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

Table 1. Selected indigenous 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 the functional 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 for twenty one (21) days (Figures 1a,b). The dried leaves were pulverized and preserved in air tight containers until further use [15,16].

![Canna indica leaves](image1)

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 powdered *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.

|                     | 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 = Flavanoids, 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.
Figure 3. 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

| Fraction       | 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)         | -         |
| 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 formula | Molecular weight | Chemical structure |
|----------------|--------|------------------------|-------------------|------------------|--------------------|
| 37.304         | 0.573  | dl-.alpha.-Tocopherol  | C_{29}H_{50}O_{2} | 430.7061         | ![Chemical Structure](image) |

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.

References
[1.] Kombaiah, K., Vijaya, J. J., Kennedy, L. J., Bououdina, M., Ramalingam, R. J., & Al-Lohedan, H. A. (2017). Okra extract-assisted green synthesis of CoFe$_2$O$_4$ nanoparticles and their optical, magnetic, and antimicrobial properties. *Materials Chemistry and Physics*, 204, 410–419. https://doi.org/10.1016/j.matchemphys.2017.10.077
[2.] Bolade, O.P., Akinsiku, A.A., Adeyemi, A.O., Williams, A.B., Benson, N.U. (2018). Dataset on phytochemical screening, FT-IR and GC-MS characterisation of *Azadirachta indica* and *Cymbopogon citratus* as reducing and stabilising agents for nanoparticles synthesis, *Data in Brief*, 20, 917 – 926, https://doi.org/10.1016/j.dib.2018.08.133
[3.] Okuo, J., Emina, A., Omorogbe, S., & Anegbe, B. (2018). Synthesis, characterization and application of starch stabilized zerovalent iron nanoparticles in the remediation of Pb-acid battery soil. *Environmental Nanotechnology, Monitoring and Management*, 9, 12–17. https://doi.org/10.1016/j.enmm.2017.11.004
[4.] Sun, M., Cheng, G., Ge, X., Chen, M., Wang, C., Lou, L., & Xu, X. (2018). Environment aqueous Hg (II) immobilization by chitosan stabilized magnetic iron sulfide nanoparticles. *Science of the Total Environment*, 621, 1074–1083. https://doi.org/10.1016/j.scitotenv.2017.10.119
[5.] Cui, L., Ouyang, Y., Lou, Q., Yang, F., Chen, Y., Zhu, W., & Luo, S. (2010). Removal of nutrients from wastewater with *Canna indica* under different vertical-flow constructed wetland conditions. *Ecological Engineering*, 36(8), 1083–1088. https://doi.org/10.1016/j.ecoleng.2010.04.026
[6.] Cui, X., Fang, S., Yao, Y., Li, T., Ni, Q., Yang, X., & He, Z. (2016). Potential mechanisms of cadmium removal from aqueous solution by *Canna indica* derived biochar. *Science of the Total Environment*, 562, 517–525. https://doi.org/10.1016/j.scitotenv.2016.03.248
[7.] Samal, K., Dash, R. R., & Bhunia, P. (2017). Performance assessment of a *Canna indica* assisted vermicompost for synthetic dairy wastewater treatment. *Process Safety and Environmental Protection, 111*, 363–374. https://doi.org/10.1016/j.psep.2017.07.027
[8.] Lamaeswari G. and Ananthiss T. (2012). Preliminary phytochemical screening and physicochemical characterization of *Canna Indica* L. *International Journal of Pharmaceutical Sciences Review and Research*, 14(2), 76–79. https://doi.org/10.1002/da.22477
[9.] Ali, S., Aminu, B., Awlia, J., Anwar, Y., Baig, M., Qadri, F., … Bahadar, S. (2018). Albizia chevalier based Ag nanoparticles : Anti-proliferation, bactericidal and pollutants degradation performance. *Journal of Photochemistry & Photobiology, B: Biology*, 182(February), 62–70. https://doi.org/10.1016/j.jphotobiol.2018.03.020
[10.] Olajire, A. A., Ifediora, N. F., Bello, M. D., & Benson, N. U. (2017). Green Synthesis of Copper Nanoparticles Using Alchornea laxiflora Leaf Extract and Their Catalytic Application for Oxidative Desulphurization of Model Oil. *Iranian Journal of Science and Technology, Transactions A: Science*, 9. https://doi.org/10.1007/s40995-017-0404-9
[11.] Elias, E., Charles, O., Aleruchi, C., Ayomide, L., Mojisola, O., Rebecca, M., … Elijah, A.
(2014). Evaluation of antibacterial activities of silver nanoparticles green-synthesized using pineapple leaf (Ananas comosus). Micron, 57, 1–5. https://doi.org/10.1016/j.micron.2013.09.003

[12.] Bello, B. A., Khan, S. A., Khan, J. A., Syed, F. Q., Anwar, Y., & Khan, S. B. (2017). Antiproliferation and antibacterial effect of biosynthesized AgNps from leaves extract of Guiera senegalensis and its catalytic reduction on some persistent organic pollutants. Journal of Photochemistry & Photobiology, B: Biology, 175(July), 99–108. https://doi.org/10.1016/j.jphotobiol.2017.07.031

[13.] Ikhuoria, E. U., Omorogbe, S. O., Sone, B. T., & Maaza, M. (2018). Bioinspired shape controlled antiferromagnetic Co3O4 with prism like-anchored octahedron morphology: A facile green synthesis using Manihot esculenta Crantz extract. Science and Technology of Materials. https://doi.org/10.1016/j.stmat.2018.02.003

[14.] Nasrollahzadeh, M., Atarod, M. and M. Sajjadi (2019). Plant-Mediated Green Synthesis of Nanostructures: Mechanisms, Characterization, and Applications. An Introduction to Green Nanotechnology (1st ed., Vol. 28). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-813586-0.00006-7

[15.] Vaghasiya, Y., Dave, R. & Chanda, S. (2011). Phytochemical analysis of some medicinal plants from Western Region of India. Research Journal of Medicinal Plants, 5: 567-576.

[16.] Tegelberg R, Virjamo V, Julkunen-Tiitto R. (2018). Dry-air drying at room temperature – a practical pretreatment method of tree leaves for quantitative analyses of phenolics? Phytochemical Analysis, (2018), 1–7. https://doi.org/10.1002/pca.2755

[17.] Varadharajan, V. (2015). Physicochemical, phytochemical screening and profiling of secondary metabolites of Annona squamosa leaf. World Journal of Pharmaceutical Research, 1(4), 1143-1164.

[18.] Singh, R., Bachheti, R. K., Ck, S., & Singh, U. (2016). In-vitro antioxidant activity of Canna indica extracts using different solvent system. Asian Journal of Pharmaceutical and Clinical Research, 9(6), 4–7. https://doi.org/10.22159/ajpcr.2016.v9i6.10583

[19.] Xiao, H., Cheng, S., & Wu, Z. (2010). Microbial community variation in phytoremediation of triazophos by Canna indica L. in a hydroponic system. Journal of Environmental Sciences, 22(8), 1225–1231, https://doi.org/10.1016/S1001-0742(09)60242-4

[20] Akinsiku, A. A., Dare, E. O., Ajanaku, K. O., Ajani, O. O., Olugbuyiro, J. A. O., Siyanbola, T. O., Ejilude, O., & Emetere, M. E. (2018). Modeling and synthesis of Ag and Ag/Ni allied bimetallic nanoparticles by green method: Optical and biological properties. International Journal of Biomaterials, 2018(3), 1-17. https://doi.org/10.1155/2018/9658080

[21] Shukla, D., & Vankar, P. S. (2012). Synthesis of plant parts mediated gold nanoparticles. International Journal of Green Nanotechnology: Biomedicine, 4(3), 277–288. https://doi.org/10.1080/19430892.2012.706175

[22] Shankar, S., & Rhim, J. W. (2016). Tocopherol-mediated synthesis of silver nanoparticles and preparation of antimicrobial PBAT/silver nanoparticles composite films. Food Science and Technology, 72, 149–156. https://doi.org/10.1016/j.lwt.2016.04.054