Bio-Efficacy of Santan (Ixora coccinea) Leaf Extract against Trichophyton mentagrophytes: An Antifungal Susceptibility Testing

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Abstract

This study was conducted to determine the Bio-Efficacy of Santan (Ixora coccinea) Leaf Extract against Trichophyton mentagrophytes: An Antifungal Susceptibility Testing. The study used the experimental research design and subjected the PDA cultures of Trichophyton mentagrophytes into sterile distilled water as a negative control, Ketoconazole as the positive control, and three (3) concentrations of Santan leaf extract in three (3) trials. The study used the two-way analysis of variance as statistical analysis, followed by Bonferroni as a post hoc test. The PDA plate treated with Ketoconazole has 44 millimeters of a zone of inhibition, making it the largest inhibition among all the treatments. There was no zone of inhibition observed on the PDA plate treated with sterile distilled water. The zones of inhibition observed on the PDA plate treated with 100% concentrations of santan leaf extract in three (3) trials are 28mm, 30mm, and 31mm, which are larger than the other concentrations. The PDA plate treated with 75% concentrations of the santan leaf extract has zones of inhibition of 25mm, 27mm, and 28mm. And lastly, the zones of inhibition in the PDA plate subjected to 50% of the santan leaf extract are 21mm, 19mm, and 18mm. Most of the findings exhibited a P-value of < 0.0001, which indicates that there is a significant difference in the antifungal susceptibility of Trichophyton mentagrophytes treated with different concentrations of santan (Ixora coccinea) leaf extract compared with Ketoconazole and distilled water. Although, the santan leaf extract is not as effective as the antifungal agent for Trichophyton mentagrophytes used for positive control, which is Ketoconazole.

Keywords: Santan (Ixora coccinea); Trichophyton mentagrophytes; Antifungal; Agar Well Diffusion Method

1. Introduction

Infectious diseases in the skin, hair, and nails can be brought on by dermatophytes, a group of fungus with intimate ties to one another and the keratinase enzyme. Trichophyton mentagrophytes is the second most prevalent cause of dermatophytosis among dermatophytes (De Leon et al., 2020). In numerous animal species, particularly pets and furry animals, the polymorphic dermatophyte Trichophyton mentagrophytes complex is the predominant frequent cause of superficial mycoses. The prevalence of these zoophilic fungal infections is highest in children between the ages of 3 and 7. Most dermatophytes grow tinea capitis, or more deeply, on hair follicles, as well as present on the skins surface. This widespread infection by T. mentagrophytes is made easier by how quickly other people can catch the surface fungus illness. It has been cultivated from both human patients with ringworm symptoms and asymptomatic animal carriers. This phenomenon raises issues with diagnosis and treatment. Inappropriately handled or recurring infections from superficial mycoses can spread to other animals and people directly and indirectly (Gnat et al., 2018).

Herbal plants have vital phenolic substances, flavonoids, alkaloids, tannins, and more compounds that are utilized to cure a variety of pathological illnesses. Bioactive compounds from plants have produced a wide range of lead chemicals that can be used as models to create novel medications. Ixora coccinea shows potent lowering efficiency and complete affinity for antioxidants. Flavonoids, kaempferol, quercetin, anthocyanidins, phenolic acids, and ferulic acid are all produced by leaves. Research on phytochemistry has revealed that the principal chemicals existing in Ixora coccinea are lupeol, oleic acid, linoleic acid, stearic acid, sitosterol, oleanolic acid, and ursolic acid. Alkaloids, glycosides, flavonoids, steroids, terpenoids, tannins, and saponins are also abundant phyto-constituents (Rani, 2022).

Specifically, this study aims to seek answers to the following specific problems:

1. What are the phytochemical components of santan?
2. What are the zones of inhibition made by santan leaf extract against Trichophyton mentagrophytes?
3. Which level of concentration is the most effective against Trichophyton mentagrophytes?
4. Is there a significant difference between santan and commercial antifungal products?

2. Method

2.1 Research Design

The study utilized an experimental research design which is necessary to be conducted in the laboratory and can only be proven tested in such a manner. The establishment of an experimental design involves of three stages: (a) the utilization of a two-level design was performed: a negative and positive control; (b) the experimental work, which is the preparation of the extracts and the bioassays; and (c) the analysis, in which different concentrations are observed and calculated. All of these procedures are rightly fitted under experimental research.

2.2 Locale of the Study

The santan leaves are obtained from the backyard of one of the researchers' houses in the province of Laguna, Philippines. The extraction of the santan leaves was done in the laboratory of PHINMA Saint Jude College, while the rotary evaporation was conducted in the laboratory of chemistry at De La Salle University, Laguna. Lastly, the antifungal test was conducted at the Microbiological Research and Services Laboratory of University of the Philippines-Diliman, Quezon City.

2.3 Samples of the Study

In general, the study sought to determine the bio-efficacy of santan (Ixora coccinea) leaf extract against Trichophyton mentagrophytes through Antifungal Susceptibility Testing. Santan (Ixora coccinea) and Ketoconazole are the independent variables or the antecedents, while the antifungal activity on Trichophyton mentagrophytes cultures is the dependent variable or the consequent. Santan was observed by its potential to instigate antifungal activity in T. mentagrophytes culture. The experimentation and observation are done through the well diffusion agar method.

2.4 Data Gathering and Procedures

The study utilized an experimental research design which was necessary to be conducted in the laboratory and can only be proven tested in such a manner. Materials used include: drying oven, incubator, pipette, refrigerator, beaker, stirring rod, analytical balance, Bunsen burner, and inoculating loop.

i. Sample preparation & Extraction

The plant samples were collected in the garden of one of the researchers, then wrapped in newspaper, and transported to the PHINMA Saint Jude College laboratory. The different parts of the plant were separated, and leaves were collected. The gathered leaves were washed using tap water to remove residues and debris. It is then air dried for 3 days and moisture removal will be observed. The electric blender was used to ground the leaves into a powder, and 150g of the powder is soaked in 500 mL of ethanol for 24 hours. The supernatant was collected and filtered through a Whatman filter paper number 1. The collected sample is then delivered to the De La Salle University – Laguna Campus for a rotary evaporator at 50°C that removes the solvent from the mixture. The final sample is then subjected to antifungal testing. The plant extracts are prepared based on the reviewed studies gathered, with minor changes. For the phytochemical test, a dried leaves sample was used.

ii. Simple Dilution

Prepared santan extract was mix with saline in a clean new tube: 1st test tube (label 100%) - 1 mL santan extract ONLY, 2nd test tube (label 75%) – 750 ul santan extract plus 250 ul saline, 3rd test tube (label 50%) – 500 ul santan extract plus 500 ul saline and 4th test tube (label 25%) – 250 ul santan extract plus 750 ul saline.

iii. Phytochemical analysis & Plant Identification

The dried leaves were sent to Department of Science and Technology-Taguig and Bureau of Plant Industry-Manila for the analysis to check for the presence of an antimicrobial class of compounds and plant identification, respectively.

iv. Microbial Suspension

Microbial suspensions were prepared from a 7- day culture of the test organism. The suspending medium used was 0.1% peptone water. Pre-poured Potato Dextrose (PDA) plates, approximately 3 mm thick, were inoculated with microbial suspension by swabbing the agar surface. The cotton swab on an applicator stick was dipped into the microbial suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The swab was streaked over the entire agar surface. This procedure was repeated two more times rotating the plate 60 degree each time to ensure the even distribution of the inoculum.

v. Antifungal testing

The Agar well diffusion method was utilized to screen the antibacterial and antifungal activities of different solvent extracts shown by (Daoud et al., 2015). Three (3) equidistant wells were made on the agar plates using a sterile cork borer (10 mm in diameter). Then, 200 µl of each extract was added to respective wells. PDA plates were incubated at room temperature for 7 days. After incubation, antimicrobial activity was

detected by measuring the zones of inhibition in millimeter (including the diameter of the wells). Sterile water was employed as a negative control, and an antifungal agent Ketoconazole as positive control.

2.5 Data Analysis

In order to calculate and determine the efficacy of Ixora coccinea leaf extract as an inhibitory for Trichophyton mentagrophytes, researchers investigated the differences in the mean crude extract concentrations between the groups, a two-way ANOVA was used. The connection between the zone of inhibition across concentration groups, positive and the negative control was compared using a Bonferroni post hoc test. A difference was deemed statistically significant using p-value of less than 0.05. This will be employed to measure the ability of the Ixora coccinea leaf extract to incapacitate the Trichophyton mentagrophytes. This study will use a significance level of 0.05 to analyze the experimental findings. The Antimicrobial Index is obtained using this formula:

$$AI = \frac{Diameter\ of\ clearing\ zone - Diameter\ of\ sample}{Diameter\ of\ sample}$$

The following descriptions are used for the effectiveness of Santan leaf extract as antifungal substance:

Table 1. Description of Effectiveness

	Breakpoints	
Interpretive Category	**MIC (ug/mL)**	**Zone Diameter (mm)**
Susceptible	≤4	≥20
Susceptible-dose dependent	8-16	15-19
Intermediate	8-16	15-19
Resistant	≥32	≤14

3. Results & Discussion

As part of the experimentation, the researcher treated the Trichophyton mentagrophytes with Santan (Ixora coccinea) leaf extract as the experimental group; with ketoconazole as the positive group, and sterile distilled water as a negative control. The antifungal activities of Santan leaf extract at 50% concentrations in three trials presented different results. Trial 1 (21mm zone of inhibition) and Trial 2 (19mm zone of inhibition) are interpreted as “Susceptible”, while Trial 3 (18mm zone of inhibition) is interpreted as “Intermediate”, with a 0.9 antimicrobial index (AI). In Santan leaf extract at 75% concentrations, Trial 1 (25mm zone of inhibition), Trial 2 (27mm zone of inhibition), and Trial 3 (28mm zone of inhibition) are all interpreted as “Susceptible with a 1.7 antimicrobial index (AI). The obtained results in 100% concentrations are also “Susceptible” in Trial 1 (28mm zone of inhibition), Trial 2 (30mm zone of inhibition), and Trial 3 (31mm zone of inhibition) with an antimicrobial index (AI) of 2.0. The positive control (ketoconazole) showed the largest zone of inhibition of about 44mm, which has a 3.4 antimicrobial index (AI).

Table 2. The Total Antifungal Activities of Treatments in Different Trials

ZONE OF INHIBITION (mm)					
SAMPLE	**TRIAL 1**	**TRIAL 2**	**TRIAL 3**	**MEAN**	**AI**
50%	21	19	18	19.33	0.9
75%	25	27	28	26.67	1.7
100%	28	30	31	29.67	2.0
Ketoconazole (+ control)	44	44	44	44	3.4
Steril Distilled water (- control)	0	0	0	0	0

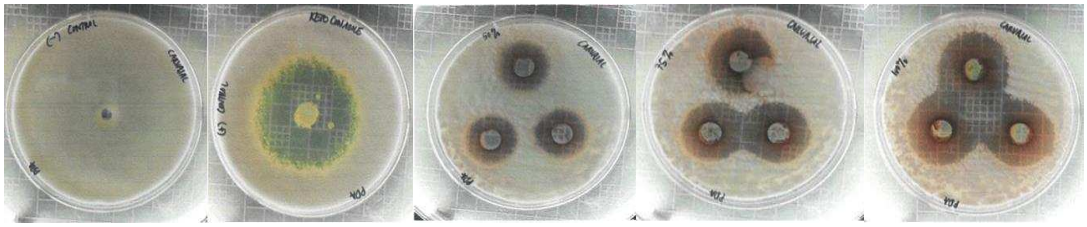

Figure 1. Antifungal Susceptibility Results

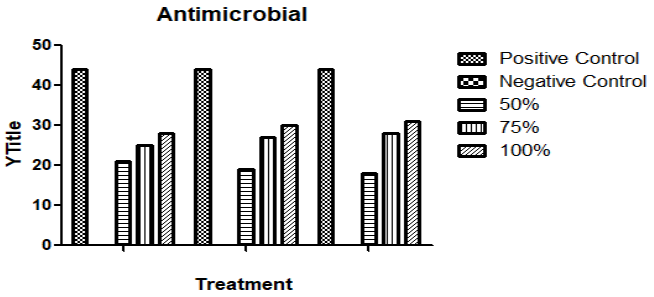

Figure 2. Graph of Antimicrobial Treatments and Results

Table 3. Statistical Analysis of the Antifungal Activities of Experimental Group in Different Concentrations, Positive Control, and Negative Control using Two-way Analysis of Variance

SOURCE OF VARIANCE	% OF TOTAL VARIATION	P VALUE	MEAN SQUARE	F	P VALUE SUMMARY
Treatment	99.55	< 0.0001	780	480	Significant
Trial	0.03	0.7588	0.47	0.29	Not Significant

Table 3 shows the two-way ANOVA test of the Antifungal Activities of Experimental Group which is the santan extract in different concentrations, Positive Control which is Ketoconazole, and Negative Control which is distilled water. The P-value of less than 0.0001 which is lower than the confidence level of 0.05

making the effect considered extremely significant while within the different concentrations, the P-value of 0.7588 is greater than the confidence level of 0.05 making the effect considered not significant.

4. Conclusion

The different concentrations of Santan (Ixora coccinea) leaf extracts (50%, 75%, and 100%) showed antifungal and antimicrobial activities, which gives an indication of the presence of promising bioactive compounds for the potential use as an inhibitory agent against Trichophyton mentagrophytes. Most of the finding exhibited a P-value of < 0.0001, which indicates that there is a significant difference in the antifungal susceptibility of Trichophyton mentagrophytes treated with different concentrations of santan (Ixora coccinea) leaf extract compared with Ketoconazole and distilled water. Therefore, the santan leaf extract is not as effective as the antifungal agent for Trichophyton mentagrophytes used as positive control, which is Ketoconazole.

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