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# Design and evaluation of antifungal vaginal suppository using coconut oil as base for vulvovaginal candidiasis

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## Abstract

**Background** The emergence of antimicrobial resistance to antifungals has made vulvovaginal candidiasis a concern. Coconut oil has antimycotic properties that could have a synergistic effect when combined with antifungals. Thus, clotrimazole suppositories were prepared using coconut oil as a base to improve its use and delivery in antimycotic treatment and the suppositories were evaluated for their physicochemical, mechanical, and drug release properties, and the antimycotic effect of clotrimazole and coconut oil was examined singly and in combination, as well as their formulations using the agar-well diffusion method in comparison with polyethylene glycol (PEG), and cocoa butter bases. Using the fusion method, coconut oil was solidified with beeswax (20–50%) to prepare 100 mg clotrimazole suppositories. Surfactants (4% $^{w}/_{w}$); Tween 20®, Span 20®, sodium lauryl sulphate, and their combinations (3% $^{w}/_{w}$ ratio 1:1 of Tween 20® and Span 20®) were used to improve the rate of drug release from the suppository.

**Results** The suppositories had a pH of 4.1–6.0 and crushing strengths of 0.53 ± 0.07–32.56 ± 5.42 N. Suppositories containing surfactants and those prepared from PEG had significantly ($p < 0.05$) lower disintegration times ranging from 35 to 90 min than those without surfactants ranging from 305 to 388 min. Drug release ($t_{80}$) was the fastest from the suppositories containing 40% coconut oil, Tween 20, and PEG. Using the Korsmeyer–Peppas' model, suppositories made from PEG had a non-Fickian diffusion, while those containing 40% coconut oil, and Tween 20 had a super case II transport mechanism. The combination of clotrimazole and coconut oil gave higher zones of inhibition against *Candida* species compared to either clotrimazole or coconut oil alone. The formulations had higher antimycotic activities against *Candida albicans* than *Candida krusei* and *Candida tropicalis*.

**Conclusion** The optimized formulation with the desired physicochemical and drug release properties was obtained with coconut oil (40% $^{w}/_{w}$) solidified with beeswax (50% $^{w}/_{w}$) as a base. Coconut oil appeared to possess a synergistic antimycotic effect on clotrimazole. Thus, clotrimazole vaginal suppositories with coconut oil as a base in the treatment of vulvovaginal candidiasis showed potential against *C. albicans* and other Candida species.

**Keywords** Antifungal, *Candida albicans*, Coconut oil, Vaginal suppository, Surfactants, Vulvovaginal candidiasis

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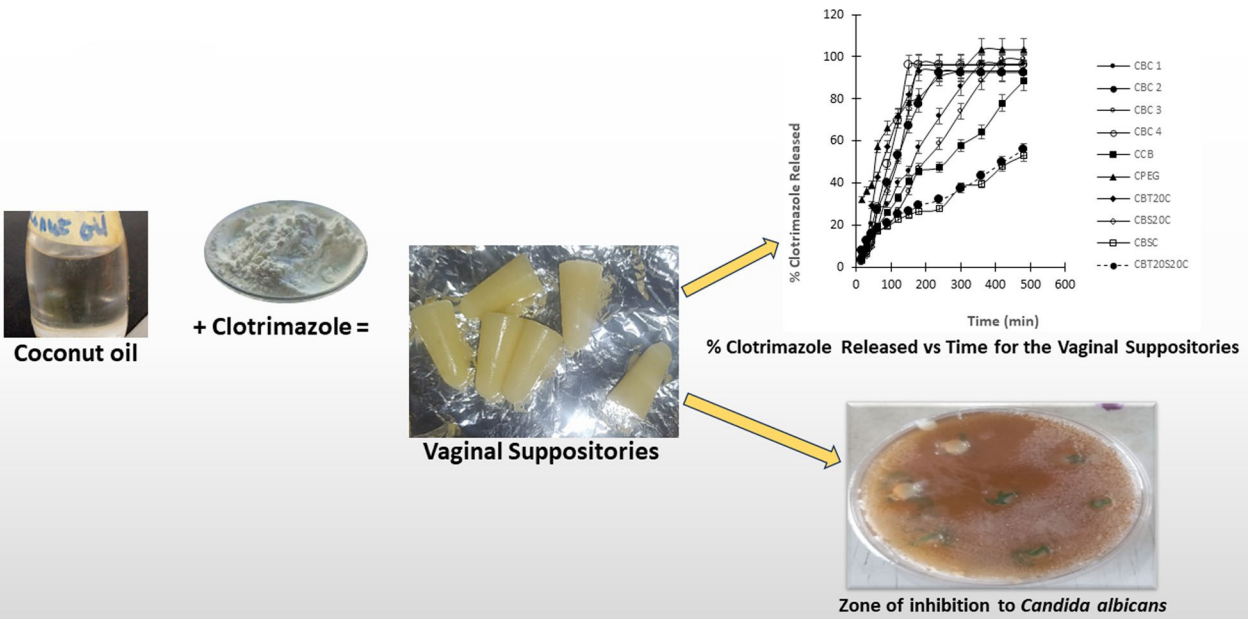
**Graphical abstract**




## Background

Vulvovaginal Candidiasis (VVC) is an opportunistic fungal infection, that in most cases is caused by Candida species with *Candida albicans* accounting for 85–95% [1, 2]. The five most associated species with vaginal candidiasis are *C. albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis,* and *Candida krusei* [3]. The pathological hallmark of the disease is an acute inflammatory condition of the vulva and vaginal mucosa induced and accompanied by the overgrowth of Candida organisms that normally exist in the vaginal mucosa as quiescent symbiotes, forming a complex vaginal ecosystem with other bacteria [4–6]. The disease is characterized by vulvar erythema, excoriation, pruritus, and an abnormal vaginal discharge, which can be "cheese-like" or watery though it has been estimated that about 33% of all women exhibit no symptoms [2, 7]. An estimated 138 million women globally experience recurrent VVC annually with associated morbidity of pain, altered self-esteem, poor work performance, discomfort, interference with sexual and affective relations, and mental distress [2, 8–10]. The main predisposing factor to VVC is the use of antimicrobial medications that destroy beneficial Lactobacillus bacteria of normal vaginal microbiota while other factors are; uncontrolled diabetes mellitus, immunosuppressive conditions, genetic predispositions, the use of glucocorticoids, oral contraceptives, or hormone replacement therapy, pregnancy, sexual activities and tight-fit clothing [4, 8, 11–13]. According to historical data, the prevalence of VVC is higher in the age group of 25–34 years, with an estimated 70% of all women experiencing at least one episode throughout their reproductive years [4, 7, 10, 13, 14].

The vaginal route for drug administration has many potential benefits because it has a large surface area for drug absorption, relatively low enzymatic activity, avoids first-pass effects, and has ease of administration [15]. Like topical Candida infections, vulvovaginal Candidiasis is treated effectively with azole-based antifungal drugs. However, such therapy can be complicated by the emergence of drug-resistant yeasts [16, 17]. This emergence of antimicrobial resistance to antifungals has made vulvovaginal candidiasis a concern [18]. The issue of drug-resistant Candida species may have been exacerbated by using over-the-counter (OTC) products for self-treatment [16, 17]. Most of the OTC drugs are azole-based, thus, the frequent or prolonged use of these products may have the potential for widespread drug resistance [19].

The composition of the suppository base is an important factor in the release process [20]. Suppository bases have been classified into oleaginous, aqueous, and emulsifying bases. The oleaginous bases are known for their ease of formulation, non-irritating, and non-sensitizing nature, though they will require refrigeration in the tropics due to their melting point ranges [21].

Coconut oil (CO), is an edible oil obtained from *Cocos nucifera* Linn, family Arecaceae [22] with saponification and iodine values of 248–268 mgKOH/g and 4.1–11.0, respectively [23, 24]. Coconut oil has been reported to have antimicrobial properties against gram-positive and gram-negative bacteria, fungi, protozoa, and viruses [25] due to the presence of medium-chain fatty acids—lauric, caprylic and capric acids [26]. It has been suggested that the mechanism for the antimycotic effect of coconut oil is that the membrane lipids of the fungi are solubilized as the fatty acids integrate into the membrane. The antimycotic effect of the coconut oil alone, however, is not adequate to treat VVC. Thus, combined with antifungals, coconut oil's antimycotic properties could be synergised [27].

Clotrimazole an azole antifungal drug belonging to the imidazole group is well-tolerated with broad-spectrum antimycotic activity majorly used for the treatment of *C. albicans* and other fungal infections [28]. It acts by inhibiting the synthesis of the fungal membrane's sterol components, thereby impairing ergosterol synthesis and leading to a cascade of membrane abnormalities in the fungus [29].

In the present study, clotrimazole suppositories were designed for the immediate release of clotrimazole using coconut oil as a base to improve their use in antimycotic treatment in comparison with polyethylene glycol and cocoa butter bases.

## Materials and methods

### Materials

Virgin coconut oil (obtained from a market in Akobo, Ibadan, Nigeria). Beeswax, cocoa butter (purchased from Showcrown Laboratory Technologists, Ibadan, Nigeria), Tween 20®, and Span 20® (BDH Chemical Ltd. Poole, England), Sodium Lauryl Sulphate, polyethylene glycol (MW 8000) (Loba Chemie Pvt. Ltd. Mumbai, India), Sabouraud dextrose agar (LAB M Ltd, Lancashire, UK) and Clotrimazole (a gift from Bond Chemical Industries Limited Awe, Oyo State, Nigeria). *Candida albican* was obtained from the Medical Microbiology Laboratory, University College Hospital Ibadan, while *C. tropicalis* and *C. krusei* were obtained from the Pharmaceutical Microbiology Laboratory, University of Ibadan, Nigeria.

### Methods

#### *Preparation of suppository*

Coconut oil was solidified with different concentrations (20, 30, 40 and 50%) of beeswax to formulate 100 mg clotrimazole suppositories using the fusion method [21]. The displacement value of the suppository base was determined using a 1 g mould. Batches of suppositories made from cocoa butter, polyethylene glycol, and those containing 4% $^{w}/_{w}$ Tween 20®, Span 20®, sodium lauryl sulphate and a combination of 3% $^{w}/_{w}$ Tween 20® + Span 20®, were prepared by adding them into the modified base before the addition of clotrimazole. The molten preparation was carefully poured into pre-calibrated stainless-steel moulds and cooled in the refrigerator to solidify at 4 °C. It was then stored at room temperature (30 ± 2 °C) for 24 h after removal from the mould to allow for uniform solidification and crystal

**Table 1** The formula of vaginal suppositories

| S/N | Composition (% w/w) | Code |
|---|---|---|
| 1 | Clotrimazole (10%) + beeswax (20%) + coconut oil (70%) | CBC1 |
| 2 | Clotrimazole (10%) + beeswax (30%) + coconut oil (60%) | CBC2 |
| 3 | Clotrimazole (10%) + beeswax (40%) + coconut oil (50%) | CBC3 |
| 4 | Clotrimazole (10%) + beeswax (50%) + coconut oil (40%) | CBC4 |
| 5 | Clotrimazole (10%) + cocoa butter (90%) | CCB |
| 6 | Clotrimazole (10%) + beeswax (20%) + Tween 20 (4%) + coconut oil (66%) | CBT20C |
| 7 | Clotrimazole (10%) + beeswax (20%) + Span 20 (4%) + coconut oil (66%) | CBS20C |
| 8 | Clotrimazole (10%) + PEG | CPEG |
| 9 | Clotrimazole (10%) + beeswax (20%) + sodium lauryl sulphate (4%) + coconut oil (66%) | CBSC |
| 10 | Clotrimazole (10%) + beeswax (20%) + Tween 20 + Span 20 + coconut oil (66%) | CBTS20C |

transformation [21]. The suppositories were then stored in a desiccator wrapped in aluminium foil in a refrigerator until needed. The formulation composition as well as the formulation codes are shown in Table 1.

#### *Evaluation of clotrimazole suppositories*

*pH* The pH of the molten suppositories was determined using a calibrated pH meter (PHS-25CW Series bend atop pH/mv meter, Shanghai, China).

*Weight variation* The weight variation was carried out as described by the British Pharmacopoeia 2013 [30]. Suppositories (20) selected from each batch were individually weighed, and the mean weight and per cent deviation was determined.

*Melting point* The melting points of the suppositories were determined using the method of Coben and Lieberman [31].

*Crushing strength* The crushing strength of the suppositories was determined using the hardness tester (R00060 model: EH01, DBK Instruments, Mumbai, India). Six suppositories were selected from each batch and the force under which each of the suppositories collapsed was recorded.

*Disintegration time* The disintegration time for the suppositories in acetate buffer, pH 5.2, was determined as the time taken for the suppository to melt or disperse when immersed in a disintegration DBK test apparatus (Mumbai, India) maintained at 37 ± 0.5 °C [30].

*In vitro release of clotrimazole from suppositories* *In vitro* drug release tests were carried out according to the USP XX basket method [21]. Each suppository was placed in the basket and lowered into a flask containing 500 ml of acetate buffer pH 5.2 rotated at a constant speed of 100 rpm and maintained at a constant temperature of 37 ± 0.5 °C. At specified time intervals, 5 ml samples were withdrawn and replaced with the same volume of fresh dissolution medium maintained at the same temperature. The amount of clotrimazole released was analysed spectrophotometrically at 259 nm using a UV–visible spectrophotometer (Spectrum lab 752 s UV–VIS spectrophotometer, Shanghai, China). The mean of three determinations was used in calculating drug release from each batch of suppositories. The clotrimazole release kinetics were investigated by fitting the release data into the Korsmeyer-Peppas equation using the DD Solver software programme (Microsoft Excel add-in, Excel 365 New Mexico, USA).

*Assessment of antimycotic activities* The antimycotic activity was assessed using the agar-well diffusion technique. The prepared agar medium was autoclaved at 121 °C for 20 min. Using the McFarland dilution, cultures of *C. albicans* and *C. spp* were inoculated in sterile water. This was flooded onto the plated Sabouraud dextrose agar using a sterile swab. A sterile 6 mm cork borer was employed to bore 7 wells into each plate of solidified media. The clotrimazole-loaded suppository (100 mg) was suspended in 10 ml acetate buffer pH 5.2 and shaken at room temperature at 50 rpm. Samples were filled into the different wells using sterile syringes, under laminar airflow. The same was repeated for clotrimazole, CO, and coconut oil diluted to 50% with 1% ethanol. The antimycotic activity of the combination of clotrimazole and CO was carried out at ratios 1:1 and 1:2. The zones of inhibition around the wells were examined after 72 h at 25 °C, and their diameters were measured [32].

*Stability study* The vaginal suppositories were kept at room temperature (30 ± 2 °C) and in the refrigerator (4 °C) for 12 weeks. The suppositories were assessed for their visual appearance—colour, odour, and consistency.

*Data presentation and analysis* All determinations were conducted in triplicate and the data is presented as mean ± SD. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) using GraphPad Prism® 5 (GraphPad Software Inc., San Diego, USA). Values with $p < 0.05$ were regarded as significant. In addition, the drug release mechanism was analysed using the DD Solver (Microsoft Excel add-in, Excel 365).

## Results

The physicochemical, mechanical, and drug release properties, as well as correlation coefficients and n obtained by fitting the release data into the Korsmeyer-Peppas kinetic model, of the vaginal suppositories, are presented in Table 2. The pH of the vaginal suppositories ranged from 4.08 to 5.98, while their weight was generally between 0.883 and 1.220 g. The melting point generally ranged from 40 to 60 °C and the crushing strength was 0.46–37.98 N.

The disintegration or liquefaction time, followed the rank order of CPEG < CBT20S20C < CBS20C < CBSC < CBT20C < CBC1 < CBC2 < CBC3 < CCB < CBC4, indicating that CPEG vaginal suppositories had the lowest disintegration time, while CBC4 vaginal suppositories took the longest time to liquefy. The plots of % clotrimazole released over time for suppositories containing coconut oil solidified with beeswax, reference bases, and surfactants are shown in Figs. 1, 2 and 3, respectively. The

**Table 2** Physicochemical properties and release kinetics of the vaginal suppositories using the Korsmeyer–Peppas model

| Formulation | pH | Weight (g) | Melting point range (°C) | Crushing strength (N) | Disintegration time (min) | $t_{50}$ (min) | $t_{80}$ (min) | Korsmeyer–Peppas | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $r^2$ | $n$ |
| CBC 1 | 5.25 | 0.983 ± 0.058 | 49–55 | 0.80 ± 0.00 | 305.0 ± 4.1 | 156.9 | 275.9 | 0.965 | 0.832 |
| CBC 2 | 5.21 | 1.000 ± 0.043 | 45–56 | 1.47 ± 0.28 | 312.0 ± 2.4 | 114.3 | 172.3 | 0.992 | 1.144 |
| CBC 3 | 4.62 | 0.967 ± 0.065 | 50–55 | 5.53 ± 0.99 | 322.0 ± 6.2 | 204.3 | 336.0 | 0.981 | 0.945 |
| CBC 4 | 5.98 | 0.950 ± 0.067 | 50–56 | 8.87 ± 0.28 | 388.0 ± 14.3 | 95.0 | 129.4 | 0.987 | 1.517 |
| CCB | 5.33 | 0.983 ± 0.039 | 40–49 | 7.90 ± 0.00 | 326.0 ± 13.1 | 237.3 | 483.2 | 0.983 | 0.661 |
| CPEG | 4.08 | 1.175 ± 0.045 | 50–60 | 32.56 ± 5.42 | 35.0 ± 2.9 | 52.5 | 139.7 | 0.887 | 0.480 |
| CBT20C | 4.95 | 0.950 ± 0.052 | 49–52 | 0.59 ± 0.05 | 90.0 ± 8.2 | 76.7 | 115.6 | 0.963 | 1.145 |
| CBS20C | 5.12 | 0.975 ± 0.045 | 49–55 | 0.53 ± 0.07 | 77.0 ± 1.7 | 115.0 | 156.1 | 0.997 | 1.538 |
| CBSC | 4.39 | 0.992 ± 0.051 | 49–52 | 1.24 ± 0.30 | 88.0 ± 15.2 | 475.8 | – | 0.972 | 0.605 |
| CBT20S20C | 5.20 | 1.000 ± 0.043 | 46–50 | 1.04 ± 0.05 | 63.0 ± 0.8 | 442.7 | – | 0.980 | 0.558 |

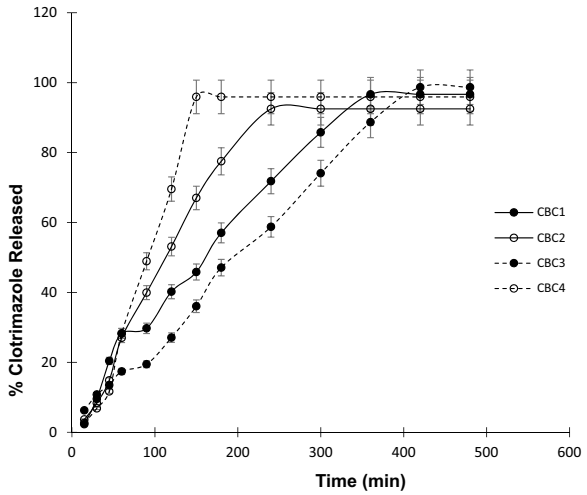



**Fig. 1** Plots of percentage clotrimazole released against time (min) for the vaginal suppositories containing coconut oil and beeswax

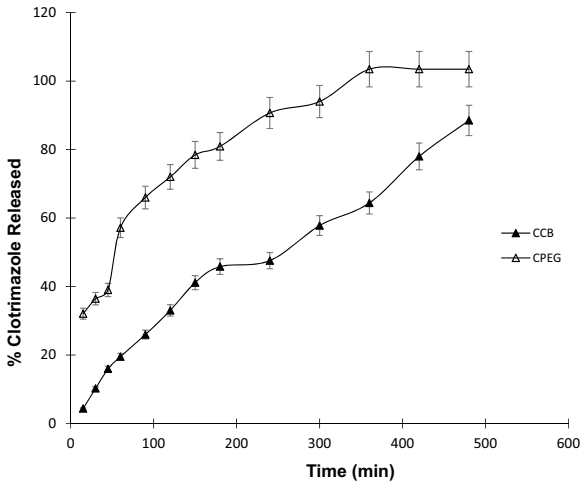



**Fig. 2** Plots of percentage clotrimazole released against time (min) for the vaginal suppositories containing either cocoa butter or PEG

time taken for 80% clotrimazole release (t80) is shown in Table 2. The results showed that CBT20C vaginal suppositories showed the fastest release while CCB exhibited the slowest.

The antimycotic activity of the coconut oil and the API (clotrimazole) tested, singly and in combination are presented in Table 3 and the antimycotic activities of the vaginal suppositories are presented in Table 4. Generally, antimycotic activity increased with an increase in concentration of the clotrimazole in the formulation, with higher activity against *C. albicans*.

The stability test showed that there was no change in the colour, odour, and consistency of the vaginal suppositories after 12 weeks.

## Discussion

pH is a measure of the acidity or basicity of a medium, and the pH of the vaginal suppositories was acidic to mildly acidic with pH ranging from 4.08 to 5.98. The vaginal pH ranges from 3.8 to 5.0 [33]. This acidic pH is important in protecting the vaginal mucosa from pathogenic organisms [34].

The clotrimazole suppositories all had a good appearance and passed the weight uniformity test i.e. they met the BP specification for weight uniformity, which is a permissible percentage deviation of 5% [30].

Coconut oil in the tropics is liquid (room temperature being 30 ± 2 °C), hence the need to solidify it with beeswax. The melting point ranges of the vaginal

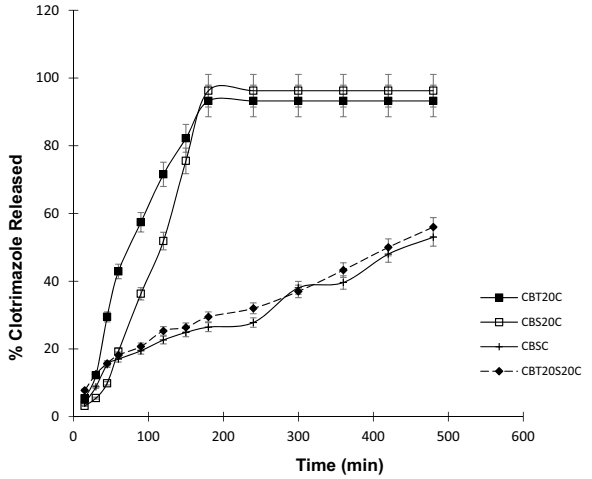



**Fig. 3** Plots of percentage clotrimazole released against time (min) for the vaginal suppositories containing coconut oil and surfactants

suppositories indicate that none of the suppositories had melting points close to the body temperature, rather they had a general melting point range of 40–60 °C. This was probably because beeswax has a melting point of 62–65 °C, thus minimizing the effect of coconut oil [35, 36]. Suppositories prepared with cocoa butter had the lowest melting temperature while PEG had the highest because of its molecular weight. Oily bases have been known to melt at body temperature while aqueous bases disperse in the vaginal fluid. The majority of the time, melting and dispersion occur simultaneously in medicated suppositories depending on the base used and the drug present in the formulation [37]. The melting range also decreased with the addition of surfactants with suppositories containing the combined surfactants exhibiting lower melting temperatures than those containing a single surfactant. The melting point range of those without surfactants increased with an increase in the concentration of beeswax, suggesting that the presence of beeswax did influence the melting point ranges.

The crushing strength of suppositories is used to determine their mechanical strength during handling, packaging, and transportation. Although there is no set standard for what constitutes acceptable or "good" crushing strength levels for suppositories, the range is generally considered to be between 17.7 and 19.6 N [38]. Even though most vaginal suppositories showed lower crushing strength, it was observed that they remained solid and non-brittle at room temperature for the duration of the investigation, indicating that they may endure the mechanical strains of handling and transit. The crushing strength of the clotrimazole suppositories increased as the concentration of beeswax increased, and coconut oil decreased. Suppositories prepared with either Tween 20 or Span 20 had the lowest

**Table 3** Antimycotic Activities of API and Coconut Oil

| Ingredient | Composition (%) | Zone of inhibition (mm) | |
|---|---|---|---|
| | | *Candida albicans* | Candida spp |
| Clotrimazole | 50.0 | 18.0 ± 0.9 | 13.0 ± 0.6 |
| | 100.0 | 20.0 ± 1.0 | 14.0 ± 0.0 |
| Coconut oil | 50.0 | 14.0 ± 0.7 | 12.0 ± 0.6 |
| | 100.0 | 18.0 ± 0.0 | 14.0 ± 0.7 |
| Coconut oil + clotrimazole | 50:50 | 35.0 ± 1.7 | 20.0 ± 1.6 |
| | 33:67 | 30.0 ± 1.5 | 18.0 ± 1.9 |
| Water | 100.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |

crushing strength (0.59 ± 0.05 and 0.53 ± 0.07, respectively), while the ones prepared from PEG had the highest crushing strength (32.56 ± 5.42, $p < 0.05$). According to previous reports, surfactants promote the spread of the melted suppository, increasing the contact surface, decreasing the viscosity of the molten mass, and decreasing the pathway of drug particles to the interface. Thus, it is expected that the suppositories in which they are present will be softer [39].

The disintegration time, which is the same as liquefaction has been used in this study; oily bases have been known to liquefy, and aqueous bases such as PEG disperse during the disintegration test [38]. Clotrimazole suppositories containing surfactants and the ones prepared from PEG had significantly lower disintegration/liquefaction time ($p < 0.05$) in the rank order CPEG < CB TS20C < CBS20C < CBSC < CBT20C, than suppositories without surfactants and those prepared with cocoa butter, which ranged between 305 and 388 min. The lower disintegration/liquefaction time of suppositories with surfactants was expected as they had very low crushing strengths. The combination of Tween 20 and Span 20 significantly reduced the liquefaction time of the vaginal suppositories, suggesting improved drug release. For suppositories without surfactants, liquefaction time increased with an increase in the concentration of beeswax and a decrease in the concentration of coconut oil.

Clotrimazole release from the suppositories followed the rank order CPEG > CBC3 > CBC1 > CBS20C > CB C4 > CBT20C > CBC2 > CCB > CBTS20C > CBSC. This shows that suppositories made from PEG had the fastest drug release, possibly because clotrimazole is lipophilic in nature and thus, it does not bind to PEG; a water-miscible base, unlike in the case of cocoa butter in which its release was poor [40]. Besides, PEG is known to improve the solubility of therapeutic agents [41]. The presence of beeswax did not hinder drug release and the addition of surfactants did not appear to greatly improve the release of clotrimazole. Surfactants increase drug release by reducing the interfacial tension between mucosal fluids and the

**Table 4** Antimycotic activity of the suppositories using Zone of inhibition (mm)

| Organisms | Concentration (mg/ml) | CBC 1 | CBC 2 | CBC 3 | CBC 4 | CCB | CPEG | CBT20C | CBS20C | CBSC | CBT20S20C | Tioconazole (0.7 mg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Candida albicans* | 2.5 | 0.0 ± 0.0 | 10.0 ± 0.0 | 10.0 ± 0.0 | 11.0 ± 1.4 | 14.0 ± 0.0 | 14.0 ± 0.0 | 10.5 ± 0.7 | 12.0 ± 2.8 | 11.0 ± 1.4 | 12.0 ± 2.8 | 30.0 ± 0.0 |
| | 5.0 | 12.0 ± 2.8 | 12.0 ± 0.0 | 14.0 ± 0.0 | 15.0 ± 1.4 | 17.0 ± 1.4 | 17.0 ± 1.4 | 14.0 ± 0.0 | 16.0 ± 2.8 | 15.0 ± 0.0 | 14.0 ± 2.8 | |
| | 10.0 | 13.0 ± 1.4 | 14.0 ± 0.0 | 17.0 ± 1.4 | 18.0 ± 0.0 | 19.0 ± 1.4 | 20.0 ± 0.0 | 17.0 ± 1.4 | 19.0 ± 1.4 | 17.0 ± 1.4 | 17.0 ± 1.4 | |
| | 20.0 | 15.0 ± 1.4 | 17.0 ± 1.4 | 19.0 ± 1.4 | 21.0 ± 1.4 | 23.0 ± 1.4 | 24.0 ± 0.0 | 19.0 ± 1.4 | 22.0 ± 2.8 | 19.0 ± 1.4 | 20.0 ± 0.0 | |
| *Candida tropicalis* | 2.5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 10.0 ± 0.0 | 11.0 ± 1.4 | 13.0 ± 1.4 | 11.0 ± 1.4 | 10.0 ± 0.0 | 11.0 ± 1.4 | 10.0 ± 0.0 | 10.0 ± 0.0 | 30.0 ± 0.0 |
| | 5.0 | 10.0 ± 0.0 | 10.0 ± 0.0 | 12.0 ± 0.0 | 15.0 ± 1.4 | 15.0 ± 1.4 | 14.0 ± 0.0 | 14.0 ± 0.0 | 15.0 ± 1.4 | 13.0 ± 1.4 | 13.0 ± 1.4 | |
| | 10.0 | 12.0 ± 0.0 | 12.0 ± 0.0 | 14.0 ± 0.0 | 17.0 ± 1.4 | 18.0 ± 0.0 | 14.0 ± 0.0 | 14.0 ± 0.0 | 15.0 ± 1.4 | 13.0 ± 1.4 | 13.0 ± 1.4 | |
| | 20.0 | 14.0 ± 0.0 | 15.0 ± 1.4 | 17.0 ± 1.4 | 19.0 ± 1.4 | 19.0 ± 1.4 | 20.0 ± 0.0 | 21.0 ± 1.4 | 19.0 ± 1.4 | 20.0 ± 0.0 | 20.0 ± 0.0 | |
| *Candida krusei* | 2.5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 11.0 ± 1.4 | 13.0 ± 1.4 | 13.0 ± 1.4 | 13.0 ± 1.4 | 10.0 ± 0.0 | 13.0 ± 4.2 | 10.0 ± 0.0 | 10.0 ± 0.0 | 30.0 ± 2.8 |
| | 5.0 | 11.0 ± 1.4 | 12.0 ± 2.8 | 15.0 ± 1.4 | 16.0 ± 2.8 | 15.0 ± 1.4 | 13.0 ± 1.4 | 13.0 ± 1.4 | 17.0 ± 4.2 | 13.0 ± 1.4 | 14.0 ± 0.0 | |
| | 10.0 | 14.0 ± 0.0 | 12.0 ± 0.0 | 18.0 ± 0.0 | 19.0 ± 1.4 | 17.0 ± 1.4 | 19.0 ± 1.4 | 17.0 ± 1.4 | 21.0 ± 4.2 | 16.0 ± 0.0 | 17.0 ± 1.4 | |
| | 20.0 | 16.0 ± 0.0 | 15.0 ± 1.4 | 19.0 ± 1.4 | 23.0 ± 1.4 | 21.0 ± 1.4 | 23.0 ± 1.4 | 20.0 ± 0.0 | 24.0 ± 2.8 | 18.0 ± 0.0 | 19.0 ± 1.4 | |

suppository base [41]. Interestingly, drug release from the vaginal suppositories without the surfactants did not show a clear trend despite the correlation between their crushing strength and disintegration time. Based on the $t_{80}$, the ranking of drug release from the formulation was from CBT20C > CBC4 > CPEG > CBS20C. Thus, showing that clotrimazole partitioned through the oil/water interface fastest through CBT20C and CBC4. It has been reported that drug release from suppositories occurs through the initial melting, spreading of the melted mass, settling of the drug particles, movement of the drug particles through the oil/water interface, and dissolution of the drug particles in the mucosal fluid [21, 42]. Drug release from all the vaginal suppositories had regression coefficients, $r^2 \geq 0.887$ and diffusion constant, $0.5 < n < 1$ (0.480–0.945), indicating non-Fickian anomalous diffusion i.e. both diffusion and relaxation (erosion) for CPEG, CBTS20C, CBSC, CCB, CBC1 and CBC3 while it was $n > 1$ (1.144–1.538) for CBC2, CBT20C, CBC4 and CBS20C, which is Super Case II transport mechanism (relaxation), meaning that drug release accelerated towards the endpoint of the drug release [43].

The antimycotic properties indicate that the zone of inhibition produced against *C. albicans* and *Candida* spp by the combination of the coconut oil and the API was higher than the activities of the coconut oil and API singly, thus suggesting a synergistic effect. In the suppository formulations, the rank of the zone of inhibition against *C. albicans* was in the order CPEG > CCB > CBS20C > CBC 4 > CBT20S20C > CBC3 = CBT20C > CBC2 > CBC1. The antimycotic activity also increased with an increase in the concentration of clotrimazole in the formulation. The antimycotic activities of the formulations against *C. tropicalis* and *C. krusei*, which are both implicated in VVC, were in the rank order of CBT20C > CPEG = CBSC = CB T20S20C > CBC4 = CCB = CBS20C > CBC3 > CBC2 > CBC 1, and CBS20C > CBC4 = CPEG > CCB > CBT20C > CBC3 = CBT20S20C > CBC1 > CBC2, respectively.

It is impressive that CBC4 with the least concentration of coconut oil exhibited higher antimycotic activity and faster release ($t_{50}$ and $t_{80}$) of clotrimazole than the other suppositories without surfactants, but comparable activities to the known bases, PEG and cocoa butter. This suggests that the concentration of coconut oil in CBC4 (40%) might just be optimal for the formulation, as it had activity against the three Candida species and generally had better activity than cocoa butter. Coconut oil contains lauric and capric acid, which confer antimycotic properties on it thus synergizing the activity of clotrimazole [44].

## Conclusion

The clotrimazole vaginal suppositories met BP specifications for weight uniformity and had a pH that conformed with vaginal pH. The addition of surfactants singly rather than in combination improved the rate of drug release. The presence of surfactants in the suppositories reduced disintegration time and enhanced drug release. The formulation containing 40% $^{w}/_{w}$ coconut oil had comparable drug release properties and antimycotic activity with those prepared with PEG and cocoa butter. The optimized formulation with the desired physicochemical and drug release properties was obtained with coconut oil (40% $^{w}/_{w}$) solidified with beeswax (50% $^{w}/_{w}$) as a base. Coconut oil appeared to have a synergistic effect on clotrimazole. Thus, clotrimazole vaginal suppositories with coconut oil as a base in the treatment of vulvovaginal candidiasis showed potential against *Candida* species.

#### Abbreviations

| | |
|---|---|
| VVC | Vulvovaginal candidiasis |
| OTC | Over the counter |
| CO | Coconut oil |
| CBC1 | Clotrimazole + beeswax 20% + coconut oil 70% |
| CBC2 | Clotrimazole + beeswax 30% + coconut oil 60% |
| CBC3 | Clotrimazole + beeswax 40% + coconut oil 50% |
| CBC4 | Clotrimazole + beeswax 50% + coconut oil 40% |
| CCB | Clotrimazole + cocoa butter |
| CPEG | Clotrimazole + polyethylene glycol |
| CBT20C | Clotrimazole + Tween 20 + coconut oil |
| CBS20C | Clotrimazole + Span 20 + coconut oil |
| CBSC | Clotrimazole + sodium lauryl sulphate + coconut oil |
| CBTS20C | Clotrimazole + Tween 20 + Span 20 + coconut oil |
| PEG | Polyethylene glycol |
| $t_{80}$ | Time taken to release 80% of the drug |
| $t_{50}$ | Time taken to release 50% of the drug |
| API | Active pharmaceutical ingredient |

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#### Author contributions

OAO contributed to the design of the study; FMO and ADM collected the samples and performed the experiments; ODA analysed the data and drafted the paper; all authors read and approved the final manuscript.

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#### Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

## Declarations

#### Ethics approval and consent to participate

This work did not require ethical approval.

#### Consent for publication

The authors declare no conflict of interest.

#### Competing interests

The authors declare that they have no competing interests.

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