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Antifungal Activity and Phytochemical Screening of *Cymbopogon citratus, Cajanus cajan* and *Plectranthus amboinicus* Leaves Collected in Guyana, South America

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Authors' contributions

This work was carried out in collaboration among all authors. Author DM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors GS, RK and AAA performed the statistical analysis, overall analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Medicinal plants have been a fundamental part of the human health since existence. Guyana is surrounded high in the green shoulder of northern South America and shares Amazon River and Amazon Forest. South American population use plant extracts obtained from traditional medicinal plants as treatment for many infectious diseases. The study aimed to estimate antifungal property and chemical composition of the three medicinal plants *Cymbopogon citratus* (lemongrass), *Cajanus cajan* (pigeon pea) and *Plectranthus amboinicus* (thick leaf thyme) leaves collected from the coastal areas of Guyana.
Study Design: Experiment based study.

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Place and Duration of Study: Plants were gathered along the East Coast of Guyana and identified at the Biodiversity Center, University of Guyana, Georgetown, Guyana between January 2017- May 2017.
Methodology: Phytochemical extraction was conducted using the soxhlet and rotovap apparatus and an aqueous extraction method. Data analysis of the study was done using R-Studio Program for statistical computing and graphics. A Tukey test was done along with ANOVA and Boxplots.
Results: Qualitative analysis of phytochemicals was carried out and the presence of terpenoids, steroids, glycoside, alkaloid, tannins and saponins were positive in some plants. Antifungal activity was tested using the poisoned food and well diffusion techniques.
Conclusion: In conclusion, *C. cajan* showed significant zones of inhibition using a well diffusion technique whereas hexane extract showed significant inhibition with poisoned food technique.

Keywords: Antifungal; plant extract; inhibition; phytochemical; poison food technique; well diffusion technique.

1. INTRODUCTION

Nature always has a wide variety of therapeutically valued bioactive compounds to offer mankind. Plant products are considered safe for their pharmacological activities and widely recognized for their vast diversity [1,2]. Human beings are dependent on medicinal plants ever since existence. The past two decades showed an increasing interest in the investigation of different medicinal plant extracts [3]. World Health Organization (WHO) estimates that about 80% of people are still dependent on traditional herb-based medications globally due to their low cost, easy accessibility and likely negligible side effects in comparison to allopathic medicines [4,5]. Before the advent of synthetic fungicides, plant derivatives were commonly used for fungal control in developing countries. Plant derivatives as fungicides were relatively cheap compared to imported synthetic fungicides [6]. Damage to crops by fungal pathogens has pressured farmers to use antifungal control agents [7]. Several of the synthetic fungicides are reported to cause adverse effects on treated soil ecosystems because of their non-biodegradable nature [8]. Substantial use of chemical pesticides induces health problems and environmental hazards in the agricultural system, therefore it is of no doubt that natural products of antimicrobial activity are the best bio rational alternatives today [9].

C. citratus, commonly called lemongrass is a tall perennial grass that grows in tropical and subtropical habitats [10]. *C. citratus* is well known to have antidepressant, antioxidant, antiseptic, sedative, nervine, bactericidal, and fungicidal properties [11,12]. It grows well in tropical and subtropical regions of Asia, South America, and Africa [13]. *C. cajan* is known to grow well in tropical and subtropical regions with wide medicinal use [14]. *C. cajan* is an annual perennial shrub typical to dry climates and few parts of South America [15]. Next plants of genus Plectranthus included in this study have over 3000 recognized species, spread along with countries in Africa, South America, Asia, and Australia [16,17,18]. The present study aimed to identify phytochemicals and antifungal activity of *C. cajan, C. citratus,* and *P. amboinicus* leaf extracts.

2. MATERIALS AND METHODS

2.1 Plant Collection

Plants were gathered along the East Coast of Guyana and identified at the Biodiversity Center, University of Guyana, Georgetown, Guyana between January 2017- May 2017. The collected leaves of each plant were washed under running tap water, dried, and placed in brown paper bags. The paper bags were weighed and placed in a hot air oven, drying at a constant temperature of 55°C until a constant weight was recorded. The dried leaves were powdered using a mixer grinder at GuySuCo Laboratory and stored for the next step [19].

2.2 Soxhlet Apparatus

Adhering accordingly to the method followed at the Pesticides and Toxic Chemicals Control Board (PTCCB), 64 grams of dried plant material were weighed and placed in a thimble. The thimble (a thick porous cellulose container) was placed into the extraction chamber. The selected solvent was slowly poured through the condenser opening. The boiling flask was heated by a heating mantle. The boiling flask collected the extracted phytochemicals with each evaporation that passed through the siphon arm and the solvent vapor was rapidly cooled in the

condenser's cooler. Each plant used a new thimble and fresh solvent.

2.3 Rotary Evaporator (ROTOVAP)

The rotary evaporator reduces the solutions down to a solid-state. The extract containing either hexane or methanol as a solvent was collected in the boiling flasks from the soxhlet apparatus. Each flask with residue was labelled; depicting solvent used and plant species.

2.4 Aqueous Extraction

The aqueous extraction was carried out using the standard method [20]. About 15 grams of grounded leaves from each plant species were extracted by successive soaking for 3 days using 35 ml of distilled water in separate containers. The extracts were filtered using Whatman No. 1 filter paper using a vacuum. The filtrates were concentrated by evaporation at a low temperature of 30^{o}C using a water bath. The concentrated samples were used to make a 50% stock solution from which the tested concentrations were created. Phytochemical residue of 5 grams was added to 5 ml of the indicated solvent to make a 50% stock solution. From the stock solution, 300µl, 400µl, and 500µl were used to check the antifungal property. Each test was done in triplicate per plant and solvent.

2.5 Qualitative Analysis of Phytochemicals

Phytochemical analysis was done to identify the presence of the phytochemicals; tannins, alkaloids, glycoside, saponins, flavonoids, terpenoids and steroids [21].

2.6 Culture Technique

Antifungal activity was tested on one selected fungi *Aspergillus niger*, the strain was obtained from pure cultures at the University of Guyana, Berbice Campus. *A. niger* was cultured and maintained on Potato Dextrose Agar (PDA). Antifungal activity was performed by Well Diffusion and Poisoned Food technique. Measurements for the Poisoned Food Technique was done following Akhila [13], between five to seven days or once the control was completely covered.

Percentage of mycelial growth inhibition was calculated from the formula:

Mycelial growth inhibition= (diameter of control-diameter of sample/ diameter of control) x100

2.7 Analysis

Data analysis of the study was done using R-Studio Program for statistical computing and graphics. A Tukey test was tested along with ANOVA and Boxplots were constructed. A Tukey test was used to compare concentration, plant, and solvents versus techniques. Box and whiskers plot also called boxplots were used as visual representations of the replicates.

3. RESULTS

3.1 Phytochemical Analysis

Table 1 shows the result of phytochemical screening in different plant extracts. Hexane, a solvent with a low polarity, extracted the most phytochemicals with each plant, while water, a solvent with high polarity, extracted the least. Alkaloid was only present in the water extraction of *C. cajan*, while Terpenoid was present only in *C. citratus*. Glycosides contents were extracted by hexane leaf extract of *C. cajan* and *P. amboinicus* only and steroid contents were extracted by methanol extracts of all the three plant leaves, saponins were the most common phytochemical identified by the plant extracts.

3.2 Poisoned Food Technique

Table 2 illustrates mean±SE for every plant extract with Poisoned food technique. Lower the diameter of fungal growth better the antimicrobial property of the three selected plant extract. Hexane solvents of both *C. citratus* (20±7.6) and *C. cajan* (25±4.4) showed reduced fungal growth with increased concentration of plant extracts. However, the plant extracts with water solvent didn't show much variation in inhibiting fungal growth.

Tukey test found a significant statistical difference between inhibition percentage among the three plant extracts (Fig. 1). *C. cajan* showed a significant inhibition compared to the other two plants with mean percent of inhibition couples above other two extracts. Fig. 2 shows Tukey test comparison among solvents used in the study. Overall, hexane showed significant difference when compared to methanol and water. The lowest inhibition percentage for hexane was 0% while the other solvents showed inhibition percentage with negative values.

Table 1. Results on the qualitative tests indicating the presence or absence of phytochemicals in each plant solvent

Phytochemical	*P. amboinicus*			*C. citratus*			*C. cajan*		
	Hex	Meth	Water	Hex	Meth	Water	Hex	Meth	Water
Saponins	+	+	+	+	+	+	+	+	-
Tannins	-	-	-	-	+*	-	-	-	+*
Flavonoid	+	-	-	+	-	-	-	-	+*
Alkaloid	-	-	-	-	-	-	-	-	+
Terpenoid	-	-	-	+	-	+	-	-	-
Steroid	+	+	-	+	+	-	-	+	-
Glycoside	+	-	-	-	-	-	+	-	-

**C. citratus extracted with methanol displayed a green black color that indicated catecholic tannins.*
**C. cajan extracted with water displayed a blue color that indicated gallic tannins.*
**C. cajan extracted with hexane displayed an orange color that indicated flavones*

Table 2. Measurements of fungal growth in millimeters (mm) and standard error for the poisoned food technique (mean±SE)

Conc (µl)	*P. amboinicus*			*C. citratus*			*C. cajan*		
	Hex	Meth	Water	Hex	Meth.	Water	Hex	Meth	Water
300	80±5.8	45±5.0	90±0.0	47±1.5	90±0.0	90±0.0	25±4.4	60±7.3	47±2.7
400	70±1.7	50±2.1	90±0.0	40±2.6	90±0.0	90±0.0	30±2.1	60±5.0	90±0.0
500	52±3.1	55±4.4	90±0.0	20±7.6	90±0.0	90±0.0	30±3.3	50±7.3	36±6.4

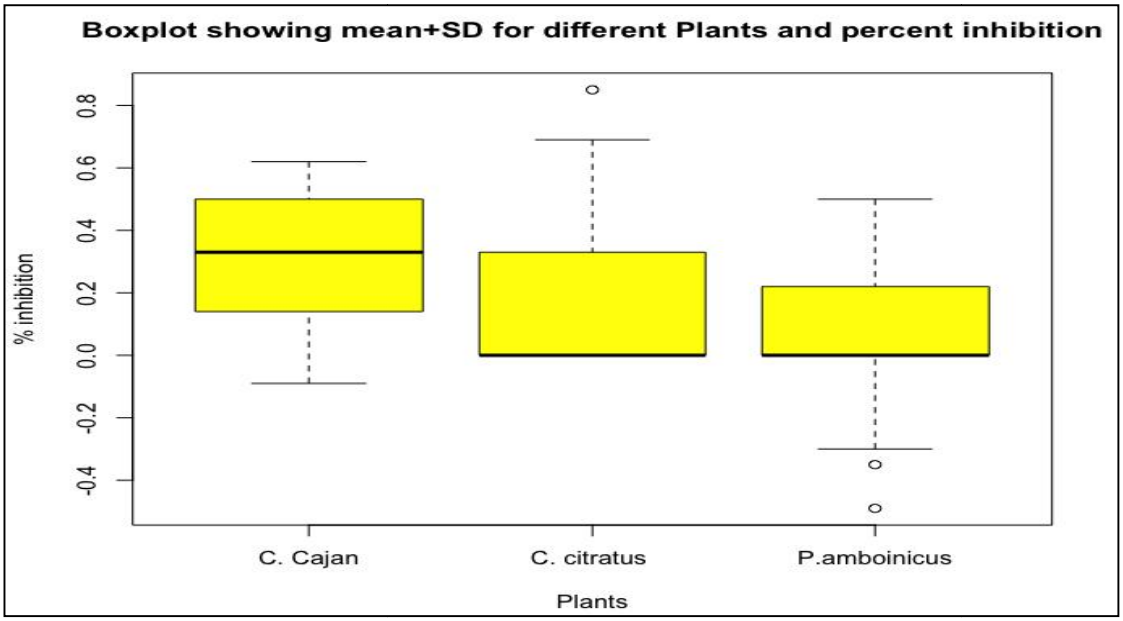

Fig. 1. Boxplot (mean±SD) for different plants and percent inhibition

The Tukey test also showed a statistically significant difference between inhibition percentage and solvents between hexane and the other two solvents methanol and water (Fig. 3). Comparison was done for inhibition percentage with plant extract and the concentration of solvent used. Percentage inhibition versus plant extract is seen between *P. amboinicus* and *C. cajanus* with hexane at 300µl concentration and *P. amboinicus* and *C. citratus* with hexane at 500µl concentration. Percentage inhibition versus solvent is clearly seen between hexane and methanol with *P. amboinicus* at 400 µl wereas concentration versus percentage

inhibition can be seen between the different methanol concentrations 300 µl and 500 µl of *C. citratus*. The concentrations and solvents those were not successful were shown as lines (---) on the graph are at zero.

3.3 Well Diffusion Technique

Table 3 on the other side demonstrate zone of inhibition of *A. fumigatus* with well diffusion technique. Water extract of *C. cajan* was the most effective with the highest zone of inhibition (30±4.4). Although water extract of *P. amboinicus* and *C. citratus* were not effective but hexane extract of *P. amboinicus* showed 23±1.8 and *C. citratus* with 21±1.8. The size of the inhibition zones was measured 48 hours after plating. The mean zone of inhibition of the three replicated tests of the plant extract was expressed in millimeter.

Tukey test comparison showed, statistically significant difference between *C. cajan* and the other two plants. Water extract of *C. cajan* was effective compared to water extracts of *P. amboinicus* and *C. citratus*. *C. cajan* showed the highest zone of inhibition was thirty-five while its lowest was three (Fig. 4). Tukey test comparison of solvents showed hexane on average produced significant zones of inhibition with all plants but water worked well with *C. cajan*, producing the highest zones of inhibition (Fig. 5). The rising gradient of *C. cajan* illustrates effective inhibition based on the various concentration of each plant (Fig. 6). Outcome of Fig. 6 shows (a) significant difference between *P. amboinicus* and *C. cajan* hexane extracts at 500µl and between *C. cajan* and *C. citratus* methanol extracts at 500µl. (b) even though the water extract for *C. cajan* had the highest zones of inhibition the solvent hexane produced zones of inhibitions in all plants at all different concentrations.

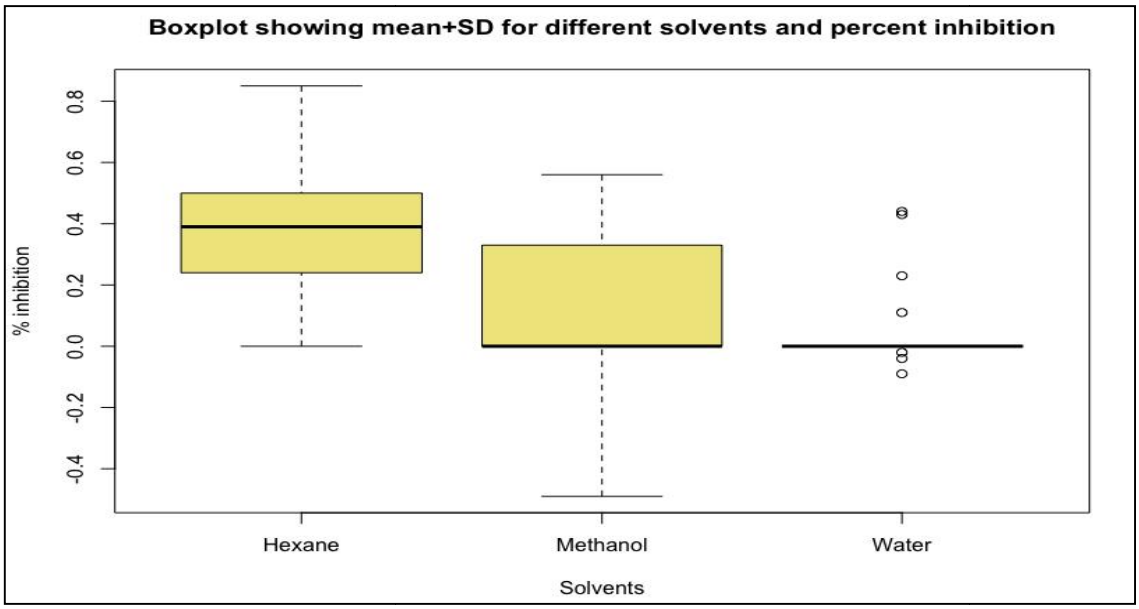

Fig. 2. Boxplot (mean±SD) for different solvents and percent inhibition

Table 3. Mean measurements of zone of inhibition in millimeters (mm) and the standard errors for the well diffusion technique

Conc (µl)	*P. amboinicus*			*C. citratus*			*C. cajan*		
	Hex	Meth	Water	Hex	Meth	Water	Hex	Meth	Water
300	6±1.9	5±0.3	0	10±0.3	1±0.6	1±0.0	3±0.3	11±0.9	20±1.7
400	16±2.2	7±0.3	0	18±0.9	7±0.3	2±0.3	5±0.3	15±1.7	25±1.7
500	23±1.8	9±0.9	0	21±1.8	9±0.3	4±0.3	7±0.9	18±3.1	30±4.4

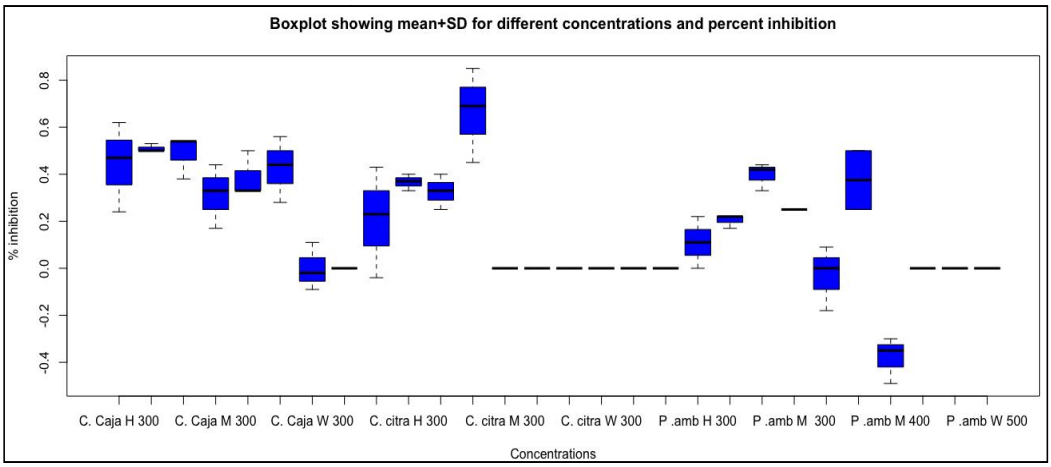

Fig. 3. Boxplot (mean±SD) between concentrations and percentage inhibition

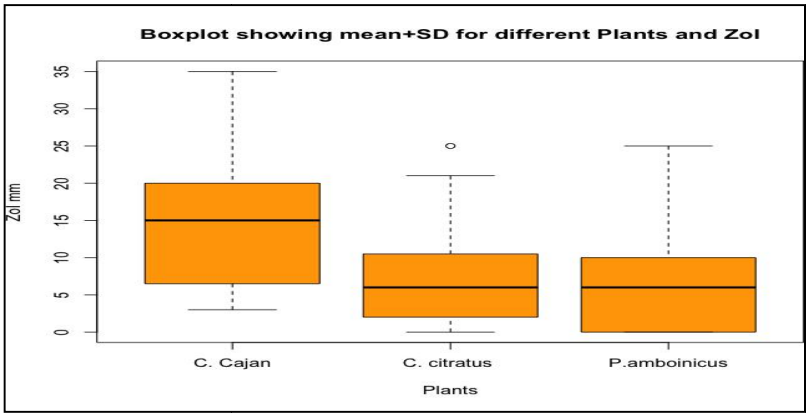

Fig. 4. Boxplot (mean±SD) for different plants and the zones of inhibition (Zol)

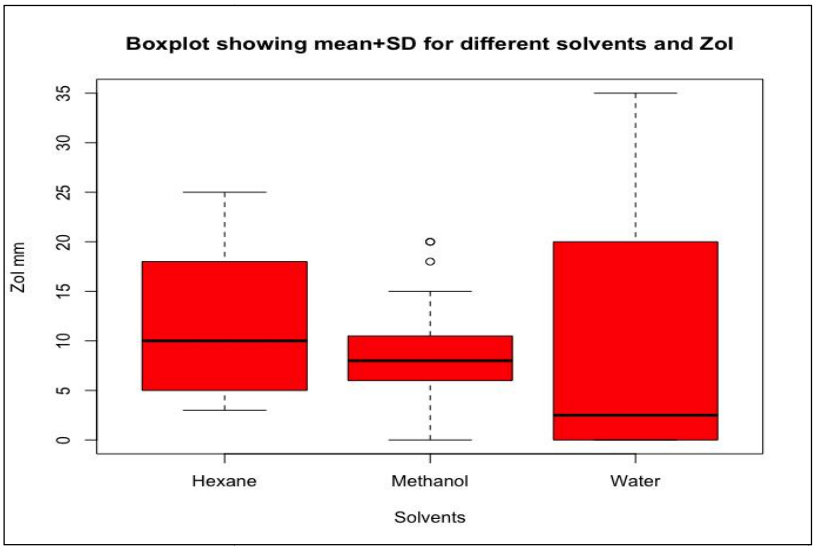

Fig. 5. Boxplot (mean±SD) for different solvents and the zones of inhibitions

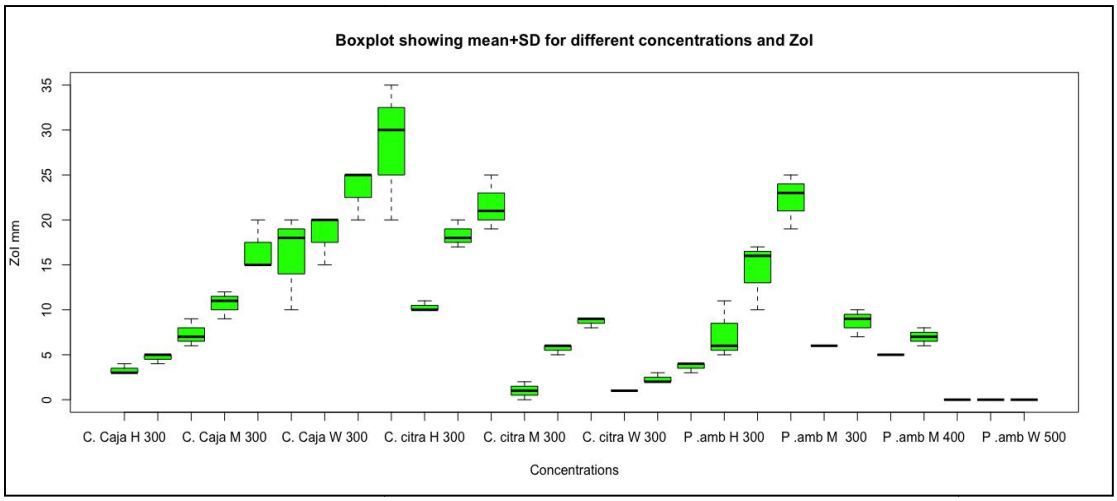

Fig. 6. Boxplot (mean±SD) for different concentrations

4. DISCUSSION

Different studies in recent years have demonstrated the importance of phytochemicals due to their outstanding health benefits. Plant metabolites are extracted by using various ways and efficiency of extraction method depends on several factors like the nature of phytochemical constituents, the method of extraction, particle size of the sample, extraction time, temperature, pH, solute to solvent ratio, and the solvent polarity [22]. It is very important to choose an appropriate solvent system to recover higher extract yield and bioactive compounds from a sample [23]. This study demonstrated a significant difference in the extract yield obtained with different solvents. Literatures have highlighted methanol as the best solvent for extraction [24,23,5]. Methanol extract of lemongrass showed greater antimicrobial properties with increasing concentration [25]. Similarly, methanol extract of *Argemone mexicana* leaves and seeds showed greater antibacterial activity than water extracts [26].

Preparation of an extract with an organic solvent was shown to have greater antibacterial activity [27]. GC-MS method showed 11 major peaks in the quantitative phytochemical analysis of methanol and ethanol of *P. amboinicus,* as well as excellent antimicrobial properties [28]. The study has reported that essential oil of *P. amboinicus* possesses antifungal activity against *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus oryzae, Candida versatilis, Fusarium moniliforme,* and *Saccharomyces cerevisiae* [29]. *C. citratus* showed antibacterial properties in two of the three main components of the oils identified i.e. the α-citral (geranial) and β-citral (neral) components [30]. The study has shown the antibacterial effect of *C. citratus* with methanol and aqueous extracts at 20 µgm [31]. The qualitative phytochemical tests of *C. citratus* showed only the presence of tannins and steroids, indicating extremely low percentage alkaloids, flavonoids, and saponins. Ethyl acetate extract of *C. citratus* showed the highest antimicrobial activity with *Aspergillus sp* and *Mucor sp* than with aqueous and methanol extracts [32].

The aqueous extraction method in this study found effective antibacterial property but poor antifungal property with *C. cajan* [33]. However, it was stated that *C. cajan* is more active against fungus *A. niger* and showed 48 components in the GC-MS spectrum [34]. Various studies in the past have demonstrated a major component in pigeon pea leaves that have potential benefits to human health with flavonoids and stilbene [35,36] including treatment of diabetes and jaundice [37,38,39].

5. CONCLUSION

All three plant extracts showed some form of inhibition against *Aspergillus niger* however the solvent hexane of *C. cajan* was most successful. The well diffusion technique showed efficient zones of inhibition with *C. cajan's* water extract. Hexane showed significant inhibition with the Poisoned Food technique especially with *C. citratus* and *C. cajan*. Overall, *C. cajan*

showed the maximum inhibition overall against *A. niger*.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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