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Antifungal activity of herbal plants against *Candida albicans*

Everlyne Kareji Amadi
Egerton University, Department of Chemistry. P.O. Box, Egerton, Kenya

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Abstract
There has been increase in use of herbal medicine by herbalists to cure diseases. They use indigenous knowledge acquired through generations. The plant parts studied were collected from Central Kenya and extracted using solvents of different polarity. Phytochemical analysis both qualitative and quantitative analysis and *in-vitro* biological activities of the plant extracts against candidiasis were determined using Disk Diffusion method. From the *in-vitro* bioassay results, it was established that ethanol extract of *Vernonia brachycalyx* (with inhibition zone of 10.5 mm) and *Aloe secundiflora* (with inhibition zone of 7.0 mm) were the most active against *Candida albicans*, the causative agent of candidiasis. The antimicrobial activities using the different solvents were statistically different ($p < 0.05$). The anti-microbial activity results of this research provided a scientific basis for the use of investigated herbal extracts in the treatment of candidiasis.

Keywords: *Aloe secundiflora*, *Vernonia brachycalyx*, candidiasis and herbal drugs

1. Introduction
Some of the medicinal plants used to treat candidiasis in Mbeere in Central Kenya included *Emilia discifolia, Leucas mollis, Senna didymobotrya, Carrisa edulis* and *Albizia gummifera*. Decoction from boiled parts including leaves, bark and whole plant are applied on the body.
Candidiasis is a condition brought about by *Candida albicans*. Candida albicans is a yeast that lives in human digestive system and can become a fungus (Enfert and Hube, 2007) [3]. The most chronic health problems related to the infection caused by this fungus include urinary problems, eczema, asthma, digestive problems, endometriosis, cough pains, sexual dysfunction and thrush, among others. Candida overgrowth manifests as hyperacidity environment which increase growth of yeast and fungus. *Candida albicans* proliferates in the intestines because of several factors including antibiotic overuse, lowered immune system, oral contraceptives and stress (Peter, 2011) [9].

2. Materials and Methods
2.1 Materials
With the help of herbalists practicing in the study area, ethnobotanical data of herbal plants were collected. Plant materials used in the preparation of the herbal drugs were collected from their sources in Mbeere district, Embu county Eastern Kenya. The specific plant parts (stem, roots, leaves and seeds) materials were air-dried on the laboratory benches away from direct sunlight then grounded into powdered form, stored in plastic bags and sealed to avoid contact with moisture.
The clinical fungal isolate *Candida albicans*, was obtained from Kenya Medical Research Institute (KEMRI) Nairobi and Yeast which was used as an anti-fungal was purchased from reputable chemists. Analytical and HPLC grade solvents, silica gel, and TLC plates were purchased from Aldrich Chemical Company Ltd., England and Merck, Germany through Kobian Kenya Ltd. All general purpose solvents were distilled prior to use.

2.2 Sensitivity Testing Using Disk Diffusion Method
Disk diffusion method was used to carry out the antimicrobial screening that was determined on Potato Dextrose Agar (PDA) using *Candida albicans* as fungal isolate. 28 g/L of PDA was dissolved and sterilized at 121°C in an autoclave. Inoculation of the test microorganism into tubes of nutrient broth was done and then incubated at 28°C for 24 hr. The media were left to cool to 40 ° C and dispensed in sterile petridishes and left to solidify. Each of the cultures was then adjusted to 0.5 McFarland turbidity standards and inoculated (0.1 ml each) onto PDA plates. This was left for 10 minutes to dry. Paper disks were diffused in each of the samples and dried at 40°C. The disks were pressed gently onto the seeded agar plates with the tips of sterile forceps. Anti-fungal served as negative controls. The plates were incubated at 37°C for 24 hours then antimicrobial activity was determined by measurement of diameter zones of inhibition (Mm) (against the test organisms) around each of the extracts and the antibiotics (Lino and Deogracious, 2006) [6].

2.3 Phytochemical Analysis
Using standard methods of analysis according to Harborne, 2005 [4], Kour and Arora (2009) [5], Okwu and Josiah (2006) [8] and Ojokuku *et al*., (2010) [7], the samples were evaluated for qualitative and quantitative determination of major phytochemicals i.e. flavones, tannins, saponins, flavonoids, alkaloids and steroids (Amadi, E.K. 2015) [1].

3. Results and Discussion
3.1 Bioassays
The bioassays carried out on the various extracts including

water, ethanol, ethyl acetate and hexane extracts of the medicinal plants studied gave different activities against strains of fungi and the inhibition diameter values obtained were as as shown in Table 3.1.

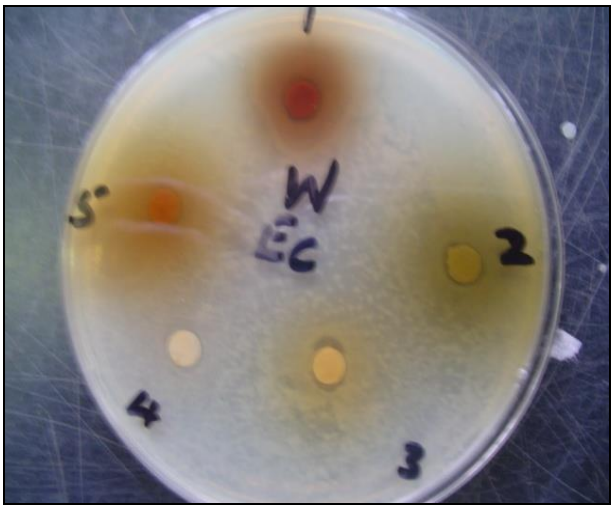

Plate 1: Water extracts against *Candida albicans*

Table 1: Anti-fungal Activities of the Solvent Extracts against *Candida albicans*

Plant name	Solvent used/ Zone of inhibition						
	Hexane	Ethanol	Ethyl acetate	Water	Control		
					ERY	GEN	YEA
Terminalia brownii	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.1±0.0
Vernonia brachycalyx	0.0 ± 0.0	7.0 ±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.1
Carrisa edulis	0.0 ± 0.0	6.8 ±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.4±0.0
Aloe secundiflora	0.0±0.0	10.5±0.0	0.0 ±0.0	0.0 ±0.0	0.1±0.0	0.0±0.0	0.4±0.0
Tithonia diversifolia	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0

*Analysis Done in Triplicates

NI- NO Inhibition; ERY- (Erythromycin 15µg); GEN-(Gentamicin 15 µg); YEA- (Yeast 10µg)

3.2 Analysis of Variance from Mean

There was significant difference ($p < 0.001$) in activity between the different solvent extracts used in the extraction and above 95% confidence interval for difference in the means. The Grand mean obtained was 1.213. The estimated standard error and the least significant differences for the samples (0.0361, 0.1033) were higher than that of the solvents (0.0323, 0.0924).

3.3 Bonferroni test for Candida albicans

There was significant variation in mean for the activity among the different extracting solvents. The deviation could be due to the type of solvent, type of herb used and the conditions of the experiment such as temperature and duration of extraction

The best solvent for extraction was ethanol. The most active extract was ethanol extract of *Aloe secundiflora* with the value 10.5 while the second was ethanol extract of *Vernonia brachycalyx* with the value of 7.767. Other extracts were inactive against *Candida albicans* except ethanol extract of *Carrisa edulis* with the value 7.0 as shown in Fig. 3.1. This showed significant differences for all the solvents ($p < 0.001$) indicating that extraction had to be done with specific solvent to obtain maximum efficiency of the herbs used for treatment of the disease.

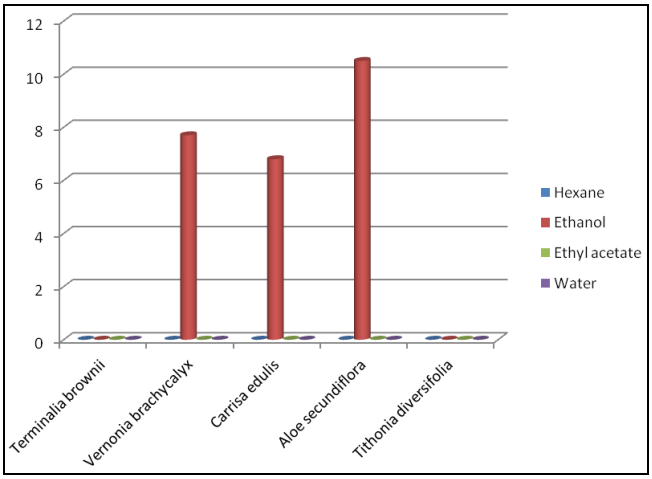

Fig 2: Activity of plant extracts against *Candida albicans*

3.4 High Performance Liquid Chromatography

The fractions of the most active compound and the second most active compound were analysed with High Performance/ Pressure Liquid Chromatography and the above retention times obtained. The retention times were compared to establish the similarities between the fractions.

The HPLC chromatograms (fingerprints) of the fractions of the most active and second most active compounds had similarities as shown in the values given in Table 3.2

Table 2: Retention time (RT) of fractions of active compounds against *Candida albicans*

RT of Ethanol extract of *Aloe secundiflora*	RT of Ethanol extract of *Vernonia brachycalyx* (mins)
3.961	3.942
4.519, 4.520, 4.632	4.518, 4.666, 4.547
7.008, 7.133, 7.277	7.084, 7.147, 7.286

The retention times for ethanol extract of *Aloe secundiflora* of 4.519 and 4.520 was very close to the retention time of ethanol extract of *Vernonia brachycalyx* of 4.518. The chromatograms obtained are as shown in Figure 3.2 and Figure 3.3. These similarities indicated the reproducibility of the extraction process (Chhetri *et al.*, 2008) [2] and similarity of the composition between the fractions of compounds studied.

The HPLC spectrum of ethanol extract of *Vernonia brachycalyx* in Fig. 3.2 with retention time of 7.286 mins shared retention times with water extract of *Carrisa edulis* and ethanol of *Aloe secundiflora*.

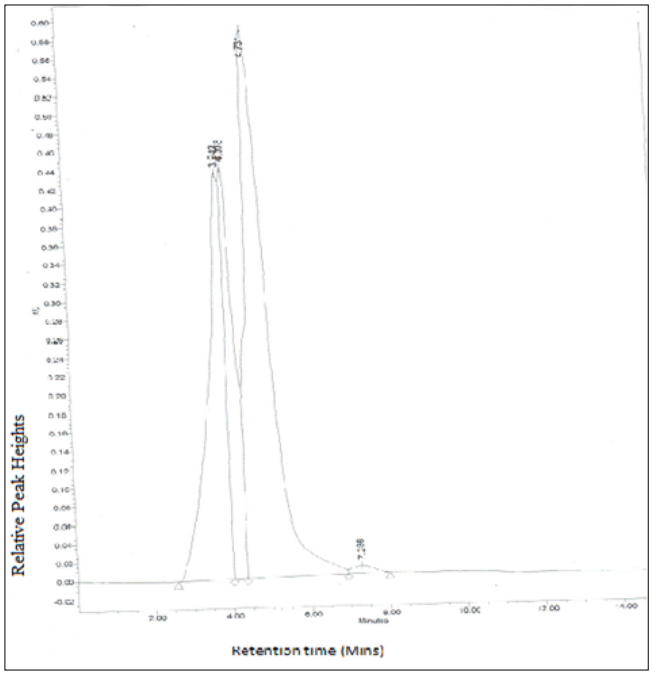

Fig 3: HPLC Spectrum of Ethanol extract of *Vernonia brachycalyx*

The spectrum of ethanol extract of *Aloe secundiflora* in Fig. 3.3 had the retention time of 3.961 mins which was very close to ethanol extract of *Vernonia brachycalyx* of 3.942 mins.

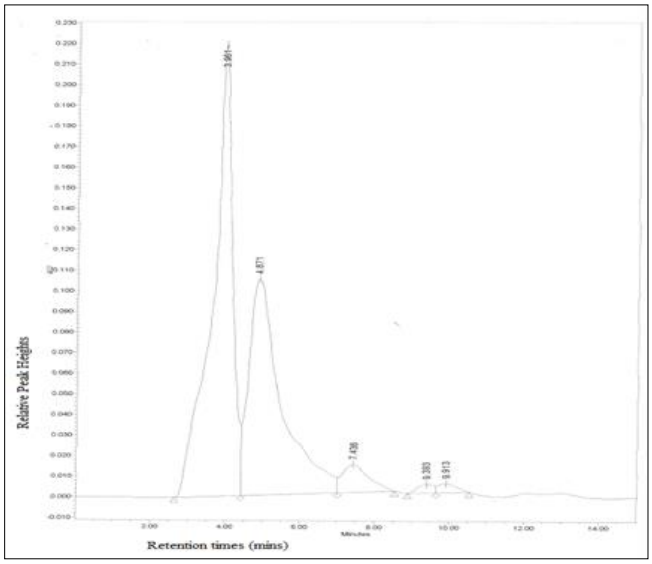

Fig 4: HPLC Spectrum of Ethanol extract of *Aloe secundiflora*

3.5 Phytochemical Results

The four different extracts of each of the medicinal plants studied showed presence of different phytochemicals as shown in Table 3.3. The different phytochemicals are responsible for the anti-microbial activity of the medicinal plants.

Table 3: Qualitative Phytochemical Results of the Herbal Plants Extracts

Plant	Extract	Saponins	Tannins	Flavonoids	Flavones	Steroids
Terminalia brownii	Hexane	-	-	-	-	++
Vernonia brachycalyx	Hexane	-	+	-	-	-
Carrisa edulis	Hexane	+	-	-	-	++
Aloe secundiflora	Hexane	-	+	+	+	++
Tithonia diversifolia	Hexane	-	+	-	-	-
Terminalia brownii	Ethanol	+++	++	++	-	++
Vernonia brachycalyx	Ethanol	++	++	-	++	++
Carrisa edulis	Ethanol	++	+++	+++	-	++
Aloe secundiflora	Ethanol	+++	+++	-	-	+++
Tithonia diversifolia	Ethanol	++	++	-	-	+++
Terminalia brownii	Ethyl acetate	-	++	++	++	+++
Vernonia brachycalyx	Ethyl acetate	++	+	-	+	++
Carrisa edulis	Ethyl acetate	-	+++	-	-	+++
Aloe secundiflora	Ethyl acetate	++	+	+	-	++
Tithonia diversifolia	Ethyl acetate	-	++	-	-	-
Terminalia brownii	Water	+++	+	-	+	+
Vernonia brachycalyx	Water	++	++	+	-	+
Carrisa edulis	Water	-	+++	-	+	-
Aloe secundiflora	Water	++	+	+	-	+
Tithonia diversifolia	Water	++	+	-	+	+

KEY: - means absent; + means present in low amount; ++ present in high amount; +++ means present in very high amounts

Very few hexane extracts showed the presence of phytochemicals. Saponins in *Carrisa edulis*, Flavonoids and flavones in *Aloe secundiflora*, Tannins in *Vernonia brachycalyx, Tithonia diversifolia* and *Aloe secundiflora,* were all present in low amounts. Steroids were in high amounts in *Terminalia brownii*, *Carrisa edulis* and *Aloe secundiflora.*

For ethanol extracts: *Terminalia brownii* and *Carrisa edulis* had all compounds present except flavones; *Vernonia brachycalyx* had all compounds present except flavonoids; *Aloe secundiflora* and *Tithonia diversifolia* had saponins, tannins and steroids.

For ethyl acetate: *Terminalia brownii* had all except saponins; *Vernonia brachycalyx* had all in high amounts except flavonoids; *Carrisa edulis* had tannins and steroids in very high amounts; *Aloe secundiflora* had all in high amounts except flavones; *Tithonia diversifolia* had only tannins in high amount.

For water extracts: *Terminalia brownii* had all except flavonoids; *Vernonia brachycalyx* had all except flavones; *Carrisa edulis* had tannins in very high amounts and flavones in low amount; *Aloe secundiflora* had all in low amounts except flavones; *Tithonia diversifolia* had all in low amounts except flavonoids.

4. Conclusion

The herbalists learned the treatment from relatives especially older in age and they used the available plant parts such as leaves, roots and barks of trees against the causative agent *Candida albicans.*

Phytoconstituents present including saponins, tannins, flavones, flavonoids and steroids were present in almost all the extracts in high amounts.

Use of High Performance Liquid Chromatography confirmed the similarities of the composition among the extracts.

The test results support traditional medicinal use of the medicinal plant extracts for the treatment of candidiasis.

5. Acknowledgements

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