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Qualitative and Quantitative Phytochemical Screening of Ethanolic Leaf Extracts of *Jatropha Tanjorensis* Ellis & Saroja and *Leptadenia Hastata* (Pers.) Decne

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ABSTRACT

The phytochemical composition of a plant extract provides the mechanistic basis for its pharmacological activity. This study screened the qualitative and quantitative phytochemical constituents of ethanolic leaf extracts of *J. tanjorensis* (*JT*), *L. hastata* (*LH*). The sample was collected, identify and authenticated at the department of Biological Sciences, Nigerian Defence Academy Kaduna. Shade-dried, powdered leaves of both plants were macerated separately in 70% ethanol for 72 hours. Qualitative screening for alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, cardiac glycosides and anthraquinones was performed using standard colorimetric and precipitation methods. Total phenolic, flavonoid, alkaloid, tannin and saponin contents were quantified spectrophotometrically and gravimetrically in triplicate. Extraction yields were 9.32% (*JT*), 10.59% (*LH*) and 9.93% (combination); only the qualitative and quantitative phytochemical profiles of the individual JT and LH extracts were determined, as the combination extract was characterised for extraction yield only. Both extracts tested positive for alkaloids, flavonoids, tannins, saponins, phenols, terpenoids and cardiac glycosides, were all present, while anthraquinones was absent from both extracts. Quantitatively, *JT* contained significantly higher total phenolics (24.18 ± 0.71mg GAE/g), flavonoids (12.76 ± 0.43mg RE/g), alkaloids (18.24 ± 0.62mg/g) and tannins (8.34 ± 0.28mg TAE/g), whereas *LH* contained markedly higher total saponins (9.12 ± 0.31mg/g; 2.18-fold higher than *JT*). *Jatropha tanjorensis* and *Leptadenia hastata* possess overlapping but quantitatively distinct phytochemical profiles, with *JT* favouring phenolic-rich constituents and *LH* favouring saponin-rich constituents.

Keywords: *Jatropha Tanjorensis*; *Leptadenia Hastata*; Phytochemical Screening; Ethanolic Leaf Extract; Medicinal Plants

INTRODUCTION

Jatropha tanjorensis Ellis & Saroja, a member of the family Euphorbiaceae and known locally as "Hospital Too Far", is a leafy vegetable widely consumed in southwestern Nigeria and traditionally reputed to possess blood-boosting properties (Oyewole & Owoyele, 2014). Preliminary phytochemical surveys have reported the presence of alkaloids, flavonoids, saponins, tannins and phenolic compounds in this plant (Nwaoguikpe *et al*., 2011; Nworu *et al*., 2012). *Leptadenia hastata* (Pers.) Decne. (Apocynaceae), known locally as "Yadiya", is similarly consumed as a vegetable across the Sahelian belt of West Africa and has been used ethnomedicinally for anaemia, fever and inflammatory conditions, with reported flavonoid, phenolic, steroidal and terpene constituents (Diallo *et al*., 2012; Nafiu *et al*., 2017). Despite these individual reports, no study has directly compared the phytochemical profiles of *J. tanjorensis* and *L. hastata* extracted under identical conditions, nor evaluated a combination of the two plants (Ibrahim *et al*., 2019; Okonkwo *et al*., 2014).

The phytochemical composition of a plant extract provides the mechanistic basis for its pharmacological activity. Flavonoids and phenolic compounds are of particular interest in haematinic research because of their antioxidant capacity and their reported role in stabilising the hypoxia-inducible factor (HIF-1α) pathway that drives erythropoietin gene expression (Tanaka *et al*., 2009). Saponins have been separately implicated in enhancing intestinal non-haeme iron absorption (Guo *et al*., 2020), while alkaloids and cardiac glycosides have been associated with erythroid progenitor stimulation and erythrocyte membrane stabilisation, respectively (Iwu, 2014; Belcher *et al*., 2014). A comparative phytochemical screening of *J. tanjorensis* and *L. hastata* is therefore a necessary first step toward establishing a mechanistic rationale for their individual and combined haematinic use.

This study therefore screened, qualitatively and quantitatively, the phytochemical constituents of ethanolic leaf extracts of *J. tanjorensis*, *L. hastata* and their 1:1 combination, to establish whether the two plants possess complementary or overlapping phytochemical profiles that justify their evaluation as a combined haematinic agent.

MATERIALS AND METHODS

Plant Material and Authentication

Fresh leaves of Jatropha tanjorensis Ellis & Saroja and *Leptadenia hastata* (Pers.) Decne were collected from the Biodiversity Conservation Centre of the Nigerian Defence Academy (NDA), Afaka and Mando respectively, both within Igabi Local Government Area of Kaduna State, Nigeria. Collections were made between 0700 and 0900 hours to minimise post-harvest metabolite degradation, and only mature, healthy, disease-free leaves were harvested. Both species were taxonomically authenticated by qualified plant taxonomists in the Department of Biological Sciences, NDA, and the Botany Department, Kaduna State University (KASU). Voucher number were assigned (*J. tanjorensis*: KASU/BSH/7792; *L. hastata*: KASU/BSH/443).

Preparation of Ethanolic Leaf Extracts

Harvested leaves were washed, shade-dried at ambient room temperature (25–30°C) for two weeks, pulverised and sieved through a 60-mesh sieve. Two hundred grams (200 g) of each powdered plant material were macerated separately in 70% ethanol for 72 hours at room temperature with intermittent agitation every 12 hours. The mixtures were filtered sequentially through muslin cloth and Whatman No. 1 filter paper, and the filtrates were concentrated by evaporation in a thermostatically controlled water bath at 40°C. The resulting semi-solid extracts were oven-dried at 40°C to remove residual solvent, weighed and stored in airtight amber vials at 4°C. Percentage yield was calculated as: Percentage Yield (%) = (Weight of dried extract ÷ Initial weight of plant powder) × 100. The combination extract was prepared by dissolving pre-weighed *JT* and *LH* extracts in distilled water in a 1:1 ratio by weight, freshly prepared on each day of use. The combination extract was characterised for extraction yield only; its qualitative and quantitative phytochemical profiling was not undertaken in this study and is addressed in a subsequent companion study.

Qualitative Phytochemical Screening

Qualitative screening of both extracts was performed in triplicate using standard colorimetric and precipitation methods described by Harborne (1992) and Trease and Evans (2002). Alkaloids were screened using Dragendorff's, Mayer's and Wagner's reagents; flavonoids by the Shinoda test and the magnesium-turnings/acidified-methanol test; tannins by 5% ferric chloride; saponins by the persistent froth test; phenols by 1% aqueous ferric chloride; terpenoids by the Salkowski test; cardiac glycosides by Baljet's reagent; and anthraquinones by Borntrager's test. Results were recorded as present (+) or absent (−) relative to positive and negative controls.

Quantitative Phytochemical Analysis

All quantitative assays were performed in triplicate and results expressed as mean ± standard error of the mean (SEM) per gram of dry extract. Total phenolic content was determined by the Folin–Ciocalteu method (Hagerman *et al.*, 2000), with absorbance read at 765 nm against a gallic acid calibration curve and results expressed as mg Gallic Acid Equivalents per gram (mg GAE/g). Total flavonoid content was determined by the aluminium chloride colorimetric method (Kumaran & Karunakaran, 2006), with absorbance read at 415 nm against a rutin calibration curve and results expressed as mg Rutin Equivalents per gram (mg RE/g). Total alkaloid content was determined gravimetrically following Harborne (1992), by acetic acid–ethanol extraction, ammoniacal precipitation, and oven-drying of the precipitate to constant weight. Total tannin content was determined by the Prussian Blue (Price–Butler) method (Van-Burden & Robinson, 1981), with absorbance measured against a ferric chloride–potassium ferrocyanide complex, and results expressed as mg Tannic Acid Equivalents per gram (mg TAE/g). Total saponin content was determined gravimetrically following the method of Obadoni and Ochuko (2001), involving aqueous-ethanol extraction, diethyl ether defatting, n-butanol partitioning, and evaporation to constant weight.

Statistical Analysis

All quantitative phytochemical data are expressed as mean ± SEM (n = 3) and were compared between *J. tanjorensis* and *L. hastata* for each parameter using Student's independent samples t-test in IBM SPSS Statistics (Version 23.0), with $p < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Percentage Yield of Ethanolic Leaf Extracts

Maceration of 200 g of dried leaf powder of each plant in 70% ethanol yielded 18.64 g (9.32%) of *J. tanjorensis* extract and 21.18 g (10.59%) of *L. hastata* extract; the combination extract yielded 19.86 g (9.93%) (Table 1). Both extracts presented as dark semi-solid pastes, fully soluble in the extraction solvent on reconstitution.

Table 1: Percentage Yield of Ethanolic Leaf Extracts of *J. tanjorensis*, *L. hastata* and Their Combination

Plant / Extract	Initial Powder Weight (g)	Dried Extract Weight (g)	Yield (%)	Appearance
J. tanjorensis (JT)	200.00	18.64	9.32	Dark green, semi-solid paste
L. hastata (LH)	200.00	21.18	10.59	Dark brown, semi-solid paste
Combination (JT+LH, 1:1)	200.00 (100 g each)	19.86	9.93	Dark greenish-brown paste

All extractions performed in triplicate from separate batches of plant material; values represent the mean of triplicate extraction yields.

The combination extract (JT+LH, 1:1) was evaluated for extraction yield only; its qualitative and quantitative phytochemical composition was not determined in the present study and is reported separately in a subsequent companion study. The qualitative and quantitative phytochemical results presented below therefore relate to the individual JT and LH extracts only.

Qualitative Phytochemical Screening

Both *J. tanjorensis* and *L. hastata* tested positive for alkaloids, flavonoids, tannins, saponins, phenols, terpenoids and cardiac glycosides. Anthraquinones were not detected in either extract (Table 2).

Table 2: Qualitative Phytochemical Screening of Ethanolic Leaf Extracts of *J. tanjorensis* and *L. hastata*

S/N	Phytochemical Class	*J. tanjorensis*	*L. hastata*
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannins	+	+
4	Saponins	+	+
5	Phenols	+	+
6	Terpenoids	+	+
7	Cardiac Glycosides	+	+
8	Anthraquinones	−	−

+ = present; − = absent. All tests performed in triplicate; results represent consistent findings across replicates.

Quantitative Phytochemical Analysis

J. tanjorensis contained significantly higher total phenolic content (24.18 ± 0.71 mg GAE/g), total flavonoids (12.76 ± 0.43 mg RE/g), total alkaloids (18.24 ± 0.62 mg/g) and total tannins (8.34 ± 0.28 mg TAE/g) than *L. hastata* ($p < 0.05$). Conversely, *L. hastata* contained markedly higher total saponins (9.12 ± 0.31 mg/g), 2.18-fold greater than the value recorded for *J. tanjorensis* (4.18 ± 0.16 mg/g; $p < 0.05$) (Table 3). These quantitative differences were consistent with the relative intensity of colour and precipitate reactions observed during qualitative screening.

Table 3: Quantitative Phytochemical Analysis of Ethanolic Leaf Extracts of *J. tanjorensis* and *L. hastata*

S/N	Parameter	*J. tanjorensis*	*L. hastata*
1	Total Phenolic Content (mg GAE/g)	24.18 ± 0.71[a]	19.46 ± 0.62
2	Total Flavonoid Content (mg RE/g)	12.76 ± 0.43[a]	10.52 ± 0.38
3	Total Alkaloid Content (mg/g)	18.24 ± 0.62[a]	14.36 ± 0.54
4	Total Tannin Content (mg TAE/g)	8.34 ± 0.28[a]	6.72 ± 0.24
5	Total Saponin Content (mg/g)	4.18 ± 0.16	9.12 ± 0.31[a]

Mean ± SEM, n = 3, mg/g Dry Extract. [a] *Significantly higher than the corresponding value for the other extract (p < 0.05, Student's independent t-test). All determinations performed in triplicate.*

Discussion

The extraction yields obtained in this study (9.32% for *JT* and 10.59% for *LH*) fall within the 7–14% range commonly reported for maceration of tropical medicinal plant leaves in 70–80% ethanol under ambient conditions, and are comparable to the 9.8% and 11.1% yields previously reported for the same species by Nafiu *et al*. (2017) and Umar *et al*. (2021), respectively. The marginally higher yield obtained from *L. hastata* is consistent with its higher saponin content, a compound class that is highly soluble in aqueous-ethanol mixtures and contributes substantially to total soluble solids (Guo *et al*., 2020).

Qualitative screening revealed that both *J. tanjorensis* and *L. hastata* share an identical phytochemical complement of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids and cardiac glycosides, consistent with earlier independent reports on each species (Nwaoguikpe *et al*., 2011; Okonkwo *et al*., 2014; Ibrahim *et al*., 2019). The detection of flavonoids by both the Shinoda and magnesium-turnings tests, and the consistent detection of saponins by the persistent froth test, and the presence of alkaloids and cardiac glycosides in both extracts are consistent with earlier phytochemical reports on the two species.

Quantitative analysis distinguished the two plants more sharply than qualitative screening alone. *J. tanjorensis* possessed significantly higher total phenolic, flavonoid, alkaloid and tannin contents, findings consistent with and extending the data of Nwaoguikpe *et al*. (2011) and Okon *et al*. (2019), who reported high total phenolic and flavonoid concentrations in ethanolic leaf extracts of this species. The elevated phenolic content measured by the Folin–Ciocalteu method indicates substantial antioxidant capacity, a property of direct relevance to haemolytic anaemia, in which oxidative stress accompanies and accelerates red blood cell destruction through lipid peroxidation of erythrocyte membranes (Smith, 1990). By contrast, *L. hastata* recorded markedly higher total saponin content (9.12 ± 0.31 mg/g), 2.18-fold greater than *J. tanjorensis*, consistent with the findings of Nafiu *et al*. (2017) and Sahabi *et al*. (2018). Saponins of this magnitude are pharmacologically relevant because they increase intestinal absorption of non-haeme iron through the formation of soluble iron-saponin complexes that resist precipitation at intestinal pH, and through modulation of gut mucosal permeability that facilitates paracellular iron transport (Guo *et al*., 2020; Verma & Mishra, 2010).

Taken together, these results indicate that *J. tanjorensis* and *L. hastata*, although qualitatively similar, are quantitatively complementary: *J. tanjorensis* is comparatively richer in antioxidant phenolic and flavonoid constituents associated with erythropoietin-mediated stimulation of red blood cell production, whereas *L. hastata* is comparatively richer in saponins associated with enhanced dietary iron absorption. This complementarity provides a clear phytochemical rationale for evaluating the two plants in combination, on the premise that a 1:1 combination extract may simultaneously deliver both the antioxidant/erythropoietic advantage of *J. tanjorensis* and the iron-absorption advantage of *L. hastata*. Detailed structural characterisation of the individual compounds underlying these phytochemical classes, together with their haematinic and toxicological evaluation in vivo, is addressed in subsequent companion studies.

CONCLUSION

Ethanolic leaf extracts of *J. tanjorensis* and *L. hastata* share a broadly similar qualitative phytochemical profile, both containing alkaloids, flavonoids, tannins, saponins, phenols, terpenoids and cardiac glycosides, and both lacking anthraquinones. Quantitatively, however, the two plants diverge in a pharmacologically meaningful way: *J. tanjorensis* is significantly richer in total phenolics, flavonoids, alkaloids and tannins, while *L. hastata* is significantly richer in total saponins. These complementary phytochemical profiles provide scientific justification for the traditional practice of combining plant-based remedies and support further pharmacological evaluation of the *J. tanjorensis–L. hastata* combination as a candidate plant-based haematinic agent.

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