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(RESEARCH ARTICLE)

Phytochemical, antioxidant, proximate and FTIR analysis of *Calopogonium mucunoides* Desv. extracts using selected solvents

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Abstract

Calopo (*Calopogonium mucunoides* Desv.), a vigorous, hairy annual trailing legume, is a cover crop in tropical tree plantations. In this study, the aerial part of calopo was extracted separately using hexane, ethylacetate and methanol. The phytochemical constituents and antioxidant activities of the extracts were determined. The nutritional value of the plant was determined by proximate analysis. The FTIR analysis was also carried out. Estimation of the phytochemical and nutritional analysis was done using the standard laboratory methods. The results showed that the total phenolic content of *C. mucunoides* was the highest (4.29 ± 0.032 mg/g). Antioxidant activity was highest in the methanol extract (65-71% inhibition). Proximate analysis revealed a high protein content (20.54%); ash content (9.86%); Fibre (21.42%); Lipid (18.62%) and carbohydrate content (21.56%). The FTIR analysis showed a broad band at 3392-3353 cm^{-1} representing bonding –OH groups. The peak around 2924-2918cm^{-1} represents aliphatic chains, -CH_2- and –CH_3. The peak around 1623 cm^{-1} (from methanol and hexane extract only) corresponds to C=O stretch. The peak observed at 1515 cm^{-1} (from ethylacetate extract) corresponds to the secondary amine group. Results from this study shows the plant contains significant phytochemical compounds and using appropriate solvent, it may serve as a source for the development of novel drugs for the treatment of various diseases as claimed by its traditional uses. The plant is also of high nutritional value, especially due to its high protein and fibre content, and therefore, may be used in feed formulation.

Keywords: Phytochemicals; Antioxidant; Proximate; DPPH; Fabaceae; Spectroscopic

1. Introduction

Overtime, plants and herbs have been proved to be of significance to the health of the individuals and communities. In recent years, many scientific investigations of traditional herbal remedies for several diseases have been carried out and it has resulted in the development of alternative drugs and therapeutic purposes. Phytochemical studies are carried out in search of new therapeutic drugs. Plants secondary metabolites have numerous health benefits such as antimicrobial, anti-inflammatory, anti-diabetic, anticancer preventive and antihypertensive properties [1]. Medicinal plants produce large range of secondary metabolites that have therapeutic potentials to cope with oxidative stress resulting from diseases [2]. Secondary metabolites have therapeutic effects which predict their specific usage [3]. Polyphenols are strong antioxidants with substantial free radicals which inhibits lipid peroxidation. They play crucial roles in pharmacology and therapeutic standpoint. Terpenoids, another class of secondary metabolites are useful f or curing obesity induced metabolic disorders [4]. Reactive oxygen species (ROS) are counteracted by phenolic compounds, which are secondary products with antioxidant capacity, in order to avoid oxidative damage [5]. Medicinal plants are known for their bio-active substances like antioxidants, anticancer, anti-inflammatory, antibacterial and anti-allergic nature as noted in flavonoid compounds. Many epidemiological studies have shown that the consumption of

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phenolics-rich foods is associated with the prevention of chronic diseases [6]. Phenolic compounds, in addition to their antioxidant properties, have been reported to be potential candidates in lowering cardiovascular diseases [7], anticarcinogenic [8, 9], antiallergenic, antiarthrogenic, antiinflammatory, antimicrobial and antithrombotic effects [10]. Plant phenolics, in particular phenolic acids, tannins and flavonoids have been shown to possess high antioxidant capacity and occur in vegetables, fruits, nuts, seeds, roots and barks [11].

Calopogoponium mucunoides Desv. is tropical forage of *Fabaceae* family. *Calopogonium mucunoides* has been reported to possess antidiarrheal activity as well as antibacteria potential against gram-positive and gram-negative bacteria, which are major causative organisms of various human diseases, and including diarrhoea [12]. The plant leaf is known to have been used in some regions as traditional medicine for the treatment of ulcer, diarrhoea and bacterial infections [13].

To the best of the authors' knowledge, there is no comprehensive information on the evaluation of the phytochemicals and assessment of the nutritional and antioxidant capacity of *C. mucunoides.* The present study is therefore aimed at investigating the phytochemistry of the extracts from different solvent polarity, nutrients and antinutrients composition, as well as the antioxidant properties of the plant. FTIR spectra of the extracts and the sample were also investigated for structural functional group elucidation.

2. Material and methods

2.1. Sample Collection

Fresh and healthy aerial plant part of *Calopogonium mucunoides* were harvested around Kwali Area Council Secretariat of FCT, Abuja.

2.2. Preparation of plant sample for extraction

The collected plant sample was screened and separated from foreign materials, air dried and pulverised using mechanical hammer mill. The powdered sample was stored in a plastic container till time of usage.

2.3. Solvents and reagents

The solvents used were of high purity and the reagents used are of analytical grade.

2.4. Extraction

200g of the powdered sample was macerated with 650 ml each of hexane, ethyl acetate and methanol in succession respectively (separately) for 48 hours. Each successive extraction was filtered using muslin cloth to separate the marc from the extract and the solvent recovered with rotary evaporator.

2.5. Phytochemical screening

Basic plants' phytochemicals screening was carried out using standard chemical tests [14, 15, 16 and 17].

2.6. Quantitative and Anti-nutrition Determination

Estimation of the quantity of Alkaloids, Tannins, Flavonoids, Phenols and Saponins was done using the spectroscopic methods described by Oloyede [18], Chang [19], AOAC [20], Obadoni *et al*. [21]. Phytate content was estimated using method described by Wheeler and Ferrel [22]. Oxalate was estimated by the method of Day and Underwood [23].

2.7. FT-IR Spectroscopy

Calopo extracts and dried powdered sample were characterized using a Nicolet IS 5 Thermo Fisher Scientific, USA FTIR spectrophotometer. The extracts and sample were scanned between the wavelength of 400 and 4000 cm^{-1}. FTIR spectra give information about the characteristic functional groups of the hexane, methanol and ethylacetate extracts of the calopo plant sample.

2.8. Antioxidant activity

Measurement of the antioxidant activity was carried by the method of Brand-Williams [24] with slight modification. This involves the discoloration of 2, 2-diphenyl-1- picryhydroxyl (DPPH) radical in methanol. The following concentrations of extract were tested (0.1, 0.3, 0.5, 0.7 and 1.0mg/ml) and the absorbance measured at 517nm against blank solution. Ascorbic acid was used as standard at same concentrations i.e., (0.1, 0.3, 0.5, 0.7 and 1.0mg/ml). The radical scavenging capacity was calculated by the formula:

$$\text{Inhibition}\% = \frac{A_b - A_s}{A_b}$$

Where, A_b = Absorbance of blank solution, A_s = Absorbance of sample

2.9. Proximate/nutrition analysis

The nutritional values of the calopo were determined by the standard method of AOAC [25]. This includes the determination of percentage moisture, ash content, crude lipid, crude fibre, crude protein and carbohydrate.

3. Results and discussion

3.1. Qualitative Phytochemical

Qualitative analysis for flavonoids, alkaloids, phytosteroids, phenols, terpenoids, steroids and tannins were carried out for the hexane, ethylacetate and methanol extracts of calopo. The result, as shown in Table 1, indicates that alkaloids, phytosterorols, phenols, steroids, tannins were present in both extracts. However, flavonoids was not detected in ethylacetate extract but present in hexane and methanol extracts. Terpenes and phlobatannins were not detected in all the three extracts. Saponin was confirmed present in hexane fraction only.

Table 1: Phytochemical screening different extracts of *Calopogonium mucunoids*

Phytochemicals	Hexane	Ethylacetate	Methanol
Flavonoids	+	-	+
Alkaloids	+	+	+
Phytosterols	+	+	+
Phenols	+	+	+
Terpenoids	-	-	-
Steroids	+	+	+
Tannins	+	+	+
Phlobatannins	-	-	-
Saponin	+	-	-

3.2. Antioxidant activities measurement

The free radical scavenging activities of the extracts of *calopo* were determined using DPPH radical scavenging assay. The result obtained is presented in Table 2.

Table 2: Antioxidant activities of the different extracts of *Calopogonium mucunoids*

Conc (mg/ml)	Hexane	Ethylacetate	Methanol	Ascorbic Acid
0.1	-	26	67.46	60.38
0.3	-	33.62	70.69	72.50
0.5	3.28	53.10	71.12	77.69
0.7	-	54.48	68.45	77.88
1.0	2.24	51.98	65.56	81.92

3.3. Phytochemicals/antinutrients estimation

Quantitative phenolic, flavonoid, tannins, alkaloid, saponins, phytate and oxalate contents of the sample leaves of calopo were determined by UV spectrophotometric method. The result is displayed in Table 3. The result showed phenols was the highest (4.290 ± 0.032mg/g) while saponin was the lowest (0.331 ± 0.025mg/g).

Table 3: Quantitative phytochemicals/antinutrients composition of *Calopogonium mucunoids*

Phytochemical	Quantity (mg/g)
Alkaloids	0.441±0.034
Tannins	3.581±0.061
Flavonoids	3.485±0.124
Phenols	4.290±0.032
Saponin	0.331±0.025
Phytate	0.171±0.041
Oxalate	0.44±0.025

The given values are mean±SD of three different determinations

Table 4: Proximate analysis of *Calopogonium mucunoids*

Component	% Composition
Crude protein	20.54±0.21
Crude fibre	21.42±0.14
Crude lipids	18.62±0.25
Moisture	8.00± 0.11
Ash content	9.86±0.32
Carbohydrate	21.56±0.15

The given values are mean±SD of three different determinations.

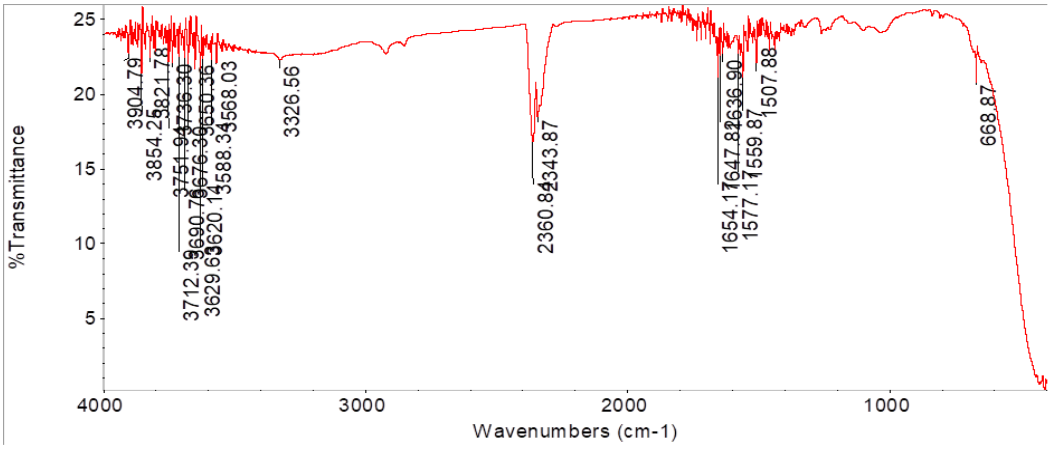

Figure 1: FTIR spectra of dried powdered sample of *Calopogonium mucunoids*

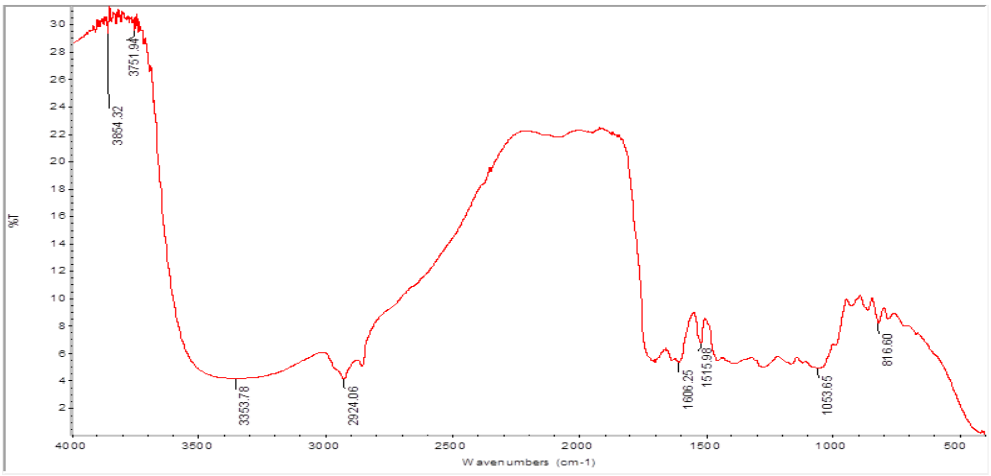

Figure 2: FTIR Spectra of the methanolic extract of *Calopogonium mucunoids*

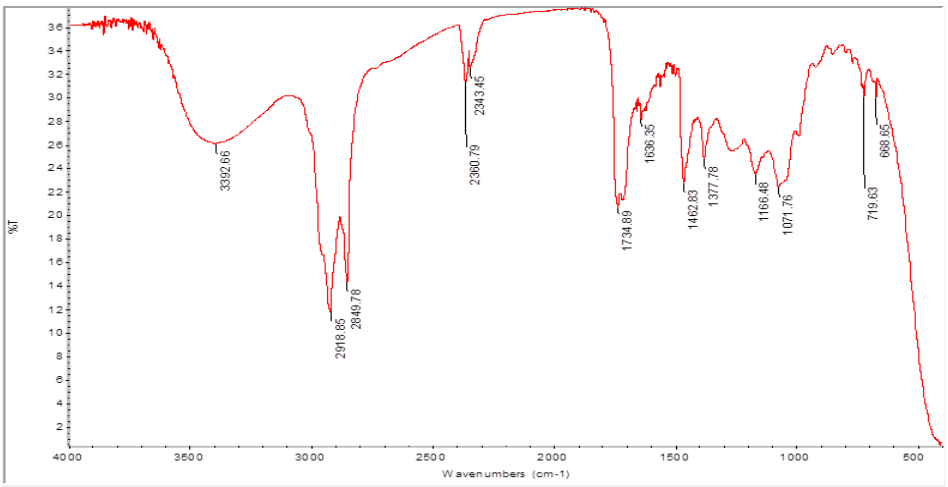

Figure 3: FTIR Spectra of the hexanic extract of *Calopogonium mucunoids*

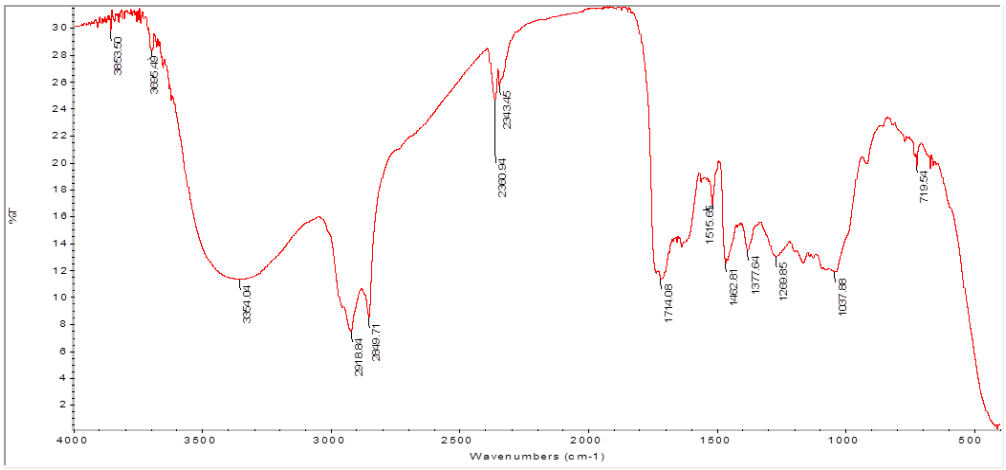

Figure 4: FTIR Spectra of the ethylacetate extract of *Calopogonium mucunoids*

Table 5: FTIR bonds' peaks for the methanol, hexane, ethylacetate extracts air dried plant of *Calopogonium mucunoids*

Functional group	Methanol	Hexane	Ethylacetate	Plant Sample
N-H stretch (Amines, amides)	3854.32(w), 3751.94(w)		3853.50(w), 3695.49(w)	3568-3904
O-H monomeric carboxylic acids hydrogen bonded alcohols, phenols	3353.78	3392.66(b)	3354.04(b)	3326.56
C-H stretch (alkanes)	2924.06	29118.85-2343.45	2918.84-2343.45(sh),	2343.87-2360.84
nitriles, carbenes (tripple bond) C=O, C=C, C=N		1734.89	1714.18	
C=O, C=C, C=N aromatic rings	1606.25, 1515.98	1636.35-1166.84	1515.66-1269.85	1507.88-1654.17
C-O, C-N, C-C (alcohols, ethers, carboxylic esters)	1053.65(b)	1071.76	1073.88	
C-C, C-H, (alkenes rock)	816.60	719.63, 668.65	719.54	668.87

4. Discussion

Plants play significant roles in discovery associated with new therapeutic agents and have continue to receive diligent attentions because of their inherent bioactive components such as antioxidants, anticancer, anti-inflammatory, antibacterial activities. The result of the analysis of the qualitative, quantitative phytochemicals and antinutritional analysis of Calopo secondary metabolites (Tables 1 and 3) showed that Calopo has alkaloids, saponins, flavonoids, phenols, tannins, steroids and phytosteroids. This shows high level of its possible medicinal and dietary values [18]. From the quantitative measurement (Table 3), phenol content is 4.290 ± 0.032mg/g. Phenolic compounds in herbs act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators [26]. The flavonoids estimation is 3.485 ± 0.124mg/g. Flavonoids prevent damage caused by free radicals in the body [27] and for the treatment of diarrhoea [28], fever-reducing (antipyretic), pain-relieving (analgesic) and spasm-inhibiting (spasmolytic) activities and anticancer activities. The phenols and flavonoids compounds are important antioxidants, antimicrobial, antiallergic, anti-inflammatory and anticancer agents. They play a vital role in reproduction and growth. They also provide protection against harmful pathogenic microbes and

predators [29, 30]. The tannins content is 3.581 ± 0.061mg/g. Tannins are known to possess immune- stimulating activities. Tannins play important role in promoting wound healing. Tannins are also known to act as primary antioxidant or free radical scavengers [31]. The antioxidant activity observed in plant extracts may be due to the presence of phenolic compounds or polyphenols or flavonoids or tannins. This is in agreement with the study conducted by [32]. Alkaloids act as stimulants, pain reliever and tranquilizer. It's used in curing hypertension. Alkaloids are organic and natural ingredients that have nitrogen, and are also physiologically active together with sedative and analgesic roles. They are found in reducing stress and depression symptoms. Alkaloids tend to be poisonous when taken in bulk amount due to their stimulatory effects, producing excitation associated with cell and nerve disorders [33, 34]. Saponins are triterpenoid or steroidal glycosides proven as important phytoconstituent with various pharmacological activities such as antiallergic, antiphlogostic, cytotoxic, antitumour, antiviral, immunomodulating, antihepatotoxic, molluscicidal and antifungal effects [35]. Saponins are extensively utilized in veterinary vaccines because their character as an adjuvant and helps in the improvement of immune response. Many of them are useful in intracellular histo-chemistry staining permitting antibody access to intracellular protein molecules. The alkaloids and saponins contents of *calopo*, 0.441 ± 0.034 and 0.331 ± 0.025 mg/g respectively, are relatively low compared with phenols, flavonoids and tannins. The relative low values suggest minimum antinutrient property of the plant. Table 2 is the result of antioxidant capacity measurement. The test results revealed that the polar methanolic extract showed a higher activity than the less polar ethylacetate extract, which, in turn, was significantly more active than hexane extract. This means phytochemical soluble in polar solvents possess a stronger potential to scavenge DPPH free radicals. Comparatively, the scavenging activities of the methanol extract and the ascorbic acid used as standard showed that methanolic extract of *calopo* compared very favourable with the standard, especially at lower concentrations. Hence calopo is a good natural source of antioxidant agent. Table 4 is the result of the proximate analysis of *calopo* plant. From the result, *calopo* is shown to be rich in crude protein (20.54%), carbohydrate (21.56%), crude fibre (21.42%), and crude lipids (18.62%). The ash and moisture content are 9.86 and 8.00% respectively. This suggests that *calopo* may be utilized as a good feed ingredient for animals, coupled with the low antinutrient composition. Figures 1, 2, 3, 4 are the FTIR spectra for the dried *calopo* sample and its methanol, hexane and ethylacetate extracts respectively. Table 5 highlights the main peaks in the spectra and the bonds they represent. From the FTIR spectra, the following bonds are noticeable: 3695.49 - 3904.79cm^{-1}; This is N-H stretch of the amines and amides functional groups. This bonds were observable in all the spectra except hexanic exxtract spectra. 3326.56 - 3392.66cm^{-1}; suggesting O-H stretch of the hydrogen bonded alcohols and phenols. This cuts across all the four spectra. C-H alkane stretch in the bonds between 2924.06 - 2360.84 cm^{-1} also cut across all the spectra. 2343.87cm^{-1} bonds representing nitriles, carbenes (tripple bond). Also 1714.18 bonds of C=O, C=C, C=N were observed only in extracts of hexanic and ethylacetate spectra. C=O, C=C, C=N aromatic rings bonds were seen in the all the spectra. 1462.81 C-H alkanes bend in hexane and ethylacetate spectra. 1377.64 bonds of nitro bond (nitro compounds). 1053.65, 1071.76 and 1037.88 represents C-O, C-N, C-C for alcohols, ethers, carboxylic esters. 719.54 and 668.87 represents C-C, C-H, (alkenes rock).

5. Conclusion

Results from the study conducted showed that *Calopogonium* plant contains secondary metabolites useful for drug development in significant quantity with good antioxidant capacity which can be exploited in combating diseases related to oxidative stress. The plant may also be used as an ingredient in formulation of animal feed due to its high nutritional value and low antinutrient composition.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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