J Cosmo Tricho
Volume 7:3, 2021

Journal of Cosmetology & Trichology

Research Article

Open Access

In Vitro Antifungal Screening of UPL-Pn-2 Peanut Seeds (*Arachis hypogaea L.*) Against Dandruff-Causing fungus, *Malassezia globosa*

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Abstract

This study aimed to screen the UPL-Pn-2 peanut seed extract for antifungal efficacy against dandruff-causing fungus, *Malassezia globosa*. Quantitative approach was conducted via *in vitro* antifungal screening through Kirby-Bauer test with experimental and control groups. Experimental group has 3 set-ups of peanut seed extracts in varying concentrations (25%, 50% and 100%). For control group, 3 set-ups of commercialized tech grade boric acid were also prepared in different concentrations (25%, 50%, 100%). Discs with dried methanol were used for negative control set-up. ANOVA revealed that antifungal effectiveness of peanut seed extract varied with the concentrations, while t-test showed no significant difference between peanut extract vis-à-vis boric acid concentrations. Also, pure boric acid and peanut extract were more effective compared to lower concentrations. The screening revealed antifungal efficacy of UPL-Pn-2 peanut seeds against dandruff-causing fungus, *Malassezia globosa*, which shows promising results as organic alternative to commercialized anti-dandruff products.

Keywords: Antidandruff • Peanut • Boric acid

Introduction

It is estimated that 50% of the global population suffer from dandruff caused by fats secreted by our scalp through oil or sebum [1]. According to Moral, men are more prone to dandruff than women because their scalp typically produces more oil due to engagement into comparatively active workouts. Though in Philippines bathing has been part of the daily hygiene, survey still revealed that 77% of its infected population complained of dandruff problems. Current global marketing is producing more anti-dandruff shampoos however, 30% of these known products address issues only on flaking indicating that after successfully washing dandruff, flakes will again resurface after a few days [2].

Dandruff is a scalp problem characterized by flaking off dead cells often accompanied by itching. A literature review has suggested that it is not related to hygiene but one theory linked dandruff production to hormones during puberty [3,4]. Nonetheless, the very root cause of the existence of the condition is still unknown [5]. Clinical studies, however, have found out that dandruff is caused by a yeast-like fungus called *Malassezia globosa* also known to be the cause of most skin diseases to humans including seborrhoeic dermatitis and tinea versicolor [6,7]. The Malassezia spp. were discovered in 1904 by Louis-Charles Malassez with currently 22 recognized species throughout the world [8,9]. M. globosa species is capable of developing oleic acid by breaking down sebum [10]. This acid causes inflammatory response to sensitive people resulting to the accelerated production of dead skin cells, hence resulting to dandruff [11]. Aykut, et al. recently found out that M. globosa may promote pancreatic cancer when successfully migrated from lumen to pancreas [12].

To this day, pharmaceutical industries have already developed arrays of antifungal medicines that help treat M. globosa in a form of oral or topical usage. Some of the most important medicines that help in combating the M. globosa include hydrocortisone and boric acid as effective drugs against the infection [13,14].

One plant proven to contain boric acid is peanut at a range of 13.8 mg/L to 1.8 mg/L along with licorice, goldenrod, and red elm [15]. Peanuts are under the family of Fabaceae, or Leguminosae commonly known to people as beans, or peas. This plant is a legume and has 6 recorded useful species throughout the world. It is predominantly grown in countries with tropical climate primarily for the cultivation of its seeds. Its seeds contain a lot of oils and phytochemicals. The UPL-Pn-2 is one of major varieties of peanut in the Philippines because it is high-yielding and moderately susceptible to cercospora, peanut rust, and sclerotium leaf spot [16]. According to Palomar, UPL-Pn-2 peanut variety matures in 90 to 100 days which is a good resort for farmers to supply the legume demand. In this study, the use of UPL-Pn-2 peanut seeds will be tested against the dandruff-causing fungus M. globosa. Specifically, this study aims to attain the following goals; (a) to measure the zones of inhibition caused by peanut seeds against M. globosa in three trials, and control set-ups; (b) to test the significance of difference in the measurement of zone of inhibitions among three trials, and set-ups; and, (c) to provide recommendations for the improvement of the current study.

Materials and Methodology

Methodology

The study is quantitative using *in vitro* antifungal activity through Kirby-Bauer test to determine the effectiveness of UPL-Pn-2 peanut seeds against dandruff-causing fungus, M. globosa [17]. M. globosa strain inoculum size was 5.0 × 103 CFU/ml and was provided by SGS, Microbiological Testing for Life Sciences, as culture, to ensure the validity of the microorganism involved in the testing. A year-old UPL-Pn-2 peanut seeds variety were provided by Department of Agriculture- Bureau of Plant Industry (DA-BPI) and was identified true variety by University of Philippines Diliman, Institute of Biology. Methanol extraction procedure was performed to isolate peanut extract from seeds [18]. After which, the extracts were diluted at different concentrations. A commercialized pure boric acid was also considered in the study for control group.

The design of the study was composed of (1) experimental and (2)

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Received: May 11, 2021; **Accepted:** May 25, 2021; **Published:** June 01, 2021

control groups. Experimental group is designated with 3 set-ups of varying concentrations of peanut extract: 25%; 50%; and 100%. Concentration of 25% and 50% extract was prepared by adding 75 drops of distilled water to 25 drops of peanut extract (25%), and 50 drops of distilled water to 50 drops of pure peanut extract (50%). The same procedure was done for the preparation of 25%, 50% and 100% concentrations of boric acid for the control group. Negative control set-up using disc with air-dried methanol was prepared to test other potential extraneous variance which might possibly affect the result of testing. Three trials were conducted to determine the consistency of results. Each trial was incubated for 5 days with 3°C until zone of inhibition is visible in the plates using Leeming-Notman agar. Caliper was used to measure the zones of inhibition by the mean of longest and shortest diameters of inhibitory zones (Diameter of the discs was subtracted). Millimeter (mm) was used as the unit for measurement. Prior to the conduct of the experiment, autoclave sterilization of all the materials needed was administered to avoid contamination of data with 15 psi for 30 minutes maintaining a temperature of 12°C. Under the same criteria, the control group with 25%, 50%, and 100% boric acid, and discs with dried methanol (negative) were incubated for 5 days with 3°C. Inferential statistics like ANOVA and t-test were used to analyze the data.

Materials

Common laboratory autoclave was used for sterilization of 15 petri dishes, 15 discs, 15 medicine droppers, 15 test tubes, 15 spatulas, mortar and pestle, and the culture medium in Erlenmeyer flask. For Kirby-Bauer test, five sterilized petri dishes and discs were used for every trial. Leeming-Notman agar was used as culture medium for the 5.0 × 103 CFU/ml of M. globosa strain. Mortar and pestle were used for pounding the 0.25 kg of UPL-Pn-2 peanut seeds. ACS grade methanol was used to isolate the extract and waited for about 15 minutes until the methanol has completely evaporated. Medicinal dropper for the preparation of concentrations was also used. A tech grade pure commercialized boric acid and dried discs treated with methanol (negative) were also used for control group.

Results and Discussion

The Kirby-Bauer test lasted for 3 weeks for 3 consecutive trials. Close supervision of the plates was observed, and sterilization was thorough to avoid contamination. The following discussions were the findings of the study.

Measurements of zones of inhibition both in experimental and control groups

After days of experimentation, result was tabulated in Table 1 both for experimental and control groups for 3 trials.

Groups	Set-Up	Trial 1	Trial 2	Trial 3	Mean
Experimental group	Set-Up 1 (25%)	1.5 mm	2.0 mm	2.0 mm	1.8 mm
	Set-Up 2 (50%)	4.5 mm	3.5 mm	3.5 mm	3.8 mm
	Set-Up 3(100%)	6.0 mm	5.5 mm	6.0 mm	5.8 mm
Control group	Set-Up 1 (25% Bor. Acid)	2.0 mm	2.0 mm	1.5 mm	3.3 mm
	Set-Up 2 (50% Bor. Acid)	5.0 mm	4.5 mm	4.0 mm	4.5 mm
	Set-Up 3 (100% Bor. Acid)	6.5 mm	6.0 mm	6.0 mm	6.2 mm
	Negative (Dried Methanol in Discs)	0.0 mm	0.0 mm	0.0 mm	0.0 mm

Table 1. Raw Data for 3 Trials. Result of 3 trials for experimental and control group in the measurements of zone of inhibition (diameter of discs were subtracted).

Methanol isolation of UPL-Pn-2 peanut seeds were found to be effective against dandruff-causing fungus, M. globosa. Further, the 100% extract of UPL-Pn-2 peanut seeds showed maximum antifungal activity (5.8 mm ± 0.29 mm) at 5.8 mm mean across all trials in experimental group. The same results were also obtained in some earlier reports on antifungal activity of different parts of peanut [19]. This antifungal property of UPL-Pn-2 peanut seed extract is attributed to the presence of boric acid which is capable of inhibiting dermatophyte growth [20]. Since the set-up of experimental group was prepared in varying doses, the effectiveness of extract decreases as concentration decreases. The same result was attained in the varying concentrations of Boric Acid (control group). Similarly, the 100% boric acid exhibited the highest antifungal activity (6.2 mm ± 0.29 mm) at 6.2 mm mean across all trials in control group (Table 1). This was due to the susceptibility of the M. globosa to the different concentrations which damaged the microbe in experimental and control groups.

The validity of the result was supported by the negative control set-up showing no inhibition in dried discs for methanol. The result indicated the inhibitory zones across varying concentrations in experimental set-ups were caused by the phytochemical without extraneous variances contaminating the data. Figure 1 shows the effectiveness of every set-up in the experimental and control group.

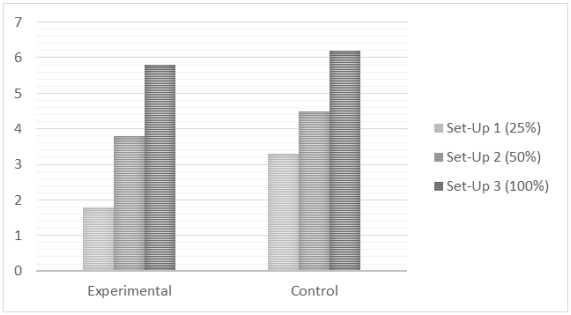

Figure 2. Mean of measurements of the zone of inhibition. The increase in the mean of measurements of zone of inhibition from 3 trials across three set-ups in experimental and control groups.

The result of Kirby-Bauer test showed that screening of UPL-Pn-2 peanut seed extract is evident as supported by the varying measurements of inhibitory zones across experimental set-ups. Though the current study presumes the active role of phytochemical boric acid in the inhibition of M. globosa, the seeds of peanut also contain other active phytochemical agents that are reported to be antifungal agents like hypogin [21]. Presence of these phytochemicals in the peanut seed extract indicated that proper screening could lead to the development of organic pharmaceutical drugs. In fact, varieties of these plants have been long used to treat conditions like, but not limited to, athlete's foot, jock itch and ringworm [22]. This result signified the antifungal efficacy of UPL-Pn-2 peanut seed extract against dandruff-causing fungus, M. globosa [23].

Significance of difference among groups and trials

ANOVA was performed to test the significance of difference of measurements of zone of inhibitions among groups of data. Table 2 shows the result of inferential test.

ANOVA	p-value* (p=0.05)	Significance	ANOVA	p-value* (p=0.05)	Significance
Comparative analysis of all set-ups in experimental group	0	Significant	Comparative analysis of all set-ups in experimental group	0	Significant
Comparative analysis of all set-ups in control group (Negative not included)	0	Significant	Comparative analysis of all set-ups in control group (Negative not included)	0	Significant

Comparative analysis of results in 3 trials	0.923	Not Significant	Comparative analysis of results in 3 trials	0.923	Not Significant

Note: *Bonferroni α correction for post-hoc analysis = 0.016.

Table 2. Comparative Analysis. ANOVA comparing the data sets in experimental, control groups, and trials.

The statistical test revealed significant difference in the measurement of zone of inhibition among set-ups in experimental group ($F(2,6)=72$, $p<0.05$). These data imply that varying concentrations of UPL-Pn-2 peanut seed extracts have different levels of effectiveness. The same result was observed from the statistical test of varying concentrations of boric acid ($F(2,6)=103.2$, $p<0.01$). Post-hoc analysis (Bonferroni correction) of experimental group revealed individual differences ($p<0.016$) in the measurements of zone of inhibitions across all set-ups. Nonetheless, the pure (100% set-up) UPL-Pn-2 peanut seed extract exhibited the maximum antifungal efficacy as shown in the mean of 3 trials (Table 1). Same post-hoc analysis result was obtained from the of control group revealing significant differences in the individual measurements of zone of inhibition in all concentrations of boric acid ($p<0.05$). The validity and reliability of these data were determined and established following the consistency of results. This was verified by the statistical no difference across the trials ($p>0.05$).

A comparative analysis of set-ups was also conducted to determine the effectiveness of varying concentrations of UPL-Pn-2 peanut seed extracts to varying concentrations of boric acid. Table 3 shows the result of the statistical test.

Experimental and Control Set-ups Comparison	p-value (p=0.05)	Significance
25%	1	Not significant
50%	0.057*	Not significant
100%	0.18	Not significant
Note: *not significant at one-tail.	0.0 mm	0.0 mm

Table 3. Comparative Analysis. Result of t-test comparing the measurements of zone of inhibitions in the set-ups of experimental and control groups.

The result of the statistical test revealed that effectiveness of the UPL-Pn-2 peanut seed extract is similar to the effectiveness of the commercialized tech grade boric acid against dandruff-causing fungus, M. globosa. This is supported by the statistical no difference ($p>0.05$) across all set-ups. These data also revealed that use of UPL-Pn-2 peanut seed extract is a good substitute to the synthetic and commercialized boric acid against M. globosa.

Recommendations for the improvement of the current study

The literature has accounts on other photochemical constituents of peanut seeds responsible for antifungal activity which might have caused extraneous variance in the result of the current study. It is therefore recommended that pure boric acid containment of UPL-Pn-2 peanut seed extract must be isolated to test its effectiveness against dandruff-causing fungus, M. globosa. Also, screening of other phytochemical constituents is needed to verify the antifungal efficacy of other UPL-Pn-2 peanut plant parts [24].

The experiment was conducted *in vitro* application. It is recommended to test the extract in vivo to determine its effect in other tissues. Therefore, toxicity levels of the UPL-Pn-2 peanut must also be considered in a future study.

Conclusion

The screening of UPL-Pn-2 peanut seed extracts across pure concentration showed maximum antifungal efficacy against dandruff-causing fungus, M. globosa. Therefore, pure concentrations of UPL-Pn-2 peanut seed extracts show promising results as organic alternative to commercialized anti-dandruff products.

Acknowledgement

The author acknowledges the help and permission of SciTeach Laboratory for allowing him to conduct the experiment.

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How to cite this article: Bibon, Michael B. "*In Vitro* Antifungal Screening of UPL-Pn-2 Peanut Seeds (*Arachis hypogaea L.*) Against Dandruff-Causing fungus, *Malassezia globosa*." *J Cosmo Trichol* 7(2021):163