

## ANTIFUNGAL EFFECT OF HENNA (*LAWSONIA INERMIS* ) EXTRACT ON PATHOGENIC FUNGI

### Abstract

The study aimed at undertaking preliminary phytochemical studies and antifungal activities of *Lawsonia inermis* leaf extracts against clinical *Candida* isolates from female patients attending Dalhatu Araf Specialist Hospital (DASH) Lafia, Nasarawa State. HVS (High Vaginal Swab) samples were collected from 185 subjects and transported to the laboratory for analysis. Microbial culture and isolations were done on Sabouraud dextrose agar (SDA), Blood agar, Potato dextrose agar (PDA) and Sabouraud dextrose broth. Identification of clinical isolates was done following standard guideline for *Candida* identification including microscopic, cultural and biochemical characteristics (sugar utilization and fermentation). Antifungal susceptibility tests of the plant extracts at different concentrations were carried out against *Candida* isolates. Distilled water and ketoconazole drug served as negative and positive control respectively. Zones of inhibitions, the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of the extracts were determined. Data were analysed on the Minitab 16.0 software for descriptive (mean with standard error) and inferential statistics and Chi Square at 95% confidence limit. In conclusion, *L.inermis* leaf has been shown to have antifungal properties since it contained quality phytochemicals in sufficient quantity that may be explored in the synthesis of drugs against some species of *Candida*. This finding is crucial in the management and control of candidiasis in the study.

**Keywords:** *Lawsonia inermis*, *Candida spp.*

### INTRODUCTION

Plants are the primary source of medicine and they provide a potent channel in the search for new drugs (Shah *et al.*, 2006). Plants and their organs house many active ingredients which are

precursors in drug synthesis Therefore, medicinal plants are important in pharmacological research and drug development (Adebolu and Oladimeji, 2005; Aguoru *et al.*, 2015a; Shah *et al.*, 2006).

*L. inermis* is a perennial shrub native to tropical and sub-tropical regions of Africa, Southern Asia, Northern Australia and semi-arid zones. The plant has been introduced to other parts of the world as exotic plants with high invasiveness and tolerance to extreme conditions (Aguoru *et al.*, 2015b; Rao *et al.*, 2005). These properties make the plant suitable for research and further exploitation in both cosmetic and pharmaceutical industries. One of the challenges in public health today most especially in Nigeria and other Tropical countries is the high prevalence of infections of bacterial or fungal or protozoan or helminthic origin (Adebolu and Oladimeji, 2005; Elham and Elbasheir, 2014; Hassan *et al.*, 2004; Umeh *et al.*, 2005). One of the recalcitrant fungal infections is candidiasis caused by many species of *Candida* although *C. albicans* is considered the most clinically important pathogens among *Candida* species (Manolakaki *et al.*, 2010). Many species are harmless as normal commensals in humans especially on mucosal surfaces of the gastrointestinal and genital tracts but they are opportunistic pathogens that are infectious when opportunity present itself (Kourkoumpetis *et al.*, 2011).

An antimicrobial is an agent or a substance sourced from plants, animal and even microorganisms that have the ability to kill microorganisms or pathogens inhibit their growth (Ajayi *et al.*, 2008; Prescott *et al.*, 2002). Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterial are used against bacteria and antifungal are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbiocidal, while those that merely inhibit their growth are called microbiostatic (Ajayi *et al.*, 2008; Prescott *et al.*, 2002). In

1928, Alexander Fleming became the first to discover a natural antimicrobial fungus known as *Penicillium rubens*. He named the substance extracted from the fungus penicillin and in 1942 it was successfully used to treat a *Streptococcus* infection. Penicillin also proved successful in the treatment of many other infectious diseases such as gonorrhoea and pneumonia, which were potentially fatal to patients. Infectious diseases have continued to be a major concern for many nations all over the world, with the current increasing trends of multi-drugs resistance among emerging and re-emerging fungal pathogens to the available modern drugs or antibiotics (Prescott *et al.*, 2002).

The potential medicinal benefits of *Lawsonia inermis* have received attention in pharmaceutical biotechnology (Abdelgadir *et al.*, 2010; Aguwa, 2008; Badoni *et al.*, 2014; Erzincan, 2017). The plant is commonly known as henna, mignonette and Egyptian privet in English. In Nigeria, it is called Laali in Yoruba and Leeli in Hausa (Aguwa, 2008). The plant was reported to be used in the treatment of ulcer, anaemia, diabetes, jaundices and other ailments in India (Chauhan *et al.*, 2007). Ancient history of India describes diverse uses and appreciable role of henna in natural health medicines (Chauhan *et al.*, 2007). Henna is also well recognized as a medicinally valuable plant in other parts of the world (Borade *et al.*, 2011; Badoni *et al.*, 2014; Ali *et al.*, 2015). Varghese *et al.* (2010) described the plant as a natural dye plant of various therapeutic roles in medicine. Currently, pharmacological claims exist that *L. inermis* possesses antiviral, antibacterial, antifungal, anti-inflammatory, tuberculostatic and anticarcinogenic activities (Varghese *et al.*, 2010). In Nigeria, most especially in the Northern parts, henna plant is commonly used among women for body beautification as part of their culture. The dye obtained from the plant may be fixed on finger nails, faces, palms, hands, legs, stomach and any other part

of the body (Aguoru *et al.*, 2015b). The dye is also used in other parts of the world as part for cosmetic purposes as traditional symbols of identity (Rao *et al.*, 2005).

Many infections most especially vaginal candidiasis with high prevalence rate among women have been implicated in cases of infertility, early miscarriages and damages to the female reproductive system (Kourkoumpetis *et al.*, 2011). Drugs that are effective against pathogenic *Candida* are either expensive or being resisted by the pathogen (Cazarolli *et al.*, 2008; Kourkoumpetis *et al.*, 2011).

A major task in public health today is to search for cheap, readily available plants as sources of highly effective antifungal drugs. It is important to continuously undertake studies on the antimicrobial activities of plants in the quest for potent antimicrobial plants that can effectively combat many human pathogenic microbes including *Candida* without any form of resistance. Therefore, this research attempted to study the antifungal efficacy of *L. inermis* leaf extracts against clinical *Candida* isolates from female patients with cases of infertility currently undergoing treatments at the gynecology unit of DASH Hospital in Lafia, Nasarawa State, Nigeria.

## **Material and Method**

### **Study Area**

Nasarawa State lies within the Guinea savannah region of Central Nigeria with a total land area of 27,116.8 km<sup>2</sup>. It covers an area of 258km, extending an area of 18km from North to South, and 14km from East to West. It is 195km from Jos (in the North) and 90km from Makurdi (in the South). The study location, Dalhatu Araf Specialist Hospital Lafia, is located in Lafia Central part of the town.

## **Study Design and Sampling**

A cross sectional study design was carried out among female patients attending the Gynecology unit of the Dalhatu Araf Specialist Hospital Lafia (DASH). The hospital was specifically chosen due to its high level of patronage among women, referral cases from other hospitals on gynecological cases, quality service delivery and state of the earth facilities

## **Ethical approval**

Ethical approval was obtained from the Hospital ethical committee. Informed consent was also obtained from each participant prior to commencement of the study.

## **Clinical Isolates**

The stock isolates for this study were obtained from female patients attending Dalhatu Araf Specialist Hospital Lafia, Nasarawa State. Samples of vaginal discharge in HVS were collected using sterile disposable speculum as described by Erzincan (2017). All collected samples were accompanied by data involving patients' age, sex, sample and code in addition to date of sample collection.

## **Leaf extraction procedures**

Leaf extraction (both fresh and dried leaves) was obtained using cold maceration method as described in Umeh *et al.* (2005). Fifty (50g) of the powdered and fresh milled plant material (leaves) was weighed into clean sterile bottles separately. The weighed-out leaves plant was extracted using 250ml of water and ethanol separately in a tightly covered bottle and left for 48hours at room temperature. The resultant suspensions were filtered into sterile beakers, and filtrates collected were re-filtered using Whatmans No. 1 filter paper into sterile sample bottles. They were labeled appropriately and stored in plastic bags at -20°C for further analyses (Umeh *et al.*, 2005).

### **Preparation of stock drug solution (ketoconazole)**

Stock drug solution (1280 mg/liter) was prepared by adding 50 ml of dimethyl sulfoxide to 64 mg of ketoconazole and was allowed to stand for 30 minutes. It was then dispensed in small amount and stored at -20°C (Warnock, 2007).

### **Determination of minimum inhibitory concentration (MIC) of extract**

Theoretically, MIC is the lowest concentration of antimicrobial agent that inhibits growth which are determined visually (show turbidity) after a standard incubation period of 18-24h at 35°C (Warnock, 2007). MIC was taken as measurement parameter to quantify the effects of antifungal agents. Based on the preliminary screening, the extract that revealed potent antifungal activity was further tested to determine the minimum inhibitory concentration (MIC) for each fungal sample. The MICs of these extracts was determined by Sabouraud broth micro dilution method (Warnock, 2007). Zones of inhibitions of the extracts at different concentrations were measured using the metre rule in milimetre. Inhibition (susceptibility) and resistance level of the treatment levels were noted.

### **Determination of minimum fungicidal concentration (MFC)**

The MFC was taken as the lowest concentration of the extract that showed no growth after a sub-culture showed no growth in the MIC test. In order words, sub-culturing of the preparations made on Sabouraud dextrose agar plate that showed no evidence of fungal growth in the MIC determination assay was taken as the minimum fungicidal concentration (MFC) as described by Warnock (2007).

## Statistical Analysis Data Analysis

Data were analysed on the Minitab 16.0 software. Quantitative data were subjected to descriptive and inferential statistics. One Way ANOVA, T-tests and Chi Square were applied appropriately at ( $P > 0.05$ ) was considered significance.

### Sample size

Sample size was determined using a conventional sample size calculator adopted in Emeribe *et al.* (2015) as given below:

$$n = \frac{Z^2 pq}{e^2}$$

n= sample size,

Z= the value of the z-table at 95% with confidence interval of 1.96

p=prevalence of *Candida albicans* infection among women at 14% (0.14) (Emeribe *et al.*, 2015)

q= 1-p

d= sampling error or degree of precision at 5%

$$n = \frac{1.96^2 \times 0.14 (0.86)}{0.05^2} = \frac{3.8416 \times 0.1204}{0.0025} = \frac{0.4625}{0.0025} \quad n = 185$$

## RESULT

Prevalence of pathogenic *Candida* (*C. albicans*) and the age distribution of female patients with vaginal candidiasis. A total of 72 females were infected resulting in prevalence of 38.9% out of the total sample size of 185 female patients. Among them, 28.1% were less than 20 years of age while 23.6% were between 31 and 40 years. 20 and years had high Infection (52.8%). *C. albicans* infection was associated with age group ( $\chi^2 = 61.1944$ ,  $P < 0.05$ ) as shown in table 1.

Table 2 gives the results of quantitative phytochemical analysis of ethanolic leaf extracts of *L. inermis*. Tannin concentration recorded the highest value among the phytochemicals quantified

with an average value of  $5.21 \pm 0.01$  while glycoside had the lowest amount recorded with an average of  $0.21 \pm 0.00$ . Alkaloid ranged from 2.40% to 2.46% with a mean of  $2.44 \pm 0.02$ . Terpenes ranged from 2.15% to 2.26% with a mean of  $2.21 \pm 0.03$ . Flavonoid ranged from 0.31% to 0.36% with a mean of  $0.34 \pm 0.01$ . Reducing sugar had an average of  $1.13 \pm 0.01$  ranging from 1.12% to 1.14%. Steroid and anthraquinone had average values of  $0.33 \pm 0.00$  and  $0.45 \pm 0.01$  respectively. Saponin ranged from 1.30% to 1.34% with a mean of  $1.32 \pm 0.01$ . Differences recorded in the quantity of phytochemicals in the *L.inermis* leaf were significant ( $F= 12121.96$ ,  $P<0.05$ ). Tannin, alkaloid and terpenes were the first three highest active ingredients while flavonoid, steroid and glycoside were the least three active ingredients present (Table 3). The hierarchical relationship is given below:

Table 3 showed the zones of inhibition and the minimum inhibitory concentration (MIC) of fresh and dried ethanolic extracts of *L. inermis* at different concentrations against *Candida* isolates. FLEE (Fresh leaf ethanolic extract) at 500mg/ml gave the highest zone of inhibition of 30mm with a mean value of  $28.7 \pm 0.88$  while DLEE (Dry leaf ethanolic extract) of the same concentration gave its highest zone of inhibition of 28mm with an average of  $23.7 \pm 2.33$  inhibition. At 250mg/ml, FLEE and DLEE yielded average zones of inhibition of  $20.0 \pm 0.58$  and  $18.0 \pm 0.56$  respectively. Similar result was observed in the positive control (ketoconazole) which gave 15mm zone of inhibition at 200mg/ml. At 125mg/ml of leaf extract, zone of inhibition was  $16.7 \pm 1.20$  in FLEE and  $15.0 \pm 1.15$  in DLEE. From the results FLEE gave higher values than DLEE on the average of inhibition capacity as shown in Table 4. Also zones of inhibition reduced as the extract concentration reduced. The minimum concentration of extract where inhibition was observed was 62.5mg/ml. Here, FLEE and DLEE gave average zone of inhibition of  $7.0 \pm 0.58$  and  $5.0 \pm 1.00$  respectively. No inhibition was observed at extract concentration

below 62.5mg/ml in both FLEE and DLEE. Also, no inhibition was recorded under distilled water employed as negative control. No significant difference was observed in the inhibitory capacity of FLEE and DLEE as both extract types gave similar results ( $T= 0.24$ ,  $P= 0.813$ ,  $P>0.05$ ). However, significant positive relationship was established between extract concentration and inhibition in FLEE ( $r =+0.945$ ,  $P<0.05$ ) and DLEE ( $r =+0.932$ ,  $P<0.05$ ). In the two types of extracts, coefficients of determination  $R^2$  were 89.3% and 86.9% respectively.

Table 4 revealed the susceptibility and resistance of five species of *Candida* to different concentrations of the ethanolic extracts of *L. inermis*. *Candida albicans* tested and identified were 72 isolates. Extract concentration of 250mg/ml gave the highest percentage of inhibition at 27.8% and 15.6mg/ml followed by 25% inhibited. Three (3) isolates showed resistance at 7.8mg/ml with 4.2%. The species of *C. glabrata* identified and tested were 45 isolates out of which 8 isolates (17.8%) showed resistance at the lowest extract concentration. 15 (33.3%) and 10 (22.2%) of the isolates were inhibited by 250mg/ ml of the extract while 62.5mg/ml of the extracts were also inhibited respectively. A total of 30 isolates were identified as *C. Krusei*. Out of these, 19 (63.3%) showed resistance at the lowest concentrations while 250mg/ml inhibited 11 (36.7%) of the isolates. There were 24 *C. parapsilosis* identified and tested. Out of these 29.2% were inhibited by 62.5mg/ml of the extract while 20.8% were inhibited at 250mg/ml. only 8.3% of the isolates showed resistance to the extract at the lowest concentration used (7.8mg/ml). *C. tropicalis* isolates were 14 in number out of which 28.6% showed resistance at the lowest concentration used but 35.7% were inhibited by 125mg/ml of the extract.

From the 185 *Candida* species isolated, 52(29.2%) were inhibited by 250mg/ml as the highest inhibition recorded followed by 32 (17.3%) isolates inhibited by 62.5mg/ml of the extracts. The

lowest concentration of 7.8mg/m resulted in 36 (19.5%) isolated which showed resistance. Using Chi square analysis, resistance and susceptibility to extracts were significantly associated with specific *Candida* species ( $\chi^2 = 9.87065$ ,  $P= 0.043$ ,  $P<0.05$ ) and the extraction concentration used ( $\chi^2 = 241.497$ ,  $P= 0.000$ ,  $P<0.05$ ).

**Table 1: Prevalence of *C. albicans* Vaginal Candidiasis and Age Distribution of Patients**

Age (years)	Number	Percentage from <i>C. albicans</i> infected subjects (n=72) (%)	Percentage from total sample size (n=185) (%)
<20	13	18.1	7.03
20-30	38	52.8	20.54
31-40	17	23.6	9.19
41-50	4	5.6	2.16
>50	0	0	0
Total	72		38.92

$\chi^2$  (age and Infection) = 61.1944,  $P=0.000$ ,  $P<0.05$

**Table 2: Quantitative Phytochemical Analysis of *L. inermis* Ethanolic Leaf**

	Alk	Flav	Reds	Glyc	Phen	Sap	Tan	Ste	Terp	Anth
Rep 1	2.45 <sup>b</sup>	0.31 <sup>h</sup>	1.14 <sup>f</sup>	0.21 <sup>i</sup>	1.89 <sup>d</sup>	1.30 <sup>e</sup>	5.21 <sup>a</sup>	0.32 <sup>h</sup>	2.15 <sup>c</sup>	0.47 <sup>g</sup>
Rep 2	2.40 <sup>b</sup>	0.36 <sup>h</sup>	1.13 <sup>f</sup>	0.2 <sup>i</sup>	1.89 <sup>d</sup>	1.32 <sup>e</sup>	5.20 <sup>a</sup>	0.33 <sup>h</sup>	2.26 <sup>c</sup>	0.44 <sup>g</sup>
Rep 3	2.46 <sup>b</sup>	0.34 <sup>h</sup>	1.12 <sup>f</sup>	0.21 <sup>i</sup>	1.93 <sup>d</sup>	1.34 <sup>e</sup>	5.23 <sup>a</sup>	0.33 <sup>h</sup>	2.22 <sup>c</sup>	0.44 <sup>g</sup>
	2.44 <sup>b</sup>	0.34 <sup>h</sup>	1.13 <sup>f</sup>	0.21 <sup>i</sup>	1.90 <sup>d</sup>	1.32 <sup>e</sup>	5.21 <sup>a</sup>	0.33 <sup>h</sup>	2.21 <sup>c</sup>	0.45 <sup>g</sup>
Mean										
S.E	±0.02	±0.01	±0.01	±0.00	±0.01	±0.01	±0.01	±0.00	±0.03	±0.01

Means that do not share a letter are significantly different

F= 12121.96,  $P= 0.000$  ( $P<0.05$ )

**Legend:**

Alk= Alkaloids

Flav= Flavonoids

Reds= Reducing sugar

Glyc= Glycoside

Phen= Phenol

Sap= Saponins

Tan= Tannins

Ste= Steroid

Terp= Terpens

Anth= Anthraquinones

UNDER PEER REVIEW

**Table 3: Minimum Inhibitory Concentration (MIC) of *L. inermis* Ethanolic Extracts and Zone of Inhibition (*Candida* isolates)**

Concentration of Extract (mg/ml)	Zone of Inhibition zone (mm)							
	FLEE	FLEE	FLEE	Mean ±S.E	DLEE	DLEE	DLEE	Mean ±S.E
500	29	30	27	28.7±0.88	23	28	20	23.7±2.33
250	21	20	22	20.0±0.58	19	18	17	18.0±0.56
125	16	19	15	16.7±1.20	13	15	17	15.0±1.15
62.5	6	8	7	7.0±0.58	3	6	6	5.0±1.00
31.3	NI	NI	NI	NI	NI	NI	NI	NI
15.6	NI	NI	NI	NI	NI	NI	NI	NI
7.8	NI	NI	NI	NI	NI	NI	NI	NI
3.9	NI	NI	NI	NI	NI	NI	NI	NI
1.95	NI	NI	NI	NI	NI	NI	NI	NI
0.98	NI	NI	NI	NI	NI	NI	NI	NI
Distilled water	0	0	0	0.0±0.00	0	0	0	0.0±0.00
Ketoconazole (200mg/ml)	15				15			

**Key:**

NI = No Inhibition

FLEE= Fresh leaf ethanolic extract

DLEE= Dried leaf ethanolic extract

FLEE and DLEE: T= 0.24, P= 0.813 (P>0.05) DF = 17

FLEE: Pearson correlation (R) of Extract Concentration and Inhibition = 0.945, P = 0.000 (R<sup>2</sup>= 89.3%)

DLEE: Pearson correlation (R) of Extract Concentration and Inhibition = 0.932, P = 0.000 (R<sup>2</sup>= 86.9%)

**Table 4: Measurement of Susceptibility and Resistance of *Candida* Species to Different Concentrations of *L.inermis* Ethanolic Extracts**

Inhibitory diameter zone	S 5mm	S 10mm	S 15mm	S 20mm	S 25mm	S 30mm	R 00mm
Concentration of extract	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.3 mg/ml	15.6 mg/ml	7.8 mg/ml
Isolates	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<i>C. albicans</i>	72	5 (6.9) 20 (27.8)	8 (11.1)	13 (18.1)	5 (6.9)	18 (25)	3 (4.2)
<i>C. glabrata</i>	45	0 (0.0)	15 (33.3)	12 (26.7)	10 (22.2)	0 (0.0)	0 (0.0)
<i>C. krusei</i>	30	0 (0.0)	11 (36.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>C. parapsilosis</i>	24	3(12.5)	5 (20.8)	4 (16.7)	7 (29.2)	0 (0.0)	3(2.5)
<i>C. tropicalis</i>	14	0 (0.0)	3 (21.4)	5 (35.7)	2 (14.3)	0 (0.0)	0 (0.0)
Total	185	8(4.3)	54(29.2)	29(15.6)	32(17.3)	5(2.7)	21(11.4)

$\chi^2$  (Inhibition and Species) = 9.87065, P= 0.043 (P<0.05)

$\chi^2$  (Inhibition and Extract concentration) = 241.497, P= 0.000 (P<0.05)

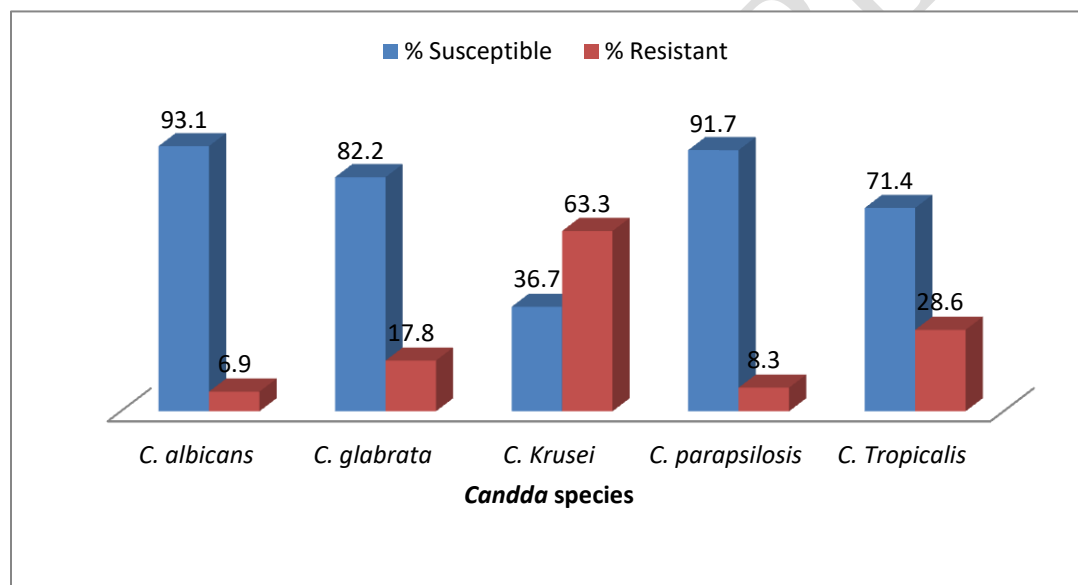


Figure 1: Percentage Resistance and Susceptibility of *L. inermis* Extracts against *Candida* species

## DISCUSSION

In this work, *L. inermis* leaf was very high in tannin (>5%). This is a powerful active principle found in plants that possess amazing healing properties and those that are highly poisonous to insects as a defensive mechanism (Talib, 2011). Tannin exerts biocidal effects through diverse mechanisms possibly through microbial enzyme inactivation. In the work of Cowan (1999), condensed tannins glued to the cell walls of bacteria; prevent growth and protease activity. These are the likely mechanisms of tannin against pathogenic fungi. The antifungal activity of *L. inermis* leaf reported in this study may be attributed to the high quantity of tannin and the moderate level of alkaloid, terpene, phenol and saponin.

It is also possible that other phytochemicals that were present in low quantity in *L.inermis* leaf might possess antifungal properties regardless of their level. This finding aligns with some phytochemical studies where active ingredients such as such as reducing sugar, anthraquinone, flavonoid, steroid, glycoside were found in low quantity (Khan *et al.*, 2015; Mudi *et al.*, 2011). In other studies, some of these active ingredients were found in large amount in all parts of the plant (Kelmanson *et al.*, 2002). Regardless of the sources, these are active principles with known antimicrobial properties. For instance, flavonoid protects plants against predation due to their antimicrobial nature (Dixon and Steale, 1999). The marked antimicrobial property of flavonoids against a variety of bacterial and fungal pathogens is mediated by their action on the microbial cell membranes (Cazarolli *et al.*, 2008).

Results have pointed out the degree of relatedness among the active ingredients. This is important in the screening of varieties and cultivars of *L.inermis* for their phytochemicals. This

approach is popular in plant studies most especially in plant breeding programme (Olasan *et al.*, 2018). In this case, the presence of one phytochemical may indicate the presence of the other within the leaf as in the case of flavonoid and terpene (+0.999), alkaloid and glycoside (+0.988), anthraquinone and terpene (+0.933), saponin and phenol (+0.866) and alkaloid and tannin (+0.849).

The present report aligns with previous investigation on the antifungal activity of *L.inermis*. In previous work for instance, ethanolic extract of the whole plant showed antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, *Microsporiumcanis* and *Aspergillus niger* (Muhammad and Muhammad, 2005). Bark decoction of henna inhibited the activity of protopectinase and polygalactouronase enzymes present in fungi (Bhakuni *et al.*, 2000).

Both fresh and dried leaf ethanolic extracts at 500mg/ml and 250mg/ml were able to inhibit fungal isolates as high as 18-30mm zones of inhibition. The extracts were as effective as ketoconazole drug at these concentrations. Thus inhibition of fungal isolated was tied to the concentration of the extracts in either fresh or dried leaf. In this work, extract concentration explained fungal inhibition by 89%. Other unforeseen factors such as nutrient availability and other environmental factors accounted for the remaining 11% of the observed inhibition not attributed to the *L. inermis* extracts. Similar findings were reported in the antimicrobial activities of other plants where effective inhibition of pathogens was observed only at high concentrations of the extracts used (Kurek *et al.*, 2011). In the present study, the minimum fungicidal concentration (MFC) of leaf extract (prepared in either fresh or dried form) ranged from 22.7mg/ml to 47.0mg/ml. The observed significant differences in the MFC of the extracts could

be attributed to the differences in the inherent resistant level of the isolates of different species with different genetic make-up.

From the results, five species of *Candida* were isolated and identified among the female patients attending gynaecology unit of Dalhatu Araf Specialist Hospital Lafia. This shows that candidiasis is still rampant and it is caused by diverse species including *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei* and *Candida parapsilosis*. Also, each of the species may be associated with different pathological effects in the female genital tract. Although only *C.albican* is considered pathogenic, all the *Candida* species identified are opportunistic infectious yeasts (Erzincan, 2017). The most common species isolated was *C.albicans* (38.9%) followed by *C. glabrata* (24.3%) and *C. krusei* (16.2%). *C. parapsilosis* and *C. tropicalis* accounted for 12.9% and 7.5% respectively. Result was consistent with existing reports in literature that *Candida albicans* is the most commonly isolated species in human genital tract causing vaginal candidiasis (Manolakaki *et al.*, 2010). The prevalence of candidiasis caused in Lafia was higher than the 6.5% *C. albicans* and 7.5% non *C. albican* prevalence rates reported by Emeribe *et al.* (2015) among non-pregnant women in Abuja, Nigeria. In this work, results have shown that those within 20-30 years are more at risk. Similar finding was reported by Emeribe *et al.* (2015) when candidiasis was more pronounced among non-pregnant women of 20-30 years of age. In this age group, women are sexually active group and they are within the optimal state of conception and child bearing. These are those with more cases of infertility at DASH Lafia. From this work, high prevalence of approximately 39% of pathogenic vaginal candidiasis might be responsible for the inability of the affected patients to conceive. Moreover, other non pathogenic opportunistic *Candida* species with high prevalence (such as *C.glabrata* and *C. krusei*) might become pathogenic in immune-compromised female patients. Results are

consistent with previous claims that there is an increasing global prevalence of infections caused by various *Candida* species (Spivak *et al.*, 2017).

The five species of *Candida* isolated among 185 subjects may pose serious epidemiological consequence because cases of synergistic interaction among pathogenic and non-pathogenic *Candida* species are possible in female genital tract. This condition may present serious opportunistic infections. Synergism may also make candidiasis difficult to treat because the organisms are likely to escape drug therapy through enhanced resistance. Differences in the genetic make-up of the species may impact on drug resistance. This view is widely upheld in epidemiological studies of many infectious diseases caused by multiple species of a genus (Mishra *et al.*, 2011). Medically important species of fungi such as *Candida* species have been reported to have multiple drug resistance and this has led to the search for plants that can effectively combat fungal infections including candidiasis (Mishra *et al.*, 2011). In this work, *C. albicans* showed the highest susceptibility to *L. inermis* extract followed by *C. parapsilosis* and *C. glabrata* meanwhile many isolates of *C. krusei* and *C. tropicalis* displayed high resistance to the extract regardless of the solvent type (ethanol or petroleum ether). This shows the extent of applicability of drug synthesis from *L.inermis* leaf against the five tested species of *Candida*, although biostatic potency may be improved during drug synthesis. These findings aligned with the work of Elham and Elbasheir (2014) in the invitro activity of *Lawsonia inermis* against some pathogenic fungi where inhibition was found to depend on species of fungi.

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