



Formulation Evaluation and *In- Vitro* Antioxidant activity of Microparticles of *Syzygium Cumini* Plant Extract

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Authors' contributions

This work was carried out in collaboration among all authors. Author SSK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PKS and VS managed the analyses of the study. Author JV managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i27B31504

Editor(s):

(1) Dr. Prem K. Ramasamy, Brandeis University, USA.

Reviewers:

(1) Lenard Farczadi, "G.E. Palade" University of Medicine, Pharmacy, Science, and Technology of Târgu Mureş, Romania.

(2) Rosario Nicoletti, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia, Italy.

(3) Renan Campos E Silva, Federal University of Pará, Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/67927>

Received 20 February 2021

Accepted 26 April 2021

Published 01 May 2021

Original Research Article

ABSTRACT

Syzygium cumini, commonly known as Malabar plum, Java plum, black plum, jamun or jambolan, is an evergreen tropical tree in the flowering plant family Myrtaceae. The present study the antioxidant effects of microparticles of *S. cumini* plant extract were evaluated using *in vitro*, 2, 2-diphenylpicrylhydrazine photometric assay, total antioxidant activity and super oxide free radical scavenging activity. The microparticles showed different levels of radicals scavenging activity in a dose-dependent manner over the range of 50–150 µg/mL concentration, indicating the high antioxidative capacity of the extract. In all the experimental screening models microparticles of aqueous extract show the maximum inhibition of free radicals comparison of other experimental model at different concentrations. These findings suggest that the seed of *Syginium cumini* has potent antioxidant activity which may be responsible for some of its reported pharmacological activities and can be used as antioxidant supplement.

Keywords: *Syginium cumini*; microparticles; antioxidant; DPPH; super oxide.

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1. INTRODUCTION

The term “medicinal plant” includes various types of plants used in herbalism (“herbology” or “herbal medicine”). It is the use of plants for medicinal purpose, and the learning of such uses. The word Herb has been derivative from the Latin word, “*herba*”. Currently, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Previously, the term “herb” was only applied to Non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or fragrance and also in certain spiritual activities [1]. The oral medication is the most important method of administering drugs for systemic effects. The parenteral route is not routinely used for self-administration of medication. The topical route of administration has only recently been employed to deliver drugs to the body for systemic effects. It is probable that at least 90 % of all drugs used to produce systemic effect are administered by the oral route. An ideal dosage regimen in the drug therapy of any disease should immediately attains the desired therapeutic concentration of drug in plasma and maintains a constant level for the entire duration of treatment. This is possible through the administration of conventional dosage forms in a particular dose and at particular frequency. The frequency of administration or dose interval of any drugs depends upon its half-life or mean residence time and its therapeutic index. In most cases, dosing interval is much shorter than the half-life of the drug, resulting in number of limitations associated with such a conventional dosage form. Microparticles offer a method to deliver macromolecules by a variety of routes and effectively control the release of such drugs. They may also be used in the delivery of vaccines and molecules such as DNA for use in gene therapy. Microparticles offer effective protection of the encapsulated agent against degradation (e.g. enzymatic), the possibility of controlled and local delivery of the drug over periods ranging from few hours to months, and easy administration (compared to alternative forms of controlled release parenteral dosages, such as macro sized implants). Controlled drug delivery systems could be extremely useful in providing the optimal therapy for a given drug [2-5]. Each drug has a characteristic ‘minimum effective concentration’, below which no therapeutic effect is observed and a characteristic ‘minimum toxic concentration’ above which undesired side effects occur. The

range in between is called the ‘therapeutic range’ or ‘therapeutic window’. Depending upon the type of drug and physiological factors, this therapeutic window could be narrow. The optimum effect of many medical treatments is obtained by maintaining the drug concentration in the therapeutic range over a sustained period of time. This is especially true for highly potent drugs [5]. *Syzygium cumini*, commonly known as Malabar plum, [6] Java plum, black plum, jamun or jambolan, is an evergreen tropical tree in the flowering plant family Myrtaceae, and favored for its fruit, timber, and ornamental value. It is native to the Indian Subcontinent, adjoining regions of Southeast Asia, including Myanmar, Sri Lanka, and the Andaman Islands. It can reach heights of up to 30 metres (98 ft) and can live more than 100 years. A rapidly growing plant, it is considered an invasive species in many world regions [7-10].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Seeds Powder of *Syzygium cumini* was collected from local market of Dewas, Madhya Pradesh India. The powder was identified by its morphological study and stored in air tight container at room temperature.

2.2 Preparation of Extract

2.2.1 Aqueous extraction of *Syzygium cumini*

100 g of dried seeds powder of *Syzygium cumini* were subjected to cold maceration with purified water at room temperature for 120 hrs. After that the aqueous extract was filtered using wett man filter paper, and the filtrate was further concentrated and dried by using hot air oven at 50 °C, and crushed in mortar pestle to obtain fine powder.

Percentage of yield is calculated as below:

$$\% \text{ Extract yield} = (W1/W2) \times 100.$$

W1 = weight of extract obtained after cold maceration, W2 = Total weight of powder used for extraction.

2.2.2 Phytochemical screening

The seed extracts of *Syzygium cumini* were analyzed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols,

saponins, terpenoid, cardiac glycosides and tannins according to standard methods [11].

2.2.3 Alkaloids [Mayer's test]

1.36 g of mercuric chloride dissolved in 60 mL and 5 g of potassium iodide were dissolved in 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100 mL using distilled water. Few drops of reagent were added to 1 mL of acetic aqueous solution of sample. added. Formation of a white or pale precipitate showed the presence of alkaloids.

2.2.4 Flavonoids

In a test tube containing 0.5 mL of aqueous extract of the samples, 5 to 10 drops of diluted HCl and a small amount of Zn or Mg were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown color indicated the presence of flavonoids.

2.2.5 Glycosides

A small amount of alcoholic extract of samples were dissolved in 1 mL water and then aqueous sodium hydroxide was added. Formation of a yellow color indicated the presence of glycosides.

2.2.6 Steroids [Salkowski's test]

About 100 mg of dried extract was dissolved in 2 mL of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interface was an indicative of the presence of steroidal ring.

2.2.7 Cardiac glycosides [keller killiani's test]

About 100 mg of extract was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution and 1mL of concentrated Sulphuric acid was added. A brown ring obtained at the interface indicated the presence of a de oxy sugar characteristic of cardenolides.

2.2.8 Saponins

A drop of sodium bicarbonate was added in a test tube containing about 50 mL of an aqueous extract of sample. The mixture was shaken vigorously and kept for 3min. A honey comb like broth was formed and it showed the presence of saponins.

2.2.9 Resins

To 2 mL of chloroform or ethanolic extract 5 to 10 mL of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5 mL of H₂SO₄ was added. Bright purple colour was produced. It indicated the presence of resins.

2.2.10 Phenols [Ferric chloride test]

To 1ml of alcoholic solution of sample, 2 mL of distilled water followed by a few drops of 10 % aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

2.2.11 Tannins [Lead acetate test]

In a test tube containing about 5 mL of an aqueous extract, a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins.

FeCl₃ test: A 2 mL filtrate 200 mg of plant material in 10 ml distilled water, filtered, and 2 mL of FeCl₃ were mixed. A blue or black precipitate indicated the presence of tannin.

2.2.12 Microscopy of extract

The microscopy of the extract of *Sygygium cumini* was studied under Projection Microscope. The extract was dissolved in purified water and a specimen was prepared by air dry process. on the screen.

2.3 Formulation of Microparticles

Formulation of microparticles of *Sygygium cumini* was done by using solvent evaporation method. This method involves the emulsification of an organic solvent containing dissolved polymer and dissolved dispersed drug in an excess amount of continuous phase, with the help of an agitator. The concentration of the emulsifier present in the aqueous phase affects the particle size and shape. When the desired droplet size is formed, the stirring rate is reduced and evaporation of the organic solvent is realized under atmospheric or reduced pressure at an appropriate temperature. Subsequent evaporation of the dispersed phase solvent yields solid polymeric micro particles entrapping the drug. The solid microparticles are recovered from the suspension by filtration, centrifugation, or lyophilization. In the solvent evaporation technique, there are basically two systems which include single and multiple emulsion solvent evaporation. [12].

2.3.1 Single emulsion solvent evaporation technique

In the case of single emulsion solvent evaporation technique, there are also two systems such as oil-in-water (o/w) and water in oil (w/o). For insoluble or poorly water-soluble drugs, the (o/w) method is frequently used [12]. This method is the simplest and effective method for the preparation of floating microspheres. In this method, the polymer is dissolved in organic solvents [13].

2.3.2 Formulation of microparticles

300 mg of glycerol monostearate (GMS) were dissolved in 10 mL of heated ethyl acetate and was stirred for 30 sec. 700 mg of extract of (*S. cumini*) were dissolved in 20 ml of distilled water. After 1 min poly vinyl alcohol (PVP) was added to the solution and stirred for 3 min to make saturated solution. Then this above solution of drug extract and PVP was added to ethanolic GMS solution drop wise and emulsified on magnetic stirrer for 3 hours to form an emulsion. The primary emulsion was subjected to homogenization resulting in the formation of a dispersion, the emulsion was then subjected to solvent removal by solvent evaporation. Finally the microparticles were collected by filtration and washed with demineralized water [14].

2.4 Evaluation of Microparticles

2.4.1 Microparticle yield

This study aimed to determine of the amounts of microparticles at the end of preparations and of polymer and drug that are consumed. It can be calculated as follows.

Percentage yield = practical yield / theoretical yield × 100

Practical yield of microparticle = amount of entrapped drug / amount of drug added.

2.4.2 Entrapment efficiency

To calculate the entrapment efficiency % (EE), 10 mg prepared microspheres were dissolved in 2 mL ethyl acetate diluted up to 10 mL with distilled water. This solution was subjected to centrifugation at 1000 rpm. Supernatant was filtered through 0.45 µm filter. The absorbance of this solution was noted using ultraviolet (UV) spectrophotometric method at λ max 200 – 400 nm and the % EE was calculated using the following equation;

%Entrapment efficiency = Amount of entrapped drug / Amount of total drug used ×100.

2.5 Microscopy of Microparticles

The microscopy of the extract of *Sygygium cumini* was done under the projection microscope. The microparticles were dissolved in the purified water and specimen was prepared by air dry process and visualized on the screen.

2.5.1 In-vitro release of *Sygygium cumini* from microparticles

In-vitro drug release studies were performed in USP Type II dissolution apparatus at rotation speed of 200 rpm. The prepared microparticles (10 mg) were immersed in 900 ml of Basic medium (pH 7.4) and (10 mg) in Acidic medium of pH 3.4 solution in a vessel, and temperature was maintained at 37±0.20°C. Required quantity 5 mL of the medium was withdrawn at specific time periods and the same volume of dissolution medium was replaced in the flask to maintain a constant volume (Sink Condition). The withdrawn samples were analyzed using UV spectrophotometer.

2.5.2 Thin layer chromatography (TLC)

Preparation of TLC plates slurry of silica gel G was prepared in water and poured on glass plates. The plate was air dried and activated at 105°C for 30 min.

Preparation of sample solution- The microparticles were dissolved in water: ethylacetate solution.

Sample spotting- The sample was applied over plate in form of band using 1ml syringe.

Development of chromatogram- The plate was allowed to run in solvent system composed of n-butanol:water 10:10). The TLC plate was observed in a UV chamber.

2.6 Qualitative Analysis and *In-vitro* Antioxidant Activity

2.6.1 Sample preparation

About 10–50 mg of the extract were dissolved in 5 mL methanol and sonicated for 45 min. at 40°C followed by centrifugation at 1000 rpm for 10 min. The clear supernatant was collected and stored in an amber bottle for analysis.

2.6.2 Determination of Total Phenolic Content

The total phenolics were determined in the *S. cumini* extracts (aqueous) using Folin-Ciocalteu reagent method, employing gallic acid as standard. Briefly, 200 μ l of aqueous extracts (2 mg/ml) were made up to 3 mL with distilled water, then mixed thoroughly with 0.5 mL of Folin-Ciocalteu reagent. After mixing for 3 min, 2 mL of 20 % (w/v) sodium carbonate was added and allowed to stand for further 60 min in the dark. The absorbance of the reaction mixture was measured at 650 nm, and the results were expressed as mg of gallic acid equivalent (GAE)/g of dry weight.

2.6.3 Determination of total flavonoid content

Total flavonoid content of crude extracts was determined using the aluminium chloride colorimetric method as described by Chang *et al* [15]. In brief, 50 μ L of aqueous extracts (20 mg/mL) were made up to 1 mL with methanol, then mixed with 4 mL of distilled water and subsequently with 0.3 mL of 5 % NaNO_2 solution. After 5 min incubation, 0.3 mL of 10 % AlCl_3 solution was added and allowed to stand for 6 min, followed by adding 2 mL of 1 M NaOH solution. Then water was added to the mixture to bring the final volume to 10 ml and the mixture was allowed to stand for 15 min. The absorbance was measured at 510 nm. Total flavonoid content was calculated as quercetin from a calibration

curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg mL^{-1} in methanol. The result was expressed as mg quercetin equivalent (QUE)/g of dry weight [15].

2.6.4 Free radical scavenging activity (DPPH)

Quantitative measurement of radical scavenging properties of *S. cumini* extracts was carried out according to the method of Blois. Briefly, 0.1 mM solution of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH.) in methanol was prepared and 1 mL of this solution was added to 3 mL of aqueous extracts at different concentrations (1-15 $\mu\text{g/mL}$). Ascorbic acid was used as a positive control. After incubation for 30 min in the dark, the discoloration was measured at 517 nm. Measurements were taken in triplicate [16]. The capacity to scavenge the DPPH* radical was calculated and expressed as inhibition percentage using the following equation:

$$\% = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

The IC_{50} values (concentration of sample required to scavenge 50 % of free radicals) were calculated by the regression equation prepared from different concentrations of aqueous extracts.

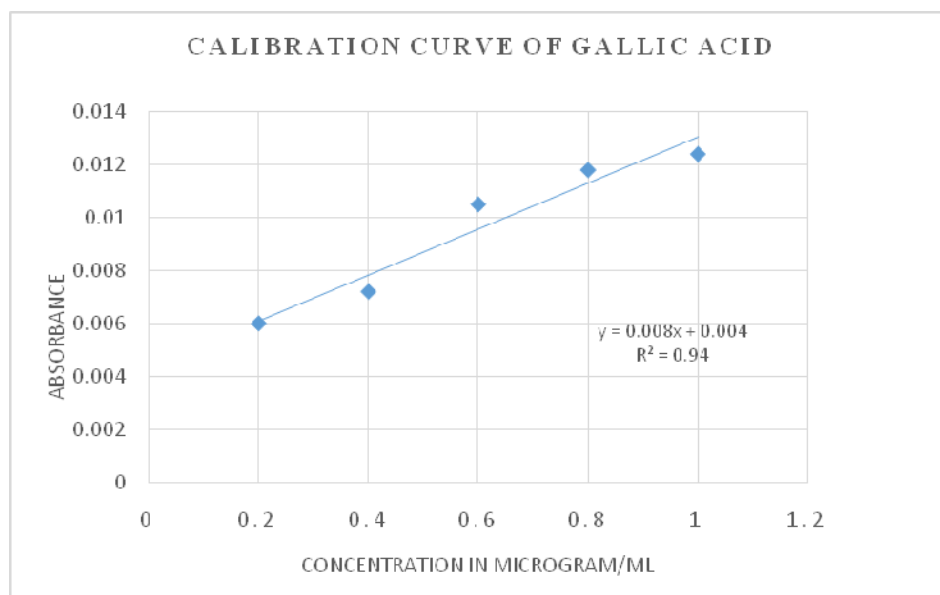


Fig. 1. Gallic acid curve

2.6.5 Total antioxidant capacity

The antioxidant activity of the extract was evaluated by the phosphomolybdate method according to the procedure describe by Prieto et al. (1999) [15]. The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A 0.3 mL extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. Then absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The antioxidant activity is expressed as number of gram equivalents of ascorbic acid.

$$(\%) \text{ Inhibition} = A_0 - A_1 / A_0 \times 100.$$

where A0 is the absorbance of the control (blank, without extract) and A1 is the absorbance in the presence of the extract [15].

2.6.6 Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity was based on the method described by Salar & Seasotiya (2011) [17]. In these experiments the superoxide anion was generated in 3 mL of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 µM) solution, 0.75 mL of NADH (936 µM) solution and 0.3 mL of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation.

$$(\%) \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract [17].

3. RESULTS AND DISCUSSION

3.1 Morphological Study

Plant morphology majorly deals with physical form and external structure of plants.

Morphological study provides suitable parameter for identification and authentication of crude drugs. The result for seed morphology is given in Table 1.

Table 1. Results for morphological studies of seeds of *Syginium cumini*

S. No	Parameter	Result
1	Color	Light Brown
2	Odour	Mild Iron rust
3	Taste	Mild Bitter
4	Size	0.1-100 mm
5	Shape	Irregular
6	Appearance	Rough

3.2 Phytochemical Screening

The aqueous extract of *S. cumini* was subjected to various phytochemical tests to detect the presence of secondary metabolites like tannins, alkaloids, flavonoids and saponins. as described earlier in methodology. The results obtained, are shown in Table 2.

Table 2. Phytochemical screening of extract of *Syzygium cumini*

S. No	TEST	RESULT
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Steroids	+
5	Cardiac glycosides	-
6	Saponins	+
7	Resins	+
8	Phenols	+
9	Tannins	+

3.3 Evaluation of Microparticles

3.3.1 Microparticle yield

The yield of microparticles of *Syginium cumini* is 90 %.

3.3.2 Drug entrapment and drug efficiency

The entrapment efficiency of Mmicroparticles of *S. cumini* is 85.71 %. The entrapment efficiencies are ranging from 75 to 85.7 %. The entrapment efficiency of *Syginium cumini* is dependent upon its solubility in the solvent and continuous phase. An increase in the concentration of polymer (4 % to 12 % w/v) in a fixed volume of solvent resulted in an increase in entrapment efficiency.

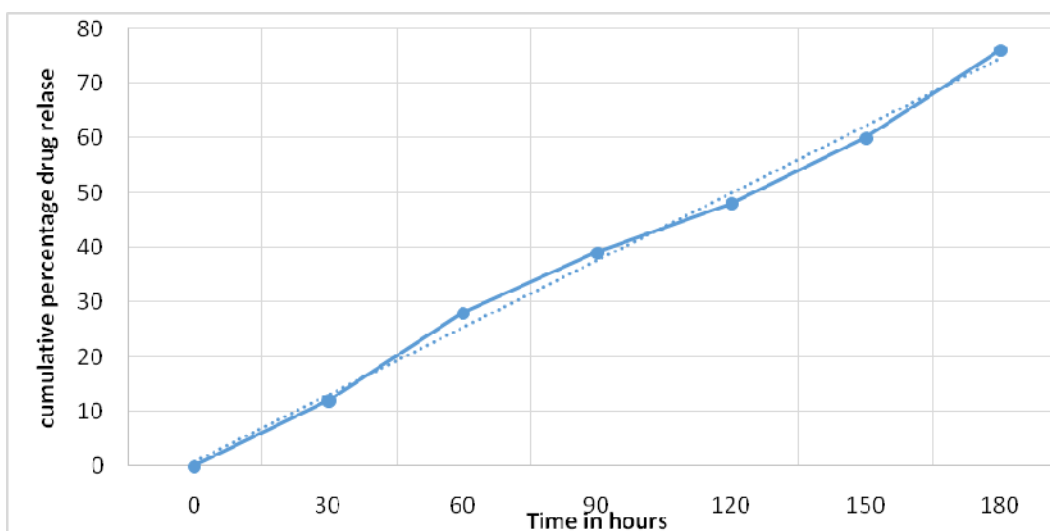


Fig. 2. Drug entrapment and drug efficiency

3.3.3 Microscopy of microparticles

The microscopy of microparticles of *S. cumini* was done, and it was found that it had, the characteristics of microparticles.

3.3.4 *In-vitro* release of microparticles from *S. cumini*

The release of microparticles from *S. cumini* depended on the polymer concentration used and amount of drug extract used.

3.3.5 Qualitative analysis and *In-vitro* antioxidant activity

Results concerning total phenolic and flavonoid content of microparticles from seeds of *S. cumini*.

3.3.6 TLC (thin layer chromatography)

The R_f Value of the Microparticles of *S. cumini* was 0.90

3.3.7 DPPH Radical scavenging activity

The DPPH radical scavenging activity of aqueous extract of *S. cumini* is shown in Table 4. It was observed to be maximum at the highest concentration. The extract showed encouraging

response in quenching DPPH radical with an IC_{50} value of 94.25 $\mu\text{g/mL}$.

3.3.8 Total antioxidant activity

The total antioxidant activity determined by the phosphomolybdate method was found to be 91.35 $\mu\text{g/ml}$ and 84.22 $\mu\text{g/mL}$ respectively.

3.3.9 Superoxide anion scavenging activity

The aqueous extract of microparticle had a strong superoxide radical scavenging activity. The IC_{50} value was found to be 61.55 $\mu\text{g/mL}$ for microparticles and 74.22 $\mu\text{g/mL}$ for standard ascorbic acid.

In the current study, the *in-vitro* antioxidant activities of microparticles from seeds of *Syginium cumini* were assessed against DPPH, as total antioxidant and super oxide free radical scavenging activity. The microparticles showed different levels of radicals scavenging activity in a dose-dependent manner over the concentration range of 50–150 $\mu\text{g/mL}$ concentration, aqueous extract. In all the experimental screening models microparticles of aqueous extract shows the maximum inhibition of free radicals comparison of other experimental model at different concentrations.

Table 3. Total phenolic and flavonoid content of microparticles of seeds of *S. cumini*

S. No	Antioxidant Test	Results
1	Total Phenolic Content	0.0969 mg/gm. GAE
2	Total Flavonoid Content	0.0903 mg/gm. QUE

Table 4. DPPH Radical Scavenging activity of micro particles of *S. cumini*

Concentration ($\mu\text{g/mL}$)	% scavenging (aqueous extract)	% scavenging (std)
50	27.57 \pm 0.43	31.01 \pm 0.62
75	38.40 \pm 0.33	47.13 \pm 0.58
100	53.63 \pm 0.34	63.65 \pm 0.36
125	63.29 \pm 0.77	71.75 \pm 0.59
150	78.34 \pm 0.61	80.14 \pm 0.66
IC ₅₀ ($\mu\text{g/ml}$)	94.25 \pm 0.84	82.22 \pm 0.65

Standard = Ascorbic acid, Values are mean \pm SEM (n=3)

Table 5. Percentage of total antioxidant activity of aqueous extract of microparticles at different concentrations

Concentration ($\mu\text{g/mL}$)	% scavenging (aqueous extract)	% scavenging (std)
50	23.47 \pm 0.23	33.01 \pm 0.22
75	33.10 \pm 0.33	48.13 \pm 0.38
100	52.23 \pm 0.24	64.65 \pm 0.16
125	61.49 \pm 0.47	72.75 \pm 0.49
150	75.24 \pm 0.61	81.14 \pm 0.26
IC ₅₀ ($\mu\text{g/ml}$)	91.35 \pm 0.44	84.22 \pm 0.45

Standard = Ascorbic acid, Values are mean \pm SEM (n=3)

Table 6. Percentage of superoxide radical scavenging activity of aqueous extract of microparticles at different concentrations

Concentration ($\mu\text{g/mL}$)	Aqueous extract	% scavenging (std)
50	12.37 \pm 0.23	23.01 \pm 0.42
75	13.30 \pm 0.33	28.13 \pm 0.18
100	22.13 \pm 0.24	44.65 \pm 0.26
125	41.49 \pm 0.47	52.75 \pm 0.49
150	55.34 \pm 0.61	66.14 \pm 0.16
IC ₅₀ ($\mu\text{g/ml}$)	61.55 \pm 0.44	74.22 \pm 0.45

Standard = Ascorbic acid, Values are mean \pm SEM (n=3)

4. CONCLUSION

Herbal plants are potential source of phytoconstituent with varied pharmacological activities. In formulation of microparticles of *Syginium cumini*, the extraction and microscopy phytoconstituent analysis were evaluated through several parameters. Assays for *in-vitro* antioxidant activity were also carried out showing considerable antioxidant activity by the microparticles.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rajak AR, Singh R. Contribution of medicinal plants in economic growth. *World J Pharma Pharma Sci.* 2017;6:367-72.
2. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *J Ethnopharmacol.* 2006;103(1):25-35.
3. Barbosa LN, Rall VL, Fernandes AA, Ushimaru PI, da Silva Probst I, Fernandes A Jr. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. *Foodborne Pathog Dis.* 2009;6(6):725-8.
4. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol.* 1990;186:1-85.

5. Miller AL. Antioxidant flavonoids: Structure, function and clinical usage. *Altern Med Rev.* 1996;1:103-11.
6. Sinha K, Mishra NP, Singh J, Khanuja SP. *Tinospora cordifolia* (Guduchi): A reservoir plant for therapeutic applications: A review. *Indian J Trad Know.* 2004;3:257-70.
7. Singh S, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. Chemistry And Medicinal Properties Of *Tinospora cordifolia*. *Indian J Pharmacol.* 2003;35:83-91.
8. Mervat MMEF, Hanan AAT. Antioxidant activities, total anthocyanins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol. *Australian J Basic Applied Sc.* 2009;3:3609-3616.
9. Patel RK, Patel JB, Trivedi PD. Spectrophotometric method for the estimation of total alkaloids in the *Tinospora cordifolia* M. and its herbal formulations. *Inter J Pharm Pharm Sci.* 2015;7:249-51.
10. Lee JY, Yoon JW, Kim CT, Lim ST. Antioxidant activity of phenylpropanoid esters isolated and identified from *Platycodon grandiflorum* A. DC. *Phytochemistry.* 2004;65:3033-39.
11. Lai HY, Lim YY, Tan SP. Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. *Biosci. Biotech. Biochem.* 2009;73:1362-1366.
12. María G Nava-Arzaluz, Elizabeth Piñón-Segundo, Adriana Ganem-Rondero, David Lechuga-Ballesteros. Single emulsion-solvent evaporation technique and modifications for the preparation of pharmaceutical polymeric nanoparticles. *Recent Pat Drug Deliv Formul.* 2012;6(3):209-23.
13. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents.* 2005;26:343-356.
14. Berkada B. Preliminary report on warfarin for the treatment of herpes 210 simplex. *J Irish Coll Phys Surg.* 1978;22:56.
15. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *Journal of Food and Drug Analysis* 2002;10(3):178-182
16. Ravi SBESa, Raman Shanthi Iyer, Sundara Rajan Subbaramaiah, S Austin Richard, Bhadrappura Lakkappa Dhananjaya. Antioxidant activity of *Syzygium cumini* leaf gall extracts. *Bioimpacts.* 2014;4(2):101–107.
17. Raj Kumar Salar, Leena Seasotiya. Free radical scavenging activity, phenolic contents and phytochemical evaluation of different extracts of stem bark of *Butea monosperma* (Lam.) Kuntze. *Frontiers in Life Science.* 2011;5;3-4:107-116.

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Peer-review history:

The peer review history for this paper can be accessed here:
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