



PHYTOCHEMICAL SCREENING AND THE ETHNOBOTANICAL IMPORTANCE OF THE LEAVES OF WILD SAGE (*Lantana camara* L.)

Aliyu R.H., Dasikwo S.Y., Alanana J.A and Ombugadu N.A

*Department of Plant Science and Biotechnology, Nasarawa State University,
P.M.B 1022 Keffi, Nasarawa State, Nigeria.*

Corresponding E-mail: joshua_abu@yahoo.com

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ABSTRACT

Leaf extract of *Lantana camara* was screened for active bioactive phytochemical compounds. The leaves were extracted using different solvent (distilled water, ethylacetate and methanol). Phytochemical analysis of the extracts were performed using thin layer chromatography (TLC). The extracts were loaded on TLC plates and developed in two solvent systems that is Hexane: Ethylacetate and Ethylacetate: Methanol at ratios 1:4 and 1:9 respectively. The phytochemical screening result indicated presence of alkaloids, tannins, saponins, carbohydrates and sterols and the absence of flavonoids and glycosides in the distilled water extract; presence of flavonoids, tannins, saponins, carbohydrates, terpenoids and steroids and absence of glycosides and alkaloids in Ethylacetate extract; presence of flavonoids, alkaloid, tannins, carbohydrates, steroids and saponins and absence of terpenoids and glycosides. The R_f values calculated from TLC indicated that the leaf have different active compounds, which show 5 spots for ethylacetate and 3 for methanol extracts. However, methanol was the best extract, followed by ethylacetate. The leaves are used to treat, ulcers, malaria, cancer, chicken pox, asthma, eczema and measles. From the outcome of this study, it can be concluded that the presence of the secondary metabolites observed, and the ethnobotanical importance of the leaves of *Lantana camara* will contribute immensely toward the developments of drugs

Keywords: *Lantana camara, Methanol, ethylacetate, distilled water*

INTRODUCTION

Plants and plant based medicaments are the basis of many of the modern pharmaceuticals we use today for various ailments (Himal *et al.*, 2008). Plants derived substances have recently become of great interest owing to their versatile applications. Therefore the medicinal value of plants lies in some chemical constituents which produce a definite physiologic action in the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Thamotharan *et al.*, 2010).

Lantana camara L. is one of the well-known medicinal plants in traditional medicine which belongs to the family Verbenaceae, also known as Lantana, Wild Sage, Surinam Tea Plant, Spanish flag and West Indian lantana (Sanjeeb *et al.*, 2012). The ability of *L. camara* to rapidly colonize areas of land which has been disturbed has allowed it to proliferate in countries where activities like logging, clearance for agriculture and forest fitters are common, it also grows at forest edge (Day, 2003).

Phytochemicals are chemical compounds that occur naturally in plants and are responsible for colour and other qualities such as taste, odour and texture etc. Phytochemical chemical composition of *Lantana camara* has been reported include essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, phenyl ethanoid, oligosaccharides, anthraquinone, saponins, steroids, triterpens, sesquiterpenoids and tannin as major phytochemical groups (Sanjeeb *et al.*, 2012). *Lantana* species are widely spread weeds in warm climates but poisonous to livestock. These plants are continuously being suppressed and destroyed because it is toxic in high percentage (invasive) and subsequent destruction will lead to the plant going into extinction. This research was therefore carried out to identify the secondary metabolites (Phytochemicals) present in *Lantana* leaves and to document the Ethnobotanical significance of *L. camara* for conservation and future use.

MATERIALS AND METHODS

This project was carried out at National Institute for Pharmaceutical Research and Development (NIPRD), Idu Industrial Area, Abuja.

Fresh leaves of *Lantana camara* were collected from Chazza Suleja, Niger State, identified in the NIPRD Herbarium and authenticated with voucher specimen number 5619.

The Ethnobotanical data collection were semi structured interviews as described by Cotton C.M (1996). The respondents background, health problems treated, diagnosis and treatment methods using the leaves of *Lantana camara*. The frequencies of the citation for each respondent was used as a basis to identify its ethnobotanical importance in the study area.

Plant preparation

The leaves were fully air-dried at room temperature for two weeks, after which it was finely grinded using mortar and pestle in order to increase the surface area and was stored in an air tight container for further study.

Phytochemical procedures

About 100g of the plant materials were macerated successively for 24 hours using ethyl acetate and absolute methanol, after which each extract was filtered and the resulting filtrate were concentrated using rotary evaporator and subsequent drying of each concentrated extract was done using water bath. The dry extracts were taken and used for further phytochemical analysis. The following tests were carried out using standard methods as described by Evans and Trease (2002); Sofowora (2008); Agrawal *et al.*, 2009 and Sanjeeb *et al.*, 2012.

Test for carbohydrate

3g of the powdered leaf extract was boiled in 50ml of distilled water on a water bath for three minutes. The mixture was filtered while hot and the resulting filtrate cooled, 3-4 drops of molisch reagent was added to the water extract obtained, and then a small quantity of concentrated sulphuric acid was added and allowed to form a lower layer. A purple ring at the interface of the liquid indicates the presence of carbohydrates the mixture was then shook, allowed to stand for 2 minutes and then diluted with 5ml of water. A purple precipitate also indicates the presence of carbohydrate.

Test for tannins

Ferric chloride test: 3g of the stems extracts was boiled in 50ml of distilled water for 3 minutes on a water bath. The mixture was filtered and a portion of the water extract was diluted with distilled and a few drops of 10% ferric chloride solution were added. A blue or green color indicates the presence of tannins.

Test for alkaloids

An extract was prepared by macerating 3g of the powdered sample in 50ml methanol. The extract was evaporated to dryness. 0.5g of the residue was mixed with 10ml of 1% aqueous hydrochloric acid on water bath. 1ml each of the filtrate was treated with a few drops of the following reagents:

- Meyers reagent - (potassium mercuric iodine solution)
- Dragendoffs reagent- (potassium bismuth iodine solution)
- Wagners reagent - (solution of iodine in potassium iodide)
- Hasgers reagent - (a solution of picric acid)

Turbidity or precipitation is indicative of the presence of alkaloids in the extract.

Test for saponins

Froth test: To a small quantity of the stems extract 95% ethanol was added and boiled, the mixture was filtered and 2.5ml of the filtrate was added to 10ml of distilled water in test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Persistent honeycomb froth is indicative of the presence of saponin.

Test for terpenes and sterol

5g of the powdered leaf was extracted by the maceration with 50ml of ethanol (95%), filtered and the filtrate was evaporated to dryness. The residue was dissolved in 10ml of anhydrous chloroform and then filtered. The filtrate was divided in to two equal portions and the following tests were carried out.

Salkowskis test: The second portion of the solution was mixed with 2ml of concentrated sulphuric acid carefully so that the acid forms a lower layer. A reddish-brown color at the interface indicates the presence of a steroid.

Test for flavoids

5ml of 10% sodium hydroxide was added to an equal volume of the de-tanned water extract. A yellow solution indicates the presence of flavonoids.

Test for carbohydrates**Molisch test**

3g of the powdered sample were boiled separately in 50ml of distilled water on a hot plate for three minutes, the mixture was filtered while the hot plate and the resulting filtrate cooled. A few drops of molisch reagent was added to 2ml of the water extract, and then a small quantity of concentrated sulphuric acid is added and allowed to form a lower layer. A purple ring at the interphase indicated the presence of carbohydrates.

Benedict's solution test

10g of the plant extract was dissolved in 5ml of water and filtered. To 0.5ml of the filtrate, 0.5ml of Benedict's reagent was added and heated on a boiling water bath for 3 minutes. A characteristic color indicates the presence of carbohydrates.

Thin Layer chromatography

Chromatographic plates were used for thin layer chromatography. The starting line was marked two centimeter above from the base. To obtain the desired concentration of the extract on the paper, the spots were applied repeatedly at the same point. The spots were kept at a distance of two centimeter apart for the acid identification.

Mobile phase: Solvent system employed for TLC were Hexane: Ethylacetate at ratio 1:4 and Ethylacetate: Methanol at ratio 9:1.

Retardation factor can be calculated using:

$$\text{Retardation factor (Rf)} = \frac{\text{distance moved by solute}}{\text{distance moved by solvent}}$$

Plate type: Normal face k5 (what man).

Spaying agent: 10% H₂SO₄ in ethanol (C₂H₅OH)

RESULTS AND DISCUSSION

The results presented in Table 1 show the presence of secondary metabolites like alkaloids, tannins, saponins, carbohydrates and sterols and the absence of flavonoids and terpenes in the distilled water extract. Presence of flavonoids, tannins, saponins, carbohydrates and sterols and the absence of alkaloids in the ethylacetate leaf extract while the methanol leaf extract shows presence for flavonoids, alkaloids, tannins, saponins, carbohydrates and sterols and the absence of terpenes.

The thin layer chromatography technique of the ethyl acetate extracts used to separate non-volatile mixtures, indicates five spots (Rf values) and three spots (Rf values) for absolute methanol, as presented in table 2 with solvent fronts of 7.6cm and 7.5cm respectively. This was performed on a sheet glass coated with a thin layer absorbent material usually silica gel which it was later dipped in a mobile phase of hexane/ethylacetate at (1:4 and of) ethylacetate/methanol at ratio (1:4).

Table 1: Phytochemical screening results of *Lantana camara L.*

Parameters	Ethylacetate	Methanol	Distilled water
Flavonoids	+	+	-
Tannins	+	+	+
Alkaloids	-	+	+
Carbohydrates	+	+	+
Terpenoids	+	-	-
Steroids	+	+	+
Glycosides	-	-	-
Saponins	+	+	+

Key: Present +

Absent -

Table 2: Retardation factor (Rf) of *Lantana camara L.* leaf extracts in solvent system Hexane:Ethylacetate (1:4)

Number of spots	Distance moved by solute	Distance moved by solvent	Rf value
1	0.9	7.6	0.118
2	2.2	7.6	0.289
3	2.9	7.6	0.382
4	4.5	7.6	0.592
5	5.8	7.6	0.763

Table 3: Retardation factor (Rf) of *Lantana camara* L. leaf extracts in solvent system Ethylacetate: Methanol (9:1)

Number of spots	Distance moved by solute	Distance moved by solvent	Rf value
1	1.8	7.5	0.240
2	5.3	7.5	0.707
3	5.8	7.5	0.773

As shown in Table 1, the extracts of *L. camara* contain many phytochemicals as revealed by phytochemical analysis of plant extract and TLC analysis. The results presented in Table 1 shows the presence of secondary metabolites like alkaloids, tannins, saponins, carbohydrates and sterols and the absence of flavonoids and terpenes in the distilled water extract; presence of flavonoids, tannins, saponins, carbohydrates and sterols and the absence of alkaloids in the ethylacetate leaf extract while methanol leaf extracts showed presence of flavonoids, alkaloids, tannins, saponins, carbohydrates and sterols and the absence of terpenes. This is supported by Degenhardt *et al.*, 2003 that reported the presence of some phytochemicals in some plants.

These secondary metabolites are known to be biologically active and play significant roles in bioactivity of medicinal plants, because the medicinal values of medicinal plant lies in these phytochemical compounds which produce a definite and specific action on the human body. The presence of saponins, phenols and alkaloids could confer antibiotic property on the plant. The natural tendency of saponins toward off microbes makes them good candidates for treatment of fungal and yeast infections (Eka, 1998). These compounds serve as natural antibiotics, which help the body to fight infections and microbial invasion (Sodipo *et al.*, 2000).

CONCLUSION

This study has revealed the presence of many secondary metabolites in the leaves of *Lantana camara*. It has further confirmed that *Lantana camara* is one of the well-known medicinal plant in traditional medicines. It is also popular in folkloric treatment as are to various ailments including ulcers, malaria, cancer, chicken pox, asthma, eczema and measles. So, new strategies should be developed to optimized the usefulness of this plant such as explaining its pharmacologic potentials.

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Table 4: An Ethnobotanical study of *Lantana camara* leaves showing frequencies of diseases.

Disease type	Frequencies
Ulcer	25
Chicken pox	24
Measles	23
Malaria	22
Asthma	21
Eczema	21
Cancer	14

The result presented in table 4 indicates Ulcer as the most common diseases with the highest frequency followed chicken pox, measles, malaria asthma, Eczema and cancer in which patient commonly visit the Traditional Medical Practitioners (TMPs) for treatment. The interviews with the TMPs found different diagnosis and treatment methods depending on the types of ailments. The practitioners commonly diagnose each health problems by an interview and visual inspection of the patients. Patients are interview for symptoms observed and the duration of the health problem, changes in eye and skin colour, body temperature and status of sores are all visually inspected by the practitioners and the remedy is prescribed.

Internal ailments were commonly treated by making the patients drink herbal preparation, skin infections such as eczema were treated by rubbing and painting herbal preparations on an infected skin. Sores by chewing and spitting remedial plant leaves on the sore, similar result was reported by Sunita verma (2008). Though special care was taken, some herbal preparation had side effects and resulted in diarrhea and vomiting when such happened, antidotes like coffee, milk, honey were used to ordered by most of the practitioners to reverse the condition.

The medicinal plant preparation given does not have standardized doses. In most cases dosages were determined according to age, sex and physical appearance of the patients.

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