**EVALUATING THE ANTIOXIDANT ACTIVITY AND IDENTIFYING POTENT COMPOUNDS USING GC-MS ANALYSIS OF *Allium cepa* (ONION), *Allium sativum* (GARLIC) AND *Zingiber officinale* (GINGER) METHANOL EXTRACTS**

**Abstract**

**Background:** Candidiasis caused by *Candida albican* species is one of the major health problems in immune-compromised patients in developing countries. *Candida* species are involved in the main opportunistic yeast infection in the world, but among the species of the genus, *Candida albicans* continues to be the most common. The *in vitro* antioxidant activity of methanol extracts of *Alliumcepa* (Onion), *Alliumsativum* (Garlic) and *Zingiber officinale* (Ginger) on *Candidaalbicans* was tested on the test drug Fluconazole.

**Aim:** The study was carried out to assess the quality and quantity of the secondary metabolites present in *Alliumcepa* (Onion), *Alliumsativum* (Garlic) and *Zingiber officinale* (Ginger) plant extracts using standard methods.

**Method:** The plant extract was subjected to thin-layer chromatography, and further subjected to column chromatography using standard methods and two fractions, F1 and F2 were obtained. The *in vitro* antioxidant activities of the plant extracts were determined using FRAP, DPPH and TBARS models, the antifungal activities and the minimal inhibitory concentration of plant extracts and its fractions using standard methods were assessed, and the combined plant extracts with the highest activity were also determined using a 2:2mg/ml ratio, and finally the phytoconstituents of the most active fraction was identified using GC-MS.

**Results:** The secondary metabolites detected were tannins, reducing sugars, phenols, saponins, terpenoids, steroids, alkaloids and anthraquinones in both the aqueous and methanol extracts. However, flavonoids were absent in both aqueous and methanol extracts of *Allium cepa*, anthraquinones were absent in aqueous extract of *Allium cepa* and aqueous extracts of *Zingiber off*icinale, terpenoids were found in the aqueous extracts of *Allium cepa* while steroids were absent in methanol extracts of *Zingiber officinale* and aqueous *Allium sativum*. The antioxidant powers of both fractions (F1 and F2) were dose-dependent. Significantly higher (p<0.05) antioxidant power was assessed at 100 mg/ml while the least antioxidant power was assessed at the lowest concentration (20mg/ml). No significant (p<0.05) difference was assessed in the antioxidant power of the fraction 1 and ascorbic acid at the concentrations of 20 and 40mg/ml. The fraction 2 significantly (p<0.05) exhibited a high radical scavenging activity at the different concentrations of (40, 60, 80 and 100mg/ml) compared to ascorbic acid at the same concentrations. Significantly higher (p<0.05) scavenging activity was assessed at the highest concentration of fraction 2 (100mg/ml) while the lowest activity was assessed at 20mg/ml of fraction 2. No significant (p<0.05) difference was assessed in the antioxidant power of the plant extract and ascorbic acid at the concentrations of 20mg/ml.

**Conclusion:** The fractions were subjected to GC-MS analysis and they showed the presence of some important compounds which include oleic acid, cis-Vaccenic acid, pentadecanoic acid, octadec-9-enoic acid, 5-Eicosene, to mention a few.

**KEY WORDS:** *Candida albican*, Phytochemicals, *Allium cepa*, *Allium sativum*, and *Zingiber officinale*.

**Introduction**

*“Candida* is an opportunistic fungus that is capable of inducing acute and chronic infections of the mouth, vagina, lung and gastrointestinal tract with different body reactions such as severe infection, purulent and granulomatous inflammation” (Azene *et al*., 2021). “Candida fungi cause candidiasis and almost 200 various species are included in the genus Candida but only a minute quantity of them are harmful and can lead to infections which can either be external or internal and severe” (Asif and Muhammad, 2019).

*“Candida albicans* are pathogens that are of much significance and are present everywhere and mostly reside in the gastrointestinal tract, skin and vagina. The immune system regularly monitors the *Candida* and any impairment in the immune system can cause an infection. However, a person with any other serious condition like cancer or HIV whose internal defense system is already much weakened can develop a much more severe one” (Sharanappa and Vidyasagar, 2017).

“The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide” (Yasin *et al.,* 2019). “This has become an important cause of morbidity and mortality in immuno-compromised patients in developing countries. Although a large number of antimicrobial agents have been discovered, pathogenic microorganisms are constantly developing resistance to these agents” (Azene *et al*., 2021). “Vaginal candidiasis has adverse effects on the health of affected women. Infertility and sexual problems associated with dissatisfaction are the unfavorable outcomes of the infection” (Azhrak *et al.*, 2020). “About 75% of adult women have at least one episode of vulvovaginal candidiasis (VVC) during their life, with the prevalence of *C. alb cans* in 70–90%” (Forche *et al*., 2018). “It also causes a variety of infections that range from non-life threatening mucosal candidiasis like vaginal yeast infections, thrush, skin and diaper rash to lethal disseminated candidiasis in those with compromised immune systems who have an implantable medical device such as a pacemaker or artificial joint, or who use broad-spectrum antibiotics” (Singh *et al*., 2019).

“The development of drug resistance in pathogens and the increasing interest of consumers in safe food forces researchers to explore new antimicrobial agents. This has given rise to a shift from the prescription of antibiotics to the use of medicinal plants and spices. It is estimated that there are about 250,000 to 500,000 species of plant on earth and a relatively small percentage of them are used as food by both human and other animal species). These plants fall under the natural products which are a major source of new natural drugs and their use as an alternative medicine for treatment of various diseases has been increased in the last few decades. In the Nigerian health care system, the role of plants/herbs as medicines is presently well recognized, and nearly all plants are associated with medicinal uses” (Anyawu and Okoye, 2017).

“Onion (*Alliumcepa*) and Garlic (*Alliumsativum*) belong to the genus *Allium*, amonocotyledonous genus of flowering plants informally referred to as the onion genus found in the family*Alliaceae*. The generic name *Allium* is the Latin word for garlic” (Balach*etal*., 2020).”Onion (*Alliumcepa.*) is considered as the largest and important representative genus of the *Liliaceae* family comprising of 450 species” (Enejiyon*et al*., 2020). “Onion *(Allium cepa )* is a bulbous plant broadly cultivated in almost every nation of the world. They are easily propagated, transported and stored. It has diverse biological importance like the treatment of cold, heart disease, diabetes, coughs and sore throat. Onions and its extracts are known to decrease blood lipids levels, increase fibrinolysis and decrease platelet aggregation and also lower blood pressure” (Michael T. *et al.*, 2022)

“Garlic (*Alliu3msativum*) is a species in the onion genus—*Allium.* Many members of the genus Allium, including about 700 species, have been recognized as rich sources of biologically active secondary metabolites in addition to their antioxidant properties. Garlic is one of the oldest vegetative propagated horticultural crops. Garlic lowers blood pressure and stimulates the circulatory capacity of the heart. It is one of the important earliest known medicinal plants. Its usage worldwide has a long history. Being an important food spice plant, it has a significant role in disease prevention and control, many of the diseases can be cured with garlic. It has been used since longtime against human pathogens” (Abdulaziz*et al*., 2018)

“Ginger (*Zingiber officianale*) has been used as a remedy or arrange of illnesses including diarrhea, stomach aches, nausea asthma, and respiratory disorders because ginger contains components of therapeutic values” (Ibrahim*et al*., 2019). “Newly, the approval of traditional medicine as a reserve form of health care and the progress of microbial resistance to the existing antibiotics has directed researchers to explore the antimicrobial activity of medicinal plants” (Yassesn andIbrahim, 2018).

**Materials and methods**

**Reagents and plant materials**

Solvents and chemicals used in the experiment were analytical grade and purchased from yola, Nigeria. Distilled water was used throughout the experiment. Fresh matured samples of *Allium cepa* (Onions), *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) plants were purchased from Jimeta market in Adamawa State, Nigeria. The plant materials were identified and authenticated in the Plant Sciences Department of Modibbo Adama University, Yola.

**Preparation and extraction of plant material**

The crude extracts of the material were prepared using the method described by Das*etal*.,(2010). The fresh onion, garlic and ginger were washed with tap water and rinsed with distil water and allowed to dry. The dried samples were blended using the laboratory blender to increase size. The powdered plant samples were weighed and added to different solvents and left for 72 hours. The extracts were filtered with Whatman filter paper, evaporated and stored for further analysis.

**Phytochemical Analysis**

**Qualitative phytochemical analysis**

The methanolic extract of onion, garlic and ginger each were analyzed for the presence of alkaloids, terpenoids, reducing sugars, saponins, tannins, flavonoids, Phlobatannis, steroids, anthraquinone and phenols using the method described by Trease and Evans, (2002).

**Quantitative phytochemical analysis**

The total alkaloids, total phenols, total flavonoid, and total tannin was carried out accordingto the method described by Jing-Chung*etal.,* (2007).

**Determination of *In Vitro* antioxidant assay**

The quantitative antioxidant activity of the methanol extracts was determined using FRA Passay, DPPH assay and TBARS, carried out according to the method described by Sutharsingh *et al.*, (2011).

**RESULTS**

**Phytochemical constituents of the plant extracts**

**Table1** shows some phytochemicals that were found to be present in the methanol and aqueous extracts of *Alliumcepa (*Onion), *Zingiber officinale* (Ginger) and *Alliumsativum* (Garlic).Tannins, reducing sugars, phenols, saponins, terpenoid, steroid, and alkaloids were found to be present in the aqueous extract of onion, while anthraquinone, flavonoids and terpenoid were found to be absent. In the case of methanol extract of the onion, flavonoids were found to be absent, while tannins, reducing sugars, phenols, saponins, terpenoid, steroid , anthraquinone and alkaloids were all found to be present. In the aqueous extract of ginger; flavonoid, reducing sugars, phenols, saponins, terpenoid, steroids, tannins and alkaloids were all found to be present and anthraquinone was absent. While, flavonoid, reducing sugars, phenols, saponins, terpenoid, anthraquinone and alkaloids were all found to be present in the methanol extract of the ginger except steroids which were found to be absent. In the garlic aqueous extract, the steroid was found to be absent. While, phenols, saponins, terpenoid, reducing sugar, anthraquinone and alkaloids were all found to be present in the garlic aqueous extract. Terpenoid was found to be absent in the methanol extract of garlic, while tannins, reducing sugars, phenols, saponins, steroid, tannins, flavonoids and alkaloids were found to be present in the garlic methanol extract.

**Table 2** shows the quantity of some of the phytochemicals that were present in the aqueous extract of *Allium cepa (*Onion), *Zingiber officinale* (Ginger) *and Allium sativum* (Garlic). Alkaloids had the highest amount with a percentage of 4.12±0.04 % and reducing sugar was found to be least in quantity with 0.04±0.01% as quantified in *Allium cepa* extract. In the extract of *Zingiber officianale;* Tannins had the highest amount with 6.11±0.03% while steroids had the least amount with 0.06±0.01%. In the extract of *Allium sativum*; flavonoids had the highest amount of 7.18±

0.03% while reducing sugars had the least amount of 0.05 ± 0.01%.

**Table 3** shows the quantity of some of the phytochemicals that were present in the methanol extract of *Alliumcepa (*Onion), *Zingiber officinale* (Ginger) *and Allium sativum* (Garlic). Phenols had the highest amount with a percentage of 6.23 ± 0.21% and steroids were found to be least in quantity with 0.06±0.02% as quantified in *Allium cepa* extract. In the extract of *Zingiber officianale;* Flavonoids had the highest amount with 8.60±0.23% while Anthraquinone had the least amount with 0.17±0.01%. In the extract of *Allium sativum*; flavonoids had the highest amount of 6.25±0.01% while reducing sugars had the least amount of 0.08±0.03%.

**Phytochemicals Content of *Allium cepa*, *Zingiber officinale* and*Allium sativa***

**Table1.Phytochemical Content of Methanol and Aqueous Extracts of the *Allium cepa*, *Zingiber officinale and Allium sativum***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Chemical****Constituents** | ***A.A. cepa*** | ***M.A. cepa*** | ***A.Z. officinale*** | ***M.Z. officinale*** | ***A.A. sativum*** | ***M.A. sativum*** |
| Alkaloids | + | + | + | + | + | + |
| Saponins | + | + | + | + | + | + |
| Phenols | + | + | + | + | + | + |
| Tannins  | + | + | + | + | + | + |
| Flavonoids | - | - | + | + | + | + |
| Anthraquinone | - | + | - | + | + | + |
| Reducing Sugar | + | + | + | + | + | + |
| Terpenoids | - | + | + | + | + | + |
| Steroids | + | + | + | - | - | + |

**Key:**

+=present

-= absent

**A=**Aqueous

 **M**= Methanol

**Table 2. Quantitative Phytochemical Constituents of Aqueous Extract of*Allium cepa*, *Allium sativum* and Zingiber *officinale* in Percentage(%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S/N** | **Phytochemical** | ***Allium cepa*** | ***Zingiber******Officianale*** | ***Allium sativum*** |
| **1** | Alkaloids | 4.12 ± 0.04bc | 5.10 ± 0.02 | 6.20± 0.05a |
| **2** | Saponins | 3.11± 0.01b | 4.01 ± 0.05 | 4.30 ± 0.02a |
| **3** | Phenols | 0.21 ± 0.01b | 0.61 ± 0.01 | 0.80 ± 0.00a |
| **4** | Tannins | 3.7 ± 0.03b | 6.11 ± 0.03ac | 4.60 ± 0.01b |
| **5** | Flavonoids | - | 2.30 ± 0.03b | 7.18 ± 0.03ac |
| **6** | Anthraquinone | - | - | 1.40 ± 0.03 |
| **7** | Reducingsugars | 0.04 ± 0.01bd | 0.13 ± 0.02a | 0.05 ± 0.01d |
| **8** | Terpenoid | - | 0.51 ± 0.03a | 0.40 ± 0.01b |
| **9** | Steroids | 0.09 ± 0.04a | 0.06 ± 0.01bd | - |

All values are mean ± SEM for 3 determinations.

a= Significantly (p<0.05) higher compared to other group in the same row b= Significantly (p< 0.05) lower compared to other group in the same row

c= Significantly (p<0.05) higher compared to other group in the same column d = Significantly (p< 0.05) lower compared to group in the same column

**Table 3. Quantitative Constituents of Methanol Extract of *Allium cepa*, *Allium sativum* and *Zingiber officinale*in Percentage (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S/N** | **Phytochemical** | ***Allium cepa*** | ***Zingiber******Officianale*** | ***Allium sativum*** |
| **1** | Alkaloids | 5.05 ± 0.01 | 3.12 ± 0.03b | 5.30 ± 0.12a |
| **2** | Saponins | 2.14± 0.14b | 3.12 ± 0.11 | 5.11 ± 0.12a |
| **3** | Phenols | 6.23 ± 0.21ac | 2.11 ± 0.01 | 1.82 ± 0.21b |
| **4** | Tannins | 4.6 ± 0.31b | 6.11 ± 0.12a | 5.40 ± 0.15 |
| **5** | Flavonoids | - | 8.60 ± 0.23ac | 6.25 ± 0.01bc |
| **6** | Anthraquinone | 1.10 ± 0.02 | 0.17 ± 0.01bd | 1.62 ± 0.11a |
| **7** | Reducingsugars | 0.07 ± 0.01bd | 0.27 ± 0.11a | 0.08 ± 0.03d |
| **8** | Terpenoid | 0.52 ± 0.03b | 0.83± 0.12a | - |
| **9** | Steroids | 0.06 ± 0.02bd | - | 0.72 ± 0.16a |

All values are mean ± SEM for 3 determinations.

a= significantly (p<0.05) higher compared to other group in the same row b= significantly (p< 0.05) lower compared to other group in the same row

c= significantly (p<0.05) higher compared to other values in the same column

d= significantly (p< 0.05) lower compared to other values in the same column

**Antioxidant potentials of the plant extracts**

**Table 4** shows the result of fractions 1 and fraction 2 using FRAP. The antioxidant power of fraction 1 was found to be dose-dependent. Significantly higher (p<0.05) antioxidant power was observed at 100mg/ml while the least antioxidant power was observed at the lowest concentration (20mg/ml) of both the plant extract and ascorbic acid. The ferric reducing antioxidant power (FRAP), of fraction 1 was significantly (p<0.05) higher at 60mg, 80mg and 100mg compared to the antioxidant power of ascorbic acid at the same concentration. No significant (p<0.05) difference was observed in the antioxidant power of fraction 1and ascorbic acid at the concentrations of 20 and 40mg/ml.

Fraction 2 significantly (p<0.05) exhibited a high radical scavenging activity at the different concentrations of (40, 60, 80 and 100 mg/ml) compared to ascorbic acid at the same concentrations. Significantly higher (p<0.05) scavenging activity was observed at the highest concentration of fraction 2 (100mg/ml) while the lowest activity was observed at 20mg/ml of fraction 2. No significant (p<0.05) difference was observed in the antioxidant power of the plant extract and ascorbic acid at the concentrations of 20mg/ml. The radical scavenging activity was found to be dose-dependent.

**Table 5** shows the result of the antioxidant activity of fraction 1 and fraction 2 using2,2-diphenyl -2- picryl hydrazyl (DPPH). The fraction 1 significantly (p<0.05) exhibited a high radical scavenging activity at the different concentrations of (40, 60, 80 and 100mg/ml) compared to ascorbic acid at the same concentrations. The radical scavenging activity was also found to be dose-dependent. Significantly higher (p<0.05) radical scavenging activity was observed at the highest concentration of the plant extract (100mg/ml) while the lowest activity was observed at 20mg/ml of plant extract. No significant (p<0.05) difference was observed in the antioxidant power of the plant extract and ascorbic acid at the concentrations of 20mg/ml.

The fraction 2 significantly (p<0.05) exhibited a high radical scavenging activity at the different concentrations of (60, 80 and 100mg/ml) compared to ascorbic acid at the same concentrations. Significantly higher (p<0.05) scavenging activity was observed at the highest concentration of fraction 2 (100mg/ml) while the lowest activity was observed at 20mg/ml of fraction 2. No significant (p<0.05) difference was observed in the antioxidant power of the plant extract and ascorbic acid at the concentrations of 20 and 40mg/ml. The radical scavenging activity was found to be dose-dependent.

**Table 6** shows the result of the antioxidant activity of fraction 1 and fraction 2 using thiobarbituric acid reactive substances (TBARS). Fraction 1 significantly (p<0.05) exhibited a high radical scavenging activity at the different concentrations of (60, 80 and 100 mg/ml) compared to ascorbic acid at the same concentrations. The radical scavenging activity was also found to be dose-dependent. Significantly higher (p<0.05) scavenging activity was observed at the highest concentration of fraction 1 (100mg/ml) while the lowest activity was observed at 20mg/ml of fraction 1. No significant (p<0.05) difference was observed in the antioxidant power of the plant extract and ascorbic acid at the concentrations of 20 and 40mg/ml. Fraction 2 significantly (p<0.05) exhibited a high radical scavenging activity at the different concentrations of (80 and 100mg/ml) compared to ascorbic acid at the same concentrations. Significantly higher (p<0.05) scavenging activity was observed at the highest concentration of fraction 2 (100mg/ml) while the lowest activity was observed at 20mg/ml of fraction 2. No significant (p<0.05) difference was observed in the antioxidant power of the plant extract and ascorbic acid at the concentrations of 20 and 40mg/ml. The radical scavenging activity was found to be dose-dependent.

**Antioxidant Activities of the Different Fractions**

**Table 4. Percentage (%) Inhibition of Ferric Reducing Antioxidant Power**

**(FRAP)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Concentration (mg/ml)** | **F1** | **F2** | **Ascorbic Acid** |
| 20 | 25.84 ± 0.14b | 26.69 ± 0.13b | 30.23 ± 0.09 |
| 40 | 38.51 ± 0.11 | 39.22 ± 0.24a | 32.51 ± 0.11 |
| 60 | 51.73 ± 0.23a | 57.13 ± 0.03a | 41.05 ± 0.22 |
| 80 | 67.90 ± 0.19a | 68.03 ± 0.11a | 42.40 ± 0.13 |
| 100 | 71.81 ± 0.13a | 81.20 ± 0.25a | 45.09 ± 0.06 |

All values are mean ± SEM for 3 determinations

a= significantly (p˂0.05) higher compared to ascorbic acid at the same concentration.

b= significantly (p˂0.05) lower compared to ascorbic acid at the same concentration.

**Table 5. Percentage (%) Inhibition of 2,2-diphenyl-2-picryl hydrazyl (DPPH)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Concentration mg/ml** | **F1** | **F2** | **Ascorbic acid** |
| 20 | 34.93 ± 0.21b | 49.16 ± 0.05a | 40.19 ± 0.12 |
| 40 | 53.04 ± 0.16a | 52.27 ± 0.14a | 46.91 ± 0.03 |
| 60 | 60.67 ± 0.24a | 56.01 ± 0.07a | 48.05 ± 0.15 |
| 80 | 68.01 ± 0.11a | 67.57 ± 0.19a | 61.83 ± 0.20 |
| 100 | 73.53 ± 0.08a | 75.88 ± 0.04a | 64.15 ± 0.03 |

All values are mean ± SEM for 3 determinations

a= significantly (p ˂0.05) higher compared to ascorbic acid at the same concentration.

b= significantly (p ˂0.05) lower compared to ascorbic acid at the same concentration.

**Table 6. Percentage Inhibition of Thiobarbituric Acid Reactive Substances (TBARS)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Concentration Mg/ml** | **F1** | **F2** | **Ascorbic acid** |
| 20 | 35.13 ± 0.12 | 35.13 ± 0.11 | 33.12 ± 0.06 |
| 40 | 36.42 ± 0.22 | 36.55 ± 0.02 | 35.18 ± 0.12 |
| 60 | 41.08 ± 0.08a | 40.92 ±0.18a | 36.22 ± 0.08 |
| 80 | 45.13 ± 0.23 | 49.92 ± 0.23a | 38.47 ± 0.21 |
| 100 | 52.89 ± 0.14a | 55.12 ± 0.03a | 40.19 ± 0.10 |

All values are mean ± SEM for 3 determinations

a= significantly (p˂0.05) higher compared to ascorbic acid at the same concentration.

**Result of GC-MS Analysis**

The chemical compounds in fraction 1 are based on the retention time and peak area in percentage; the compound with the highest percentage peak area is 9-Hexadecenoic acid with a percentage peak area of 10.52 and a retention time of 22.879 followed by Z-(13, 14-Epoxy) tetradec-11-en-1-ol acetate with a percentage area of 8.63 and a retention time of 21.775. While, D-Allose is the compound with the least percentage area of 0.01 and a retention time of 4.849.

Some important compounds that were found to be present in fraction1 include Oleicacids,cis-Vaccenicacid,5-Eicosene, (E)-, Pentadecanoic,Octadec-9-enoicacid, Cyclopentadecanone, 2-hydroxy-,Agaricic acid,6- Nitroundec-5-ene, 1,6-Dideoxy-l-mannitol and SOxirane, tetradecyl-,as seen in table 7.

The chemical compounds in fraction 2 based on the retention time and peak area in percentage; the compound with the highest percentage peak area is 1,14-Dibromotetradecane with a percentage peak area of 11.80 and a retention time of 22.868 followed by Oleic acid with a percentage peak area of 6.69 and a retention time of 22.050. While, D-Allose and 3, 4-Altrosan are the compounds with the least percentage area of 0.06 and a retention time of 4.506.

Some important compounds that were found to be present in fraction 2 include Oleicacids,13-Tetradecenal, Eicosene, l-(+)- Ascorbic acid2,6-dihexadecanoate, 1H-Indene,5-butyl-6-hexyloctahydro-,6-Octadecenoic acid, cis-Vaccenic acid,1,2-Benzisothiazole, 3-(hexahydro- 1H-azepin-1-yl)-,1,1-dioxide, Aspidospermidin-17-ol,1-acetyl-19,21-epoxy-15,16-dimethoxy-and 15-Hydroxypentadecanoic acid, as seen in table 8.

**Table 7.Chemical Composition of Fraction 1**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S/N | Name of Compounds | Retention Time (Min) | Peak area  | Percentage peak area (%) | Molecular weight (g/mol) | Molecular formula |
| 1 | Oleic acid | 22.404 | 129335 | 1.61 | 282.47 | C18H34O2 |
| 2 | cis-Vaccenic acid | 22.612 | 129339 | 1.72 | 282.5 | C18H34O2 |
| 3 | 5-Eicosene, (E)-  | 29.368 | 127769 | 0.41 | 242.3975 | C15H300 |
| 4 | Pentadecanoic acid | 35.096 | 95854 | 0.04 |  |  |
| 5 | Octadec-9-enoic acid | 31.645 | 129341 | 0.17 | 282.46 | C18H34O2 |
| 6 | Cyclopentadecanone, 2-hydroxy- | 22.616 | 94142 | 1.72 | 240.38 | C15H28O2 |
| 7 | Agaric acid  | 17.346 | 217845 | 1.42 | 416.5 | C22H40O7 |
| 8 | 6-Nitroundec-5-ene  | 20.115 | 60376 | 2.66 | 199.29 | C11H21NO2 |
| 9 | 1,6-Dideoxy-l-mannitol  | 20.739 | 24171 | 2.30 | 150.17 | C6H14O4 |

**Table 8.Chemical Constituents of the Fraction 2**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S/N** | **Name of Compounds** | **Retention Time (Min)** | **Peak area**  | **Percentage peak area (%)** | **Molecular weight (g/mol)** | **Molecular formula** |
| 1 | Oleic acid | 22.050 | 129337 | 6.69 | 282.47 | C18H34O2 |
| 2 | 13-Tetradecenal  | 30.730 | 69490 | 1.172 | 210.3556 | C14H26O |
| 3 | Eicosene  | 25.878 | 129492 | 4.47 | 280.53 | C20H40 |
| 4 | l-(+)-Ascorbic acid 2,6-dihexadecanoate | 34.123 | 242221 | 0.14 | 652.9 | C38H68O8 |
| 5 | 1H-Indene, 5-butyl-6-hexyloctahydro- | 37.087 | 114291 | 0.13 | 264.5 | C19H36 |
| 6 | 6-Octadecenoic acid  | 29.997 | 129340 | 0.88 | 282.5 | C18H34O2 |
| 7 | cis-Vaccenic acid | 22.868 | 129339 | 11.08 | 282.5 | C18H34O2 |
| 8 | 1,2-Benzisothiazole, 3-(hexahydro- 1H-azepin-1-yl)-, 1,1-dioxide | 33.671 | 113661 | 0.30 | 264.34 | C13H16N2O2S |
| 9 |  Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy- | 34.792 | 217201 | 0.53 | 414.5 | C23H30N2O5 |

**DISCUSSION**

“A medicinal plant is any plant which contains substances that can be used for therapeutic purpose, also serve as precursors for the synthesis of useful drugs” (Erb and Kliebenstein, 2020). “Phytochemicals such as alkaloids, terpenoids, saponins and phenolics are considered as strong antimicrobial agents that can aid in solving the problem of antibiotic resistance” (Anwer*etal*., 2017). Tannins and alkaloids were found to be in high amounts in *Allium cepa* and *Zingiber officianale* respectively as found in this study. Tannins have been found to possess antifungal activity as reported by Agi and Azike, (2019). Alkaloids which are one of the largest groups of phytochemicals in plants have led to the development of powerful pain killer medications as well as antifungal as reported by (Doughari, 2016).

“Alkaloids were said to have antioxidant and antifungal properties .Indole had the ability to stop the chain reaction of free radicals efficiently” (Erb and Kliebenstein, 2020).Other alkaloids that had antioxidants properties was quinolone which could act as a buffer hydroxyl radicals and melatonin (Doughari*et al*., 2016). Guimarães*et al*., (2021) reported that alkaloids have defense mechanisms through which plants ward off pests. This suggests the medicinal properties (such as analgesic, antispasmodic and antimicrobial effects)of alkaloids from plants. It was also reported that alkaloids have a wide range of pharmacological activities including antifungal and antimalarial (Erb and Kliebenstein, 2020).

The significant effect of garlic on *Candidaalbican*s is in agreement with the findings of Krishnamanda *et al*., (2017) which reported that Garlic could be used as an effective antifungal agent. It also corroborates the position of Pavle *et al.*, (2018) who observed that garlic is effective against some fungi and some bacteria. The antimicrobial activities have been reported to increase with increase in the concentration of extracts. Therefore, the extracts activities were observed in a dose dependent manner.

“Alkaloids which had antimicrobial activities were cryptolepine. Alkaloid compounds could inhibit the synthesis of nucleic acids and ergosterolin *C.albicans.* Ergosterols are a component of *C.albican* plasma membrane. Ergosterols play a role in the formation of chitin which were polysaccharide component of the cell wall and had an important role in the germination of *C. albicans*. Triterpenoids compounds had antifungal activity by affecting the permeability of the cell membrane which could lead to cell membrane lysis” (Gorniak *et al*., 2019).

To reveal the presence of the bioactive compounds present, the fractions were further analyzed with GC-MS. The compounds identified by the GC-MS analysis are illustrated in Tables 7 and 8. Results obtained from the gas chromatography-mass detector showed the presence of a high number of bioactive constituents in both fractions.

The long-chain unsaturated fatty acids, such as oleic acids, are efficacious antifungal agents (Shallan*etal*.,2020). This information could serve as the basis for the observed antifungal activity against the *Candida albicans* by the extracts since oleic acid was found to be present in the extract as revealed by the GC-MS analysis.

The 6-Octodecadienoic acid, Pentadecanoic and Tetradecanoic acid were identified as possible antifungal agents in *C. guianensis* as reported by (Abdel-Rahman*etal*.,2018). “Therefore, it is possible that these active components in the extract were mainly responsible for the observed anti- *Candidalalbican* effects in this study. Linolenic (docosatetraenoic acid and octadecatrienoic acid) plays a role as antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, anti eczemic and anticoronary” (Yasin*etal*.,2019).

“Other compounds as cis-Vaccenic acid possess antifungal activity and hypolipidemic effect. Moreover, 1, 2-Benzenedicarboxylic acid was revealed to have antioxidant, antifouling, antimicrobial, cancer enzyme inhibitors in pharmaceutical, cosmetics and food industries” (Lee*etal.,* 2021).Among these compounds is oleic acid. Doughari and Abraham*et al.,*(2021) found that Oleic acid is capable of preventing the growth of various fungi. “It can provide an antibacterial defense to tears and can be used to develop lipid-based treatment options for eye infections helping in reducing antibiotic usage. Other studies revealed that oleic acid had different properties as antifungal, antioxidant, and anti-inflammatory, anti-androgenic, anti-cancer. Other compounds as 6-Octadecenoic acid were also said to possess anti fungal activity”(Mohamad *et al*., 2018).

**CONCLUSION**

In conclusion, anti-*Candida albican* activity of the methanol extracts of *Allium cepa* (Onion), *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) was evaluated and the results revealed that all plants act significantly against *Candida albicans*. This might be as a result of the phytochemicals found to be present and the high antioxidant activity of the plant extract; this makes it act against microorganisms. The GC-MS analysis revealed the presence of phytoconstituents which may be responsible for the observed effect against *Candida albicans*.

The research work affirms the antifungal activity of these plants extracts as its constituents are effective for the treatment of Candidiasis Albicans.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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