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Library Environment :Microbial Quality Assessment and Antibacterial Activity of Cinnamomum verum and Piper nigrum with respect to microorganisms of SRCW library

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Library Environment :Microbial Quality Assessment and Antibacterial Activity of *Cinnamomum verum* and *Piper nigrum* with respect to microorganisms of SRCW library

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Abstract

BACKGROUND:

Background research interest focuses on medicinal herbs such as *Cinnamomum verum* and *Piper nigrum* extracts can be used to trap the microbes from the atmosphere.

- To collect bark of *Cinnamomum verum* and *Piper nigrum* plants and make a dry powder.
- To perform Phytochemicals analysis for the extracted powder.
- To perform antimicrobial susceptibility test and MIC against different strains.
- To develop herbal coated tissue paper.

METHODOLOGY:

- The bark of *Cinnamomum verum* and *Piper nigrum* were collected and finely powdered.
- Different extracts of *C. verum and P. nigrum* (10 µl to 40 µl) were loaded on to the wells of MHA and incubated at 37 ˚C for 24 h to determine the MIC.
- Highest antimicrobial concentration of the herbal extract was coated on the tissue by spraying method.

Discussion:

The microbial load was significantly decreased in library after exposure of herbal extract.

CONCLUSION:

The Research work concludes that 30 µl concentration of *C.verum* and *P. nigrum* is effective to reduce the microbial load in our library atmosphere. The developed filter paper can be placed in the library environment since it is eco-friendly and cost effective.

Keywords: Library environment**,** airborne pathogens, *Cinnamomum verum, Piper nigrum* and MIC.

1 INTRODUCTION

Traditional herbal extract medicine is still relatively modest, when compared to the conventional pharmaceutical industry because of its medicinal values (Tilburt and Kaptchuk, 2008). Since ancient time herbs were used as a natural medicine against a variety of human disease and the presence of different secondary metabolites in the herbs exhibit antimicrobial properties (Hayat et al., 2017; Compean and Ynalvez, 2014). Medicinal plants play a major role in the traditional natural health care system of the rural population in developing Asian countries (Oyebode et al., 2016). Plant extracts were used to treat wide range of diseases such as asthma, urinary tract infection, intestinal disorder and fever. There has been more constant focus in the development of newer antimicrobial agent in traditional herbs (Kapailan, 2015). Cinnamomum verum is a true cinnamon tree or Ceylon cinnamon tree which belongs to the family *Lauraceae* and contains secondary metabolites such as alkaloids, steroids, flavonoids, terpenoids and tannins (Vakilwala and Trivedi, 2017). Cinnamomum has a warm, spicy, harsh odour and the major constituent of cinnamon oil is **cinnamaldehyde** (Wong et al., 2014). *Piper nigrum* called as black pepper (King of species) belongs to the family *Piperaceae*. The aerial part of the plant consists of organic compounds such as tartaric acid, acetic acid, citric acid and succinic acid. The plant also contains gums, pectin, sugars, tannins, alkaloids, flavonoids, glycosides and sesquiterpenes **(**Vastrad *et al.*, 2014**)**. The plant is known as aromatic warming herbal spice that lowers fever and improves digestion. *Piper nigrum* was used as a medicine in both allopathy and Ayurvedic system for its antimicrobial activity was recorded against a wide range of pathogens and antioxidant effects against a series of reactive oxygen and nitrogen sps including the scavenging of superoxide ion, Hydrogen peroxide, nitric oxide, DPPH,ABTS and reducing affect against ferric and Molybdenum. The plant is used to treat stomach chills, food poisoning, cholera, dysentery, diarrhoea and vomiting (Saranraj and Sivasakthi, 2014).

Microorganisms are ubiquitous, *Micrococcus sp, Staphylococcus sp,* and *Bacillus sp* (Bomala *et al.*, 2016).”Contaminants level must be maintained at low concentration for human and to guarantee the correct preservation of works of Art and cultural heritage”. Bacterial contamination in the air and settled dust were studied in libraries. The evaluation of microorganisms in air and surface dust is the first step to control microbial population in the library environment (Alghamdi *et al.*, 2014). Dust serves as a source of nutrient for microorganisms for its growth and multiplication (Suba *et al*., 2017). Bacteria consume cellulose in the paper and thrive on nutrients in pastes, binding threads serves as a growth medium for micro-organisms. The present research work focuses on aqueous and ethanol extraction of herbs such as *Cinnamomum verum* and *Piper nigrum*. The phytocompounds present in the extracts were determined by phytochemical screening. Antibacterial susceptibility test was performed for the crude extract by well diffusion method and there by Minimum Inhibitory Concentrations (MIC) was determined.

2 MATERIALS AND METHODS

2.1 Collection of samples

The bark of *Cinnamomum verum* and *Piper nigrum* were collected from local market, Coimbatore and washed three times with distilled water. The materials were air dried and kept under hot air oven at 55°C for 3 hrs and powdered. The powdered samples were sealed in separate polythene bags unit the time of the extraction. *Cinnamomum verum* and *Piper nigrum* selected for its important role in antioxidant, antimicrobial potential and gastro-protective role.

2.2 Exposure of nutrient agar plates in library environment

Nutrient agar plates were prepared, sterilized and exposed to the library environment of Sri Ramakrishna college of Arts and Science for women (Coimbatore, Tamil Nadu, India). The plates were kept opened for about 30 mns on the surface of book rack in the library. After exposure the pates were incubated at 37˚C for 24 hrs. (Musa *et al.,* 2017).

2.3 Identification and biochemical characterization of isolated strains

Four different strains were isolated. Each isolate was processed for staining, cultural characteristics and biochemical test such as Indole, MR-VP, citrate, oxidase, catalase, H_2S production, triple sugar iron test and urease test (*Cappuccino* manual 10^{th} edition). (James *et al*., 2014)

2.4 Preparation of herbal powder

The bark of *C. verum* and fruit of *P. nigrum* were shade dried for 3 days. The dried herbs were crushed and grinded to make a fine powder. (Shiva *et al*., 2013)

2.5 Exposure of herbal powder in library environment

Three g of *C. verum* and 3 g of *P. nigrum* powder were weighed and tied on to the tissue paper. The herb loaded tissues were exposed in the library environment for about 5 days. (Kumari 2016)

2.6 Preparation of *Cinnamomum verum* and *Piper nigrum* extracts

To the 10 g of *C. verum* and 10 g of *P. nigrum* 100 ml of double distilled water was added and kept in a water bath at 60˚C for 30 mins for aqueous extract. To the 10 g of *C. verum* and 10 g of *P. nigrum* 100 ml of ethanol was added and kept in a water bath at 60˚C for 30 mins for ethanol extract. (Vakilwala *et al*., 2017)

2.7 Phytochemical analysis of *C. verum* and *P. nigrum* extract

The phytochemical tests are standard test followed in order to determine the basic characteristics like presence of metabolic alkaloids, terpenoids, saponins etc.,

Test for Alkaloids (Wagner's test)

To the 0.5 ml of sample extract the Wagner's reagent (solution of potassium iodide) was added. A reddish-brown precipitate confirms the presence of alkaloids.

Test for Flavonoids (Alkaline reagent test)

To the 0.5 ml of sample extract few drops of sodium hydroxide solution was added. A yellow colouration which turns to colourless by addition of few drops of dilute acetic acid indicates the presence of flavonoids.

Test for Phenolic compound test (Ferric chloride test)

To the 0.5 ml of sample extract, few drops of 5%ferric chloride solution were added. A dark green colour indicates the presence of phenolic compounds.

Test for Saponins (Froth test)

A pinch of the dried powdered was added to 3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicates the presence of saponins.

Test for Terpenoids (Salkowski test)

0.5 ml of extract was treated with chloroform and few drops of concentrated sulphuric acid and shaken well, red colour at the lower layer indicates the presence of sterols and formation of yellow coloured lower layer indicates the presence of terpenoids.

Test for Tannins (Lead acetate test)

To the 0.5 ml of sample extract, a few drops of 10% lead acetate were added. Formation of precipitate indicates the presence of tannins.

Test for Resins

Sample extract of 0.5 ml was treated with a few drops of acetic anhydride solution followed by 1ml of concentrated sulphuric acid. The orange to yellow colour indicates the presence of resins.

Test for carbohydrate (Bardford Test)

To the 0.5 ml of sample extract, 1 ml of Bardford reagent was added in a test tube and then heated in a water bath for period of 2 minutes. A reddish precipitate indicates the presence of carbohydrates (Mekala *et* al., 2019).

2.8 Antibacterial susceptibility test and minimum inhibitory concentration of *C. verum* extract

The aqueous and ethanol extract was subjected to antibacterial activity against microorganisms isolated from library environment. The Muller Hinton agar plates were prepared and the test strain was swabbed on to it. Wells were made using sterile cork borer. Different concentration of *C. verum* extract such as 10 µl,20 µl, 30 µl and 40 µl were loaded on to the wells and incubated at 37 ˚C for 24 h. (Ganesh *et al*., 2014)

2.9 Antibacterial susceptibility test and minimum inhibitory concentration of *Piper nigrum* extract

The aqueous and ethanol extract was subjected to antibacterial activity against microorganisms isolated from library environment. The Muller Hinton agar plates were prepared and the test strain was swabbed on to it. Wells were made using sterile cork borer. Different concentration of *P. nigrum* extract such as 10 µl,20 µl, 30 µl and 40 µl were loaded on to the wells and incubated at 37 ˚C for 24 h. (Ganesh *et al*., 2014)

2.10 Development of herbal extract loaded on the tissue Paper

Highest antimicrobial concentration (seems to destroy the air born strains) of the herbal extract, subjected to coat on the tissue by spraying method and to the developed herb with coated tissue was placed on the book shelf in order to trap microbial population. The minimum inhibitory concentration of *C. verum* (30 µl) and *P. nigrum* (30 µl) has higher antibacterial activity against the test strain. 5 ml of 30 µl concentration of *C. verum* and 5 ml of 30 µl concentration of *P. nigrum* was separately sprayed on a sterile tissue and air-dried for activating the product.

3 RESULTS AND DISCUSSION

3.1 Exposure of nutrient agar plates in library environment

After incubation of exposed nutrient agar plates for 30 minutes in library environment, four different isolates were selected and subjected to characterization. Similarly, Muhammad *et al*. (2017) reported that 64 isolates were isolated from nutrient agar plates after the plates exposed to air for 30 minutes at different sites in respective lecture rooms.

3.2 Identification and biochemical characterization of isolated strain

The selected four strains were subjected to microscopic, macroscopic and biochemical characterization. Strain 1 observed to be gram positive cocci in clusters and positive for methyl red and catalase test. Strain 2 observed to be gram positive cocci in pairs and positive for methyl red, H_2S production, oxidase, catalase and H_2S. Strain 3 observed to be gram positive rods and positive for H_2S. Strain 4 observed to be gram negative rods and positive for methyl red, citrate, urease, catalase, and H_2S. Similarly, Muhammad *et al*., 2017 reported that the presence of *Staphylococcus aureus, Streptococcus* sp, *Escherichia coli* and *Bacillus* sp in the various lecture rooms respectively. Bomala *et al.*, 2016 reported that the presence of *Micrococcus sp, Staphylococcus sp, Pseudomonas sp and Klebsiella sp* in the various class rooms respectively.

3.3 Exposure of herbal powder in library environment

Prepared herbal powder was exposed in the library environment to decrease the microbial load. Plate 1 represents the exposure of herbs in library environment. The microbial load was significantly decreased after exposure of herbs in the library environment. Similarly, Kumari *and Shashirekha*, (2016) reported that the extract of *Dhupana* decreases the microbial load in 4^{th} day of exposure at indoor environment of central hospital.

3.4 Identification and biochemical characterization of isolated strain after exposure of herbs

The selected two strains were subjected to microscopic, macroscopic and biochemical characterization. Strain 1 appeared to be gram positive cocci in clusters and positive for methyl red and catalase test. Strain 2 appeared to be gram positive cocci in pairs and showed positive for methyl red, oxidase, catalase, and H_2S. Similarly, Muhammad *et al*. (2016) reported and that the presence of *Staphylococcus aureus, Streptococcus* sp, *Escherichia coli* and *Bacillus* sp in the various lecture rooms respectively. Bomala *et al.,* (2016) reported and detected the presence of *Micrococcus sp, Staphylococcus sp, Pseudomonas sp and Klebsiella sp* in the various class rooms respectively.

3.5 Phytochemical analysis of *Cinnamomum verum* and *Piper nigrum* extract

Different phytochemical compounds were present in the aqueous and ethanol extract of *C. verum* and *P. nigrum* were analysed and the results were tabulated in table 1. The phytochemical analysis of *C. verum* extract reveals the presence of carbohydrate, flavonoids, glycosides, steroids, tannins, phenolic compound, amino acid, saponins and absence of alkaloids. *P. nigrum* extract shows the presence of alkaloids, glycosides, steroids, tannins, amino acid, saponins and absence of carbohydrate, flavonoids, and phenolic compound respectively. Similarly, James *et al.,* 2014 reported that the phytochemical analysis of ethanol extract of *C. verum* reveals the presence of alkaloids, flavonoids, saponins, terpenoids, and tannins. Ganesh *et al.,* 2014 reported that the phytochemical analysis of ethanol extract of *P. nigrum* reveals the presence of tannins, alkaloids, and flavonoids respectively.

3.6 Antibacterial susceptibility test and minimum inhibitory concentration of *C. verum* extract:

The antibacterial susceptibility test was carried out and zone of inhibition in the plate represents that the extract of *C. verum* has antibacterial activity against the test organisms. The zone of inhibition was tabulated in Table 2. Plate 2(A) represents the minimum inhibitory concentration of *C. verum* extract. 10µl ethanol extract of *C. verum* was found to be resistant for strain 1 and strain 2. 20 µl ethanol extract of *C. verum* was found to be sensitive (12 mm,13 mm) against strain 1 and strain 2. 30 µl ethanol extract of *C. verum* was found to be sensitive (15 mm,15 mm) against strain 1 and strain 2. 40 µl ethanol extract of *C. verum* was found to be sensitive (18 mm,19 mm) against strain 1 and strain 2. Similarly, Yi-sub *et al.,* (2017) reported that the ethanolic extract of *C. verum* has 20 mm zone of inhibition in 15 mg/disk against gram positive bacteria such as *B. cereus*, *S. aureus* and *E. coli*.

3.7 Antibacterial susceptibility test and minimum inhibitory concentration of *P. nigrum* extract

The antibacterial susceptibility test was carried out and zone of inhibition in the plate represents that the extract of *P. nigrum* has antibacterial activity against the test organisms. The zone of inhibition was tabulated in table 3. Plate 2(B) represents the minimum inhibitory concentration of *P. nigrum* extract. 10 µl ethanol extract of *P. nigrum* was found to be resistant for strain 1 and strain 2. 20 µl ethanol extract of *P. nigrum* was found to be sensitive (13 mm, 14 mm) against strain 1 and strain 2. 30 µl ethanol extract of *P. nigrum* was found to be sensitive (15 mm, 17 mm) against strain 1 and strain 2. 40 µl ethanol extract of *P. nigrum* was found to be sensitive (19 mm, 20 mm) against strain 1 and strain 2. Similarly, Shiva *et al.*, (2013) reported that the ethanolic extract of *P. nigrum* has 8 mm – 18 mm zone of inhibition against gram positive bacteria *S. aureus* (18 mm) and *Bacillus* sp (14 mm).

3.8 Development of herbal extract loaded on filter paper

Development of filter paper material with herbs (*C. verum* (30 µl) and *P. nigrum* (30 µl)) after drying was placed in each book rack of library environment. Plate 3 represents the development of herb extract loaded in filter paper and placed in book shelf.

4.CONCLUSION

The present Research work focuses on isolation of air borne microorganisms present in the SRCW (Sri Ramakrishna College of Arts and Science for Women) library environment. The amount of the microbial content of indoor air and surface of library were students spend much of the time is an important parameter because it has a direct impact on the mental health, physical development and performance of the students. More 100 sterile nutrient agar plates on exposure to the library environment for 30 minutes, (Interval of 15 days) the airborne microbes (*Bacillus, Micrococcus, Staphylococcus, Pseudomonas, Klebisella*). The selected herbs such as *C.verum* and *P. nigrum* coated on tissue or filter paper strip which absorbs or traps the microbes from the library atmosphere. The loaded dry herbal powder in filter bags placed in book tracks. The gradual decrease in microbial load were recorded in library atmosphere. The Research work concludes that 30 µl concentration of *C.verum* and *P. nigrum* is effective to reduce the microbial load in our library atmosphere. The developed filter paper can be placed in the indoor environment since it is eco-friendly, pollutant free and cost effective.

Table 1. Phytochemical analysis of herb extract

S.NO	Phytochemical	*Cinnamomum verum*	*Piper nigrum*
1	Alkaloids	-	+
2	Carbohydrates	+	-
3	Flavonoids	+	-
4	Glycosides	+	+
5	Steroids	+	+
6	Tannins	+	+
7	Phenolic compound	+	-
8	Amino acid	+	+
9	Saponins	+	+

"+"-Positive "_"-Negative

Table 2 Minimum inhibitory concentration of *Cinnamomum verum* extract

S.NO	Concentration	Strain-1	Strain-2
1.	10µl	-	-
2.	20µl	12mm	13mm
3.	30µl	15mm	15mm
4.	40µl	18mm	19mm

Table 3 Minimum inhibitory concentration of *Piper nigrum* extract

S.NO	Concentration	Strain-1	Strain-2
1.	10µl	-	-
2.	20µl	13mm	16mm
3.	30µl	15mm	17mm
4.	40µl	19mm	20mm

Pates

Plate A&B Sri Ramakrishna College of Arts and Science for Women Library

Plate 1: Exposure of herbal powder to library environment A-*Cinnamomum verum* B-*Piper nigrum*

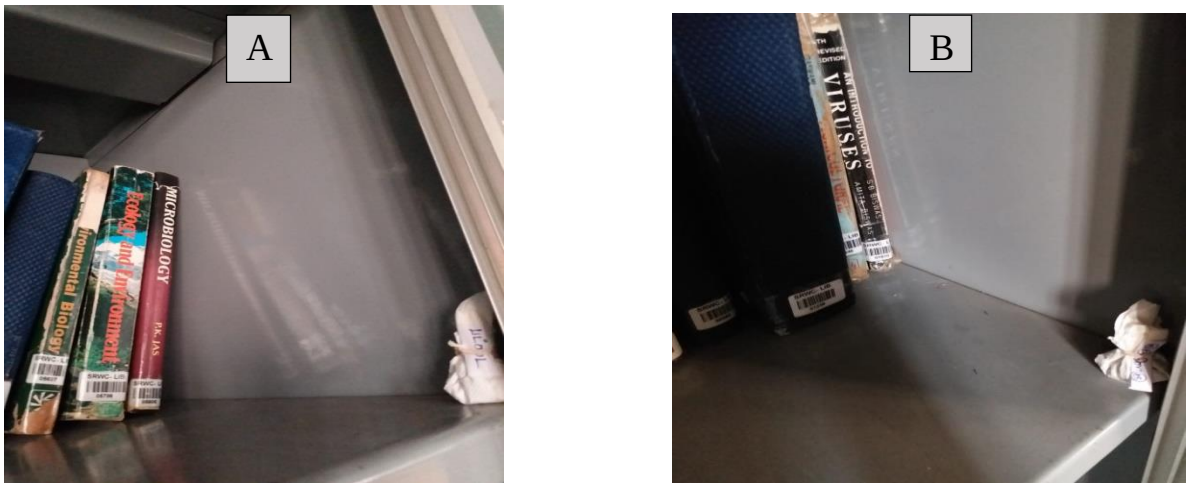

Plate 2: Minimum inhibitory concentration of herb extracts A-*Cinnamomum verum* B-*Piper nigrum*

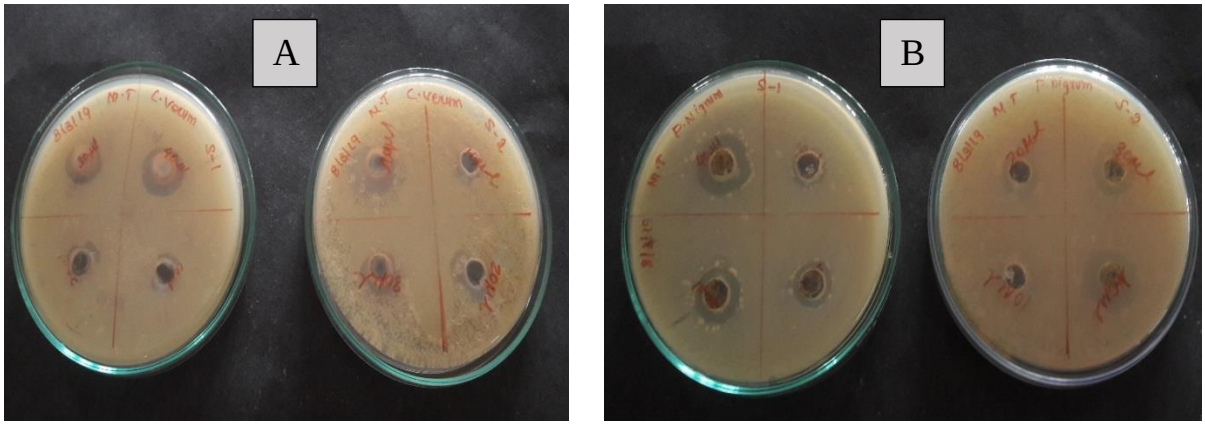

Plate 3: *Cinnamomum verum* B-*Piper nigrum* herbs coated on Filter paper

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