The effect of*Morindacitrifoliaethanolic*extractagainst*Malassezia globosa*obtained from the scalp of individuals between the ages of 18-24

Abstract

Dandruff, a common scalp condition affecting 50% of the world's population, is mainly caused by the fungus *M. globosa.* In this study, the antifungal effect of *M. citrifolia*, commonly known as noni, was tested against *M. globosa.M. citrifolia* fruit, leaf, and stem contain phytochemicals including alkaloids, phenols, flavonoids, and glycosides. Extracts of the three plant parts were obtained by maceration method using ethanol, and prepared in concentrations of 100%, 75%, 50% and 25%. The scalps of 6 individualswere swabbed to obtain a sample of *M. globosa.* The susceptibility of *M. globosa* to *M. citrifolia* extracts was tested in triplicate using the disc diffusion method, and a measure of the extent of susceptibility was ascertained by the size of the inhibition zones. It was found that of the 3 extracts, noni fruit showed the highest inhibitory effect with 20/48 discs having inhibition zones, noni stem showed moderate inhibition with 12/48 inhibition zones, while noni leaf exhibited no inhibition zones, indicating its lack ofinhibitory effect against *M. globosa.* Notably, noni fruit at 100% and 75% concentrations proved to be most effective against *M. globosa*, and at 25% concentrations, noni stem was most effective. However, statistical analysis using both ANOVA and Kruskal Wallis tests indicated that the observed data was insignificant since the *p*-values were > 0.05. It can therefore be concluded that noni fruit, leaf, and stem extracts have little to no significant inhibitory effect on the growth of *M. globosa*, notwithstanding, a change in various experimental factors including agar type and extract preparation may prove otherwise.

Keywords: Antifungal properties, inhibition zones, maceration, noni, *M. citrifolia, M. globosa*

1. INTRODUCTION

Dandruff, which is essentially dry, dead, scaly skin, affects one in two persons in the world and occurs because of the activity of *Malassezia* on the scalp. Glands attached to hair follicles, secret an oil called sebum which is composed of saturated and unsaturated fatty acids, *Malassezia* feeds on the saturated fatty acids, leaving the unsaturated fatty acid behind (Dolenc-Voljč, 2017). These unsaturated acids have a sharp twist in their structure in their double bonds, which irritates by

way of small breaches in the scalp when it is absorbed. Water escapes from these breaches, the body reacts defensively, and the skin cells prematurely proliferate to correct the breaches, denying them the chance to mature and differentiate properly. Consequently, these cells clump together, on the scalp around the hair follicles and are eventually shed as visible flakes (Vijaya Chandra et al., 2021). Many remedies are used to treat dandruff. Synthetic treatments include the use of various brands of anti-dandruff shampoos, like Head and Shoulders, Nizoral, Selsun and Neutrogena T, whose inhibitory components are zinc pyrithione, ketoconazole, selenium sulfide and coal tar respectively. Botanical treatments include *Hibiscus rosa-sinensis* flower, *Mangifera indica* kernel, *Camellia sinensis* leaf and *Citrus aurantifolia* juice (Dikshit et al., 2012; Mahantesh et al., 2018).

Three common microbes readily inhabit the scalp's microbiome, they include*Staphylococcus epidermidis*,*Propionibacterium acnes*, which are bacteria, and *Malassezia* species, specifically *M. restricta* and *M. globosa*(Saxena et al., 2018). In humans, *Malassezia* yeasts are found on the cutaneous microflora and populate areas of the skin that have an abundance of sebaceous glands. There are 14 various identified species of *Malassezia*. *Malassezia furfur, Malassezia globosa and Malassezia restricta* are most likely to cause skin diseases in humans. Diseases associated with *Malassezia* include psoriasis, pityriasis, atopic dermatitis and dandruff (Dolenc-Voljč, 2017).

Noni, an evergreen shrub that is thought to be a miracle plant, has its origin in Southeast Asia, where it has been used as traditional medicine. Various parts of the plant are used in different capacities and have varying therapeutic attributes. Of the leaf, stem, fruit, root, flower and bark, the fruit is thought to have chemical compounds that are of higher value. *M. citrifolia* has been discovered to have a therapeutically high effect against viruses, bacteria, fungi, tumours, internal parasites, hypertension, inflammation, immune deficiency, and pain (Adeel et al., 2023; Mahantesh et al., 2018). To be more precise, noni has approximately 29 beneficial acids including oleic acid, lauric acid and octanoic acid. Additionally,*M. citrifolia*contains other beneficial biochemicals such as anthraquinones, sterols, glycosides, terpenes, and amino acids. Anthraquinones help to fight inflammation, tumours, bacteria, and parasites. Compounds found in anthraquinones are also thought to function as pain relievers, being more effective than morphine (Elvis et al., 2023; Royani et al., 2023).Ambarwati et al. (2015)found that 96% ethanol extract of noni fruit juice inhibited the growth of 14 of the fungal isolates that cause dandruff in their experiment and

concluded that noni can be used to treat dandruff. Kumar et al. (2010) andSharma and Smita (2010)positedthat noni leaf properties extracted with ethyl acetate prevent the growth of *Candida albicans* at a diameter of 17 mm. Nidhi et al. (2020)showed that the growth of *C. albicans* and *A.niger*are inhibited in the presence of a 10 mg/ml concentration of petroleum ether extract of noni leaves at 22 mm and 26 mm respectively.

Also, Srinivasahan and Durairaj (2014)used 100 µg of hydroethanolic extract from noni fruit to prevent the 5 fungi from growing, namely, *C. albicans, A. niger, Monoscuspurpureus, Monoscucs and A. fumigatus.* Additionally, Kakad et al. (2015) research enhances the claim of noni's antifungal properties, showing that methanol extract of noni leaves inhibits the growth of *D. flavida, A. niger and C. albicans* at 17 mm, 22 mm, and 20 mm respectively. Yukti (2011)showed that noni leaf extract inhibits the growth of *Malassezia furfur*, where the concentration of the leaf extract was 25%. Soraya et al. (2011) results showed that selenium sulfide works against the growth of*M. furfur* a fungus, which is one fungus that causes dandruff. Moreover, Barani et al. (2014)proved that *C. albicans* growth can be hindered by 1000 µg/ml of noni fruit extract. Purwantiningsih and Suranindyah (2014)research highlighted phenol and flavonoid as two components of noni that function as antibacterials. Finally,Kakad et al. (2015) showed various compounds that are present in noni leaf extract, including glycosides, phenols, terpenoids, alkaloids, steroids, flavonoids, and tannins. The research highlighted the inhibitory effects of noni fruit and leaf extracts at different concentrations on dandruff-causing fungi. The same idea would be tested in this research; however, the effects of noni fruit, leaf, and stem extracts will be used against specific dandruff-causing fungi, *M. globosa.*

2. MATERIAL AND METHOD

This study was conducted in the Biology Laboratory of the University of Guyana.

2.1 Plant specimen collection and extract preparation

Plant samples were collected from two noni trees, one at Liliendaal Railway Embankment Road, ECD and the other at Block R North Sophia. Leaves, fruits and stem were taken to the biology

laboratory, where they were cut into smaller pieces to increase surface area, the fruit was placed into pans labelled with the respective date, the researcher's initials and the number according to the number of fruit pans (e.g. NF #1 17/04/23 XH), leaves were placed into paper bags and labelled using the same format, the stem was placed in a pan and a paper bag and labelled as was the fruit and leaf. The samples were weighed using a 300g electronic scale, weights were recorded, and the samples were placed in an incubator to dry at 45^{o}C for two weeks to retain phytochemical properties. The samples were then transferred into an oven at 45^{o}C for additional drying. Weighing was done every day or sometimes every two days until the weights of the samples remained constant, this indicated that the samples were finished drying. After drying the samples were ground using a hand mill. The maceration method includes 50g of the ground solute was weighed and placed in large jars, to which 300 ml of ethanol was added (Ambarwati et al., 2015; Duraipandiyan et al., 2006). The mixture was stirred and allowed to sit for 7 days while being stirred occasionally. After 7 days, the mixture was strained using gauze, placed into smaller jars, and taken to the natural products laboratory of the chemistry department to remove the ethanol from the extract using a rotary evaporator. Subsequently, a crude extract was obtained for all 3 mixtures, then divided into 75%, 50% and 25% extracts, using $m_1v_1 = m_2v_2$ formula, the concentrations were placed into theory respective glass vials with appropriate labelling (NF:100%, NL: 100, NS: 100% etc.). Paper discs were made from Watman #1 filter paper using a paper puncher, the discs were then sterilized in the autoclave and inserted into the vials in the sterile environment of the fume hood, the discs were soaked for 24 hours(Ambarwati et al., 2015; Shettima et al., 2023).

2.2 Controls Preparation

For the negative control, a ketoconazole tablet was crushed, dissolved in water, and placed in a glass vial. The positive control was placed in a glass vial as well, and paper discs were added to both controls in the same way they were added to the extracts.

2.3 Media Preparation

Potato Dextrose Agar (PDA)from commercial media powder was used. The agar needed for 60 plates was calculated with reference to the instructions on the agar bottle. Using an 800 ml and a 400 ml flask, 31.2g of powder and 15.6g of powder were used, respectively. The mixture of powder and water was boiled and mixed until the powder was fully dissolved. The solution was

sterilized for 30 minutes in the autoclave, cooled then poured into the Petri plates, in a sterile fume hood in laminar airflow. The agar was allowed to solidify, the plates were then invertedly stored in a fridge until they were ready to be used(Majumdar & Mandal, 2019; Patil & Pangrikar, 2023).

2.4 Sample Collection and Identification

To obtain a sample of *M. globosa* the scalps of 7 individuals between the ages of 18-24 were swabbed in the biology building, 4 had dandruff and 3 had healthy scalps. Verbal consent was given by each participant. Using examination gloves, sterile combs, sterile tipped applicators and the fume hood, the temporal and vertex regions of healthy scalps were swabbed, while the region of the scalp having the most dandruff was swabbed, this method followed the steps ofRudramurthy et al. (2014), samples were immediately swabbed were labelled and placed prepared agar plates. The plates were then incubated at 37^{o}C for 48 hours (Ambarwati et al., 2015). Fungal growth was observed for all 7 plates, and *M. globosa* was isolated based on colony morphology and the use of literature by Ramadán et al. (2012).

2.5 SusceptibilityTesting

The McFarland standard was made using Barium Chloride dihydrate and sulphuric acid. 44 plates were dried, sterilized in the fume hood using a UV light for 20 minutes, thenlabelled with the researcher's initial, the date, the name of the organism + the plant part and its concentration and the plate number (Cardiliya et al., 2023; Yildirim et al., 2023). Six of the 44 plates were used for the positive control (ethanol) and the negative control (ketoconazole). Ten ml sterilized water and a small portion of *M. globosa*, formed the inoculum which was standardized using the McFarland standard(Hudzicki, 2009; Yildirim et al., 2023). The inoculum was then swabbed onto the 44 prepared and labelled PDA plates. Four paper discs with their respective concentrations were added in their respective quadrats, except in the case of the negative control, to which 2 paper discs were added. The plates were taped, incubated invertedly for 1 day at 35^{o}C and 3 days at 37^{o}C. The plates were observed for zones of inhibition, these were recorded in mm in a data sheet (Ambarwati et al., 2015).

3. STATISTICAL ANALYSIS

Three different plant parts were used in this study therefore, one-way ANOVA was employed, followed by a Tukey test. A Kruskal Wallis test was subsequently used since the data obtained was not normally distributed. The statistical software RStudio: Integrated Development for R. RStudio (Version 2, PBC, Boston, MA, and the Statistical Package for Social Sciences (Version 21 for Windows, SPSS Inc., New York, NY, USA) were used to perform the analyses.

4. RESULTS AND DISCUSSION

Noni fruit had the most inhibition zones of the three extracts (20/48 discs), while noni stem had the second most inhibition zones (12/48 discs) while noni leaf had no inhibition zones. Noni fruit at 100% and 75% had the better inhibitory effect on *M. globosa* of all the fruit concentrations while noni fruit at 50% and 25% had the least effect for the fruit concentrations. Noni stems at 25% displayed the best inhibitory effect against *M. globosa* of all the stem extracts. To ascertain if the values and observations were significant, a one-way ANOVA test was conducted, the *p*-value of which was 0.0887. A Tukey test was conducted on the results of the ANOVA, the *p*-values of which ranged from 0.4043331 - 1.0000000, this shows that there is no significant difference in the inhibitory effects of the 3 three extracts when compared with each other. The data was not normally distributed, hence, a Kruskal Wallis test was conducted, the *p*-value of which was 0.09786. Both the ANOVA and Kruskal Wallis test yielded a *p*-value>0.05 indicating that there was no significant difference in the data.

As noted earlier noni fruit and stem displayed some inhibitory effect against *M. globosa*, while the leaf did not, the properties in the stem and fruit that could have possibly been responsible for the inhibition zones observed.Ranvir et al. (2017)indicatedthat the hydroethanolic extract of noni fruit has phenols, alkaloids, tannins, proteins, diterpenes, terpenoids, carbohydrates, saponins, flavonoids, anthraquinone, glycosides, steroids, amino acids, and tannins. Jose and Maya (2020)found that aqueous extracts of noni fruit had carbohydrates, protein, steroids, phenol, alkaloids, cardiac glycosides, saponins, terpenoids and tannins(Ranvir et al., 2017). One or several of these bioactive compounds found in noni fruit is responsible for inhibiting the growth of *M. globosa*, to be certain which one did each compound would need to be tested against the fungus of focus.

Shettima et al. (2023) revealed that the phytochemicals found in methanolic extract of noni stem are alkaloid, tannin, flavonoid, terpenoid and steroid. Alkaloid, tannin, terpenoid, saponin, and glycosides were found in petroleum ether extract of noni stem. The acetone extract of the noni had terpenoid, steroid and glycosides present while the ethyl acetate extract of the noni stem had alkaloid and tannin present, carbohydrates were absent from all the stems when the extracts were used. Carbohydrates were, however, present in noni fruit, this may account for the difference in the number of inhibition zones between the two extracts(Abdillah et al., 2020). The negative controlketoconazole showed no inhibitory effect, however, as stated in the introduction, it is a potent antifungal compound. Moreover,the researcher conducted the test of keto against *M. globosa* againusing Mueller Hinton (MH) agar and it was indeed ascertained that it can inhibit the growth of *M. globosa* given the large diameter of the inhibition zone. The same cannot be said for the extracts since the inhibition zones remain the same when using MH agar(Åhman et al., 2020; Jalal et al., 2023).

Setyani and Setyowati (2018)found that the active secondary metabolites found in noni leaf were flavonoids, tannins, alkaloids, steroids, and saponins. Pandey and Rizvi (2009)indicate that flavonoids have subclasses, those subclasses can further be identified by direct examples, which are found in a specific fruit. For example, a subclass of flavonoids is flavones; an example of a flavone is apigenin which is found in lemon. It can therefore be inferred that the bioactive compounds in the leaves are groups and may further be subdivided, so that the specific type of a subdivision of a compound may be present in the fruit and/or stem and absent in the leaf, thus yielding a different result. Additional phytochemicals in noni leaf as found by are carbohydrates, terpenoids, reducing sugar, α-amino acids, phenolic compounds, and glycosides(Yee, 2019).

Ambarwati et al. (2015) noted that ethanol extract of noni fruit inhibited the growth of 14 of 18 dandruff-causing fungal isolates, and 15 of the 18 isolates were yeasts. The inhibition zones of noni ethanol extract ranged from 10mm - 35mm, and 96% ethanol extract exhibited the strongest inhibitory effect against the isolates; they subsequently concluded that noni fruit can be used to treat dandruff. In this research when *M. globosa* yeast was specifically targeted with noni fruit extracts, it proved to have little to no inhibitory effect and the diameter of inhibition zones ranged from 0mm-9mm, less than the minimum inhibitory zone Ambarwati et al. (2015). Additionally, the concentrations used in this research were 100%,75%, 50% and 25%, none of

which had a significant effect on *M. globosa.* It can therefore be inferred that a concentration of 96% or untested concentrations may prove to be effective.Also,the difference in preparation of the extracts may account for the difference in results as well.

5. CONCLUSION

This research focused on the antifungal properties of *M. citrifolia* against *M. globosa.* After conducting susceptibility tests in the laboratory, it was found that ethanolic extract of noni fruit displayed the strongestinhibitory effect against *M. globosa* at 100% and 75% concentration, ethanolic extract of noni stem showed moderate inhibitory effect against *M. globosa* the largest zones being observed at 25% extract concentration, while noni leaf had no inhibitory effect against *M. globosa.* Statistical analysis conducted on the data record of inhibition zones, using one-way ANOVA and Kruskal Wallis tests, yield a p-valueof > 0.05, therefore it was deduced that ethanolic extracts of noni fruit, leaf, and stem, have little to no effect on the growth of *M. globosa*.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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