Qualitative analysis, total phenolic content, FT-IR and GC-MS characterisation of *Canna indica*: bioreducing agent for nanoparticles synthesis

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Qualitative analysis, total phenolic content, FT-IR and GC-MS characterisation of *Canna indica*: bioreducing agent for nanoparticles synthesis

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Abstract. Within the framework of determining capping and stabilizing bioactive components present in *Canna indica* towards nanoparticles synthesis, phytochemical screening, total phenolic content, infrared spectroscopy and chromatographic characterisation were carried out on the locally sourced plant. Extracts were prepared from *C. indica* leaves using ethanol, de-ionised water (DW) and ethanol/DW in ratio 1:1. Qualitative screening showed the presence of saponins, alkaloids, terpenoids, phenols and coumarins. Highest total phenolic content (TPC) was observed in the aqueous fraction and least in ethanol fraction. Characterisation was carried out using Fourier Transform - Infrared spectroscopy (FT-IR) and Gas Chromatography - Mass Spectrometry (GC-MS). Absorption bands observed from FT-IR analysis showed presence of aromatic O-H stretch (3300 cm^{-1}) and aromatic C=C stretch (1451 and 1640 cm^{-1}) respectively. GC-MS analysis of ethanolic extract indicated the presence of dl-.alpha.-tocopherol – a phenolic compound.

Keywords: Phytochemicals; *Canna indica*; Total phenolic content (TPC); Gas chromatography mass spectrometry (GC-MS); Fourier transform infrared spectroscopy (FT-IR)

1. Introduction

In recent years, metallic nanoparticles have gained prominence in the field of environmental studies due to their unique features, such as magnetic susceptibility, low toxicity, among others [1,2]. Researchers have explored physical and chemical syntheses of nanomaterials over the years; however, these involve the use of toxic reagents and the production of agglomerated nanomaterials which futher require stabilization using chemical reagents and polymeric materials [3,4]. Plant-mediated synthesis of nanoparticles presents a framework for energy efficiency, use of less hazardous chemicals and use of renewable feedstock which align with the priciples of green chemistry. *Canna indica* (Indian shot) locally known as “Ido” in South-west Nigeria is a medicinal plant found in parts of Africa and Asia. It

is used medicinally (for treatment of gonorrhea and dermatoses), as well as for phytoremediation and wastewater treatment [5-8].

Table 1. Selectedindigenous plants reported for synthesis of nanoparticles

Scientific name	Part of plant	Precursor	Type of NPs	References
Albizia chevalier	Bark	Silver nitrate	Silver	[9]
Alchornea laxiflora	Leaves	CuSO4.5H2O (Copper (II) sulphate pentahydrate)	Copper	[10]
Ananas comosus	Leaves	Silver nitrate	Silver	[11]
Guiera senegalensis	Leaves	Silver nitrate	Silver	[12]
Manihot esculenta Crantz	Root	Cobalt chloride	Cobalt oxide	[13]

Synthesis of nanoparticles using plant parts (such as stem, leaf and bark) obtained from locally sourced and readily available plants have been reported (Table 1). Secondary metabolites found in these plants such as phenols, alkaloids, saponins, flavonoids etc. act as stabilising and capping agents during synthesis, thereby reducing aggregation of nanoparticles. However, not much has been reported on the specific biomolecules responsible for reduction of metal precursors during nanoparticles synthesis and resultantly, the mechanistic pathways for these reactions [14].

Thus the aim of this study was to determine the phytochemical composition of *C. indica* (Indian shot) and characterize thefunctional groups and chemical structures of the specific phenolic moieties present using Fourier Transform - Infrared spectroscopy (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS), respectively. This will (i) provide information on phenolics responsible for capping/stabilisation of synthesised nanoparticles and (ii) facilitate understanding of reaction pathways during nanoparticles synthesis.

2. **Materials and method**

2.1 Sample collection

Fresh leaves of *Canna indica* were collected from Covenant University, Ota and identified by a botanist. The leaves were thoroughly washed in distilled water and air-dried at room temperature fortwenty one (21) days (Figures 1a,b). The dried leaves were pulverized and preserved in air tight containers until further use [15,16].

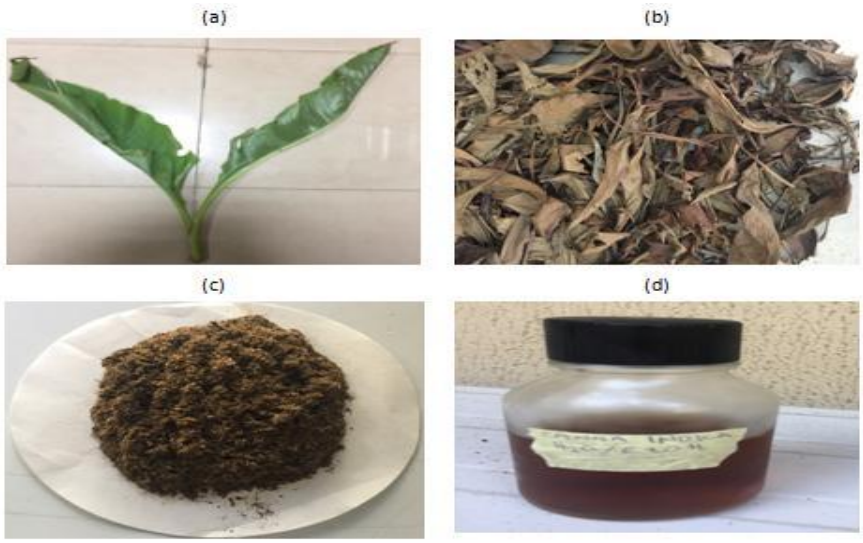


Figure 1.*Canna indica* (a) Fresh leaves (b) Dried leaves (c) Powdered leaves (d) Ethanol/deionised water extract (1:1).

2.2. Sample preparation and characterisation

Powdered leaves of *C. indica* (25 g) was extracted with 125 mL solvent to obtain three extracts: ethanolic, aqueous and ethanol/water (1:1) (Figure 1). After filtration, extracts were concentrated with rotary evaporator (BUCHI). Phytochemical screening was carried out following a procedure described by [17].

Total phenolic content (TPC) of extracts was determined by Folin-Ciocalteau reagent method described by [15]. Different aliquots of each concentrated extract were measured and made up to 3 mL in test tubes, and then mixed with 0.5 mL Folin-Ciocalteau reagent. The tubes were placed in boiling water for 60 seconds, then left to cool. Absorbance of the treated samples was taken on a UV/VIS spectrophotometer using reagent blank at 650 nm wavelength. Gallic acid standards were prepared and used for calibration. The phenolic content of each sample is reported as mg/g gallic acid equivalent (GAE). Samples were analysed in triplicates.

For FT-IR and GC-MS characterisation, 10 g of powered *C. indica* leaves was extracted with ethanol, deionised water and ethanol:water (1:1). Extracts were filtered and concentrated using rotary evaporator. 1 mL of each extract was taken for FT-IR analysis while 1 mL of ethanolic extract was packed for GC-MS analysis.

3. Results and discussion

3.1 Phytochemical screening of Canna indica leaves extracts

The results of the qualitative screening of *C. indica* extracts indicating the presence of phenols, saponins, alkaloids, terpenoids and coumarins are presented in Table 2.

Table 2. Qualitative analysis of *Canna indica* extracts.

	TAN	SAP	FLA	ALK	ANTHO	BETA	QUIN	GLY	CARD GLY	TER	TRI-TERP	PHE	COU	STE	ACIDS
Ethanolic extract	-	+	+	+	-	-	-	-	-	+	-	+	-	-	-
Ethanol:DW extract	-	++	+	-	-	-	-	-	-	+	-	+	+	-	-
Aqueous extract	-	++	+	-	-	-	-	-	-	+	-	+	-	-	-

+: Presence; -: Absence
TAN = Tannins, SAP = Saponin, FLA = Flavonoids, ALK = Alkaloids, ANTHO = Anthocyanins, BETA = Betacyanin, QUIN = Quinones, GLY = Glycosides, CARD-GLY = Cardiac Glycosides, TER = Terpenoids, TRI-TERP = Triterpenoids, PHE = Phenols, COU = Coumarins, STE= Steroids

3.2 Determination of total phenolic content

Figure 2 provides a summary of the total phenolic content (TPC) in each fraction. TPC is expressed as milligrams of gallic acid equivalents (GAE) per gram.These results are in agreement with findings on phytochemical screening of *C. indica* reported by [18,19]. Aqueous extracts of *C. indica* contained highest phenolic content (0.80 mg GAE/g) in comparison with ethanolic and ethanol/DW fractions.

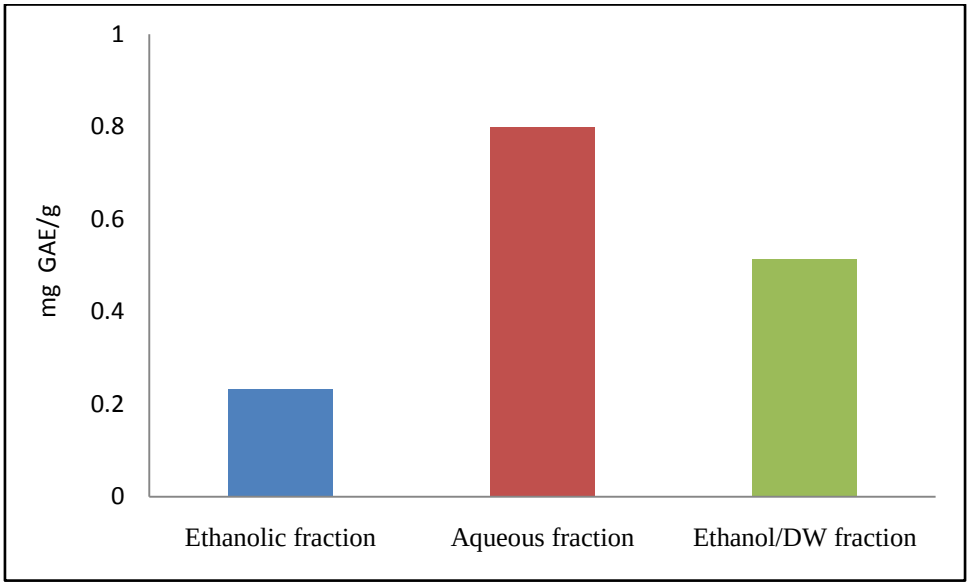


Figure 2. Total phenolic content of *C. indica* leaf extracts

Phenolic compounds are antioxidants known to be present in several medicinal plants. Biogenic antioxidants have attracted much interest in nanoparticles synthesis because of their reducing capabilities on metallic precursors such as ferric chloride and silver nitrate. Thus, the use of indigenous herbal plants with high antioxidant composition has gained traction in recent years. Also found in *C. indica* extracts were saponins and terpenoids, which are widely distributed natural products. Common examples of saponins are resveratrol and flavones. Terpenoids are usually classified based on the number of isoprene units. [20] and[21] have reported *C. indica*-mediated synthesis of silver and gold nanoparticles, respectively. Characterisation of synthesised nanoparticles was carried out with techniques such as FT-IR, field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), x-ray diffractometer (XRD) and atomic force microscopy (AFM).

3.3 FT-IR Characterisation

Figure 3 presents the FT-IR spectra of ethanol, aqueous and ethanol/water (1:1) extracts of *C. indica* leaves. The absorption frequency of each band and their corresponding intensity is further detailed in Table 3. The main absorption band, which is present in all three fractions is the strong, broad band at around 3300 cm^{-1}. This can be attributed to O-H present in phenols. The intensity of the phenolic O-H band increases with increasing polarity from ethanol to water. The presence of phenolic component is further corroborated by the weak-medium absorption band observed at 1451 and 1640 cm^{-1} in the ethanolic fraction. These bands can be attributed to aromatic C=C stretch. The band at 1451 cm^{-1} reduces with increasing polarity and is absent in water. The absorption band observed at 1048 cm^{-1}, which is absent in the aqueous fraction can be allocated to C-O found in primary alcohols. The presence of weak absorption bands at 2925, 2974 and 2985 cm^{-1} found in ethanolic and ethanol/DW fractions respectively can be attributed to C-H stretching vibration.

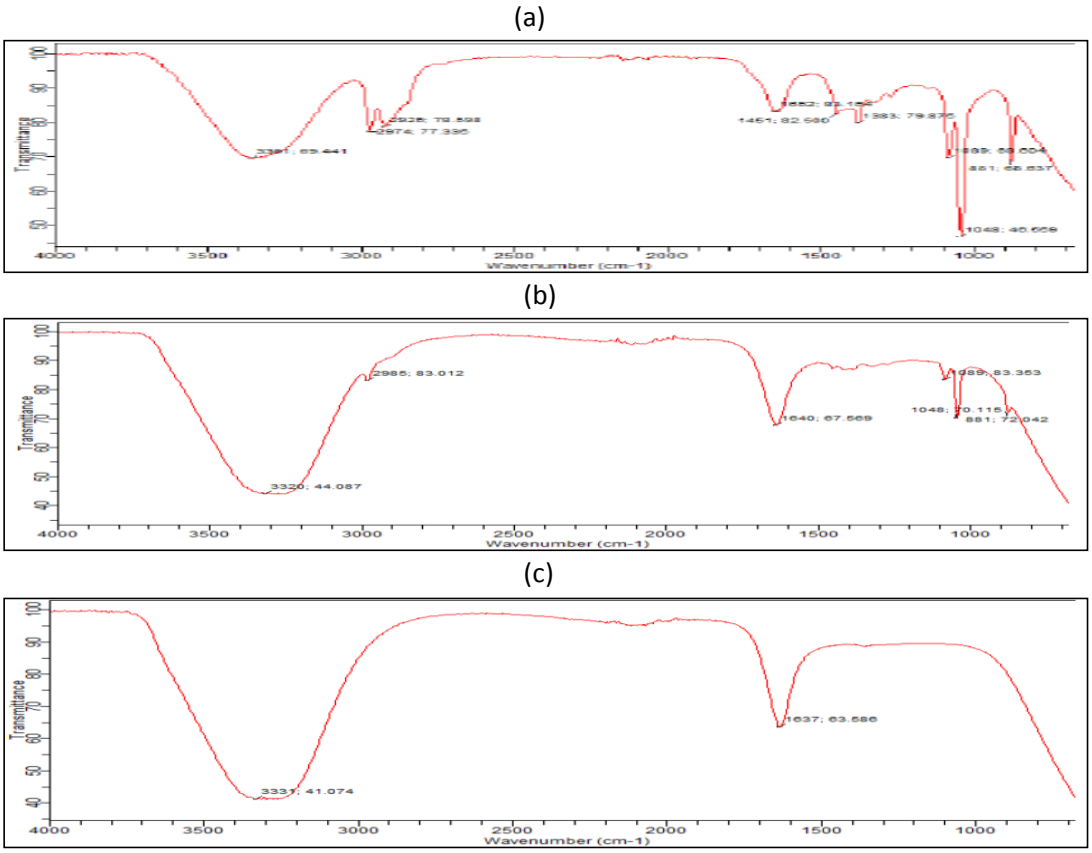


Figure3. FT-IR spectra of: (a) ethanolic fraction (b) ethanol/DW fraction (1:1) (c) DW fraction

Table 3.FT-IR frequency/intensity for three fractions of *Canna indica* leaves

	FT-IR Absorption frequency (cm^{-1})/intensity								
Ethanol	881 (m)	1048 (s)	1089 (m)	1383 (w)	1451 (w)	1652 (w)	2925 (w)	2974 (w)	3361(m,b)
Ethanol/water	881 (w)	1048 (m)	1089 (w)	-	-	1640 (m)	-	2985 (w)	3320 (s,b)
Water	-	-	-	-	-	1637 (m)	-	-	3331 (s,b)

m – medium, s – strong, w – weak, b – broad

3.4 GC-MS Analysis of ethanolic extract of Canna indica leaves

The GC-MS chromatogram obtained for analysis of ethanolic fraction of *C. indica* extract is shown in Figure 4. The only phenolic compound identified in the ethanolic extract of *C. indica* leaves is dl-.alpha.-Tocopherol, which eluted after thirty-seven minutes and with area percent of 0.57% as presented in Table 3. In addition, the structural composition of phenolic component identified in *Canna indica* is shown in Table 4. However, it is a naturally occurring form of vitamin E and possesses stabilizing and radical scavenging properties, thus making it a potent antioxidant. Figure 5 shows the TIC and chemical structure of dl-.alpha.-Tocopherol. [22] reported the synthesis of spherical silver nanoparticles using tocopherol as reducing and stabilizing agent. The synthesized nanoparticles subsequently displayed strong antimicrobial properties.

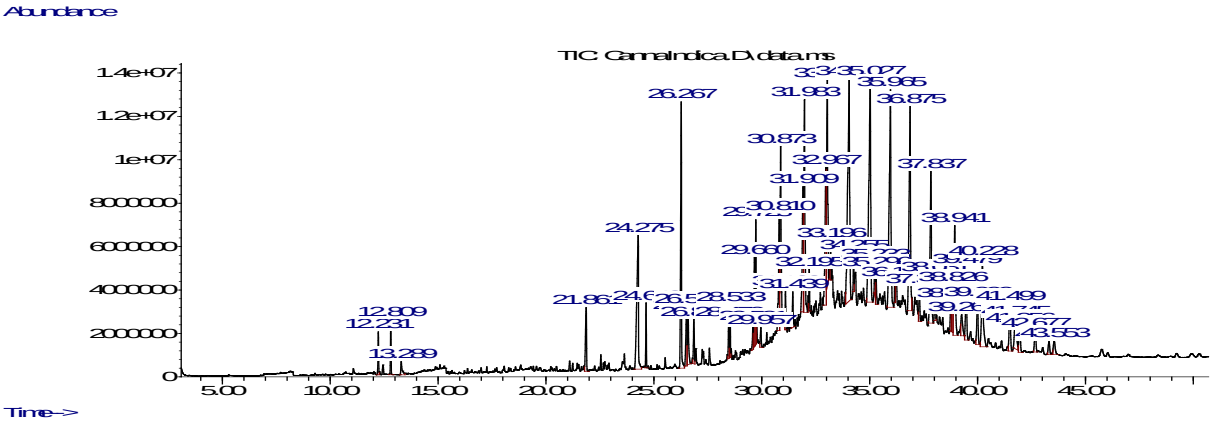
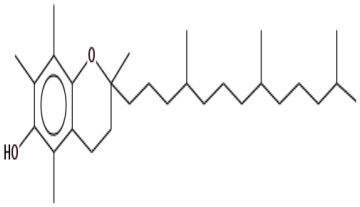


Figure 4. GC-MS Chromatogram of *Canna indica* leaves (ethanolic extract)

Table 4: Structural composition of phenolic component identified in *Canna indica* leaves extract

Retention time	Area %	IUPAC name of compound	Molecular formular	Molecular weight	Chemical structure
37.304	0.573	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430.7061	

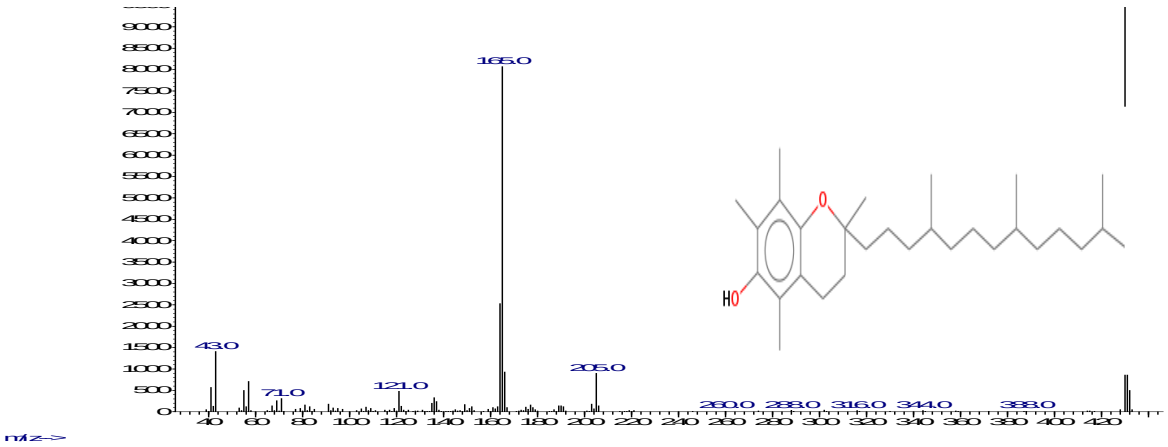


Figure 5. Total ion chromatogram (TIC)dl-.alpha.-Tocopherol

4. Conclusion

Phytochemical components found in *Canna indica* leaves based on phytochemical, spectral and chromatographic characterisations make it a potential source for biosynthesis of nanoparticles such as iron oxide, silver and gold. These nanoparticles will find wide application in drug delivery, environmental remediation and electronics.

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Conflict of interest

The authors declare no conflict of interest.

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