# Vulvovaginitis in Women: Risk Factors and Antifungal Effect of Two Selected Plant Extracts on *Candida* species

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**Background and study aim:** *Candida* species is a leading cause of recurrent inflammation of both the vagina and vulva known as vulvovaginitis. The increasing occurrence of vulvovaginitis among women of reproductive years makes it important to investigate the risk factors associated with vulvovaginitis and the antifungal effect of selected plant extracts on *Candida* species associated with vulvovaginitis.

**Materials and Methods:** Three hundred women of reproductive age (15 – 50 years) with complaints suggesting vulvovaginitis were assessed for possible risk factors associated with vulvovaginitis. High Vaginal swabs (HVS) were collected and cultured for the presence of *Candida* species on Sabouraud Dextrose Agar (SDA). Colonies were identified based on colony morphology, germ tube test, and biochemically using API 20C AUX. Agar-well diffusion was used to determine the effect of *Gomphrena celosioides* and *Vernonia perrottettii* extracts on the isolates. Chi square ($P<0.05$) was used to determine the factors associated with vulvovaginitis.

**Results:** There was a significant association ($P<0.05$) with the use of birth control pills, tight underwear, tight clothing, pregnancy and vulvovaginitis. ($\chi2= 82.78$, $P<0.001$, $\chi2= 23.06$ $P<0.001$, $\chi2= 8.292$ $P<0.004$, $\chi2 = 30.95$ $P\leq0.0001$ respectively). Twenty-one (21) isolates of *Candida* species were identified as *Candida tropicalis* 13(59.1%)), *Candida glabrata* 3(13.6%), *Candida albicans* 3(13.6%), *Candida parasiplosis* 1(4.6%) and *Candida lambica* 1(4.6%)..

**Conclusion:** The study demonstrates that birth control pills, tight underwear, tight clothing and pregnancy had a significant association with vulvovaginitis. All *Candida* species isolates were resistant to plant extracts. Women should be educated on the risk factors associated with vulvovaginitis.

## INTRODUCTION

Vaginal candidiasis is a common cause of gynecological infection among women worldwide, and up to 70% of women of reproductive age have at least, at a time experienced symptomatic vaginal candidiasis **[1]**. Vaginal candidiasis is caused by yeast, predominantly *Candida albicans* **[2]**. Invasive candidiasis was estimated to cause a mortality of over 70% **[3]**. *Candida albicans* is said to be responsible for 90% of cases of infectious vaginitis **[4]**. Nevertheless, over the last decades, there have been reports demonstrating an increase in the frequency of cases caused by non-albicans species with *Candida glabrata* consistently being the lead species after *Candida albicans* **[2,5]**.

In recent times, relative proportions of organisms causing nosocomial bloodstream infections have changed over the last decade, with *Candida* species now firmly established as one of the most frequent agents **[6]**. The symptoms manifest in many ways, and it is now postulated that vaginal candidiasis in human have increased at an alarming rate, mainly among immune-compromised individuals **[2]**. A study in Brazil confirmed 66.66% vulvovaginal candidiasis and recurrent vulvovaginal candidiasis in diabetic women in comparison with 33.33% in the non-diabetic women **[7]**. These conditions result in severe genital itching, vaginal odour and severe irritation with discharge. Occasionally, there may be no

discharge or there may be discharge without inflammation. The problem of vaginal discharge is probably the most frequently narrated complaint of women of reproductive age **[8]**. The experience of candidiasis with *Candida albicans*, being the most implicated species is suggestive of predisposing factors such as prolonged or repeated use of antibiotics, steroids hormones medication, hormone replacement therapy (HRT), ontraceptives with high estrogen content, pregnancy, diabetes mellitus, and changes in mucus lining of the vaginal which encourage *Candida* to flourish **[9,10]**.

Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites. These medicinal plants are believed to be a potential source for the discovery of new drugs **[11,12]**. A number of active compound classes like alkaloids, terpens, flavonoids, glycosides, lignans, phenolics and saponins have been used in the modern medicine for their wide therapeutic activities **[13]**. With the emergence of high resistance of microorganisms to antifungal drugs **[14]**, efforts are geared towards a search for more potent antimicrobial agents which is a global challenge preoccupying research institution, pharmaceutical companies **[15]**. *Gomphrena celosioides* belongs to the Amaranthacea family and over 120 species which have been applied to gangrenous wounds by natives as a result of the antimicrobial properties they possess **[16]**. *Vernonia perrottettii* belongs to the family Asteracea. A decoction of the whole plants with *Senecio madagascariensis* and *Piliostigma. thonningii* is used as a fever remedy. The recurrent nature of vulvovaginitis, the emergence of antimicrobial resistance and the quest for treatment options make it crucial to investigate the risk factors and the antifungal effect of *Gomphrena celosioides* and *Vernonia perrottettii* extracts on *Candida* species associated with vulvovaginitis in women of child-bearing period.

## MATERIALS AND METHODS

### Description of the Study Area:

The study was conducted at Ahmadu Bello University Health Centre (Sick Bay), Zaria, Kaduna State, Nigeria. The health centre occupies 2600 square metres and serves staff, students and neighbouring communities. The study targeted apparently healthy women between age group 15-50 years with complaints of vaginitis at the Ahmadu Bello University Health Service (Sick Bay), Zaria, North-Central Nigeria.

### Sample Collection:

Following informed consent from participants, 300 structured questionnaires were administered using the method described by [17]. Apparently healthy women attending the Ahmadu Bello University Health Services with complaints of vulvovaginitis were randomly assessed for the sociodemographic characteristics and possible risk factors associated. Sterile swab sticks were used to obtain high vagina swabs (HVS) from the symptomatic women, with the assistance of certified medical personnel in the hospital and transported immediately under ice to the Department of Microbiology laboratory, Faculty of Sciences, Ahmadu Bello University, Zaria, Kaduna State Nigeria.

### Identification of *Candida* isolates:

The swabs were inoculated unto the surface of sterile Sabouraud Dextrose Agar (Oxoid Ltd, England) plates for *Candida* species and incubated at $25^0$C for 48hours. Distinctive colonies that are creamy white with fermentative odours were subcultured for further purification and characterized using biochemical test such as Grams reaction, germ tube tests, then confirmed using the API 20C AUX System (Analytab Products, France) as described by **[18]**.

### Antifungal Susceptibility of Isolates:

This was carried out using agar well diffusion method. Washed overnight broth culture of isolate was diluted appropriately using sterile distilled water to 1.0 McFarland scale ($1.0\times10^8$cfu/ml). Sterile Sabouraud Dextrose Agar (15 ml) was poured into sterile petri dishes and allowed to set, then flooded with 0.1ml of the standardized isolates of *Candida* isolated species and this were spread uniformly using spread plate method. Wells of 6mm diameter were bored on the agar medium using a sterile cork-borer (No.1). Aqueous solution of five standard antifungal drugs namely: Nystatin (100 iu), Mycotene 100mg), Ketoconazole 200mg, Itraconazole (100mg) and Fluconazole (500mg) were prepared which served as positive control **[19]**.

Exactly 0.1ml of the different concentrations of the standard antifungal drugs was placed in each well. The plates were allowed to stand for one

hour at room temperature to allow diffusion of the substrates to proceed before the growth of the organism commenced. Reference strain *C. parapsilopsis* ATCC 22019 was included in each test as quality control isolates. The plates were finally incubated at room temperature for 48 hours. Presence of zone of inhibition was measured using a transparent ruler and expressed in terms of zone of inhibition (mm) for susceptibility.

**Plant Preparation:**

The plant materials were collected from the botanical garden and environment of the main campus of Ahmadu Bello University, Zaria, Nigeria. The plants were brought to the Department of Biological Sciences for identification. The whole plant of *Gomphrena celosioides*, and *Vernonia perrottettii* were air dried for 2-3 weeks, ground to powder, and stored in an air tight container for future use.

**Extraction of Plant Materials:**

The extraction of the plant material of *Gomphrena celosioides* and *Vernonia perrottettii* were carried out using known standard procedures **[20]**. Aqueous and methanolic extraction of individual plants were carried out using a total of 100g powdered sample of the whole plant part in distilled water for 24hrs and 70% methanol for 3 days respectively. Each extract was filtered and solvent evaporated under reduced pressure in a rotary evaporator and weighed.

**Phytochemical Screening of the Extracts:**

Phytochemical screening of aqueous and methanol extracts of *Gomphrena celosioides* and *Vernonia perrottettii* were carried out using standard phytochemical procedure of **[21]**. Different phytochemical constituents were tested which includes: carbohydrates, tannis, reducing sugar, saponins, flavonoids, alkaloids, phenols, anthraquinones and steroids.

**Determination of Antifungal Activity of the Extracts:**

Approximately 0.1ml of the different concentrations (400mg/ml, 300mg/ml, 200mg/ml and 100mg/ml) of the extract was placed in each well in the agar medium containing the culture singly and in combination using a sterile Pasteur pipette. The plates were allowed to stand for one hour at room temperature to allow diffusion of the substrates to proceed before the growth of the organisms commence. The plate was finally incubated at $25^0$C for 48hours for *Candida* spp. like appearance. The presence of zone of inhibition around the hole containing the extracts indicates the antimicrobial activity against the test organisms and this was measured and expressed in terms of diameter zones of inhibition (mm).

**Statistical Analysis:**

Chi square was used to determine the association between the risk factors and vulvovaginitis at $P<0.05$ using SPSS (version 23).

## RESULTS

**Association between Risk Factors and Vulvovaginitis:**

Majority of the study participants were single women 161(53.7%). The potential risk factors associated with vulvovaginal candidiasis were assessed (birth control pills, tight dresses, tight pants and pregnancy). Out of the 300 participants, 90(30%) users of birth control pills, 58(19.3%) users of tight dresses, 59(19.7%) users of tight pants, 97(32.3%) pregnant women, 52(17.3%) users of vaginal creams and 124(41.3%) users of antibiotics participated (Table 1).

**Identification of *Candida* Isolates:**

Twenty-one isolates of *Candida* species were identified as *Candida tropicalis* (13), *Candida albicans* (3), *Candida glabrata* (3), *Candida parasiplosis* (1) and *Candida lambica* (1) (Table 2).

**Antifungal Sensitivity of Isolates:**

The sensitivity of *Candida* isolates to standard antifungal drugs (Table 3) shows that all *Candida* spp. showed 100% resistance against Ketoconazole. *Candida albicans* isolates exhibited resistance against all the antifungal agents tested. The non- *C. albicans* showed 100% susceptibility to Nystatin.

**Phytochemical Screening of Plant Extracts:**

Extracts of *Gomphrena celosioides* and *Vernonia perrottettii* contained bioactive compounds.

**Antifungal Sensitivity of Candida Isolates to Extracts.**

The sensitivity of the *Candida* species to the extracts revealed that, some of the isolates

exhibited multiple resistances to the aqueous and methanolic extracts of *Gomphrena celosioides* and *Vernonia perrottettii*, even the extract combination.

**Table 1: Risk factors associated with vulvovaginitis.**

| Factor | | No examined | N(%positive) |
|---|---|---|---|
| **Marital status** | Single | 161 | 81(50.31) |
| | Married | 139 | 72(51.80) |
| **Total** | | 300 | 153(51.0) |
| | | Df =1 | |
| **Use of birth control pill** | Use | 90 | 82(91.11) |
| | Don't use | 210 | 71(33.81) |
| **Total** | | 300 | 153(51.0) |
| **$\chi^2$ = 82.78** | p< 0.0001 | OR =20.07 | CI =9.192 to 43.79 |
| **Use of tight clothing** | Wear | 58 | 46(79.31) |
| | Don't wear | 242 | 107(44.21) |
| **Total** | | 300 | 153(51.0) |
| **$\chi^2$ = 23.06** | p<0.0001 | OR = 4.836 | CI=2.440 to 9.587 |
| **Use of tight underwear** | Wear | 59 | 40(67.80) |
| | Don't wear | 241 | 113(46.89) |
| **Total** | | 300 | 153(51.0) |
| **$\chi^2$=8.292** | p = 0.004 | OR= 2.385 | CI=1.306 to 4.354 |
| **Pregnancy** | Pregnant | 97 | 72(74.23) |
| | Not pregnant | 203 | 81(39.90) |
| **Total** | | 300 | 153(51.0) |
| **$\chi^2$= 30.95** | p< 0.0001 | OR = 4.338 | CI = 2.541 to 7.405 |
| **Use of vaginal cream** | Use | 52 | 32(61.54) |
| | Don't use | 248 | 121(48.79) |
| **Total** | | 300 | 153(51.0) |
| **$\chi^2$= 2.796** | p = 0.0945 | OR = 1.679 | CI = 0.9108 to 3.096 |
| **Use of antibiotics** | Use | 124 | 60(48.39) |
| | Don't use | 176 | 93(52.84) |
| **Total** | | 300 | 153(51.00 |
| **$\chi^2$= 0.5774** | p = 0.4473 | OR = 0.8367 | CI = 0.5281 to 1.326 |

OR = odds ratio; CI = confidence interval

**Table 2: Distribution of *Candida* species among the study population.**

| Yeast Isolates | Occurrence (n) | % |
|---|---|---|
| ***Candida tropicalis*** | 13 | 61.90 |
| ***Candida glabrata*** | 3 | 14.29 |
| ***Candida albicans*** | 3 | 14.29 |
| ***Candida parasiplosis*** | 1 | 4.76 |
| ***Candida lambica*** | 1 | 4.76 |
| **Total** | 21 | 100 |

**Table 3: Sensitivity assay of *Candida* species.**

| Antifungal agent | *C. tropicalis* | | *C. albicans* | | *C. glabrata* | | *C. parasiplosis* | | *C. lambica* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (n=13) | | ( n=3) | | ( n=3) | | ( n=1) | | ( n=1) | |
| | Percentages | | | | | | | | | |
| | S | R | S | R | S | R | S | R | S | R |
| **Nystatin(100 iu)** | 85 | 15 | 0 | 100 | 100 | 0 | 100 | 0 | 100 | 0 |
| **Mycotene (100mg)** | 54 | 31 | 0 | 100 | 67 | 33 | 100 | 0 | 100 | 0 |
| **Ketoconazole (200mg)** | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| **Itraconazole (100mg)** | 62 | 23 | 0 | 100 | 33 | 67 | 100 | 0 | 0 | 100 |
| **Fluconazole (500mg)** | 54 | 38 | 0 | 100 | 100 | 0 | 0 | 100 | 100 | 0 |

n= number of isolates; R = Resistant; S = Susceptible

**Table 4: Phytochemical screening of plant extracts**

| Phytochemicals | Methanol extracts | | | Aqueous extracts | | |
|---|---|---|---|---|---|---|
| | *Gomphrena celosioides* | *Vernonia perrottettii* | Combination of *G. celosioides* and *V. perrottettii* | *Gomphrena celosioides* | *Vernonia perrottettii* | Combination of *G. celosioides* and *V. perrottettii* |
| **Carbohydarate** | + | + | + | + | + | + |
| **Reducing sugar** | ++ | + | ++ | ++ | + | ++ |
| **Tannins** | + | ++ | + | + | ++ | + |
| **Saponins** | + | ++ | ++ | + | ++ | ++ |
| **Flavonoids** | + | + | + | + | + | + |
| **Alkaloids** | ++ | + | ++ | ++ | + | ++ |
| **Phenols** | - | - | - | - | - | - |
| **Anthraquinones** | - | - | - | - | - | - |
| **Steroid and Triterpene** | - | + | - | - | + | - |
| **Cardiac glycoside** | + | + | + | + | + | + |

+ = positive; ++ =highly positive; - = negative

**Table 5: Antifungal activity of plant extracts against *Candida tropicalis*.**

| Plant extracts | Concentration | Organisms | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *C.t* | *C.t* | *C.t* | *C.t* | *C.t* | *C.t* | *C.t* | *C.t* | *C.t* | *C.t* | *C.t* | *C.t* | *C.t* |
| **GC(ME)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **CM(ME)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **VP(AQ)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | 9 |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **VP(ME)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | 10 |
| | 200 | NS | NS | 5 | NS | NS | 10 | NS | NS | NS | NS | NS | NS | NS |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **CM(AQ)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **GC(AQ)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

GC(ME) = *Gomphrena celosioides*, CM(ME) = Combination (Methanol), VP(AQ) = *Vernonia perrottetii* (Aqueous), CM(AQ) = Combination (Aqueous), GC(AQ) = *G. celosioides* (Aqueous), C.t = *Candida tropicalis*, NS = Not sensitive.

**Table 6: Antifungal activity of plant extracts against *Candida glabrata*, *C. albicans*, *C. parasiplosis* and *C. lambica***

| Plant extract | Concentration | Organism | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *C.g* | *C.g* | *C.g* | *C.a* | *C.a* | *C. a* | *C.p* | *C.*l |
| **GC(ME)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS |
| **CM(ME)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | 10 | NS | NS |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS |
| **VP(AQ)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | NS | 10 | NS |
| | 300 | NS | NS | NS | NS | NS | 8 | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS |
| **VP(ME)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | 5 | NS | NS | NS | 5 | NS |
| | 300 | NS | NS | NS | NS | NS | 10 | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS |
| **CM(AQ)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS |
| **GC(AQ)** | 100 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 200 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 300 | NS | NS | NS | NS | NS | NS | NS | NS |
| | 400 | NS | NS | NS | NS | NS | NS | NS | NS |

GC(ME) = *Gomphrena celosioides*, CM(ME) = Combination (Methanol), VP(AQ) = *Vernonia perrottetii* (Aqueous), CM(AQ) = Combination (Aqueous), GC(AQ) = *G. celosioides* (Aqueous), C.g = *Candida glabrata*, C.a = *Candida albicans*, C.p = *Candida parasiplosis*, C.l = *Candida lambica.*

## DISCUSSION

The use of birth control pills, wearing tight dresses, wearing tight pants and pregnancy had significant association *(P<0.05)* with vulvovaginitis. It could be inferred that these factors increase the risk of vulvovaginitis. Pregnancy and birth control pills could cause hormonal changes that result in vulvovaginitis. This finding is similar to the report of **[22]**, who observed the use of birth control pills to be significantly associated *(P<0.05)* with vulvovaginitis. Similarly, our study corroborates the findings of **[23]**, who in a study of prevalence of vulvovaginal candidiasis in pregnancy observed an association of vulvovaginal candidiasis with pregnancy. Consequently, hormonal changes caused by pregnancy and the use of birth control pills have been linked to the overgrowth of *Candida* species **[24]**. However, no significant association *(P>0.05)* was observed between marital status, use of vaginal creams, use of antibiotics and vulvovaginitis. This is in contrast with the previous work of **[25]** who reported the use of antibiotics to be associated with vaginal candidiasis. This suggests that the reduction of vaginal bacteria by antibiotics does not promote vulvovaginal candidiasis. For instance, *Candida* colonizations of the vagina environment are promoted by estrogen **[26]**. Hence, should not be antagonistic with bacterial colonization of the vagina.

In addition, this study is in contrast with the work of **[27]** who reported that *Candida tropicalis* was predominant, accounting for 61.90% of the *Candida* species. The predominance of *Candida tropicalis* could be as a result of abuse of antifungal drugs causing an increase in the occurrence of non- *C. albicans* in vulvovaginal candidiasis. Jimoh *et al*. **[28]** reported a similar finding of the emergence of non- *C. albicans* in vulvovaginal candidiasis. Our finding of the predominance of *Candida*

*tropicalis* among the non-*albicans* is also similar to the reports by **[29]**.

Furthermore, Ketoconazole, which is a drug of choice for treating fungal infections, was least effective therapy thereby limiting its use in the treatment of vulvovaginal candidiasis. This finding is in line with earlier reports of **[27,30,31]**. The resistance exhibited by *Candida albicans* isolates against all the antifungal agents is suggestive of a growing resistance to commonly used antifungal drugs, thus making treatment options for *Candida* infections difficult. Similarly, **[32]** reported an increasing resistance of *Candida* species to azoles. The non-*albicans* displayed the highest degree of susceptibility to Nystatin. This can be inferred that Nystatin may be a drug of choice in treating infections caused by non-*C. albicans*. This finding of the effectiveness of Nystatin in the treatment of vulvovaginal candidiasis caused by non-*albicans* is in line with the earlier report of **[33]**.

The phytochemical screening of the extracts of *Gomphrena celosioides* and *Vernonia perrottettii* reveals that the extracts contain bioactive compounds such as tannins, flavonoids and glycosides which are known to possess antifungal activity **[34]**. Similarly, De Andarde Monteiro and Santos **[35]** also reported tannins, flavonoids and glycosides to have antifungal activity against *Candida* species. All the plant extracts demonstrated no effectiveness against *C. tropicalis*, suggestive of an emerging resistance of non-*albicans* to plant extracts as well as antifungal drugs, although *Candida* species showed a very weak sensitivity to the aqueous and methanolic extracts of *V. perrottettii* at 200mg/ml. The resistance displayed by *Candida* species to the combination of the extracts may be explained in two ways; one, due to phytochemical antagonism of the extract combination **[36]**. The intrinsic properties of *Candida* species related to the permeability of their surface to extracts may be another reason for the low effectiveness of plant extracts against *Candida* species **[37,38]**. Similarly, this study observed no antifungal activity of both aqueous and methanolic extracts of *Gomphrena celosioides* against *Candida* species. It can be inferred that *Candida* species are widely resistant to extracts of *Gomphrena celosioides.* This finding is in line with the report of **[16]** who observed no antifungal activity of both aqueous and methanolic extracts of *Gomphrena celosioides* against *Candida* species.

## CONCLUSION

This study reveals that vulvovaginitis is associated with the use of birth control pills, tight underwear, tight clothing and pregnancy. Also, *Candida* species isolates showed growing resistance to antifungals, including plant extracts. Public enlightenment on the risk factors associated with vulvovaginitis should be carried out in order to decrease the occurrence and reoccurrence of vulvovaginitis in women. Other medicinal plants should be investigated for their antifungal effects on *Candida* species in order to discover an effective treatment option for vulvovaginal candidiasis.

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### Conflict of Interest:

The authors declare there are no conflicts of interest.

### Ethical Approval:

Ethical approval was granted by the authorities of the Ahmadu Bello University Health Services, Zaria, Nigeria.

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### HIGHLIGHTS

- Vulvovaginitis is significantly associated with the use of birth control pills, tight underwear, tight clothing and pregnancy.
- *Candida* species isolates showed resistance to antifungals, including plant extracts.

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