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Original Research Article

Allium cepa and Beta Vulgaris Extracts and Their Synergistic Activity with Antifungal against *Candida albicans*

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Abstract: ***Background*:** Due to their high mineral and vitamin content, beetroot and onions, which are frequently utilized as antibiotics, antioxidants, and nutrients, are generating growing interest in their potential medical applications. ***Objective*:** The current study's is to separate the active components from beetroot and onions and use them as antimicrobial agents. ***Materials and Methods***: A mixture of beetroot and onion in varying ratios, as well as fungal and bacterial cultures, were created for testing. Various procedures, including hot water, were utilized to extract the active components. ***Results*:** According to the findings, hot alcohol was used to extract the components that were the most active. Depending on the concentration of the active ingredients in the extract solution, the extracts exhibit varying bioactivities. Conclusions: Extracts of beetroot and onions can be used to treat Candida albicans. ***Conclusions*:** The antifungals nystatin, miconazol, and clotremazol as well as beetroot and onion demonstrated the best efficacy against Candida albicans at 95/5, 50/50, and 75/25, respectively.

Keywords: C. albicans, water extraction, beetroot and onion.

1. INTRODUCTION

A range of edible taproots developed in the Middle East are included in beetroot, an annual or biennial cultivar of Beta vulgaris subsp. vulgaris conditiva that has spread throughout the world from the Americas to Europe and Asia [1, 2]. It is thought to possess health-promoting qualities, anti-oxidant and anti-inflammatory effects, anti-carcinogenic and anti-diabetic activities, hepatoprotective, hypotensive, and wound healing properties as a rich and nutritional source [3-5]. It is noteworthy that most recent studies on beetroot supplementation, especially those addressing its hypotensive and ergogenic properties, emphasized the critical role of inorganic NO3 on the clinical effect of this vegetable and its byproducts. As a result, beetroot is currently being applied as a functional ingredient in the development of various meals [6-8]. Traditional herbal remedies for a variety of diseases have included onions and plants from the Allium genus because of their links to numerous pharmacological effects [9].

Commonly, thiosulfinates, a volatile sulfur-containing molecule that gives onions their distinctive flavor, scent, and lachrymatory properties, have been blamed for the biological impacts of onions [10]. When tissues are damaged, the enzyme alliinase is released from cell vacuoles, and this result in the formation of these chemicals from precursors to cysteine sulfoxide [11].

Albicans Candida

At some point in their lives, over 70% of people and about 75% of women have the innocuous commensal fungus Candida albicans living in their gastrointestinal and genitourinary tracts [12]. It turns into an opportunistic pathogen in patients with immune system problems, some people who have weak immune systems, and called candidiasis. Based on the severity of the illness, there are two kinds of candidiasis.

The most well-known mucosal infection in the first group is thrush, which is identified by white patches on infected membranes. The most frequent infections are those that affect the oropharyngeal, vaginal, or gastrointestinal epithelial cells. In addition, Vulvo Vaginal Candidiasis (VVC) is quite common in women, and some of them experience recurrent VVC (RVC) (RVVC). On the other hand, it has a high fatality rate of roughly 30% and produces life-threatening, systemic infections in very unwell individuals. Systemic Candida infections can occur in HIV-positive individuals, transplant recipients, chemotherapy patients, and low-birth-weight infants [13].

The objectives of this study are to:

(1) Identify the total active ingredient in onions and beets and the antioxidant capacity of crude onion extracts;
(2) Assess the efficacy of onions and beets against candida; and
(3) Examine the synergistic effects of onions and beets.

2. MATERIALS AND METHODS

Preparing of Plant Extracts

Three extracts of Beetroot & onion are prepared as fallowing:

100 grams of beetroot and onion powder were combined with 500 milliliters of sterile, deionized water to create Hot Water Extract. The mixture was then placed in a saxolith device for 24 hours, filtered twice—once through gauze and once through the filter unit (Whatman1)—and collected in appropriate glass containers. When it is dry and ready for use, it is placed in the incubator at a temperature of 40 to 45 degrees Celsius.

Candida Suspension Activation and Preparation

A few colonies were selected by loop and placed in tubes containing Muller-Hinson broth to activate the fungus. The tubes were then cultured for 18 hours to create the suspension where it is preferable that the fungal number be near to (1.5 x 108) cells/ml for each 1 ml of the suspension.

The agar well diffusion method was used to grow the isolates for our study. (12)A well of 10 mm was then created after spreading 0.1 ml of the fungal suspension with 108 cells per ml across the surface of the culture medium. Then, equal amounts of various plant extract concentrations were added to these wells in the culture medium, then left "until the extract was absorbed" for 15 minutes. The inhibitory zone was then measured in mm by Vernia after being incubated at 37 °C for 24 hours.

Qualitative Analysis of Some Bioactive Components of Plant Extracts

Glycosides

A test tube containing 1 ml of the plant extract is mixed with 2 ml of Benedict's reagent, agitated thoroughly, and placed in a pot of boiling water for 5 minutes. After the tube has cooled, a crimson residue is visible, which indicates the presence of glycosides [14, 15].

Tannins

When a few drops of a 1% FeCl3 solution are added to a test tube containing 0.5 ml of the extract, a bluish green hue develops as proof that tannins are present [14, 15].

Phenols

The ferric chloride (Ferric Chloride) solution, which is made by dissolving ferric chloride salt in distilled water at a ratio of (1%), was used to detect it, when this reagent is combined with the extract in the watch glass that contains the phenolic compounds, a green or blue color result [16].

Flavonoids

Equal parts of the solution and the plant extract are combined to create the detection solution, which is made by mixing 10 ml of a potassium hydroxide (KOH) solution at a concentration of 50% with 10 ml of ethyl alcohol at a concentration of 50%. The presence of the yellow color indicates the presence of flavonoids [16, 17].

Saponin

The test is performed by following the two methods below: [18]

A- An aqueous solution of plant powder is prepared in the study and separately. The solution was placed in a test tube and was shaken very strongly. The appearance of thick and persistent foam for a long time was evidence of the presence of soap.
B- (1-3) ml of mercuric chloride solution (HgCl2) at a concentration of 1% is added to 5 ml of the plant extract, and a white precipitate appeared as evidence of positive detection.

Coumarins

According to what was stated in, coumarin was discovered [18]. For each of the aforementioned extracts, a small amount of plant extract was added to test tubes. After covering the tubes with filter papers that had been moistened with diluted sodium hydroxide (NaOH) solution, the tubes were submerged in a pot of boiling water for a short period of time, and the filter papers were then exposed to an ultraviolet light source. The presence of coumarin is indicated by the presence of a vivid greenish-yellow tint.

Resins

Twenty ml of distilled water that had been acidified with hydrochloric acid (4% HCl) were added to ten ml of each extract. Turbidity appeared which suggested the presence of resins.

Alkaloids

There are two ways to detect something: Take one mL of the extract and a few drops of Marquis Reagent to get started. Alkaloids were present, as evidenced by the hue changing to an abrasive gray color.

Antimicrobial activity

Twenty ml of distilled water that had been acidified with hydrochloric acid (4% HCl) were added to ten ml of each extract. Turbidity appeared, which suggested the presence of resins.

Microorganisms

The antibacterial activity of the extracts was tested against Candida albicans, Klebsiella spp., Staphylococcus aureus, and Escherichia coli through the inhibitory zone technique.

Analysis of Beetroot & Onion Powder's Synergistic Effect with Anti-Fungals against C. Albicans

In order to ensure proper dissolution, 0.01 g of each of the three anti-fungals (nystatin) used in this study is dissolved in 10 ml of distilled water (pH: 6). Following the successful dissolution, we create a number of dilutions, which are as follows:

50µl of the beetroot and onion powder extract and 50µl of the anti-fungals are taken for the first dilution and added to culture media, and on culture media, 75µl of beetroot and onion powder extracts and 25µl of an anti-fungal are diluted.

In order to ensure proper dissolution, 0.01 g of each of the three anti-fungals (nystatin) used in this study is dissolved in 10 ml of distilled water (pH: 6). Following the successful dissolution, we create a number of dilutions, which are as follows:

50 ul of the beetroot and onion powder extract and 50 ul of the anti-fungals are taken for the first dilution and added to culture media, and on culture media, 75 ul of beetroot and onion powder extracts and 25µl of an anti-fungal are diluted.

Antifungal drugs have been utilized (nystatin) It operated as follows:

a. In 10 ml of distilled water, 0.1 gram of dried samples of beetroot and onion (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a transparent, polystyrene tube measuring 12 mm by 75 mm. The DensiChek turbidity meter is used to adjust turbidity. For Gram-positive bacteria, the McFarland turbidity ranges are 0.50 to 0.63. Inoculation: Using an integrated vacuum system, bacterial suspensions are inoculated onto reagent cards. The bacterial suspension-containing can tube is put into a specific rack (cassette) and the transfer tube is inserted into the matching suspension tube while the reagent card is positioned in the next slot. A vacuum chamber station receives the loaded cassette. The organism suspension is driven via the transfer tube into micro-channels that fill all of the test wells once the vacuum is removed and air is reintroduced into the station.
b. Card sealing and incubation: Before being loaded into the incubator, inoculated cards are passed via a mechanism that seals them and cuts off the transfer tube. At 35.5 (1.0) oC, all card kinds are incubated. Once every 15 minutes, each card is taken out of the incubator, brought to the optical system for reaction readings, and then put back until the next read time. Within 4 to 8 hours, the entire analyzed set's results were acquired.

Ruch *et al.*, (1989) 11 employed the DPPH method to estimate the free radical scavenging activity. Using ascorbic acid as the reference, a stock solution (10 mg/Ml) of the extract was created and diluted to a final concentration of (100-400) g/Ml. 1,1 difenyl-2-picryl-hydrazyl (DPPH) was used to assess the antioxidant activity of the extract. To 2.5 Ml of the sample in various concentrations, 1 Ml of 0.3 Mm DPPH in methanol was added. The sample was then left to stand in the dark for 30 min at room temperature. The absorbance of all the samples was measured at 517 nm against methanol as a blank and the inhibitory effect of DPPH was calculated according to the following equation:

% of free radical scavenging activity = $[(Ac - As)/Ac] \times 100$

$$\%\ of\ free\ Radical\ Scaving\ Activity = \frac{(Ac - As)}{Ac}\ X\ 100$$

Where: Ac = Absorbance of control and As = Absorbance in presence of extract

RESULTS AND DISCUSSION

Table 1: The chemical constituents of leaf extracts of Beet & Onion extract

	Detection type	Water extract Beet	Water extract Onion	Water extract Beet :onion
1	*Tannins Test*	+	+	+
2	*Carbohydrate Test*	_	_	_
3	*Glycosides Test*	+	+	+
4	*Phenols Test*	+	+	++
5	*Resins Test*	+	_	_
6	*Flavonoids Test*	+	+	+
7	*Saponin Test*	+	+	+
8	*Alkaloid Test*	+	+	+
9	*Protein Test*	+	+	+
10	*Coumarins Test*	+	+	+
11	*TerpenesTest*	_	_	_
12	*Steroids Test*	_	_	_

The results are shown in Table 1, and the phytochemical analysis of beet and onion leaf extracts found some bioactive substances such alkaloids, tannins, phenol, flavonoids, glycosides, saponins, tannins, and protein. The existence of several of these bioactive elements supports earlier research [19].

Table 2 displays the findings of our evaluation of the effectiveness of beet and onion extracts against pathogenic microorganisms. The result revealed that the mixture of beet and onion extract gave the maximum inhibition zone on Candida albicans, with 100% concentration 5mg/ml giving the highest activity in inhibiting pathogenic bacteria and Candida albicans.

Table 2: Evaluation of the effectiveness of beet and onion extracts against pathogenic microorganisms

Strains	**S1**				**S2**				**S3**			
	100	75	50	25	100	75	50	25	100	75	50	25
Staphylococcus aureus	28	26	24	22	30	27	25	20	36	34	31	29
Staphylococcus Epidermidis	24	21	19	14	28	26	22	18	30	27	23	24
Escherichia coli	19	15	11	8	26	21	17	12	33	29	26	20
Klebsiella spp.	17	15	11	6	26	22	18	13	27	22	18	14
Candida albicans	33	28	26	24	35	31	28	25	37	35	33	30
S1: Beet extract, S2 onion extract, S3 mixture of Beet and Onion extracts												

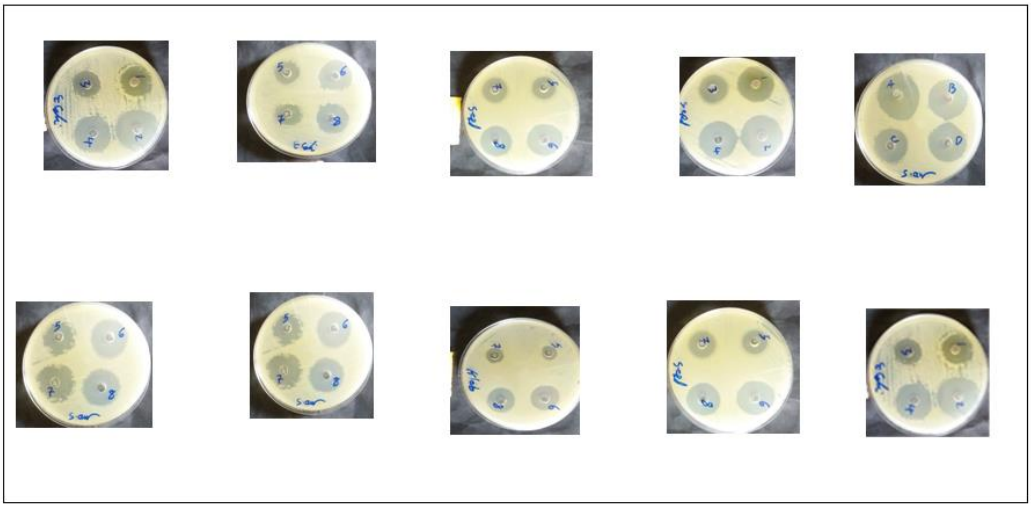

Figure 1: Showed the inhibition zone of Beet& Onion extracts on pathogenic microbe

Table 3: The results of the synergistic efficacy of Beet& Onion extracts with the antifungal nystatin on *Candida albicans*

Dilution for Nystatin on c. albicans				
Number	**Extracts**	**50:50**	**75:25**	**95:5**
1.	Beet water extract	33	30	27
2.	Onion Water Extract	35	33	29
3.	Beet: onion	39	35	33

The outcomes seen in Table 3 When nystatin and beet and onion extracts were diluted 50:50, the results revealed that Beet: Onion water extract had the most synergistic activity, followed by beet water extract and onion water extract. Beet: Onion water extract had the highest level of synergistic activity, followed by Onion Water Extract and Beet Water Extract, according to the results of diluting nystatin and Beet & onion extracts at a ratio of 75:25.

The findings of diluting Beet: Onion water extract with Nystatin at a ratio of 95:05 revealed the highest synergistic activity, followed by Onion Water Extract, and Beet water extract. Finally, the highest synergistic effect is beet& onion extract with Nystatin.

Table 4: The results of the synergistic efficacy of Beet & Onion extracts with the antifungal Miconazole nitrate on Candida albicans

Dilution for miconazole nitrate on c. albicans				
Number	***Extracts***	***50:50***	***75:25***	***95:5***
1	Beet water extract	*29*	*25*	*23*
2	Onion Water Extract	*34*	*30*	*27*
3	Beet: onion	*38*	*36*	*34*

The outcomes seen in Table 4 When nystatin and beet and onion extracts were diluted 50:50, the results revealed that beet: onion water extract had the most synergistic activity, followed by beet water extract and onion water extract. Beet: Onion water extract had the highest level of synergistic activity, followed by Onion Water Extract and Beet Water Extract, according to the results of diluting nystatin and Beet& onion extracts at a ratio of 75:25. The findings of diluting Beet: Onion water extract with Nystatin at a ratio of 95:05 revealed the highest synergistic.

Table 5: The results of clotremazole on c. albicans

Dilution for clotremazole on c. albicans				
Number	**Extracts**	**50:50**	**75:25**	**95:5**
1	Beet water extract	27	25	21
2	Onion Water Extract	29	23	19
3	Beet: Onion	33	30	26

The outcomes shown in Table 5 When clotremazole and beet: onoin water extract were diluted 50:50, the results revealed that beet: onoin water extract had the strongest synergistic action when compared to other extracts.

Table 6: The results of vitek test for *candida albicans*

Biochemical Details																	
3	LysA	-	4	lMLTa	-	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	+
13	TyrA	-	14	BNAG	-	15	ARBa	+	18	AMYa	-	19	dGALa	+	20	GENa	-
21	dGLUa	+	23	LACa	-	24	MAdGa	+	26	dCELa	-	27	GGT	-	28	dMALa	+
29	dRAFa	-	30	NAGA1	-	32	dMNEa	+	33	dMELa	-	34	dMLZa	-	38	lSBEa	-
39	lRHAa	-	40	XLTa	+	42	dSORa	+	44	SACa	+	45	URE	-	46	AGLU	+
47	dTURa	+	48	dTREa	+	49	NO3a	-	51	lARAa	-	52	dGATa	+	53	ESC	-
54	lGLTa	+	55	dXYLa	+	56	LATa	+	58	ACEa	+	59	CITa	+	60	GRTas	+
61	lPROa	+	62	2KGa	+	63	NAGa	+	64	dGNTa	+						

The vitec analysis of beet and onion extracts against Candida albicans revealed that it is favorable for the following substances. (dGLUa, dTURa, lGLTa, lPROa, XLTa, dTREa, dXYLa, 2KGa, LeuA, ARBa, MAdGa, dMNEa, dSORa, LATa, NAGa, ARG, SACa, ACEa, dGNTa, dGALa, dGATa, CITa, GLYLa, dMALa, AGLU and GRTas).

As evident Phytic testing of beet and onion extracts against Candida albicans revealed that they are negative for the following substances. (LysA, TyrA, dRAFa, lRHAa, lMLTa, BNAG, LACa, NAGA1, NO3a, AMYa, dCELa, dMELa, lARAa, ERYa, GGT, dMLZa, URE, GENa, lSBEa and ESC).

Table 7: Estimation of free Radical scavenging activity by DPPH methodof the extracts of Beet& onion extracts

Concentration (µg/ml) extract	Beet extract	Onion extract	Beet: Onion Extract
100	24.33 ± 2.52 A a	17 ± 1 A b	36.33 ± 1.53 A c
200	36.67 ± 1.15 B a	35 ± 2 B a	45.33 ± 2.52 B b
300	46.33 ± 2.08 C a	45.33 ± 2.08 C a	58.67 ± 0.58 C b
400	67.67 ± 1.53 D a	59.67 ± 4.04 D b	74.67 ± 2.08 D c
Ascorbic acid 100	73 ± 1.73 E a	73 ± 1.73 E a	73 ± 1.73 D a
P-value	**Significant**	**Significant**	**Significant**

CONCLUSION

- The existence of certain bioactive components with antibacterial action has been shown in beet and onion extracts. This therefore supports the use of beet and onion extracts by traditional healers to treat particular diseases. However, the required dosage and purification are still challenging.
- As a result, I recommended doing more investigation into the dosage and in-vivo evaluation of the beet and onion extracts.
- Candida albicans is particularly susceptible to the effects of antifungal medications and beet and onion extracts (Nystatin, Miconazole nitrate, Clotremazole).

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