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ANTIFUNGAL ACTIVITIES OF UNRIPE GRAPE (*Vitis venifera)* AND PAWPAW *(Carica papaya)* FRUIT EXTRACT: A COMPARATIVE STUDY.

***[1]Ukonu, C.U. and [2]Abdulsalam, F.**

[1]Science Laboratory Technology Department (Biochemistry unit),
Federal Polytechnic, Ilaro, Ogun State.

[2]Science Laboratory Technology Department (Environmental Biology unit),
Federal Polytechnic, Ilaro, Ogun State.

***Corresponding Author:** Christian.ukonu@federalpolyilaro.edu.ng, **Phone no:** 08063389016

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ABSTRACT

*Pathogenic fungi are harmful organisms that causes diseases in human and had the potential of resulting to death, due to negligence of its capacity to do so. This work is undertaken to evaluate the anti-fungi activity of unripe fruits of Carica papaya and Vitis venifera**:** The phytochemical compounds of the whole unripe fruits were determined using standard procedure. The anti-fungi activity was Carried out using zone of inhibition/susceptibility assay. The result of the phytochemicals obtained showed that Vitis venifera had ten (10) compounds which includes Alkaloids, flavonoids, terpenoids, phenol while the Carica papaya had nine (9) compounds present, only Phlobatamine is absent. The quantitative phytochemicals result revealed that reducing of the sugar has the highest value of 90.99 ± 0.16 and 84.83 ± 0.28 in both Vitis venifera and Carica papaya**.** This was followed by saponin with 44.48 ± 0.47 and 44.20 ± 3.78 in Vitis venifera and Carica papaya respectively. It is observed that all the phytochemicals in Vitis venifera are higher than that of Carica papaya**.** The zone of inhibition/susceptibility of the fungi strains revealed that Carica papaya had high zone of inhibition against two strains namely; A. Flavus and R. oligosporus with 40mm each than Vitis venifera and the anti-fungi control (Ketoconazole) Vitis venifera showed higher zone of inhibition against A. niger than the Carica papaya and the anti-fungi control. This result implied that despite the high concentration of the phytochemicals in Vitis venifera, Carica papaya had better anti-fungi effect than the standard anti-fungi control ketoconazole and Vitis venifera.*

Keyword: Antifungi, phytochemicals, fungi, unripe fruits, susceptibility, inhibition

INTRODUCTION

Fungi are distinctive eukaryotic organisms which could be single celled or multicellular with diversity in their mode of action or activity which can be beneficial or harmful to human, plants and animals (Willis, 2018; Vernon, David & Constantine, 2019; Kohler, Casadevall & Perfect, 2015). The harmful fungi are pathogenic organisms that cause diseases or infections in human and are difficult to manage (Kohler, Casadevall & Perfect, 2015). The iniquitousness of fungi in the environment increases the frequency of fungal infections (Walsh & Groll, 1999).

An annual worldwide report of about 1.5 million deaths as a result of deadly fungal infections have been attributed to the negligence of the society to these life-threatening pathogens (Sanglard, 2002). Skin infections among others are one of the major causes of diseases by pathogenic fungal (Hay et al., 2013).

Pathogenic fungi are classified based on their mode of infection to human or their host, these include the fungal group that infect healthy human (immunocompetent human),

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among them is the disease causing *Conidiobolus spp* (entomophthoromycete) which causes nose, sinuses and central face submucosal disease with difficulty in breathing, submucosal swelling and disfurement (Prabhu & Patel, 2004). Pathogenic *Onygenales* (Ascomycota) infects the lungs, causing illness such as influenza or pneumonia (Queiroz-Telles & Escuissato, 2011) and *crptococci spp* (Basidiomycota phyla) is implicated with cases of *cryptococcal* meningitis linked with AIDS amounting to about 957,900 which was reported to results to about 624,700 deaths (Part et al., 2009).

The second class of fungi are those that infect immune-compromised human (opportunistic pathogens), these includes *Candida species* (Ascomycota phyla) which live in the gastrointestinal tract as a commensal and on the mammalian mucous membrane (Wrobel et al., 2008). *Candida abican* is the most common human commensal and opportunistic pathogen ever isolated (Krcmery & Bans, 2002) and associated with candidiasis which causes abscesses in the brain of premature new-born; it also infects the mucous membrane in individuals with irregular cellular immunity (Koher, Casadevall & Perfect, 2015). *Aspergillus species* are another example of fungi responsible for approximately 90% of invasion aspergilloisi resulting to relentless pneumonia and abscesses of brain in neutropenic patient (Schimitt et al., 1990). Spellberg et al. (2005) reported that *Rhizopus* spp associated with virulence factor which include its iron scavenging activity from the host, among which *Rhizopusoryzae*is is responsible for about 70% of the diseases in human. The increase in the frequency of opportunistic infections of fungi and resistance has arouse concern and increased the search for novel antifungal agents especially of ethno-botanical background, due to their enrichment with bioactive metabolite like saponin, alkaloids, flavonoid and terpens among others, with antifungal properties (Arif et al., 2009; Webster et al., 2008).

Grape (*Vitis venifera*) and pawpaw (*Carica papaya*) have been reported to be medicinal plants because of their potentials demonstrated in traditional and fork medicine in treating various diseases (Basan, Abdul, Rasool & Hassan, 2012); and the abundance of the phytochemicals such as phenolic compounds, alkaloids, terpenoid etc (Yogiraj & Besser, 2017). These phytochemicals have been reported to be good antioxidants and implicated in the treatment of fever, cancer, wound healing, anti-hypertensive (Yogiraj & Besser, 2017; Bergonion & Perez, 2016).

Various researches has been done using different parts of the grape and pawpaw plant, but little information is available with respect to the antifungal activity of aqueous extract of unripe whole fruit of grape and pawpaw. These therefore, necessitated this research to investigate and compare the antifungal activity of the whole unripe fruits.

METHODOLOGY

CHEMICAL AND REAGENTS

Sabouraud Dextrose Agar (Beijing Salanbio Sci and tech, Co Ltd, Beijing, China), RPM K1640 MEDIUM (Gibco, life technologic corporation,MA, USA)

SAMPLE COLLECTION

Two (2) whole unripe fruits of pawpaw (*Carica papaya*) and grapes (*Vitis venifera*) samples were gotten from a local market in Ilaro.

PREPARATION OF EXTRACT USING HOT WATER EXTRACTION

The whole unripe fruit samples of both pawpaw and grape were washed under running (tape water), then with distilled water against any contamination. The fruits were diced to smaller pieces and weighing balance was used to obtain the weight. The 250g of the weighed sample was boiled in a clean pot with two (2) liters of distilled water for 25 minutes. After cooling, it was sieved and filtered with a muslin cloth and filter paper. The filtrate was poured into a beaker and a rotary vacuum evaporator was used to concentrate it at a temperature below 40^{o}C.

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The resultant crude extract obtained was then exposed to UV-rays for 24hour to check for sterility.

QUALITATIVE AND QUALITATIVE EVALUATION OF THE PHYTOCHEMICAL CONSTITUENTS OF THE UNRIPE FRUITS

Phytochemical screening and identification of bioactive chemical constituents from the extracts of the whole unripe fruit samples were carried out using the standard procedures as described by Evans (2002).

TANNIN TEST

To a test tube, 2.5 ml of the sample was poured and in a water bath, was boiled for 10 minutes. Thereafter, Whiteman filter paper was used to filter the boiled sample. Three (3) drops of 0.1% of ferric chloride was mixed with 2 ml of the filtrate. Positive test is established when brownish-green or a blue-black colouration is observed.

SAPONIN TEST

To 2.5ml of the sample, 5ml distilled water was added, mixed and boiled in a water bath for 10 minutes. To obtain a stable persistent froth, vigorous agitation of the mixture was done. Positive result showed emulsion formation when three drops of olive oil was added.

STEROID TEST

In a test tube containing 2.5ml of the sample, 5ml of ethanol was added and mixed to extract the components in a period of 2 hours. To the extracted sample, 2 ml acetic anhydride and 2 ml of concentrated tetraoxosulphate (VI) acid were added. The presence of steroid is established when a violet to blue or green colouration was obtained.

TERPENOIDS TEST

Measured 2.5ml of the Sample was poured into a test tube; 1 ml chloroform and 1.5ml of concentrated tetraoxosulphate (VI) acid were added. Reddish brown coloration indicated the presence of terpenoid.

FLAVONOIDS TEST

Four pieces of magnesium ribbon were used to extract the sample (1.5 ml), and a few drops of strong hydrochloric acid were also added. Flavonoids are present when orange, pink, and red to purple coloration form.

ALKALOIDS TEST

A test tube was filled with one (1 ml) of each sample extract and 0.1 ml of 1% HCl. After adding a few drops of Dragendorff's reagent (Bismuth Potassium Iodide solution), a brownish red or orange precipitate that indicates the presence of an alkaloid was formed.

PHENOLS TEST

The presence of phenols in the sample extract is indicated by the production of a blue color when 1ml of each extract is combined with 1ml of 5% aqueous ferric chloride.

GLYCOSIDE TEST

A test tube containing 0.5 ml of each sample extract, 2 ml of chloroform, and a few drops of strong sulfuric acid were mixed together. The development of reddish-brown steroid rings is a sign of glycoside presence.

PHLOBATANNIN (HCL TEST) TEST

The existence of a crimson precipitate, a sign of phlobatannin, was checked after adding 2 ml of the extract to diluted HCl.

QUINONES TEST

To one (1) ml of concentrated sulfuric acid, 1 ml of extract of sample was added. Quinine presence was identified by the formation of a red color.

COUMARINS TEST

To one (1) ml of sodium hydroxide, 1 ml of extract of the sample was added. Yellow color formation showed the presence of coumarins.

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SYSTEMS TEST

The test organisms were received from the Federal Polytechnic in Ilaro's Department of Microbiology after being clinically isolated.

FUNGI STRAINS

In this study, pathogenic fungus isolates from four different strains were used. *Aspergillus niger, Aspergillus flavus, Candida albicans, and R. oligosporus are these.*

ANTIMICROBIAL SCREENING

Four (4) clinically significant fungus isolates in all were used. The inoculums were made using microbial cultures that had been cultivated for 24 hours at 37 degrees Celsius in fungal Sabouraud Dextrose Broth (SDB) and that had been adjusted to contain 2x108 colony-forming units (CFU) and 2x105 spores per milliliter using a 0.5 M Macfalard standard solution. The test organisms were examined for antibacterial activity with fruit extract using agar-well diffusion method as described by Clinical Laboratory Standard Institute (CLSI). On 20 ml of solidified Sabouraud Dextrose Agar for Fungi, an inoculum was swabbed uniformly, and the medium was allowed to absorb the inoculum. On the seeded medium, 8-mm-diameter holes were bored, and they were left there for an hour so that the seeds could germinate properly.

THE FUNGI ISOLATES' MINIMUM Inhibitory Concentration (MIC)

The isolate that displayed extract susceptibility was added to broths with various extract concentrations (serial dilutions of the extract equivalent to 200, 10, 50, 25, and 1.25 g/ml). At 28 °C, the tubes were stored for another 24 hours. The plate with low concentration (without visible growth) was considered to be the minimum inhibitory concentration (MIC) (Owoseni & Ajayi, 2010). Following that, a loopful of each test tube's MIC test sample was smeared onto Sabouraud dextrose agar, incubated for 24 hours at 28 OC, and then examined. The presence of a growth suppression of 80% when compared to the RPMI negative control well was regarded as proof of microbial activity.

RESULT

Table 1: The qualitative phytochemical constituents of the extracts of unripe *Carica papaya* and *Vitis venifera*

Phytochemicals	Unripe (*carica papaya*)	Unripe *Vitis venifera*
Tannins	+	+
Saponins	+	+
Phenols	+	+
Glycosides	+	+
Alkaloids	+	+
Terpenoids	+	+
Flavonoids	+	+
Steriod	+	+
Reduced Sugar	+	+
phlobatamine	-	+

Presence of constituent = +Absence of constituents = -

The table above showed the presence of ten (10) phytocompound in the extract of unripe *Vitis venifera*, while only phlobatanin is absent in the extract of *Carica papaya.*

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Table 2: The quantitative concentration of phytocompounds in unripe *Carica papaya* and*Vitis venifera* extracts in Mean±SD

Phyto-compounds	*Carica papaya*	*Vitis venifera*
Tannins	**19.88±0.08**	**22.80`±0.37**
Saponins	**44.20±3.78**	**44.48±0.47**
Phenols	**32.16±0.11**	**34.55±0.39**
Glycosides	**28.56±0.18**	**31.13±0.23**
Alkaloids	**30.05±0.19**	**32.61±0.31**
Terpenoids	**13.33±0.18**	**14.35±0.13**
Flavonoids	**20.12±0.33**	**35.38±0.32**
Steriod	**16.72±0.11**	**17.75±0.17**
Reduce sugar	**84.83±0.28**	**90.99±0.16**

Table 2 above revealed the quantitative concentrations of the phytocompound in the extracts of the unripe fruits. Reducing sugar has the highest mean value of 80.83±0.28 and 90.99±0.16 in both *Carica papaya* and *Vitis venifera* respectively, followed by saponin, phenol, alkaloid; while terpenoid is found to be the lowest in both the extract of the unripe pawpaw and grape fruits.

Table 3: Zone of Inhibition/ Susceptibility of the fungi strain to the extracts of unripe *Carica papaya* and *Vitis venifera* fruits.

Fungal Isolates	*Carica papaya* (mm)	*Vitis venifera* (mm)	Ketoconazole (mm)
Aspergillus niger	**30**	**35**	**32**
Aspergillus flavus	**40**	**30**	**30**
Candida albicans	**R**	**R**	**36**
Rhizopus oligosporus	**40**	**35**	**30**

DISCUSSION

Medicinal and healing activities of plant or herbs look similar in chemical composition; they are classified into major groups such as alkaloid, terpenoids, phenol, tannins, quinines, saponin, glycoside which are dependent on the method of extraction of the compounds from the plants (Nisa et al., 2013; Monishi & Meng, 2017). The qualitative screening of phytochemicals in the extract of unripe *Carica papaya* and *Vitus veniferal* fruits as showed in table1 revealed the presence of ten (10) phytochemicals in *Vitis veniferal* and nine (9) in *Carica papaya* with phlobatamine absent. The presence of these phytocompounds makes them potential herbs for medicine as antimicrobial and antifungi agent. (Robinso, 1995; Nayan, Moreno & Sanchez, 2016).

The quantitative concentration of the phytochemicals in both *Carica papaya* and *Vitis veniferal* (table 2) revealed that reducing sugar has highest value of 84.83±0.28 and 90.99±0.16 respectively with *Vitis veniferal* higher than the former. This is followed by saponin which is higher in both extracts with concentration values of 44.20±3.76 and 44.48±0.47 respectively and phenol in both with concentration value of 32.16±0.11 for *Carica papaya* and

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34.55±0.39 for *Vitis veniferal*. Terpenoid is the phytochemical with the lowest concentration value of 13.33±0.18 and 14.35±0.13 for *Carica papaya* and *Vitis veniferal* respectively.

The concentrations of all the phytochemicals analyzed were formed to be higher in *Vitis veniferal* than those of *Carica papaya*, hence, presenting it as desirable than *Carica papaya.* Silva et al. (2007) stated that plants extracts with the presence of phenol, tanine and flavonoids poses medicinal potential towards oxidative damage that are capable of causing inflammation and cancer. Alkaloids, nitrogen containing secondary metabolite have been reported to have many pharmacological activities which include anticancer, antimalarial and antihypertensive. Terpernoid also has medicinal potential such as antimicrobial activity, antiulcer, antimalarial and anti-inflammation effect (Wink, Schemeller & Latz, 1998; Dudareva et al., 2004). The result of this work agreed with the work done by Kone et al. (2004) who demonstrated the presence of saponin, alkaloid, tannin and phenol in *Carica papaya* extract.

The zone of inhibition of the extract of unripe fruit of *Carica papaya* and *Vitis venifera* were tested against four pathogenic fungal isolates namely *Aspergillisniger, Aspergillus flavus, Candida albicans and Rhizopus oligosporus* measured in millimeter (mm) and using Ketoconazole as a standard drug (control antifungal). The result obtained showed that *Vitisvenifera* had a higher zone of inhibition of 35mm, followed by Ketoconazole the standard drug with 32mm and the least *Carica papaya* with 30mm all against *Aspergillus niger* also represented in the descending order *Vitisvenifer*>Ketoconazole>*Carica papaya. Aspergillus niger* is a pathogenic fungi implicated with the cause of otomylosis a fiungi ear infection associated with temporary difficulty in hearing, pain, tympanic membrane effect and in serious cases, ear canal damage (Schuster, Dunn-coleman, Frisvad & Van-Dijck, 2002).

A higher zone of inhibition of 40mm was observed with *Carica papaya* against *Aspergillus flavus, than Vitus venifera* with 30mm inhibition and katoconazole (antifungi control) with inhibition zone of 30mm. Katoconazole, the antifungi control had zone of inhibition of 36mm against *Canida albican* which showed resistance to both *Carica papaya* and *vitis venifera* extract. The zone of inhibition of *rhizopus oligosporus was* observed to be 40mm with the extract from *Carica papaya* while that of *vitis venfera* is 35mm lower than the former. The least is ketoconazole (antifungi control) with zone of inhibition of 30mm.

The result obtained implies that *Carica papaya* had a higher potential power to inhibit *Aspargillus flavus*and *Rhizopusoligoporus* than *Vitis venferal* and the standard drug, ketoconazole. This is followedby *Vitis venferal* which showed a higher zone of inhibition against *Aspergillus niger* more than *Carica papaya* and ketoconazole.

CONCLUSION

In this study *Vitis venfera* reveled higher composition of the phytochemicals but notwithstanding, *Carica papaya* showed higher potential power in inhibiting the tested fungi especially *Aspargillus flavus* and *Rhizopus oligoporus than* ketoconazole (antifungi control) and *Vitis venfera;* except against *Aspergillus niger* where *Vitis venfera* had more inhibitory activity than *Carica papaya* and ketoconazole. This implies that the extract of *Carica papaya* and *Vitis venfera* are potential medicinal plant with great potential for pharmacological importance in the management of fungi related diseases.

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