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PHYSICOCHEMICAL AND PHYTOCHEMICAL PROFILES OF AERIAL PARTS OF *LIPPIA NODIFLORA* L.

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ABSTRACT: *Lippia nodiflora* L. is an important medicinal plant of Verbenaceae family and are used traditionally for several ailments. The present study deals with the detailed physicochemical, fluorescence and phytochemical evaluation of aerial parts of *Lippia nodiflora* L., in order to explore the authentic plant material suitably for its traditional claims. The physicochemical evaluations and fluorescence analysis were determined as per standard protocols. Phytochemical constituents of dried plant material were carried out by both qualitative and quantitative methods. Gas Chromatography–Mass Spectrometry (GC-MS) was used to analyze the phytochemical constituents of the methanol extract. Analysis of physicochemical parameters of the powdered aerial parts showed that total ash was approximately two times more than water insoluble ash and alcohol soluble extractive value was higher than water soluble extractive value. Preliminary phytochemical screening showed the positive result for the presence of flavonoids, steroids, glycosides, alkaloids, terpenoids, tannins and phenolics. Quantitative analysis showed that the methanolic extract consist of high phenolic compounds (98.31 ± 0.004 mg GAE/g) followed by total flavonoids (60.88 ±0.001mg QE/g) and flavonols (27.46 ± 0.002 mg QE/g). Total tannin and saponin content was found to be 5.97 ± 0.021mg TAE/g and 3.52 ± 0.017mg DE/g respectively. The major chemical components of methanolic extract of *L. nodiflora* determined by GC-MS were 2, 7-dioxatricyclo [4.3.1.0 (3, 8)] decan-4-one (35.75%), stigmasterol (16.86%), benzoic acid, 4-etoxy-, ethyl ester (13.73%), azacyclotridecan-2-one (11.86%) and n-hexadecanoic acid (10.12%). The results of the present study provide preliminary referential information of the plant and also serve as an investigative tool for the appropriate identification. The presence of various chemical constituents suggests that this plant exhibit rich phytopharmaceutical importance.

INTRODUCTION: About 75 to 80% of the world population was comprised of medicinal plants and especially in developing countries, the herbal drugs play a central role in many health care programs. The broad definition of medicinal plants has been incorporated in an ancient Indian literature which portrays that "all plant parts to be potential sources of medicinal substances" [1].

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The lack of citations and inflexible quality control has hindered the acceptance of the alternative medicines in the developed countries by serving a lead obstacle. Hence, documentation is very essential part of research work to be carried out on traditional medicines [2]. It becomes extremely important to make an attempt towards consistency

of the plant material to be used as medicine in this scenario. WHO has also recommended the evaluation of physicochemical and phytochemical parameters of medicinal plants for its efficacy, due to lack of confined synthetic drugs [3]. These evaluation parameters help in identification and authentication of the plant material. The safety and efficacy of herbal medicine depends mainly upon the exact identification and quality assurance of the starting materials.

Lippia nodiflora L. (Verbenaceae), a creeping perennial herb, grows in wet places along irrigation channels, canal edges and river banks. It is commonly called as Poduthalai in Tamil, Bhujokra in Hindi, Ratoliya in Gujarati and Jalpippali in Sanskrit and the plant is distributed in tropical and subtropical region [4, 5]. According to Ayurveda and Unani system of medicine, the plant possess acrid, aphrodisiac, diuretic, stomachic properties and useful in heart diseases, ulcers, bronchitis, fevers and cold, knee joint pain and in lithiasis [5, 6].

From the literature review, it was found that the aerial parts were used as anodyne, antibacterial, emmenogogue, parasiticide, refrigerant, febrifuge and cooling [7]. This plant is very useful in treatment of wounds, asthma, thirst and loss of consciousness. Many chemical constituents like flavone glycosides including lippiflorin A & lippiflorin B, nodiflorin A & B, alkaloids, flavonoids like nepetin, jaceosidin, hispidulin, 6-hydroxyluteolin, flavone monosulfates and flavone disulfates, essential oil, stigmasterol, beta-sitosterol has been previously reported from this plant [8].

L. nodiflora has been reported to possess several pharmacological properties like antimalarial, [9] anti-inflammatory, analgesic and antipyretic, [10] antinociceptive, [11] gastroprotective, [12] antibacterial, [13] antioxidant, free radical scavenging and protective effect, [14] diuretic effect [15] and antidiabetic [16]. Even though *L. nodiflora* was traditionally used in the treatment of various ailments for a long time, the physico-chemical standardization was inadequate.

Therefore, the aim of the present work was to ascertain chemical standards like physicochemical and phytochemical testing of aerial parts and also the chemical constituents of methanolic extract of aerial parts were determined by GC-MS analysis.

MATERIALS AND METHODS:

Plant material: *Lippia nodiflora* L. was freshly collected from Karaikudi, Sivagangai District of Tamil Nadu, India. It was taxonomically identified and authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore and the voucher specimen was deposited with register number BSI/SRC/5/23/2012-13/Tech-19. The voucher specimen of the plant *L. nodiflora* was preserved in the Department of Bioinformatics, Alagappa University, Karaikudi, Sivagangai, Tamil Nadu, India.

Physicochemical analysis: The aerial parts of *L. nodiflora* were cut and dried under shade. These dried aerial parts were mechanically powdered by using blender, passed through 60 mesh sieve and stored in an airtight container for further use. The various physicochemical parameters such as moisture content, total ash, acid insoluble ash, water soluble ash, water soluble extractive value and alcohol soluble extractive value of powdered plant materials were determined by the methods described in Indian Pharmacopoeia [17] and WHO guidelines [18].

Fluorescence analysis: The plant powder was subjected to fluorescent analysis, as it is and also after treating with various chemical reagents like aqueous 1N sodium hydroxide, conc. hydrochloric acid, acetic acid, ferric chloride, ammonia, iodine etc. against visible and UV light (shorter wavelength-254nm and longer wavelength-365nm). A fluorescent analysis of the plant material was carried out according to the methods of Chase and Pratt [19] and Kokoshi *et al* [20].

Phytochemical screening:

Qualitative analysis: For preliminary phyto-chemical screening, the powdered aerial parts (100g) were sequentially extracted with petroleum ether, chloroform, ethyl acetate, methanol and water respectively in Soxhlet apparatus and the solvent extracts were concentrated using a rotary vacuum evaporator and stored at 4ºC until further use. The extracts thus obtained were subjected to different qualitative analysis to ascertain the bioactive components. The presence of flavonoids, alkaloids, saponins, tannins, carbohydrates, protein

and amino acids, terpenoids, steroids, anthra-quinones, glycosides, fixed oils and fats, gum and mucilages were determined according to the method described by Kokate [21] and Harborne [22].

Quantitative analysis:

1. **Determination of Total Phenolic content:** The total phenolic content of the plant extract was estimated using Folin-Ciocalteu phenol reagent (FCR) based assay [23]. For 200µl of the crude extract (1mg/ml), 2.45 ml of water and 150µl of FCR were added. The mixture was incubated at room temperature for 5 min and then 300µl of 1N sodium carbonate solution was added. The mixture was incubated again at room temperature for 30 min and the color developed was recorded at 765nm. Total phenols (mg/g) in the crude extract was expressed as gallic acid equivalent (GAE), using a standard curve prepared from gallic acid (0.1mg/ml) solution.

2. **Determination of Total Flavonoids:** Colorimetric aluminum chloride method was used for the determination of flavonoids [24]. 0.5 ml of plant extract (1mg/ml) was mixed with 1.5ml of methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water separately. The mixture was incubated at room temperature for 30 min and the absorbance was measured at 415nm with UV/Visible spectrophotometer. The calibration curve was prepared by using serial dilutions of quercetin (100µg/ml) and total flavonoids were expressed as mg/g of quercetin equivalent.

3. **Determination of Total Flavonols:** The content of flavonols was determined by the method of Kumaran and Karunakaran [25]. 0.2ml of the aliquot was taken from a stock solution (5mg/ml) of the extract and 2ml of aluminium trichloride (20mg/ml) and 6ml of sodium acetate solution (50mg/ml) were added.

   The reaction mixture was incubated for 2.5hr at 20ºC and the absorbance was measured at 440nm. The calibration curve was prepared by using serial dilutions of quercetin (100µg/ml) and total flavonol content was calculated as mg/g of quercetin equivalent.

4. **Determination of Total Tannins:** The tannins were determined using the Folin-Ciocalteu Phenol reagent as reported by Amorim *et al* [26]. For 0.2ml of the aliquot taken from a stock solution (10mg/ml) of the extract, 7.5ml of distilled water, 0.5ml of Folin-Ciocalteu Phenol reagent, 1ml of 35% sodium carbonate solution were added and diluted to 10ml with distilled water. The mixture was shaken well, incubated at room temperature for 30 min and absorbance was measured at 725nm. Total tannin content (mg/g) was expressed as tannic acid equivalent (TAE), using a standard curve prepared from tannic acid (1mg /ml) solution.

5. **Determination of Total Saponins:** The total saponin content of plant extract was estimated by the method described by Hiai *et al* [27] with some modifications. 250µl of the aliquot was taken from a stock solution (10mg/ml) of the extract and mixed with an equal volume of vanillin reagent (8g/100 ml ethanol) followed by 2.5ml of sulphuric acid (72% wv/v). The mixture was mixed with a vortex and placed in a water bath at 60°C for 10 min. The tubes were cooled in an ice bath for 3 to 4 min and absorbance was read at 544nm against a blank that contained methanol instead of sample extract. Diosgenin was used as a reference standard (0.25mg/ml), and the total saponin content was expressed as diosgenin equivalents (DE, mg/g extract) calculated from a standard curve.

GC-MS analysis: Twenty gram of powdered plant material was soaked in methanol overnight and filtered through a Whatman No. 41 filter paper along with sodium sulphate to remove the sediments and traces of water in the filtrate. The filtrate was then concentrated to 1ml by bubbling nitrogen gas into the solution. The extract contains both polar and non-polar components of the material. GC-MS technique was carried out at Indian Institute of Crop Processing Technology (IICPT) Thanjavur, Tamil Nadu. GC-MS analysis of the extract was performed using a Perkin Elmer GC Claurus 500 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (30 m x 1µ Mdf. composed of 100% Dimethyl poly siloxane).

For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min. and an injection volume of 2µl was employed (split ratio of 10:1). Injector temperature and Ion-source temperature was 250°C; 280°C. The oven temperature was programmed from 110°C (isothermal for2 min.), with an increase of 10°C /min to 200°C, then 5°C/ min to 280°C, ending with a 9 min isothermal at 280°C.

Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Turbo Mass Ver5.2.0 software was adopted to handle mass spectra and chromatograms. The identification of components was carried out by referring to NIST mass spectral database.

RESULTS:

Physicochemical constants: The total ash content of *L. nodiflora* is 14.35% and water soluble ash is 7.5%. The acid insoluble ash is 10.75% and it is greater than that of water soluble ash value. Percent weight loss on drying or moisture content was found to be 18.91 % w/w. The result of percentage extractive yield indicate that crude powder was highly soluble in alcohol (17.36%) than water (8.16%) [**Table 1**]. The behavior of various solvent extracts of *L. nodiflora* viz., colour, consistency and odour and outcomes of extractive value of powdered drug in different solvent obtained by successive extraction were reported in **Table 2**. Successive solvent extraction reveals that methanol extract showed higher extractive values (10.21%) followed by aqueous extract (8.46). The least yield was noticed in ethyl acetate extract (2.84%).

TABLE 1: PHYSICOCHEMICAL PARAMETERS OF AERIAL PARTS OF *L. NODIFLORA* L.

Parameters	Yield (%)
Moisture content	18.91
Total ash	14.35
Acid-insoluble ash	10.75
Water-soluble ash	7.5
Alcohol soluble extractive	17.36
Water soluble extractive	8.16

TABLE 2: BEHAVIORS AND EXTRACTIVE VALUES OF VARIOUS SOLVENT EXTRACTS OF AERIAL PARTS OF *L. NODIFLORA* L.

Extract	Color	Consistency	Odor	Extractive values (% w/w)
Petroleum ether (60-80ºC)	Yellowish green	Oily	Characteristic	4.78
Chloroform	Green	Resinous	Leafy	6.23
Ethyl acetate	Dark green	Semi-solid sticky	Characteristic	2.84
Methanol	Blackish green	Semi-solid	Agreeable	10.21
Aqueous	Brown	Sticky solid	Characteristic	8.46

Fluorescence analysis: The results of fluorescence characteristics of powdered plant materials were presented in **Table 3** and they exhibit different colors with different chemical reagents under visible light, short and long ultra-violet light.

TABLE 3: FLUORESCENCE ANALYSIS OF POWDER OF AERIAL PARTS OF *L. NODIFLORA L.*

S. No.	Powder Treatment	Color observed		
		Visible light	UV light (254nm)	UV light (365nm)
1.	Powder as such	Green	Dark green	Brown
2.	Powder + Conc. HCl	Pale yellow	Dark green	Light green
3.	Powder + Conc. HNO_3	Reddish orange	Yellowish green	Brown
4.	Powder + Conc. H_2SO_4	Reddish brown	Blackish green	Blackish brown
5.	Powder + Glacial acetic acid	Yellowish orange	Dark green	Bright yellow
6.	Powder + 5% NaOH	Dark yellow	Greenish yellow	Brown
7.	Powder + 5% KOH	Pale yellow	Brownish yellow	Light green
8.	Powder + 5% $FeCl_3$	Brown	Green	Coffee brown
9.	Powder + picric acid	Yellowish green	Black	Blackish green
10.	Powder+NH_3	Pale yellow	Fluorescent Green	Greenish black
11.	Powder + 5% iodine solution	Dark brown	Black	Blackish green
12.	Powder+aqueous1N NaOH	Yellowish brown	Brown	Greenish black

Phytochemical analysis: The powdered plant sample of *L. nodiflora* was extracted with various solvents viz. petroleum ether, chloroform, ethyl acetate, methanol and water. These extracts were subjected to qualitative chemical tests and the results were compiled in **Table 4**. The preliminary phytochemical analysis has revealed the presence of various phytochemicals such as alkaloids, flavonoids, glycosides, steroids, saponins, phenols and tannins. The methanol extract was rich in flavonoid content and only moderate amount of flavonoids were present in ethyl acetate, chloroform and water extract. Higher concentrations of tannins, phenols and glycosides were also present in the methanol extract when compared to the other extracts. Anthraquinones, gums and mucilages were absent in all extracts. Steroids were present in all extracts moderately except water extract. Petroleum ether and chloroform extracts alone exhibit positive for fixed oils and fats. The result shows that maximum constituents were present in considerable amounts in methanolic extract compared to other extracts.

TABLE 4: PHYTOCHEMICAL SCREENING OF AERIAL PART EXTRACTS OF *L. NODIFLORA* L.

S. No.	Test for	Test applied/ Reagents used	Observation	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
1.	Flavanoids	a) $FeCl_3$	Intense green	--	--	+	+ + +	+ +
		b) NaOH-HCl	Yellow to colorless	--	--	+	++	+
2.	Alkaloids	a) Dragendorff‘s	Orange precipitate	--	+	--	+	++
		b) Hager’s	Yellow precipitate	--	++	--	+	+
3.	Carbohydrates	a) Fehling’s	Brick red	--	--	--	+	+
		b) Benedict’s	Brick red	--	--	--	+	+
4.	Steroids	Liebermann-Burchard’s Test	Bluish green	+	++	+	++	--
5.	Terpenoids	Noller’s test	Purple	+	+	--	+	+
6.	Tannins and phenols	a) 10% Lead acetate solution	White precipitate	--	--	+	+++	+
		b) 5% Ferric chloride solution	Intense color	--	--	+	+++	+
7.	Saponins	Foam test	Formation of honey comb like froth	--	--	--	+	+
8.	Fixed oils and fats	Spot test	Oily stain	+	+	--	--	--
9.	Gums and mucilage	Alcohol	Precipitation	--	--	--	--	--
10.	Protein and amino acids	a) Biuret	Violet	--	--	--	+	+
		b) Xanthoprotein	Yellow	--	+	--	+	--
		c) Ninhydrin	Purple	--	+	--	+	--
11.	Glycosides	Keller-killiani test	Brown ring	--	+	++	+ + +	+
12.	Anthraquinones	Borntrager’s test	Pinkish red	--	--	--	--	--

+++ - high concentration; ++ - moderate concentration; + - small concentration; -- completely absent

The qualitative phytochemical analysis reveals the presence of various secondary metabolites in considerable amounts in methanol extract and hence this extract alone was used for further analysis. The quantitative estimation of the phytochemicals of *L. nodiflora* was presented in **Table 5**. The methanolic extract of aerial parts of *L. nodiflora* exhibit highest phenolic content (98.31 ± 0.004mg GAE/g), using the standard curve of gallic acid. Using the standard curve of quercetin, the total flavonoid and flavonol content of methanolic crude extract was found to be 60.88 ±0.001mg QE/g and 27.46 ± 0.002 mg QE/g. The total tannin and saponin content was calculated as 5.97 ± 0.021mg/g of tannic acid equivalent and 3.52 ± 0.017mg/g of diosgenin equivalent.

TABLE 5: TOTAL AMOUNT OF PHENOLIC, FLAVANOID, FLAVANOL, TANNIN AND SAPONIN CONTENT OF THE METHANOLIC EXTRACT OF *L. NODIFLORA* L.

Phytochemical constituents	*L. nodiflora*
Total phenolic (mg/g) content (in GAE*)	98.31 ± 0.004
Total flavonoid (mg/g) content (in QE*)	60.88 ±0.001
Total flavonol (mg/g) content (in QE*)	27.46 ± 0.002
Total tannin (mg/g) content (in TAE*)	5.97 ± 0.021
Total saponin (mg/g) content (in DE*)	3.52 ± 0.017

Results are mean of triplicate determinations on basis of reference standard ± standard deviation. GAE*- Gallic acid equivalent; QE*- Quercetin equivalent; TAE*- Tannic acid equivalent; DE*- Diosgenin equivalent

GC-MS analysis: GC-MS analysis gives idea about the chemical characters of compounds present in the extract. The spectrum profile of GC-MS analysis confirmed the presence of eight major constituents in the methanolic extract of aerial parts of *L.nodiflora*. Based on the peak area (%), retention time (RT), molecular weight (MW) and molecular formula (MF), active principles were identified and presented in **Table 6**.

The major five detected constituents showed retention times of 6.86, 7.58, 8.17, 13.07 and 30.97 min, respectively [**Figure 1**]. However, based on the matching library the molecular components were recognized as: (1) 2, 7-Dioxatricyclo [4.3.1.0(3, 8)] decan-4-one (35.75%), (2) Azacyclotridecan-2-one (11.86%), (3) Benzoic acid, 4-etoxy-, ethyl ester (13.73%), (4) n-Hexadecanoic acid (10.12%) and (5) Stigmasterol (16.86%). The presence of hexanedioic acid, bis (2-ethyl hexyl) ester (2.85%), phytol (3.68%) and dodecanoic acid (5.15%) were reported in minor quantities.

TABEL 6: PHYTOCONSTITUENTS IDENTIFIED IN THE METHANOL EXTRACT OF *L.NODIFLORA* L.

S. No.	Retention time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)	Nature of compound
1.	6.86	2, 7-Dioxatricyclo [4.3.1.0(3, 8)] decan-4-one	$C_8H_{10}O_3$	154	35.75	Glucoside
2.	7.58	Azacyclotridecan-2-one	$C_{12}H_{23}NO$	197	11.86	Homopolymer
3.	8.17	Benzoic acid, 4-etoxy- ethyl ester	$C_{11}H_{14}O_3$	194	13.73	Esters
4.	11.54	Dodecanoic acid	$C_{12}H_{24}O_2$	200	5.15	Lauric acid
5.	13.07	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.12	Palmitic acid
6.	14.87	Phytol	$C_{20}H_{40}O$	296	3.68	Isoprenoid alcohols
7.	18.70	Hexanedioic acid, bis(2-ethyl hexyl) ester	$C_{22}H_{42}O_4$	370	2.85	Esters
8.	30.97	Stigmasterol	$C_{29}H_{48}O$	412	16.86	Phytosterol

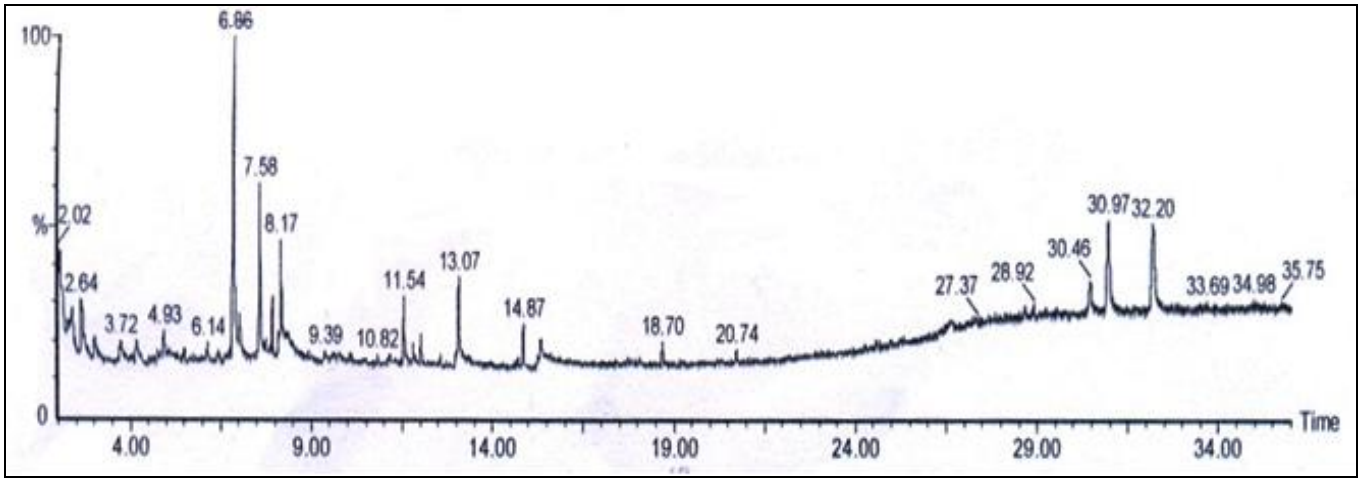

FIGURE 1: GC-MS CHROMATOGRAM OF METHANOLIC EXTRACT OF *LIPPIA NODIFLORA* L.

DISCUSSION: The most important parameter in detecting adulteration or improper handling of drugs was based on the evaluation of physicochemical parameters of the particular drug [28]. Since the plant, *L. nodiflora* has been widely used in traditional medicine to treat various ailments, it is very essential to standardize the drug for its use. The physicochemical analysis was carried out with reference to dried powder. Ash values of drug provide knowledge about the non-volatile inorganic components and some other impurities present in it.

The evaluation of purity of drugs mainly depends on the total ash present in the plant material, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica [29, 30]. The amount of inorganic elements present in drugs was determined by means of water soluble ash. From this study, the results of various types of ash values may provide a basis to identify the purity and quality of the drug. The low moisture content of drug in this result (8.91 % w/w) reveals its capability to cast down the reduction of bacteria, yeast or fungi through storage.

Extractive value of crude powder helps in the assessment of their nature, especially when their constituents cannot be readily valued by any other means. Moreover they assist in the evaluation of solubility of specific constituents in a particular solvent [31]. The percentage extractive yield was higher in alcohol than water in the present study, which signifies that the large amount of phytoconstituents of aerial parts was soluble in alcohol than water.

Several drug materials furnish a fluorescence tinge when they are exposed to chemical reagents. This might be due to the interaction of the phytoconstituents present in the crude drug and the fluorescence pattern is specific for each compound.

When the substances are not fluorescent by themselves, the chemical reagents facilitate their conversion into fluorescent derivatives and by this way some crude plant materials were qualitatively assessed. The powder of aerial parts of *L. nodiflora* exhibit various range of colors under visible -UV light and suggests probably that the plant contains active agents which help in the management of many diseases.

The phytochemical analysis expected marked importance, because the crude drugs possess varied composition of secondary metabolites which varies qualitatively and quantitatively from plant to plant and also in different samples of the same species based upon the atmospheric factors and storage conditions [32].

The prediction of chemical nature and detection of different constituents present in different polarity of solvent were revealed in the course of such preliminary phytochemical screening. Moreover the existing phytoconstituents might be accountable for the bioactivity of the plant extracts and the result of this study shows that maximum constituents were present in considerable amounts in methanolic extract compared to other extracts. Among the five groups of phytochemicals determined quantitatively, the phenolic content was found to be high in the plant extract followed by flavonoids and flavonols, while saponins and tannins were present in low concentrations. Phenolic compounds have predictable significant attention because of their potential antioxidant activities and act as free radical terminators [33].

Flavonoids are considered as one of the most varied and prevalent group of natural compounds and probably they are one of the most important natural phenolics. From literature point of view, it has been recognized that many flavonoid compounds demonstrated a wide range of activities including antioxidant properties [34] and their effects on human nutrition and health were also significant. Hence it was essential to calculate the total amounts of phenolics, flavonoids, and flavonols in specific extract.

The concentration of these compounds as shown in this study correlates to the significant antioxidant activity of the plant as reported earlier [18]. Tannins are a polyphenol group with high molecular weights and in general possess antioxidant, anticarcinogenic, antimutagenic and antimicrobial activities and it can also assist in the prevention and treatment of diseases [35]. Saponins are glycosides of both triterpenes and sterols and have rich pharmaceutical importance. About seventy families of higher plants were evidenced for its saponin contents [36]. The composition of saponin observed in this study may justify its traditional usage for the management of knee joint pain.

The chemical constituents of *L. nodiflora* were characterized by GC-MS analysis. The relative concentration of compounds eluted on the basis of retention time was shown in gas chromatogram. Among the identified phytochemicals, except stigmasterol other components were documented for the first time in this plant material. Dodecanoic acid and n-Hexadecanoic acid have the property of antioxidant and antimicrobial activities [37] and n-Hexadecanoic acid has also been reported to possess larvicidal effect [38].

Stigmasterol, a potential anti-inflammatory compound and its action is mediated by the inhibition of several pro-inflammatory and matrix degradation mediators involved in osteoarthritis-induced cartilage degradation [39]. Phytol belongs to reactive oxygen species-promoting substances and it represents a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases [40].

The biological property of other compounds identified in this study: 2, 7-Dioxatricyclo [4.3.1.0(3, 8)] decan-4-one, Azacyclotridecan-2-one, Benzoic acid, 4-etoxy-, ethyl ester, hexanedioic acid, bis (2-ethyl hexyl) ester were not assessed in a specific manner. GC-MS analysis of essential oils and hexanic fraction of *Lippia* species were previously reported [41, 42].

CONCLUSION: The physicochemical and phytochemical analysis focused in this study provides apparent information with regards to its identity and also helps to differentiate from other species. The phytochemical screening assessment may be useful in the exposure of the bioactive principles and subsequently may lead to the drug discovery and development. The data generated from quantitative estimation have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments and also revealed that the methanolic extract contains significant amount of phenols and flavonoids.

Further research is in progress regarding isolation, purification and characterization of therapeutically potent compounds from methanolic extract, which could be subjected to pharmacological analysis in order to understand the exact mechanisms of action.

CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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