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IN VITRO EVALUATION OF ANTI-FUNGAL ACTIVITY OF AQUEOUS EXTRACTS OF ROOT BARK POWDER OF AZIMA TETRACANTHA AGAINST CANDIDA ALBICANS

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ABSTRACT

The side effects of synthetic fungicides and the development of fungicide-resistant pathogens have led to an intense search for naturally occurring compounds with anti-candida activity. Consequently, there has been a significant focus on identifying Antifungal activity in recent years. As a result, researchers around the world conducted extensive searches for plant species with antifungal activity against Candida albicans. The primary objective of this study was to evaluate the anti-candida properties of the Azima tetracantha (L) Kurz plant against Candida albicans. The effectiveness of hot and cold extracts was tested using dry root bark at different concentrations (100%, 50%, and 25%) in this study. Samples of Candida albicans were obtained from Eastern University and the Anti-Candida activity was analyzed using Sabouroud Dextrose Agar media. The presence or absence of inhibition zones in the disc diffusion method was utilized to evaluate the antifungal activities. The displayed zones of inhibition were dose-dependent, except for the hot extract tested against Candida albicans. The hot aqueous extract showed a 100% maximum zone of inhibition against Candida albicans (3.73±0.59cm) followed by 50% (2.4±0.54cm), 25% (1.64±0.24cm) then compared to the Cold extract 100% (3.11±1.25cm), 50% (2.35±0.64cm), 2.5% (1.45±0.43cm). The results indicated that the root bark powder was effective among the plant extracts. 10 mg/10 ml of hot extract proved to be more effective than cold extract. Compared to Fluconazole (10 mg/10 ml), the positive control, Azima tetracantha (L) Kurz exhibits more potent anti-candida activity. The findings suggest the usefulness of root aqueous extracts of Azima tetracantha (L) Kurz against pathogenic fungal (Candida albicans) strains.

Keywords: Anti candida activity, Azima tetracantha, Candida albicans, Sabouroud dextrose agar, Concentration, Zone of inhibition

INTRODUCTION

Known as a commensal pathogen, Candida albicans colonizes mucousal surfaces asymptomatically as a commensal, as it causes a variety of diseases. [1]

Plants with preservation and therapeutic properties were treasured in ancient cultures worldwide. Since the late 1800s, there have been scientific studies on the antibacterial abilities of plants and their constituents. Researchers have discovered that plants possess small molecules that can impede the growth of fungus in laboratory settings. [2]

Despite the availability of several antimycotics, it is not uncommon for the treatment of candidiasis to fail because of environmental factors that lower the drug concentration to sub-therapeutic levels.

[3] In many cases, herbal-based interventions are used to treat Candida infections without being scientifically validated. This will benefit the patient and will improve the Siddha system by providing a unique drug that is highly effective. Azima tetracantha, also known as Mulchangan in Tamil, is a sprawling, spiny shrub that belongs to the Salvadoraceae family. In Sanskrit, it is also referred to as Kundali. This species' presence of quadrangular spines at the nodes is one of its distinctive features. [4] It is a potent diuretic and a stimulant tonic in cases of rheumatism, dropsy, dyspepsia, and chronic diarrhea. Traditionally, Indian doctors have treated acute phase inflammation with Azima tetracantha, an anti-inflammatory drug. Azima tetracantha is used to treat diarrhea, coughs, phthisis, smallpox, and asthma. The decoction of stem bark is astringent, expectorant, and antiperiodic; it is also thought to have antibacterial properties. The leaves contained isorhamnitine-3-O-rutinoside, carpine, azecarpine, and azimine. Azima tetracantha leaf extract contains the following compounds: β-sitosterol, glutinol, lupeol, and friedelin. Some new findings have been made as a result of fatty acid extraction from this plant's seeds. Plant extracts and essential oils have antifungal properties against a variety of fungi, according to Duraipandiyan's (2010) research. [5]

Since ancient times, people have utilized plants as both a preventative and therapeutic measure for illnesses. The study of medicinal plants and how they are used in various nations has attracted more attention lately. Nevertheless, having scientific proof to back up the use of a plant or its active ingredients is now crucial. Azima tetracantha is one such plant that is used to treat a wide range of conditions, including diabetes, dysentery, fever, asthma, colds, coughs, rheumatism, toothaches, dog bites, snake bites, and liver diseases. When taken with food, the root, root bark, and leaves are used as a remedy for rheumatism. [6]

There have been numerous studies conducted on Azima tetracantha's pharmacological properties. The plant has been shown in experiments to have pharmacological properties such as antimicrobial activity in the leaves, antioxidant, anti-inflammatory, cytotoxic, antivenom, hepatoprotective, antiepileptic, diuretic, antiulcer, antiasthmatic, antidiarrheal, analgesic, nephroprotective, antipyretic, and insecticidal activity. The nanoparticles made from Azima tetracantha have antibacterial, antioxidant, and insecticidal characteristics. Friedelin, a plant-derived pentacyclic triterpenoid, has been demonstrated to have numerous bioactivities, including antibacterial, hypolipidemic, antidiarrheal, anti-inflammatory, antipyretic, insecticidal, gastroprotective, and antiradical action. [2] Further, Azima tetracantha has been reported for its antimicrobial (Mohamed et al., 2007), analgesic, [7] anti inflammatory, [8] and wound healing activity. [9] The roots and leaves were used as stimulant and tonic.

Abirami (2016) published research on a fast, effective, and eco-friendly method for producing gold nanoparticles from gold chloride solution, using leaf extract from the plant Azima tetracanthaLam. The synthesized gold nanoparticles displayed antibacterial properties against several bacterial pathogens, such as Aeromonas liquefaciens, Enterococcus fecalis, Micrococcus luteus, and Salmonella typhimurium, as well as fungal pathogens including Candida albicans, Cryptococcus sp, Microsporum canis, and Trichophyton rubrum. These findings suggest that gold nanoparticles could provide a safer alternative to current antibacterial treatments. [10] The assertion that Azima tetracantha root bark has anti-fungal properties was found to be unsupported by scientific data. The study's objective was to ascertain whether Azima tetracantha's

root bark possesses any antifungal properties against Candida albicans. The potential anti-fungal effects of Azima tetracantha's root bark have not been investigated. Several antifungal drugs derived from plants, minerals, and animals are described in the Siddha textbooks. With this knowledge in hand, the researchers decided to look into Azima tetracantha Linn's root bark as a possible anti-fungal remedy. This study aimed to investigate the potential anti-fungal properties of Azima tetracantha root bark.

MATERIAL AND METHODS

Collection of Plant materials

The collection of Azima tetracantha root bark took place in the Trincomalee District, specifically in the Muthunagar and Kappalthurai areas. To ensure accurate identification, the plants and species were submitted to Gunapadam laboratory and will undergo taxonomic authentication by the Gunapadam laboratory of Unit of Siddha Medicine, Trincomalee Campus, Eastern University, Sri Lanka. Once collected, the root bark was thoroughly washed using tap water and any excess water was removed by blotting the plant material with a filter paper. The root bark was then either shade-dried or air-dried at a temperature of 28°C for 2 hours. To make it into a powder, a grinder was used. Finally, the powder was stored in an airtight glass container and labeled appropriately.

Figure 2.1 - Collected root bark of Azima tetracantha

Extraction process

The extraction of the plant was performed using both hot and cold distilled water, and it was carried out by triturating the dry powder with a motor and pestle, as detailed in Claire, 2018. [11]

Cold water: The plant's stem, roots, and leaves were all grounded into ten (10) mg of powder each. Using a motor and pestle, distilled water was added to each and thoroughly mixed. After that, the mixture was put on a shaker and left for a full day. It was then centrifuged for ten minutes at 10,000 rpm. A careful separation and room temperature storage were done with the resultant supernatant. Hot water: After being separately boiled in 10 ml of distilled water, 10 mg of plant leaves, stem, and root were thoroughly crushed with a motor and pestle. After that, the mixture was heated to 1000C for five minutes in a water bath. It was centrifuged for ten minutes at 10,000 rpm after it had cooled. After careful separation, the resultant supernatant was kept at room temperature. The two extracts were diluted using a simple dilution method to achieve concentrations ranging from 10 to 2.5 mg/ml. The final volume of the solution was 10, resulting in concentrations of 100%, 50%, and 25%.

Source of Micro-organism

A fungal culture was procured from the Department of Microbiology at Eastern University in Sri Lanka to assess the efficacy of the extracts against fungi. Candida albicans was the specific type of fungus that was utilized. After being subcultured onto Sabouraud Dextrose Agar, the culture was incubated for 24 hours at 37°C. Once created, the sub-cultures were kept in storage at 5°C until needed.

Sterilization

Every piece of apparatus and glassware that was used underwent heat sterilization, which required using moist heat in an

autoclave set at 121°C for 20 minutes. A Bunsen burner's blue flame was used to sterilize both the needle loop and the agar medium. 70% ethanol was used to clean the workbench because it is known to destroy bacteria by dissolving their proteins.

Preparation of fungal culture

A commercially available dehydrated base was used to make Sabouraud Dextrose Agar. Following the manufacturer's instructions was done. The prepared medium was autoclaved for 20 minutes at 15lb/. at 121 degrees Celsius. The medium was cooled in a water bath that was kept at a temperature between 45°C and 50°C after the autoclaving process was finished. Petri dishes (100x15 mm) were filled with freshly prepared and cooled medium and placed on a level, horizontal surface. The medium was added to the mixture until it reached a consistent depth of about 4 mm. 60 ml of medium was required for plates with diameters of 150mm, while plates with a diameter of 100mm require 25-30 ml. The medium was transferred ascetic method, ideally in a sterile cabinet (Laminar air flow cabinet). pH was maintained at 5.6 +/- 0.2 at 25ºC. Following preparation, the agar medium was allowed to reach room temperature and, if not using it right away, kept in a refrigerator (2°C to 8°C). Each batch of plates was a representative sample checked for sterility by incubating at 30 to 35 degrees Celsius for at least 24 hours.

Transferring slant tube culture of Fungi

Both the sterile agar slant tube and the stock culture tube were held in the palm of one hand. After burning the inoculating loop to sterilize it, take the caps off of each tube. Lightly burn the tube mouths and remove a tiny amount of fungus from the stock culture tube. To remove the agar block containing mycelium from other fungi, use a half spear point needle. After inserting the agar block face down near the bottom of the slant into the sterile tube, remove the needle or loop. After replacing the caps and flame-testing the tube mouths once more, flame the loop ultimately. [12]

Preparation of Inoculum

Candida albicans culture was freeze-dried, and a standard protocol was followed to prepare a suspension. After the culture was freeze-dried, 5 ml of sterile water were taken and placed inside a microcentrifuge tube. After that, a vortex mixer was used to homogenize the mixture for 15 seconds in order to create a consistent suspension. [13]The suspension was mixed and then left to stand for 20 minutes before being placed on solid media. Using the 0.5 MacFarland standard as a reference, the fungal concentration of 4x 106 colony-forming cells/ml was determined. Subsequently, the mixture was applied to the solidified media and allowed to incubate for a period of 24 to 48 hours at 37°C. [14]

Growth method

A sterile wire inoculation loop was used to gather a population of microorganisms. Less organisms were deposited on the surface of the agar that had solidified in a petri dish after the loop was diluted by smearing it back and forth. Sabouraud dextrose agar (SDA) medium (HiMedia, Mumbai, India, Catalogue No. M063) should be incubated at 37°C for 48 hours on petri plates. After that, it should be incubated at 35°C for 24 to 48 hours to yield white circular colonies against a yellowish background. [11] Using the single streaking method, microorganisms were subcultured regularly and maintained to obtain pure isolation on the SDA for further drug sensitivity testing. Test organism loops were inoculated, and the resulting sterilized loop was then burned to create the fungal inoculation.

Figure 2.5 – Growth of Fungus (Candidaalbicans)

Determination of Anti-candida Activity by Disc Diffusion method

A reference antibiotic was applied to each plate using the Stokes Disc diffusion sensitivity testing method. Chloramphenicol (50.0 mg) was included in the reference antibiotic disc to prevent the growth of other molds. The discs were created by using a perforator to cut discs (5–6 mm) from filter paper. Three of these discs were then placed in a vial, and 10 mg/10 ml of each extract solution—diluted with distilled water for the extraction of varying concentrations—was added. The vial was then allowed to dry. [15]

Figure 2.6 – Preparation of Discs

Additionally, control discs were prepared, with pure water serving as the negative control and fluconazole (10 mg in 10 ml) serving as the positive control (Standard). The appropriate concentration of the anticipated antifungal plant extract was impregnated into each disk. The test organism Candida albicans was then cultured on a sensitivity testing SDA plate after this was transferred. For twelve, twenty-four, and forty-eight hours, the incubation process was carried out at 37°C. [13]

The antifungal compound was spreaded outward from a disc into the surrounding medium. Following an overnight incubation period, the culture was inspected to look for a zone of inhibition—a region surrounding the disc where no growth occurs. Using a vernier caliper scale, the radius of this zone was calculated from the disc's edge to the zone's edge. Growth starts at the point of inhibition's end. Increased inhibition zone diameter wad correlated with increased antifungal activity. Antifungal activity of the plant extract was expected to stop any growth around the disc. Fungal strains that are sensitive to antifungals were inhibited at a distance from the disc, whereas resistant strains grow up to the disc's edge. The discs were placed on plates that have fungal streaks on them already. [13]

Figure 2.7: Zone of inhibition

Clean-up& Disposal

After completing the transfer work, the area was cleaned with a disinfectant solution containing 70% ethanol. All used glass materials were autoclaved.

Statistical analysis

A Vernier calliper was used to measure the diameter of the zone of inhibition for fungi following the incubation period. The

measurement was taken in centimetres (cm). The results were then compared with the control to evaluate them. [16] The statistical software SPSS was used to enter all of the data into a database. The data will next be examined using the Independent Sample T-test and a one-way analysis of variance (ANOVA) to compare the mean inhibitory zones. When the mean +/- standard deviation is less than 0.05, it is deemed that the values are statistically different.

RESULTS

Zones of inhibition For Azima tetracantha hot & cold extract against the Candida albicans

The homogeneity test and one-way ANOVA were used to examine the anti-fungal activity in relation to the concentration of the hot and cold extracts (100%, 50%, and 25%). Extracts from the powdered root bark of Azima tetracantha exhibited a moderate to potent (partial to complete inhibition of fungal growth) anti-fungal activity against Candida albicans.

Table 1: Zones of inhibition For Azima tetracanthahot & cold extract against the Candida albicans

Fungus - Candida albicans		
	Mean inhibitory zones(cm) (without radius)	
Concentration	Azimatetracantha root bark Hot method extraction	Azimatetracantharootbark Cold method extraction
100%	3.73±0.59	3.11±1.25
50%	2.4±0.54	2.35±0.64
25%	1.64±0.24	1.45±0.43

Data expressed as mean ±SD

The extract displayed zones of inhibition in a dose dependent manner, with an exception for the hot extract tested against Candida albicans. Results from the hot aqueous extract tested against Candida albicans, exhibited the maximum zone of inhibition for 100% (3.73±0.59cm) followed by 50% (2.4±0.54cm) , 25% (1.64±0.24cm) then compared to the Cold extract 100% (3.11±1.25cm) , 50% (2.35±0.64cm), 2.5% (1.45±0.43cm).

Results of 25% concentration of root bark powder of Azima tetracantha

Table 3.2: Significance of correlation between the standard drug and the test drug and the Antifungal activity

		Sum of Squares	df	Mean Square	F	Sig
Hot 25%	Between groups	0.546	1	0.546	69.342	0.685
	Within groups	0.079	10	0.008		
	Total	0.625	11			
Cold 25%	Between groups	1.920	1	1.920	274.286	0.786
	Within groups	0.070	10	0.007		
	Total	1.990	11			

One way-ANOVA analysis was done using SPSS Version 23, between the standard group and the test group at 25% concentration gradient. The significant values for both the hot extract and the cold extract were as 0.685 and 0.786 which indicates that these values were higher than the p value of 0.05 (p>0.05). There is no significant correlation between the standard drug and the test drug and the antifungal activity.

Table 3.3. Descriptive Statistics

	N	Range	Minimum	Maximum	Mean		Standard Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std Error	Statistic	Statistic
Hot 25%	12	0.62	1.28	1.90	1.6567	0.08879	0.25830	0.067
Cold 25%	12	0.90	1.00	1.90	1.4500	0.12278	0.42553	0.181
Valid N (list wise)	12							

The hot test drug (Azima tetracantha root bark powder) At the 25% concentration gradient was with the mean

value and standard deviation of 1.63±0.23cm and the cold test drug at the concentration gradient of 25% concentration was with the mean value and standard deviation of 1.45±0.42cm.

Results of 50% concentration of root bark powder of Azima tetracantha

Table 3.4 Significance of correlation between the standard drug and the test drug and the Antifungal activity

		Sum of Squares	df	Mean Square	F	Sig
Hot 50%	Between groups	2.651	1	2.651	482.104	0.038
	Within groups	0.054	10	0.005		
	Total	2.705	11			
Cold 50%	Between groups	2.613	1	2.613	12.727	0.056
	Within groups	2.053	10	0.205		
	Total	4.667	11			

One way-ANOVA analysis was done using SPSS Version 23, Between the standard group and the test group at 50% concentration gradient. The significant values for both the hot extract and the cold extract were as 0.038 and 0.056, which indicates that the hot extract significant value (0.038) was $p<0.05$ that shows that there is a significant correlation between the test drug and the standard drug for the antifungal activity. Whereas the cold extract showed a significant value (0.056) of $p> 0.05$ which indicates that the cold extract does not show any significant correlation with the standard drug to show the antifungal activity. Moreover, compared to the cold extract of the test drug, the hot extract of the test drug is an efficient and effective for the treatment of candida infection.

Table 3.5 Descriptive statistics

	N	Minimum	Maximum	Mean		SD	Variance
	Statistic	Statistic	Statistic	Statistic	Std Error	Statistic	Statistic
Hot 50%	12	1.60	3.00	2.4000	0.15765	0.54606	0.298
Cold 50%	12	1.00	3.00	2.3500	0.18907	0.64456	0.415
Valid N (listwise)	12						

The hot test drug (Azima tetracantha root bark powder) At the 50% concentration gradient was with the mean value and standard deviation of 2.4±0.54cm and the cold test drug at the concentration gradient of 50% concentration was with the mean value and standard deviation of 2.35±0.64cm.

Results of 100% concentration of root bark powder of Azima tetracantha

Table 3.6: Significance of correlation between the standard drug and the test drug and the Antifungal activity

		Sum of Squares	df	Mean Square	F	Sig
Hot 100%	Between groups	3.000	1	3.000	31.690	0.008
	Within groups	0.947	10	0.095		
	Total	3.947	11			
Cold 100%	Between groups	14.963	1	14.963	61.493	0.046
	Within groups	2.433	10	0.243		
	Total	17.397	11			

One way-ANOVA analysis was done using SPSS Version 23, Between the standard group and the test group at 100% concentration gradient. The significant values for both the hot extract and the cold extract were as 0.008 and 0.046. which indicates $p<0.05$. which shows that there is a significant correlation between the test drug and the standard drug. As the standard drug the test drug is an effective and efficient antifungal agent to treat candida infections.

Table 3.4. Descriptive statistics

	Statistic	Statistic	Statistic	Statistic	Statistic	Std Error	Statistic	Statistic
Hot 100%	12	1.60	3.00	4.60	3.7333	0.17291	0.59899	0.359
Cold 100%	12	1.60	1.96	4.60	3.1167	0.34983	1.23758	1.532
Valid N (list wise)	12							

The hot test drug (Azima tetracantha root bark powder) At the 100% concentration gradient was with the mean value and standard deviation of 3.7±0.59cm and the cold test drug at the concentration gradient of 100% concentration was with the mean value and standard deviation of 3.11±1.2cm.

The data presented in Table 3.2, 3.3, 3.4, 3.5 and 3.6 indicated that the Azima tetracantha root bark hot water extract exhibited the highest zone of inhibition 100% (3.73±0.59cm) for Candida albicans which subsequently reduced with the decrease in concentration of extract 50% (2.4±0.54cm), 25% (1.64±0.24cm). The Comparison of means were displayed a statistically significant difference ($p<0.05$) at each concentration of Azima tetracantha 's root bark Hot extract against the Candida albicans , among Azima tetracantha 's root bark Cold extract the highest zone of inhibition was also exhibited at 100% (3.11±1.25cm), followed by 50 %(2.35±0.64Cm), & 25%(1.45±0.43cm) The Comparison of means were displayed a statistically significant difference ($p<0.05$) at each concentration of Azima tetracantha's root bark Cold extract against the Candida albicans. There was significant difference between the Azima tetracantha'sroot bark hot & cold extract concentration of 100% against Candida albicans.

Effectiveness of Azima tetracantha's root bark against Candida albicans in different concentration of Hot & Cold extract

Figure 5.5.1 shows histogram analysis standard value of inhibition for each extract was 2.2cm. Compared with the hot & cold method, the hot method is efficient for inhibit the growth of Candida albicans. Among the three different Concentration (100%, 50%, 25%) 100% was elicited the highest mean rate of inhibition for both hot and cold extract.

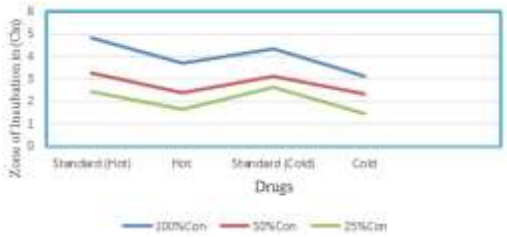

Figure 5.5.1 - Effectiveness of Azima tetracantha's root bark against Candida albicans in different concentration of hot &cold

Effectiveness of Azima tetracantha's root bark against Candida albicans in different concentration of hot extract and Positive control

Table 5.6 Zones of inhibition for hot extract of Azima tetracantha against the Candida albicans and zone of inhibition of Standard drug Flucanazole

	Mean inhibitory zones(cm) (without radius)	
Concentration	*Azimatetracantha* root bark Hot method extraction	Standard drug Fluconazole (Positive control)
100%	3.73±0.59	4.83±0.28
50%	2.4±0.54	3.27±0.21
25%	1.64±0.24	2.42±0.12

Table 5.6 indicates the mean value and standard deviation of the test drug's hot extract at different concentration gradients and Standard drug at different concentration gradients. It indicates that both the hot extract of the test drug and the standard drug has the anti-fungal activity against Candida albicans but the standard drug is more effective than the test drug.

Effectiveness of Azimatetracantha's root bark against Candida albicans in different concentration of Cold extract and Positive control

Table 5.7 Zones of inhibition for cold extract Azima tetracantha against the Candida albicans and zone of inhibition of Standard drug Flucanazole

	Mean inhibitory zones(cm) (without radius)	
Concentration	*Azimatetracantha* root bark Cold method extraction	Standard drug Fluconazole (Positive control)
100%	3.11±1.25	4.32±1.12
50%	2.35±0.64	3.11±0.76
25%	1.45±0.43	2.64±0.53

Table 5.7 indicates the mean value and standard deviation of the test drug's cold extract at different concentration gradients and Standard drug at different concentration gradients. It indicates that both the cold extract of the test drug and the standard drug has the anti-fungal activity against Candida albicans but the

standard drug is more effective than the test drug.

DISCUSSION AND CONCLUSION

Discussion

Azima tetracantha is a unique traditional herbal plant used in the Siddha medical system that is highly efficacious against the majority of microbial infections. As a result, in this study, the antifungal properties of Azima tetracantha root bark powder were evaluated against the Candida albicans fungal strain. The plant Azima tetracantha exhibits pharmacological activities such as antimicrobial activity of leaves, antioxidant, anti-inflammatory, cytotoxic, antivenom, hepatoprotective, antiepileptic, diuretic, antiulcer, antiasthmatic, antidiarrheal, analgesic, nephroprotective, antipyretic, and insecticidal activity. The nanoparticles synthesized from Azima tetracantha have shown antimicrobial, antioxidant, and insecticidal properties. Phytochemicals such as pentacyclic, triterpenoid, friedelin isolated from the plant is responsible forits wide range of pharmacological actions such as antimicrobial, hypolipidemic, antidiarrheal, anti-inflammatory, antipyretic, insecticidal, gastro protective and antiradical activity. [17]

According to the current study, components of Azima tetracantha have strong antifungal effects on Candida albicans. A maximum zone of inhibition against fungal growth (Candida albicans) was revealed by the plant Azima tetracanthar's anti-fungal activity at three different concentrations: 100%, 50%, and 25%. Based on statistical analysis, it was found that the root's hot and cold extracts exhibit anti-fungal activity against Candida albicans at varying concentrations (100%, 50%, and 25%). The results of this study also reveal that different concentrations of hot and cold extracts of this plant showed statistical significance ($P<0.05$). The aqueous extracts (hot and cold) of Azima tetracantha were found to have potent antifungal properties at different concentrations. Therefore, the hot extract is more effective than the cold extract. Azima tetracantha has an effective anti-fungal activity since 100% of its concentration is more potent than 50% and 25%. The zone of inhibition of fluconazole is 4.32±1.12cm, but the root bark of the plant Azima tetracantha showed a smaller zone of inhibition against Candida albicans. It showed antifungal activity to some extent, but was not as effective as the standard.

The aim of this study was to investigate the antifungal effect of hot and cold aqueous extracts of the root bark of Azima tetracantha on Candida albicans. An antifungal effect was observed at 100% of the hot aqueous extract of the root bark of Azima tetracantha (10 mg/10 ml), and also at 100% of the cold extract of the root bark of Azima tetracantha. However, it was slightly less than the hot method. In this study, the minimum inhibitory concentration was not determined; nevertheless, the zone diameter of various concentrations, hot and cold extracts were reported in (Table-5.1.1). The hot aqueous extract showed the highest zone of inhibition against Candida albicans (3.73±0.59cm), followed by 50% (2.4±0.54cm) and 25% (1.64±0.24cm), compared to the cold extract (3.11±1.25cm), 50% (2.35±0.64cm), and 25% (1.45±0.43cm).

In this investigation, the antifungal impact of hot and cold aqueous extracts of root bark of Azima tetracantha on Candida albicans was slightly lower than that of the positive control. At 100% (10mg/10ml), the positive control had a maximum zone of inhibition against Candida albicans of 4.32±1.12cm. Tables 5.6 and 5.7 show that the positive control tested against Candida albicans had the highest zone of inhibition at 100% (4.32±1.12cm), followed by 50% (3.11±0.76cm) and 2.5% (2.64±0.53cm).

There were no research evidences from the literature, no actions proved in root bark of plant Azima tetracantha extract, so the process of zone of inhibition (anti-fungal activity) is compared to the effects of Trihumors, suvai, veeriyam and vibakam. Effect on Dosham, It pacifies vitiated Kaphaand Vatadosha (later stage it will be aggravated) due to its Kaippusuvai and UshnaVeeryam. [18] This plant Azima tetracantha (Mutchangan) is an anti-fungal plant and also it has an Ushna veeriyam.[19]so far, the plant (Mutchangan) can pacify the Kapha dhosha and cure the Canndida albicans based diseases. Plant Azima tetracantha has Kaippu suvai and they reduce the vitiated Kapha dhosha. Kaippu suvai consists of Vaayu and Visumbu so it pacifies the Kapham. Kaippu suvai acts as an anti- infectious. [18] Therefore Kaippu suvai could act against the fungal infection.

Each taste has an energetic effect on digestion called "Veeriyam" or "Potency". The plant Azima tetracantha possesses the Ushna veeriyam, the increased kapha can be reduce by characters such as laghu (Shanmugavelu, 1988). Based on the Ushnaveeriyam where it increases the Pitham and reduces the both Vatham and Kapham. [18] Pungent, bitter, and astringent plants have a pungent vipakm. This plant owes to the action of pungent (kaarpu), also it is pacifying the kapham. Vipakam is the outcome of digestion and metabolism occurs due to digestive enzymes. The drugs which made from the plant Mutchangan (Azima tetracantha) can be cure the fungal infections.

Conclusion

The root bark of Azima tetracantha plant (Extract) has anti-fungal activity on Candida albicans. The hot extract is more effective than the cold extract at 100% concentration, with a zone of inhibition of 3.73±0.59cm and a p-value of 0.008. The cold extract has anti-fungal action at 100% concentration, with a zone of inhibition measuring 3.11±1.25cm and a p-value of 0.046.

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