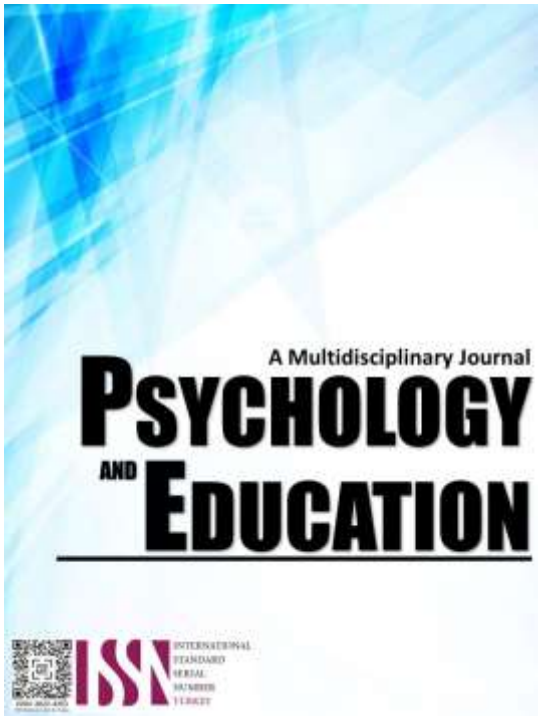
ANTI FUNGAL PROPERTY OF CALABASH, CRESCENTIA CUJETE, FRUIT IN BREAD MOLDS



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Research Article

Anti Fungal Property of Calabash, Crescentia Cujete, Fruit in Bread Molds

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Abstract

Fungal growth is the most frequent cause of spoilage in bread products. To prevent economic losses associated with spoilage, producers or the bread industry is now looking for alternatives that are plant derived sources to the currently used preservatives. This study evaluated the antifungal property of the calabash fruit *Crescentia cujete* extract at 100, 75 and 50% concentration on the growth of fungi causing bread spoilage. Complete Randomized Design was used in three petri plates containing a pure culture of bread molds that was subjected to 100, 75 and 50% concentrations of *C. cujete* extract, and acetic acid as the control employing the Kirby-Bauer Disc Diffusion technique. There was a significant difference ($P<0.05$) in the antifungal property of *C. cujete* extract (100, 75 and 50%) and the acetic acid on the growth of fungi in bread. However, there were no significant differences ($P>0.05$) between these three concentrations. The fruit extract of *C. cujete* at various concentrations and control treatments against bread molds showed weak antifungal activity. Although the fruit extract of the *C. cujete* showed weak antifungal activity, this implies that it has a potential to be developed as an antifungal agent against bread molds. Further study is necessary to determine the optimum concentration of the fruit extract that can inhibit the fungal growth in bread products.

Keywords: *anti-fungal, calabash fruit, bread molds, fungal growth, spoilage*

Introduction

Bread is considered as one of the most important staple foods worldwide and has been a major part in the diets of most populations for thousands of years. Hence, there is an increase in bread consumption with the passage of time where it has become an integral and established staple part of the diet of the populace. However, bread can be spoiled by molds (Legan, 1993). Bread mold is a kind of fungus that is commonly found on the surfaces of baked products. It takes food and nutrients from the bread and causes damage to the surface where it lives. Losses due to mold spoilage vary between 1% and 5% of bread products depending on season, type of product and method of processing (Legan 1993; Guynot *et a*l. 2002). Moreover, a shelf life of only 3-4 days in unpreserved bread may be expected especially if the level of hygiene in the preparation is not high (Bluma *et al*. 2008; Gutierrez *et al*. 2009). In order to solve the economic losses associated with spoilage, producers of the bread products are now looking for alternatives to the currently used preservatives and to the high cost of production of these synthetic compounds (Sahan 2011). Further concern is that mycotoxins produced by the molds may cause public health problems (Cauvain 2003; Dal Bello *et al*. 2007; Gutierrez *et al*. 2009). A wide range of microorganisms like bacteria, yeasts and molds can cause spoilage and food safety issues in baked products.

The use of weak organic acids such as acetic, propionic, benzoic and sorbic, modified atmosphere packaging, irradiation and pasteurization of packaged bread and biopreservation are the known methods applied to prevent or minimize microbial spoilage of bread (Cauvain 2003; Dal Bello *et al*. 2007; Gutierrez *et al*. 2009). The continuous usage of chemical preservatives for longer shelf life is widespread but these are responsible for many carcinogenic and teratogenic attributes. With growing concern of microbial resistance towards conventional preservatives, consumers tend to be apprehensive of chemical additives. At present, consumers prefer products without preservatives but microbial growth free, toxins-free and other quality worsening factors while maintaining freshness and good qualities (Nielsen and Rios 2000; Guynot *et al*. 2002; Gutierrez *et al*. 2009).

Plant derived products such as spices, and fruit and vegetable preparations or extracts have been used for the preservation and extension of the shelf life of foods for centuries (Chattopadhyay and Bhattacharyya 2007). Thus, natural derived plant products receive increasing attention (Viacava et. al 2013) as non-toxic alternatives. One of the potential plant sources is the calabash tree *Crescentia cujete* that belongs to Family Binoniacea. The tree ranges from 6 to 10 m tall with a wide crown and long branches, and gourd-like fruit. It is native to Caribbean region, Mexico, Northern and Southern American and later introduced to tropical Africa from Senegal to Cameroon then to other parts of Africa (Arbonnier 2004). This tree has also been

introduced in the Philippines. The phytochemicals like saponins, flavonoid, cardenolides, phenol and tannins have been reported to be present in the fruit of calabash. Phenol and phenolic compounds have been used in disinfection and remain the standard with which other bactericides are compared in some tests (Cater 1979). Likewise, the extracts/fractions of leaves and stem bark of *C. cujete* showed antibacterial activity against two pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli* (Parvin *et al*. 2015). Several reports have shown the anti-inflammatory, antibacterial and other medicinal benefits of the leaves, bark and fruit of *C. cujete* (Morton 1968; Mahbub *et al*. 2011; Ejelonu *et al*. 2011; Parvin *et al*. 2015; Magno *et al*. 2021). At present, studies on the antifungal property of *C. cujete* has not been explored. Hence, the present study evaluated the antifungal property of the calabash fruit extract at concentrations of 100, 75 and 50% on the growth of fungi causing the spoilage in bread.

Literature Review

Mahbub et al. (2011) studied the antibacterial activity Crescentia cujete against Shigella dysenteriae, Bacillus cereu, Bacillus subtilis, Bacillus megaterium and Staphylococcus aureus wherein the ethanolic extract showed significant results. However, the extract failed to show any effect against Salmonell yphi, Salmonella paratyphi-A, E. coli and Sarcina lutea. The study revealed that Crescentia cujete could be good sources of drugs that may be used in bacterial infection treatment if it is found effective and non toxic in animal trials.

Antibacterial activity test has been carried out from extracts of leaves, bark and fruit of (Crescentia cujete) on Vibrio alginolyticus, using disc diffusion method and dilution. The results of this research showed that the extract of fresh leaves forming the largest clear zone of 19.8 mm. While the dry leaves of 11.1 mm, fresh bark of 9.4 mm, dry bark of 9 mm, and fresh fruit of 8.8 mm. Only the extract of dried fruits that negative clear zone (Rinawati, n.d.) However, no researches found about the antifungal property of Crescentia cujete.

Methodology

Collection and Preparation of Samples

Fresh mature fruit of *C. cujete* was plucked from the tree in Midsayap, North Cotabato. After washing the fruit, it was cut open and the pulp was scooped out. The seeds were carefully removed from the pulp. The pulp (4000 g) was then blended to obtain a homogeneous mixture. Using a clean cloth, the mixture was squeezed out and strained to have a residue-free extract. The extract was used for antifungal experiment. Concentrations of 100 ml, 75 ml:25 ml and 50 ml:50 ml of the extract mixtures and distilled water were obtained. Acetic acid was as used the control. The extracts were collected and placed in separate clean containers.

Collection of Bread Molds

Five slices of fresh bread were put in a sterile and wide-mouthed glass container. The bread was moistened with 10 ml tap water. The container was left open and put in a safe place with room temperature of 29°C. After one week, mold that grew on the bread was streaked into a standard medium of potato dextrose agar (PDA) in a zigzag pattern. Aseptic technique was observed in the entire processing of the samples. The cultures were incubated at room temperature of 32°C. The samples were then observed after 24 and 48 h. To obtain a pure culture, the tip of the growing hyphae was cut off with a sterile blade and transferred to a new dish of PDA. Isolated bread molds were randomly picked and purified three times after 24 h of incubation to produce pure culture.

Evaluation of Antifungal Property Using Kirby-Bauer Disc Diffusion Method

A fungal suspension for cultured bread molds was made based on 0.5 McFarland Turbidity Standard, which is presumed to have approximately $1.5x10^8$ cells/ml in screw-capped tubes. A sterile cotton swab was dipped into the tubes containing the fungal suspension and streaked unto the surface of the properly labeled PDA plates.

The antifungal susceptibility of the cultured bread molds was tested by screening their phenotypic susceptibility to prepared calabash fruit extract. Each petri plate containing pure culture of bread molds was subjected to 100, 75 and 50 % calabash fruit extract, and acetic acid as control by using the Kirby-Bauer Disc Diffusion technique. Acetic acid is the commonly used preservative in bread and is the by-product of a fermentation, hence, its pH level can be compared with the *C. cujete* concentrations. The standard treated paper disks with fruit extract were aseptically placed at a 26 mm distance from each other. There were three replicates for each concentration using a completely

randomized design. The plates were then incubated for 24 h at room temperature of 32°C. The diameter of the zone of inhibition produced in each treated disc was measured and recorded based on the standard Zone Diameter Interpretation Chart. The sensitivities of the molds to the plant extracts were determined by measuring the sizes of inhibitory zones on the agar surface around the disks. The following interpretative range of standard zone was adopted from Ontengco (1992) cited by Segismundo et.al 2008.

Results

Table 1. Antifungal activity of calabash fruit *Crescentia cujete* extract against bread mold. Means are from 3 replicates of each treatment. Mean diameter of inhibition (± SE) with the same letter superscripts are not significantly different (P>0.05). Antifungal activity: Strong (+++), Moderate (++), Weak (+), Negative (-)

Treatment	*Mean Diameter of Inhibition (mm)*	*Antifungal Activity*
% calabash fruit extract		
50	7.3 ± 0.83^{a}	+
75	7.9 ± 0.15^{a}	+
100	8.3 ± 0.06^{a}	+
Acetic acid	12.1 ± 8.2^{b}	+

Table 2. *Tukey HSD computation of different treatments*

(I) Treatment (% Calabash Fruit Extract)	*(J) Treatment*	*Mean Difference (I-J)*	*Std. Error*	*Sig.*
50	75% calabash	-.55556	.67928	.846
	100%calabash	-1.00000	.67928	.466
	Acetic acid	-3.88889*	.67928	.000
75	50% calabash	.55556	.67928	.846
	100% calabash	-.44444	.67928	.913
	Acetic acid	-3.33333*	.67928	.000
100	50%calabash	1.00000	.67928	.466
	75%calabash	.44444	.67928	.913
	Acetic acid	-2.88889*	.67928	.001
Acetic acid	50% calabash	3.88889*a	.67928	.000
	75% calabash	3.33333*b	.67928	.000
	100%calabash	2.88889*c	.67928	.001

Table 3. *ANOVA showing the significant differences of the antifungal activity of different treatments*

Inhibition	*Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Between Groups	81.194	3	27.065	13.035	0.000
Within Groups	66.444	32	2.076		
Total	147.639	35			

Discussion

The fruit extract of *C. cujete* at various concentrations of 100, 75 and 50%, and control treatments against bread molds showed weak antifungal activity (Table 1). The zone of inhibition showing the antifungal activity had diameters of 8.3, 7.9, and 7.3 mm at 100, 75 and 50% concentration, respectively and 11.2 mm for acetic acid. (Fig. 1). Based on the Tukey HSD, there was significant difference (P<0.05) on the antifungal effect of the calabash extract at different concentrations of 100, 75 and 50% against the acetic acid, which is the commonly used biopreservative for bread (Table 2). This has the same findings with Legaspi *et. al* (2020) and Magno *et. al* (2021) that Calabash extracts possess high antibacterial effects against *E. coli* and exhibited better antimicrobial activity compared to chloramphenicol. However, there were no significant differences (P>0.05) among the three concentrations of the fruit extract when subjected to ANOVA (Table 3). The f -value of 13.035 is higher than the tabular value of 5.14 at 0.05 level of

significance with degrees of freedom (3, 32) = 5.14, hence, there are significant differences on the antifungal effect of the calabash extract in different concentrations 100, 75 and 50 % to acetic acid. Although the calabash fruit extract showed weak antifungal activity, this implies that it has a potential to be developed as an antifungal agent against bread molds. This agrees to the findings of Legaspi *et. al* (2020) that Calabash is a promising antimicrobial potential against two common pathogens: *S. aureus and E. coli.* Further, extracts/fractions of leaves and stem bark of *C. cujete* showed antibacterial activity against two pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli* (Parvin *et al*. 2015). The plant has rich of secondary metabolites that have several antimicrobial activities: saponin, polyphenol, tannin, alkaloid and flavonoid (Cater 1979, Cushine *et. al 2* 005). The extract may require a higher concentration to show a strong antifungal activity.

The bread mold observed in this study were grown in spore that grows like a brush-like structure and its color is white with black or grey. There are various types of molds and some of the common bread molds include *Penicillium*, *Aspergillus*, *Rhizopus*, *Monascus*, and *Fusarium* (Durnovtseva, 2017). However, the type of bread mold in this study was not identified.

The phytochemicals found in the calabash fruit include tannins and phenols, saponins, alkaloids, flavonoid, anthraquinone, and cardenolides (Ejelonu *et al.* 2011). Phenols and phenolic compounds are used as disinfectant and bactericides in emollient healing and burns (Arbonnier 2004). Tanins have astringent properties. Saponins can be used as natural antibiotics, and can boost energy (Lipkin, 1995 cited by Billacura *et. al* 2017). These are also useful in reducing inflammation of the upper respiratory passage (Cankaya *et al.* 2021). Likewise, various minerals have been found in the fruit extract (Ejelonu *et al.* 2011). The protein content of the fruit is 8.38%. There are many more good properties of the chemical components found in calabash fruit. The incorporation of the fruit extract in the bread products is an advantage. However, its strong antifungal property should be established. Other parts of the fruits may also be tried for its antifungal property.

Further study is necessary to determine the optimum concentration of the fruit extract that can inhibit the fungal growth in bread. The active components with potential for antifungal use or bio preservatives in the fruit should be determined. Other parts of the calabash tree such as the extracts of leaves and barks may also be worth testing for antifungal activity against bread molds.

Conclusion

The phytochemicals found in the calabash fruit include tannins and phenols, saponins, alkaloids, flavonoid, anthraquinone, and cardenolides (Ejelonu et al. 2011). Phenols and phenolic compounds are used as disinfectant and bactericides in emollient healing and burns (Arbonnier 2004; Burkill 1985; Morton 1981). Tanins have astringent properties. Saponins can be used as natural antibiotics, and can boost energy (Lipkin, 1995 cited by Billacura et. al 2017). These are also useful in reducing inflammation of the upper respiratory passage (Cankaya et. al 2021). Likewise, various minerals have been found in the fruit extract (Ejelonu et al. 2011). The protein content of the fruit is 8.38%. There are many more good properties of the chemical components found in calabash fruit. The incorporation of the fruit extract in the bread products is an advantage. However, its strong antifungal property should be established. Other parts of the fruits may also be tried for its antifungal property.

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