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Synergistic Antifungal Analysis of *Carica papaya* Leaf and *Solanum melongena* Peel Ethanolic Extracts Against *Candida albicans*

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ABSTRACT

Infectious diseases are responsible for most mortalities and morbidities globally. They are estimated to cause 17 million deaths annually. *Candida albicans* is a microflora found in mucous membranes of the gastrointestinal tract and vaginal orifice. Though a commensal organism, it can cause either oropharyngeal or vaginal candidiasis in immunocompromised states. It thus, is a common opportunistic infection especially in patients living with HIV/AIDS. Management of candidiasis requires use of antifungal agents but some are costly or drug-resistant. Therefore, there is need for exploration of novel and affordable treatment modalities. Medicinal plants offer alternative therapeutic modalities. Against this backdrop, we sought to evaluate synergistic antifungal activity of ethanolic extract of *Carica papaya* leaves and *Solanum melongena* fruit peel against *Candida albicans*. Ethical approval was sought from School of pharmacy, Kabarak University Research Ethics Committee (KUREC) and National commission for science, technology, and innovation (NACOSTI). Experimental research design was employed. Green leaves of *Carica papaya* were obtained from the school's herbarium while the eggplant fruit was purchased from Nakuru town market. The sample plants were dried and powdered. Phytochemical analysis was carried out and antifungal activity evaluated via disk diffusion method. Phytochemical studies showed presence of tannins, alkaloids and flavonoids in both plants. Anthraquinone glycosides were absent in both samples. *Carica papaya* and *Solanum melongena* ethanolic extracts respectively showed activity against *Candida albicans* at different concentrations with zones of inhibition of 8.34mm and 7.98mm, respectively, at the highest concentrations. *S. melongena* extracts showed a lower zone of inhibition against *Candida albicans* compared to *C. papaya* extracts. Combined ethanolic extracts of *Solanum melongena* and *Carica papaya* showed higher activity at all concentrations than individual extracts, implying synergism between the two plant extracts. The difference in zones of inhibition was significant at different concentrations($P<0.05$). The study findings have demonstrated that *Carica papaya* and *Solanum melongena* extracts have remarkable antifungal activity against *Candida albicans.* This makes them potential efficacious antifungal agents against *Candida albicans*.

Keywords: ***Candida albicans, Carica papaya, Solanum melongena, Phytochemicals and Zones of inhibition.***

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I. INTRODUCTION

Infectious diseases constitute a major public health concern estimated to cause about 50,000 daily fatalities (Arum, 2017). From a historical perspective, the trend in infectious diseases makes the future worrisome. Prior to the world wars, inadequacy of effective antimicrobial agents made treatment options challenging. Most antimicrobial agents emerged during the world wars to facilitate treatment of soldiers from secondary infections. Since then, more potent and efficacious agents have been discovered. However, resistance to these agents has emerged and is continually on the rise. This is mainly attributed to irrational use of such medicines.

Fungi are a group of microorganisms that though mostly harmless, are associated with serious infections

among immunocompromised patients. In particular, HIV/AIDS patients have the highest risk burden. Among the opportunistic fungal infections, *Candida albicans* is a commonly encountered pathogen. It causes oropharyngeal and vaginal candidiasis (Santos et al., 2018). Candida is a commensal organism found on mucosal surfaces of vagina, throat, mouth and gut. Infections result from altered microenvironment in immunocompetent patients and also from weakened immune systems among immunocompromised patients. Globally, approximately 2 million cases are reported annually and a rise in numbers is being seen in low- and middle-income countries (Bongomin et al., 2017; Mushi et al., 2017). Coupled with emerging resistance to conventional remedies, secondary candidiasis treatment becomes not only a challenge but also makes palliative care for the immunocompromised patients a burden. Moreover, the cost of treatment is higher as price-controlled antifungals are less commonly used. Against this backdrop, we sought to evaluate alternative modalities for treatment, in this case, use of plant extracts.

Plants have been documented to have phytochemical compounds with antimicrobial properties. *Carica papaya* is a common nutritional plant with various medicinal properties (antidiabetic, antimicrobial, contraception use) attributed to phytochemicals such as glycosides, flavonoids, alkaloids, saponins and triterpenes (shubham et al., 2019). *Solanum melongena* (aubergine/eggplant) is a food additive used as a spice; it has been shown to contain flavonoids, alkaloids, tannins, proteins and saponins as major phytochemicals (Tiwari et al., 2009). In this study, we sought to evaluate antifungal activity of ethanolic extracts of *Carica papaya* leaves and eggplant fruit peels using the disk diffusion method. Since these plants are commonly encountered, their concoctions may serve as potential alternative treatment options for oropharyngeal candidiasis.

II. METHODS

Experimental design was employed for this research (Librarians, 2006). The study was carried out at the pharmacognosy and pharmaceutical microbiology laboratories, school of pharmacy, in Kabarak University.

A. Sample preparation

Green leaves of pawpaw were collected from a single tree within the Kabarak University School of Pharmacy herbarium. Purple eggplant fruits were purchased from Nakuru town market. Both the samples were taken to the pharmacognosy lab and authenticated by the school's botanist. Samples were washed thoroughly to remove debris and dust. Green pawpaw leaves were dried at room temperature on the laboratory's bench for four days and powdered afterwards using mortar and pestle. The eggplant fruit was peeled and peels dried for six days in a locked cabinet to prevent bioactive degradation and afterwards powdered using a mortar and pestle.

B. Extraction

Soxhlet extraction method was used to extract active constituents (Redfern et al., 2014). Fifty grams of each sample was separately weighed after sieving their respective powders. The masses were then mixed each with 500ml of ethanol within the thimble and heated at 50^{o}C. Sample extracts left in the thimble after all the solvent had evaporated were collected separately and transferred into two clean weighing beaker and dried in an oven at 45^{o}c. percentage yield was then calculated.

C. Phytochemical analysis

Alkaloid: 0.590g of *C. papaya* powdered leaves and 0.512g of S. *melongena* powdered peels were separately analyzed for alkaloids using Mayer's, Dragendroff's and Wagner tests.

Flavonoids: 0.520g of *C. papaya* powdered leaves and 0.534g of S. *melongena* powdered peels were separately analyzed for flavonoids using Shinoda and ammonium fumes tests

Phenols: 0.562g of *C. papaya* powdered leaves and 0.550g of S. *melongena* powdered peels were separately analyzed for phenols using ferric chloride and lead acetate tests.

Tannins: 0.519g of *C. papaya* powdered leaves and 0.506g of S. *melongena* powdered peels were separately analyzed for tannins using ferric chloride and lead acetate tests.

Saponins: 0.531g of *C. papaya* powdered leaves and 0.533g of S. *melongena* powdered peels were separately analyzed for saponins using the foam test (Vrunda, 2008).

Anthraquinones: 0.525g of *C. papaya* powdered leaves and 0.535g of S. *melongena* powdered peels were separately analyzed for anthraquinones using Bontrager's and Modified Bontrager's tests.

Terpenoids: 0.519g of *C. papaya* powdered leaves and 0.505g of S. *melongena* powdered peels were separately analyzed for terpenoids using Libbermann's and Salkowski tests.

D. Disk diffusion analysis

Disks prepared by punching filter papers using a sterile paper punch were sterilized by autoclaving at 121^{o}C for 15 minutes and dried in the oven. Four concentrations for each sample were prepared: 2 g/ml,1 g/ml,0.5 g/ml and 0.25 g/ml. For each concentration, four sterilized disks were impregnated. Negative and positive controls were made by impregnating two disks with 99% DMSO and 200µl nystatin respectively. All disks were incubated at 20 ^{o}C for 24hours.

Isolates of *Candida albicans* were obtained from Kenya medical research institute via an authorized personell and kept at 4 ^{o}C. The isolates were culture using SDA medium at 37 ^{o}C for 24-48hours. Chloramphenicol was added to the Petri dish to prevent bacterial growth. A few colonies from the culture were transferred using a sterile wire loop to a boiling tube having 0.9% normal saline. The boiling tube was incubated at 30 ^{o}C and process repeated till it achieved 0.5 Mac Farland standard turbidity. Colonies from the broth were then cultured in newer SDA growth media. Using a pair of forceps, three discs (negative, positive control and sample disc) were placed on each plate sufficiently distant from each other and incubated at 37 ^{o}C for 24hours. Tests were repeated three times and average diameter of zone of inhibition for each concentration calculated. In addition, the fourth disk comprising of a mixture of the sample at the different concentrations was prepared and evaluated.

Approval for data collection was sought from the pharmacy school. Ethical approval was acquired from Kabarak University Research Ethics Committee (KUREC-030722). Permission to conduct research was obtained from National Commission for Science, Technology, and Innovation (NACOSTI/P/22/19313).

III. RESULTS

Yield of Extract

Table 1

Percentage Yield of Plant Extract

Sample extract	Percentage yield
C. papaya	9.11%
S. melongena	12.93%

Qualitative Phytochemical Analysis of Carica Papaya Leaves and Solanum Melongena Peel Extracts

Table 2

Phytochemical Constituent Analysis

Phytochemical compound	Tests	C. papaya leaves	S. melongena peels
Alkaloids	Dragendroff's test	+++	+++
	Mayer's test	-	++
	Wagner's test	++	+
Saponins	Froth test	-	++
Tannins	Ferric chloride test	+++	++
	Lead acetate test	++	++
Phenols	Ferric chloride test	-	++
	Lead acetate test	++	+
Anthraquinone glycosides	Borntrager's test	-	-
	Modified Borntrager's test	+	-
Terpenoids	Libbermann's test	-	++
	Salkowski test	-	++
Flavonoids	Ammonium fumes test	+++	++
	Shinoda test	+++	+++

Antifungal Activity of Solanum melongena Peel and Carica Papaya Leaves Ethanolic Extracts Against Candida Albicans

According to results in **table 3**, *Solanum melongena* ethanolic peel extracts and *Carica papaya* ethanolic leaf extracts showed antifungal activity at all concentrations against *Candida albicans*. At 2g/ml average zone of inhibition was 7.98mm (*Solanum melongena*) and 8.34mm (*Carica papaya*) while at 0.25g/ml average zone of inhibition was 5.48mm (*Solanum melongena*) and 5.84mm (*Carica papaya*). Significant difference in the zones of inhibition for both *Solanum melongena* ethanolic peel extracts and *Carica papaya* ethanolic leaf extracts at different concentrations were noted; (P=0.0005, df=5, F=4.3) and (P=0.0003, df=3, F=4.0) respectively.

Table 3

Activity of S. melongena Peels and C. papaya Leaves Ethanolic Extracts Against Candida albicans

Concentration (g/ml)	Zone of inhibition (mm)							
	C. papaya leaves				*S. melongena peels*			
	1	2	3	Avg	1	2	3	Avg
2	8.41	8.39	8.22	**8.34**	7.79	8.09	8.06	**7.98**
1	7.42	6.70	7.90	**7.34**	6.91	6.96	7.07	**6.98**
0.5	6.68	6.85	6.81	**6.78**	6.50	6.47	6.29	**6.42**
0.25	5.85	5.77	5.89	**5.84**	5.69	5.59	5.18	**5.48**

Synergistic Antifungal Activity of *Solanum melongena* and *Carica papaya* Ethanolic Peel and Leaves Against *Candida Albicans*

According to results in **table 4**, *Carica papaya* leaf extracts and *Solanum melongena* peel ethanolic extracts were combined to establish a synergism antifungal effect. They showed a higher zone of inhibition against *Candida albicans* than the individual extracts at different concentrations. At 2g/ml they showed average zone of inhibition of 10.73mm while at 0.25g/ml they showed an average zone of inhibition of 7.14mm. Findings showed no significant difference in zones of inhibition at different concentrations (P=0.0016, F=4.06, df=3).

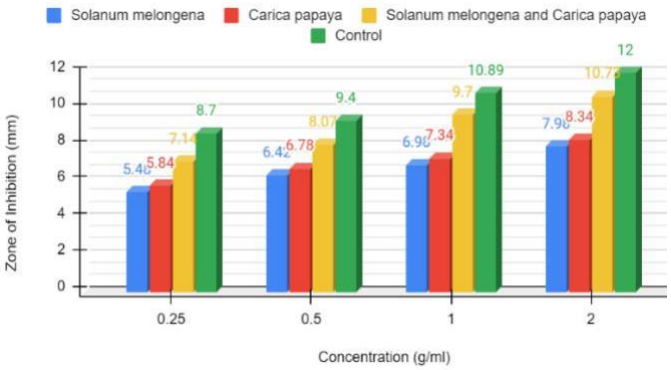
Table 4

Activity of Combined *Solanum melongena* Peel and *Carica papaya* Leaf Ethanolic Extracts Against *Candida albicans*

Concentration (g/ml)	Zones of inhibition (mm)			
	1	2	3	Average
2	11.05	10.52	10.63	**10.73**
1	9.12	9.68	10.31	**9.70**
0.5	8.27	8.19	7.75	**8.07**
0.25	7.01	7.54	6.88	**7.14**

Figure 1

Comparison of Average Antifungal Activity of Carica papaya, Solanum melongena, Combined Extracts, and Nystatin at Different Concentrations Against Candida albicans



IV. DISCUSSION

The main factor that enables plants to possess medicinal properties is presence of phytochemical compounds. Though the primary function of such compounds is to ensure plant survival (in terms of evading pathogens and herbivores, impacting colour for pollination and to cope with environmental stress), their structural orientation enables them to interact with biomolecules thus their therapeutic applications (Nilofer et al., 2020). Phytochemical analysis as presented in table 2 shows C. papaya leaves had highest concentrations of alkaloids, flavonoids and tannins. Only alkaloids and flavonoids were in highest concentration in the peels of S. melongena with the rest of phytochemicals being in moderate concentration. Peels of S. melongena had more phytochemical compounds at moderate to high concentration compared to leaves of C. papaya. Notably, C. papaya leaves were negative for saponins which contrasts the study done by Adeyeye (2010) that concluded C. papaya leaves have saponins. In addition, phenols were shown to be present by the lead acetate test only unlike study done by Swati et al. (2018) that reported presence of phenolic compounds by both lead acetate and ferric chloride test. While this analysis reported absence of anthraquinone glycosides in the peels of S. melongena, study by Edeke et al. (2021) showed the opposite. It has been shown that various environmental factors influence concentration of phytochemicals compounds in a plant during a particular growth or developmental stage. Thus, from an analytical point of view, the differences being noted may be attributed such factors like climatic conditions the plant was grown, developmental stage of the plant, time of harvesting among others.

The antifungal activity of both plant species was noted to increase with increase in concentration of extract as shown in table 3. Notably, the antifungal activity of C. papaya was higher than that of S. melongena at each concentration as demonstrated by the difference in values of the average zone of inhibitions. This demonstrates that C. papaya leaves are more efficacious than S. melongena peels. Evidently, while peels of S. melongena had more phytochemical compounds at moderate to high concentration compared to leaves of C. papaya, it is the latter that showed more efficacy. This indicates that the antifungal activity is not depended on presence of different phytochemical compounds rather on which specific class is present. C. papaya leaves had more tannins and flavonoids in general than S. melongena peels. These two phytochemical compounds have been shown to disrupt cell membrane formation and integrity leading to cytolysis in a similar manner as azole antifungals and amphotericin B respectively (Ami et al., 2021, Cyuzuzo et al., 2020).

Discs that were impregnated with both sample plant extracts showed a higher antifungal activity than

individual sample extracts at each concentration but was less than the combined added activity of both sample extracts. This suggest that use of both sample extracts exhibits synergistic antifungal action which may be beneficial in reducing side effects due to additive effect. The average zone of inhibition for the combined extracts was however below that of nystatin (positive control). Moreover, the synergistic activity increases with increase in concentration of the extracts thus at higher concentrations, it might be suggested that combining the plant extracts exhibits more potent activity than nystatin.

V. CONCLUSION

i. Both *Carica papaya and Solanum melongena* leaf and peel extracts respectively contain phenols, tannins, flavonoids and alkaloids. Anthraquinone glycosides were absent in both plant extracts.

ii. Both *Carica papaya* and *Solanum melongena* leaf and peel ethanolic extracts showed antifungal activity against *Candida albicans* at different concentrations.

iii. Antifungal activity of Carica papaya was however higher than that of Solanum melongena at each concentration

iv. Combined ethanolic extracts of *Solanum melongena* peels and *Carica papaya* leaf showed a higher antifungal activity against *Candida albicans* implying increased sensitivity of the test organism to combined extracts than individual extracts.

VI. RECOMMENDATIONS

Based on the conclusions, we therefore recommend a standardized concoction (cream or mouth paste) of the two plant extracts can be prepared at concentrations slightly above 2g/ml to be used for managing oropharyngeal candidiasis.

VII. CONFLICT OF INTEREST

Authors declare no conflict of interest.

VIII. REFERENCES

Adeyeye, A. (2010). Phytochemical and nutrient evaluation of carica papaya (pawpaw) leaves. *International Journal of Research and Reviews in Applied Sciences*, *5*, 325–328.

Ami, F., Shelli, F., Yusqadriani, Y., & Vivien, K. (2021). *Antifungal Effects of Solanum Melongena L Peel Extract Against Candida Albicans: In Vitro Study*.1(1), 5–6. https://www.greennetwork-events.com/all_images/file_690.pdf

ARUM, S. F. (2017). *Phytochemical analysis and antimicrobial activity of Carica papaya l. Var. Wainamalo extracts against selected microorganisms in Maseno, Kenya*.1(1),1-6. https://repository.maseno.ac.ke/bitstream/handle/123456789/3659/THESIS.pdf?sequence=1&isAllowed=y

Cyuzuzo, C., Nsanzimana, B., & Henry, A. (2020). *Phytochemical Screening and Antimicrobial Activities of Methanolic and Aqueous Leaf Extracts of Carica papaya Grown in Rwanda*.4(1),1–6. https://doi.org/10.21705/mcbs.v4i1.74

de Oliveira Santos, G. C., Vasconcelos, C. C., Lopes, A. J. O., de Sousa Cartágenes, M. do S., Filho, A. K. D. B., do Nascimento, F. R. F., Ramos, R. M., Pires, E. R. R. B., de Andrade, M. S., Rocha, F. M. G., & de Andrade Monteiro, C. (2018). Candida Infections and Therapeutic Strategies: Mechanisms of Action for Traditional and Alternative Agents. *Frontiers in Microbiology*, *9*, 1351. https://doi.org/10.3389/fmicb.2018.01351

Edeke, A., Uchendu, N., Omeje, K., Odiba, A. S. (2021). Nutritional and Pharmacological Potentials of Solanum melongena and Solanum aethiopicum Fruits. *The Journal of Phytopharmacology*, *10*(1), 61–67. https://doi.org/10.31254/phyto.2021.10112

Librarians, R. (2006). Research Guides: Organizing Academic Research Papers*: Types of Research Designs*. https://library.sacredheart.edu/c.php?g=29803&p=185902

Mushi, M. F., Bader, O., Taverne-Ghadwal, L., Bii, C., Groß, U., & Mshana, S. E. (2017). Oral candidiasis among African human immunodeficiency virus-infected individuals: 10 years of systematic review and meta-analysis from sub-Saharan Africa. *Journal of Oral Microbiology*, *9*(1), 1317579. https://doi.org/10.1080/20002297.2017.1317579

Nilofer, N., & Chenthamarai, G. (2020). Anti-fungal activity of Carica papaya leaf extract against candida albicans and its synergy with fluconazole*: An in-vitro study*. *Vol 10(1)*, 3–4. https://dx.doi.org/10.18203/2319-2003.ijbcp20205547

Olum, R., Baluku, J. B., Okidi, R., Andia-Biraro, I., & Bongomin, F. (2020). Prevalence of HIV-associated esophageal candidiasis in sub-Saharan Africa: A systematic review and meta-analysis. *Tropical Medicine and Health*, *48*(1), 82. https://doi.org/10.1186/s41182-020-00268-x

Redfern, J., Kinninmonth, M., Burdass, D., & Verran, J. (2014). Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties†. *Journal of Microbiology & Biology Education*, *15*(1), 45–46. https://doi.org/10.1128/jmbe.v15i1.656

Shubham, S., Shubham, S., Mishra, R., Gautam, N., Nepal, M., Kashyap, N., & Dutta, K. (2019). *Phytochemical Analysis of Papaya Leaf Extract: Screening Test*.6(7),350 https://www.semanticscholar.org/paper/Phytochemical-Analysis-of-Papaya-Leaf-Extract%3A-Test-Shubham-Mishra/6c9a20a92f22d646a36c5d4548c80fddcff608e8

Swati, K., & Shivam, P. (2018). Total phenolics & flavonoid content of the leaves of carica papaya & syzygium cumini. *World Journal of Pharmaceutical Research*.7(14),5-6.https://doi : 10.20959/wjpr201814-12858

Tiwari, A., Jadon, R., Tiwari, P., & Nayak, S. (2009). Phytochemical Investigations of Crown of Solanum melongena fruit. *Phytomedicine*, *1*, 9–11. https://doi.org/10.5138/ijpm.2009.0975.0185.05787

Vrunda, S., & K.R, K. (2008). Practical Pharmacognosy. *Scientfic research journal*1(1),149-153.https://www.scribd.com/document/276643293/Practical-Pharmacognosy