Phytochemical, Heavy Metals and Antimicrobial Study of the Leaves of *Calopogonium Mucunoides*

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Research Article

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Abstract

Background: In this study, the phytochemical, heavy metals, and antimicrobial characteristics of the leaves of *Calopogonium mucunoides* were investigated in other to determine its therapeutic potentials. The phytochemicals present in the leaves were extracted with *n*-hexane, methanol and ethyl acetate, and the extracts were used to investigate for the phytochemical constituents and antimicrobial activity. The methanol extract was used to test for the concentration of five heavy metals.

Results: The result of the phytochemical screening confirmed the presence of alkaloids, anthocyanins, flavonoids, glycosides, phenols, reducing sugars, saponins, steroids, tannins, and terpenoids in various quantities. The heavy metals analysis result revealed the presence of lead (0.08 mg/kg) and iron (0.08 mg/kg) well below the acceptable limits set by the World Health Organization for heavy metals in plants, while cadmium, nickel and zinc were found to be below detectable limits. The extracts were tested against thirteen human pathogens (ten bacteria and three fungi) using the disk diffusion method. The extracts possessed a broad range of microbial activities, with the methanolic extract reportedly showing the highest zone of inhibition (31 mm) against *Bacillus sp.* while the *n*-hexane extract did not show any antimicrobial activity in the whole test organisms.

Conclusion: The results obtained revealed that the leaves of *C. mucunoides* has some therapeutic values and could be exploited in the preparation of herbal drugs for the treatment of various ailments.

Highlights

- The Phytochemical, Heavy Metal, and Antimicrobial properties of the leaves of *Calopogonium mucunoides* were studied.
- The Components in the leaves were extracted with three solvents; *n*-hexane, methanol and ethyl acetate.
- The result of the phytochemical screening confirmed the presence of alkaloids, tannins, saponins, steroids, flavonoids, glycosides, terpenoids, anthocyanins and reducing sugars in various quantities.
- The result obtained revealed the presence of lead (0.08 mg/kg) and iron (0.08 mg/kg) well below the acceptable limits set by WHO for heavy metals in plants, while cadmium, nickel and cadmium were found to be below detectable limits.
- The extracts possessed a broad range of microbial activities, with the methanolic extract reportedly showing the highest zone of inhibition (31 mm) against *Bacillus sp.* while the *n*-hexane extract did not show any antimicrobial activity.

1. Background

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern pharmaceuticals have been isolated from natural sources (Boominathan and Ramamurthy 2009). Medicinal plants are the best natural source of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Bakht, Ali et al. 2011). In African countries, herbal formulations have for ages been used for the treatment of various diseases and is still the most affordable and accessible healthcare system (Soladoye, Ikotun et al. 2013). Plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment, hence the basic reason for the recent increase in the demand for herbal drugs (Borokini and Omotayo 2012). Secondary plant metabolites, otherwise known as phytochemicals, previously with unknown pharmacological activities, have recently been investigated extensively as a source of medicinal agents (Parekh and Chanda 2007). Phytochemicals are non-nutritive chemical compounds that occur naturally in plants, and have protective or disease preventive properties (Harbone 1998, Iwuozor 2019, Temitope, Olugbenga et al. 2020). These compounds vary in plants depending on their growing conditions, plant species, location of the plant, extraction methods, soil topography, and age of the plant (Afiomah and Iwuozor 2020, Temitope, Olugbenga et al. 2020).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments (Prusti 2008). In most developing countries where the locals do not have access to the quite expensive synthetic drugs, traditional plants becomes the alternative, and investigations into the phytochemical and antimicrobial activities of these plants have been ongoing (Bakht, Islam et al. 2011). However, Onyema and Ajiwe (2014) noted that the illegal use of these plant medications without the prescription coupled with the adaptability of bacteria has resulted to an increase in the number of drug resistant organisms, and there have been growing concern about the era of antimicrobial medications coming to an end. As a result, researchers are increasingly turning their attention to folk medicine in a bid to developing good and efficacious drugs against microbial infections (Parekh and Chanda 2007).

Despite the economic and nutritional values of medicinal plants, there has been an increasing concern about their safety and toxicity. Studies have shown that heavy metal concentrations in animal sex organs such as testicles and ovaries leads to a loss of sexual drives in the animals (Solomon, SA et al. 2014, Iwuozor 2019). Industrial spills and discharges, indiscriminate dumping of wastes and sewages, agricultural practices and general environmental degradation has resulted to an increase in harmful heavy metals in the soil (Souza, Camargos et al. 2014, Iwuozor 2018, Iwuozor and Gold 2018, Iwuozor, Oyekunle et al. 2021, Ogunlalu, Oyekunle et al. 2021). Plants on the other hand remain the major link in the transfer of these heavy metals from the contaminated soil to humans.

Calopogonium mucunoides Desv (*C. mucunoides*) is a vigorous, hairy annual trailing legume belonging to the family of fabaceae. It is a woody vine listed in the global compendium of weeds as an invasive weed impacting primarily agricultural and semi-natural ecosystems (Randall 2012). Cook, Pengelly et al. (2005) reported that *C. mucunoides* is commonly planted as a pioneer species and as a nitrogen fixing species to reduce erosion and improve soil fertility. Like many other legume forages, the nutritional potential of *C. mucunoides* lies in its protein content (Obua, Okocha et al. 2012, Oyaniran, Ojo et al. 2018). The plant leaf has been used in Nigerian folk medicine for the treatment of ulcer, bacterial infections

(Enechi, Odo et al. 2014, Fadeyi, Akiode et al. 2020), diarrheal and scurvy (Borokini and Omotayo 2012). The presence of various secondary metabolites of pharmacological importance in the plant makes *C. mucunoides* a potential alternative drug for the treatment of such ailments like skin diseases, diarrhoea, and ulcer (Fitriarni and Kasiamdari 2018).

The phytochemical constituents of the plant leaf have been investigated (Borokini and Omotayo 2012, Fadeyi, Akiode et al. 2020), likewise the proximate and anti-nutritional compositions (Obua, Okocha et al. 2012, Oyaniran, Ojo et al. 2018). However, from published literature, the heavy metal content and antimicrobial activity of any part of the plant have not been studied. Bada and Olarinre (2012) in their study: characterization of soils and heavy metal content of vegetations in oil spill impacted land in Nigeria, included *C. mucunoides* as part of the vegetations investigated, but the heavy metal composition of the plant have not been properly and exclusively analyzed.

In view of this, the aim of this study was to elucidate on the phytochemical components of *C. mucunoides* leaves, to determine the concentration of heavy metals present in the leaves, and finally, the analysis of the anti-microbial activity of the plant leaf against some selected human pathogens.

2. Methodology

2.1. Sample Collection and Extraction

The leaves of *C. mucunoides* were collected from a farmland near the laboratory of the department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka. The plant was identified and authenticated by Dr I.E Mbaenwe, taxonomist from the department of Botany of the said institution. The sample specimen is kept at the herbarium of the Botany department. They were then air dried for two weeks, after which it was ground in a clean electric motor grinder to get the powered sample which was analysed. Finely ground sample was used to make three different kinds of extracts using methanol, *n*-hexane and ethyl acetate. In each extract, 10 g of sample was soaked in 100 ml of solvent and allowed to stand for 24 h after which the solutions were filtered. The filtrates were then used to determine qualitatively, the presence of the phytochemical components.

2.2. Phytochemical Screening

2.2.1. Qualitative Analysis

Qualitative analysis was carried out on the leaf extracts of *C. mucunoides* for the presence of phytochemicals such as alkaloids, anthocyanins, flavonoids, glycosides, phenols, reducing sugars, saponins, steroids, tannins, and terpenoids; using the methods described by Harbone (1998), Trease and Evans (1996), and Akpuaka (2009).

Test for Alkaloids (Mayer’s reagent)

1 ml of Mayer's reagent was added to 1 ml of each of the extract in a test tube; and a creamy precipitate indicated the presence of alkaloid.

Test for Anthocyanin

2 ml of 2 M HCl and 2 ml of ammonia solution were added to 1 ml each of the extracts in test tubes. The appearance of pink-red colouration which turns blue-violent indicated a positive test for anthocyanin.

Test for Flavonoids (Sodium hydroxide Test)

2 drops of 10% NaOH solution was added separately to 1ml of each extract on 3 different test tubes; and the presence of yellow precipitate revealed the presence of flavonoids.

Test for Glycosides

2 ml of each extract in three different test tubes were added 5 ml of distilled water, 5 ml of concentrated H_2SO_4 and boiled on a water bath for 15 min. The test tubes were then allowed to cool and each was neutralized with 20% NaOH, after which 1 ml of Fehling's solution was added and boiled for another 15 min. A brick-red precipitate indicated the presence of glycosides.

Test for Phenols

1 ml of distilled water was added to 1 ml of each of the extracts, followed by the addition of few drops of 5% NaOH solution. A colour change from yellow to bright orange indicated the presence of phenol.

Test for Reducing Sugars

To the test tubes containing 2 ml of the different extracts were added 5 ml each of Fehling's solution A and B. They were then heated on a water bath for 10 min at 80 ^{o}C. A brick-red precipitate indicated the presence of reducing sugars.

Test for Saponins (Frothing Test)

8 ml of distilled water was used to dilute 2 ml of each of the extracts and the content was vigorously shaken for 2 min. A persistent frothing indicated the presence of saponins.

Test for Steroids (Salkowski Test)

2 ml of chloroform and 2 ml of concentrated H_2SO_4 were added to 1 ml of the extracts in different test tubes. The chloroform layer appeared red, while the acid layer showed green fluorescence. This indicated the presence of steroid.

Test for Tannins (Potassium hydroxide Test)

0.5 ml of 20% freshly prepared KOH was separately added to 1 ml of each extract in different test tubes; and the presence of dirty white precipitate indicated the presence of tannins.

Test for Terpenoids

To 2 ml of each of the extracts, 2 ml of acetic anhydride and 2 ml of concentrated H_2SO_4 were added; and the formation of blue-green rings showed a positive test for terpenoid.

2.2.2. Quantitative Analysis

The quantitative phytochemical analysis of the leaves was determined using the method by Harbone (1998).

2.3. Determination of Heavy Metals

The Atomic Absorption Spectrometer (AAS, VGP-210) was used to test for the presence of five heavy metals (cadmium, iron, lead, nickel, and zinc) in the plant using the methanol extract. 5 g of the ground sample was soaked in 50 ml of methanol and allowed to stand for 3 h. The solution was filtered, and the residue was left to dry for 72 h at room temperature. After expiration of the time, 2 g of the dried residue was weighed into a platinum crucible and heated to ash using a bunsen burner. The ash was then dissolved with deionized water, shook thoroughly, filtered, and made up to 100 ml mark with deionized water. This filtrate served as the analyte for the heavy metal determination.

2.4. Antimicrobial Screening

The antimicrobial activities of methanol, n-hexane, and ethyl acetate extracts of the leaves of *C. mucunoides* were tested against human pathogens (bacterial and fungal) using the disk well diffusion control. A total of thirteen organisms were used, ten pathogenic bacteria namely: *Bacillus sp, Clostridium sp, Escherichia coli, Klebsieela pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella sp, Serratia marcescens, Staphylococcus aureus*, and *Streptococcus sp;* and three pathogenic fungi, *Aspergillus fumigates, Candida albicans*, and *Penicillium cyclopium*. These organisms were maintained on Nutrient Broth for bacteria and Sabouraud Dextrose Broth for fungi.

Antimicrobial Susceptibility Test

This was determined by the modified method described by Agu, Igweoha et al. (2013). Plates that had confluent and/or semi-confluent growth were selected for the antimicrobial susceptibility tests. The disk diffusion method was used to assay the effect of the extracts on the various microorganisms. Mueller Hinton Agar was used for the bacteria and Sabouraud Dextrose Agar (SDA) for the fungus. 24 h broth cultures of the test organisms were serially diluted, then 10^{-1} and 10^{-2} dilutions were used to seed the fungal isolates, while 10^{-2} was used to seed the bacteria plates. Then 0.1 ml of the appropriate dilution of the broth culture of each microorganism was uniformly spread using a sterile glass spreader on the surface of the media, and sterile filter papers were soaked in the extracts (*n*-hexane, methanol, and ethyl acetate extracts) and placed on two points on each petri-dish. Clear zones of inhibition around the wells indicated antimicrobial activities of the extracts against the test organisms. The diameter of the zone of inhibitions were measured and recorded in millimetre. All experiments were done in duplicates and the mean taken. Negative controls were set up with sterile water and positive controls were set up using 0.5% Nystatin for fungi and 0.5% Ciprofloxacin for bacteria.

3. Results

3.1 Result of the Phytochemical Analysis

Table 1
Result of qualitative phytochemical analysis

Phytochemicals	Ethyl acetate	Methanol	*n*-Hexane
Alkaloids	+	-	++
Anthocyanins	+	+	++
Flavonoids	-	+	++
Glycosides	-	+	++
Phenols	-	-	-
Reducing sugars	-	+	++
Saponins	+	++	+
Steroids	++	+	-
Tannins	++	++	+
Terpenoids	+	+	++

++ = Present; + = Mildly present; - = Below detectable limit

Table 2
Result of quantitative analysis

Phytochemicals	Alkaloids	Flavonoids	Glycosides	Saponins	Steroids	Tannins
Concentration (%)	36.8	1.40	19.6	4.10	19.6	14.2

The qualitative phytochemical screening of the *n*-hexane extract of the leaves of *C. mucunoides* as summarized in Table 1 above revealed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, terpenoids and reducing sugars, with the exception of phenols, steroids and anthocyanins. The methanolic extract revealed the presence of all the phytochemicals tested for except for alkaloids and phenols; while ethyl acetate extract shows a positive result for alkaloids, tannins, saponins, steroids, terpenoids and anthocyanins. The result of the quantitative analysis as shown in Table 2 revealed that alkaloids has the highest concentration (36.8%) while flavonoids has the least concentration (1.40%).

3.2 Result of the Heavy Metal Analysis

Table 3
Concentrations of heavy metals

Heavy metals	Cadmium	Iron	Lead	Nickel	Zinc
Concentration (mg/kg)	-	0.08	0.08	-	-
*Permissible limit (mg/kg)	0.02	20	2	35	0.60

*World Health Organization (Organization 1996)

The result of the heavy metal analysis is presented in Table 3. It showed the presence of iron and lead in a concentration of 0.08 mg/kg each, while cadmium, nickel and zinc were found to be below detectable limits.

3.3 Result of the Antimicrobial Screening

Table 4
Antibacterial susceptibility screening

Organisms	Ethyl acetate (mm)	Methanol (mm)	*n*-Hexane (mm)	0.5% Ciprofloxacin (mm)
Bacillus sp	28.5	31	-	37
Clostridium sp	24	28	-	36.5
Escherichia coli	-	12.5	-	37
Klebsiella pneumonia	22	29.5	-	36
Proteus mirabilis	7	8.5	-	34.5
Pseudomonas aeruginosa	-	-	-	34.5
Salmonella sp	15	19.5	-	37
Serratia marcescens	15.5	14	-	38
Staphylococcus aureus	22	14.5	-	40.5
Streptococcus sp.	8	16	-	37.5

*0.5% Ciprofloxacin was used as the positive control.

Table 5
Antifungal susceptibility screening

Organism	Ethyl acetate (mm)	Methanol (mm)	*n*-Hexane (mm)	0.5% Nystatin (mm)
Aspergillus fumigates	-	-	-	34.5
Candida albicans	-	-	-	41
Penicillium cyclopium	26	26.5	-	36.5

0.5% Nystatin was used as the positive control.

4. Discussion

Table 1 presents the results of the secondary metabolites obtained in the various solvents. The result is in tandem with that obtained by Fadeyi, Akiode et al. (2020), except for the absence of phenols in all the extracts. Phenol is responsible for the colouring of fruits in plants, thus, its absence is not a problem as *C. mucunoide* has low palatability. The quantitative values as shown in Table 2 and Figure 1 revealed that alkaloids have the highest concentration (36.80%). Similar result was reported by Enechi, Odo et al. (2014) and Borokini and Omotayo (2012), they noted that the phytochemical screening of the leaves of *C. mucunoides* revealed a copious presence of alkaloids and flavonoids among other secondary metabolites. Alkaloids confer to many plants their medicinal efficacy, from treatment of diarrhoea, reduction and stopping of pains and regurgitation, and many more. Flavonoids have anti-microbial potentials and can be used in the treatment of dropsy, hay fever and ulcer (Finar 2006), and also reduces the risk of cardiovascular diseases and stroke (Trease and Evans 1996). Table 2 also showed the presence of glycosides (19.60%), tannins (14.21%), steroids (19.60%) and saponins (4.10%) in relative quantities. Saponins are used in the treatment of fungal and bacterial infections. Since *C. mucunoides* serves as fodder for livestock, the low value obtained for saponins may be ideal. According to Bassey, Peters et al. (2014), high saponin content in plants may be responsible for the swelling of rumen in domestic animals. Tannins serves as metal chelators and has anti-inflammatory properties (Okerulu, Onyema et al. 2017). Its presence in livestock feeds, however, poses nutritional risk such as stunted growth, indigestion, among others (Onyema, Ofor et al. 2016). Glycosides are used in the treatment of heart-related diseases such as arrhythmia. Steroids helps in the development of male and female sex hormones, and its presence in *C. mucunoides* leaves could be the reason for the morphological deficiencies observed in the testis and ovaries of rabbits after being fed for long with the plant leaves (Solomon, SA et al. 2014). Hence, the need to always ascertain the presence and concentrations of these phyto compounds in such forages as *C. nucunoides* before using them. The medicinal values of plants and their derivatives is dependent on the compositions of these secondary metabolites (Edeoga, Okwu et al. 2005), thus, the presence of these phyto compounds in the leaves of *C. mucunoides* confer on it its medicinal value.

The result of the heavy metals determination of the methanolic leaves extract of *C. mucunoides* is shown in Table 3. Cadmium, nickel and zinc were found below detectable limit. This however, contrast with the result of Bada and Olarinre (2012) for Cd (0.21 mg/kg) and Zn (1.44 mg/kg). Lead and iron on the other hand, were present in mild concentrations of 0.08 mg/kg each. This value is well below the permissible limit of 2 mg/kg for Pb and 20 mg/kg for Fe set by WHO (Organization 1996) for heavy metals in plants. Higher value was reported for Pb (0.39 mg/kg) by Bada and Olarinre (2012), but their study centered on plants obtained from oil contaminated soil, a factor that might have contributed to the high levels of these metals. Pb is a non-essential element that has no known biochemical benefit to both plants and animals (Emenike, Iwuozor et al. 2021). It readily accumulates in different parts of the plant and gets to human by biomagnification (Sharma and Dubey 2005). Higher concentrations of Pb causes several deleterious effects to plants, animals and human; some of which include chlorosis, blackening of root system, decreased survival rate, retarded development, impaired mobility and reduced egg production (Sharma and Dubey 2005, Liu, Shang et al. 2020, Shilpa, Anupama et al. 2021). However, the low concentration of Pb in the leaf extract of *C. mucunoides* implies that the plant is relatively safe, either to be used as fodder for livestock or for the preparation of herbal drugs for the treatment of the said ailments. Nevertheless, Pb have been reported to pose adverse health concern even at levels considered acceptable (Bouchard, Bellinger et al. 2009), hence, the need to constantly check the level of Pb concentrations and other heavy metals in plants used for herbal formulations. Fe on the other hand, is an essential element needed for the production of red blood cell. It aids photosynthesis in plants, helps in the growth of tissues in animals and in the movement of oxygen in the body (Mensah, Antwi-Akomeah et al. 2020).

Antimicrobial features of plants are wanted tools in the control of unwanted microorganisms particularly in the treatment of contagious diseases (Temitope, Fasusi et al. 2016). The *n*-hexane extract of the leaves of *C. mucunoides* has no antibacterial activity against the organisms tested as shown in Table 4 and graphically represented in Figure 2. The methanolic extract on the other hand showed antibacterial activity on the whole organisms except in *Pseudomonas aeruginosa*, with the most effective being in *Bacillus sp*, and the least effective being against *Proteus mirabilis.* Ethyl acetate extract also showed antibacterial activity against the organisms except in *Pseudomonas aeruginos* and in *Escherichia coli.* It is most effective against *Bacillus sp.* and least effective against *Proteus mirabilis.* When compared to the positive control, 0.5% Ciprofloxacin, which served as the standard, the methanolic extract appeared to be the most effective, except in *S. aureus* and *Serratia marcescens* where the ethyl acetate extract was more effective. Another point worth noting from the result is the absence of *Pseudomonas aeruginosa* in the three extracts. This implies that none of the extracts can be used in the treatment of bacterial infections related to *Proteus mirabilis.* According to Onyema and Ajiwe (2014), an organism is susceptible to an extract if the inhibitory zone is ≥16 mm in diameter, while 11-15 mm indicates an intermediate effect. Thus, the methanolic and ethyl acetate extracts of the leaves of *C. mucunoides* have some levels of inhibition against most of the test organisms and can be used in the treatment of bacterial infections caused by them except *Pseudomonas aeruginosa* and *Proteus mirabilis*, while *n*-hexane extract of the plant leaf should not even be considered at all for any such antibacterial drug because of its inactivity in all the tested organisms.

The result of the three pathogenic fungal tested for was reported in Table 5 and Figure 3. It revealed that the three extracts do not show antifungal activity against *Aspergillus fumigatus* and *Candida albicans*. However, while n-hexane extract was also absence in *penicillium cyclopium*, the methanolic and ethyl acetate extracts proved to be highly effective against *Penicillium cyclopium* with high diameter zones of inhibition. This is in agreement with the result obtained in antibacterial screening; thus, *n*-hexane extract of *C. mucunoides* leaves is not a good solvent for antimicrobial activity while methanolic extract is a better solvent compare to ethyl acetate. While none of the extracts is up to the standards used (0.5% ciprofloxacin for antibacterial and 0.5% nystatin for antifungal), the high zones of inhibitions shown is a proof that the leaves of *C. mucunoides* is a potential herbal medicine for microbial infections.

5. Conclusions

This work exploited the leaves of *Calopogonium mucunoides* using different solvents in a bid to finding the medicinal value of the plant. The findings support the knowledge that *C. mucunoides* appears in the hierarchy of medicinal plants in Nigeria and is indeed a very useful potential in the demand of substitute natural medicine for the treatment of various ailments such as ulcer and diarrhoea. This has been proven by the results of the phytochemical screening of the plant leaves that revealed many secondary metabolites of medicinal value. The treatment of bacterial infections using the plant extracts is also possible as seen in the results of the methanolic and ethyl acetate extracts of the plant leaves which showed high zones of inhibition against most of the pathogenic bacteria tested. The presence of toxic heavy metal such as lead is well below the permissible limit set by WHO. All these makes the leaves of the plant an ideal potential source of drug. Consequently, further exploitation of this plant for probable preparation of cheap and effective drugs is hereby recommended. Further research on other parts of this plant is recommended so as to probe further into the therapeutic potentials of the plant.

Abbreviations

C. mucunoides
Calopogonium mucunoides
WHO
World Health Organization

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data and materials are available

Competing interests

The authors declare that they have no competing interests

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Authors’ contributions

ECE designed and performed the experiments, and wrote the manuscript. OC conceived the idea, collected the sample, revised and edited the manuscript, and supervised the experiments. All authors read and approved the final manuscript.

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Figures

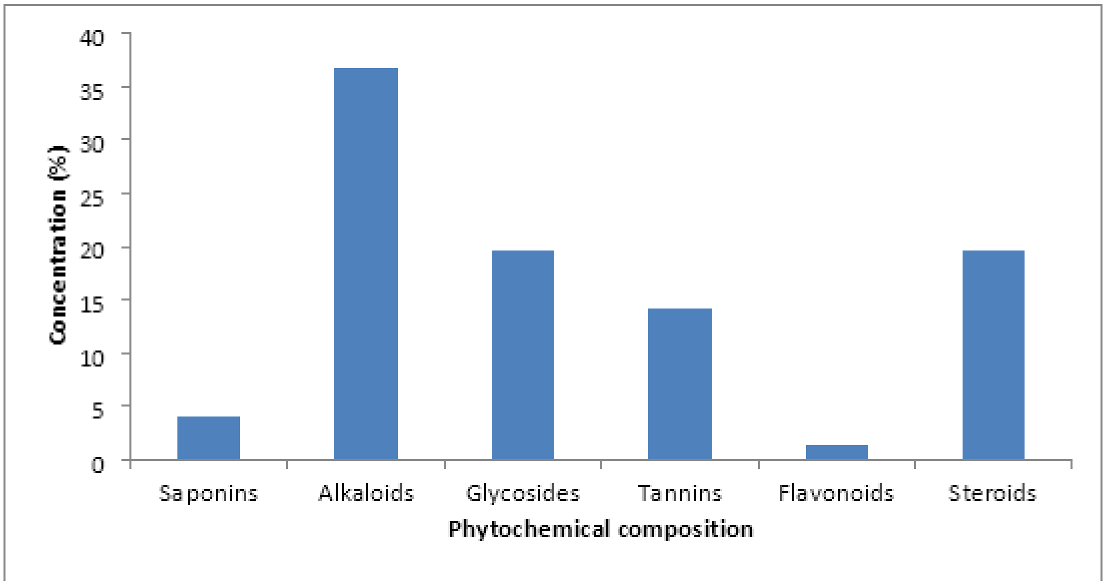

Figure 1

Quantitative phytochemical compositions of C. mucunoides

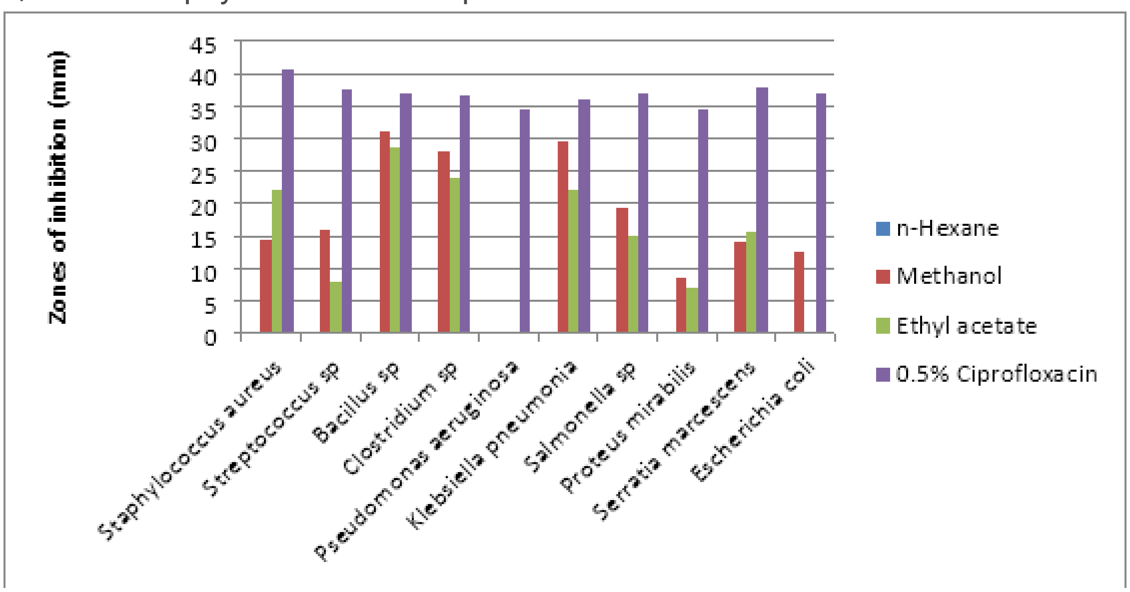

Figure 2

Graphical representation of antibacterial activities of C. mucunoides leaf extract against selected pathogenic bacterial

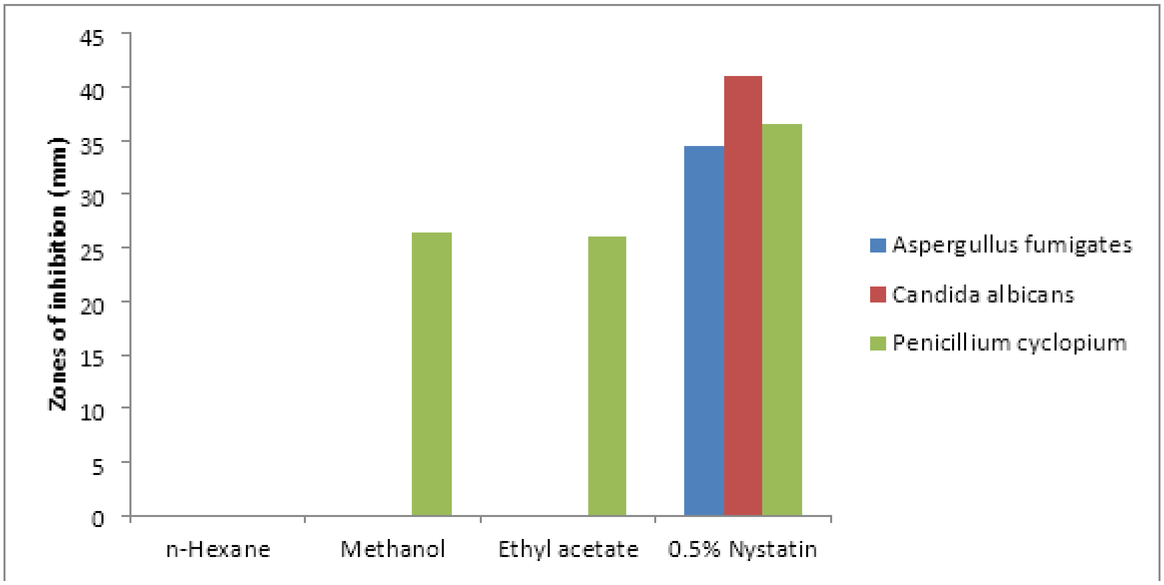

Figure 3

Graphical representation of antifungal activities of C. mucunoides leaf extract against selected pathogenic fungal

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