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Selection of Effective Plant Extract as A Disinfecting Agent using Hot and Cold-water Extraction

Haorongbam Joldy Devi[1*], Prathiba Gnanasekaran[2] and Yumnam Asha Devi[1]

[1]Department of Microbiology, Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Chennai 600 119, T.N., India
[2]Department of Microbiology, Sathyabama Dental College and Hospital, Jeppiaar Nagar, Chennai 600 119, T.N., India

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ABSTRACT

The purpose of the study was to determine the antibacterial activity of the cold and hot water extract of the *Mangifera indica*, *Morinda citrifolia* and *Syzygium cumini* leaves by agar well diffusion method. Among the plant extracts that showed the best antibacterial activity was further used to evaluate the colony count reduction of plant extracts after application on the floor surfaces and to analyse the phytochemical present The study showed that hot water extracts of *S.cumini* leaves showed the best antibacterial activities against all the test organisms and it was also observed that the number of microorganisms reduced significantly after application on the floor surfaces. The phytochemical analysis of hot water extract of *S.cumini* leaves showed the presence of tannin, flavonoids, terpenoids, saponin, phenol but absence of alkaloids. Thus, hot water extract of *S.cumini* leaves can be selected as the best plant extract for plant-based disinfecting agent among the other plant extracts. This is the first report for hot water extract of *S.cumini* leaves which is tested for efficacy of plant-based disinfecting agent.

Key words: *Plant-based disinfecting agent, Plant extracts, Antibacterial activity, Colony count reduction, Phytochemical analysis.*

Introduction

Environmental cleanliness is an important way to prevent infection. Floor surfaces are often covered by the dust containing of organic pollutants, fibres, particles, polycyclic aromatic compounds, minerals, metals, bacteria, pollen, allergens, etc. (Vong *et al.,* 2018; APIC). Some of the clinically important bacteria found mainly in the indoor environment floors are *Staphylococcus aureus, Bacillus sp., Pseudomonas aeruginosa, Escherichia coli, Salmonella* sp., *Klebsiella pneumonia*, *etc.* (Gupta, 2017; North Star Mat Services, 2019). Disinfection is very much necessary in the cleaning process which contributes to broad microbiological sterilisation (Vong *et al.,* 2018). The term 'disinfection' means the process of reducing or eliminating the harmful microorganisms from inanimate objects and surfaces (Mc Keen, 2012). Proper cleaning and disinfection can reduce the risk of infection (Medline Plus, 2020). There is indirect evidence suggesting environmental contamination leads to nosocomial infection (Hota, 2004). So, chemical disinfectants which are commonly available in the market are used.

Alcohol-based chemical disinfectantshave negative effects such as irritation of the skin and microbial resistance (Pandya *et al.,* 2017). Often used of chemical cleaning agents can cause serious health

related problems viz. allergies, asthma, eczema, including cancer. Allergic reaction can be mediated by exposure to skin or inhalation of least amounts of allergen contain in a chemical disinfectant. As chemical disinfectants are formulated in concentrated form, handlers should wear gloves. (Wolkoff *et al.,* 1998; American Lung Association, 2020; Safety Data Sheet Considering the possible health risks caused by chemical disinfectants, an alternative natural plant-based disinfectant is needed as it is non-toxic, eco-friendly, cost effective and less side effects when compared to other commercial chemical disinfectant available today (Nusrat; Maid 4 Condos, 2019).

Medicinal plants like, *Syzigium cumini* (Jamun), *Mangifera indica* (Mango) and *Morinda citrifolia* (Noni) have been reported to possess good antimicrobial activity (Imran *et al.,* 2017; Hannan *et al.,* 2013; Serafini *et al.,* 2011). *S. cumini* leaves have been reported to possessed medicinal properties such as antibacterial, antidiabetic and widely used to treat constipation, leucorrhoea, gastralgia, pyrexia, gastropathy, dermatopathy, inhibit rectal bleeding, strengthen teeth and gums (Gowri and Vasantha, 2010). Study have reported that *M. indica* leaves possessed medicinal properties such as antimicrobial, antioxidant, anthelminthic, antidiabetic, antiallergic, etc (Hannan *et al.,* 2013). *M. citrifolia* leaves have been reported to possess antibacterial, antioxidant, anti-inflammatory, antinociceptive properties, etc (Serafini *et al.,* 2011).

Water is recommended as the best solvent for bioactive compounds extraction or herbal formulation (Pranabesh, 2019). Studies have shown that water extracts show less toxicity in animal study than other solvents such as N-Haxane, acetone, ethanol, etc. (Olasehinde *et al.,* 2016). This study therefore aimed to determine the antibacterial activity of cold and hot water extract of the *S.cumini*, *M.indica* and *M. citrifolia*. The plant extracts showing best antibacterial activity was further used to evaluate the colony count reduction after application on the floor surfaces and analysed for the presence of phytochemicals.

Materials and Methods

Collection of different plants

Fresh and healthy leaves of *Mangifera indica* (Mankani illai in Tamil), *Morinda citrifolia* (Nuna illai in Tamil) and *Syzygium cumini* (Naavar illai in Tamil), were collected in and around Chennai.

Preparation of plant extracts

The collected leaves were properly washed with running tap water in order to remove the dirt. (Saleem and Saeed, 2020). The cleaned leaves were shade dried for 10 days (Das and Goswami, 2019). The dried leaves were cut into small pieces and powdered using grinder (Saleem and Saeed, 2020). Then the powdered leaves were subjected to extraction using distilled water as a solvent (Abdelgadir *et al.,* 2015).

Cold water extract

25 g of dried leaves powder were mixed with 250 ml of distilled water in Erlenmeyer flaskplugged with cotton and kept under shaking condition for 3 days. Then it was strained and filtered using muslin cloth and Whatman no.1 filter paper respectively. The filtrates were evaporated. Then the dried filtrate was collected in the sterile screw capped tube and stored at 4 °C until use (Mummed *et al.,* 2018).

Hot water extract

25 g of dried leaves powder were mixed with 250 ml of distilled water. The mixture was heated at 60 ^{0}C for 2hrs and kept for 24 vhrs in room temperature (Sabandar, 2016). Then it was strained and filtered using muslin cloth and Whatman filter paper respectively. The filtrates were evaporated and dried filtrate was collected in the sterile screw capped tube. Then filtrate was stored at 4 °C until use.

Cultures

Bacterial cultures such as *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus* sp., *Klebsiella* sp., *Salmonella* sp. and *Escherichia coli* were collected from Institute of Basic Medical Sciences, Taramani, Chennai.

Media used

Brain heart infusion broth, Muller Hinton agar and Nutrient agar

Inoculum preparation

Bacterial strains were inoculated into the BHI broth and incubated at 37 ^{o}C for 3 hrs.

Antibacterial activity of the plant extracts

The antibacterial activity of the plant extracts was

determined by agar well diffusion method (Balouiri *et al.,* 2016). The lawn culture was made with the test organisms such, as K*lebsiella* sp., *S. aureus*, *P. aeruginosa*, *Bacillus* sp., *E. coli* and *Salmonella* sp. Onto the Muller Hinton agar using sterile cotton swab. The plates were then punched using gel puncture. A stock solution of 1000 mg/ml was prepared. From that, 100 µl and 200 µl of different concentration of plant extract were loaded into the wells using sterile micropipette. Ciprofloxacin antibiotic (5µg/ml) was used as a positive control. As a negative control, distilled water was used. The plates were then incubated at 37 °C for 24 hrs. After incubation, diameter of the zone of inhibition was measured. The assay was conducted in three replicates.

Preliminary study of plant extracts as a disinfecting agent

Three floor surfaces from hospital and laboratory were randomly selected and marked. The marked area was applied for 5 minutes with sterile distilled water, commercial disinfectant and hot water extract of *S.cumini* leaves as a negative control, positive control and test respectively. After 5 minutes, surface samples were collected with sterile moistened swab for negative control. For positive control and test, the surface was wiped with distilled water and samples were collected similarly. The surface swabbed sample of the respective areas were diluted respectively in 10 ml sterile saline and then 1ml of each sample was pour plated into the nutrient agar plate in two replicates. The plates were then incubated at 37 °C for 24 hrs (Vong *et al.,* 2018; Choudhari; Choudhari). The number of colony forming unit per area sample=CFU/cm^2 was calculated using the formula (National Infection services, 2017).

$$\text{Count per swab} = \frac{C}{v(n_1+0.1n_2)d} X\, n_3$$

where, C= total colonies on all plates, v= volume applied to each plate, n_1= total plates counted at 1st dilution, n_2= total plates counted at 2nd dilution, n_3= volume of suspension, d= 1st dilution

For CFU/cm^2, count per swab should be divided by area swabbed

The percentage reduction of microorganisms on the floor surfaces before and after disinfectant application was calculated using the following formula (Saad *et al.,* 2011).

$$\% \text{ Reduction} = \frac{\text{Control(CFU/cm}^2\text{)-Test(CFU/cm}^2\text{)}}{\text{Control(CFU/cm}^2\text{)}} \times 100$$

A colony from the respective agar plates was smeared onto a clean glass slide respectively and Gram staining was performed to examine the microorganisms on the plates.

Phytochemical Analysis

The Phyto chemical analysis of prepared plant extract was carried out by following standard procedures

Tannins test: To 1ml of the extract, 5 ml of distilled H_2O was added. Then, 2 drops of $FeCl_3$ solution were then added. The presence of tannins was indicated by transient greenish to black colour changed (Ukoha *et al.,* 2011).

Saponin test: 0.5g of each extract was diluted with 2ml of distilled water. Then, it was shaken vigorously. Formation of foam after shaking indicates the presence of saponin (Junaid and Patil, 2020).

Flavonoids test: A few drops of NaOH solution was added to 50mg of plant extract. After the addition of diluteacid, turning of the intense yellow colour to colourless indicates the presence of flavonoids (Bandiola, 2018).

Phenol test: To 2 ml of extract, 2 ml of distilled was added. Few drops of 10% $FeCl_3$ solution were then added to it. The presence of phenol was indicated by the appearance of bluish black colour (Shah and Yadav, 2015).

Terpenoids test: 0.2 g of plant extract was added in a test tube. 2 ml of chloroform and 3 ml conc. H_2SO_4 were added into the test tube containing the plant extract. The presence of terpenoids was indicated by reddish-brown coloration (Alamzed *et al.,* 2013).

Alkaloids test: Tothe extract, 1-2 drops of Meyer's reagent was added. The presence of alkaloids was indicated by the yellow precipitation (Junaid and Patil, 2020).

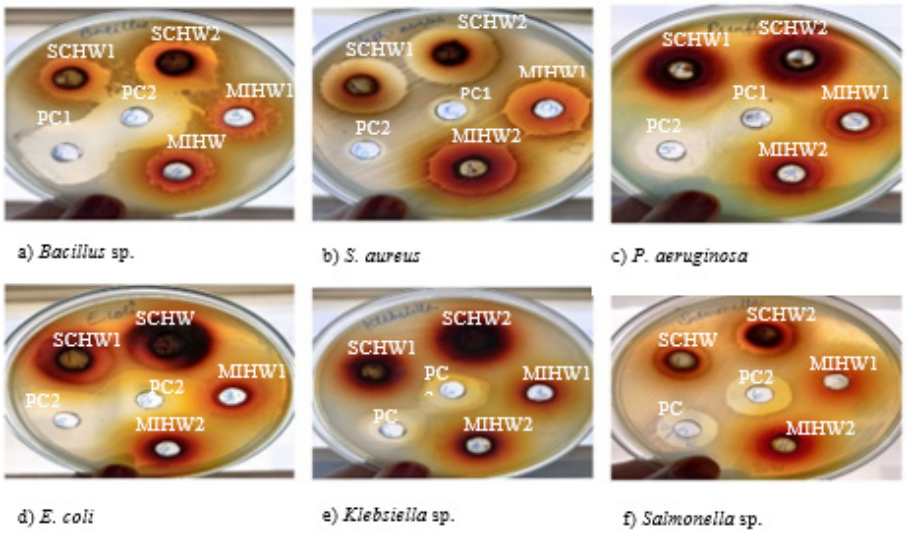
Results

Antibacterial Activity

The antibacterial activity of cold and hot water extracts of *S.cumini* leaves, *M.indica* leaves and *M.citrifolia* leaves were examined against the test

organisms such as *S. aureus*, *Klebsiella* sp., *P. aeruginosa*., *Bacillus* sp., *Salmonella* sp. and *E. coli*which was shown in Fig. 1.

Zone of inhibition (mm) of cold-water and hot water extract of the plants against the test organisms were summarized in Table 1 and 2 respectively. The data represent as mean ± SD (standard deviation) and P- value. The results were analysed using student's t-test and found to be significant at P-value <0.05. The graphical representation of comparison



SCHW1- *S. cumini* hot water extract (100 µl), SCHW2- *S. cumini* hot water extract (200 µl), MIHW1- *M. indica* hot water extract (100 µl), MIHW2- *M. indica* hot water extract (200 µl), PC1- Positive Control (100 µl), PC2- Positive Control (200 µl)

Fig. 1. Antibacterial activity test

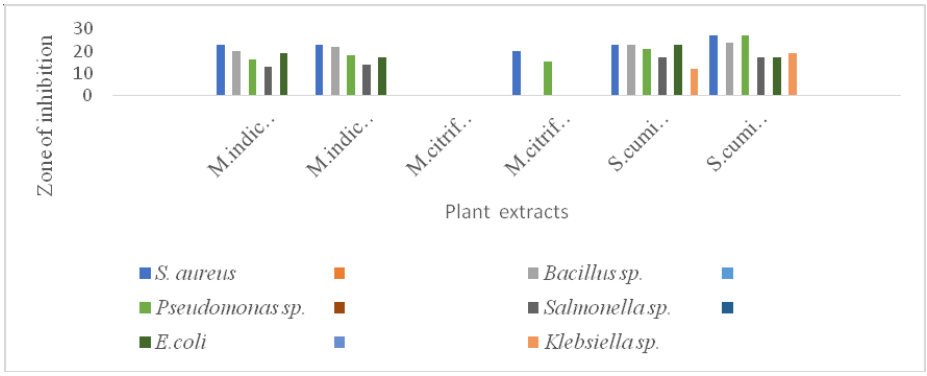


Fig. 2. Antibacterial activity comparison of cold and hot-water extracts of three plants against the test organisms

Table 1. Zone of inhibition (mm) of cold-water extract of plants

Organisms	Plants used						Positive control (Ciprofloxacin) (200 µl)	
	S. cumini (200 µl)		*M.indica* (200 µl)		*M. citrifolia* (200 µl)			
	Mean±SD	P-value	Mean±SD	P-value	Mean±SD	P-value	Mean±SD	P-value
S. aureus	23±1	0.0012	23±1	0.0012	-	-	17±0.5	0.0007
Bacillus sp.	23±1	0.0012	20±1.5	0.0038	-	-	36±2.783882	0.0029
P. aeruginosa	21±0.5	0.0004	16±1	0.0033	-	-	34±2.645751	0.0030
Salmonella sp,	17±0.5	0.0007	13±0.5	0.0017	-	-	21±2	0.0059
E. coli	23±0.5	0.0003	19±2	0.0078	-	-	39±1.5	0.0007
Klebsiella sp.	12±1.5	0.0202	-	-	-	-	21±0.5	0.0004

Table 2. Zone of inhibition (mm) of hot-water extract of plants

Organisms	Plants used						Positive control (Ciprofloxacin) (200 µl)	
	S. cumini (200 µl)		*M. indica* (200 µl)		*M. citrifolia* (200 µl)			
	Mean±SD	P-value	Mean±SD	P-value	Mean±SD	P-value	Mean±SD	P-value
S. aureus	27±1	0.0026	23±1	0.0012	20±1	0.0017	17±0.5	0.0007
Bacillus sp.	24±2	0.0041	22±2	0.0052	-	-	36±2.783882	0.0029
P. aeruginosa	27±0.5	0.0002	18±0.5	0.0006	15±1	0.0041	34±2.645751	0.0030
Salmonella sp,	17±0.5	0.0007	14±1	0.0052	-	-	21±2	0.0059
E. coli	17±1	0.0027	17±1	0.0027	-	-	39±1.5	0.0007
Klebsiella sp.	19±0.5	0.0005	-		-	-	21±0.5	0.0004

of antibacterial activity of cold and hot-water extracts of three plants against the test organisms were shown in Fig 2.

The cold water and hot water extracts of *S.cumini* leaves showed antibacterial activity against all the test organisms.

In case of *M.indica* leaves, both the cold and hot water extracts showed sensitive to *Staphylococcus aureus, Bacillus sp., P. aeruginosa, Salmonella* sp, and *E. coli* but resistant to *Klebsiella pneumonia.*

However, *M.citrifolia* leaves showed sensitive to *S. aureus* and *P. aeruginosa* in hot water extract but resistant to *Bacillus sp., Salmonella* sp., *E. coli* and *Klebsiella* sp. Meanwhile, cold water extracts of *M.citrifolia* leaves found to be not sensitive to *S. aureus, Bacillus sp., P. aeruginosa, Salmonella* sp, *Klebsiella* sp. and *E. coli.*

Preliminary study of plant extract as a disinfecting agent

The total colony count in CFU/cm^2 of the floor surfaces were estimated to study the efficacy of the plant extract as a disinfecting agent. The total colony count of the distilled water (control) was 25 × 10^2 CFU/cm^2 that got reduced to 2.95×10^{2}CFU/cm^2 and 2.20 × 10^{2}CFU/cm^2 upon disinfecting with *S. cumini* extract and commercial disinfectant respectively. A graphical representation of the data indicating that disinfecting with *S. cumini* extract and commercial disinfectant significantly reduced CFU/cm^2 of the floor surface were shown in Fig. 3. The statistical analysis was carried out using t-test and P- value was found to be significant (<0.05).

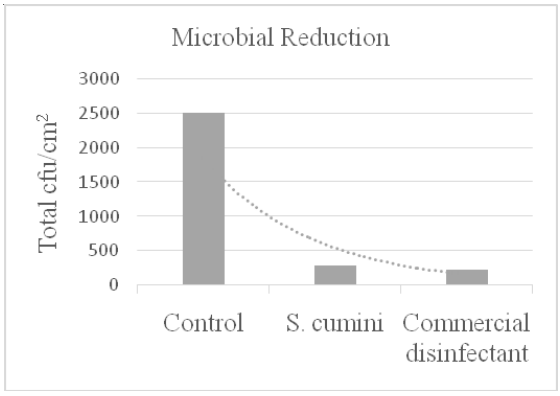


Fig. 3. Effectiveness of commercial disinfectant and *S. cumini* extract as a disinfecting agent.

The percentage reduction of microorganisms on the floor surfaces before and after disinfection with commercial disinfectant and cold and hot water extracts of *S. cumini* leaves were calculated. The reduction percentage of commercial disinfectant was 91.2% and hot water extracts of *S.cumini* leaves was 88.2%.

Gram staining revealed the presence of 4 Gram-positive cocci and 5 Gram-positive bacilli.

Phytochemical Analysis

Table 3 summarized the phytochemical screening of chemical constituents of hot water extracts of *S. cumini* leaves under study on the qualitative basis. Tannin, flavonoids, terpenoids, saponin and phenol were detected in hot water extract.

Table 3. Phytochemical analysis of *S.cumini* leaves extract

Phytochemical constituents	*S. cumini* leaves extract
Tannins	+
Alkaloids	-
Flavonoids	+
Terpenoids	+
Saponin	+
Phenol	+

Discussion

In the present study, the antibacterial activity of the cold and hot water extract of three plants leaves such as *S.cumini*, *M.indica* and *M.citrofolia* were

tested against organisms such as *S. aureus*, *Bacillus* sp., *P. aeruginosa*, *Salmonella* sp., *E. coli* and *Klebsiella* sp. Ciprofloxacin antibiotic is used as a positive control. As a negative control, distilled water was used. The cold water and hot water extracts of *S.cumini* leaves were found to be sensitive against all test organisms. The previous study also reported that water extract of *S. cumini* leaves were sensitive to *S. aureus*, *E. coli* and *Bacillus* sp. but resistant to *Pseudomonas* sp. (Abdelgadir *et al.,* 2015). Jagetia, 2017 also revealed that water extract of *S. cumini* leaves has antibacterial activity against *S. aureus*, *Pseudomonas* sp., *Bacillus* sp. and *E. coli* (Jagetia, 2017). In case of *M. indica* leaves, both the cold and hot water extracts showed sensitive to *Staphylococcus aureus, Bacillus sp., P. aeruginosa, Salmonella* sp, and *E. coli* but resistant to *Klebsiella* sp. *and* produced a comparatively lesser zone of inhibition than *S. cumini* leave extracts. Garga *et al.* 2020 also reported that water extract of *M.indica* leaves was sensitive to *S. aureus* and *Pseudomonas* sp. (Garga *et al.,* 2020). Meanwhile, *M. citrifolia* leaves showed sensitive to *S. aureus* and *P. aeruginosa* in hot water extract but resistant to *Bacillus sp., Salmonella* sp., *E. coli* and *Klebsiella* sp. in both cold and hot water extracts and produced a comparatively lesser zone of inhibition than *S. cumini* leave extracts. Ranaweera *et al.* 2016 also revealed that water extract of *M.citrifolia* leaves was not sensitive to *S. aureus* and *E. coli* (Ranaweera *et al.,* 2016).

Floor surface swab samples were taken to determine the percentage reduction of microbial count before and after disinfection with commercial disinfectant and hot water extracts of *S. cumini* leaves. To the best of our consciousness, this is the first report for hot water extract of *S.cumini* leaves which is tested for the efficacy of plant-based disinfecting agent. This study revealed that hot water extracts of *S.cumini* leaves showed 88.2% reduction of microbial count which is closed to that of the commercial disinfectant microbial count reduction percentage (91.2%). The use of chemical disinfectant has negative effects, such as allergies, eczema, asthma, cancer and even become resistant by the pathogen. Considering the potential health risks caused by chemical agents, hot water extracts of *Syzigium cumini* leaves can be used as alternative natural plant-based disinfectant which are non-toxic, eco-friendly, cost effective and less side effects when compared to other commercial chemical disinfectant available today (Nusrat; Maid 4 Condos, 2019).

S. cumini leaves extract showed the highest antibacterial activity against all the test organisms as well as significant reduction of microorganisms present on the floor surfaces upon application. Phytochemical constituents of *S. cumini* leaves extract were analysed. Tannin, flavonoids, terpenoids, saponin and phenol were detected in hot water extract of *S. cumini* leaves. Satyavati *et al.,* 2014 also reported the presence of tannin, saponin, flavonoids and phenol for water extract of *S. cumini* leaves (Satyayathi and Bhavani, 2014). Jagetia 2017 also reported that water extract of *S. cumini* leaves possessed tannin, saponin, alkaloids, flavonoids and phenol (Jagetia, 2017). The presence of tannin, flavonoids, terpenoids, saponin and phenol are responsible for their antimicrobial activity (Mayuri *et al.,* 2019; Akhtar *et al.,* 2018; Yang *et al.,* 2020).

Conclusion

The hot water extract of *S.cumini* leaves extract showed the best antibacterial activity against all tested organisms except *E. coli* as compared to the other plant extracts. Moreover, it showed the significant reduction of microorganisms present on the floor surfacesupon application.The phytochemical constituents present in it is responsible for their antibacterial activity. Thus, hot water extracts of *S.cumini* leaves could be used as an alternative plant-based disinfecting agent. Further studies should focus on toxicity test, carcinogenicity test and skin irritation test of the *S. cumini* leaves extract.

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