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Full Length Research Paper

Phytochemical Screening Chromatographic Profiling and Pharmacognostic Analysis on Leaves of *Lantana camara Linn.*

***Egharevba Henry Omoregie[1], Ibrahim Jemilat Aliyu[1], Ebere Uchechukwu Doris[2], Ugbabe Grace[1] and Wudil Muazzam Ibrahim[1]**

[1]Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

[2]Department of Chemistry, Faculty of Science, Imo State University Owerri, Imo State, Nigeria.

****Corresponding author: Egharevba Henry Omoregie***

Abstract

The ethnomedicinally highly dependable and toxic herb Lantana camara was studied for its phytochemical pharmacologic profile to establish some of the profile of the variety found in North-central Sahel Savana region of Nigeria. Extraction was done on the leaves of Lantana camara using ethylacetate and methanol as solvents respectively. Extraction of the volatile oil from the leaves was also carried out using the Clevenger's hydro distillation apparatus. Phytochemical screening analysis test was carried out on the crude extract of L. camara, using standard protocol and the MPR & TM manual, indicating the presence of carbohydrates, tannins, steroids, reducing sugar, flavonoids, terpene and saponin. TLC was conducted on the volatile oil, ethyl acetate and methanolic extracts of L. camara using hexane: ethyl acetate (5:1), hexane: ethyl acetate (1:4), ethyl acetate: methanol (9:1). The volatile oil and ethyl acetate extracts showed 5 spots while 3 spots were seen on the chromatogram of the methanolic extract. The Pharmacognostic analysis carried out on the plant sample indicated 18% moisture content, 15.2% water extractive value and 9% alcoholic extractive value. Its ash content was 11.25%. The leave microscopy indicated the presence of stomata, irregular epidermal cells, glandular trichomes, and trichome types on the abaxial layer and abundant stomata, glandular trichome, striated cell, wavy epidermal cells and abundant oil globules on the adaxial layer.

Keywords: *Lantana camara, Phytochemistry, chromatography, leaf microscopy, pharmacognosy*

Introduction

The use of in ethnomedicine has long been established since ancient times. Also, the fact that most modern medicines have their origin from plant cannot be overemphasized. Thus the use of plants in traditional medicinal system to cure a variety of diseases such as fever, cough, cold, pain, headache, etc., and as potential source of orthodox medicine continues to generate interesting research in the field. Within the last century, plants has been reported scientifically to possess various medicinal properties such as antibacterial, antifungal, anticancer, anti-inflammatory, anthelmintic, antioxidant, larvicidal activity, etc. These reports continue to support the possible use of plants for the development of new therapeutic compounds in in the drug discovery process (Kalita, *et al.*, 2011).

Lantana camara Linn (Figure 1) is a species of flowering plant within the verbena family (verbenaceae), that is widely used in ethnomedicine. The plant is native to American tropics and has spread from its native central and South America to around 60 subtropical and tropical countries worldwide where it has become an invasive species. *L. camara* Linn will often out-compete other more desirable species, leading to a reduction in biodiversity. The plant is commonly called Wild sage, common lantana, red sage, yellow sage, shrub verbena (Kalita *et al.*, 2012). Locally, the plant is known in Igbo as *"Anya nnunu",* in Yoruba as *"Ewonadele"* and in Hausa as "Kimbamahalba" (Gabi, *et al*., 2011; Ezeike, 2014). Its toxicity to livestock such as sheep, cattle, horses, dogs and goats, and ability to form thickets and slow down forest regeneration could causes serious agricultural productivity challenges. *Lantana camara* Linn is one among the most toxic plants known so far, possibly within top ten. The active substances causing toxicity in grazing animals is believed to be a pentacyclic triterpenoids which results in liver damage and photosensitivity. It excretes toxic chemicals called allelopathy which reduces the growth of surrounding plants by inhibiting germination and root elongation. The toxicity of the plant in humans has not been well established (Kalita *et al.*, 2012; Kumarasamyraja *et al*., 2012).

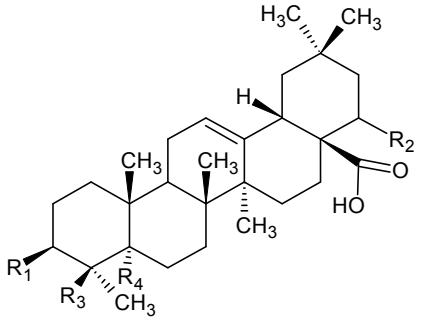
The main uses of *L. camara* have historically been medicinal and ornamental. Studies conducted in India have found that leaves of *Lantana camara* Linn exhibit antimicrobial, fungicidal and insecticidal activities. It has also been used in traditional herbal medicines for treatment of variety rheumatisms, ulcers, catarrhal infection, tetanus, malaria, tumour, cancer, chicken pox, asthma, ulcer, swelling, eczema, measles, fevers, cold, bilious fever, ataxy of abdominal viscera, sores, and high blood pressure (Kalita *et al.*, 2012). The plant had been scientifically proven to possess antibacterial, antifungi, antifilerial, antihypertensive, hemolitic, antiulcerogenic, antihelmentic, anti-inflammatory, antiproliferative, antifertility, and antioxidant activities (Barreto *et al.,* 2010; Kalita *et al.*, 2012;

Kumarasamyraja *et al*., 2012). The plant had also been reported to possess anti-motility activity, antipyretic activity, anti-cancer activity, anti-mutagenic activity, antihyperglycemic activity, hepatic protective activity, larvicidal activity and termiticidal activity (Kalita *et al*., 2011).

Fig 1: Picture of *L. camara*

Phytochemical composition of *Lantana* camara Linn has been reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, anthraquinone, saponins, steroids, triterpenes, sesquiterpenoides and tannin as major phytochemical groups (Kalita *et al*., 2012; Sousa and Costa, 2012). The main constituents of the essential oil of the leave which had been reported to possess adulticidal activity against *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles culicifacies*, *An*. *Fluvialitis* and *An*. *stephensi* mosquitoes, include bicyclogermacrene (19.42%), isocaryphyllene (16.70%), valecene (12.94%), and germacrene D (12.34%) were the main constituents (Sousa *et al*., 2010; Kalita *et al*., 2012; Jawonisi and Adoga, 2013; Reddy, 2013).

Oleanonic acid (**1**) (Figure 2) had been isolated from *Lantana* camara Linn and found to exhibits promising cytotoxicity against A375 cells (malignant skin melanoma) (Ghosh, *et al*; 2010).



1R_1=O; R_2=R_4=H; R_3=CH_3
2R_1=β -OH; R_2=β -OCOCH=$C(CH_3)_2$
3R_1=O; R_2=β-OCOC(CH_3)=$CHCH_3$; R_3=CH_3; R_4=H
4R_1=O; R_2=β-OCOCH=$C(CH_3)_2$; R_3=CH_3; R_4=H
5R_1=O; R_2=β-OCOCH(CH_3)CH_2CH_3; R_3=CH_3; R_4=H
6R_1=O; R_2=β-OCOCH(CH_3)$_2$; R_3=CH_3; R_4=H
7R_1=O; R_2=β-OCOC(CH_3)=$CHCH_3$; R_3=CH_2OH; R_4=H

Fig 2: Structures of some compounds reported from L. camara

22-acetoxylantic acid (Figure 3) and 22-dimethylacryloyloxy lantanolic acid (**2**) (Figure 2) from *Lantana camara* Linn has been reported to show antimutagenic activity. The antimutagenicity test was performed by micronucleus test in Swiss mice. Both compounds exhibited high antimutagenic activity in Mitomycin C induced mutagenesis in mice (Kalita, *et al*; 2012). The plant had been reported to contain lantadene A (**3**), B (**4**), C (**5**) and D (**6**) (major toxins involved in poisoning),as well as other structurally and toxicologically related pentacyclic triterpene acids, including reduced Lantadene A, dihydrolantadene A, and icterogenin (**7**) (Figure 2). Foliage and ripe berries has also been reported to contain the toxic substances with the toxin being in higher concentration in green berries. These compounds are toxic to domestic animals when taken in significant amount (Ross, 1999). Sheep, cattle and goats had been reported to be susceptible to lantadenes A, B, D and icterogenic acid toxicity, whereas horses, rats, neonatal calves and lambs were not susceptible to lantadene A. The prominent clinical sign of poisoning include photosensitization and jaundice as well as loss of appetite. The animals die within 2 days of severe poisoning but usually death occurs 1-3 weeks after poisoning (Kalita*, et al;* 2012).

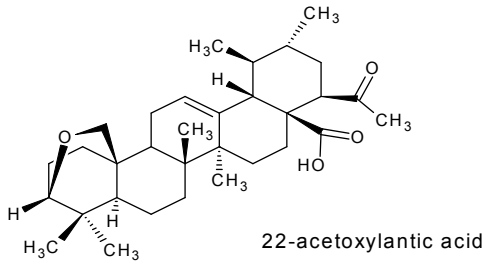


Fig 3: Structure of 22-acetoxylantic acid

Materials and Methods

Materials

All reagent used were of analytical grade and according to equipment specification. Other materials include mortar and pestle, razor blade, transparent nail polish, microscope, glass slides, cover slips, soft Carmel hair brush, Clevenger's hydro-distillation apparatus, and TLC plates.

Plant material collection and preparation

Fresh leaves of *Lantana camara* was collected from Chazza Suleja, Niger state on 2nd of July, 2015 at about 6:30am. The plant identified at the NIPRD Herbarium with the voucher specimen number 5619 of 2005, at the Department of Medicinal Plant Research & Traditional Medicine, NIPRD Idu- industrial area, Abuja, Nigeria.

Fresh leaves were separated from the stock and air-dried for seven days. Dried leaves were uniformly grinded using local mortar and pestle to make fine powder. The pulverized material was kept in airtight container until required.

Extraction

The leaf powder (20 g) each was macerated separately using ethyl acetate and methanol as solvents respectively for 24 hours. The extracts were filtered and the filtrates were concentrated on a water bath and left to dry. The dried extract was collect in an air tight container and stored at up for further use.

Essential oil extraction

The powdery form of the leaves of *L. camara* was subjected to hydro distillation using the Clevenger-type apparatus. The extracted essential oil was dried over anhydrous sodium sulphate and collected in sterile airtight glass sample bottle for GC-MS analysis. The percentage yield was calculated based on the dry weight of the leaf (450g).

Phytochemical screening

Phytochemical screening on the leaves of *L. camara* was carried out by using the standard protocols. Plants were screened for carbohydrates, reducing sugar, saponins, terpenes, steroids, alkaloids, flavonoids, glycosides and tannins (Kalita, *et al.,* 2012; Sofowora, 2008; Evans, 2002).

Thin layer chromatography

TLC profiling was carried out on the volatile oil, ethyl acetate and methanolic extracts of the leaves of *L. camara.* The solvent systems used were hexane: ethyl acetate (ratio 5 : 1) for the volatile oil, hexane : ethyl acetate (ratio 1:4) for ethyl acetate extract and ethyl acetate : methanol (ratio 9:1).

Pharmacognostic analysis

Pharmacognostic parameters such as moisture content, extractive values of water and alcohol and ash content were determined according to the British Pharmacopoeia (1980).

Microscopic examination of Lantana camara Linn (procedure)

The microscopic leave examination was conducted according to the methods of African Pharmacopoeia (1986) as described by Egharevba *et al.* (2015). Briefly, fresh leaves of *Lantana camara* were cut into considerable sizes and placed in a petri dish which was soaked in concentrated nitric acid for 4hours in order to macerate the mesophyll. When bubbles were observed, they were removed and placed a different petri dish containing distilled water. The Abaxial surface (upper layer) was then separated from the Adaxial surface (lower layer) using a forceps.

The Abaxial epidermis was teased off with a soft camel hairbrush in order to remove the tissue debris, and was placed in a cleaned petri dish containing water. On the other hand, the mesophyll on the Adaxial surface was teased off, picked and placed in another petri dish containing distilled water. The camel hair brush was used to pick the Abaxial and Adaxial epidermis which was later placed on separate clean glass slide, labelled and then dehydrated with tissue paper. Specimen were stained with cephranone A stain for 2minutes, excess stain was washed off with distilled water, drained and placed back on the slides. A drop of glycerol was added on the slide for moulting and covered with a coverslip and then observed under the binocular microscope. All measurement was made using a calibrated eyepiece micrometer (x10 and x40) objective.

Results and Discussion

The results of phytochemical screening of the crude extract of *Lantana camera* leaves showed the presence of carbohydrates, reducing sugar, sterols, saponins, flavonoids, terpenes, and tannins (Table 1).Alkaloid and glycosides were not detected as reported by other workers in literature (Kalita *et al.*, 2012). This may an indication of specie variation and call for further chemotaxonomic scrutiny to determine fundamental change or modification.

Table 1: Phytochemical analysis of *Lantana camara* leaves

Phytochemicals	Results
Phenolic compounds	+
Flavonoids	+
Alkaloids	-
Tannins	+
Saponins	+
Reducing sugar	+
Carbohydrates	+
Steroids	+
Terpenes	+
Glycosides	-

Key: + means present; - means not detected

The result of TLC conducted on the essential oil of leaves of *Lantana camara*Linn, the ethyl acetate extract and the methanolic extracts gotten from direct solvent maceration of the plant on a normal phase (K_5) TLC plate as as depicted in Figures 4 and Table 2. There was no spot observed on the chromatogram of the volatile oil when viewed under daylight. However, 5 clear spots were seen after spraying with 10% H_2SO_4 in ethanol and drying at a temperature of 105°C. For the ethyl acetate extract, 3 spots were seen in daylight and two more were seen after charring with the spray reagent. Two spots were observed on the chromatogram of the methanolic extract and one after charring. Thus, 5 spots were observable on the volatile oil and ethyl acetate extracts and 3 were seen on the methanolic extract. The retention factors were calculated as presented in Table 2. The profile presented could serve as a means of sample standardization and authentication.

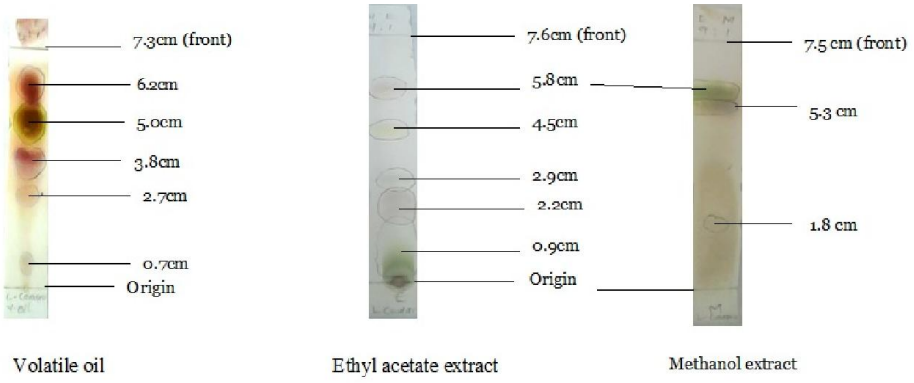


Fig 4: TLC Chromatogram of *L. camara* leaf volatile oil and extracts

Table 2: Retention Factors of the TLC spots on the chromatogram

No. of spot	Retention factor (R_f)		
	Volatile oil	Ethyl acetate Extract	Methanol Extract
1	0.096	0.118	0.240
2	0.370	0.289	0.707
3	0.521	0.382	0.773
4	0.685	0.592	
5	0.850	0.763	

The result of pharmacognostic analysis revealed high percentage moisture content of 18%, which is above the normal range of 8-14% prescribed for vegetable drug (African Pharmacopeia, 1986). This implies that the plant may retain sufficient moisture to support microbial growth which would make the plant susceptible to microbial degradation under storage. For effective storage, further drying may be needed by means of applied heat or drying over a longer period to reduce the moisture content, and also storing under specified humidity or packaging /treatment with a suitable drying agent. The water extractive value 15.9% was higher than the alcoholic extractive value of 9%, indicating that more the plant constituents were more soluble in water than in an alcoholic solvent. The total ash value was 11.25% and is indicative of a good source of mineral elements. However, the type of mineral elements contained should be verified to avoid intake of harmful metals.

Table 3: Results for Pharmacognostic analysis conducted on the leaves of *Lantana camara*

Parameter	Moisture Content.	Water EV.	Alcohol EV.	Total Ash.
Values	18%	15.9%	9%	11.25%

The leaf microscopy was as depicted in Figures 5a & 5b. The upper layer (abaxial layer) showed trichomes surrounded with four and seven epidermal cells (Figure 5a, images A, B, and C). Slides D, E and F show abundant trichomes and their bases present in the upper layer. The type of trichome presented in the cell was glandular trichome, the cells surrounding the base of the trichome are irregular in shape with undulate cell wall. There were also stomata in the upper layer (Figure 5a, image G).

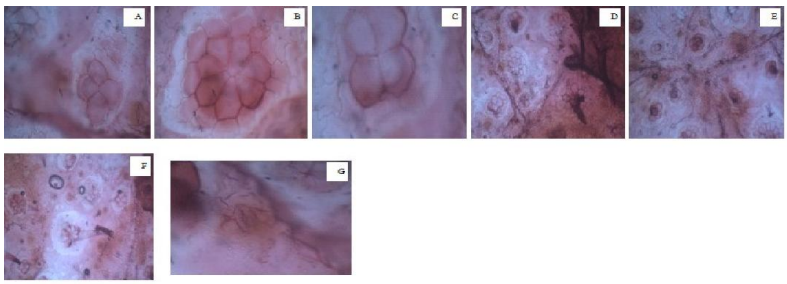


Fig 5a: Abaxial layer (Upper layer)

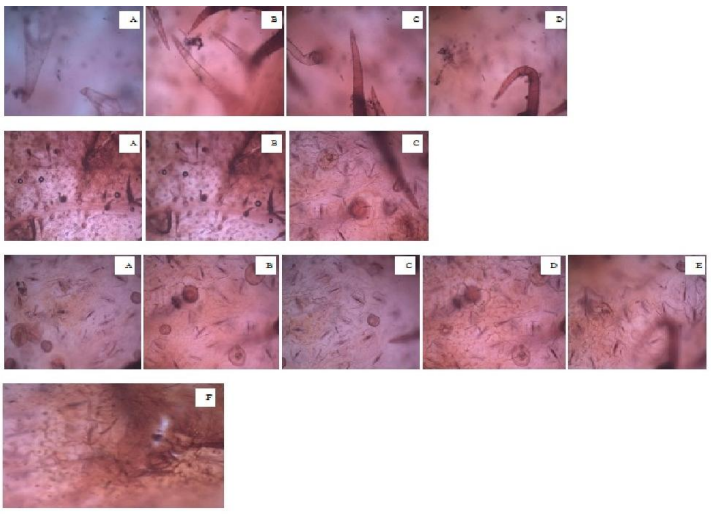


Fig 5b: Adaxial layer showing abundant stomata, trichomes and trichomes with single bases (slides A-E), as well as oil gland (slide F).

The adaxial layer showed presence of glandular and wavy trichomes. The two types of trichomes are depicted on the B slide (Figure 5b). The presence of abundant stomata and trichome with single trichome bases could be seen on the enlarge slide. Slide F show presence of oil globules on the adaxial layer.

Table 4: Microscopic character of leaf of *Lantana camara* Linn.

Parameters	Abaxial Layer	Adaxial Layer
Cell type	Epidermal	Epidermal
Cell shape	Irregular	Wavy
Trichome number	Two	Three
Trichome type	Glandular	Wavy
Stomata type	Vigna- paracytic	Vigna- paracytic
Anticlinal wall pattern	Straighted	Straighted & undulated

Table 4 showed the difference in foliar epidermal characters observed on the both surfaces include cell shape, trichome, trichome type, stomata, stomata type, anticlinal wall pattern and periclinal wall pattern, in which the cell shape in the abaxial surface observed were irregular epidermal cell shape with undulating cell wall (Figure 5a), while that of the adaxial surface was wavy and polygonal epidermal cell shape. The trichome present in the abaxial surface was glandular while the adaxial surface was wavy. Straighted anticlinal wall pattern was observed for both surfaces. Oil globules were observed on the adaxial surface. This is the first time the internal structure of the Nigerian species would be described and this could help in taxonomic identification and monograph development.

Conclusion

The results of this study showed that the plant is rich in essential oils and secondary metabolites like phenols, flavonoids, tannins, saponins and steroids which had been reported to exhibit various pharmacological activities and are believed to be responsible for the plant's pharmacological activities as observed in ethnomedicine and some scientific work. The TLC, pharmacognostic parameters and miscroscopic characteristics could provide basic information for identification and authentication of samples and development of monograph for the Nigerian species.

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