

ANALYSIS OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT OF *LANTANA CAMARA* LEAVES AND FLOWERSJIGISHA ANAND^{1*}, SHANU CHAUDHARY², NISHANT RAI¹¹Department of Biotechnology, Graphic Era (Deemed to be University), Dehradun – 248 002, Uttarakhand, India. ²Department of Microbiology, Uttaranchal College of Science and Technology, Dehradun – 248 001, Uttarakhand, India. Email: jiggs21@gmail.com

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ABSTRACT

Objective: The present study aims at screening phytochemicals present in *Lantana camara* leaves and flower extracts.**Methods:** Methanolic extracts of leaves and flower of *L. camara* were prepared by the solvent extraction method. The preliminary qualitative photochemical analysis of leaves and flower extract was done following standard procedures. For quantitative phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and free radical scavenging activity of *L. camara* was estimated.**Results:** The preliminary qualitative analysis showed the presence of flavonoids, phenols, terpenoids, anthocyanins, carbohydrates, and proteins in the methanolic extracts of *Lantana* leaves and flower, while saponins, glycosides, and alkaloids were not detected in both the extracts. Quantitative assays were done for determination of TPC, TFC, and free radical scavenging activity of *L. camara*. The methanolic extract of *Lantana* flowers depicted highest TFC (15.76±0.005 mg of hydrated catechin equivalent/ml of sample) and TPC (17±0.005 mg of Gallic acid equivalents equivalent/ml of sample), while both leaves and flower extracts revealed extortionately high 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging activity.**Conclusion:** The present work depicted that *L. camara* leaves and flowers are rich in antioxidant activity and have a promising application as nutraceuticals for herbal drug formulations.**Keywords:** *Lantana camara*, 2, 2-diphenyl-1-picrylhydrazyl, Folin–Ciocalteu, Antioxidant activity.© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11i4.23900>

INTRODUCTION

Lantana camara is a popular ornamental garden plant and commonly known as wild sage or *Lantana*. It is a tropical origin plant and native to Central and Northern South America and Caribbean. *L. camara* is reported in Mexico, Florida, Trinidad, Jamaica, Brazil, Kenya, Uganda, Tanzania, and South Africa [1].

It is a woody straggling plant with small flower held in clusters (called umbels). The color usually is pink, white, yellow, violet, orange, and sometimes varying from white to red in various shades (Fig. 1). The leaves are arranged in opposite pairs and are broadly oval, rough with short hairs, with finely toothed edges along with a number of veins giving a wrinkled appearance. The leaves are 3–8 cm long and 3–6 cm wide, green in color. Leaves and stem are covered with rough hairs (Fig. 2).

L. camara is an important medicinal plant with several medicinal uses in the traditional medication system. It has been used to cure many health problems in different parts of the world [1]. The leaves have medicinal properties and exert a therapeutic effect against cuts, ulcers, catarrhal infection, tetanus, rheumatism, malaria, cancer, chicken pox, asthma, ulcer, swelling, eczema, tumor, high blood pressure, sores, measles, and fevers.

The whole plant infusion is used to cure bronchitis, and the powdered root in milk is given to children for stomachache and as a vermifuge. *Lantana* oil is used in the treatment of skin, itches, and as an antiseptic for wounds [2]. The fruits are useful in fistula, pustules, tumors, and rheumatism.

Different parts of *L. camara* are reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids,

glycosides, quinine, saponins, steroids, triterpenes, and tannin as major phytochemical groups [3,4]. These diversified phytochemicals attribute to multiple health potentials of plants and their extracts [5]. These phytoconstituents are used for the treatment of various human ailments and possesses wound healing, allelopathic, anthelmintic, anticancer, antifungal, nematostatic, insecticidal, and antihyperglycemic activity [6-9]. Thus, it is pertinent that these herbal sources are optimistic nutraceuticals and can have wide pharmacological application in herbal drug development.

To the best of our knowledge, flowers (pink turn yellow colored) of *L. camara* have not been much explored for their total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. Thus, the aim of the present study was to assess TPC, TFC, and antioxidant activity of methanolic extract of *L. camara* leaves and flowers (pink turn yellow color) and comparing their pharmacological activity. The rationale for the present investigation was to evaluate active phytochemicals in leaves and flower extracts of *L. camara* which will assist in the future determination of their efficacy as a potent antimicrobial herbal drug against clinical and drug-resistant pathogens.

MATERIALS AND METHODS

Collection of *L. camara* leaves and flowers

L. camara leaves and flowers (yellow and pink colored) were collected from a nearby locality of Gujrara, Sahastradhara Road, Dehradun (30°38'72.31"N, 78°13'16.06"E) and were authenticated at the Department of Botany, Uttaranchal College of Science and Technology, Dehradun. The leaves and flower samples have been indicated as *Lantana* leaves (LL) and *Lantana* flowers (LF), respectively. The LL and LF samples were thoroughly washed, dried, and ground into fine powder and were kept in sealed polyethylene bags in a refrigerator at 4°C.



Fig. 1: *Lantana camara* flower



Fig. 2: *Lantana camara* leaves

Preparation of extracts

The leaves and flower extract was individually prepared in 70% methanol (plant: solvent ratio [1: 10]) following maceration technique. The crude extracts of LL and LF, left as a viscous mass was weighed and their respective percentage yield was evaluated. The extracts were stored at 4°C in a refrigerator [10].

Preliminary qualitative phytochemical analysis

Phytochemicals flavonoids, alkaloids, steroids, terpenoids, glycosides, saponins, catechol tannins, anthocyanin, carbohydrates, and amino acid were traced in the methanolic extract of LL and LF following standard procedures [11].

Preliminary quantitative phytochemical analysis

Determination of TFC

The TFC was determined by the aluminum chloride colorimetric method. 100 µl of the 1 mg/ml stock solution of LL sample was gently diluted with 400 µl of distilled water in a test tube. Thereafter, the diluted sample was subsequently mixed with 30 µl of 5% NaNO₂ solution and kept at rest. After 5 min of incubation, 30 µl of 10% AlCl₃ was incorporated into the mixture and allowed to stand for another 5 min. Thereafter, 20 µl of 4% NaOH was added, and the final volume of solution was made up to 1 ml with distilled water. Absorbance of the mixture was immediately measured against blank at 510 nm. The standard curve of hydrated catechin was used for quantification of TFC (R²=0.916) [12]. The test was repeated for LF sample (1 mg/ml). Duplicate measurements were taken. Results were expressed as milligram of catechin equivalents per

gram of samples and calculated as hydrated catechin × V/M where, hydrated catechin equivalent (mg/ml), V = total volume of sample and m = weight of sample.

Determination of TPC

The TPC present in LL and LF extracts was assessed by following Folin–Ciocalteu Reagent (FCR) assay. A stock solution of methanolic extract of LL and LF (1 mg/ml) was prepared in methanol. The stock solution (20 µl) was diluted in 80 µl of water in a test tube. Then, 500 µl of FCR was added to the mixture and was kept for incubation in the dark at room temperature for 5 min. 400 µl of 7.5% sodium carbonate solution was incorporated into the mixture, agitated and left for further incubation for 30 min in the dark at room temperature. The test was repeated for LF sample as well. Absorbance of all the treated extracts was measured at 765 nm against blank using colorimeter. Water was used as blank while quantification of TPC was done on the basis of a standard curve of Gallic acid (R²=0.944) [13]. Measurements were carried in duplicates. Results were expressed as milligram of Gallic acid equivalents (GAE) per gram of samples and calculated as GAE × V/M where, GAE = Gallic acid equivalent (mg/ml), V = total volume of sample and m = weight of sample.

Determination of the Antioxidant Activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant potential of both LL and LF extracts against DPPH free radical was assessed using DPPH free radical scavenging activity. The LL and LF solvent extracts (100 µl each) were separately mixed with 1900 µl of the DPPH solution and allowed to incubate for 2 h in the dark. Absorbance was measured against blank in duplicates at 515 nm. Methanol was taken as blank while ascorbic acid was used as a standard for plotting of the calibration curve (R²=0.919) [14].

The percentage scavenging effect was calculated as:

Scavenging rate (%) = [(A₀ - A₁)/A₀] × 100, where, A₀ was the absorbance of the control (without extract), and A₁ was the absorbance in the presence of the extract.

RESULTS

Data interpretation

Statistical analysis was carried out using Windows Excel 2007. Experimental results were expressed as the mean ± standard deviation (SD). The significance of screened TPC, TFC, and antioxidant activity was determined using one-way analysis of variance followed by *t*-test. The results were considered significant at p ≤ 0.05.

Preparation of extracts

Methanolic extracts of LL and LF were prepared, and their percentage yield was evaluated. Between the methanolic extract of LL and LF, maximum % yield was depicted in LF extract (Table 1).

Preliminary qualitative phytochemical analysis

The qualitative phytochemical screening of solvent extracts of LL and LF indicated the presence of phenols, terpenoids, flavonoids, carbohydrates, amino acids, anthocyanin, and betacyanins while saponins, alkaloids, and glycosides were not detected in the test samples (Table 2).

Preliminary quantitative phytochemical analysis

There was a significant difference in TFC and TPC observed between LL extract and LF extract (p ≤ 0.05); however, there was the non-significant difference in free radical scavenging activity of LL and LF extracts and both the extracts showed favorably higher percentage of antioxidant activity (Table 3).

The TFC in methanolic extracts of LL and LF was quantified from the standard calibration curve of hydrate catechins (Fig. 3). The TFC has been recorded highest in LF with a concentration of 15.76 ± 0.005 mg

Table 1: Nature and color of methanolic extracts and their percentage yield

Methanolic extract	Nature and color of extract	% yield
LL	Lustrous thick, dark green	57.53
LF	Coal tar form, black	33.48

LL: *Lantana* leaves, LF: *Lantana* flowers

Table 2: Qualitative estimation of phytochemicals in solvent extracts of LL and LF

S. No.	Qualitative test	Leaves extract	Flower extract
	Carbohydrates		
1.	Benedict's test	+	+
	Fehling's test	+	+
	Bar ford's test	+	+
2.	Protein		
	Biuret test	+	+
3.	Saponins	-	-
4.	Alkaloids	-	-
5.	Anthocyanin and betacyanins	+	+
6.	Flavonoids	+	+
7.	Glycosides	-	-
8.	Terpenoids	+	+
9.	Phenols	+	+

Where, +: Indicates presence of the phytochemical and -: Indicates absence of the phytochemical. LL: *Lantana* leaves, LF: *Lantana* flowers

hydrated catechin equivalent/g of sample ($p \leq 0.05$).

The quantitative estimation depicted a significant difference in TPC of LL and LF extracts ($p \leq 0.05$). Methanolic extract of LF showed higher TPC at a concentration of 17 ± 0.005 mg of GAE/g of sample with reference to a standard curve (Fig. 4).

The antioxidant potential of LL and LF was quantified using the standard calibration curve of ascorbic acid (Fig. 5) and showed a non-significant ($p \geq 0.05$) difference in antioxidant potential of methanolic extract of LL and flower extracts.

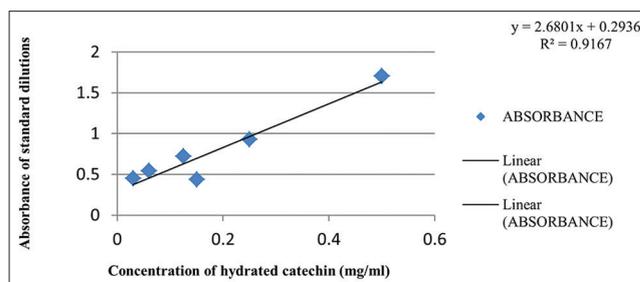
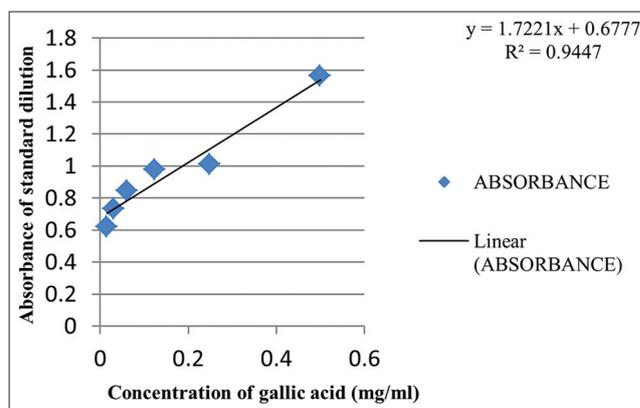
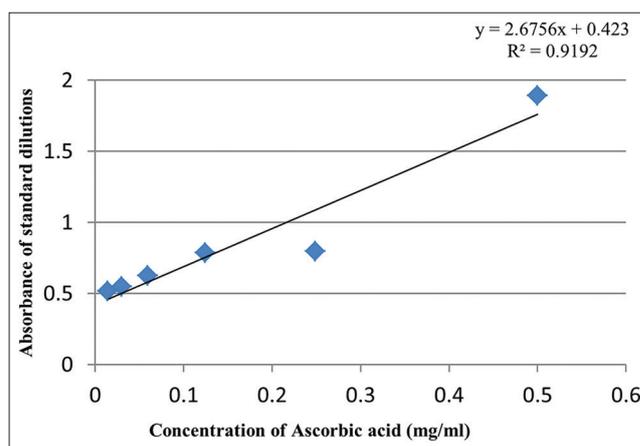
DISCUSSION

The variation in the nature and solubility of plant secondary metabolites in different solvents contributes differences in percentage yield of the plant [15]. In the present study, we also observed variation in % yield of solvent extracts of LL and flowers.

The phytochemical review of *Lantana* has indicated the presence of quinones, terpenoids, flavonoids, steroids, and glycosides as the main class of components with relevant biological activity [16]. Our findings correlate with the previous studies in which flavonoids, terpenoids, phenols, carbohydrates, amino acids, and anthocyanins were reported to be present in *Lantana* while alkaloids, saponins, and glycosides were reported absent in solvent extracts of LL [17-19].

In comparison to the present observation, Kumar *et al.* have reported higher TFC and TPC in methanolic extracts of *Lantana* plant with pink turn yellow flower [20]. It is noteworthy that maceration time for extraction was longer in our study, which could have affected the extraction of flavonoids and phenolics in the extracts [21].

With respect to the free radical scavenging activity, the presence of higher polyphenolic compounds attributes in favorable reducing capability of plants and indicates their antioxidant potential [22-24]. In the present investigation, the quantitative phytochemical analysis of leaves and flower extracts of *Lantana* exhibited a substantial amount of TFC and TPC which could have significantly contributed to their

**Fig. 3: Calibration curve of hydrated catechin as a reference for total flavonoid content****Fig. 4: Calibration curve of gallic acid as a reference for total phenolic content****Fig. 5: Calibration curve of ascorbic acid for 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging activity**

higher free radical scavenging activity. Our findings are consistent with those reported by Esmaili *et al.*, who has also reported that there is the existence of a strong relationship between phytochemical contents and free radical scavenging potential of natural resources [25].

The presence of flavonoids, phenolics compounds, and antioxidant activities in *L. camara* extracts (LL and LF) signify broad-spectrum antibacterial potential and confer their implementation as alternative therapeutic agents against pathogenic as well as antibiotic-resistant bacteria [26].

CONCLUSION

The present investigation concludes that the LL and flowers have rich antioxidant efficacy with promising nutraceuticals potency which

Table 3: Quantitative estimation of TFC, TPC, and free radical scavenging activity in methanolic extracts of LL and LF

Extracts	TFC (mg of hydrated catechin equivalent/ml of sample)	TPC (mg of GAE equivalent/ml of sample)	DPPH free radical scavenging activity (%)
LL	11.5±0.056 ^a	9.16±0.016 ^b	86.3±0.354 ^c
LF	15.76±0.005 ^a	17±0.005 ^b	87.2±0.003 ^c

a, b, and c represent significant difference in student's *t*-test. Values are expressed as mean±SD (n=2). For, TFC and TPC, means in the same column with same letters are significantly different (p≤0.05), while for DPPH free radical scavenging activity, means in the same column with same letters are statistically non-significant (p>0.05).

Row (degree of freedom=3), column (degree of freedom=1). LL: *Lantana* leaves, LF: *Lantana* flowers, TFC: Total flavonoid content, TPC: Total phenolic content, GAE: Gallic acid equivalent, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, SD: Standard deviation

confers its pharmacological application in herbal drug development against debilitating diseases.

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AUTHOR'S CONTRIBUTION

JA designed the study and wrote the manuscript. SC and JA performed the experiments. JA and NR analyzed and verified the data.

CONFLICT OF INTEREST

The author's declares no conflict of interest.

ABBREVIATIONS

TFC- Total flavonoid content
 TPC- Total phenolic content
 GAE- Gallic acid equivalent
 DPPH- 2, 2-diphenyl-1-picrylhydrazyl
 LL- *Lantana* leaves
 LF- *Lantana* flowers
 FCR- Folin ciocalteu reagent
 SD- Standard deviation
 ANOVA- Analysis of variance

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