Effects of Simulated Preparations of Plants used in Nigerian Traditional Medicine on *Candida* spp. Associated with Vaginal Candidiasis

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Research

Abstract

Some Nigerian medicinal plants are popular among traditional producers of phytotherapies in the treatment of sexually related infections. For this study we used modified agar disk, agar spot and agar well-diffusion methods, preparations of simulated crude aqueous and ethanolic extracts of 11 traditionally used medicinal plants for *in vitro* antimicrobial activities against seventy five strains of *Candida* species associated with *Candida* vaginitis and 37 vaginal *Lactobacillus* species. *Candida pseudotropicalis* (Castell.) Basgal were minimally inhibited by the plant extracts, while the rate of inhibition of other *Candida* strains by the ethanolic extracts of the plants were, *Ageratum conyzoides* L. (44.4 - 66.7%), *Anthocleista djalonensis* A. Chev. (57.1 - 66.7%), *Senna alata* (L.) Roxb. (44.4 - 75.0%) *Ficus exasperata* Vahl. (44.4 - 62.5%), *Gliricidia sepium* Kunth ex Steud. (64.3%-75.0%) *Chromolaena odorata* (L.) R.M. King & H. Rob.(57.1%-62.5%) and *Rauwolfia vomitoria* Afzel. (62.5%). Apart from *Aspilia africana* (Pers.) C.D. Adams (24.3%) and *Ageratum conyzoides* L. (35.1%), very low *in vitro* inhibitory activities of between 5.4% and 16.2% were produced by the medicinal plants against the vaginal *Lactobacillus* species indicating their ethnophytotherapeutic safety.

Background

Sexually transmitted diseases (STDs) are a major cause of morbidity and mortality in developing countries (Mayaud *et al*. 1995), and a growing list of fungi has been implicated in sexually transmissible infections (STI) such as vaginal candidiasis. This is one of the most frequent infections of the female genital tract with high incidence rates. A vast majority of cases are known to be caused by various species of *Candida* (Adad *et al*. 2001, Armstrong-James 2007; Asmundsdottir *et al*. 2002, Colombo *et al*. 2006, Fan *et al*. 2008, Hospenthal *et al*. 2004, Jones 1998, Larone 1995, Mashburn 2006, Pfaller *et al*. 2001). Approximately 75% of sexually active women have been found to suffer at least one episode of *Candida* vaginitis and 10% of them have been reported to have recurrent episodes (Richter *et al*. 2005, Saporiti *et al*. 2001).

In spite of the fact that there is a limited but growing number of antimicrobials that can be used to treat mycotic infections such as candidiasis (Fleck *et* al. 2007, Goa & Barradell 1993, Graybill 1996, Hsueh *et al*. 2005, Kauffman 1994, Ostrosky-Zeichner *et al*. 2003, Pfaller *et al*. 2005), there is rapid increase in the patronage of herbal remedies as an alternative form of therapy in Nigeria, especially among the low and middle class citizenry (Ogunshe & Kolajo 2006; Ogunshe *et al*. 2006; Ogunshe 2007), just as the use of traditional herbal remedies is becoming increasingly popular all over the world (Kofi-Tsekpo 2004, Steenkamp 2003). Since traditional remedies are part of the cultural and religious lives of Africans, which may be attributed to accessibility, affordability (Steenkamp 2003) and ancestral beliefs, several medicinal plants of Africa have been investigated for their chemical components, and while some of the isolated compounds have been shown to possess interesting biological activities, a few

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have been found to be active even in controlled clinical evaluations.

The majority of information about herbs is however, based on experience from their historical use (Kambizi & Afolayan 2001, Vidyasagar & Prashantkumar 2007). It is however, necessary that treatment of any infectious disease should be supported by scientific evidence; therefore, this present pilot study tries to provide information on the ethnophytotherapeutic use of certain extracts of medicinal plants on pathogenic *Candida* species implicated in STI. This paper reports an antimicrobial study of 11 selected medicinal plants that are common among the Nigerian traditional herbal practitioners, in the preparation of herbal remedies for treatment of *Candida* vaginitis and other STIs.

Methods

Sourcing of information

In order to obtain ethnobotanical information on the use of herbal treatments for various male and female reproductive infections and disorders, twenty six traditional herbal practitioners/healers from eight Nigerian states, Lagos, Ogun, Oyo, Osun, Ekiti, Kwara, Edo and Delta states were informally interviewed, especially during national herbal trade fairs. Information on methods of the herbal preparations, doses, durations and local names of the medicinal plants was also obtained.

Medicinal plant materials

Crude aqueous and ethanolic leaf extracts of plant species, A*spilia africana* (Pers.) C.D. Adams, *Newbouldia laevis* (P. Beauv.) Seem. ex Bureau, *Ficus exasperata* Vahl., *Senna alata* (L.) Roxb., *Chromolaena odorata* (L.) R.M. King & H. Rob., *Gliricidia sepium* Kunth ex Steud., *Bixa orellana* L., *Ageratum conyzoides* L., *Anthocleista djalonensis* A. Chev., *Kalanchoe pinnata* (Lam) pers. (formerly *Bryophyllum pinnata*) and *Rauwolfia vomitoria* Afzel., were screened against the test *Candida* and *Lactobacillus* strains. The part of the plant (leaves) used was based on the information obtained from traditional practices of the Nigerian medicinal plant sellers, traditional practitioners and herbalists. Botanical identification of the plant materials was confirmed at the Herbarium of Department of Botany and Microbiology, University of Ibadan, Nigeria and the plant materials were later deposited there.

Preparation of crude plant extracts

The simulated preparations of the leaf samples were according to the methods for the herbal preparations by the traditional herbal practitioners and sellers. The leaves were collected, first washed with tap water and then sterile distilled water. One kilogram of each fresh leaf samples were crushed in a mortar and the plant fluids were then extracted with 0.1 liter of hot, sterile distilled water. The plant extracts were then sieved with sterile Whatman filter paper. Another set of each plant was macerated and extracted in 0.1 liter of 95% ethanol for 24 hours.

Microorganisms

A total of 54 *Candida* and 37 *Lactobacillus* isolates were used in this study. The *Candida* spp. were isolated from high vaginal swabs (HVS) and endocervical swabs (ECS) of patients at the Special Treatment Centre, University College Hospital, (UCH), Ibadan, while the *Lactobacillus* strains were isolated from the human vaginal samples of healthy, pre-menopausal women that had not been on antimicrobial therapy in about six months prior to the collection of the specimens.

Culture media and methods

The *Lactobacillus* spp. were grown on de Man, Rogosa and Sharpe (MRS) medium (Lab M, England). The *Lactobacillus* species from the vaginal specimens from non-diseased subjects were obtained by cutting the swab sticks into modified MRS broth at pH 5.3 - 5.5 and incubating anaerobically in 5% CO_2 (Gas Pak Anaerobic System, Oxoid) at 32°- 35°C for 24-48 hours. The broth cultures were subsequently streaked unto MRS agar and incubated anaerobically in 5% CO_2 (Gas Pak Anaerobic System, Oxoid) at 32°- 35°C for 24-48 hours. Representatives of each different colony type were randomly picked from the primary plates and sub-cultured by repeated streaking onto sterile MRS agar plates to assure purity. The *Lactobacillus* species were characterized based on standard phenotypic taxonomic tools (Antonio *et al*. 1999). The *Lactobacillus* strains were kept in triplicates on MRS agar slants as bench cultures, while the *Lactobacillus* stock cultures were stored in Hogness freezing medium (3.6mM K_2HPO_4; 1.3mM KH_2PO_4; 2.0mM Na-citrate; 1.0mM $MgSO_4$; 12% glycerol) and kept frozen.

The cervical swabs (CS) and high vaginal swabs (HVS) of the female patients were streaked directly unto the prepoured, sterile modified Sabouraud Dextrose Agar (SDA) plates and then incubated aerobically at 30°C for 24-48 hours to isolate the *Candida* species. Representatives of each different colony type were randomly picked from the primary plates and sub-cultured by repeated streaking onto sterile SDA agar plates to assure purity. The *Candida* species were characterized based on standard phenotypic taxonomic tools. The strains were kept in triplicates on SDA agar slants as bench and stock cultures and kept at 4°C.

Antimicrobial bioassay

Each *Candida* and *Lactobacillus* strain was suspended in SDA and MRS broth respectively and incubated at 30°C

and 35°C for 18-24 hours. The crude aqueous and ethanolic extracts of the leaves (500µl and 1000 µl) were screened against the test *Candida* spp. and vaginal *Lactobacillus* spp. and the detection of antagonistic activity was determined by the modified agar spot-diffusion, agar disc-diffusion and agar well-diffusion methods of Tagg *et al*. (1976).

Agar spot-diffusion method

Sterile SDA and MRS were separately poured into each sets of sterile Petri plates and allowed to set at room temperature after which the agar surfaces were inoculated by streaking with 500µl of each *Candida* and *Lactobacillus* strain. Plant extracts of 500µl and 1000µl in which 1% sterile agar was homogenised to prevent spreading of the extracts on the agar surface, were separately spotted (dispensed) into the pre-seeded SDA and MRS agar plates. The plates were then incubated un-inverted at 32°C for the *Candida* and 35°C for the *Lactobacillus* strains for 24-48 hours. Sterile, distilled water incorporated into sterile soft agar served as control. The experiment was performed in duplicates and the mean of zones of inhibition were determined and recorded.

Agar disc-diffusion method

Sterile SDA and MRS were separately poured into each sets of sterile Petri plates and allowed to set at room temperature after which the agar surfaces were inoculated by separately streaking with 500µl of each *Candida* and *Lactobacillus* strains. Sterile 6 mm in diameter sterile filter paper discs were separately impregnated with 500µl and 1000 µl of each plant extracts. The impregnated wet discs were placed on the pre-seeded agar plates and then incubated un-inverted at 32°C for the *Candida* and 35°C for the *Lactobacillus* strains for 24-48 hours. Sterile, distilled water-impregnated discs were used as control. Zones of inhibition were measured and recorded in mm diameter. The experiment was performed in duplicates and the mean of zones were determined and recorded.

Agar well-diffusion method

Sterile SDA and MRS were separately poured into sterile Petri plates and allowed to set at room temperature, after which holes 6mm in diameter were bore into the set agar plates. The agar surfaces were flamed with inverted Bunsen flame and the agar surfaces inoculated separately by streaking with 500µl of each *Candida* and *Lactobacillus* strains. Plant extracts of 500 µl and 1000 µl was separately introduced into the SDA and MRS agar wells. The plates were then incubated un-inverted at 32°C for the *Candida* and 35°C for the *Lactobacillus* strains for 24-48 hours. Sterile, distilled water incorporated into sterile soft agar served control. The experiment was performed in duplicates and the mean number of zones of inhibition were determined and recorded.

Results

Among the crude aqueous extracts of the screened medicinal plants, only *B. orellana* (13.0% inhibition), *R. vomitoria* (3.7% inhibition) and *S. alata* (1.85% inhibition) exhibited very minimal inhibitory activities against the *Candida* species, while other plants displayed no inhibition against the test *Candida* strains. The zones of inhibition by the aqueous leaf extracts of the plants were between 15.0 - 25.0 mm. However, results of Tables 1 to 4 indicated that high inhibitory activities were exhibited against the test *Candida* species by the ethanolic extracts of *A. conyzoides*, *A. djalonensis*, *S. alata*, *F. exasperata*, *B. orellana*, *G. sepium* and *C. odorata*.

Candida albicans (C.P. Robin) Berkhout strains were highly inhibited by *G. sepium* (64.3%), *S. alata* (64.3%) and moderately inhibited by *A. djalonensis* (57.1%), *C. odorata* (57.1%), *N. laevis* (57.1%) and *R. vomitoria* (57.1%). Among the *Candida glabrata* (H.W. Anderson) S.A. Mey. & Yarrow strains, very high inhibitory activities were exhibited by *S. alata* (75.0%), *G. sepium* (75.0%), *A. conyzoides* (62.5%), *A. djalonensis* (62.5%), *C. odorata* (62.5%), *F. exasperata* (62.5%) and *R. vomitoria* (62.5%). *Candida tropicalis* strains were also highly inhibited by *A. conyzoides* (66.7%), *A. djalonensis* (66.7%) and *F. exasperata* (53.3%) while minimally inhibited by *A. conyzoides* (44.4%), *F. exasperata* (44.4%) and *S. alata* (44.4%). None of the strains of *C. pseudotropicalis* was inhibited by the crude ethanolic extracts of *C. odorata* and *K. pinnata* but very minimal to moderate inhibitory activities (11.1 – 44.4%) were exhibited by the other crude ethanolic plant extracts.

With the exception of *A. africana* (24.3%) and *A. conyzoides* (35.1%), very low *in vitro* inhibitory activities between 5.4% and 16.2% were displayed by the medicinal plants against the vaginal *Lactobacillus* species (Table 5).

Discussion

Sexually transmissible Infections (STIs) are currently gaining significant importance due to rapid spread of the diseases, high cost of treatment and increased risk of transmission of other STDs. Meanwhile, vulvovaginal candidiasis has also been reported to be a common cause of vaginitis (Hildago 2006, Spinillo 1992). Several species of *Candida* have been implicated in vulvovaginal candidiasis, which has been and continues to be a major feminine disease. The *Candida* strains which were assayed for in this study were *C. albicans* (25.9%); *C. glabrata* (28.9%); *C. pseudotropicalis* (16.7%) and *Candida tropicalis (*Cas-

Table 1. *In vitro* inhibition of *Candida albicans* strains by 500µl and 1000µl crude ethanolic extracts of 11 traditional Nigerian medicinal plants using modified agar spot-diffusion, well-diffusion and agar disk-diffusion methods. Keys: *Fic = Ficus exasperata*; *Ager = Ageratum conyzoides*; *Chro = Chromolaera odonata*; *Anth = Anthocleista djalonesis*; *Gliri = Gliricida sepium*; *Senn = Senna alata*; *Bixa = Bixa orellana*; *Kalan = Kalanchoe pinnata*; *Newb = Newbouldia levis*; *Rouv = Rauvolfia vomitoria*; *Aspi = Aspilia africana*. Low susceptibility = 10.0 - ≤14.0 mm diameter zone of inhibition; [1]moderate susceptibility (15.0 – 24.0 mm diameter zone of inhibition); [2]high susceptibility (25.0 – ≥35.0 mm diameter zone of inhibition). R = no zones of inhibition or ≤10.0 mm diameter zone of inhibition.

S/N	Laboratory codes of *C. albicans* strains	Ager	Fic	Anth	Chrom	Senna	Gliric	Kalan	Bixa	Rauv	Newb	Aspil
1	*C. albicans* AC1	15.0[1]	R	15.0[1]	28.0[2]	15.0[1]	16.0[1]	R	18.0[1]	R	25.0[2]	17.0[1]
2	*C. albicans* AC2	R	22.0[1]	16.0[1]	R	25.0[2]	15.0[1]	15.0[1]	20.0[1]	18.0[1]	R	R
3	*C. albicans* BC1	R	R	R	R	30.0[2]	18.0[1]	R	12.0	R	R	R
4	*C. albicans* FC1	R	R	R	R	R	R	R	R	R	R	R
5	*C. albicans* FC2	R	R	R	R	R	R	R	R	R	R	R
6	*C. albicans* GC1	R	R	R	R	R	R	R	R	15.0[1]	R	R
7	*C. albicans* GC2	24.0[1]	28.0[2]	15.0[1]	R	17.0[1]	28.0[2]	23.0[1]	28.0[2]	20.0[1]	25.0[2]	R
8	*C. albicans* 1C1	28.0[2]	28.0[**]	32.0[2]	26.0[2]	32.0[2]	30.0[2]	32.0[2]	30.0[**]	32.0[2]	30.0[2]	32.0[2]
9	*C. albicans* 4C2	R	R	30.0[2]	R	30.0[2]	R	R	R	30.0[2]	R	30.0[2]
10	*C. albicans* CA2	R	R	20.0[1]	R	R	R	R	R	15.0[1]	R	20.0[2]
11	*C. albicans* CA13	R	23.0[*]	R	R	R	R	R	R	R	20.0[1]	R
12	*C. albicans* CA23	23.0[1]	R	R	R	20.0[1]	R	30.0[2]	R	30.0[1]	R	R
14	*C. albicans* CA29	25.0[2]	35.0[2]	17.0[1]	R	15.0[1]	R	R	R	R	R	R
15	*C. albicans* CA51	R	12.0	18.0[1]	R	15.0[1]	R	R	R	15.0[1]	10.0	R
Number of susceptibility		5	5	8	2	9	5	4	5	8	5	4
% susceptibility		35.7	35.7	57.1	14.2	64.2	35.7	28.5	35.7	57.1	35.7	28.5

Table 2. *In vitro* inhibition of *Candida glabrata* strains by 500µl and 1000µl crude ethanolic extracts of 11 traditional Nigerian medicinal plantsusing modified agar spot-diffusion, well-diffusion and agar disk-diffusion methods. Keys: *Fic = Ficus exasperata*; *Ager = Ageratum conyzoides*; *Chro = Chromolaera odonata*; *Anth = Anthocleista djalonesis*; *Gliri = Gliricida sepium*; *Senn = Senna alata*; *Bixa = Bixa orellana*; *Kalan = Kalanchoe pinnata*; *Newb = Newbouldia levis*; *Rouv = Rauvolfia vomitoria*; *Aspi = Aspilia africana*. Low susceptibility = 10.0 - ≤14.0 mm diameter zone of inhibition; [1]moderate susceptibility (15.0 – 24.0 mm diameter zone of inhibition); [2]high susceptibility (25.0 – ≥35.0 mm diameter zone of inhibition). R = resistant (no zones of inhibition or ≤10.0 mm diameter zone of inhibition). R = no zones of inhibition or ≤10.0 mm diameter zone of inhibition.

S/N	Laboratory codes of *Candida glabrata* strains	Ager	Fic	Anth	Chrom	Senna	Gliric	Kalan	Bixa	Rauv	Newb	Aspil
1	*C. glabrata* BC2	15.0[1]	18.0[1]	20.0[1]	R	R	16.0[1]	R	16.0[1]	35.0[2]	R	R
2	*C. glabrata* HC	R	R	20.0[1]	15.0[1]	30.0[2]	R	R	R	R	R	15.0[1]
3	*C. glabrata* 1C2	20.0[1]	35.0[2]	28.0[2]	35.0[2]	30.0[2]	30.0[2]	35.0[2]	35.0[2]	30.0[2]	30.0[1]	35.0[2]
4	*C. glabrata* 1TC	R	R	30.0[2]	R	21.0[1]	R	R	R	12.0	R	R
5	*C. glabrata* X1C	20.0[1]	30.0[2]	R	30.0[2]	25.0[2]	30.0[2]	R	30.0[2]	R	R	R
6	*C. glabrata* CA3	R	R	15.0[1]	R	14.0	R	R	R	17.0[1]	R	R
7	*C. glabrata* CA6	10.0	15.0[1]	20.0[1]	R	15.0[1]	R	R	R	25.0[2]	R	35.0[2]
8	*C. glabrata* CA10	R	R	R	R	R	R	R	20.0[1]	R	R	R
9	*C. glabrata* CA12	24.0[1]	28.0[2]	R	R	10.0	R	R	30.0[2]	R	18.0[1]	R
10	*C. glabrata* CA27	25.0[2]	28.0[2]	20.0[1]	R	15.0[1]	R	R	R	17.0[1]	R	R
11	*C. glabrata* CA34	28.0[2]	30.0[2]	R	R	R	R	R	R	R	R	R
12	*C. glabrata* CA36	25.0[2]	35.0[2]	12.0	R	R	R	R	R	R	R	R

S/N	Laboratory codes of *Candida glabrata* strains	Ager	Fic	Anth	Chrom	Senna	Gliric	Kalan	Bixa	Rauv	Newb	Aspil
13	*C. glabrata* CA42	R	35.0[2]	R	R	19.0[1]	R	R	R	25.0[2]	R	R
14	*C. glabrata* CA43	32.0[2]	25.0[2]	R	R	20.0[1]	R	R	R	28.0[2]	R	R
15	*C. glabrata* CA44	R	R	15.0[1]	R	25.0[2]	R	20.0[1]	R	32.0[2]	R	R
16	*C. glabrata* CA61	35.0[2]	R	30.0[2]	R	30.0[2]	R	20.0[1]	R	35.0[2]	R	R
Number of susceptibility		10	10	10	3	12	3	3	5	10	2	3
% susceptibility		62.5	62.5	62.5	18.8	75.0	75.0	18.8	31.2	62.5	12.5	18.8

Table 3. *In vitro* inhibition of *Candida tropicalis* strains by 500µl and 1000µl crude ethanolic extracts of 11 traditional Nigerian medicinal plants using modified agar spot-diffusion, well-diffusion and agar disk-diffusion methods. Keys: *Fic = Ficus exasperata*; *Ager = Ageratum conyzoides*; *Chro = Chromolaera odonata*; *Anth = Anthocleista djalonesis*; *Gliri = Gliricida sepium*; *Senn = Senna alata*; *Bixa = Bixa orellana*; *Kalan = Kalanchoe pinnata*; *Newb = Newbouldia levis*; *Rouv = Rauvolfia vomitoria*; *Aspi = Aspilia africana*. Low susceptibility = 10.0 - ≤14.0 mm diameter zone of inhibition; [1]moderate susceptibility (15.0 – 24.0 mm diameter zone of inhibition); [2]high susceptibility (25.0 – ≥35.0 mm diameter zone of inhibition). R = resistant (no zones of inhibition or ≤10.0 mm diameter zone of inhibition). R = no zones of inhibition or ≤10.0 mm diameter zone of inhibition.

S/N	Laboratory codes of *C. tropicalis* strains	Ager	Fic	Anth	Chrom	Senna	Gliric	Kalan	Bixa	Rauv	Newb	Aspil
1	*C. tropicalis* 2TC	R	25.0[2]	R	10.0	R	R	R	R	R	20.0[1]	R
2	*C. tropicalis* 4C1	R	15.0[1]	20.0[1]	R	R	18.0[1]	R	R	25.0[2]	20.0[1]	20.0[1]
3	*C. tropicalis* 6C	21.0[1]	18.0[1]	18.0[1]	R	15.0[1]	14.0	R	R	R	20.0[1]	10.0
4	*C. tropicalis* 6C1	10.0	R	10.0	30.0[2]	20.0[1]	R	R	25.0[2]	18.0[1]	22.0[*]	18.0[1]
5	*C. tropicalis* 10C	20.0[1]	R	20.0[1]	R	30.0[2]	R	R	20.0[1]	15.0[1]	R	20.0[1]
6	*C. tropicalis* CA4	35.0[2]	R	20.0[1]	R	14.0	R	R	R	R	R	R
7	*C. tropicalis* CA8	35.0[2]	R	23.0[1]	R	30.0[2]	R	15.0[1]	20.0[1]	35.0[2]	R	R
8	*C. tropicalis* CA9	R	R	R	R	R	R	R	R	R	R	R
9	*C. tropicalis* CA14	24.0[1]	24.0[1]	20.0[1]	R	30.0[2]	R	R	R	R	R	R
10	*C. tropicalis* CA20	25.0[2]	25.0[2]	R	R	R	R	R	R	R	R	R
11	*C. tropicalis* CA26	24.0[1]	28.0[2]	20.0[1]	R	15.0[2]	R	R	R	17.0[1]	R	R
12	*C. tropicalis* CA28	32.0[2]	30.0[2]	R	R	R	R	R	R	R	R	R
13	*C. tropicalis* CA31	R	30.0[2]	23.0[1]	R	R	R	R	R	25.0[2]	R	R
14	*C. tropicalis* CA52	20.0[1]	R	28.0[2]	R	20.0[1]	R	23.0[1]	R	35.0[2]	R	R
15	*C. tropicalis* CA53	R	R	R	R	20.0[1]	R	R	R	R	R	R
Number of susceptibility		10	8	10	2	9	2	2	3	7	4	4
% susceptibility		66.7	53.3	66.7	13.3	60.0	13.3	13.3	20.0	46.7	26.7	26.7

tell.) Berkhout (27.8%). Similar *Candida* species had earlier been obtained from vaginal samples in cases of vaginitis (Buscemi *et al*. 2004, Konje *et al*. 1991, Saporiti *et al*. 2001, Verghese *et al*. 2001, Vráblik *et al*. 2007).

Some previous studies on antifungal drugs indicated that most of the antimycotic drugs have one or more limitations, such as profound side effects, a narrow antifungal spectrum, poor penetration of certain tissues, development of resistance (Bastert 2001, Kauffman 1994), as well as toxicity. Therefore, new approaches to the design of novel drugs seem to be necessary, more so, since many patients of STIs are seeking help from alternative systems of medicines (Vermani & Garg 2002). The use of traditional medicine was also observed to be widespread (Ogunshe *et al*. 2006, Ogunshe 2007) and prevalent over orthodox medicine in many parts of Nigeria (Igoli *et al*. 2004) due to several problems associated with the primary health care delivery systems in the country, as well as the increased

Table 4. *In vitro* inhibition of *Candida pseudotropicalis* strains by 500µl and 1000µl crude ethanolic extracts of 11 traditional Nigerian medicinal plants using modified agar spot-diffusion, well-diffusion and agar disk-diffusion methods. Keys: *Fic* = *Ficus exasperata*; *Ager* = *Ageratum conyzoides*; Chro = Chromolaera odonata; Anth = *Anthocleista djalonesis*; *Gliri* = *Gliricida sepium*; *Senn* = *Senna alata*; *Bixa* = *Bixa orellana*; *Kalan* = *Kalanchoe pinnata*; *Newb* = *Newbouldia levis*; *Rouv* = *Rauvolfia vomitoria*; *Aspi* = *Aspilia africana*. Low susceptibility = 10.0 - ≤14.0 mm diameter zone of inhibition; [1]moderate susceptibility (15.0 – 24.0 mm diameter zone of inhibition); [2]high susceptibility (25.0 – ≥35.0 mm diameter zone of inhibition). R = no zones of inhibition or ≤10.0 mm diameter zone of inhibition.

S/N	Laboratory codes of *C. pseudotropicalis* strains	Ager	Fic	Anth	Chrom	Senna	Gliri	Kalan	Bixa	Rouv	Newb	Aspi
1	*C. pseudotropicalis* 2C1	R	R	R	R	R	R	R	R	R	R	R
2	*C. pseudotropicalis* 2C2	R	R	20.0[1]	R	25.0[2]	R	R	25.0[2]	R	R	R
3	*C. pseudotropicalis* 6C2	R	R	R	R	R	R	R	R	R	R	R
4	*C. pseudotropicalis* 9C	R	20.0[1]	20.0[1]	R	R	12.0	R	R	R	20.0[1]	20.0[1]
5	*C. pseudotropicalis* X7C	19.0[1]	24.0[1]	R	R	R	22.0*	R	16.0[1]	R	R	R
6	*C. pseudotropicalis* CA16	25.0[2]	23.0[1]	R	R	15.0[1]	R	R	R	R	R	20.0[1]
7	*C. pseudotropicalis* CA25	23.0[1]	23.0[1]	R	R	R	R	R	R	R	R	R
8	*C. pseudotropicalis* CA48	R	R	R	R	12.0	R	R	15.0[1]	R	R	R
9	*C. pseudotropicalis* CA65	20.0[1]	R	25.0[2]	R	12.0	R	R	R	10.0	R	R
Number of susceptibility		4	4	3	0	4	2	0	3	1	1	2
% susceptibility		44.4	44.4	33.3	0.0	44.4	22.2	0.0	33.3	11.1	11.1	22.2

Table 5. *In vitro* antifungal susceptibility profiles of *Candida* species implicated in sexually transmissible infections using antifungal agents. Keys: CNSTT = canesten tablet; CNSTC = canesten cream; = FLGY = flagyl; MYCS = mycostatine; INTZ = interzol; FLMD = flucamed; MYCT = mycoten tablet; MYCC = mycoten cream; TRDX = tetradox.

	% Antifungal susceptibility profiles								
Pathogens	**MYCT**	**MYCC**	**CNSTT**	**CNSTC**	**FLMD**	**TRDX**	**INTZ**	**MYCS**	**FLGY**
Candida albicans	92.3	88.4	88.4	88.4	96.1	88.4	96.1	100.0	84.6
Candida glabrata	33.3	33.3	27.8	5.6	-	11.1	33.3	38.9	5.6
Candida tropicalis	33.3	33.3	27.8	5.6	-	11.1	33.3	38.9	5.6
Candida pseudotropicalis	27.5	30.0	27.5	-	2.5	2.5	25.0	20.0	10.0

beliefs in the potency of the herbal remedies due to series of classical advertisements by the various traditional herbal practitioners (Ogunshe 2007).

Phytotherapeutic studies on candidal vaginitis in Nigeria has been less studied and therefore, very limited documented information (Buwa & van Staden 2006) is available on the use of certain extracts of medicinal plants on pathogenic *Candida* species associated with sexually transmissible infections. However, information from the herbal practitioners, medicinal plant sellers and the local populace indicated the use of some plants for the treatment of vaginal infections in Nigeria. It was observed in this study that *F. exasperata*, *A. conyzoides*, *A. djalonensis*, *S. alata* and *R. vomitoria* exhibited the most pronounced inhibitory activities against the *Candid*a species *in vitro*. The findings of this present study also indicated that the ethanolic crude extracts of the plants were more inhibitory towards the *candida* species than the aqueous extracts.

Ageratum conyzoides L., Asteraceae, is an annual herbaceous plant (Menut *et al*. 1993, Ming 1999) of valuable agricultural resources and a long history of traditional medicinal uses in several countries of the world. It is widely utilized in folk/traditional medicinal practices and pharmacological studies by various cultures in Central Africa (Durodola 1977), traditional communities in India (Borthakur & Baruah 1987), and in Asia, South America, and Africa (Almagboul *et al*. 1985), although uses vary by regions. It was also reported by Lans (2007) that the plant is useful in the treatment of prostrate problems and venereal diseases. The results obtained in this study therefore, confirmed that *A. conyzoides* has strong *in vitro* anti-candidal activities against *Candida* strains implicated in sexually transmissible infection.

Table 6. *In vitro* inhibition of vaginal *Lactobacillus* strains by 500µl and 1000µl crude ethanolic extracts of 11 traditional Nigerian medicinal plants using modified agar spot-diffusion, well-diffusion and agar disk-diffusion methods. Keys: *Fic* = *Ficus exasperata*; *Ager* = *Ageratum conyzoides*; *Chro* = *Chromolaera odonata*; *Anth* = *Anthocleista djalonesis*; *Gliri* = *Gliricida sepium*; *Senn* = *Senna alata*; *Bixa* = *Bixa orellana*; *Kalan* = *Kalanchoe pinnata*; *Newb* = *Newbouldia levis*; *Rouv* = *Rauvolfia vomitoria*; *Aspi* = *Aspilia africana*. Low susceptibility = 10.0 - ≤14.0 mm diameter zone of inhibition; [1]moderate susceptibility (15.0 – 24.0 mm diameter zone of inhibition); [2]high susceptibility (25.0 – ≥35.0 mm diameter zone of inhibition). R = no zones of inhibition or ≤10.0 mm diameter zone of inhibition.

S/N	Laboratory codes of *Lactobacillus* strains	Fic	Ager	Chrom	Anth	Gliric	Senna	Bixa	Kalan	Newb	Rauv	Aspil
1	*L. casei* VL	R	R	R	R	R	R	28.0[2]	R	R	R	R
2	*L. plantarum* VL2	R	R	R	R	R	R	R	25.0[2]	R	R	35.0[2]
3	*L. delbrueckii* VL3	R	22.0[1]	R	R	R	R	R	R	R	R	R
4	*L. brevis* VL5	R	27.0[2]	R	R	R	R	R	R	R	R	R
5	*L. fermentum* VL6	R	12.0	R	35.0[2]	R	R	R	R	10.0	10.0	R
6	*L. plantarum* VL7	R	R	R	R	R	R	R	R	R	R	R
7	*L. fermentum* VL9	R	R	R	R	R	R	R	R	R	30.0[2]	20.0[1]
8	*L. acidophilus* V12	R	R	R	25.0[2]	R	20.0[1]	R	R	R	R	25.0[2]
9	L. *casei* VL122	R	R	R	28.0[2]	R	R	R	R	R	R	28.0[2]
10	L. *fermentum* VL13	R	R	R	R	R	R	R	R	R	R	30.0[2]
11	*L. acidophilus* VLX4	R	28.0[2]	R	R	R	R	R	R	R	R	R
12	*L. casei* VL15	R	25.0[2]	R	R	R	R	R	R	R	R	30.0[2]
13	*L. fermentum* VL16	R	10.0	R	R	R	R	R	R	R	R	R
14	*L. acidophilus* VL18	R	25.0[2]	R	R	R	R	R	R	R	R	20.0[1]
15	*L. acidophilus* VL18	R	30.0[2]	R	R	R	R	R	25.0[2]	R	R	R
16	*L. fermentum* VL20	R	30.0[2]	R	R	R	R	R	R	R	R	R
17	*L. fermentum* VL20	R	R	R	R	R	R	R	R	R	R	35.0[2]
18	*L. plantarum* VL21	R	20.0[1]	R	R	R	20.0[1]	20.0[1]	20.0[1]	R	20.0[1]	R
19	*L. fermentum* VL22	R	R	R	R	R	R	R	R	R	R	R
20	*L. acidophilus* VL222	R	20.0[1]	R	R	R	R	20.0[1]	20.0[1]	R	R	R
21	*L. reuteri* VL23	R	30.0[2]	R	R	R	R	30.0[2]	30.0[2]	18.0[1]	18.0[1]	R
22	*L. reuteri* VL225	R	R	R	R	R	R	R	R	20.0[1]	20.0[1]	R
23	*L. acidophilus* VL27	R	R	R	R	R	R	R	R	R	R	R
24	*L. casei* VL28	R	R	R	R	R	R	R	R	R	R	20.0[1]
25	*L. reuteri* VL29	R	R	R	R	R	R	R	R	R	R	R
26	*L. reuteri* VL30	R	R	R	R	R	R	R	R	R	R	R
27	*L. casei* VL32	R	R	R	R	R	R	R	R	R	R	R
28	*L. casei* VL34	R	R	R	R	R	R	R	R	R	R	R
29	*L. acidophilus* VL35	R	R	R	R	R	R	R	R	R	R	R
30	*L. plantarum* VL36	R	R	R	R	R	R	R	R	R	R	R
31	*L. acidophilus* VL54	R	R	R	R	R	R	R	R	R	R	R
32	*L. casei* VL562	R	R	R	R	R	R	R	R	R	R	R
33	*L. casei* VL59	R	R	R	R	R	R	R	R	R	R	R
34	*L. plantarum* VLB	R	R	R	R	R	R	R	R	R	R	R
35	*L. plantarum* VLF	R	R	R	R	R	R	R	R	R	R	R
36	*L. casei* VLH	R	22.0[1]	R	R	R	R	R	28.0[2]	R	R	R

S/N	Laboratory codes of *Lactobacillus* strains	Fic	Ager	Chrom	Anth	Gliric	Senna	Bixa	Kalan	Newb	Rauv	Aspil
37	*L. casei* VLR	R	R	R	R	R	R	R	R	R	R	R
Number of susceptibility		0	13	0	3	0	2	4	6	3	5	9
% susceptibility		0.0	35.1	0.0	8.10	0.0	5.40	10.8	16.2	8.10	13.5	24.3

Similarly, *S. alata* (L.) Roxb. (syn. *Cassia alata*) leaves have been selected and recommended for the treatment of dermatomycotic infections (Phongpaichit *et al*. 2004, Thirach *et al*. 2003, Wuthi-udomlert *et al*. 2003;). Crude ethanolic and water extracts of leaves and barks from the plant were tested *in vitro* against *Aspergillus fumigatus* Fresen. and *Microsporum canis* E. Bodin ex Guég, *C. albicans*, *Staphylococcus aureus* and *Escherichia coli* by Somchit *et al*. (2003). The extracts were also reported to be traditionally used in Ivory Coast, West Africa to treat bacterial infections caused by *E. coli* and fungal infections caused by *C. albicans* and some dermatophytes (Crockett *et al* 1992). It was also observed in this study that the inhibitory effects of ethanolic leaf extracts of *F. exasperata*, *A. conyzoides*, *A. djalonesi*s, *S. alata* and *R.vomitoria* plants were comparable with those of standard antifungals.

There is a classic view that the vagina of healthy women is colonized by a homogeneous population of *Lactobacilli* (Balow *et al*. 1990) and the predominance of *Lactobacillus* species present in the vagina has been found to confer protective activities against urinary tract infections. For example, adherence of *Lactobacillus acidophilus* to the vaginal cell wall has been known to block the attachment of uropathogenic bacteria to the surface of the uroepithelial cells. The prevention and control of infections by *Lactobacillus* species is due to the production of some metabolites such as bacteriocins, aminoglycosides, hydrogen peroxide, diacetyl, and lactic acid, which act as an inhibitory barriers against some pathogenic microorganisms (Boris and Barbés 2000, Falagas *et al*. 2006).

Most of the previous ethnobotanical/ethnomedicinal studies usually considered the antimicrobial activities of medicinal plants on pathogenic flora without considering the effect of the medicinal plants on other microflora that inhabit the same ecological niche where the plants may be effective *in vivo*. Combining the strong *in vitro* inhibitory activities of the selected medicinal plants on the *Candida* strains, and the less inhibitory activities of the plant extracts on the vaginal *Lactobacillus* strains, it can be deduced that *A. djalonensis*, *R. vomitoria*, *F. exasperata* and *S. alata* are the best potentials in the ethnophytotherapeutic treatment of STI caused by pathogenic *Candida* strains.

Collaborative or multidisciplinary studies would assist in this recently noted area of concern, so that, while potent medicinal plants are curing some infections, they are not creating certain conditions for opportunistic infections. For example, if the vaginal *Lactobacilli* are eliminated by any of the medicinal plants during phytotherapy, the candidal flora would be on the increase, thereby creating an enabling environment for the proliferation of opportunistic *Candida* in the vagina and other urogenital pathogenic bacterial strains, which can ultimately lead to vaginal candidasis and bacterial vaginitis.

Conclusions

Historical attachments to indigenous therapy in Nigeria cannot be overlooked, and this is an additional reason for the increase in the higher patronage of traditional (phytotherapy) in the country. A good understanding of peoples' choice of ethnophytotherapy would therefore, support insight into the need for research-based studies on medicinal plants that are very popular among different nations. Scientific support based on research findings pending clinical trials on which plants are effective is therefore compulsory, since many Nigerians still depend largely on medicinal plants as complementary or alternative form of therapy in diseased conditions. This study concludes that simulated ethanolic preparations of *A. djalonesis*, *R. vomitoria*, *F. exasperata* and *S. alata* had very minimal inhibitory effects on the vaginal *Lactobacillus* species, indicating their ethnophytotherapeutic safety.

Further studies are in progress to determine the comparative inhibitory effects of the traditional simulated and laboratory extraction methods of the plants on the *Candida* species and some other clinical pathogens implicated in STI infections. This nature of research study will aid in preserving the indigenous knowledge of ethnobotany in relation to scientific findings, so as to improve the human public health importance, especially from the phytotherapeutic point of view.

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