



## **Phytochemical Screening and Antifungal Activity of *Samadera indica***

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### **Authors' contributions**

This work was carried out in collaboration between both authors. Author RAG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SJ managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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### **ABSTRACT**

*Samadera indica* plant is an antifungal and bitter plant spread all over India. The applications of NDDS in phytopharmaceuticals have been widely investigated and various commercial formulations of plants are available in the global market. The goal of nano drug delivery system of herbal extract is to reduce toxicity, to improve drug efficacy, to improve better therapeutic effect, etc. Nanomicelles of *samadera indica* were successfully prepared by solvent evaporation method and studied various parameters such as Preliminary phytochemical screening, Scanning electron microscopy, and antifungal study. In present methodology, it was concluded that the Nanomicelles of methanolic extract of *samadera indica* plant was successfully prepared. It was safe and non-toxic. Plant extract contained flavonoids, triterpenes, and alkaloids which is responsible for antifungal activity. This research work concludes that the *samadera indica* is used as antioxidant, antimicrobial as well as antifungal compound. Novel drug delivery system is a advanced technology for delivering the drug with their so many advantages. Nanomicelles is an excellent pharmaceutical carriers by using bioactive constituents of plant having no side effects, effective formulation for various diseases especially in case of skin diseases. Novel formulation enhanced the topical drug delivery system of *Samadera indica* in order to treat skin infections was developed.

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## 1. INTRODUCTION

In ancient times herbal remedies contains hundreds of phytopharmaceuticals of plants and it has been increasing day by day among physicians and patients. Various dietary products and supplements are also derived from the natural origin and gaining more interest in the industry and the global market for phytopharmaceuticals [1,2]. *Samadera indica* is a medicinal plant obtained from western ghats of India including Kerala, Ernakulam and Karnataka forest region in India. *Samadera indica* is also known as Neipa bark tree, *Quassia indica*, karinjotta, karingota etc. It is bitter in taste and is a small tree upto 11 m in height with stout branches and pale yellow bark. It is widely distributed in evergreen forests and along backwaters of south India. *Samadera indica* leaves are used for the treatment of number of diseases. It is traditionally used in vitiated conditions of kapha and pita, leprosy, scabies, pruritis, skin diseases, for treating burns, inflammations, bacterial infections, gonorrhoea, asthma etc. [1,3,4]. A typical extraction process may contain following steps [4] Collection and authentication of plant material & drying, Size reduction, Extraction, Filtration, Concentration, Drying & reconstitution [5,6]. Quality of an extract is influenced by several factors such as, plant parts used as starting material, solvent used for extraction, extraction procedure and plant material: solvent ratio etc. From laboratory scale to pilot scale all the parameters are optimized and controlled during extraction.

Extraction techniques separate the soluble plant metabolites through selective use of solvents. It is the oldest method of preparation of plant extract. It is a main constituents source of bioactive compound which are used for medicine and other applications. Plant extract contains many bioactive compounds like as flavonoids, steroids, tannins, alkaloids, fixed oils, volatile oils etc. All of these bioactive compounds are obtained from specific parts of plants like as flowers, leaves, fruits, barks, seeds etc. [6-8]. Now a day's herbal formulations are mostly used which is converted into novel herbal formulations and extraction method is one of the important method for obtaining bioactive compounds. There are number of methods available for extraction- maceration, percolation, infusion, decoction, soxhlet extraction etc. [9-11].

It should be noted that choice of appropriate solvent is of essential importance along with application of a compatible extraction method. For selection of solvents 'like dissolves like' principle is applicable. Thus polar solvents will extract out polar substances and non-polar material will be extracted out by non-polar solvents. Solvent extraction is the most popular method of extraction [7,10-13].

### 1.1 Micelles

A sudden change in many physicochemical properties is seen in solutions of amphiphilic molecules or surfactant monomers that possess a polar head and a lipophilic tail. The change in physicochemical properties is associated with the orientation and association of amphiphilic molecules in solution resulting in the formation of structures called micelles. The micelles internally have a hydrophobic core and externally a hydrophilic surface. Micelles are generally made up of 50 to 200 monomers. The radius of a spherical micelle is almost the same as the length of a fully extended surfactant monomer, which mostly is 1-3 nm, and thus micelles lie in the colloidal range [14-17].

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The plant is generally found in South, Ernakulam district Kerala during the month of February. But For the study, plant was obtained from DKC Agrotech Pvt. Ltd., New Delhi, India.

### 2.2 Chemicals

The Vitamine E, Petroleum ether, Methanol and ethanol used in this study were obtained from laboratory of Dr. APJ Abdul Kalam University, Indore, [M. P.], India.

### 2.3 Plant Profile

**Common name:** *Samadera indica*

**Synonyms:** Karinjotta, Quassia Indica, Lokhandi, Niepa bark tree

**Family:** Simaroubaceae

**Medicinal use:** for fever, skin disease, rheumatic, febrifuge, erysipelas, anti inflammatory.



Fig. 1. *Samadera indica* plant

## 2.4 Preparation of Plant

Leaves were carefully collected and separated out and washed to remove impurities. Leaves were dried in presence of sunlight, then leaves are blend in the mechanical grinder and converted into fine powder then passed through sieve no 40 and stored in an airtight container for further use. 50 gm of fine powder was taken in round bottom flask and were soaked in 250 ml of methanol for 24 hours with intermittent shaking. The extraction process was done by hot extraction method using soxhlet apparatus. This process was continued until the solvent became clear and collected the sample in container. After the extraction, the extract was kept in heating mentle for evaporating the solvent until the extract given in crude form.

## 2.5 Determination of Extraction Yield (% Yield)

The yield (% W/W) from the dried extracts was calculated as

$$\% \text{ Yield} = (W_1 \times 100) / W_2$$

Where  $W_1$  is the weight of extract after evaporation of solvent and  $W_2$  is the weight of the plant powder.

## 2.6 Preliminary Phytochemical Investigation

After the crude drug obtained or methanolic extract was used in preliminary phytochemical

studies [5] for presence of various phytochemical constituents. The extract of *Samadera indica* leaves was found to contain some bioactive compounds such as flavonoids, tannins, alkaloids, phenolic compounds, triterpenes, resins, proteins and carbohydrates in methanolic extract.

## 2.7 Preparation of Nanomicelle (NMI)

Plant extract was taken with Vitamin E TPGS in beaker and a mixture was made using the solvent evaporation method where the organic solvent was removed through evaporation. The solvent evaporation method [5] includes drug and polymer which are dissolved in a volatile organic solvent. This volatile organic solvent mix with aqueous phase by using sonication [6]. After the evaporation of the organic solvent, a thin film of drug/polymer was formed at the bottom of the flask. This film was reconstituted by shaking in an aqueous phase. Solvent evaporation may provide an effective variable for tailoring micelle morphology. Vitamin E TPGS (7 gm) and 2 mL of acetone were added in it. When a clear solution was obtained, 25 mL of distilled water was added. Then the organic solvent was removed gradually through evaporation. Change of solvent quality and selectivity from organic to aqueous was gradual; the polymer and the extract were able to aggregate into micelles rather than precipitating from the solution into the bulk. The solvent of choice was acetone, due to its high water miscibility and low vapor pressure, which simplified the solvent removal.

## 2.8 Evaluation Nanomicelle (NMI)

### 2.8.1 SEM (Scanning Electron Microscope)

It is a type of microscopy which shows image of a sample by scanning the surface with a focused beam of electrons. This interacts with atoms in the sample, producing various signals that contain information about the surface topography sample content. It is used for surface fracture, flaws, contaminants or corrosion. Scanning electron microscope is a specialized field of science that employs the electron microscope as a tool and uses a beam of electrons to form an image of a specimen allowing imaging and quantification of surface topographic features.

### 2.8.2 Determination of antifungal activity of Nanomicelle

This is the most important study for plant extract or plant parts due to presence of microorganism such as bacteria, archaea, fungi, viruses and protozoa. The presence of higher number of spores bacteria could be explained by the some of the organism like as *Bacillus cereus* and *Clostridium perfringens* and the fungal contamination of extract such as *Aspergillus niger* and *Candida albicans*.

## 3. RESULTS AND DISCUSSION

A medicinal plant is any plants in which one or more of its organs contains active ingredients which can be used for therapeutic purposes or contain compounds that can be used for synthesis of useful drugs. *Samadera indica* plants belong to the family of simaroubaceae and taste of this plant is bitter due to presence of Quassanoids [7]. *Samadera indica* was carried out and used for preparing [8] novel drug delivery system to develop Nanomicelle. Nanomicelles were prepared by using methanolic extract of *Samadera indica*. Methanolic extract of plants was prepared by soxhlet apparatus kept for 24 hours. Evaporation method was used to obtain crude dry form of drug. The yield of crude extract was obtained by measuring its dry weight. % yields of extract may be Determined with help of following formula:

$$[\% \text{ Yield} = (W_1 \times 100)/W_2]$$

The value of percentage yield was obtained 4.

Preliminary phytochemical screening of the extracts shows the presence of active constituents of methanolic extract of plant. Phytochemical

screening of *Samadera indica* confirmed the presence of triterpenes, steroids, gums and mucilages, steroids and carbohydrates as its major chemical constituents (Table 1). It was observed that alkaloids, flavanoids, tannins, phenolic compounds, triterpenes, protein were present in the extracts of the plant. While resins and carbohydrate was absent. Result of preliminary phytochemical screening of plant extract as given in below table:

**Table 1. Phytochemical screening of *Samadera indica***

S. No.	Phytochemicals	Observation
1	Flavanoids	+
2	Tannins	+
3	Alkaloids	+
4	Phenolic Compounds	+
5	Triterpenes	+
6	Resins	-
7	Proteins	+
8	Carbohydrates	-

+ Present, - Absent

Nanomicelle was successfully prepared by solvent evaporation method under the required condition.

### 3.1 Scanning Electron Microscopy

By means of evaluating the SEM images, the particle size of formulation was determined. SEM plays a paramount role for assessing the microbial populations, three-dimensional structure, physiology, thickness, etc. SEM image shows the morphological character, size and surface of Nanomicelles formulation. The size of nanomicelles is 10 to 15 nm with the mixture of many shapes i.e. spherical and needle like shaped are clearly observed. SEM enabled us to detect the existence of divergences from the expected results, allowing for a better quality evaluation of the herbal formulation to production technology.

Prepare different concentrations of methanolic extracts of *Samadera indica* for antifungal activity. Comparison of methanolic extract with standard sample of fluconazole. There are strain of fungi such as *Aspergillus niger*, *Candida albicans*.

### 3.2 Antifungal Properties by MIC Method

The culture of saboraaud dextrose agar medium (Fungi) plates were stored in slants as stock

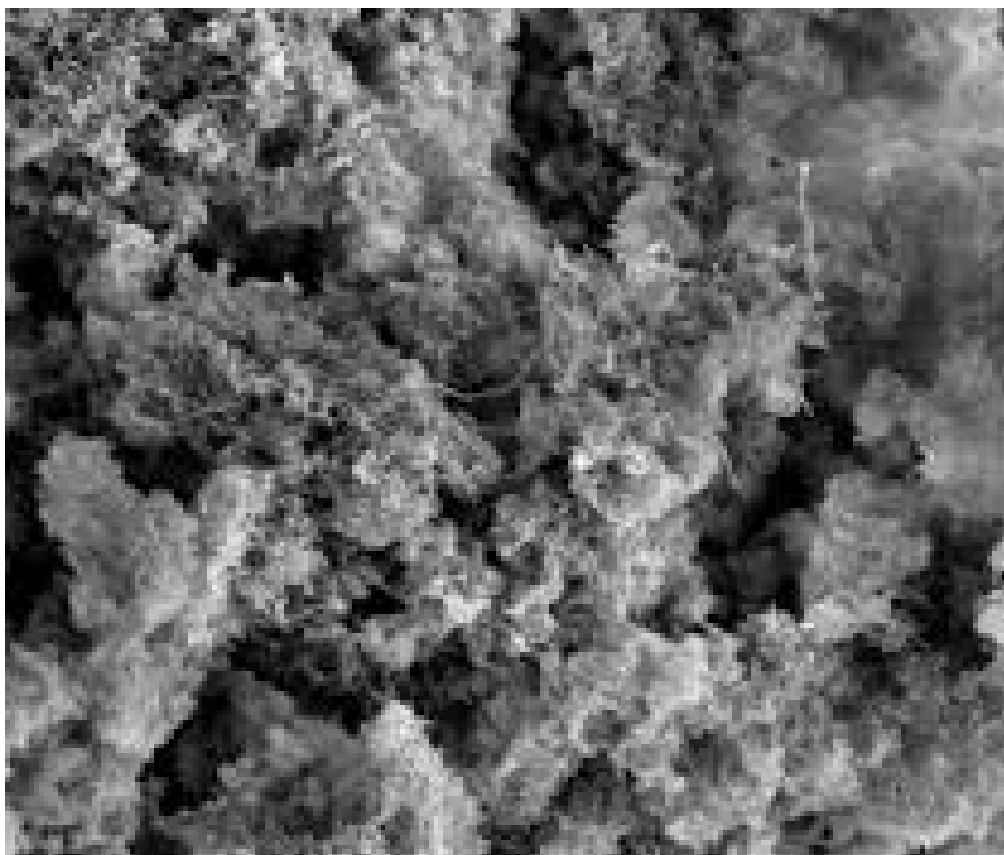


Fig. 2. SEM of Nanomicelle of methanolic extract of *Samadera indica*

Table 2. Antifungal activity

Organism	Zone of inhibition (mm)			
	Standard (Fluconazole)	Methanolic extract		
	10 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml
<i>Candida albicans</i>	19.9±0.02	-	-	12.28±0.19
<i>Aspergillus niger</i>	21.22±0.02	-	10.9±0.04	16.29±0.72

cultures and incubated at 27°C for 48 h for fungi. It was determined by liquid broth method of two fold serial dilution technique. In this assay, the minimum concentration of each test substances required to inhibit the growth of microorganism was determined by the production of turbidity. The activity was found to be more for the fungus *C. albicans*, *Aspergillus Niger* indicating that they would be a potent antifungal drug. The standard used in the study was fluconazole (10 µg/ml).

#### 4. CONCLUSION

In present methodology, it was concluded that the Nanomicelles of methanolic extract of

*Samadera indica* plant was successfully prepared. It was safe and non- toxic. The selected plant extract shows the presence of flavonoids, triterpenes, alkaloids which was responsible for antifungal activity. Current research in drug discovery from medicinal plants involves a multidisciplinary approach combining botanical, phytochemical, biological and molecular techniques. There is a need to improve technology for the rapid isolation of active compounds in large quantities for evaluation with the scientific collection of plant material and maintenance of biodiversity. This research work conclude that the *Samadera indica* is used as antioxidant, antimicrobial as well as antifungal compound. Novel drug delivery

system is an advanced technology for delivering the drug with their so many advantages. Nanomicelles is an excellent pharmaceutical carriers by using bioactive constituents of plant having no side effects, effective formulation for various diseases especially in case of skin diseases. Novel formulation enhanced the topical drug delivery system of *Samadera indica* in order to treat skin infections was developed. Nanomicelles of plant extract are used to prevent or minimize lower adverse side effects, drug degradation, better therapeutic response, enhanced bioavailability etc. Considerable effort has been made by researchers to find efficient extraction methods in order to get high efficiency and efficacy.

### CONSENT

It is not applicable.

### ETHICAL APPROVAL

It is not applicable.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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