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Exploring The Antioxidant Potential of Angaya Chooranam: Investigating Mechanism of Action in Siddha Medicine

Nalina Saraswathi K*[1], Ramani M[2], Kabilan N[3], Velpandian V[4]
[1]Lecturer, Department of Gunapadam, Government Siddha medical College, Arumbakkam, Chennai, Tamilnadu, India – 600106.
[2]Research Scholar, Department of Siddha, The Tamil Nadu Dr. M. G. R. Medical University, Guindy, Chennai, Tamilnadu, India – 600032.
[3]Professor & Head, Department of Siddha, The Tamil Nadu Dr. M. G. R. Medical University, Guindy, Chennai, Tamilnadu, India – 600032.
[4]Professor (Rtd), Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai, Tamilnadu, India – 600106.

Corresponding Author:
Dr.K.Nalina Saraswathi
Lecturer, Department of Gunapadam, Government Siddha medical College, Arumbakkam, Chennai,
Email: knalina.s@gmail.com

ABSTRACT:
Siddha medicine is one of the oldest medical systems in the world. It originated in Southern part of Tamilnadu. Traditional Siddha medicines are recently attaining worldwide acceptance for its health benefits, rejuvenation and synergistic properties, less side effects, cost effectiveness and it is easily available. 'Angaya Chooranam' comes under the division 'Kaya karpam'. The word "Kayakarpam" means the Prevention of body from diseases. Gastric ulcer is caused by an imbalance between the action of aggressive and defensive factors on the gastric mucosa. Gastric mucosal damage can be induced by aggressive factors such as hydrochloric acid, pepsin, leukotrienes, free radicals, nonsteroidal anti-inflammatory drugs (NSAIDs), ischemia, dysmotility, ethanol, nicotine, and stress. In this research article Gastric ulcer correlated with the Angaya Chooranam Anti-Oxidant property. Angaya Chooranam (AC) was prepared as per the Siddha literature Marunthu Sei Iyalum Kalaiyum. "Angayam" is a thogai sarakku which possess mainly 5 ingredients: Mustard, Asafoetida, Fenugreek, Garlic and Ajwain. In traditional preparation method dried Neem flowers, dried Turkey berries, and dried black night shade fruits are added to it. Angaya Chooranam is a Siddha poly herbal formulation containing about eight herbal Ingredients indicated for treating gastric ulcer, improves appetite, heals mouth ulcers and expels intestinal worms. The above herbs listed in this article does contain free radical scavenging activity, due to its total phenolic content, a potent anti-oxidant relating to the concept of kaya karpam regimen stated in Siddha literatures thereby leading to anti-ageing effect. This research paper exhibits in vitro Anti-oxidant activity of the test drug Angaya Chooranam through DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl), Nitric Oxide Radical Scavenging, ABTS, Hydrogen Peroxide Radical Scavenging activities and the results show significant free radical scavenging properties.
Keywords: *Angayam Chooranam, Siddha Medicine, Anti-oxidant, Kayakarpam.*

INTRODUCTION

The Siddha medicine is an ancient system of medicine that is followed in Southern part of India especially in Tamil Nadu. Siddha medicine was founded by Siddhars, who believe in the art of immortalizing the corporeal human body. Kayakarpam is the Hallmark of Siddha system which imparts immunity to diseases and responds the aging process which is attained through 'karpavizhtham' (medicines) and 'karpayogam' (regimens of life). The word "Kayakarpam" means the Prevention of body from diseases. Kayakarpam make our body competent and strong.

The antioxidants are present in several herbs disable the hazardous effects caused by free radical. Free radical cause age related problems, cancer, atherosclerosis, and arthritis...etc. [1] Siddha is the medical science dedicated to the longevity of life, for the wellbeing of humanity. Kayakarpam is the set of specific therapies, which can actually alter the metabolism of body and thereby reverses the physical degeneration. [2]

All living things naturally produce free radicals as part of their physiological processes, which are produced during regular cellular operation or from external sources like pollution, medication, cigarette smoke or through radiation. They act as both beneficial as well as toxic compounds to the body [3]. When an

overload of free radicals cannot gradually be destroyed, they get accumulated and lead to phenomenon called "Oxidative damage" or "Oxidative Stress" [4].

An antioxidant is a chemical substance that, in small quantities relative to an oxidizable substrate, considerably inhibits or delays the substrate's oxidation. Biologically significant reactive oxygen species (O2−, H2O2, OH, HOCl, ferryl, peroxyl, and alkoxyl) can be scavenged by antioxidants, or they can act to stop their creation or repair any damage they already cause [5].

Anti-oxidants are essential in slowing down the aging process by scavenging the damaging free radicals and lowering the oxidative stress. Free radicals have the potential to harm the cells and accelerate aging [6]. Antioxidant Supplements can prevent or reverse Age related alterations in Antioxidant defence. Oxygen derived free radicals (ORFs) are thought to play a role in age related cellular damage, and interventions that target ORFs may help slow the aging process [7]. Due to the complex nature of anti-oxidants and ROS, high dose of a particular anti-oxidant like Vitamin C, Vitamin E and β carotene resulted in no effect. Thus an alternate approach is required and anti-oxidant rich foods would be the promising approach as they contain a large number of different antioxidants that have been carefully selected by plant evolution to protect all of the plants cells from oxidative damage [8]. In order to analyse the anti-oxidant capacity of various kinds of anti-oxidants, the Oxygen Radical Absorbance Capacity (ORAC) was developed by the National Institute on Aging in the year 1992 [9].

Gastric ulcer is a relatively health problem, since it occurs from 12 to 17% in developed countries [10]. This gastrointestinal disorder affects 10% of the population at some period of life [11]. Gastric ulcer is caused by an imbalance between the action of aggressive and defensive factors on the gastric mucosa [12]. Gastric mucosal damage can be induced by aggressive factors such as hydrochloric acid, pepsin, leukotrienes, free radicals, nonsteroidal anti-inflammatory drugs (NSAIDs), ischemia, dysmotility, ethanol, nicotine, and stress [13].

There are several effective drugs in the treatment of gastric and duodenal ulcers, among which are inhibitors of proton pump and antagonists of histamine H_2 receptor that interfere with the secretion of acid, and antacids that neutralize acid secretion [14, 15]. However, many of these drugs besides having different adverse effects including hypersensitivity, arrhythmias, hematopoietic disorders, erectile dysfunction, and gynecomastia [16, 17].

Many medicinal plants extracts are used in folk medicine in Brazil to treat diverse types of digestive disorders [18]. Several different substances found in these plants have gastroprotective effects [19] and among the major classes of compounds related to this activity, there are the terpenes, triterpenes, flavonoids, alkaloids, glycosides, saponins, and polysaccharides [20]. Since natural products represent a promising and renewed therapeutic strategy in gastroprotection.

Gastric lesions are among the main diseases of the gastrointestinal tract, and its pathogenesis is related to a complex multifactorial process resulting from an imbalance between the protective and aggressive factors on the gastric mucosa [21, 22].

NSAIDs are drugs routinely used throughout the world; however, they are known to induce gastric mucosal damage including gastritis, bleeding, ulceration, and perforation in humans and experimental animals [23]. These effects occur through the inhibition of the cyclooxygenase (COX) enzymes, resulting in a marked decrease in the levels of prostaglandins [24], but the reactive-oxygen-species (ROS)-induced enhancement in lipid peroxidation plays an important role in the mechanism of gastric damage induced and the increase in free radical metabolites depends possibly upon neutrophil activation and is associated with the significant increase in lipid peroxidation, the fall in the gastric blood flow at ulcer margin, and the excessive release of the proinflammatory cytokine such as IL-1b [23].

The imbalance between the oxidant and antioxidant systems leads to a biological condition defined as oxidative stress that contributes to the formation of reactive oxygen species, which are unstable molecules resulting from various processes in the body that can be highly harmful and cause a series of diseases [25]. Oxidative stress is closely related to the pathogenesis of various diseases, and it is postulated that it plays a crucial role in the induction of gastric mucosal lesions induced by ethanol. ROS promotes oxidative stress, causing deleterious effects and cell death, leading to degenerative diseases, cancer, ulcers, and atherosclerosis [26, 27, 28].

Several plants, plant extracts, and phytochemicals with antioxidant activity, such as many phenolic compounds, flavonoids, and terpenoids, play an important role in preventing oxidative damage, whereas these agents neutralize and break the free radical chains and can contribute to control the oxidative stress-

initiated disease [29, 30]; they have potential utility as pharmacotherapeutic intervention to scavenge ROS and ameliorate effects against mutagens, carcinomas, and inflammatory pathological processes [29, 31, 32].

The anti-oxidant capacity is also analyzed through in vitro assays like DPPH radical scavenging assay, Hydroxyl radical scavenging assay, Superoxide radical scavenging assay, Nitric oxide radical scavenging assay, Hydrogen peroxide scavenging assay and Total Phenolic Contents. The phenolics are the potent anti-oxidants present in various plants. [33]

MATERIALS AND METHODS

Angaya Chooranam was prepared as per the Siddha literature Marunthu Sei Iyalum Kalaiyum. ANGAYAM is a thogai sarakku which possess mainly 5 ingredients: Mustard (Kadugu), Asafoetida (Perungayam), Fenugreek (Vendhayam), Garlic (Vellai poondu) and Ajwain (Omam). In traditional preparation method dried Neem flowers (Vepam poo), dried Turkey berries (Sundai vatral), and dried black night shade fruits (Manathakkali) are added to it.

Table no 1: Ingredients of Angaya Chooranam

S.No	Vernacular name	English name	Botanical Name	Family	Part used
1.	Kadugu	Mustard	Brassica juncea	Brassicaceae	Seed
2.	Perungayam	Asafoetida	Ferula asafoetida	Apiaceae	Gum resin
3.	Vendhayam	Fenugreek	Trigonella foenum – graecum	Fabaceae	Seed
4.	Vellai Poondu	Garlic	Allium sativum	Alliaceae	Bulb
5.	Omam	Ajwain	Trachyspermum ammi	Apiaceae	
6.	Vepam poo	Neem flowers	Azadirachta indica	meliacea	Flower
7.	Sundai vatral	Turkey berries	Solanum torvum	Solanaceae	Fruit rind
8.	Manathakkali	Black night shade	Solanum nigrum	Solanaceae	Fruit

The raw drugs were selected, authenticated and purified as per the SOP. All the ingredients were mildly roasted and powdered well and filtered with cloth (Vasthira kayam). Finally weighed and stored in an air tight container marked as Angaya Chooranam (AC). This end product was used for analysis. It is indicated for treating gastric ulcer, improves appetite, heals mouth ulcers and expels intestinal worms. Invitro Anti-oxidant studies of the test drug "Angaya Chooranam" were done at Noble Research Solution, Perambur, Chennai.

EVALUATION OF ANTIOXIDANT ACTIVITY

Anti-oxidant activity was carried out by In-vitro cell line studies using the following assays:

1) DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay
2) Nitric Oxide Scavenging Assay
3) ABTS Assay
4) Hydrogen Peroxide Radical Scavenging Assay

1. DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay [34]

The antioxidant activity of test drug sample Angaya chooranam (AC) was determined using the 2, 2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample AC at the concentration of 10 µg/ml to 100 µg/ml, was admixed with 95% methanol. Ascorbic acid was used as standard and was prepared in same concentration as that of the test drug by using methanol as solvent. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample AC at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100µg/ml) was noted after 15 min incubation period at 37^0C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

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

The effective concentration of test sample AC required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations

2. Nitric Oxide Radical Scavenging Assay [35]

The concentrations of test sample AC are made into serial dilution from 10—100 µg/mL and the standard gallic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the test drug (10—100 µg/mL) and incubated at 25°C for 180 mins. The test drug AC was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the test drug but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The absorbance was measured at 546 nm using a Spectra Max Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Gallic acid was used as the positive control. The percentage inhibition of the test drug AC and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug AC and gallic acid were calculated using the following formula:

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.

3. ABTS Assay [36]

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug AC against 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radicals. The ABTS radical cation method was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1 : 44, v/v). To determine the scavenging activity, 100 µL ABTS reagent was mixed with 100 µL of test sample (10-100µg/ml made with DD water) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734 nm. 100% methanol was used as a control. Gallic acid with same concentrations of test drug AC was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample AC was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

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

4. Hydrogen Peroxide Radical Scavenging Assay [37, 38]

A hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the test sample AC (different concentration ranging from 10-100µg/ml) were transferred into the test tubes and their volumes were made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4). After adding 0.6 mL hydrogen peroxide solution, tubes were vortexed and the absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. BHA was used as the positive control. The percentage inhibition of the test drug AC and standard was calculated and recorded. The percentage radical scavenging activity of the test drug AC and BHA were calculated using the following formula:

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

RESULTS AND DISCUSSION

Antioxidant potential of Angaya Chooranam

Table no 2: Percentage inhibition of test drug AC on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of AC	% Inhibition of Ascorbic Acid
10 µg/ml	3.975 ± 0.9969	10.4 ± 1.369
20 µg/ml	7.906 ± 4.109	15.84 ± 0.6317
40 µg/ml	14.44 ± 5.888	40.32 ± 3.78
60 µg/ml	19.95 ± 6.504	50.55 ± 4.378
80 µg/ml	26.43 ± 5.033	63.65 ± 3.288
100 µg/ml	29.85 ± 7.816	81.26 ± 0.9563

Data are given as Mean ± SD (n=3)

Table no 2a: IC50 Values for DPPH radical scavenging Assay by AC and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
AC	169.1 ± 36.17
ASCORBIC ACID	59.77 ± 1.302

Data are given as Mean ± SD (n=3)

Table no 3: Percentage inhibition of test drug AC on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of AC	% Inhibition of Gallic Acid
10 µg/ml	3.333 ± 2.122	25.19 ± 9.235
20 µg/ml	7.274 ± 1.005	38.12 ± 5.401
40 µg/ml	12.06 ± 2.78	52.16 ± 2.42
60 µg/ml	15.22 ± 3.243	59.4 ± 2.907
80 µg/ml	17.11 ± 4.251	68.58 ± 4.585
100 µg/ml	19.8 ± 3.235	85.85 ± 1.134

Data are given as Mean ± SD (n=3)

Table no 3a: IC50 Values for Nitric Oxide radical scavenging assay by AC and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
AC	276.6 ± 61.6
GALLIC ACID	43.14 ± 5.793

Data are given as Mean ± SD (n=3)

Table no 4: Percentage inhibition of test drug AC on ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of AC	% Inhibition of Gallic Acid
10 µg/ml	3.649 ± 1.737	15.41 ± 5.423
20 µg/ml	8.549 ± 3.046	39.58 ± 5.919
40 µg/ml	12.67 ± 2.023	53.53 ± 3.084
60 µg/ml	17.19 ± 2.029	69.25 ± 1.717
80 µg/ml	21.9 ± 2.989	76.63 ± 3.114
100 µg/ml	25.04 ± 1.312	88.55 ± 1.81

Data are given as Mean ± SD (n=3)

Table no 4a: IC50 Values for ABTS radical scavenging assay by AC and standard.

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg/ml)
AC	205.6 ± 18.18
GALLIC ACID	41.83 ± 5.002

Data are given as Mean ± SD (n=3)

Table no 5: Percentage inhibition of test drug AC on Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of AC	% Inhibition of BHA
10 µg/ml	3.101 ± 0.7684	14.17 ± 4.026
20 µg/ml	7.838 ± 2.71	27.3 ± 7.953
40 µg/ml	14.04 ± 3.6	42.35 ± 3.62
60 µg/ml	16.45 ± 3.073	54.76 ± 8.28
80 µg/ml	19.18 ± 3.842	67.62 ± 7.574
100 µg/ml	22.68 ± 2.181	83.62 ± 5.807

Data are given as Mean ± SD (n=3)

Table no 5a: IC50 Values for Hydrogen peroxide radical scavenging assay by AC and standard.

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg/ml)
AC	231.4 ± 28.13
BHA	54 ± 5.155

Data are given as Mean ± SD (n=3)

I. DPPH radical scavenging activity

The DPPH radical scavenging activity of methanolic extract of the Siddha drug AC were detected and compared with standard Ascorbic acid Table 2. The percentage inhibition (% inhibition) at various concentration (10-100 µg/ml) of drug as well as standard Ascorbic acid (10-100 µg/ml) were calculated. The test drug shows higher inhibition 29.85 ± 7.816% at 100µg/ml. At the same time, the standard Ascorbic acid exhibits 81.26 ± 0.9563% of inhibition at 100µg/ml. In lower concentration 10 µg/ml, the test drug shows 3.975 ± 0.9969% of inhibition Table 2. The IC50 values are calculated and were found Ascorbic acid (59.77 ± 1.302 µg/ml), and test drug exhibits (169.1 ± 36.17 µg/ml) Table 2a.

II. NO radical scavenging activity

The Nitric Oxide radical scavenging activity of methanolic extract of the Siddha drug AC were detected and compared with standard Gallic acid Table 3. The percentage inhibition (% inhibition) at various concentration (10-100 µg/ml) of drug as well as standard Gallic acid (10-100 µg/ml) were calculated. The test drug shows higher inhibition 19.8 ± 3.235% at 100µg/ml. At the same time, the standard Gallic acid exhibits 85.85 ± 1.134% of inhibition at 100µg/ml. In lower concentration 10 µg/ml, the test drug shows 3.333 ± 2.122% of inhibition Table 3. The IC50 values are calculated and were found Gallic acid (43.14 ± 5.793 µg/ml), and test drug AC exhibits (276.6 ± 61.6 µg/ml) Table 3a.

III. ABTS radical scavenging activity

The ABTS radical scavenging activity of methanolic extract of the Siddha drug AC were detected and compared with standard Gallic acid Table 4. The percentage inhibition (% inhibition) at various concentration (10-100 µg/ml) of drug as well as standard Gallic acid (10-100 µg/ml) were calculated. The test drug shows higher inhibition 25.04 ± 1.312% at 100µg/ml. At the same time, the standard Gallic acid exhibits 88.55 ± 1.81% of inhibition at 100µg/ml. In lower concentration 10 µg/ml, the test drug shows 3.649 ± 1.737% of inhibition Table 4. The IC50 values are calculated and were found Gallic acid (41.83 ± 5.002 µg/ml), and test drug AC exhibits (205.6 ± 18.18 µg/ml) Table 4a.

IV. Hydrogen peroxide radical scavenging activity

The Hydrogen peroxide radical scavenging activity of methanolic extract of the Siddha drug AC were detected and compared with standard BHA Table 5. The percentage inhibition (% inhibition) at various concentration (10-100 µg/ml) of drug as well as standard BHA (10-100 µg/ml) were calculated. The test drug shows higher inhibition 22.68 ± 2.181% at 100µg/ml. At the same time, the standard BHA exhibits 83.62 ± 5.807% of inhibition at 100µg/ml. In lower concentration 10 µg/ml, the test drug shows 3.101 ± 0.7684% of inhibition Table 5. The IC50 values are calculated and were found BHA (54 ± 5.155 µg/ml), and test drug AC exhibits (231.4 ± 28.13µg/ml) Table 5a.

The trial drug, 'Angaya Chooranam' exhibits significant antioxidant activity which helps to treating gastric ulcer, improves appetite, heals mouth ulcers and expels intestinal worms, retard the aging process, boosts immunity, revitalizes body, mind, soul and repairs the worn out tissues.

The free radical DPPH, which is widely used to evaluate the ability of compounds to operate as free-radical scavengers and hydrogen suppliers, is a rapid, simple and low-cost method for testing antioxidant capabilities. The DPPH test relies on the elimination of DPPH, a stabilized free radical. DPPH is actually a dark coloured crystalline compound which is made up of free radical particles that are stable. Particularly, it is a well-known radical and a popular antioxidant test. The DPPH test is used to estimate antioxidant activity based on the process through which antioxidants limit lipid oxidation, resulting in DPPH free-radical scavenging and therefore determining free radical scavenging potential. [39, 40]

Hydrogen peroxide (H_2O_2), a biologically relevant, non-radical oxidizing species, may be formed in tissues through oxidative processes. Hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals (OH) resulting in initiation and propagation of lipid peroxidation. The hydrogen peroxide scavenging activity of methanol extract test drug were detected and compared with standards. The IC50 values for hydrogen peroxide scavenging activity of the test drug AC were found significant when compared to the Standard. The ability of the extracts to quench OH_ seems to be directly related to the prevention of the lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction.

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity.

It is a diffusible free radical that plays major roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities. [41] The Nitric oxide scavenging activity of methanol extract of test drug AC were detected and compared with standards. The IC 50 values for nitric oxide scavenging activity of AC were significant when compared to Standard. Though the test drug AC showed potent nitric oxide activity in the natural sector. Thus Nitric oxide scavenging exhibits potent oxidant scavenging activity than others.

CONCLUSION

The in vitro antioxidant activity showed that the test drug AC can reduce the actions of oxidative stress as a triggering effect during gastric ulcer. The above findings indicate the therapeutic potential of anti-oxidant to be used as an effective gastroprotective agent. The drug quenches the free radicals. The result of this study drug proved the trial drug AC to be very effective in gastric ulcer. Based on the results obtained from the In vitro anti-oxidant assay for the sample AC, it was concluded that the Siddha formulation AC has potential anti-oxidant activity in the estimated assays.

Siddha system concentrates mainly on preventing the disease and enhancing a healthy aging. This can be achieved through consumption of Angaya Chooranam during gastric ulcer. The modern researches have paved the way for the better understanding of the benefits of this AC with regards to the active constituents. While the role of anti-oxidants in combating age-related ailments is promising, further research is needed to understand their effectiveness in day-to-day basis and optimal use in promoting healthy aging. Embracing a balanced diet rich in anti-oxidants along with healthy lifestyle remains crucial for overall wellbeing and pacify the impact of free radicals on human body.

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AUTHORS CONTRIBUTION

Conceptualization of the study and formal analysis were performed by Dr.N.Kabilan, The original draft was prepared by Dr.K.Nalina Saraswathi, while writing, editing and methodology were handled by Dr.M.Ramani, Visualization, Validation and supervision were conducted by Dr.V.Velpandian. All authors have reviewed and approved the final version of the manuscript for publication.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

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