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Anitfungal and Phytochemical Screening of Carica Papaya Seed Extract

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Abstract
Carica papaya is a plant known globally for its nutritional properties. The various part of the plant has therapeutic potential. The seed was analyzed to determine its proximate, phytochemical in-vivo activity against fungi isolates using methanol, n-hexane, ethanol and aqueous extract of the seed. The proximate analysis showed that the seed contains protein, carbohydrate, fiber, ash. The phytochemical contents tested were alkaloids, saponin, phenol, flavonoids, glycoside, tannin, anthraquinone and steroid**.** The in-vitro antifungal analysis activity of *Carica papaya* seed was determined using agar well diffusion method. The fungal isolates tested were *Aspergillus niger, Candida albicans, Aspergillus flavus, Fusarium and Penicillium*. All the isolates were sensitive to both extracts at different concentration. The highest zone of inhibition recorded was against *Candida albicans* and the least was against *Fusarium 6mm*. The methanol and ethanol extract had a better zone of inhibition as antifungal at the 100mg/ml than the n-hexane and aqueous extract. The seed of *Carica papaya* has antifungal property and could serve as alternative antifungal agent.

Keywords: *Carica papaya*, fungal isolates, antifungal, proximate, phytochemical

Introduction

Carica papaya is among the plants whose parts (leaf, seed, stem bark, root) are believed to possesses medicinal benefit. The seed are black, tuberculous and enclosed in a transparent aril. The seed is considered as carminative, abortifacient, emmenagogue, counter irritant and vermifuge (Singh and Ali, 2011). The seed of *carica papaya* has both nutritional and therapeutic benefits (Joachim *et al*., 2013). The seed is reported to have antibacterial and antifungal properties (Kanadi *et al.,* 2021). Kanadi *et al. (2*019) in their report wrote that *C. papaya* seed possesses preventive measures against potassium bromated induced renal toxicity in rats. It is of great importance to assess the nutritive and medicinal use of this plant for improved usage.

Fungi causes harm to different parts of the human body such as the foot, tongue, skin, nail, cardiovascular, nervous digestive and system (He *et al.,* 2017). Fungal infections like candidiasis can be very severe and even fatal in immunocompromised patients (Wirth *et al.,* 2008, Morace *et al.,* 2014). There has been an increasing report of resistance of drugs to fungal infections due to drug abuse and wide usage of antifungal drugs broad spectrum like fluconazole, itraconazole and ketoconazole (Sheikh *et al.,* 2018). Most antifungal agents are known to be toxic and produce numerous side effects such as Amphotericin B (Laniado-Laborín *et al.,* 2009). This has given rise to search and study on the efficacy of medicinal plant for curing and healing of various diseases that is less toxic and cost-effective drugs that are readily available with proven efficacy and bioactive components from plant origin. It is important to assess the scientific basis of its use by traditional medicinal practitioners

Plant material collection

The fruits of Carica papaya were collected from a family garden and transported to the laboratory. The seed were removed from the fruit and placed in a sterile beaker. The seed were thoroughly washed several times with clean distilled water and spread under shade at room temperature to dry. The dried seeds were grounded to fine powder using clean laboratory blender and mortar and is placed in sterile container and kept in the refrigerator at 4°C till usage.

Preparation of plant for extraction

Solvent – solvent extraction method was used to extract the seed of Carica papaya. One Hundred grams (100g) of powder was dissolved in 500ml of water and partitioned exhaustively with Methanol, n- Hexane distilled water and Ethanol using Soxhlex apparatus. The solvent was evaporated using rotary vacuum evaporator for the solvent and water bath for water extract. The extracts were stored in sterile bottles at 4°C in the refrigerator. The extracts were tested for sterility by plating it out on Sabouraud Dextrose Agar. The absence of growth after 48 hours' incubation indicates sterility.

Proximate Analysis screening

Proximate composition of the extract was determined using the method of (AOAC,2019).

Phytochemical Screening

The extracts were screened for phytochemicals using the method described by Isela et al. (2019) to identify the phytochemical constituents present. Phytochemical screening was performed to test for alkaloids, saponin, phenol, flavonoids, glycoside, tannin, anthraquinone and steroid.

Isolation of Test Organism

The isolates used for this study were Aspergillus niger, Candida albicans, Aspergillus flavus, Fusarium and Penicillium and were obtained from pure culture plates of Department of Medical Microbiology University of Nigeria. The microorganisms were isolated on Sabouraud dextrose agar (SDA) and were sub cultured twice before use to ensure pure isolates were obtained.

Media preparation

The media, Sabouraud dextrose agar were prepared and treated according to the manufacturer's instruction. About 20ml of the sterilize medium cooled to about 50^0c was aseptically poured into 90 mm diameter sterilized Petri-dishes and allowed to set. Sterility of the prepared media was assessed by incubation of randomly selected 3 plates from the batch at 37°C for 24hours.

Antifungal susceptibility testing of the extracts

The antifungal activity potentials of the extract were done using the method described by Jayshree et al. (2013). Sterile cotton swab was used to inoculate each of the (already standardized to 0.5 McFarland) fungal suspension aseptically and pressing the swab on the inside of the fungal suspension to remove excess fluid and was rotated several times on the surface of sterilized SDA to ensure uniform spreading. A 100 mg/ml of the extracts prepared by dissolving 200 mg of the crude extracts into 2 ml of Dimethyl sulfoxide (DMSO) was used. A sterile cork borer of 6 mm diameter was used to make 2 well on each agar plate then loaded with 60 µl of each of the stock concentration of the crude extracts of the test samples, it was allowed to stand for 30 minutes to allow diffusion of the extracts into the agar and incubated at 28 ± 2°C for 3 – 20 days with daily inspection. Each test was done in triplicate. The zone of inhibition was observed and recorded to the nearest millimeters using a transparent meter rule results expressed as mean. Fluconazole 50mg/ml was used as the standard positive control against the fungal isolates.

Statistical Analysis

The experiment results were expressed as mean. Data obtained were statistically analyzed using one-way Analysis of Variance (ANOVA), a tool in Statistical Packages for Social Sciences (SPSS 17.0). The level of significance was set at $p \leq 0.05$.

Result

Table 1: Proximate composition of *Carica papaya* seed

Proximate	Amount (mg per 100 g)
Moisture	7.40
Carbohydrate	22.45
Fiber	20.25
Protein	27.30
Ash	8.94
Magnesium	224.81
Calcium	765.00
Zinc	5.25
Iron	5.35
Potassium	845.20

Table 2: Phytochemical contents of different crude solvent extracts of *Carica papaya* seed

Phytochemical	Flavonoid	Phenol	Saponin	Alkaloid	Tannin	Glycosides	Anthraquinones	Steroid
Methanol	+	+	+	+	+	+	+	+
Aqueous	+	+	+	+	+	-	+	-
n-Hexane	+	+	+	+	+	+	+	+
Ethanol	+	+	+	+	+	+	-	-

Table 3: Antifungal activity of different solvent extracts of *Carica papaya* seeds zone of inhibition (mm) at 100mg/ml

Fungal isolates	Methanol	Aqueous	n-Hexane	Ethanol
A. niger	14	7	8	11
C. albicans	16	9	8	14
A. flavus	14	7	9	11
Fusarium	12	6	9	12
Penicillium	12	8	8	11

Discussion

Carica papaya seed is found to contain numerous rich nutritional components. The proximate analysis of Carica papaya seed extract was shown in table 1. It has a high content of protein which is essential for body building, carbohydrates and fat which makes it a good source of energy that will be of good help in malnourished persons. Our finding is in agreement with previous reports (Azevedo *et al.,* 2014; Oche *et al.,* 2017). It also contains vital minerals like Ca, Mg, K, Zn and P. They are essential for the improved metabolic and physiological functioning of the body (Morais *et al.,* 2016). Dietary fiber is found in the seed and fiber helps in the removal of toxins from the digestive system, help in lowering of cholesterol (Wulansari *et al.,* 2019) and proper functioning of large intestine and protect the gastrointestinal tract from disorders and cancer cells (Otles and Ozgos, 2014). It has a low moisture content which could help in maintain the shelf life

(Kanadi *et al.,* 2019) reported that moisture content of foodstuff affects its shelf life and less moisture withstand early spoilage of food by reducing microbial growth.

Carica papaya seed were found to be a rich source of phytochemicals and these phytochemicals are helpful as anti- proliferation of cancer cells, shielding against cellular oxidative injury and help in reducing the risk of non-communicable diseases (Olcum *et al.,* 2020). Phytochemical of *Carica papaya* seed is presented in table 2**.** It shows that aqueous, methanol, ethanol and n-hexane extracts of *C. papaya* seed contain different amounts of phytochemicals like alkaloid, tannin, phenol, flavonoid, glycosides etc. Tannin helps in the coagulation of the cell wall proteins. Alkaloids have an anti-bacteria and anti-analgesic properties hence its use in preparation of medicinal agents (Okwu and Okwu, 2004). Alkaloids are the most important secondary metabolites and have a therapeutic potential of curing diseases (Olanitola *et al.,* 2018). Flavonoids are lipophilic, capable of interrupting cell wall and membrane formation with inhibition of enzymatic activity by formation of complexes with cell wall of the bacteria (Olanitola *et al.,* 2018). Phenol and anthraquinones serves as scavenger molecule that helps in protecting the cellular environment from damage against activities of inflammatory and oxidative agents (Olcum *et al*., 2020). Saponin enhances the entry of toxic material or seepage of important contents of the cell (Ajiboye *et al*., 2020**)** and also has a cytotoxic effect like permealization of the small intestine and inhibit growth of a range of cells leading to its anti-cancer and anti-inflammatory action (Yildirims *et al.,* 2015). Glycosides plays a role in restoring cardiac rhythm disorders because of its ability to improve the output force of the heart thereby increasing the rate of heart contractions by enhancing pumping of sodium- potassium cellular ATPase (Jing *et al.,* 2019).

The antifungal susceptibility testing of *Carica papaya* seed was presented in table 3. The extracts have different zone of inhibition on the test isolates. The highest zone of inhibition recorded was for methanol extract on *C. albicans* 16mm and the least 6mm for aqueous extract of fusarium. The highest zone of inhibition for methanol was 16mm and the least was 12mm. The highest zone of inhibition for ethanol was 14mm and the least was 11mm. The highest zone of inhibition for n-hexane was 9mm and the least was 8mm. The highest zone of inhibition for aqueous was 9mm and the least was 6mm. Our finding is consistent with previous findings (Noshad *et al*., 2018; Baskaran *et al.,* 2012). The antifungal susceptibility of the methanol and ethanol extracts had better antifungal properties compared to n- Hexane and Water extract. Choice of solvents during extraction plays an important role on the extraction, because each solvent has different polarity and range of solubility for phytochemical components (Arvind Kumar *et al.,* 2014). Therefore, the selective extraction of bioactive compounds from medicinal plants requires the use of suitable solvents in order to obtain the biological active components that can be of therapeutic use (Dagne *et al.,* 2021; and Calzada *et al.,* 2007). The mode of action of antifungal activity of *Carica papaya* seed extract could be by production of oxygen radicals that cause breakdown of mitochondrial membrane and reducing the activities of the respiratory mitochondria chain enzymes (Zhang *et al.,* 2017). *C. papaya* seed is reported to contain Benzyl isothiocyanate (BITC) compound which is responsible for its inhibitory effect (He *et al.,* 2017). However, it is advisable that more research be done on it.

Conclusion

This study shows that *Carica papaya* seed contain many nutritional and phytochemical compounds essential to human health. The antifungal susceptibility testing of all the extract used shows that *Carica papaya* seed has antifungal properties suggesting that it could be used as a possible potential source of alternative natural anti-fungal agent.

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