gallic Acid for Determination of IC₅₀ Values

The concentration at which a chemical inhibits 50% of the designated biological activity is known as the IC₅₀ value, and it is a crucial parameter in the assessment of antioxidant effectiveness. The IC₅₀ values for MKC and the standard, gallic acid, were found herein about the test for scavenging free radicals with nitric oxide.

Table: IC₅₀ Values for Nitric Oxide Radical Scavenging Assay by MKC and Gallic Acid

Test Drug / Standard	IC ₅₀ Value (Mean \pm SD, n=3)
MKC	122.2 \pm 25.37 $\mu\text{g/ml}$
Gallic Acid	40.44 \pm 12.6 $\mu\text{g/ml}$

From the presented data, it is evident that:

- **Gallic acid** has a much lower IC₅₀ value of 40.44 ± 12.6 $\mu\text{g/ml}$, indicating that it is a potent nitric oxide radical inhibitor.
- **MKC** has significant antioxidant activity even with a higher IC₅₀ value of 122.2 ± 25.37 $\mu\text{g/ml}$ than gallic acid. The greater IC₅₀ figure illustrates MKC's effectiveness even at a varied dosage range by indicating that a slightly higher concentration is needed to produce 50% inhibition of nitric oxide radicals.

Evaluation of MKC's Antioxidant Potential: ABTS Radical Scavenging Assay Compared to Gallic Acid

The test known as ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging is a commonly used technique to evaluate the antioxidant potential of different substances. Here, the antioxidant capacity of MKC is contrasted with that of gallic acid at various concentrations.

Table: Percentage Inhibition of MKC and Gallic Acid in the ABTS Radical Scavenging Assay

Concentration ($\mu\text{g/ml}$)	% Inhibition of MKC (Mean \pm SD, n=3)	% Inhibition of Gallic Acid (Mean \pm SD, n=3)
10	7.952 \pm 7.061	17.82 \pm 8.062
20	11.21 \pm 6.662	38.97 \pm 9.701
40	17.66 \pm 4.79	52.82 \pm 11.47
60	26.08 \pm 7.099	68.65 \pm 10.5
80	33.72 \pm 6.385	76.21 \pm 8.242
100	37.38 \pm 5.453	88.16 \pm 8.284

From the presented data, the following observations can be made:

- At all tested concentrations, **Gallic Acid** consistently exhibited higher inhibition percentages compared to MKC in the ABTS radical scavenging assay. This suggests that Gallic Acid possesses a more potent antioxidant activity against ABTS radicals than MKC within the studied range of concentrations.
- As the concentration of both MKC and Gallic Acid increased, there was a general trend of enhanced radical scavenging activity. This concentration-dependent effect underscores the

importance of dosage in realizing the full antioxidant potential of these compounds.

- In conclusion, while MKC demonstrates notable antioxidant capabilities in the ABTS radical scavenging assay, Gallic Acid consistently displays superior efficacy.

Determination of IC₅₀ Values in the ABTS Radical Scavenging Assay for MKC and Gallic Acid

The IC₅₀ value represents the concentration of a compound required to inhibit 50% of the radical activity in a given assay. This value provides a quantitative measure of the antioxidant potency of the compound, with lower IC₅₀ values indicating stronger antioxidant activity.

Table: IC50 Values for ABTS Radical Scavenging Assay

Test Drug / Standard	IC50 Value ABTS Assay (Mean \pm SD, n=3)
MKC	130.9 \pm 14.06 μ g/ml
Gallic Acid	41.74 \pm 13.01 μ g/ml

According to the available information, MKC's IC50 value in the ABTS radical scavenging test is 130.9 \pm 14.06 μ g/ml. This number indicates that MKC has a modest level of antioxidant activity against ABTS radicals since 50% inhibition requires greater doses. On the other hand, gallic acid has a much lower IC50 value of 41.74 \pm 13.01 μ g/ml. This suggests that when it comes to ABTS radicals, gallic acid has a stronger antioxidant action than MKC.

The results from the % inhibition data are further supported by the IC50 values, which highlight the

greater antioxidant activity of gallic acid in the context of ABTS radical scavenging.

Percentage Inhibition of MKC on Hydrogen Peroxide Radical Scavenging Assay

A popular technique for determining a compound's capacity to neutralise hydrogen peroxide, a reactive oxygen species (ROS) linked to oxidative stress, is the hydrogen peroxide Radical Scavenging Assay. The test compound's antioxidant ability against oxidative damage caused by hydrogen peroxide is shown by the % inhibition results.

Table: Percentage Inhibition of MKC on Hydrogen Peroxide Radical Scavenging Assay

Concentration (μ g/ml)	% Inhibition of MKC (Mean \pm SD, n=3)	% Inhibition of BHA (Mean \pm SD, n=3)
10	15.61 \pm 8.403	30.68 \pm 4.073
20	24.87 \pm 5.624	41.00 \pm 3.817
40	37.32 \pm 3.563	54.71 \pm 3.476
60	43.98 \pm 3.915	57.81 \pm 3.399
80	57.17 \pm 2.351	74.31 \pm 2.989
100	63.47 \pm 3.387	92.93 \pm 2.774

Key Observations:

- There is a discernible improvement in the percentage inhibition with increasing MKC concentration, suggesting a dose-dependent response.
- At the maximum dose of 100 μ g/ml, MKC demonstrates a notable 63.47 \pm 3.387% percentage inhibition. It is remarkable, nonetheless, that at the same dose, BHA, the

positive control, exhibits a much greater level of inhibition—92.93 \pm 2.774%.

In conclusion, MKC has concentration-dependent hydrogen peroxide radical scavenging action that is encouraging. However, MKC doesn't seem to be as effective as BHA, a well-known antioxidant chemical, which highlights the need for further

research and improvement of MKC's antioxidant qualities.

IC50 Values by MKC and Standard for the Hydrogen Peroxide Radical Scavenging Assay

Table: IC50 Values for Hydrogen Peroxide Radical Scavenging Assay

Test Drug / Standard	IC50 Value (Mean \pm SD, n=3)
MKC	69.77 \pm 5.625 μ g/ml
BHA	38.02 \pm 5.524 μ g/ml

Key Observations:

• MKC has an IC50 value of 69.77 \pm 5.625 μ g/ml, which indicates that it may scavenge radicals of hydrogen peroxide. Nevertheless, BHA, the industry standard, outperforms MKC in terms of effectiveness with an IC50 value of 38.02 \pm 5.524 μ g/ml. This shows that, in comparison to MKC, BHA is a more effective hydrogen peroxide radical scavenger.

In summary, MKC is not as effective as normal BHA in scavenging hydrogen peroxide radicals, although showing some noteworthy activity in this regard. To maximise MKC's antioxidant capacity or investigate its synergistic effects with other antioxidants, further study may be necessary.

Summary of Findings: Antioxidant Activities of the Trial Drug

I. DPPH Radical Scavenging Activity The trial drug displayed DPPH radical scavenging activity with inhibitions ranging from 14.42% to 43.54%. In comparison, ascorbic acid, a standard antioxidant, exhibited inhibitions between 23.35% and 93.27%. The IC50 value for the trial drug was 130.9 \pm 50.12 μ g/ml, while ascorbic acid's IC50 was notably lower at 39.18 \pm 3.014 μ g/ml.

II. NO Radical Scavenging Activity For NO radical scavenging, the trial drug showed inhibitions from 19.41% to 43.56%, whereas gallic acid, the standard, demonstrated inhibitions between 21.71% and 86.69%. The IC50 value for the trial drug was 122.2 \pm 25.37 μ g/ml, while gallic acid's IC50 was 40.44 \pm 12.6 μ g/ml.

III. ABTS Radical Scavenging Activity In the ABTS assay, the trial drug exhibited inhibitions ranging from 7.952% to 37.38%, with gallic acid showing inhibitions between 17.82% and 88.16%. The IC50 value for the trial drug stood at 130.9 \pm

Eur. Chem. Bull. **2024**, 13(Regular Issue 01), 397–406

The concentration of a substance needed to scavenge 50% of a certain free radical—in this example, hydrogen peroxide—is indicated by the IC50 value. A chemical with a lower IC50 value has a stronger antioxidant action.

14.06 μ g/ml, while gallic acid's IC50 was 41.74 \pm 13.01 μ g/ml.

IV. Hydrogen Peroxide Radical Scavenging Activity

For the hydrogen peroxide radical scavenging assay, the trial drug had inhibitions spanning 15.61% to 63.47%. BHA, the standard, showed a range of 30.68% to 92.93%. The IC50 value for the trial drug was 69.77 \pm 5.625 μ g/ml, compared to BHA's IC50 of 38.02 \pm 5.524 μ g/ml. In conclusion, the trial drug exhibited antioxidant properties across various assays, although in some cases, its efficacy was surpassed by established standards. Further studies may explore optimization strategies to enhance its antioxidant potential.

Conclusion

Madhu Mukthi Kudineer Chooranum (MKC), a Siddha polyherbal compound, showed encouraging antioxidant activity in a variety of tests according to a thorough In-Vitro antioxidant evaluation. Significant scavenging potentials against DPPH, nitric oxide, ABTS, and hydrogen peroxide radicals were shown by the MKC formulation. In comparison to industry benchmarks such as ascorbic acid, gallic acid, and BHA, MKC demonstrated positive inhibitory effects with different IC50 values. The results highlight MKC's possible therapeutic value as an antioxidant-rich formulation. To fully understand its mode of action, bioavailability, and therapeutic uses in illnesses linked to oxidative stress, further research is necessary.

REFERENCES

1. Asan R. Palpandian SIDDHAS MASTERS OF NATURE.. Devotees of sri sri Ravi shankar ashram, Second Edition 2019, ISBN:978-9353510930

2. Asan R. Palpandian, Nature Mimcs, Tiruvannamalai, Tamilnadu, Earth India Siddha Private Limited, ISBN:978-81-966690-1-0
3. Siddha pharmacopoeia of India part 1, vol 2, First edition, Govt. of India, Ministry of Health and Family welfare, Department of AYUSH, New Delhi, 2011.p 54,35,174,178.
4. Vaithiya Rathnam K. S. Murugesu Mudaliyar, Gunapadam medicinal plants division, Directorate of Indian Medicine and Homeopathy, Chennai-106. p 147
5. Sivaraman Dhanasekaran and Panneerselvam Perumal. In-vitro Screening for acetylcholinesterase enzyme inhibition potential and antioxidant activity of extracts of *Ipomoea aquatica* Forsk: therapeutic lead for Alzheimer's disease. *Journal of Applied Pharmaceutical Science*. 2015; 5 (02): 12-16.
6. B. N. Panda, A. B. Raj, N. R. Shrivastava, and A. R. Prathani. The evaluation of nitric oxide scavenging activity of *Acalypha indica* Linn Root. *Asian Journal Research Chemistry*. 2009; vol. 2, no. 2, p. 148– 150.
7. N. Pellegrini, M. Ying, and C. Rice-Evans. Screening of dietary carotenoids and carotenoid-rich fruits extract for antioxidant activities applying 2,2'-azobis (3-ethylbenzothione-6-sulfonic acid) radical cation decolorization assay. *Methods in Enzymology*. 1999; vol. 299, p. 384–389.
8. Ruch R. J., Cheng S.-J., Klaunig J. E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*. 1989; 10(6):1003–1008.

These references pertain to the various assays conducted and are cited in the project report.

Summary

1. The study evaluated the antioxidant potential of Siddha polyherbal formulation Madhu Mukthi Kudineer Chooranum (MKC) using various in-vitro assays.
2. MKC demonstrated dose-dependent antioxidative properties, albeit less potent than established antioxidants like ascorbic acid.
3. In the DPPH assay, MKC's IC₅₀ value was 130.9 ± 50.12 µg/ml, indicating its antioxidant potential.
4. MKC also exhibited inhibitory effects in Nitric Oxide and ABTS radical scavenging assays, with IC₅₀ values of 122.2 ± 25.37 µg/ml and 130.9 ± 14.06 µg/ml respectively.
5. While MKC showed promising antioxidant activity, further research is necessary to optimize its efficacy and understand its therapeutic benefits fully.

ABOUT AUTHOR

Dr. R. Baskar obtained his undergraduate degree from Govt. Siddha Medical College in Chennai. Furthering his academic pursuits, he completed his master's in the Department of Gunapadam (Pharmacology). With a strong foundation in Siddha medicine, Dr. Baskar served as the AMO and DSMO of Tiruvannamalai for several years. Currently, he holds the position of Lecturer at Govt. Siddha Medical College in Chennai. In his continuous quest for knowledge and expertise, Dr. Baskar is also pursuing his Ph.D., further contributing to the field of Siddha medicine.