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Original research Article

Qualitative and Quantitative Phytochemicals Studies of Ethanol Stem bark Extracts of *Isoberlinia doka* Craib & Stapf and *Isoberlinia tomentosa* (Harms) Craib & Stapf

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ABSTRACT

Isoberlinia doka and *Isoberlinia tomentosa* are plant used in traditional treatment of various kinds of diseases in Nigeria without scientific validation on the phytochemicals responsible for their actions. The present work was aimed to perform phytochemical analysis on the stem bark crude extracts and fractions of *I. doka* and *I. tomentosa*. The qualitative and quantitative phytochemicals studies were conducted on *I. doka* and *I. tomentosa* based on standard method. The findings from qualitative phytochemical analysis showed the presence of flavonoids, tannins, terpenoid/steroid, saponins and alkaloids whereas anthraquinones were absent in all the extracts and fractions of *I. doka* and *I. tomentosa*. The phytochemicals content of the crude extract and fractions of *I. doka* were found to be higher than *I. tomentosa* except for total saponin content. The crude extract of *I. doka* has the highest total alkaloids content (42.1 mg/g), the highest total flavonoids and phenol contents were obtained from n-Butanol fraction of *I. doka* (139.1 mg/g and 310.0 mg/g, respectively). However, the highest total saponin content was obtained as 170.6 mg/g in *I. tomentosa* crude ethanol extract. Therefore *I. doka* and *I. tomentosa* were very rich in similar phytochemical which could be responsible for their claimed efficiency in traditional medicine for the prevention of several diseases.

Keywords: UV-VIS Spectroscopy, Phytochemical screening, Leaf extracts, Isoberlinia.

Introduction

Isoberlinia doka and *Isoberlinia tomentosa* belong to the family fabaceae (ceasalpinoideae). They exist as shrub or tree measuring up to 10-20 m tall with a trunk of about 40-50 cm diameter, branching from about 5 m upwards.[1] The leaves member type of *Isoberlinia* genus are pinnate and paripinnate types of compound leaves. The leaflets few per leaf (2–5 pairs); opposite or sub-opposite in arrangement; petiotulated with short stalk.[2] The fruits are two-valved pod which twist during dehiscence; obliquely oblong, compressed, becoming woody. The mature valves with conspicuous, prominent and raised venation. Seeds not arillate.[2] *Isoberlinia* is a hardwood tree native to African tropical savannas and guinea forest savanna mosaic dry forest. To the north of the Equator, there are only three species: *I. doka, I. tomentosa* and *I. paradoxa.* To the south of the Equator, *I. tomentosa, I. doka, I. angolensis* and *I. scheferi* are found. In Benin, *I. doka* and *I. tomentosa* are the dorminant species.[3] However, *I. doka* and *I. tomentosa* are major constituents of the woodland belt in Northern Nigeria Guinea Savanna. They also occur sparsely in the south of these woodlands.[4]

However, due to over exploitation for human uses in Northern Nigeria open woodlands, these plants has been reduce to herbaceous state as a result, they grow annually from regeneration of old stocks.[4]

I. doka and *I. tomentosa* are widely used by traditional medical practitioners for the treatment of diabetes, ulcer, arrow poison, wound, stomach pain, jaundice and cough.[5-7] *I. doka* is also used to treat infectious diseases, and scientific investigations have confirmed its antibacterial activity. Other researchers showed scientific evidence that the stem bark extract of *I. doka* enhanced sexuality in male Wistar rats.[5,8] Studies have shown the antioxidant activity and mineral elements profiles of *Isoberlinia doka* leaves.[6] The root, stem bark and leaves extract of *Isoberlinia doka* has been reported to be active in brine shrimp lethality test.[7] Scientific evaluations of *I. tomentosa* are still scanty in literature. Phytochemical screening of *I. doka* leave extract has been reported to reveal the presence of saponins, flavonoids, alkaloids, terpenoids and tannins.[6,9,10] Members of the fabaceae family show variability of secondary metabolites in their plants as a result of several factors like geographical location, soil and different environmental conditions.

Several studies have been carried out on herbal medicines, as an alternative approach to avoid the side effects of synthetic medicines to achieve high effectiveness, low cost, improve patient compliance and therapeutic efficacy in treatment of various kinds of diseases like cancer, neurodegenerative, cardiovascular diseases, type 2 diabetes, etc.[11-13]

It is important to determine the phytochemical constituents of medicinal plants, thus knowing the type of biological activity which might be exhibited by the plant.[14,15] Several researchers have reported significant phytochemicals from various medicinal plants across the globe that may serve as potentials for novel drugs for the management of different varieties of ailments.[11-16] Studies have also shown that phytochemicals in fruits of medicinal plants are potential polyphenol anti-oxidants.[15] Phytochemicals are extracted from medicinal plants through different techniques, isolated, then screened for their biological activity.[17] Therefore, this study was aimed to determine the qualitative and quantitative phytochemicals of the stembark extract

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and fractions of *I. doka* and *I. tomentosa* to provide scientific validation to the enormous traditional uses of this plants in Nigeria.

Materials and Methods

Collection and identification of plant samples

The fresh plants of *I. doka and I. tomentosa* were first identified on the field using their morphological features, samples were collected from Shika village, with the Coordinates (11° 12' 14.80" N & E 007° 33' 45.40"). Giwa local Government Area, Kaduna State in January 2018. The plant were taxonomically authenticated by Namadi Sanusi with Voucher specimen numbers 022478 and 016280 for *I. doka and I. tomentosa*, respectively deposited at the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria, Nigeria.

Preparation of plant materials

The two plants (5 kg each) were collected after authentication, the stem barks of *I. doka* and *I. tomentosa*, were cut from the plant and washed in clean water in order to remove all foreign matters. They were then sliced into pieces and air-dried under shade until a constant weight was obtained, pulverized into coarse powder with mortar and pestle and stored in cellophane bags at room temperature until required for the experiment.

Extraction and fractionation of plant materials

The air-dried powder of *I. doka* (1.5 kg) and *I. tomentosa* (1 kg) stem barks were first extracted with 70% ethanol (5 L) at room temperature by maceration for 48 h. The extracts were filtered using whatman filter paper No 1 and filtrate were concentrated to dryness using a rotary evaporator at reduced pressure and temperature (60°C). The percentage yields of the crude extracts were recorded. Fifty gram (50 g) of the crude ethanol extract of *I. doka* and 40 g of *I. tomentosa* were suspended separately in distilled water (500 mL). The aqueous portion was extracted with ethyl acetate (5 x 400 mL) and *n*-butanol (5 x 400 mL).[18,19]

Qualitative phytochemical screening

The plant materials were screened for phytochemicals using standard methods. [20-22]

Thin layer chromatographic studies (TLC) of the extracts of I. doka and I. tomentosa

The extracts and fractions were subjected to TLC which is a qualitative method used to identify and confirm the presence of some of the secondary metabolites obtained in the preliminary phytochemical tests using standard protocol.[23] Pre-coated TLC plate (silica gel $G_{600.}$ 0.25 mm thickness) were used, Samples were prepared by diluting the crude extracts and fractions with respective solvent and then applied (usually 1-10 µL volumes) to the origins of a TLC plate, 10 mm above the lower edge of the plate using a capillary tube. The plate was dipped into a suitable solvent system (mobile phase) and placed in a closed chamber. The following solvent systems were used: Ethyl acetate (100%), Chloroform:Methanol (9:1), Hexane: Ethyl acetate (7:3) and Chloroform: Ethyl acetate:Methanol:Water (15:8:4:1). The solvents were allowed to ascend through adsorbent phase up to three quarter of the plate. At the end of the chromatographic development, the plate was removed from the chromatographic tank, solvent front was marked, allowed to evaporate at room temperature and the separated spots were visualized in UV chamber (365 nm) and p-anisaldehyde has standard spraying reagent for TLC.[23] Number of spots and retention factors (R_f) were examined. R_f value or the ratio of the distances moved by a given compound and a stated reference substance (the R_f value) as follows:

$$R_= \frac{a}{b}$$

Spraying of the TLC plates was done using various chemical reactants such as Dragendroff`s spray for alkaloids, ferric chloride spray for tannins, libermann burchard spray for terpenoids, aluminium chloride spray or 5% NaoH for flavonoids and bontrager for anthraquinones.

Quantitative phytochemical analysis

Determination of total phenolic content, total tannin content, total alkaloid content and total flavonoid content were done by spectrophotometric methods as previously described.[24,25]

a. Total Alkaloid Contents (TAC)

The crude extract and fractions of *I. doka* and *I. tomentosa* (1 mg/mL) were dissolved separately in dimethylsulphoxide) and added 1 mL of 2 N HCl and filtered. This solution was transferred to a separating funnel. Five mililitre (5 mL) of bromocresol green (BCG) solution and 5 mL of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 mL of chloroform by vigorous shaking and collected in a 10 mL volumetric flask and diluted to the volume with the chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described already. The absorbance for standard solutions and test solutions were determined on the reagent blank at 470 nm with a UV/Visible spectrophotometer. The content of alkaloids was expressed as mg of AE/g of plant extract.

b. Total Flavonoids Contents (TFC)

Colorimetric assay was used to determine the total content of flavonoid using aluminium chloride for the reaction, the plant extract/fractions (1 mL) and distilled water (4 mL) was taken in a 10 mL flask. Precisely 0.30 mL of 5% sodium nitrite was added to the flask and 5 min after, 0.3 mL of 10% aluminium chloride was mixed in the flask. 5 min later, 2 mL of prepared sodium hydroxide (1 M) was added and made up to 10 mL using distilled water. A set of standard solutions of quercetin (20, 40, 60, 80 and 100 µg/mL) were prepared as mentioned earlier. The absorbance was measured for test and standard solutions using reagent blank at 510 nm wavelength by UV-Visible spectrophotometer. The total flavonoid contents was denoted as mg of QE/g of extract. This process was done for crude extract and fractions of *I. doka* and *I. tomentosa.*

c. Total Tannins Content (TTC)

The tannin content was estimated by Folin-Ciocalteu method. About 0.1 mL of the extract/fractions was treated with 7.5 mL of distilled water, with 0.5 mL of prepared Folin-Ciocalteu (10%) reagent and 1 mL of the prepared sodium carbonate solution (35%) were added and diluted to 10 mL using distilled water. The entire reaction mixture was mixed well and kept at room temperature for 30 min. The gallic acid was used as the standard and prepared to various concentrations like 20, 40, 60, 80 and 100 µg/mL with methanol. Finally, the absorbance of all the samples and gallic acid standards were determined using an UV or Visible spectrophotometer at 725 nm. The tannin content obtained was expressed as mg of gallic acid equivalent (GAE) per gram of extract. This process was done for crude extract and fractions of *I. doka* and *I. tomentosa*

d. Total Phenolic Contents (TPC)

The phenolic compounds concentration in extract was quantified by Spectrophotometry method. Folin-Ciocalteu method was employed for the quantification of total phenolic content. The reaction mixture contains 1 mL of plant extract/fractions and 9 mL of distilled water. 1 mL of Folin-Ciocalteu phenol reagent was treated with the mixture and well shaken. After 5 min, 10 mL of 7% Na_2CO_3 solution was treated with the mixture. A set of gallic acid standard solutions (20, 40, 40, 60, 80 and 100 µg/mL) were prepared as earlier. After incubation for 90 min at 30°C and absorbance was read for test and standard solutions with reagent blank at 550 nm using UV-Visible spectrophotometer. This process was done for crude extract and fractions of *I. doka* and *I. tomentosa*. The content of total phenolic compound was expressed as mg of GAE/gm of extract.

e. Total Saponin Contents (TSC)

The estimation of total saponins content was determined based on vanillin-sulphuric acid colorimetric reaction with some modifications. [26] Exactly 5000 µL of water was added to 100 µL of diosgenin. Five hundred microlitre of vanillin reagent (8 g of vanillin in 100 mL of 99.5% ethanol) was added and 5 mL of 72% sulphuric acid was added and mixed well. Saponin standard solutions (20, 40, 40, 60, 80 and

100 µg/mL) were prepared. This solution was kept in a water bath at 60°C for 10 min. After 10 min, it was cooled and the absorbance was read at 544 nm and recorded. This process was done for crude extract and fractions of *I. doka* and *I. tomentosa*. All measurements were performed in triplicate for each analysis. The total saponins content was determined from the linear equation of a standard curve prepared with diosgenin and expressed as mg/g diosgenin equivalent (DE) of dry extract.

Preparation of standard calibration curve

Calibration curve was prepared by taking 2 mL, 4 mL, 6 mL, 8 mL and 10 mL solutions from the concentration of 10 µg/mL and applying the procedure for the estimation of the studied phytochemicals and the standard curve was plotted by taking the sample concentration on X-axis and absorbance values on Y-axis, [27] the graphs are shown in Figure 1.

Statistical analysis

Results generated were presented as Mean ± Standard Deviation and Graphs of the standard and absorbance values were plotted using the Microsoft Excel spreadsheet application.

Results and Discussion

Qualitative and quantitative analysis of secondary metabolites are very essential for identifying the presence of phytochemicals which is important for the contribution of medicinal and physiological properties to the plants. The pharmacological actions of medicinal plants are due to quality and quantity of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, and steroids, etc. present in them.[22]

Percentage yield of the stem bark extract and fractions of *I doka* and *I tomentosa* are shown in Table 1.

Qualitative Phytochemical Screening of Ethanol Stem Bark Extract of I doka and I tomentosa

The results of the phytochemical screening are shown in Table 2. The result revealed the presence of similar phytochemicals such as phenols, flavonoids, saponins, tannins, alkaloids, steroids/terpenoids, glycosides and triterpenes for *I. doka* and *I. tomentosa* crude extracts and fractions only. Steroids/terpenoids where found to be absent in the aqueous fraction of *I. doka* as well as anthraquinones.

Preliminary phytochemical screening of the extracts and fractions of *I. doka and I. tomentosa* showed similar phytochemicals which are responsible for their pharmacological actions. This may be due to the fact that the two plant are closely related from the same genus *Isoberlinia* and were obtained from the same geographical locations in Nigeria, or as a result of similiar eco-physiological conditions, a minor difference in the qualitative polyphenolic content in the leaves of cecropia species collected at the same location.[28] Flavonoids, terpenoids/steroids, tannins, alkaloids, saponins, and phenols were found to be present in both plants, this also supported previous findings.[6,10]

The TLC finger printing profile for *I. doka and I. tomentosa* extract and fractions were revealed in plate I. This showed the presence of various phytochemicals in the extracts and fractions of *I. doka and I. tomentosa* as indicated by different coloured spots and R_f values using various solvent systems and visualizing spray reagents as indicated in Table 3 for general spray and specific spray in Table 4. The solvent system CHL:EAA:MEOH:W (15:8:4:1) revealed eleven spots for crude *I. doka.* However, butanol, aqueous and crude *I. tomentosa* revealed ten spots each with green, brown and blue colours. This study has clearly shown that the crude extracts and fractions were very rich in phytoconstituents. The solvent system. Chloroform:Methanol (9:1) revealed six spots for *I. tomentosa* ethyl acetate fraction only and Hexane:Ethyl acetate (7:3) revealed six spots for *I. doka* ethyl acetate fraction. The TLC profiles of *I. doka and I. tomentosa* when sprayed with specific spraying reagent for phytoconstituents detection confirmed the presence of some of the phytochemicals with changes in colour such as orange colored spots were observed in all the extracts and fractions when sprayed with dragendorff's reagent with R_f values 0.88, 0.36 and 0.44, confirmed the presence of alkaloids. To detect the presence of flavonoids using separately developed TLC plate, Aluminum chloride spray did not revealed any spots at day light but after UV observation at 366 nm the whole extracts and fractions using the three solvent systems showed yellow fluorescent constituents, the yellow colored spots with R_f values 0.84, 0.44, 0.17, 0.36, 0.12 and 0.04 as shown in Table 4 confirmed the presence of flavonoids in all the extracts/fractions of the two plants. Blue-black coloured spots with R_f values 0.82, 0.41, 0.55, 0.64 and 0.79 were identified as tannins after spraying with ferric chloride reagent. Purple/green coloured spots were identified as triterpenes when sprayed with Liebermann-Burchard reagent. The confirmatory phytochemical test using TLC did not show any spots when sprayed with Bontrager's reagent this indicated the absence of anthraquinones in all samples. These findings are also in support with earlier report on the leaves extract of *I. doka.*[6] TLC confirmed the presence of alkaloids, flavonoids, tannins and triterpenes in the two studied samples of *Isoberlinia*. TLC profiles determination are valuable tools for the qualitative determination of small amount of impurities present in medicinal plants and preliminary steps for identification and isolation of active compound in drugs.[29]

Quantitative Phytochemical Screening of Ethanolic Stem Bark Extract of I. doka and I. tomentosa

The standard calibration curves obtained for the estimation of total alkaloids, flavonoids, phenols, tannins, and saponin content in the extract and fractions of *I. doka and I. tomentosa* are shown in Figure 1.

Majority of the phytochemicals content of the crude extract and fractions of *I. doka* were found to be higher than *I. tomentosa*. *I. doka* crude extract has the highest TAC as 42.1 mg/g, the highest TFC was obtained from n-Butanol fraction of *I. doka.* The highest TPC was 455.1 mg/g and 170.6 mg/g in *I. tomentosa* crude ethanol extract as indicated in Table 5.

Despite the similarities in the qualitative phytochemicals observed in *I. doka and I. tomentosa*, their quantitative phytochemicals have varied concentrations. The crude ethanol extracts of *I. doka and I. tomentosa* contain high amount of phenolic compounds but the TFC, TPC and TTC were very high in the butanol fractions, these compounds possesses an antioxidant activity, they are the main dietary phenolic compounds which serve as protection against pathogens and microbial attack in plants, this findings is also in support of earlier report that *I. doka* is involved in scavenging free radicals from tissues, thus reducing the oxidative stress.[6] Several studies have revealed that phenolic compounds are a class of phytoconstituents with powerful antioxidant activity. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers .[10] Flavonoids in plants possess medicinal benefits which includes antioxidant and anticancer activities, the flavanol and xanthone glycosides isolated lend support to it activities by scavenging hydroxyl radicals, superoxide anions and lipid peroxy radicals.[15,27] Flavonoids have been reported to promote human health and help to reduce the risk of diseases as potential antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anticancer and antiviral properties.[29-33]

Various classes of terpenoids have been identified and isolated from medicinal plants to be useful in the prevention and therapy of several diseases, including cancer. They act as vitamins, as regulators of metabolism and play a protective role as antioxidants.[27,28] Saponins are naturally occurring structurally and functionally diverse phytochemicals that are widely distributed in medicinal plant. Saponins also act as antitumor agents by inhibiting tumor cell growth and induce apoptosis.[32] Alkaloids have been reported to possess various pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial and anticancer activity.[13] Secondary metabolites in plant produces some biological activities responsible for their potential use as drugs.[27]

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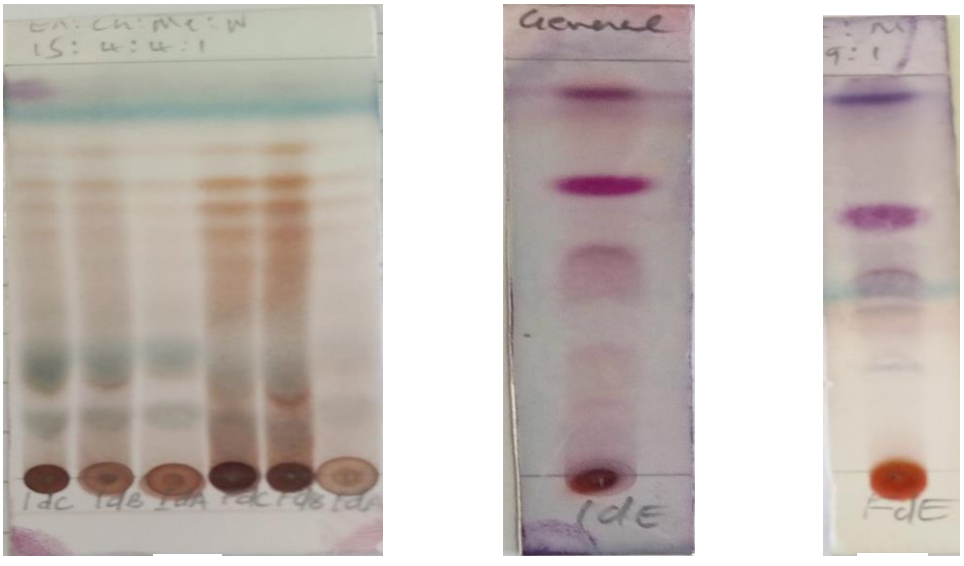
Table 1: Percentage yield of the Ethanol Stem Bark Extract of *I doka* and *I tomentosa*

	Colour	Yield (g)	% yield
Ethanol crude *I. doka*	Coffea brown	140.0	14.0
50 g of dry crude extract			
Ethylacetate fraction *I. doka*	Light brown	4.0	8.0
n-butanol fraction *I. doka*	Light brown	4.0	8.0
Aqueous fraction *I. doka*	Coffea brown	40.0	80.0
Ethanol crude *I. tomentosa*	Light brown	104.2	10.4
40 g of dry crude			
Ethylacetate fraction *I. tomentosa*	Light brown	4.0	10.0
n-butanol fraction *I. tomentosa*	Light brown	6.0	15.0
Aqueous fraction *I. tomentosa*	Light brown	30.0	75.0

Table 2: Qualitative phytochemical screening of ethanol stem bark extract of *I doka* and *I tomentosa*

		Isoberlinia doka				***Isoberlinia tomentosa***			
Phytochemicals	**Test**	**Crude**	**EAA**	**NB**	**AQ**	**Crude**	**EAA**	**NB**	**AQ**
Cardiac glycoside	kelle killiani	+	+	+	+	+	+	+	+
Glycoside	Fehling	+	+	+	+	+	+	+	+
Flavonoids	Shinoda	+	+	+	+	+	+	+	+
Saponins	Frothing	+	+	+	+	+	+	+	+
Triterpenes	Libermann-buchard	+	+	+	+	+	+	+	+
Steroids/terpenoids	Salkwoski	+	+	+	-	+	+	+	+
Phenols	Ellagic acid test	+	+	+	+	+	+	+	+
Alkaloids	Wagner/Dragendorff	+	+	+	+	+	+	+	+
Anthraquinones	Bontragers	-	-	-	-	-	-	-	-

EAA: ethyl acetate, NB: n-butanol, AQ : Aqueous fraction



A B C

Plate I: TLC profiles developed with different mobile phase and sprayed with p-anisaldehyde (general sray)
A. Chromatogram of *I. doka* crude, butanol, Aqueous fractions represented as IDC, IDB&IDA respectively and *I. tomentosa* crude, butanol, Aqueous fraction as FDC,FDB &FDA respectively, developed in Ethylacetate:chloroform:methanol:water (15:4:4:1).
B. Chromatogram of *I. doka* , Ethylacetate fractions represented as IDE, developed in Hexene: Ethylacetate: (7:3).
C. Chromatogram of *I. tomentosa* Ethylacetate fractions represented as FDE, developed in Chloroform: Methanol: (9:1).

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Table 3: TLC of *I. doka and I. tomentosa* spray with p- anisaldehyde (general spray)

Solvent system	**Crude**	**Ethylacetate**	**Butanol**	**Aqueos**
CHL: EAA: MEOH:W	Eleven spots	Not spotted	Ten spots	Ten spots
(15 : 8 : 4 : 1) for *I. doka*	0.16 (green)		0.16 (green)	0.16 (green)
	0.27 (green)		0.27 (green)	0.27 (green)
	0.24 (green)		0.24 (green)	0.24 (green)
	0.41 (brown)		0.41 (brown)	0.41 (brown)
	0.55 (brown)		0.55 (brown)	0.55 (brown)
	0.59 (brown)		0.59 (brown)	0.59 (brown)
	0.64 (brown)		0.64 (brown)	0.64 (brown)
	0.71 (brown)		0.71 (brown)	0.71 (brown)
	0.79 (brown)		0.79 (brown)	0.79 (brown)
	0.86 (blue)		0.86 (blue)	0.86 (blue)
	0.93 (pink)			
CHL: EAA: MEOH:W	Ten spots	Not spotted	Ten spots	Ten spots
(15 : 8 : 4 : 1) for *I. tomentosa*	0.16 (green)		0.16 (green)	0.16 (green)
	0.27 (green)		0.27 (green)	0.27 (green)
	0.24 (green)		0.24 (green)	0.24 (green)
	0.41 (brown)		0.41 (brown)	0.41(brown)
	0.55 (brown)		0.55 (brown)	0.55 (brown)
	0.59 (brown)		0.59 (brown)	0.59 (brown)
	0.64 (brown)		0.64 (brown)	0.64 (brown)
	0.71 (brown)		0.71 (brown)	0.71 (brown)
	0.79 (brown)		0.79 (brown)	0.79 (brown)
	0.86 (blue)		0.86 (blue)	0.86 (blue)
Hexane: Ethylacetate(7 : 3) for		Six spots		
I.doka		0.11 (brown)		
		0.17 (brown)		
		0.29 (brown)		
		0.56 (pink)		
		0.68 (pink)		
		0.93 (pink)		
Chloroform: Methanol: (9:1) for *I.*		Six spots		
tomentosa ethylacetate fraction		0.30 (blue)		
only		0.40 (brown)		
		0.45 (blue)		
		0.50 (purple)		
		0.63 (purple)		
		0.91 (purple)		

CHL: EAA: MEOH: W; Chloroform: Ethylacetate: Methanol: Water

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Table 4: TLC profiles of *I. doka and I. tomentosa* spray with specific spray for some major phytochemicals

Phytochemicals	Detection reagent	No of spots	R_f value	Colour of spot after spray	Extracts	Solvent system
Flavonoids	Aluminium chloride	2 spots each	0.86,0.44	yellow	IDC,IDB,IDA, FDC, FDB & FDA	Chloroform:Ethylacetate: Methanol:Water (15:8:4:1)
		1	0.17	yellow	IDE	Hexane :Ethylacetate (7:3)
		3	0.36, 0.12 0.04	yellow	FDE	Chloroform: Methanol: (9:1)
Triterpenes	Liebermann-Buchard	3 each	0.93,0.27 0.16	Purple,Green,Green	IDC,IDB,IDA, FDC, FDB & FDA	Chloroform:Ethylacetate: Methanol:Water (15:8:4:1)
		4 each	0.3,0.4,0.63, 0.91	Green,green,purple,purple	FDE	Chloroform: Methanol: (9:1)
		2		Purple.purple	IDE	Hexane :Ethylacetate (7:3)
Tannins	Ferric chloride	1	0.82	Blue black	FDE	Chloroform:Ethylacetate: Methanol:Water (4:8:4:1)
		4 each	0.41,0.55,0. 64,0.79	Blue-black	IDC,IDB,IDA, FDC, FDB & FDA	Chloroform:Ethylacetate: Methanol:Water (15:8:4:1)
Alkaloids	Dragendorff's	1	0.88	orange	IDC,IDB,IDA, FDC, FDB & FDA	Chloroform:Ethylacetate: Methanol:Water (15:8:4:1)
		1	0.30	orange	FDE	Chloroform: Methanol: (9:1)
		1	0.46	orange	IDE	Hexane :Ethylacetate (7:3)
Anthraquinones	Bontrager's	-	-	-	-	-

I. doka crude, butanol and aqueous fractions represented as IDC, IDB and IDA, respectively; *I. tomentosa* crude, butanol and aqueous fractions represented as FDC, FDB and FDA, respectively.

Table 5: Quantitative phytochemicals of ethanolic stem bark extract of *I doka* and *I tomentosa* in mg/g

	Isoberlinia doka				***Isoberlinia tomentosa***			
Phytochemicals	**Crude**	**EAA**	**NB**	**AQ**	**Crude**	**EAA**	**NB**	**AQ**
Alkaloids	42.1	38.7	9.2	10.3	28.2	30.6	8.5	7.4
Flavonoids	93.4	35.1	139.1	19.1	75.6	15.2	92.4	12.2
Phenols	254.2	141.6	310.0	210.0	231.5	149.1	247.2	171.5
Tannins	455.1	165.2	318.8	426.1	455.1	108.3	231.9	376.8
Saponins	155.2	96.9	142.9	115.3	170.6	93.8	158.3	100.0

EAA: Ethyl acetate, NB: n-Butanol, AQ: Aqueous fraction.

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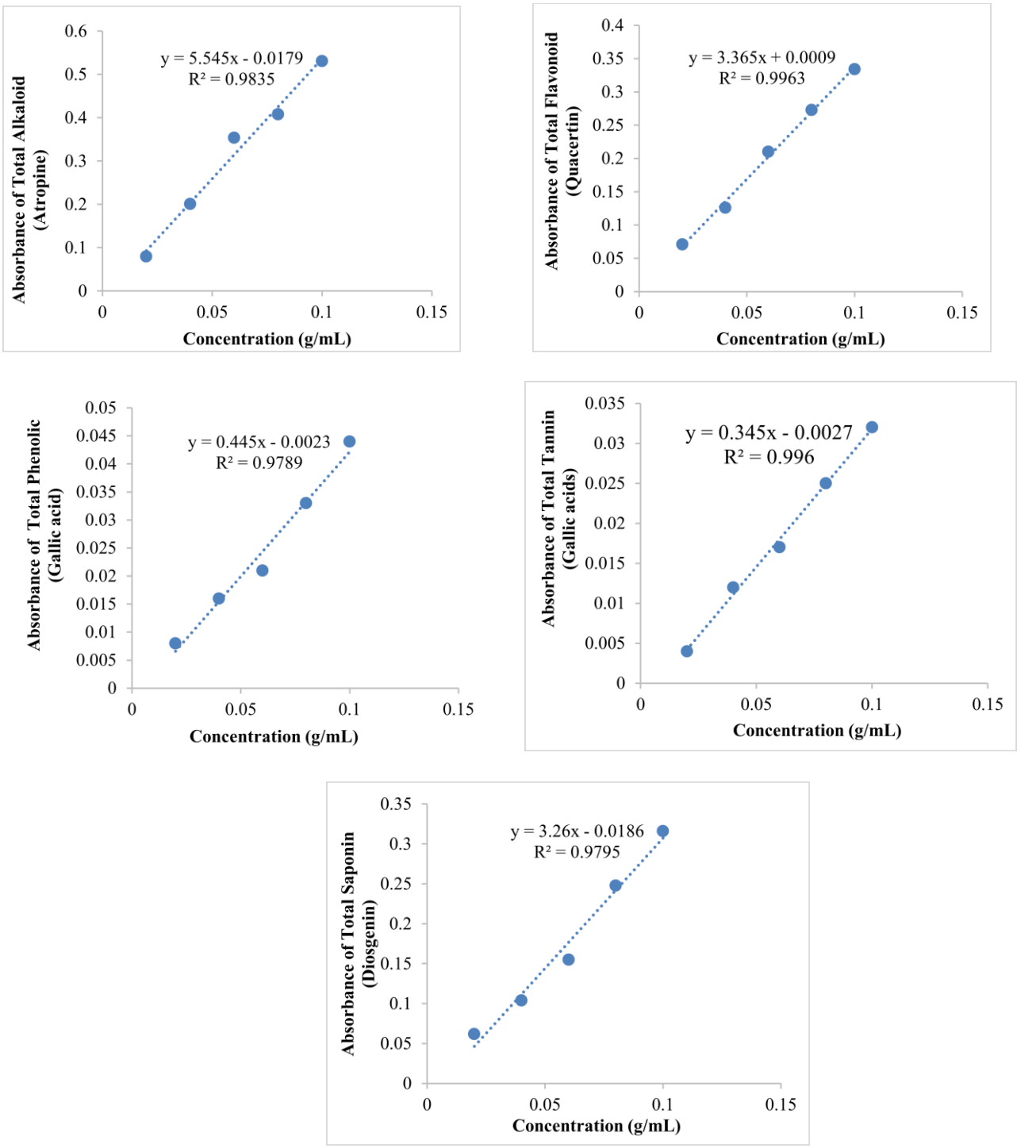

Figure 1: Calibration curve of total alkaloid, flavonoid, phenol, tannin and saponin contents of *I. doka and I. tomentosa* stem

Conclusion

The qualitative phytochemicals of *I. doka* and *I. tomentosa* of this study revealed the presence of enormous phytochemicals such as glycosides, steroids/terpenoids, alkaloids, flavonoids, phenols and saponins which are responsible for their therapeutic efficiency. Quantitative analysis showed that that ethanol extract, ethyl acetate and butanol fractions of *I. doka* total alkaloids content, total flavonoids content and total phenol content contains higher amounts than *I. tomentosa* extracts and fractions. The TLC analysis also help to confirmed these compounds and verify adulteration in quality control of crude extract as primary step for isolation of compounds. Further studies are ongoing on these plants to isolate, characterize and elucidate the major compounds responsible for their biological activities.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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