Original Research Article

Antifungal activity and phytochemical screening of *Cymbopogon citratus, Cajanus cajan*, and *Plectranthus amboinicus* leaves collected in Guyana, South America

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.
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. MATERIAL 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].

Comment [DS1]: Add Duration of Study

Comment [RK2R1]: Correction done

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.

Comment [DS3]: It was possible to test more than one fungi, not one.

Comment [RK4R3]: Remodifed the sentence!

Percentage of mycelial growth inhibition was calculated from the formula:

$$Mycelial\ growth\ inhibition = \frac{diameter\ of\ control - diameter\ of\ sample}{diameter\ of\ control} * 100$$

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.

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*

3.2 POISONED FOOD TECHNIQUE

Table 2 illustrates mean±SE for every plant extract. Tukey test found a significant statistical difference between inhibition percentage and plant extract (Figure 1). *C. cajan* showed a significant inhibition compared to the other two plants. Hexane extract of *P. amboinicus* and *C. cajan* showed statistical significance inhibition at 300µl concentration were as hexane extract of *P. amboinicus* and *C. citratus* showed inhibition at 500µl concentration (Figure 2). The Tukey test also showed a statistically significant difference between inhibition percentage and solvents between hexane and the other two solvents -methanol and water (Figure 3). Table 3 shows the inhibition percentage between the tree leaf extract.

Table 2 Measurements of fungal growth millimeters (mm) and standard error for the Poisoned Food Technique (mean±SE).

Conc (µl)	*P. amboinicus*			*C. citratus*			*C. cajan*		
	Hex	Meth	Water	Meth	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

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

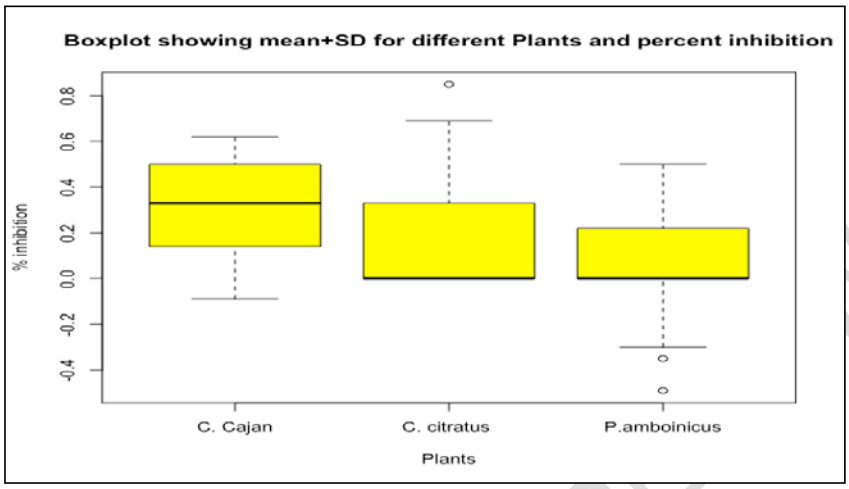

Figure 1. Boxplot (mean±SD) for different plants and percent inhibition.

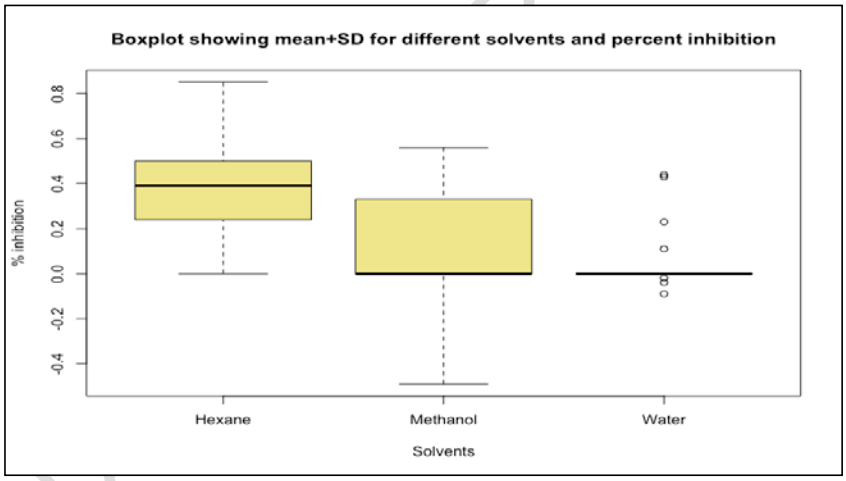

Figure 2 Boxplot (mean±SD) for different solvents and percent inhibition.

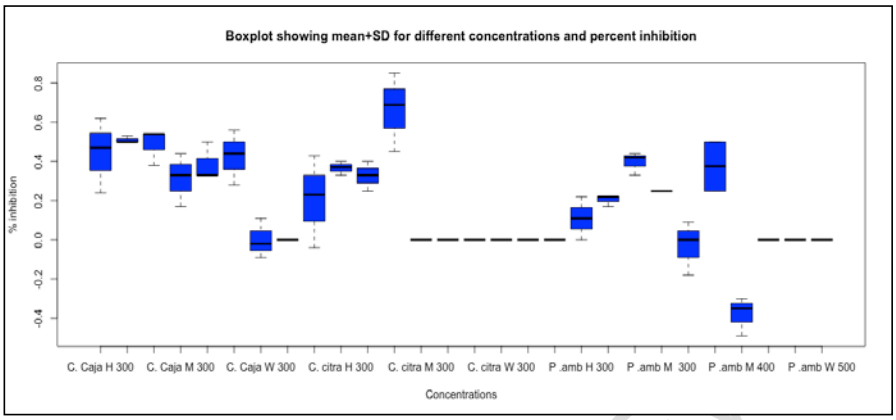

Figure 3 Boxplot (mean±SD) between concentrations and percentage inhibition.

3.3 WELL DIFFUSION TECHNIQUE

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. According to the Tukey test, there is a 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*. There is a significant difference between *C. cajan* and the other two plants. *C. cajan* showed the highest zone of inhibition was thirty-five while its lowest was three (Figure 4). Hexane on average produced significant zones of inhibition with all plants but water worked well with *C. cajan*, producing the highest zones of inhibition (Figure 5). The rising gradient of *C. cajan* illustrates effectiveness inhibition based on the various concentration of each plant (Figure 6).

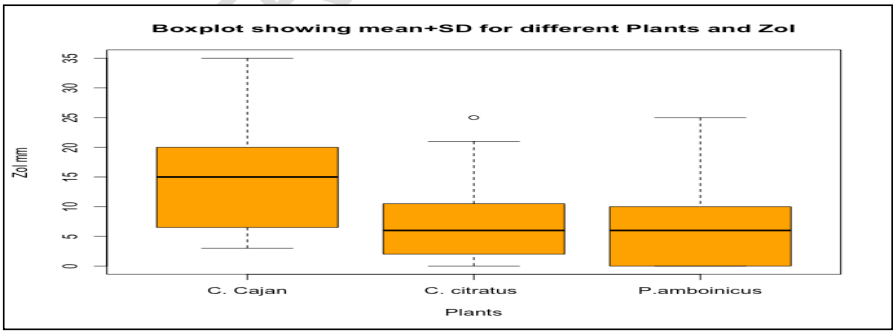

Figure 4 Boxplot (mean±SD) for different plants and the zones of inhibition (ZoI).

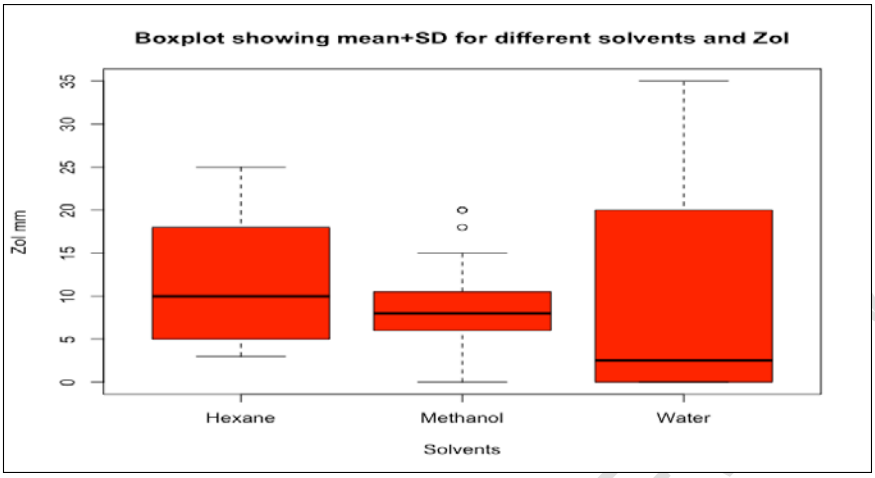

Figure 5 Boxplot (mean±SD) for different solvents and the zones of inhibitions

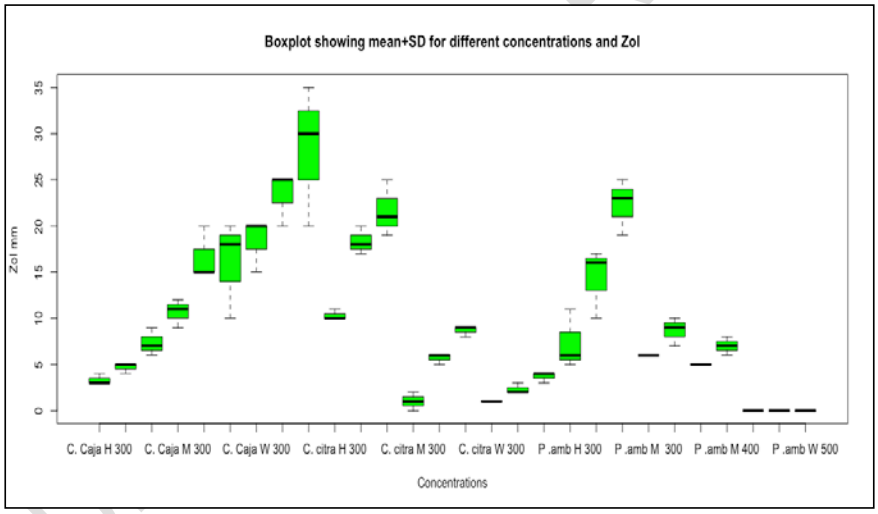

Figure 6 Boxplot (mean±SD) for different concentrations

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 and that *A. niger* 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

Some form of inhibition was shown by all three plants and 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*.

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